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TABLE OF CONTENTS OF VOLUME IX (1940/41)

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ALCOHOLISM IN THE RURAL ENVIRONMENT[1]

By

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Among the factors influencing the economic standards and the state of health of rural populations, the consumption of alcohol is by no means the least important. If the question of alcoholism is apt to receive less than its due share of attention in plans to improve the health standards of the countryside, this is doubtless owing to the complexity of the problem and to the tragic conflict between the economic and the health interests which are bound up with its production on the one hand and its consumption on the other.

The matter is one of special interest to rural populations, for a very considerable portion of the produce grown is intended for the distillation of alcohol, and a fair number of farmers are equipped for this purpose. In many wine-growing districts, alcohol represents the one source of income on which the well-being of the population rests. In areas where the land is suitable for potato- and wheat-growing, some farms use the whole of their surplus crops for distillation. This enables them to employ a great deal of labour and to make considerable profits, for alcohol is always readily saleable. Despite its high price, a really palatable alcohol always finds a market, and this is why, at a certain stage of their development, farm estates always tend to take up alcohol production as a means of securing their income. The consequence is an increase in this type of production to the detriment of that of agricultural

[1] Report drawn up for the European Conference on Rural Life.

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produce for direct consumption, which may indirectly affect the price of the latter and thereby the standard of living.

It is but natural that the public authorities should have turned their attention to the alcohol market, with its high turnover and its great possibilities as a source of Government revenue. In many countries, State intervention has been carried to the length of instituting a Government monopoly for the production and sale of alcoholic liquor. Where such conditions prevail, not only the grower, manufacturer and dealer, but the Treasury as well is an interested party in the production and sale of alcohol. And so, whilst acknowledging the soundness of the case for prohibition, it is averse from taking the risk of looking for some other source of revenue capable of the same yield as liquor taxes. In the wine-growing countries in particular, the Government could not contemplate promoting the transition to some other type of agricultural production with any real hope of its proving as lucrative as the wine grape. For such countries, wine is frequently the main export item which keeps their foreign trade exchanges going and stabilises their currency. In the wheat- and potato-growing countries, on the other hand, the situation is not so difficult ; nevertheless, if the sale of agricultural produce intended for the distillation of alcoholic liquor were not ensured in advance, it would lead to a substantial reduction in the output of such liquor, which might unbalance the budget and produce an agricultural crisis.

The question of alcohol must therefore always be approached from the twofold standpoint of the producer and the consumer. It might be thought an easy matter to calculate alcohol consumption figures and watch their fluctuation. It is not.

Even surveys limited to small groups and conducted by highly accurate methods do not yield exact consumption figures, for, even in countries where the consumption of alcohol is high, the investigating officers find difficulty in eliciting the real facts, and the information they are given is usually unreliable. None the less, approximate though they be, the figures obtained point definitely to the conclusion that the consumption of alcohol raises an extremely serious health, economic and social problem and that, so long as this problem remains unsolved, there can be no prospect of an improvement of standards of living in the rural environment.

In order to obtain accurate information as to the present position in the various countries, an enquiry was carried out last year through the agency of the International Bureau against Alcoholism. Twelve countries sent in replies ; some of them supplied very precise details. This enquiry, which was based on a questionnaire, comprised thirteen main and five supplementary questions, as follows :

Main Questions.

1. Number of inhabitants.
2. Percentage of the rural population.
3. *Per caput* consumption of alcohol.
4. Quantity of alcohol consumed by the rural population.
5. Are the interests of the rural population involved in any way in the production of alcoholic liquor ?
6. What percentage of the total income of rural populations is derived from the production of alcoholic liquor ?
7. Number of cases of illness or death attributable to alcohol.
8. Number of offences or crimes committed under the influence of alcohol.
9. Has the situation changed during the past few years, and if so, in relation to what factors ?
10. Number of accidents of various kinds due to the consumption of alcohol.
11. Did the economic position change when the consumption of alcohol was reduced or increased, and if so, in what way ?
12. What means are applied by the public authorities and temperance societies in their campaign against alcohol, more especially in the rural areas ?
13. Number of public bars.

Supplementary Questions.

1. What approximately is the percentage of the total income of country dwellers which is laid out on :

   (*a*) Alcoholic liquor ? (*b*) Tobacco ?

2. Is alcoholic liquor given to children ?
3. What are the traditional occasions, festivities, customs, etc. which furnish an opportunity for the consumption of alcohol ?
4. Which category of rural inhabitants is most addicted to alcohol : farmers, agricultural labourers, artisans, etc. ?

5. Is the production of fruit beverages widespread in the country (sweet cider, verjuice, etc.) ?

The above enquiry could not be expected to yield accurate figures of unimpeachable scientific value. Indeed, the figures cannot be so regarded, for they are intended only to be generally descriptive of the situation. None the less, quite definite conclusions can be drawn from the opinion of persons who are experienced in this field and can speak with authority.

Quantities of Alcohol consumed by the Rural Populations

In all countries, the rural population is an important consumer of spirits and other alcoholic liquor. There is considerable difficulty in calculating what percentage of the total population it represents, for the very concept of "rural population" is difficult to define in a satisfactory manner. In the case of those countries which answered the questionnaire, the percentage varies between 15 (Denmark) and 77 (Yugoslavia). Immediately after Yugoslavia comes Poland (74). The figures for the remaining countries are :

	%		%
Latvia	59.0	Finland	34.0
Hungary	51.8	Germany	29.0
France	48.8	Switzerland	23.0
Sweden	37.7	England and Wales	18.0
Scotland	36.7	Denmark	15.2

Other things being equal, the countryman will undoubtedly drink no less alcohol than the townsman, but the impoverishment of the rural population consequent on the severe economic depression that has prevailed in the agricultural industry has led to a more marked reduction of alcoholic consumption in the country than in the town. Whilst precise figures as to the amount of this reduction are not obtainable, the consumption of alcohol in the rural environment can undoubtedly be estimated from the total figure for the country as a whole. In countries in which the latter is high, the proportion accounted for by the country districts is greater than in those with a lower all round annual *per caput* consumption.

According to the data collected by the Central Statistical Office of Poland, the mean annual *per caput* consumption of the principal alcoholic beverages during 1932 to 1934 was as follows (compared with the years 1929 to 1932).

Wine	1929-1932	1932-1934	Beer	1929-1932	1932-1934	Alcohol	1929-1932	1932-1934
	Litres			Litres			Litres	
France	166.9	176.4	Belgium ...	210.5	182.1	France	2.75	2.54
Italy	89.8	84.3	England ..	68.7	71.9	Denmark ..	2.10	2.10
Spain	74.7	76.1	Switzerland	64.8	60.5	Belgium ...	1.50	1.12
Switzerland	43.8	41.9	Denmark .	58.2	53.5	Holland ...	1.25	1.07
Hungary ..	35.2	28.7	Germany ..	54.8	42.1	Switzerland	1.09	1.05
Austria ...	21.0	15.8	Sweden ...	47.1	41.7	Norway ...	1.11	1.00
Belgium ...	4.9	3.4	Austria ...	71.2	38.5	Poland	1.23	0.77
England ..	1.4	1.3	France	34.6	35.2	Germany ..	0.94	0.69
Norway ...	1.6	1.2	Holland ...	26.9	19.4	England ..	0.70	0.57
Denmark ..	1.6	1.1	Norway ...	17.3	14.6	Italy	0.45	0.30
Holland ...	1.7	1.0	Poland	7.4	3.6			
Poland	0.1	0.0	Hungary ..	5.2	1.9			

The replies to the enquiry have confirmed these particulars, as will be seen from the following table :

Country	Population (in millions)	Rural population %	*Per caput* consumption of alcoholic liquor				
			Pure alcohol	Wine	Cider and fruit wines	Beer	Spirits
					Litres		
Germany	79.4	29.0	4.1	7.3	?	56.4	3.1
England	40.8	18.0 [1]	4.9	?	?	93.1	1.0
		1937					
Scotland	4.8	36.7	3.0	?	?	36.1	11.8
Denmark	3.7	15.2	2.4	0.2 [2]	0.1 [2]	1.7 [2]	0.4 [2]
Finland	3.8	34.0	1.4	0.4	?	5.7	27.6
France	42.0	48.8	20.	165.0 [3] / 117.0 [4]	11.0	34.	2.6
			1932/33				
Hungary	9.0	51.8	4.3	41.9	0.5	2.7	0.8
Latvia	2.0	59.0	4.5	0.1	—	6.0	5.4
Poland	35.0	74.0	1.2	0.1	?	3.6	?
Sweden	6.3	37.7	3.5	0.7	?	21.4	4.5
	1930						
Switzerland	4.1	23.0	12.3	47.0	40.0	64.0	6.0
Yugoslavia	15.4	76.4	6.0	27.4	?	2.6	6.5

[1] The rural population is not wholly agricultural.
[2] In terms of pure alcohol.
[3] Figures given by the *Office du vin*.
[4] Figures given by the *Office fiscal du Trésor*.

As might be expected, the great consuming countries are those which produce the largest quantities of alcoholic liquor and whose agricultural produce is intended for conversion into alcohol. Foremost among these are the wine-growing countries. For instance, the consumption per head of 100° alcohol is 20 litres in France, 12.3 litres in Switzerland and 6 litres in Yugoslavia. Being itself a producer of alcoholic beverages, the rural population in these countries has greater facilities for consumption than the population of the towns. Hence there is reason to suppose that the consumption of the country districts is not merely not smaller, but is indeed greater than that of the towns. According to M. BASTIDE, of the International League against Alcoholism, 40% of the rural population in France distil alcohol for their private use. The 1934 statistics show that, out of a total number of 3,104,534 distillers, 3,102,440 are merely *bouilleurs de cru* (owners of stills operating with their own produce) who are entitled to put aside for their own use 10 litres of pure (palatable) alcohol tax-free. It may be assumed that this figure is frequently exceeded, and it is impossible to determine what quantity of alcohol is illegally produced and held.

In France, 19% of the rural population are engaged in wine-growing, whilst, at the same time, ranking first as consumers of their own products. Cider is produced and stored more especially by the farmers of Britanny and Normandy. Except for *apéritifs*, spirits and fruit liqueurs are consumed in larger quantities in the country than in the towns.

The Swiss Rural Office has published the results of its research on the agricultural returns for the years 1933/34 ; certain figures are given which indicate the level of consumption of alcohol in the countryside. These data, which have been obtained from selected estates, are only of limited value : they have reference to private consumption.

	Annual *per caput* consumption	Value (Swiss francs)
	Litres	
Cider	153.4	26.70
Wine	17.5	12.70
Spirits	2.5	4.90

The author is moreover of the opinion that these figures cannot be taken as standards, since the consumption of alcohol assumes many diverse forms.

The consumption also varies according to the size of the estate concerned. According to the data referred to above, it works out as follows :

Size of the estate (in hectares)	Cider	Wine (in litres)	Spirits
3-5	107.8	37.5	1.8
5-10	137.6	17.2	2.1
10-15	158.4	15.1	2.3
15-30	170.9	14.7	3.2
Over 30	160.2	18.5	3.2

The great variability according to district is well illustrated by the figures supplied by the Federal Alcohol Department. They relate to the tax-free spirits kept by the producers for so-called " family " consumption during the period 1932/33.

	Litres per estate
Central Switzerland	25
Lucerne	74
Fribourg	43
Berne	25
Zurich	16
Ticino	9

Since the Federal Monopoly is unable to exercise any réally reliable supervision over the total amount of spirits kept by producers or over the uses to which it is put, the mean figures given above may be assumed not to represent the real facts of the case and to indicate rather less than the true amount involved.

In Sweden, according to Axelman, the consumption of alcohol in the country is lower than in the town, so far at least as wine, beer and spirits are concerned. The quantity of cider consumed, on the other hand, is higher.

In Poland, the consumption of alcohol is lower in the rural than in the urban districts. Wine is practically a negligible quantity. The quantity of beer consumed is extremely small. The only real item is spirits, and even these are consumed in lesser quantities than in the towns. In actual fact, we have no precise figures, for no statistics of sales to the rural population are kept. There is reason to suppose, however, that they fluctuate between 0.2 and 1 litre of 100° alcohol, and, in the large cities, between 3 and 4 litres per head per annum.

Considerable differences are to be observed, moreover, according to regions. Less alcohol is drunk in the eastern and southern areas than in the western. The highest consumption is recorded in the central voivodships, where we find the two largest cities of Poland—Warsaw and Lódź. The highest consumption of beer occurs in the southern voivodships and, after them, in the western districts bordering on Germany.

Allowance should be made for a certain quantity of alcohol obtained either by illicit distilling or smuggled in from abroad, especially in the frontier districts. The poor population of the east sometimes drinks denatured alcohol. The clandestine distillation of alcohol, which is severely punished by law, is only a minor factor in the general standard of health. Smuggling, on the other hand, despite the close supervision exercised on the frontiers, is still very widespread ; contraband is carried on not only with alcohol, but also with ether, which, in certain areas, is consumed in the same way as alcohol.

Are the Interests of the Rural Population involved in any Way in the Production of Alcoholic Liquor ?

It is clearly to the interest of the rural population to convert some part, or the whole, of its produce into alcoholic liquor, this being more profitable than the sale of the raw produce, and a market being assured. At the same time, the interests of the whole of the population are not involved in the same measure. In Switzerland, for instance, there are areas which afford no opportunities for making extra profits in this manner, the soil being unsuitable for either fruit- or wine-growing, and distillation from potatoes and grain being prohibited. There are, on the other hand, areas in which more than 10% of total gross income is derived from fruit-growing and the subsequent conversion of the fruit into cider or spirits.

Taking Switzerland as a whole, the mean return from fruit represents 7% of total gross income. It is difficult, however, to estimate both the quantity intended for direct consumption and that converted into alcoholic beverages. Finally, in certain wine-growing areas, the production of wine is the only revenue-yielding enterprise of the rural estates. In Switzerland, viticulture accounts, on the average, for 4%-5% of gross income.

Of 238,469 rural estates (1929 census) more than 200,000 possess orchards and 41,800 have vineyards. The number of estates of which more than 25% of the total cultivated area is under vines is 6,128, including 2,466 in which the proportion exceeds 75%.

In England and Wales, the interest which the rural population has at stake in the production of alcohol also varies according to districts. In Devonshire, Herefordshire and Gloucestershire, as in certain other counties, the rural population produces cider for its own consumption and for sale. Hop-growing is an important industry in certain parts of Kent, in which there are large malt factories, breweries and distilleries. The rural population also works in these establishments.

In France, alcohol produced from fruit is subject to a very high tax (2,500 francs per hectolitre), the effect being to induce farmers to produce alcohol for their own needs and to become, as already mentioned, *bouilleurs de cru*. The wine and cider taxes are less heavy. The French farmer makes a large quantity of wine for his private use and for that of his labourers, who are supplied with wine in part payment of their work.

According to the 1935 statistics, this production (139,103,000 francs) represents more or less the only source of income of 1,658,000 farmers. The production of cider, perry and especially spirits of various kinds yields an important part of the revenue of the remainder of the population.

For France as a whole, the total outlay for the purchase of alcoholic liquor amounts to 25,000 million francs.

In Sweden, only the southern areas are interested in alcohol production, for it is only there that the potatoes and barley suitable for the purpose are grown.

In Latvia, the production of alcohol offers very little prospect of advantage to the rural population. In 1936, the distilleries converted 68,475 tons of potatoes. The farmer receives on an average 40 to 44 lats per ton. In addition to this, 4,036 tons of malt were sold to the breweries at about 200 lats a ton.

In Poland, Government revenue reaches an annual total of about 2,426 million złoty. Of this sum, the portion contributed by the Alcohol Monopoly amounts to 291 millions; in other words, one-eighth of the Government revenue is derived from the sale of alcoholic liquor. If account is also taken of the various intermediate sources

of income which this industry affords to the Treasury in a number of ways, it will be realised at once that the real importance of this branch of production in the national economy and in the budget is even greater. For the financial year 1937/38, the total gross income from the sale of alcohol for consumption amounted to 383 million złoty. To this there should be added a further sum of nearly 2 millions representing the tax yield from the alcohol supplied to manufacturers of spirituous liquor and the duty on imported alcohol. The operating costs of the Alcohol Monopoly for the same financial period having amounted to 151 million złoty, the difference represents the net profit accruing to the Treasury, which is still further increased by the sale of alcohol for purposes other than consumption. It has, moreover, to be considered that the employment which is afforded to a certain number of employees and workers and the payments received by certain branches of industry furnishing essential supplies for the manufacture and sale of alcohol (bottles, corks, etc.) are among the many other obstacles which stand in the way of a transformation of this branch of production on lines more conformable to the requirements of public health.

The situation presents itself in a still more serious light when another aspect of the consumption of alcoholic liquor is considered. Poland does not rank as one of the large beer-consuming countries; yet, in 1937, 1,317,000 hectolitres were drunk there. The bulk of this was home produced, for imports are very small. This production represents 60 million złoty. The other alcoholic drinks, such as wine, fruit wines and mead, which play a much more important part in other countries, are not a very large item in the Polish budget; none the less, their value amounts to several million złoty. It is generally true to say that this type of production may be of economic importance to countries which are not in a position to produce wine in large quantities.

Moreover, all these branches of production give employment to a substantial number of employees and workers. The middleman and the retailer also provide work for large numbers. The following table gives the relevant particulars for the distilleries and breweries in Poland.

	Agricultural and industrial distilleries	Breweries and malt factories
Number of establishments	1,375	166
Number of workers	2,915	4,750
Number of employees	1,496	921
Aggregate annual payroll (in zloty)	4,501,000	9,975,000

To these there should be added the persons employed by the State Alcohol Monopoly, who number 783 and whose pay totals 4,286,000 złoty per annum. About 10,000 persons in all are engaged in Poland in the manufacture of alcoholic liquor and receive annual wages totalling 20 million złoty.

It is impossible to give precise statistical data on the number of persons working as middlemen and in the retail trade. The 6,038 industrial licences issued to commercial undertakings in 1937 do not give an adequate idea of the number of employees of all kinds occupied in the handling of and the trade in alcoholic liquor. The number of workers occupied in this branch is, in any case, high; so much is certain, and any declaration of prohibition forbidding the production of and trade in alcoholic liquor would effect a serious disturbance in several departments of economic life. This disturbance would be particularly grave in the trade in agricultural produce, especially potatoes, of which a large proportion is converted into alcohol.

In 1937, the potato harvest yielded 402,210,000 quintals. The quantity supplied by small-holders (farms of less than 50 hectares) was 346,975,000 quintals. In 1936/37, 6,121,000 quintals of potatoes, or $1\frac{1}{2}\%$ of the total harvest, were used for the manufacture of 77,782,000 litres of alcohol. This would admittedly be of no great consequence if the marketing of potatoes were not difficult and if the price of agricultural produce were sufficiently high to yield an adequate income. That, however, is not always the case. In countries whose economic structure is agricultural, the price of the produce of the soil remains low and frequently falls below the cost of production. When one portion is converted into alcohol and paid for at high rates, the general level of the farm income is raised, even though the proportion of the whole which is thus converted may be small. In practice, however, the distillation of alcohol from potatoes takes place more especially on the large industrialised rural estates.

It may therefore be inferred that, in Poland, the small-holders (estates of less than 50 hectares) have but a limited economic inducement to produce alcohol. This is also true of beer. The advantage that may accrue to the agricultural labourer is also very small, since, as will appear from the figures quoted above, only a tiny number find employment in the distilleries and breweries.

It would be extremely useful if some idea could be gained of the illicit production of alcoholic liquor and of the share of the rural population in this. Obviously, the higher the tax on alcohol, the greater the inducement to evade the law, and the more frequent the attempts at illicit manufacture. Offences of this kind are, however, comparatively infrequent in non-wine-growing countries. In Poland, cases of consumption of denatured alcohol, previously freed from its unpalatable ingredients, are fairly frequent. The process involved does not eliminate the methyl alcohol, and poisoning causing blindness has sometimes occurred. Among 44 cases of blindness observed, Dr. Rostkowsky[1] found 9 which were due to denatured alcohol.

In the United Kingdom, according to Weeks, the illicit production and sale of contraband alcohol is negligible, save indeed for small quantities fraudulently imported by travellers. During the period 1936/37, a total quantity of 128 gallons (581 litres) of alcohol was confiscated. During the period 1932-1937, 68 cases of illicit distillation were brought to light in England, 57 in Scotland and 400 in Northern Ireland. This gives a total of 525 cases, mostly concerned with the production of very small quantities.

Quite obviously, a rural population will tend to engage in the manufacture of alcoholic liquor only in such measure as it can derive a profit therefrom. It is, however, scarcely possible to determine the rate of profit with accuracy, for it depends upon the kind of agricultural production.

In areas in which the rural population is engaged in wine- and fruit-growing, the abolition of these forms of agricultural production would endanger their vital interests ; where beer and alcohol are produced, the interests of the population are less involved, for the agricultural produce intended for conversion into alcohol is generally

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[1] Dr. Rostkowsky : " Contribution to the Campaign against Blindness in Poland " (*Review of Trachomatology*, 1936, No. 2).

grown upon large and better equipped estates and represents, moreover, but a small proportion of the total output. In such cases, the production of alcohol could be reduced without dangerously disturbing the living conditions of the rural population.

Number of Offences and Crimes committed under the Influence of Alcohol

In the light of the above considerations, it may therefore be concluded that the rural populations of all countries have some interest at stake in the manufacture of alcohol in conjunction with their agricultural production, and that the proportion whose interests are involved is in any case larger than among the urban populations. In the matter of consumption, the country-dwellers usually fall behind the town-dwellers ; only where the production of alcoholic liquor is carried on by small producers, who are entitled to reserve a portion of their output for themselves free of tax, does the rural consumption probably stand higher than the urban.

The countryman therefore spends less money on drink than the townsman. Even in countries in which the grower-producer consumes a large proportion of his own wine, he is less likely to involve hinself in ruinous expenditure than is the town-dweller. An exception must be made, however, in the cases in which the rural population is exploited by innkeepers who supply alcohol on credit and obtain payment in kind in the form of work. This relegates the agricultural labourer to a condition of dependence which is akin to slavery, for he can never, in practice, free himself from his debt towards his creditor, and so frequently brings both himself and his family to ruin.

The increase of criminality caused by alcohol is certainly one of its important ill effects. There can be no doubt that alcohol is the direct or indirect cause of a substantial number of offences. One class of offence, in particular, is closely connected with the consumption of alcohol. In periods during which the drink evil declines, the frequency of such offences is similarly diminished. The subjoined statistical tables taken from C. C. Weeks's book, *Alcohol and Human Life*, may be taken as an example for England, Scotland and Wales.

SCOTLAND
(per 100,000 population)

Class of offence	Annual average 1910-1914	1932	1933	1934	1935	1936
Offences against the laws on alcoholic liquor : drunkenness and various disturbances	870.0	243.0	240.0	251.4	268.2	291.8
Disorderly conduct	1,007.0	349.4	367.0	388.7	390.6	408.1
Cruelty to children	14.3	4.1	5.0	5.4	6.3	5.9
Indecent behaviour not induced by drink	31.5	16.3	14.8	17.2	16.0	19.4
Indecent behaviour	52.2	7.5	7.7	6.2	6.3	7.2
Consumption of alcohol, in gallons per head	1.29	0.329	0.308	0.341	0.344	0.360
Beer, in gallons per head	—	6.4	6.7	7.1	7.4	7.6
Pure alcohol, in gallons per head .	—	0.5	0.5	0.6	0.6	0.6

ENGLAND AND WALES
(per 100,000 population)

Convictions for drunkenness.....	332.2	72.1	49.7	49.1	48.2	46.5
Cruelty to children	9.4	1.8	1.6	2.0	2.3	2.3
Indecent assaults (not accompanied by drunkenness) .	10.0	8.7	8.7	9.2	9.0	9.7
Consumption of alcohol, in gallons per head	0.6	0.2	0.2	0.2	0.2	0.2
Beer, in gallons per head	32.5	16.4	17.2	18.3	19.2	19.6
Wine, in gallons per head	0.25	0.3	0.3	0.3	0.3	0.4
Pure alcohol, in gallons per head .	—	0.8	0.9	0.9	1.0	1.0

The position in Sweden is much the same (Swedish Statistical Office) :[1]

Alcohol and Criminality in Sweden.

Years	Prisoners (male)	Percentage of crime due to drunkenness	Prisoners (female)	Percentage of crime due to drunkenness	Annual consumption of alcohol per head of population (litres)
1887-1897	24,398	72.2	2,766	13.4	4.9 (1886-1890)
1898-1907	28,267	71.6	2,559	18.0	—
1908-1917	29,848	59.0	2,084	21.9	—
1918-1926	27,086	35.7	1,734	20.5	2.9 (1923)
1927-1935	21,083	42.5	1,130	14.7	3.5

Although it is difficult to draw the line of demarcation between offences committed in the country and in the town, the figures available nevertheless warrant certain conclusions.

Criminal statistics for 46 districts in England and Wales during the year 1935 provide interesting data in this respect (WEEKS).

(*a*) England (alone) :

Male population : 18,331,600, including 2,901,360 (15%) belonging to the rural population. Of 4,191 cases of rape and sexual offences, 37% were committed in rural areas. Of 18,708 cases of assault, 30% occurred in the country. Of 934 cases of ill-treatment of children, 30% are spread over the country districts.

(*b*) Wales (alone) :

Male population : 1,269,400, including 743,240 (59%) belonging to the rural population. Of 177 cases of rape and other sexual offences, 60% were committed in rural districts. Of 7,274 cases of assault, 70% took place in rural districts. Of 29 cases of ill-treatment of children, 70% occurred in the country. It should be pointed out, however, that, in the countryside, offences are more readily discovered than in the town. Moreover, a certain proportion are committed by mentally afflicted persons, of whom a greater number are at large in the countryside than in the towns.

[1] Table quoted from WEEKS.

Drunkenness : Number of Convictions in 1936.

	Males	Females	Total	Per 100,000 population
England :				
Rural districts	10,295 (336)	703 (26)	10,998 (362)	5.12
Urban districts + London	26,281 (242)	5,740 (72)	32,021 (314)	13.07
Wales :				
Rural districts	1,061 (24)	40 (6)	1,101 (30)	5.97
Urban districts	380 (32)	25 (8)	405 (40)	8.75
		Total	44,525 (746)	

The figures in brackets indicate methyl-alcohol poisoning (estimated). As for the data for previous years, these show that the average proportion of convictions for drunkenness in rural districts as a whole is 16%, a lower figure than might have been expected, having regard to the conditions of the rural population.

The data relative to Scotland are no less interesting. In 1936, the total number of offences and crimes was 128,889, the number of arrests for drunkenness and for offences or crimes committed under the influence of drink was 16,184, whilst arrests for a breach of the peace totalled 20,269. There were 21 cases of criminal assault, 32 cases of murder and 1,646 cases of assault and battery. All the breaches of the peace were, of course, not committed under the influence of drink. In Scotland, however, it is the practice not to discriminate between these various offences, so that alcohol may be assumed to have been a factor in 28.3% of all the offences recorded.

Scotland has 41 towns of over 10,000 inhabitants, with a population of 2,847,901, or 58.8% of the total population. The remainder of the population (small towns and villages) totals 1,995,079, or 41.2%.

The total number of crimes, offences and petty offences was 88,405. 40,494, or 45.6%, were committed by the populations of small towns and villages.

Arrests for drunkenness numbered 29,170 for the population as a whole, and 7,263, or 24.7%, for the small towns and villages.

In the case of Denmark, the figures are more favourable to the rural population. In 1935, the number of arrests made for offences

or crimes committed under the influence of drink were as follows :

At Copenhagen and Frederiksborg	6,188
In the rural districts	664

In Finland, the number of offences committed under the influence of alcohol is slightly larger in the country than in the towns—*i.e.*, 3,943 as against 2,946. Unfortunately, the year to which the figures relate is not stated.

In France, in the course of 1933, 1,202 delinquents, including 147 minors, were convicted before the courts. The offences in question are, of course, only those connected with the abuse of alcohol. 480 persons were condemned for (voluntary) homicide—committed under the influence of drink in 78 cases—whilst 898 were sentenced for blows and wounds (158 of the offenders being in a state of inebriation) and 354 for theft, arson and breach of trust (37 of these offences being committed under the influence of alcohol).

The correctional tribunals condemned 222,090 delinquents, of whom 20,577 were under age. Of these young people, 80% were probably children of alcoholic parents. Of the offenders in question, 8,877 were condemned for delinquencies or crimes committed under the influence of drink, and 5,875 for miscellaneous offences and drunkenness. The record for these offences is held by the Department of Finistère, which had 5,875 charges of drunkenness brought by the police.

In Hungary, in 1938, there were recorded 53,402 offences and crimes probably connected with excessive consumption of alcohol. 67.8% of the offences took place at Budapest, and the remainder are spread over the whole population, which is mainly rural.

The data concerning the mainly agricultural countries are particularly interesting. It is among these that Latvia should undoubtedly be classified. In 1937, 444 persons were sentenced for various crimes and offences committed whilst in a state of inebriation, 37 of them being charged with murder. In addition, 15,551 charges were made in respect of various offences connected with the abuse of alcohol, 13,807 relating to drunkenness, 1,253 to the illicit trade in alcoholic liquor and 491 to the unlawful manufacture and sale of alcohol. Unfortunately, no details are available to show what proportion of these offences were committed by the rural population.

In Poland, no data are available which would make it possible to

judge how far alcohol helps to increase the number of offences and crimes in the rural environment. The abridged *Statistical Year-Book, 1938*, gives the following figures for offences likely to be connected with alcoholism :

	1935	1936	1937
Obstructing the police	6,003	5,616	5,300
Blows and wounds	17,215	19.531	18,416
Drunkenness	61,980	69,266	72,488
Disturbance of the peace	107,344	120,326	124,651

From these figures it will appear that, save for " obstructing the police ", there is a tendency for all these offences to increase in number, which is proof of the growing danger of alcoholism.

It should be mentioned that the majority of the offences were committed in the towns and, to be more precise, in the two great industrial centres of Warsaw and Łodz. A similar tendency is observable, however, in rural areas. Day-by-day experience shows, moreover, that the village inn is frequently the source of various kinds of offence, especially blows and wounds—indeed, even murder —and that these occur more especially before and during high days and holidays, which rarely pass off without quarrels and violence. The growth in the number of offences is connected, in the first place, with the improvement of wage levels in town and country alike. When agricultural produce is selling well, the consumption of alcohol tends to increase in the countryside, leading to excesses which are reflected in an increased incidence of crime and disease.

Among the offences which occur as a result of the abuse of alcohol, reference may be made to road traffic accidents. This is a matter which concerns, more especially, the towns, for motor-cars are less numerous in the country. It is not proposed, therefore, to go into it here, although it is of major importance. In any case, it has been discussed with great thoroughness in the above-quoted book of Dr. Weeks.

Number of Cases of Death or Disease attributable to Alcohol

It does not seem that any useful purpose would be served by quoting all the publications which deal with the relationship between the use of alcohol and morbidity and mortality rates, especially since

we are only concerned here with the question whether the frequency of sickness attributable to the use of alcohol is greater in the country or in the town. Accurate data on this point would enable useful conclusions to be drawn as to the frequency of alcoholism in the rural districts.

Under present circumstances, the way to obtain accurate indications as to the damage done by alcoholism will be to determine the frequency of those cases of disease and death which can be ascribed beyond doubt to the use of alcohol, more especially cirrhosis of the liver, chronic alcoholism and nephritis.

For England and Wales, the following details are available as to deaths occurring during 1932 to 1936 :

Year	Total deaths		Cirrhosis of the liver		Nephritis	
	Males	Females	Males	Females	Males	Females
1932	245,715	238,414	946	505	8,298	8,018
1933	250,625	245,840	817	424	7,687	7,786
1934	242,855	233,955	872	476	7,894	7,717
1935	243,458	233,943	898	436	8,059	7,995
1936	253,319	242,445	851	414	7,952	7,771
1937	260,057	249,517	792	441	7,593	7,472

For the rural districts outside London, the corresponding details are as follows :

Year	Total deaths		Cirrhosis of the liver		Nephritis	
	Males	Females	Males	Females	Males	Females
1932	48,216	46,263	180	81	1,723	1,565
1933	47,386	45,493	162	69	1,506	1,444
1934	45,790	43,204	150	79	1,619	1,417
1935	44,982	42,286	175	63	1,553	1,458
1936	46,418	42,902	161	68	1,561	1,445
1937	46,616	43,461	131	58	1,510	1,346

In Scotland, during 1936, 32 out of 66,749 deaths were caused by chronic alcoholism and 176 by cirrhosis of the liver.

In Denmark, in 1937, 71 deaths from alcoholism were reported, including 17 in Copenhagen, 41 in other towns and 13 in rural districts.

It would be difficult to draw conclusions from the cases of *delirium tremens* recorded in Danish statistics. Of 28 cases registered in

1935, 8 occurred in Copenhagen, 11 in other towns and 9 in the rural districts.

In the case of Poland, it has been impossible to obtain similar data. It is believed, however, that the urban population is more seriously affected by the abuse of alcohol than the rural population.

Has the Situation changed during the Past Few Years and, if so, in Relation to What Factors ?

This is a question of fundamental importance to public health. The interrelation of the factors affecting public health will obviously differ according to whether the danger is increasing or abating. For purposes of public health policy, it is moreover important not only to know the consequences entailed by a greater or lesser consumption of alcohol but, in addition, to analyse the causes of observable variations in this field.

It may be presumed that an excessive use of alcohol is closely bound up with the economic situation of the population. The well-to-do classes drink more alcohol than the poorer classes. The economic depression has exerted a quite definite influence on the quantity of alcohol consumed, and it will be readily understandable, therefore, that when the agricultural produce market is weak, the consumption of the rural population stands at a lower level than at times when sales are easy, as during the period immediately following the great war. The improvement of market conditions for agricultural produce brings about a recrudescence of the consumption of alcohol in the countryside. Similarly, the urban population, which generally earns more money than the rural, spends more on drink.

Owing to the lack of adequate data, it is unfortunately impossible to say for certain whether the slow but steady improvement of the agricultural produce market which has been taking place latterly is leading to the consumption of more alcohol.

In Germany, the increase in the consumption of alcohol appears to have run parallel to that of the national income. The latter amounted, in 1932, to 56,800 million Reichsmarks and, in 1937, to 76,300 millions. According to T. Gläs, the quantity of spirits consumed in 1931 /32 was 397,000 hectolitres, whilst, in 1937, it had risen to 769,000 hectolitres, or nearly double the amount. The

expenditure involved totalled from 3 to 4 thousand million Reichsmarks, or about 7% of the national income.[1]

In England, Wales and Scotland, consumption, which had fallen considerably between 1913 and 1932, has been rising sharply since that period, this development appearing to be connected with a reduction of unemployment and an improvement in the standard of living. The fall in the price of beer and the advertisement campaign of the brewery firms have also tended to increase the consumption of alcohol.

In the opinion of Dr. WEEKS, the relaxation of moral standards and the weakening of the sense of responsibility due to the great war, the slackening of the work of the temperance societies, and the further circumstance that the interest of the Christian churches has been concentrated on the question of peace, for instance, rather than on the campaign against alcohol, have also contributed to the increase in the consumption of alcoholic liquor. The subjoined table shows the obvious connection between such consumption and the improvement in standard of living and income.

Consumption of Alcoholic Liquor in Relation to Economic Status.

	1913	1932	1933	1934	1935	1936	1937
Beer, per head, in gallons	31.3	16.4	17.2	18.3	19.2	19.6	20.5
Spirits, per head, in gallons	0.6	0.2	0.2	0.2	0.2	0.2	0.2
Cost of maintenance (1914 = 100)	—	143.4	139.6	141.2	142.8	146.9	154.5
Cost of maintenance (1924 = 100)	—	82	79.8	80.7	81.6	84	88.3
Rate of wages (1924 = 100)	—	95	94	94	95	98	1 0
Number of unemployed registered in the last quarter of each year (000's omitted)	—	2,843	2,397	2,115	1,960	1,630	1,376

SOURCE : *Ministry of Labour Gazette,* August 1938.

[1] T. GLÄS, *Forschung z. Alkoholfrage,* 1937, page 45.

In Scotland, the *per caput* consumption of alcohol amounted, in 1920, to 0.91 gallon, and fell sharply to 0.31 gallon in 1933. Since then, it has been increasing steadily and reached 0.38 gallon in 1937.

The fall in consumption observable during the period 1920-1933 is attributed to the gradual depression through which industry passed, whilst the increase during the years 1934-1937 would appear to be due in part to improved economic conditions, and probably also to the intense advertisement campaign conducted by the liquor trade.

In the past few years, the consumption of alcohol has been increasing in Denmark, Finland, France, Hungary, Latvia, Switzerland and Poland. A decrease is recorded in Sweden and Yugoslavia.

In France, the rise in the consumption of wine has been due, in the opinion of M. BASTIDE, to the propaganda carried out by the Office du Vin with the support of the public authorities. As for the consumption of spirits, this has increased in the rural population as a result of the Decree of 1935 which did away with the supervision *in situ* of the operations of private still owners and substituted a so-called " Offsetting Tax ", which appears to have produced deplorable results.

Furthermore, the Parliamentary Decree of 1933 authorised the opening of 2,000 new sales premises. Inns have thus been established in localities where none previously existed.

The production of *apéritifs* has doubled during the past few years, according to the statements of the wholesale dealers and the proceedings of the general meetings of shareholders of the alcoholic liquor manufacturing concerns. The profits of the latter have increased in startling fashion.[1]

Imports of *aniseed*, which is one of the ingredients of most *apéritifs*, have now reached twice the 1924 figure.

Furthermore, Dr. LOGRE, the medical officer in charge of the special Police Prefecture Infirmary—who is quoted by M. BASTIDE—has found that the number of cases of *delirium tremens* has practically doubled since the application of the recent social laws.

According to M. BASTIDE, the main cause of the appalling increase of alcoholism among the working classes is precisely the bringing

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[1] See BASTIDE : "Les quarante heures et l'alcool", *L'Étoile Bleue*, October 1938.

into operation of these social laws. Hours of work having been greatly reduced, the worker has more time to spend in public houses and cafés, the days and hours of opening of which are subject to no restriction. Moreover, increased wages enable many workers to indulge their propensity for alcohol more freely.

One interesting fact, which was noted in Hungary, is the reduction of the consumption of beer following an increase in that of wine.

During 1929/30, the total quantity of wine consumed was 2,066,799 hectolitres, or 24.25 litres per head. In 1936/37, it was 3,750,198 hectolitres, or 41.91 litres per head. There was, hence, an increase of 74%.

For beer, on the other hand, the figures are as follows : in 1929/30, a total of 572,713 hectolitres, or 6.72 litres per head ; in 1936/37, a total of 240,766 hectolitres, or 2.68 litres per head. Consumption thus fell by 60.12%.

Statistics for Latvia supplied by M. Hermanis Asaris, President of the Latvijas Pretalkohola Biedziba, show that the increase in the consumption of alcohol proceeds *pari passu* with the improvement of the general economic conditions.

For this country, the consumption of alcohol was :

	Litres per head		Litres per head
1928	4.13	1933	3.87
1929	3.87	1934	3.98
1930	3.30	1935	4.19
1931	2.70	1936	4.45
1932	2.85		

The period 1931-1933 was that of economic depression. An improvement took place in 1933, which led to an increase in the consumption of alcohol.

In the case of beer, there has been a fairly sharp increase, as will be seen from the following figures :

	Hectolitres		Hectolitres
1928	99,859	1933	59,792
1929	95,355	1934	75,503
1930	92,497	1935	79,980
1931	73,641	1936	83,160
1932	53,739	1937	117,900

In Poland, an increase in the consumption of alcohol also supervened upon improved economic conditions. It appears from the subjoined figures that this increase is, comparatively speaking, larger in the voivodships in which the population is mainly rural than in the large cities.

Sale of Alcohol by the Polish State Alcohol Monopoly.

	1933/34	1934/35	1935/36	1936/37	Percentage increase from 1933/34 to 1936/37
Consumption of alcohol :	Thousands of litres				
Total for whole country	25,820	26,071	31,574	36,346	40
Distribution :					
City of Warsaw	4,126	4,014	4,884	5,255	27
Voivodship of Łódź	3,331	3,304	4,008	4,424	33
,, ,, Wilno	835	793	1,062	1,255	50
,, ,, Polesia	474	466	597	745	57
,, ,, Pomerelia ...	1,233	1,303	1,567	1,710	38
,, ,, Cracow	1,700	1,857	2,123	2,475	45
,, ,, Poznań	2,167	2,121	2,359	2,535	17
,, ,, Nowogródek .	541	590	832	1,085	100

The consumption of the typical rural voivodships of Vilna, Polesia and Nowogródek shows an increase of 50 to 100%, whereas in the large towns and in the voivodships with many urban centres, it amounted only to 17 to 45%.

In Switzerland, the economic depression has caused a reduction in the consumption of alcohol. Only that of cider is increasing as compared with pre-war years.

	Period 1910-1912 Litres	Period 1930-1932 Litres
Wine	2,500,000	1,900,000
Beer	2,900,000	2,600,000
Cider	1,000,000	1,600,000
Spirits	230,000	250,000

The comparatively small increase in the consumption of spirits makes an impression of unreality, since the population figure has increased from 3,625,000 in 1910 to 4,066,400 in 1930. It may be assumed that, as a result of the new policy concerning the sale of

alcohol, consumption decreased again during the following years. Since 1932, the cost of alcoholic liquor has increased by 50 to 100%, and this has exerted a decisive influence on consumption. The economic depression can only have still further increased the effect produced.

It may be taken, however, that an improvement of economic conditions will also be reflected in increased consumption of alcoholic liquor, as has occurred in other countries.

In Yugoslavia, compared with pre-war years, the consumption of all alcoholic drinks shows a marked reduction, which appears to have been caused by a fall in production. In certain social circles, however, there has been an increase of consumption, which is believed to have been caused by the obstacles placed in the way of the export of wine and spirits and the increase of the wine tax. This limitation of production and consumption has, in any case, only a very slight bearing upon the welfare of the rural population, since the number of persons who earn their livelihood by the production of wine does not exceed 500,000.

Number of Establishments selling Alcohol in the Towns and in the Country

The propaganda which is carried out in favour of the consumption of alcoholic liquor has beyond doubt been powerfully assisted by the large number of commercial establishments engaged in the sale of alcohol. The number of inns in relation to the number of inhabitants may offer a means of comparison enabling the extent of the danger to be gauged for a whole country or a particular area. Unfortunately, on this point, the replies to the questionnaire fail to supply sufficiently accurate information to enable the extent of the danger to the rural population to be properly assessed.

In Germany, the ratio is 1 : 272—that is to say, 36.9 establishments per 10,000 inhabitants.

In England and Wales, taken together, there were, at the end of 1936, 74,681 licences for sale for consumption on the premises (" on " licences)—*i.e.*, 18.29 per 10,000 inhabitants—24,094 licences for the sale of alcohol to be carried away (" off " licences)—*i.e.*, 5.41 per 10,000 inhabitants—and 16,297 licences held by registered clubs—*i.e.*, 3.99 per 10,000 inhabitants. The proportion in areas

including large towns, counting both " on " and "off " licences, was 25.78 per 10,000 inhabitants, and in areas not including any large towns, 23.70.

These figures do not point to any marked difference in the position between town and country.

The situation is similar is Scotland.

In Finland, the number of places for the sale of alcohol is :

(*a*) For the whole country, 1 per 5,400, or 1.85 per 10,000 inhabitants ;

(*b*) For towns, 1 per 1,000, or 10 per 10,000 inhabitants ;

(*c*) For country districts, 1 per 40,000, or 0.25 per 10,000 inhabitants.

In France, the situation is quite different. According to official statistics for 1934, there were at that time throughout the country 498,833 premises for the sale of alcohol, not including 29,381 wholesale establishments. The proportion is, therefore, 1 per 80 inhabitants, or 123 per 10,000. The proportion varies, however, as between the different departments. Thus the Seine Department, with a population of 4,962,967 inhabitants, has 49,139 inns, or 1 for every 109 inhabitants. The Nord Department, with a population of 2,022,167, has 41,027 premises for the sale of alcohol—*i.e.*, 1 for every 38 inhabitants, or 263 per 10,000. In those departments where the number of inns is relatively small, the number of home distillers of spirits is greater—*e.g.*, 91,243 in the Department of Ille-et-Vilaine.

M. S. Bastide quotes the opinion of doctors in the last-mentioned department, who, in a joint declaration, state that ". . . alcohol is to-day as essential in the eyes of the peasants as kitchen salt. It is the whirlwind which is destroying the race."

In Latvia, the position was the following at December 31st, 1937 :

	Town	Country	Total
Premises holding " off " licences	480	466	946
Premises holding " on " licences	370	209	579
Restaurants licensed to sell wines and beer	118	121	239
Other premises licensed to sell wines and beer	114	603	717
Total	1,082	1,399	2,481

	Town	Country	Total
Population at end of 1937	711,129	1,259,938	1,971,661
Number of inhabitants per licence ...	658	900	795
Number of licences per 10,000 inhabitants	15.2	11.2	12.6

As regards Poland, some idea of the number of places for the sale of alcohol may be gained from the total of licences granted during 1937. The central voivodships, which include the two largest towns, and the western voivodships, have the largest number of places of sale. The poorer voivodships, covering country districts to the east and south, show a much lower proportion.

Poland : Industrial Lieences granted to Commercial Establishments in 1937 (Smaller Statistical Annual for 1938).

	Voivodships				
	Centre	East	West	South	Total
Beer	448	189	550	532	1,719
Alcoholie beverages	2,036	759	852	672	4,319
Total	2,484	948	1,402	1,204	6,038
Number of licences per 10,000 inhabitants	1.8	1.7	3.14	1.46	1.9

In Sweden, the total number of licences for the sale of spirits and wines amounted, in 1936, to 1,854, of which 1,599 were in the hands of the " system " companies operating in Sweden and 255 in the hands of private individuals. There were, at that time, 121 " system " companies, 97 operating in towns, 20 in densely inhabited areas, and only 4 in country districts. On July 1st, 1934, there were 8,977 premises licensed to sell alcohol and beer, of which only 2,008 in country areas.

Switzerland has approximately one inn for every 770 inhabitants. There is no basis upon which to discriminate between town and country.

Generally speaking, it can be stated that the number of premises for the sale of alcohol is lower in countries of an agricultural character than in the industrial countries. The worst situation is found in those countries which are themselves producers of wine ; here, the figures reach alarming proportions.

Means for preventing and putting down the Consumption of Alcohol in Rural Areas

The importance of measures for combating addiction to alcohol in rural areas cannot be overlooked; they are a pre-requisite both of the improvement of the economic position of the populations concerned, and of any effort to arrest the process of degeneration and utter ruination. The enquiry carried out has not led to the discovery of any particular method specially adapted to the rural environment and differing from those applied more generally elsewhere. In some countries, the communes have power to limit, or even prohibit altogether, the sale of alcohol once a vote in this sense has been taken. This method, however, seldom meets with complete success, and the action so far taken is in most cases confined to various forms of propaganda by the temperance societies. Their propaganda meets with serious opposition on the part of the industries and undertakings interested in alcoholic beverages, who all advertise their products widely. A railway journey in France, Belgium or Switzerland will enable the traveller to see from the carriage window that the most numerous and most prominent advertisements are those for wines, brandy and tobacco. Not only the popular daily newspapers, but even medical journals are used for the same purpose, while both the wireless and the cinema derive considerable financial resources from advertisements for the liquor trade.

One of the most serious difficulties met with in the struggle against addiction to alcohol lies in the ambiguous position adopted by the medical profession.

Many medical treatises maintain that alcohol is a source of energy which can be substituted for carbohydrates and fats, and used, in a variety of cases, as a remedy. Those statements, baseless as they are, nevertheless create uncertainty in the mind of young doctors who have just completed their university studies and who begin to practice in town or country without knowing precisely whether the campaign against alcoholism, and against alcohol itself, is fully justifiable from a scientific standpoint. Such being the circumstances, doctors do not assist the campaign against alcohol to any appreciable extent, and in certain cases even discredit it.

The belief that alcoholic beverages are of nutritive and medical value provides one of the reasons for their growing use. It is the duty of the doctor to take the lead in counteracting that belief and the view that alcohol taken in small quantities does good to the organism and cannot be detrimental.

These erroneous views are more frequently met with in country areas than in towns, owing to the conservative character of rural opinion, which still clings to medical views long since discredited among urban populations. Notions of the merits of alcohol current a hundred years ago were very different from those obtaining to-day. It will be sufficient to recall that in almost every army spirits were issued to the soldiers as part of their normal ration. Most cases of illness were treated with doses of alcohol given as medicine. To-day, urban populations look upon such practices as wholly obsolete, but in many countries it is quite normal to find country-dwellers still convinced, as they were a century ago, that alcohol possesses some nutritive virtue. One of the most serious consequences of that ignorant belief is the practice of giving alcohol to children—a practice which is seldom met with in towns, but is still frequent in the country, where even small children are given their share of strong drink on the occasion of family celebrations. It is even quite frequent to find mothers giving infants a lump of sugar soaked in spirits, especially if the infant is restless and wakeful.

The surest means of combating this attitude is to provide a thorough medical and health organisation in rural areas. Doctors, nurses and midwives can, provided they are convinced of the need for such action, contribute powerfully to the removal of this evil. Hence, the fullest attention should be given to the problem of alcoholism in their courses of study.

Space does not allow of consideration of the important psychological and physiological problems inherent in the question of alcohol as an adjunct to relaxation or corollary to recreation. Unfortunately, it provides a ready means of satisfying the need for relief from serious work. Here, again, the phenomenon is more apparent in the country than in towns, since recreations of whatever type are, in the former case, always limited to certain fixed dates—holidays, Sundays, etc.—upon which the whole population is given over to amusement and rest ; in towns, on the contrary, the quicker pace

of life brings with it more frequent opportunities for entertainment, even on working-days.

Moreover, the town offers many more possibilities of employing leisure hours, while recreation is not necessarily linked with the consumption of alcohol. Rural populations have only one place where they may go for amusement and entertainment : that place is the inn, where dancing takes place and games—some of a semi-athletic type, such as bowls—or billiards are played. The inn is a particular attraction to youths of both sexes, and it goes without saying that every occasion on which they congregate offers an inducement to drinking, which is the *raison d'être* of the establishment.

The above considerations justify the conclusion that the most effective method of combating the drinking habit in country areas lies in an adequate organisation of the leisure hours of rural populations. The cinema, the radio, camping and, above all, sport can play a very important rôle in this connection.

A NINTH ANALYTICAL REVIEW OF REPORTS FROM PASTEUR INSTITUTES ON THE RESULTS OF ANTI-RABIES TREATMENT[1]

By

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I. GENERAL

The statistics dealt with in this review relate almost entirely to the years 1936 and 1937. A few reports relating to 1935 (Accra, Ankara, Lagos, Prague, and São Paulo) are included, together with those of Cluj for 1933, 1934, and 1935, and those of Salonica for 1931, 1932, 1933, 1934 and 1935.

The present analysis deals with a total of 304,525 treated persons. The following table gives the number of cases included in previous reviews, together with the total for all nine reviews.

Review	Persons	Review	Persons
First	31,656	Sixth	118,062
Second	69,707	Seventh	110,884
Third	69,541	Eighth	123,040
Fourth	119,433	Ninth	304,525
Fifth	115,859		
		Total	1,062,707

The general characteristics of each schedule are given below.

[1] For previous reviews, see document C.H.844, and the *Bull. Health Org.* 1932, **1**, pages 110 and 725; 1933, **2**, page 533; 1934, **3**, page 613; 1935, **4**, page 752; 1937, **6**, page 17; 1938, **7**, page 1.

Methods used and Results obtained

Schedule	Year	Vaccine	Days of treatment	Number treated	Deaths	Mortality %	Paralytic accidents
		(1) *Dried or glycerinated cords.*					
1. Alexandria	1936	Dried 2 days : glyc.	15-21	719	2	0.28	0
2. Algiers	1936	Dried 5-2 days : glyc.	15-25	2,725	9	0.33	6 (rccovered)
3. Algiers	1937	Dried 4-2 days : glyc.	15-25	3,208	9	0.28	1 (recovercd)
4. Antananarivo (Madagascar) (Europeans)	1936	Dried 5-1 days : glyc.	14-20	60	1	1.66	0
5. Antananarivo (Madagascar) (non-Europeans)	1936	Dried 5-1 days : glyc.	14-20	77	0	—	0
6. Antananarivo (Madagascar) (Europeans)	1937	Dried 5-1 days : glyc.	18-20	80	0	—	0
7. Antananarivo (Madagascar) (non-Europeans)	1937	Dried 5-1 days : glyc.	18-20	107	2	1.87	0
8. Bangkok, institutc	1936	Dried 2 days : glyc.	18-25	339	0	—	0
9. Bangkok, institute	1937	Dried 2 days : glyc.	18-25	283	1	0.35	3 (1 fatal)
10. Beirut (Lebanon)	1936	Dried 4-2 days : glyc.	15-24	104	0	—	0
11. Berne	1937	Dried 2 days : glyc.	18-21	3	0	—	0
12. Bordeaux	1936	Dried 5-2 days : glyc.	14-21	117	0	—	0
13. Bordeaux	1937	Dried 5-2 days : glyc.	14-21	88	0	—	0
14. Casablanca (Morocco)	1936	Dried 5-2 days : glyc.	15-25	1,217	12	0.98	0
15. Casablanca (Morocco)	1937	Dried 5-2 days : glyc.	15-25	880	6	0.68	0
16. Ceara (Brazil)	1936	Dried 7-1 days : glyc.	15-25	515	1	0.19	0
17. Ceara (Brazil)	1937	Dried 7-1 days : glyc.	15-25	711	0	—	0
18. Cordoba (Argentina)	(?)1936[1]	Dried 6-2 days :	26-38	418	0	—	0
19. Dakar (Senegal) (Europeans)	1936	Dried 3-1 days : glyc.	15-20	17	0	—	1 (recovered)
20. Dakar (Senegal) (non-Europeans)	1936	Dried 3-1 days : glyc.	15-20	54	0	—	0
21. Dakar (Senegal) (Europeans)	1937	Dried 3-1 days : glyc.	15-20	5	0	—	0
22. Dakar (Senegal) (non-Europeans)	1937	Dried 3-1 days : glyc.	15-20	27	0	—	0
23. Hanoi (Tongking) (Europeans)	1936	Dried 4-1 days : glyc.	15-20	201	0	—	0
24. Hanoi (Tongking) (non-Europeans)	1936	Dried 4-1 days : glyc.	15-20	1,339	14	1.04	0
25. Hanoi (Tongking) (Europeans)	1937	Dried 4-1 days : glyc.	15-20	168	0	—	0
26. Hanoi (Tongking) (non-Europeans)	1937	Dried 4-1 days : glyc.	15-20	1,116	7	0.63	0
27. Hué (Annam)	1936	Dried 6-1 days : glyc.	15-21	883	1	0.11	0

28. Hué (Annam)	1937	Dried	6-1 days : glyc.	15-20	1,026	0	—	1 (fatal)
29. Juiz de Fora (Brazil)	1936	Dried	4-1 days : glyc.	?	734	0	—	0
30. Juiz de Fora (Brazil)	1937	Dried	4-1 days : glyc.	?	636	0	—	0
31. Keijo (Chosen)	1936	Dried	3-1 days : glyc.	18	2,476	13	0.53	0
32. Keijo (Chosen)	1937	Dried	3-1 days : glyc.	18	2,989	10	0.33	0
33. Lille	1936	Dried	4-2 days : glyc.	14-24	73	0	—	0
34. Lille	1937	Dried	5-2 days : glyc.	14-24	56	0	—	0
35. Lyons	1936	Dried	4-2 days : glyc.	15-25	88	0	—	0
36. Lyons	1937	Dried	4-2 days : glyc.	15-25	115	0	—	0
37. Marseilles	1936	Dried	5-2 days : glyc.	15-25	163	0	—	0
38. Marseilles	1937	Dried	? days : glyc.	15-25	129	0	—	0
39. Montevideo	1936	Dried	8-1 days	14-21	1,060	2	0.19	0
40. Montevideo	1937	Dried	10-1 days	14-21	930	0	—	0
41. Montpellier	1936	Dried	? days : glyc.	13-24	141	0	—	0
42. Montpellier	1937	Dried	? days : glyc.	13-24	186	0	—	0
43. Paris	1936	Dried	4-2 days : glyc.	15-25	463	0	—	1 (recovered)
44. Paris	1937	Dried	4-2 days : glyc.	15-25	454	0	—	1 (recovered)
45. Pnom-Penh (Cambodia) (Europeans)	1936	Dried	6-1 days : glyc.	15-21	50	0	—	0
46. Pnom-Penh (Cambodia) (non-Europeans)	1936	Dried	6-1 days : glyc.	15-21	421	1	0.24	0
47. Pnom-Penh (Cambodia) (Europeans)	1937	Dried	6-1 days : glyc.	15-21	18	0	—	0
48. Pnom-Penh (Cambodia) (non-Europeans)	1937	Dried	6-1 days : glyc.	15-21	442	0	—	0
49. Porto Alegre (Brazil)	1936	Dried	5-2 days	15-25	664	0	—	0
50. Porto Alegre (Brazil)	1937	Dried	5-2 days	15-25	1,087	0	—	0
51. Saigon (Europeans)	1936	Dried	6-1 days : glyc.	15-21	86	0	—	0
52. Saigon (non-Europeans)	1936	Dried	6-1 days : glyc.	15-21	2,111	1	0.05	0
53. Saigon (Europeans)	1937	Dried	6-1 days : glyc.	15-21	119	0	—	0
54. Saigon (non-Europeans)	1937	Dried	6-1 days : glyc.	15-21	2,292	2	0.09	0
55. Teheran	1936	Dried	? days : glyc.	15-25	28	0	—	0
56. Tokio, Government Institute for Infectious Diseases	1936	Dried	3-1 days : glyc.	18	338	0	—	0
57. Tokio, Kitasato Institute	1936	Dried	5-1 days : glyc.	18	60	0	—	0
58. Tunis	1937	Dried	4-1 days : glyc.	16-20	1,872	4	0.21	0
59. Tunis	1937	Dried	4-1 days : glyc.	16-20	1,800	2	0.11	0
60. Vientiane (Laos)	1936	Dried	? days : glyc.	?	65	0	—	0
61. Vilno (Poland)	1936	Dried	8-2 days : glyc.	14-20	10	0	—	0
62. Vilno (Poland)	1937	Dried	8-2 days : glyc.	14-20	16	0	—	0

[1] Two schedules, the statistics in which are the same, were received from Cordoba, one labelled 1936, the other 1937.

Schedule	Year	Vaccine	Days of treatment	Number treated	Deaths	Mortality %	Paralytic accidents
		(2) *Modified dilution methods.*					
1. Certain Bulgarian institutes .	1936	Boeeker's vaccine : 90 mg.	20	2,929	6	0.20	0
2. Certain Bulgarian institutes .	1937	Boeeker's vaccine : 90 mg.	20	3,001	1	0.03	0
3. Istanbul	1937	Högyes-Philipps : 29-425 mg.	9-25	1,764	3	0.17	0
4. Madrid	1937	Högyes modified : 1/5000-1/100	14-20	1,005	3	0.30	0
5. Receife (Pernambueo, Brazil) .	1936	Högyes	12-20	1,572	1	0.06	0
6. Receife (Pernambueo, Brazil) .	1937	Högyes	12-20	1,674	2	0.12	0
7. Riga, institute	1936	Dilution	14-21	16	0	—	0
8. Riga, outside institute	1936	Dilution	14-21	195	1	0.51	0
9. Riga	1937	Dilution	14-21	128	0	—	0
				12,284	17	0.14	0
		(3-6) *Phenol vaccines : modifications of Fermi's method.*					
		(3) *Killed phenol vaccines.*					
1. Aecra (Gold Coast)	1935	? : Semple	?	45	3	6.67	0
2. Accra (Gold Coast)	1936	? : Semple	?	4	0	—	0
3. Aecra (Gold Coast)	1937	? : Semple	?	45	0	—	0
4. Ankara	1935	840-2400 mg. : Semple	14-20	1,478	7	0.47	0
5. Ankara	1936	840-2400 mg. : Semple	14-20	1,994	9	0.45	0
6. Ankara	1937	840-2400 mg. : Semple	14-20	1,900	7	0.37	0
7. Baghdad (Europeans)	1936	700-3500 mg. : Semple	?	17	0	—	0
8. Baghdad (non-Europeans) ..	1936	700-3500 mg. : Semple	?	184	0	—	0
9. Baghdad (Europeans)	1937	350-3500 mg. : Semple	?	8	0	—	0
10. Baghdad (non-Europeans) ..	1937	350-3500 mg. : Semple	7-14	343	2	0.58	0
11. Bangkok, institute	1936	Phenolised vaccine	14	1,217	1	0.08	0
12. Bangkok, outside institute ..	1936	Phenolised vaccine	14	1,797	4	0.22	0
13. Bangkok, institute	1937	Phenolised vaccine	14	1,280	5	0.39	0
14. Bangkok, outside institute ..	1937	Phenolised vaccine	14	1,593	3	0.18	0
15. Bombay (Europeans)	1936	700-7000 mg. : Semple	7-14	327	0	—	0
16. Bombay (non-Europeans) ..	1936	700-7000 mg. : Semple	7-14	7,765	31	0.39	0
17. Bombay (Europeans)	1937	700-7000 mg. : Semple	7-14	376	0	—	0
18. Bombay (non-Europeans) ..	1937	700-7000 mg. : Semple	7-14	9,482	48	0.51	0
19. Brazzaville (French Congo) .	1936	? : Semple	14	19	0	—	0
20. Brazzaville (French Congo) .	1937	? : Semple	14	27	0	—	0
21. Calcutta, institute (Europeans)	1936	350-700 mg. : Semple	7-14	111	0	—	0

22. Calcutta, institutc (non-Europeans)	1936	350-700 mg. : Semple	7-14	819	0	—	0	
23. Calcutta, out-centres (Europeans)	1936	350-700 mg. : Semple	7-14	190	0	—	0	
24. Calcutta, out-centres (non-Europeans)	1936	350-700 mg. : Semple	7-14	4,028	29	0.72	0	
25. Calcutta, institute (Europeans)	1937	350-700 mg. : Semple	7-14	160	0	—	0	
26. Calcutta, institute (non-Europeans)	1937	350-700 mg. : Semple	7-14	1,059	2	0.19	0	
27. Calcutta, out-centres (Europeans)	1937	350-700 mg. : Semple	7-14	202	0	—	0	
28. Calcutta, out-centres (non-Europeans)	1937	350-700 mg. : Semple	7-14	5,144	38	0.74	0	
29. Cernauti (Roumania) (light cases)	1936	2100-3000 mg. : Semple	14-20	575	1	0.17	0	
30. Cernauti (Roumania) (light cases)	1937	2100-2700 mg. : Semple	14-18	625	0	—	0	
31. Colombo	1936	350-900 mg. : Semple	7-18	1,993	9	0.45	0	
32. Colombo	1937	350-1000 mg. : Semple	7-21	2,173	9	0.41	0	
33. Coonoor (Europeans)	1936	700-7000 mg. : Semple	7-14	342	0	—	0	
34. Coonoor (non-Europeans)	1936	700-7000 mg. : Semplc	7-14	11,678	19	0.16	2	(1 fatal)
35. Coonoor (Europeans)	1937	700-7000 mg. : Semple	7-14	351	1	0.29	0	
36. Coonoor (non-Europeans)	1937	700-7000 mg. : Semple	7-14	11,382	22	0.19	1	(recovered)
37. Cracow	1936	2000 mg. : Semple	20	234	1	0.43	0	
38. Cracow	1937	2000 mg. : Semple	20	228	0	—	0	
39. Freetown (Sierra Leone)	1936	? : Semple	?	16	0	—	0	
40. Freetown (Sierra Leone)	1937	? : Semple	?	46	0	—	0	
41. Galatz (Roumania)	1936	2250-7500 mg. : Semple	15-25	715	2	0.28	0	
42. Galatz (Roumania)	1937	3750-12500 mg. : Semple	15-25	1,449	3	0.21	0	
43. Hong-Kong	1936	1400-2800 mg. : Semple	14	256	0	—	0	
44. Hong-Kong	1937	1400-2800 mg. : Semple	14	231	0	—	0	
45. Kasauli, institute (non-Europeans) : Class II	1936	1400 mg. : Scmple	14	168	0	—	0	
46. Kasauli, out-centres (non-Europeans) : Class II	1936	1400 mg. : Semple	14	1,964	0	—	1	(recovercd)
47. Kasauli, institutc (non-Europeans) : Class III	1936	3500 mg. : Semple	14	337	0	—	0	
48. Kasauli, out-centres (non-Europeans) : Class III	1936	3500 mg. : Semple	14	8,456	29	0.34	0	
49. Kasauli, institute (non-Europeans) : Class IV	1936	7000 mg. : Semple	14	257	3	1.17	0	

Schedule	Year	Vaccine	Days of treatment	Number treated	Deaths	Mortality %	Paralytic accidents
50. Kasauli, out-centres (non-Europeans) : Class IV	1936	7000 mg. : Semple	14	4,549	74	1.63	1 (recovered)
51. Kasauli, institute (Europeans) : Class II	1937	1400 mg. : Semple	14	6	0	—	0
52. Kasauli, institute (non-Europeans) : Class II	1937	1400 mg. : Semple	14	160	0	—	0
53. Kasauli, out-centres (Europeans) : Class II	1937	1400 mg. : Semple	14	94	0	—	0
54. Kasauli, out-centres (non-Europeans) : Class II	1937	1400 mg. : Semple	14	2,468	3	0.12	0
55. Kasauli, institute (Europeans) : Class III	1937	3500 mg. : Semple	14	32	0	—	0
56. Kasauli, institute (non-Europeans) : Class III	1937	3500 mg. : Semple	14	305	0	—	0
57. Kasauli, out-centres (Europeans) : Class III	1937	3500 mg. : Semple	14	266	0	—	1 (recovered)
58. Kasauli, out-centres (non-Europeans) : Class III ...	1937	3500 mg. : Semple	14	9,242	28	0.30	1 (recovered)
59. Kasauli, institute (Europeans) : Class IV	1937	7000 mg. : Semple	14	3	0	—	0
60. Kasauli, institute (non-Europeans) : Class IV	1937	7000 mg. : Semple	14	219	5	2.28	0
61. Kasauli, out-centres (Europeans) : Class IV	1937	7000 mg. : Semple	14	114	2	1.75	0
62. Kasauli, out-centres (non-Europeans) : Class IV	1937	7000 mg. : Semple	14	5,211	51	0.98	3 (recovered)
63. Kuala Lumpur (Malaya) ...	1936	1400-2100 mg. : Semple	14-21	21	0	—	0
64. Kuala Lumpur (Malaya) ...	1937	1400-2100 mg. : Semple	14-21	169	0	—	1 (recovered)
65. Lagos (Nigeria)	1935	? : Semple	?	156	1	0.64	0
66. Lagos (Nigeria)	1936	? : Semple	?	105	0	—	0
67. Lagos (Nigeria)	1937	? : Semple	?	380	2	0.53	0
68. Lisbon	1936	2500-10000 mg. : Semple	10-40	637	0	—	0
69. Lisbon	1937	2500-10000 mg. : Semple	10-40	933	1	0.11	0
70. Lwow (Poland), institute ...	1936	? : Semple	20	9	0	—	0
71. Lwow (Poland), out-centres.	1936	? : Semple	20	496	2	0.40	0
72. Lwow (Poland)	1937	? : Semple	20	708	0	—	0
73. Nairobi (Kenya)	1936	1400-7000 mg. : Semple	14	8	0	—	0
74. New Orleans (Europeans) ..	1936	? : Semple	15- ?	286	0	—	0
75. New Orleans (non-Europeans)	1936	? : Semple	15- ?	49	0	—	0

76. New Orleans (Europeans) .	1937	?	: Semple	14- ?	1,018	1	0.09	0	
77. New Orleans(non-Europeans)	1937	?	: Semple	14- ?	324	0	—	0	
78. New York	1936	?	: Semple	14-21	1,804	0	—	0	
79. New York	1937	?	: Semple	14-21	2,276	0	—	0	
80. Palestine	1936	?	: Semple	14	1,808	1	0.06	0	
81. Palestine	1937	?	: Semple	14	1,881	2	0.11	1	(recovered)
82. Rangoon (Europeans)	1936	700-7000 mg.	: Semple	7-14	208	0	—	1	(recovered)
83. Rangoon (non-Europeans) .	1936	700-7000 mg.	: Semple	7-14	1,487	2	0.13	2	(1 fatal)
84. Rangoon (Europeans)	1937	700-7000 mg.	: Semple	7-14	315	0	—	0	
85. Rangoon (non-Europeans) .	1937	700-7000 mg.	: Semple	7-14	1,954	2	0.10	3	(1 fatal)
86. Santiago (Chile)	1936	2240-4800 mg.	: Semple	?	726	0	—	0	
87. Santiago (Chile)	1937	2240-4800 mg.	: Semple	?	689	0	—	0	
88. Shanghai (Europeans)	1936	562-3000 mg.	: Semple	15-25	270	0	—	0	
89. Shanghai (non-Europeans) .	1936	562-3000 mg.	: Semple	15-25	614	4	0.65	0	
90. Shanghai (Europeans)	1937	1125-1800 mg.	: Semple	15-24	142	0	—	0	
91. Shanghai (non-Europeans) .	1937	1125-1800 mg.	: Semple	15-24	425	1	0.23	0	
92. Shillong, institute	1936	700-4200 mg.	: Semple	7-14	102	0	—	1	(not fatal)
93. Shillong, institute	1937	700-4200 mg.	: Semple	7-14	148	1	0.68	0	
94. Sivas (Turkey)	1937	840-2400 mg.	: Semple	14-20	77	0	—	0	
95. Teheran	1936	?	: Semple	20-30	66	0	—	0	
96. Teheran	1937	?	: Semple	20-30	188	0	—	0	
97. Toronto	1936	?	: Semple	14-21	10	0	—	0	
98. Toronto	1937	?	: Semple	14-21	19	0	—	0	
99. Warsaw, institute	1936	1400-3000 mg.	: Semple	20-25	298	0	—	0	
100. Warsaw, outside institute .	1936	1400- ? mg.	: Semple	20-25	2,634	8	0.30	0	
101. Warsaw, institute	1937	1600-4000 mg.	: Semple	20-25	377	0	—	0	
102. Warsaw, outside institute .	1937	1600-2000 mg.	: Semple	20-25	3,482	8	0.23	1	(recovered)
					135,058	487	0.36	20	(3 fatal)

(4) *Live phenol vaccines : Puntoni's modification.*

1. Florence	1936	Puntoni's modification	?	39	0	—	0
2. São Paulo (Brazil)	1935	Puntoni's modification	?	848	0	—	0
3. São Paulo (Brazil)	1936	Puntoni's modification : 3000-7500 mg.	?	1,170	0	—	0
4. São Paulo (Brazil)	1937	Puntoni's modification : 3000-7500 mg.		1,485	0	—	0
				3,542	0	—	0

Schedule	Year	Vaccine	Days of treatment	Number treated	Deaths	Mortality %	Paralytic accidents
		(5) *Fermi's original vaccine.*					
1. Alexandria	1937	Fermi : ?	15-25	834	0	—	0
2. Beirut (Lebanon)	1936	Fermi : 3750-6250 mg.	15-25	28	0	—	0
3. Budapest	1936	Fermi : 115-330 mg.	6-10	3,762	4	0.10	0
4. Budapest	1937	Fermi : ?	6-10	3,037	3	0.10	0
5. Buenos Aires, Bacteriological Institute	1936	Fermi : 6000-9150 mg.	20-28	1,214	0	—	0
6. Buenos Aires, Bacteriological Institute	1937	Fermi : 6000-9150 mg.	20-28	1,364	0	—	1 (fatal)
7. Cairo	1936	Fermi : ?	15-20	3,883	8	0.21	0
8. Cairo	1937	Fermi : ?	15-20	5,257	17	0.32	0
9. Dakar (Senegal) (Europeans)	1937	Fermi : 6000-7800 mg.	20-26	2	0	—	0
10. Dakar (Senegal) (non-Europeans)	1937	Fermi : 6000-7800 mg.	20-26	9	1	11.11	0
11. Kosice (Czecho-Slovakia)	1936	Fermi : ?	14-21	309	1	0.32	0
12. La Paz (Bolivia)	1936	Fermi-Kraus	?	16	0	—	0
13. Prague	1935	Fermi : ?	14-21	622	1	0.16	0
14. Prague	1936	Fermi : ?	14-21	453	1	0.20	0
15. Prague	1937	Fermi : ?	14-21	50	0	—	0
				20,840	36	0.17	1 (fatal)
		(6) *Fermi's sero-vaccine.*					
		This method has not been employed since 1934.					
		(7) *Heated vaccines : Babes' method.*					
1. Bucharest	1936	Dried 6-0 days + heated 50°-60°C.	10-24	7,053	20	0.28	0
2. Bucharest	1937	Dried 6-0 days + heated 50°-60°C.	10-24	6,320	26	0.40	0
3. Cernauti (Roumania) (severe cases)	1936	Semple + heated 60° and 65°C.	14-20	208	1	0.48	0
4. Cernauti (Roumania) (severe cases)	1937	Semple + heated 60° and 65°C.	14-20	385	2	0.52	0
5. Chisinau (Roumania)	1936	Högyes, in light cases, +, in severe cases, heated 60°C.	14-23	1,494	4	0.27	0
6. Chisinau (Roumania)	1937	Högyes, in light cases, +, in severe cases, heated 60°C.	14-23	1,690	2	0.12	0

7. Cluj (Roumania)	1933	Dried 4-0 days + heated 45°-60°C.	?	4,398	8	0.18	0
8. Cluj (Roumania)	1934	Dried 4-0 days + heated 45°-60°C.	?	3,525	14	0.40	0
9. Cluj (Roumania)	1935	Dried 4-0 days + heated 45°-60°C.	?	4,244	4	0.09	0
10. Cluj (Roumania)	1936	Dried 4-0 days + heated 45°-60°C.	?	5,753	10	0.17	0
11. Cluj (Roumania)	1937	Dried 4-0 days + heated 45°-60°C.	?	5,882	8	0.14	0
12. Jassy (Roumania)	1936	Heated 60°-65°C. + Fermi	8-18	3,079	1	0.03	0
13. Jassy (Roumania)	1937	Heated 60°-65°C. + Fermi	8-18	3,783	4	0.11	0
				47,814	104	0.22	0

(8) *Killed ether vaccine : Hempt's method.*

1. Novi Sad and other Yugoslav institutes	1936	Hempt's standard method	1-6	12,474	15	0.12	0
2. Novi Sad and other Yugoslav institutes	1937	Hempt's standard method	1-6	13,836	18	0.13	1 (fatal)
3. Prague	1935	Hempt's standard method	5-7	257	0	—	0
4. Prague	1936	Hempt's standard method	5-7	325	0	—	0
5. Prague	1937	Hempt's standard method	3-7	583	0	—	0
				27,475	33	0.12	1 (fatal)

(9) *Mixed treatments.*

(*a*) 1. Athens	1936	Boecker +, in severe cases, Alivisatos	20	1,692	1	0.06	1 (recovered)
2. Athens	1937	Boecker +, in severe cases, Alivisatos	20	1,596	2	0.12	1 (recovered)
3. Certain Bulgarian institutes	1936	Högyes +, in severe cases, Alivisatos : 275-3700 mg.	13-15	1,869	1	0.05	0
4. Certain Bulgarian institutes	1937	Högyes +, in severe cases, Alivisatos : 275-3700 mg.	13-15	2,195	1	0.05	0
5. Vienna	1936	Högyes + Alivisatos	?	219	0	—	0
				7,571	5	0.07	2 (recovered)

Schedule	Year	Vaccine	Days of treatment	Number treated	Deaths	Mortality %	Paralytic accidents
(*b*) 1. Buenos Aires, Pasteur Laboratory	1936	Cords dried 6-1 days : glyc. +, in severe cases, Fermi's original vaccine	16- ?	2,736	4	0.14	0
2. Buenos Aires, Pasteur Laboratory	1937	Cords dried 6-1 days +, in severe cases, Fermi's original vaccine	16- ?	1,900	3	0.15	1 (recovered)
				4,636	7	0.15	1 (recovered)
(*c*) 1. Salonica	1931	Fermi : 3200-8000 mg. +, in severe cases, Alivisatos	18-25	1,467	2	0.14	0
2. Salonica	1932	Fermi : 3200-8000 mg. +, in severe cases, Alivisatos	18-25	1,094	3	0.27	0
3. Salonica	1933	Fermi : 3200-8000 mg. +, in severe cases, Alivisatos	18-25	753	1	0.13	0
4. Salonica	1934	Fermi : 3200-9600 mg. +, in severe cases, Alivisatos	18-25	786	0	—	2 (recovered)
5. Salonica	1935	Fermi : 3200-9000 mg. +, in severe cases, Alivisatos	18-25	843	0	—	1 (recovered)
6. Salonica	1936	Fermi : 3200-9600 mg. +, in severe cases, Alivisatos	18-25	775	1	0.13	0
7. Salonica	1937	Fermi : 3200-9800 mg. +, in severe cases, Alivisatos	18-25	928	1	0.11	0
				6,646	8	0.12	3 (recovered)

The statistics included in certain schedules could not be made use of. A summary of these schedules is given below, with the reason for exclusion.

Schedule	Year	Vaccine	Days of treatment	Number treated	Deaths	Mortality %	Paralytic accidents		Reason for not using statistics
1. Dijarbekir (Turkey)	1935	Semple and Högyes	?	50	0	—	0		
2. Dijarbekir (Turkey)	1936	Semple and Högyes	?	66	0	—	0		
3. Dijarbekir (Turkey)	1937	Semple and Högyes	?	103	0	—	0		Statistics for different treatments not given separately
4. Istanbul	1935	Semple, Philipps and Pasteur-Calmette	?	2,391	10	0.41	3	(recovered[1])	
5. Istanbul	1936	Semple, Philipps and Pasteur-Calmette	?	2,113	7	0.33	2	(recovered[2])	
6. Mexico	1935	Fermi	14-21	3,910	?	?	2		
7. Mexico	1936	Fermi	14-21	3,620	?	?	0		
8. Mexico	1937	Fermi	14-21	4,517	?	?	0		Number of deaths unknown
9. Nairobi (Kenya), out-stations	1937	Semple	?	61 courses issued	?	?	?		
10. Santa Clara (Cuba)	1936	Semple : 1920-3360 mg.	14-21	247 courses issued	?	?	?		
11. Santa Clara (Cuba)	1937	Semple : 1920-3360 mg.	15-21	356 courses issued	?	?	?		
12. Shillong, out-centres	1937	Semple	?	1,661	?	?	?		
13. Sivas (Turkey)	1935	Semple and Högyes	?	239	0	—	0		Statistics for different treatments not given separately
14. Sivas (Turkey)	1936	Semple and Högyes	?	241	0	—	0		
15. Vientiane (Laos)	1937	Dried cords and phenol	?	93	2	2.15	0		
				19,668	—	—	—		

[1] 2 Högyes ; 1 Semple.
[2] Both Philipps.

The 19,668 cases in the above table are excluded from all other tables and results.

II. METHODS OF TREATMENT

The methods of treatment employed at each institute, its duration and, where available, its dosage are given in the preceding table.

For purposes of comparison, the various methods of treatment have been classified under the following headings :

1. *Dried or glycerinated cords* (Pasteur's original method and Calmette's modification).
2. *Modified dilution methods* (Högyes).

3-6. *Phenol : Modifications of Fermi's method.*

3. *Killed phenol vaccines* (Semple and Mulford's modifications).
4. *Live phenol vaccines* (Puntoni's modification).
5. *Fermi's original method.*
6. *Fermi's sero-vaccine.*
7. *Heated vaccines* (Babes).
8. *Killed ether vaccines* (Hempt).
9. *Mixed treatments :*

   (*a*) (dilutions *plus*, in severe cases, Alivisatos) ;
   (*b*) (dried cords *plus*, in severe cases, Fermi's original vaccine) ;
   (*c*) (Fermi *plus*, in severe cases, Alivisatos).

10. *Yatren vaccine.*

In certain instances, the following shortened system of classification has been employed :

(a) *Killed vaccines* : comprising 3 and 8 above.
(b) *Live vaccines* : comprising 1, 2, 4, 9(*a*) and 9(*b*) above.
(c) *Heated vaccines* :
(d) *Other* : comprising 5, 6, 9(*c*) and 10 above.

III. NUMBER OF PERSONS TREATED

The total number of treated persons regarding whom information has been received was 304,525 (excluding the 19,668 cases "not usable"). The adjoining table gives the distribution of these according to the method of treatment.

1.	Cords	38,659	8.	Killed ether	27,475
2.	Dilutions	12,284	9.	Mixed (*a*)	7,571
3.	Killed phenol	135,058		(*b*)	4,636
4.	Live phenol	3,542		(*c*)	6,646
5.	Fermi's vaccine	20,840			
7.	Heated	47,814		Total	304,525

IV. MORTALITIES FOR ALL PATIENTS

Of the total of 304,525 treated persons, 797 died of rabies. The percentage mortality was thus 0.26, as compared with 0.49 in the first, 0.48 in the second, 0.23 in the third, 0.48 in the fourth, 0.38 in the fifth, 0.35 in the sixth, 0.30 in the seventh, 0.27 in the eighth, and 0.33 over the whole period covered by the nine reviews. The detailed figures for the present statistics, and for the set of nine reviews, are as follows :

	Ninth review			All nine reviews			
					Deaths		
	Number treated	Deaths	Mortality %	Number treated	Observed	Calculated[a]	Mortality %
1. Cords	38,659	100	0.26	152,899	527	509	0.34
2. Dilutions	12,284	17	0.14	75,141	141	250	0.19
3. Killed phenol	135,058	487	0.36	490,670	2,347	1,634	0.48
4. Live phenol	3,542	0	0	7,006	6	23	0.09
5. Fermi's vacc.	20,840	36	0.17	28,159	49	94	0.17
6. Fermi's S.V.	—	—	—	390	0	1	0
7. Heated	47,814	104	0.22	140,959	258	470	0.18
8. Killed ether	27,475	33	0.12	90,919	112	303	0.12
9. Mixed (*a*)	7,571	5	0.07	57,227	65	191	0.11
(*b*)	4,636	7	0.15	7,307	14	24	0.19
(*c*)	6,646	8	0.12	6,646	8	22	0.12
10. Yatren	—	—	—	5,384	13	18	0.24
Total	304,525	797	0.26	1,062,707	3,540	3,539	0.33

$P < 1$ in a million.

[a] Calculated from the mortality of the total of the 12 groups (*i.e.*, from 0.33311).

According to the shortened system of classification, the figures are :

	Ninth review			All nine reviews			
					Deaths		
	Number treated	Deaths	Mortality %	Number treated	Observed	Calculated	Mortality %
(*a*) Killed	162,533	520	0.32	581,589	2,459	1,937	0.42
(*b*) Live	66,692	129	0.19	299,580	753	998	0.25
(*c*) Heated	47,814	104	0.22	140,959	258	470	0.18
(*d*) Other	27,486	44	0.16	40,579	70	135	0.17
Total	304,525	797	0.26	1,062,707	3,540	3,540	0.33

$P < 1$ in a million.

The percentage mortalities in the successive reviews and in the whole set have been :

	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	All nine
(*a*) Killed	0.65	0.80	0.28	0.66	0.51	0.40	0.35	0.31	0.32	0.42
(*b*) Live	0.27	0.26	0.21	0.26	0.23	0.31	0.28	0.24	0.19	0.25
(*c*) Heated	—	0.15	0.08	0.04	0.24	0.18	0.11	0.15	0.22	0.18
(*d*) Other	—	—	—	0.22	0.27	0	0	0.08	0.16	0.17
Total	0.49	0.48	0.23	0.48	0.38	0.35	0.30	0.27	0.26	0.33

The same tests of significance of differences as were employed and described in previous reviews have been used. It will be remembered that a value P is obtained from the statistics which measures the probability that equal or greater differences than those observed would be likely to occur in samples of a population which was admittedly homogeneous. The underlying idea is to test the assumption that the various figures derived from different methods of treatment refer to samples selected at random from a very large homogeneous population. When the results of the statistical analysis indicate that the figures do not agree with this assumption, the data will be referred to as *heterogeneous* or as showing a *significant difference.* The dividing-line between *homogeneity* and *heterogeneity* may conveniently be taken as being defined by $P = 0.05$, any lesser value indicating a real discrepancy and, in the present comparison, significant differences in the efficacy of the various vaccination methods employed.

V. RACIAL STATISTICS

Information regarding race type was lacking in the case of 8,403 persons, 1 of whom died of rabies.

Of the remainder, 158,354 were of European descent (53.5%) and 137,768 were of non-European birth (46.5%).

The percentages of Europeans in the various groups for this and former reviews were as follows :

	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	All nine
1. Cords	57.6	52.7	50.2	62.5	56.1	49.5	49.1	29.8	31.1	44.5
2. Dilutions	100	100	100	94.8	96.6	100	89.0	100	100	97.6
3. Killed phenol	19.7	11.9	62.1	10.3	15.6	12.9	21.5	15.6	20.1	17.5
4. Live phenol	—	100	100	100	100	100	100	100	100	100
5. Fermi's vaccine	—	—	—	100	100	100	100	100	53.9	64.8
6. Fermi's S.V.	—	—	—	100	100	—	100	—	—	100
7. Heated	—	100	100	100	100	100	100	100	100	100
8. Killed ether	99.7	—	100	100	100	100	100	100	100	100
9. Mixed (*a*), (*b*) and (*c*)	100	100	100	100	100	100	100	100	100	100
10. Yatren	—	—	—	100	100	—	—	—	—	100
Total	52.4	53.5	81.1	48.9	58.0	52.0	50.8	45.2	53.5	53.7

The figures relating to mortality are given in the following table :

	Ninth review				All nine reviews			
		Deaths				Deaths		
Europeans	Number treated	Observed	Calculated	Mortality %	Number treated	Observed	Calculated	Mortality %
1. Cords	11,252	18	18	0.16	65,025	95	100	0.15
2. Dilutions	12,284	17	20	0.14	73,364	125	113	0.17
3. Killed phenol	25,911	53	42	0.20	81,555	135	126	0.17
4. Live phenol	3,542	0	6	0	7,006	6	11	0.09
5. Fermi's vaccine	11,223	10	18	0.09	17,723	23	27	0.13
6. Fermi's S.V.	—	—	—	—	390	0	1	0
7. Heated	47,814	104	77	0.22	140,959	258	217	0.18
8. Killed ether	27,475	33	44	0.12	90,919	112	140	0.12
9. Mixed (*a*)	7,571	5	12	0.07	57,227	65	88	0.11
(*b*)	4,636	7	7	0.15	7,307	14	11	0.19
(*c*)	6,646	8	11	0.12	6,646	8	10	0.12
10. Yatren	—	—	—	—	5,384	13	8	0.24
Total	158,354	255	255	0.16	553,505	854	852	0.15
	P = 0.0004				P = 0.0009			
Non-Europeans								
1. Cords	24,908	82	98	0.33	80,952	423	452	0.52
2. Dilutions	—	—	—	—	1,777	16	10	0.90
3. Killed phenol	103,259	433	405	0.42	383,955	2,194	2,144	0.57
5. Fermi's vaccine	9,601	26	38	0.27	9,601	26	54	0.27
Total	137,768	541	541	0.39	476,285	2,659	2,660	0.56
	P = 0.015				P = 0.0001			

Information was not available regarding 8,403 treated persons, of whom 1 died of rabies.

Or, using the shortened system of classification :

	Ninth review				All nine reviews			
		Deaths		Mortality		Deaths		Mortality
Europeans :	Number treated	Observed	Calculated	%	Number treated	Observed	Calculated	%
(*a*) Killed	53,386	86	86	0.16	172,474	247	266	0.14
(*b*) Live	39,285	47	63	0.12	209,929	305	324	0.15
(*c*) Heated	47,814	104	77	0.22	140,959	258	217	0.18
(*d*) Other	17,869	18	29	0.10	30,143	44	47	0.15
Total	158,354	255	255	0.16	553,505	854	854	0.15
	P = 0.0004				P = 0.02			
Non-Europeans :								
(*a*) Killed	103,259	433	405	0.42	383,955	2,194	2,144	0.57
(*b*) Live	24,908	82	98	0.33	82,729	439	462	0.53
(*d*) Other	9,601	26	38	0.27	9,601	26	54	0.27
Total	137,768	541	541	0.39	476,285	2,659	2,660	0.56
	P = 0.015				P = 0.0002			

The mortalities compared with previous reviews are as under :

Europeans :	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	All nine
(*a*) Killed	0.10	0.14	0.10	0.12	0.19	0.22	0.14	0.09	0.16	0.14
(*b*) Live	0.20	0.11	0.13	0.15	0.17	0.22	0.13	0.11	0.12	0.15
(*c*) Heated	—	0.15	0.08	0.04	0.24	0.18	0.11	0.15	0.22	0.18
(*d*) Other	—	—	—	0.19	0.27	0	0	0.24	0.10	0.15
Total	0.17	0.13	0.10	0.14	0.21	0.21	0.13	0.11	0.16	0.15
Non-Europeans :										
(*a*) Killed	0.83	0.89	0.93	0.85	0.65	0.52	0.45	0.41	0.42	0.57
(*b*) Live	1.02	0.82	0.71	0.74	0.46	0.65	0.67	0.44	0.33	0.53
(*d*) Other	—	—	—	—	—	—	—	—	0.27	0.27
Total	0.84	0.88	0.78	0.83	0.62	0.54	0.48	0.42	0.39	0.56

It appears from the combined figures that the mortality amongst non-Europeans is 3.7 as great as that amongst Europeans.

In the statistics analysed in the previous review, it was noted that all the values of P found in the tables corresponding to those given above were greater than 0.05, and that consequently there was no evidence of significant differences in the efficacies of the various methods of treatment. In the figures relating to the present (ninth) review, this is no longer the case, all values of P being below 0.05. As the figures cover a period of two years, and form a large proportion of the combined figures for all nine reviews, this is reflected

in the combined tables relating to the whole series. The heterogeneity in the figures relating to the ninth review is mainly due to a deficiency of deaths amongst Europeans treated by Puntoni's live-phenol method, an excess amongst Europeans treated by heated vaccines, and a deficiency amongst non-Europeans treated by Fermi's vaccine. From the combined tables, it appears that, in the case of Europeans, the main heterogeneous elements are excessive deaths amongst those treated by heated vaccines and deficient deaths amongst those treated by killed ether and the mixed treatment 9(*a*) ; in the case of non-Europeans, the outstanding discrepancy is the low observed figure for those treated by Fermi's vaccine.

VI. SPECIES OF BITING ANIMAL

The percentages of persons bitten by the different species of animals are as under :

	Dog	Cat	Wolf	Jackal	Solipeds	Ruminants	Other	Human	Missing
1. Cords	85.6	5.7	0	0.1	0.6	0.3	1.5	0.3	5.9
2. Dilutions	78.5	10.2	0.4	0.1	2.9	4.4	2.1	0.5	0.9
3. Killed phenol	88.1	1.8	0.1	5.3	0.5	0.6	1.3	0.7	1.6
4. Live phenol	86.1	10.6	0	0	0.7	1.2	0.9	0.4	0
5. Fermi's vacc.	84.4	7.4	0.5	0.1	2.2	2.9	1.8	0.6	0.2
7. Heated	82.4	7.5	0.1	0	0.2	5.6	2.4	1.2	0.6
8. Killed ether	80.5	5.9	0.2	0	0.7	5.9	3.1	3.6	0.1
9. Mixed (*a*)	83.4	9.2	0.1	0	2.0	3.6	1.1	0.5	0
(*b*)	80.6	16.6	0	0	0.3	0.2	2.2	0.1	0
(*c*)	88.8	6.5	0.5	0.2	1.3	1.0	0.5	1.1	0.2
Total	85.3	4.9	0.1	2.4	0.8	2.2	1.7	1.0	1.6

The figures relating to mortality are as follows :

	(a) *Dog*			(b) *Cat*		
	Number treated	Deaths	Mortality %	Number treated	Deaths	Mortality %
1. Cords	33,108	100	0.30	2,190	0	0
2. Dilutions	9,647	15	0.16	1,259	0	0
3. Killed phenol	118,929	379	0.32	2,497	1	0.04
4. Live phenol	3,051	0	0	374	0	0
5. Fermi's vacc.	17,582	27	0.15	1,547	2	0.13
7. Heated	39,392	96	0.24	3,595	1	0.03
8. Killed ether	22,111	31	0.14	1,627	0	0
9. Mixed (*a*)	6,317	5	0.08	699	0	0
(*b*)	3,738	7	0.19	768	0	0
(*c*)	5,901	5	0.08	431	0	0
Total	259,776	665	0.26	14,987	4	0.03

(c) *Wolf*

	Ninth review			All nine reviews			
	Number treated	Deaths	Mortality %	Number treated	Deaths Observed	Deaths Calculated	Mortality %
1. Cords	0	0	0	76	4	5.9	5.26
2. Dilutions	43	0	0	100	11	7.8	11.00
3. Killed phenol	110	8	7.27	588	50	45.6	8.50
5. Fermi's vacc.	108	7	6.48	113	7	8.8	6.19
7. Heated	51	7	13.73	138	13	10.7	9.42
8. Killed ether	45	2	4.44	262	17	20.3	6.49
9. Mixed (*a*)	7	0	0	83	5	6.4	6.02
(*c*)	34	3	8.82	34	3	2.6	8.82
10. Yatren	—	—	—	23	0	1.8	0
Total	398	27	6.78	1,417	110	109.9	7.76

P = 0.65

(d) *Jackal*

	Ninth review			All nine reviews			
	Number treated	Deaths	Mortality %	Number treated	Deaths Observed	Deaths Calculated	Mortality %
1. Cords	29	0	0	154	3	2.4	1.95
2. Dilutions	17	2	11.77	94	2	1.4	2.13
3. Killed phenol	7,097	92	1.30	30,387	465	464.8	1.53
5. Fermi's vacc.	11	0	0	11	0	0.2	0
7. Heated	17	0	0	87	0	1.3	0
8. Killed ether	0	0	0	4	0	0.1	0
9. Mixed (*a*)	3	0	0	38	1	0.6	2.63
(*c*)	15	0	0	15	0	0.2	0
Total	7,189	94	1.31	30,790	471	471.0	1.53

P = 0.9

(e) *Solipeds, Ruminants, Human and Other*

(The figures in parentheses indicate deaths.)

	Solipeds	Ruminants	Other	Human	Missing
1. Cords	241	121	577	131	2,262
2. Dilutions	353	545	252	62	106
3. Killed phenol	689 (1)	765	1,822 (4)	1,012	2,137 (2)
4. Live phenol	26	43	33	15	0
5. Fermi's vacc.	454	598	373	127	40
7. Heated	73	2,677	1,156	561	292
8. Killed ether	203	1,620	843	1,002	24
9. Mixed (*a*)	153	275	82	35	0
(*b*)	15	7	104	4	0
(*c*)	86	64	33	70	12
Total	2,293 (1)	6,715	5,275 (4)	3,019	4,873 (2)

Information regarding the biting animal was lacking in the case of 4,873 persons, of whom 2 died of rabies.

The death amongst those bitten by solipeds occurred at Ankara, the animal being an ass. Four deaths were reported amongst those

treated by killed-phenol vaccines and bitten by " other animals ". These were 2 foxes (Coonoor) and 2 hyenas (Kasauli).

From the combined figures for the nine reviews, it appears that, of 26,802 persons bitten by solipeds and ruminants, only 3 cases (1 from Chosen, 1 from Tunis, and 1 from Ankara) have developed rabies. Of 8,581 persons who have come in contact with cases of human rabies, only 1 (Kasauli) has been reported as having contracted the disease.

VII. EVIDENCE OF RABIES IN THE BITING ANIMAL

The percentages of treated persons in the different categories are as follows :

	A	B	C	D
1. Cords	7.4	14.0	70.4	8.1
2. Dilutions	14.4	30.4	37.5	17.8
3. Killed phenol	6.7	5.4	85.8	2.0
4. Live phenol	1.4	78.6	20.0	0
5. Fermi's vacc.	11.7	5.6	82.7	0
7. Heated	18.7	19.4	58.0	3.9
8. Killed ether	20.7	7.1	71.2	1.0
9. Mixed (*a*)	25.8	30.8	27.6	15.7
(*b*)	2.5	16.6	81.0	0
(*c*)	11.5	26.7	61.7	0
Total	11.2	12.1	73.0	3.7

The figures relating to mortality in the different categories are as under :

Category A.

	Ninth review			All nine reviews		
	Number treated	Deaths	Mortality %	Number treated	Deaths	Mortality %
1. Cords	2,742	21	0.77	11,682	79	0.68
2. Dilutions	1,763	7	0.40	9,580	21	0.22
3. Killed phenol	8,742	22	0.25	26,056	117	0.45
4. Live phenol	50	0	0	338	0	0
5. Fermi's vacc.	2,398	9	0.38	2,995	11	0.37
6. Fermi's S.V.	—	—	—	24	0	0
7. Heated	8,947	25	0.27	15,453	48	0.31
8. Killed ether	5,694	13	0.23	14,096	33	0.23
9. Mixed (*a*)	1,956	0	0	8,134	10	0.11
(*b*)	114	0	0	774	4	0.52
(*c*)	762	2	0.26	762	2	0.26
10. Yatren	—	—	—	298	0	0
Total	33,168	99	0.30	90,192	325	0.36

Category B.

	Ninth review			All nine reviews		
	Number treated	Deaths	Mortality %	Number treated	Deaths	Mortality %
1. Cords	5,193	12	0.19	23,250	118	0.51
2. Dilutions	3,729	8	0.21	16,703	49	0.29
3. Killed phenol	7,056	22	0.31	31,043	100	0.32
4. Live phenol	2,784	0	0	3,395	2	0.06
5. Fermi's vacc.	1,153	0	0	2,969	2	0.07
6. Fermi's S.V.	—	—	—	60	0	0
7. Heated	9,268	9	0.09	33,268	40	0.12
8. Killed ether	1,940	1	0.05	8,171	9	0.11
9. Mixed (*a*)	2,335	3	0.13	22,177	36	0.16
(*b*)	769	5	0.65	830	6	0.72
(*c*)	1,774	6	0.34	1,774	6	0.34
10. Yatren	—	—	—	2,644	9	0.34
Total	36,001	66	0.18	146,284	377	0.26

Category C.

1. Cords	26,071	67	0.26	97,168	300	0.31
2. Dilutions	4,602	2	0.04	35,141	46	0.13
3. Killed phenol	111,517	438	0.39	396,230	2,073	0.52
4. Live phenol	708	0	0	2,769	3	0.11
5. Fermi's vacc.	16,940	26	0.15	18,835	29	0.15
6. Fermi's S.V.	—	—	—	52	0	0
7. Heated	27,704	70	0.25	84,016	167	0.20
8. Killed ether	19,546	19	0.09	52,627	63	0.12
9. Mixed (*a*)	2,093	2	0.10	14,461	11	0.08
(*b*)	3,753	2	0.05	5,703	4	0.07
(*c*)	4,097	0	0	4,097	0	0
10. Yatren	—	—	—	1,486	4	0.27
Total	217,031	626	0.29	712,585	2,700	0.38

Category D.

1. Cords	3,001	0	0	10,804	3	0.03
2. Dilutions	2,190	0	0	9,142	9	0.10
3. Killed phenol	2,642	1	0.04	17,904	21	0.12
4. Live phenol	0	0	0	98	0	0
5. Fermi's vacc.	0	0	0	18	0	0
6. Fermi's S.V.	—	—	—	0	0	0
7. Heated	1,844	0	0	6,738	0	0
8. Killed ether	271	0	0	13,003	0	0
9. Mixed (*a*)	1,187	0	0	8,163	3	0.04
(*b*)	0	0	0	0	0	0
(*c*)	0	0	0	0	0	0
10. Yatren	—	—	—	956	0	0
Total	11,135	1	0.009	66,826	36	0.05

Details regarding 7,187 persons, of whom 5 died of rabies, are lacking.

As in former reviews, in this and in the succeeding tables, statistics of the two race types—European and non-European—will be treated separately, as the degree of heterogeneity introduced where they are taken together is so great as to mask other effects.

The following table relates to the statistics of the two categories A and B taken together—that is, the statistics of those bitten by animals proved or certified to be rabid—and those of category C—that is, those bitten by animals suspected but not proved to be rabid—are given for the ninth and for the total of all nine reviews.

Categories A and B (combined).

	Ninth review			All nine reviews			
					Deaths		
Europeans	Number treated	Deaths	Mortality %	Number treated	Observed	Calculated	Mortality %
1. Cords	1,112	2	0.18	11,066	20	22	0.18
2. Dilutions	5,492	15	0.27	28,005	70	55	0.25
3. Killed phenol	10,002	19	0.19	30,130	59	59	0.20
4. Live phenol	2,834	0	0	4,039	3	8	0.07
5. Fermi's vacc.	2,446	5	0.20	4,041	9	8	0.22
6. Fermi's S.V.	—	—	—	84	0	0.16	0
7. Heated	18,215	34	0.19	49,731	91	98	0.18
8. Killed ether	7,634	14	0.18	25,265	49	50	0.19
9. Mixed (*a*)	4,291	3	0.07	33,239	50	65	0.15
(*b*)	883	5	0.57	1,604	10	3	0.06
(*c*)	2,536	8	0.32	2,536	8	5	0.32
10. Yatren	—	—	—	2,942	9	6	0.31
Total	55,445	105	0.19	192,682	378	379	0.20
				P = 0.0005			
Non-Europeans							
1. Cords	1,899	20	1.05	5,038	64	37	1.27
2. Dilutions	—	—	—	474	2	3	0.42
3. Killed phenol	3,881	19	0.49	14,794	82	108	0.54
5. Fermi's vacc.	0	0	0	0	0	0	0
Total	5,780	39	0.67	20,306	148	148	0.73
				P = 0.000001			

Category C.

Europeans	Ninth review: Number treated	Deaths	Mortality %	All nine reviews: Number treated	Deaths: Observed	Deaths: Calculated	Mortality %
1. Cords	3,424	1	0.03	17,030	16	25	0.09
2. Dilutions	4,602	2	0.04	33,934	37	50	0.11
3. Killed phenol	14,254	34	0.24	41,703	84	62	0.20
4. Live phenol	708	0	0	2,769	3	4	0.11
5. Fermi's vacc.	8,037	4	0.05	9,937	7	15	0.07
6. Fermi's S.V.	—	—	—	52	0	0.08	0
7. Heated	27,704	70	0.25	84,016	167	124	0.20
8. Killed ether	19,546	19	0.10	52,627	63	78	0.12
9. Mixed (*a*)	2,093	2	0.10	14,461	11	21	0.08
(*b*)	3,753	2	0.05	5,703	4	8	0.07
(*c*)	4,097	0	0	4,097	0	6	0
10. Yatren	—	—	—	1,486	4	2	0.27
Total	88,218	134	0.15	267,815	396	395	0.15
					P = 0.000001		
Non-Europeans							
1. Cords	10,917	31	0.28	26,299	100	134	0.38
2. Dilutions	—	—		680	0	3.5	0
3. Killed phenol	90,668	386	0.42	260,466	1,361	1,325	0.52
5. Fermi's vacc.	9	1	11.11	9	1	0.05	11.11
Total	101,594	418	0.41	287,454	1,462	1,462	0.51
					P = 0.003		

The values of P now obtained are all significant.

In the following table, categories A, B and C are combined. The difference between this and that in Section V is simply that category D—that is, those bitten by dogs *not* suspected to be rabid—is excluded.

Total, excluding Category D.

Europeans	All nine reviews: Number treated	Deaths: Observed	Deaths: Calculated	Mortality %
1. Cords	28,096	36	47	0.13
2. Dilutions	61,939	107	104	0.17
3. Killed phenol	71,833	143	121	0.20
4. Live phenol	6,808	6	11	0.09
5. Fermi's vacc.	13,978	16	23	0.11
6. Fermi's S.V.	136	0	0.2	0
7. Heated	133,747	258	225	0.19
8. Killed ether	77,892	112	131	0.14
9. Mixed (*a*)	47,700	61	80	0.13
(*b*)	7,307	14	12	0.19
(*c*)	6,633	8	11	0.12
10. Yatren	4,428	13	7	0.29
Total	460,497	774	772	0.17
			P = 0.001	

All nine reviews

Non-Europeans	Number treated	Deaths Observed	Deaths Calculated	Mortality %
1. Cords	31,337	164	164	0.52
2. Dilutions	1,154	2	6	0.17
3. Killed phenol	275,260	1,443	1,440	0.52
5. Fermi's vacc.	9	1	0.05	11.11
Total	307,760	1,610	1,610	0.52

P = 0.25

Also in the abbreviated form of classification :

Europeans				
(*a*) Killed	149,725	255	252	0.17
(*b*) Live	151,850	224	255	0.15
(*c*) Heated	133,747	258	225	0.19
(*d*) Other	25,175	37	42	0.15
Total	460,497	774	774	0.17

P = 0.025

Non-Europeans.				
(*a*) Killed	275,260	1,443	1,440	0.52
(*b*) Live	32,491	166	170	0.51
(*d*) Other	9	1	0.05	11.11
Total	307,760	1,610	1,610	0.52

P = 0.8

It is to be noted that the values of the Ps for categories A, B and C are higher than those for categories A and B alone. Thus the inclusion of category C has tended to decrease the evidence of heterogeneity amongst the different treatment groups. Also the values of the Ps in the above table are lower than those in the table for all cases in Section V, and so the exclusion of category D has also led to a decrease in the evidence of heterogeneity. As in the previous review, one is led to the conclusion that the important line of demarcation is between those suspected of rabies and those *not* suspected of rabies. In other words, that entrance into category C is a very strong indication that the biting animal was rabid. This is not to be wondered at, as the judgment in this case depends upon the decision, arrived at from the evidence, by the director of a Pasteur institute.

The significance of these results will be further discussed in the summary and conclusions in section XIII.

VIII. SEVERITY OF BITE

The percentage proportions in the several groups are :

	Deep	Superficial	No visible lesion
1. Cords	27.7	57.1	15.2
2. Dilutions	15.4	63.8	20.9
3. Killed phenol	48.0	45.1	6.8
4. Live phenol	32.5	67.2	0.3
5. Fermi's vacc.	26.9	61.3	11.8
7. Heated	14.6	74.6	10.6
8. Killed ether	19.3	46.2	34.6
9. Mixed (*a*)	16.6	53.2	30.3
(*b*)	5.6	94.4	0
(*c*)	65.6	26.8	7.6
Total	35.5	51.8	12.7

The percentages with no visible lesion of those treated by dilutions, killed ether and mixed (*a*) (dilution and Alivisatos) are rather high (*vide* also the sixth, seventh and eighth reviews).

The figures relating to mortality are as follows :

	(a) *Deep*			(b) *Superficial*			(c) *No visible lesion*	
	Number treated	Deaths	Mortality %	Number treated	Deaths	Mortality %	Number treated	Deaths
1. Cords	10,640	84	0.78	21,891	16	0.07	5,806	0
2. Dilutions	1,888	13	0.69	7,827	4	0.05	2,566	0
3. Killed phenol	63,422	382	0.60	59,620	101	0.17	9,045	2
4. Live phenol	1,153	0	0	2,380	0	0	9	0
5. Fermi's vacc.	4,907	28	0.57	11,180	8	0.07	2,156	0
7. Heated	3,514	36	1.02	17,905	24	0.13	2,593	0
8. Killed ether	5,296	24	0.45	12,682	9	0.07	9,491	0
9. Mixed (*a*)	1,255	5	0.40	4,025	0	0	2,291	0
(*b*)	107	2	1.87	1,793	1	0.56	0	0
(*c*)	4,358	8	0.18	1,778	0	0	508	0
Total	96,540	582	0.60	141,081	163	0.12	34,465	2

Information was not available regarding 32,439 persons, of whom 50 died of rabies.

The statistics subdivided according to race type are as follows :

(a) *Deep*

Europeans	Ninth review: Number treated	Deaths	Mortality %	All nine reviews: Number treated	Deaths: Observed	Deaths: Calculated	Mortality %
1. Cords	1,446	3	0.21	6,775	30	35	0.44
2. Dilutions	1,888	13	0.69	12,347	91	64	0.74
3. Killed phenol	3,230	32	0.99	12,427	107	65	0.86
4. Live phenol	1,153	0	0	2,074	3	11	0.14
5. Fermi's vacc.	2,573	10	0.39	3,750	17	20	0.45
6. Fermi's S.V.	—	—	—	22	0	0.1	0
7. Heated	3,514	36	1.02	19,508	108	102	0.55
8. Killed ether	5,296	24	0.45	18,289	78	95	0.43
9. Mixed (*a*)	1,255	5	0.40	15,923	47	83	0.30
(*b*)	107	2	1.87	226	3	1	1.33
(*c*)	4,358	8	0.18	4,358	8	23	0.18
10. Yatren	—	—	—	187	7	1	3.74
Total	24,820	133		95,886	499	500	0.52
				P > 1 in a million.			
Non-Europeans							
1. Cords	2,877	40	1.39	6,013	115	45	1.91
3. Killed phenol	58,004	334	0.58	170,311	1,215	1,286	0.71
5. Fermi's vacc.	8	1	12.50	8	1	0.06	12.50
Total	60,889	375	0.62	176,332	1,331	1,331	0.75
				P > 1 in a million.			

(b) *Superficial.*

Europeans	Ninth review: Number treated	Deaths	Mortality %	All nine reviews: Number treated	Deaths: Observed	Deaths: Calculated	Mortality %
1. Cords	2,080	0	0	21,018	10	18	0.05
2. Dilutions	7,827	4	0.05	40,347	29	34	0.07
3. Killed phenol	17,128	20		44,946	35	37	0.12
4. Live phenol	2,380	0	0	4,535	3	4	0.07
5. Fermi's vacc.	3,757	0		5,856	0	5	0
6. Fermi's S.V.	—	—	—	339	0	0.3	0
7. Heated	17,905	24	0.13	88,320	104	74	0.12
8. Killed ether	12,682	9	0.07	50,307	34	42	0.07
9. Mixed (*a*)	4,025	0	0	29,731	18	25	0.06
(*b*)	1,793	1	0.56	4,345	7	4	0.16
(*c*)	1,778	0	0	1,778	0	1	0
10. Yatren	—	—	—	3,334	6	3	0.18
Total	71,355	58	—	294,856	246	247	0.08
				P = 0.002			
Non-Europeans							
1. Cords	10,511	11	0.10	23,409	39	54	0.17
3. Killed phenol	35,127	72	0.20	110,166	268	253	0.24
5. Fermi's vacc.	1	0	0	1	0	0	0
Total	45,639	83	0.18	133,576	307	307	0.23
				P = 0.024			

It will be seen that all the values of P in the above tables are less than 0.05, and so indicate the presence of heterogeneity. There can be no doubt, as was noted in previous reviews, that the interpretation of the phrase " severely bitten " varies greatly in the different institutes. So much is this so that the only information to be gained from this section which is of any value at all is with regard to those showing " no visible lesion ". In the following table, persons with " no visible lesion " have been excluded.

(*c*) *Deep and Superficial Combined.*

	All nine reviews			
		Deaths		
Europeans	Number treated	Observed	Calculated	Mortality %
1. Cords	27,793	40	53	0.14
2. Dilutions	52,694	120	100	0.23
3. Killed phenol	57,373	142	109	0.25
4. Live phenol	6,609	6	13	0.09
5. Fermi's vacc.	9,606	17	18	0.18
6. Fermi's S.V.	361	0	0.7	0
7. Heated	107,828	212	206	0.20
8. Killed ether	68,596	112	131	0.16
9. Mixed (*a*)	45,654	65	87	0.14
(*b*)	4,571	10	9	0.22
(*c*)	6,136	8	12	0.13
10. Yatren	3,521	13	7	0.37
Total	390,742	745	746	0.19
		P = 0.00004		
Non-Europeans				
1. Cords	29,422	154	156	0.52
3. Killed phenol	280,477	1,483	1,482	0.53
5. Fermi's vacc.	9	1	0.05	11.11
Total	309,908	1,638	1,638	0.53
		P = 1.0 (approximately)		

The above table differs from that given in section V only in the fact that from this table the statistics regarding those showing " no visible lesion " have been excluded. In the European group, there is evidence of heterogeneity, whereas in the non-European group, there is none.

Finally, it appears that for the deeply bitten the mortality amongst non-Europeans is about 1.4 times as great as amongst Europeans, whilst for those superficially bitten the ratio is about 3 times. Also amongst Europeans, the mortality from deep bites is 6.5 times as great as from superficial, whilst in the case of non-Europeans, it is 3.3 times as great.

IX. INTERVENTION OF CLOTHING

The section relating to the effect of intervention of clothing has been discontinued. The statistics dealt with in the fifth, sixth, seventh and eighth reviews showed that amongst Europeans intervention of clothing reduced mortality to about a seventh or an eighth, and amongst non-Europeans to a third or two-fifths. Beyond the fact that the clothing of the European is more protective than that of the non-European, there does not seem to be anything to be learnt from this source.

X. POSITION OF BITE

The percentage of those bitten in the various positions are as follows :

	Head	Arm	Trunk	Leg
1. Cords	4.8	38.3	5.5	51.3
2. Dilutions	6.1	49.1	3.8	41.0
3. Killed phenol	7.2	34.3	3.9	54.5
4. Live phenol	7.5	51.0	4.2	37.3
5. Fermi's vacc.	6.3	47.8	2.8	43.1
7. Heated	6.2	51.3	4.2	38.3
8. Killed ether	6.2	40.9	3.5	49.5
9. Mixed (*a*)	4.4	49.5	15.2	30.9
(*b*)	4.6	61.2	1.7	32.5
(*c*)	6.6	41.9	3.1	48.3
Total	6.5	39.9	4.2	49.4

The figures relating to mortality are as follows :

	(*a*) Head			(*b*) Arm		
	Number treated	Deaths	Mortality %	Number treated	Deaths	Mortality %
1. Cords	1,778	46	2.59	14,132	30	0.21
2. Dilutions	619	7	1.13	4,962	9	0.18
3. Killed phenol	9,356	146	1.56	44,415	168	0.38
4. Live phenol	266	0	0	1,805	0	0
5. Fermi's vacc.	1,304	23	1.76	9,859	9	0.09
7. Heated	1,596	25	1.57	13,247	30	0.23
8. Killed ether	1,117	14	1.25	7,386	19	0.26
9. Mixed (*a*)	249	2	0.93	2,819	3	0.11
(*b*)	214	1	0.47	2,835	5	0.18
(*c*)	441	6	1.36	2,787	2	0.07
Total	16,940	270	1.59	104,247	275	0.26

	(c) Trunk			(d) Leg		
	Number treated	Deaths	Mortality %	Number treated	Deaths	Mortality %
1. Cords	2,019	2	0.10	18,925	22	0.12
2. Dilutions	381	0	0	4,140	1	0.02
3. Killed phenol	5,045	10	0.20	70,546	156	0.22
4. Live phenol	148	0	0	1,323	0	0
5. Fermi's vacc.	569	1	0.18	8,881	3	0.34
7. Heated	1,077	1	0.09	9,887	12	0.12
8. Killed ether	623	0	0	8,933	0	0
9. Mixed (*a*)	864	0	0	1,758	0	0
(*b*)	78	0	0	1,509	1	0.07
(*c*)	209	0	0	3,209	0	0
Total	11,013	14	0.13	129,111	195	0.15

Information regarding 43,214 persons, of whom 43 died of rabies, was not available.

The statistics subdivided according to race type are as follows :

(a) *Head.*

	Ninth review			All nine reviews			
					Deaths		
Europeans	Number treated	Deaths	Mortality %	Number treated	Observed	Calculated	Mortality %
1. Cords	283	2	0.71	1,939	14	25	0.72
2. Dilutions	619	7	1.13	4,756	68	61	1.43
3. Killed phenol	1,358	15	1.10	4,504	56	58	1.24
4. Live phenol	266	0	0	469	2	6	0.43
5. Fermi's vacc.	703	7	1.00	1,052	15	14	1.43
6. Fermi's S.V.	—	—	—	19	0	0.2	0
7. Heated	1,596	25	1.57	6,042	95	78	1.57
8. Killed ether	1,117	14	1.25	4,247	58	55	1.37
9. Mixed (*a*)	249	2	0.80	2,786	22	36	0.79
(*b*)	214	1	0.47	331	3	4	0.91
(*c*)	441	6	1.36	441	6	6	1.36
10. Yatren	—	—	—	180	6	2	3.33
Total	6,846	79	1.15	26,766	345	345	1.29
				P = 0.006			
Non-Europeans							
1. Cords	536	23	4.29	1,314	72	46	5.48
2. Dilutions	—	—	—	48	1	2	2.08
3. Killed phenol	4,866	128	2.63	14,549	479	506	3.29
5. Fermi's vacc.	4	1	25.00	4	1	0.1	25.00
Total	5,406	152	2.81	15,915	553	554	3.47
				P = 0.0003			

(b) *Arm.*

	Ninth review			All nine reviews			
					Deaths		
Europeans	Number treated	Deaths	Mortality %	Number treated	Observed	Calculated	Mortality %
1. Cords	3,308	0	0	20,126	19	30	0.09
2. Dilutions	4,962	9	0.18	40,403	56	60	0.14
3. Killed phenol	12,934	28	0.22	43,080	67	64	0.16
4. Live phenol	1,805	0	0	3,504	3	5	0.09
5. Fermi's vacc.	6,191	3	0.05	8,605	4	13	0.05
6. Fermi's S.V.	—	—	—	231	0	0.3	0
7. Heated	13,247	30	0.23	47,399	89	70	0.19
8. Killed ether	7,386	19	0.26	26,403	53	39	0.20
9. Mixed (*a*)	2,819	3	0.11	28,087	30	42	0.11
(*b*)	2,835	5	0.18	4,352	10	6	0.23
(*c*)	2,787	2	0.07	2,787	2	4	0.07
10. Yatren	—	—	—	3,393	5	5	0.15
Total	58,274	99	0.17	228,370	338	338	0.15
				P = 0.0015			
Non-Europeans							
1. Cords	3,095	11	0.36	7,291	37	39	0.51
2. Dilutions	—	—	—	408	1	2	0.25
3. Killed phenol	28,012	131	0.47	85,062	460	457	0.54
5. Fermi's vacc.	2	0	0	2	0	0	0
Total	31,109	142	0.46	92,763	498	498	0.53
				P = 0.8			

(c) *Trunk.*

	Ninth review			All nine reviews			
					Deaths		
Europeans	Number treated	Deaths	Mortality %	Number treated	Observed	Calculated	Mortality %
1. Cords	151	0	0	806	0		0
2. Dilutions	381	0	0	1,794	0		0
3. Killed phenol	958	0	0	2,384	0		0
4. Live phenol	148	0	0	319	0		0
5. Fermi's vacc.	194	0	0	564	0		0
6. Fermi's S.V.	—	—	—	15	0		0
7. Heated	1,077	1	0.09	6,028	1		0.02
8. Killed ether	623	0	0	2,948	0		0
9. Mixed (*a*)	864	0	0	2,588	3		0.12
(*b*)	78	0	0	236	0		0
(*c*)	209	0	0	209	0		0
10. Yatren	—	—	—	112	0		0
Total	4,683	1	0.02	18,003	4		0.02
Non-Europeans							
1. Cords	947	1	0.11	2,114	6	5	0.28
2. Dilutions	—	—	—	101	0	0.2	0
3. Killed phenol	3,638	8	0.22	10,793	23	24	0.21
5. Fermi's vacc.	1	0	0	1	0	0	0
Total	4,586	9	0.20	13,009	29	29	0.22
				P = 0.68			

(d) *Leg.*

	Ninth review			All nine reviews			
					Deaths		
Europeans	Number treated	Deaths	Mortality %	Number treated	Observed	Calculated	Mortality %
1. Cords	1,091	1	0.09	9,897	9	5	0.09
2. Dilutions	4,140	1	0.02	23,362	15	11	0.06
3. Killed phenol	7,482	6	0.08	23,542	16	11	0.07
4. Live phenol	1,323	0	0	2,677	1	1	0.04
5. Fermi's vacc.	3,514	0	0	5,816	1	3	0.02
6. Fermi's S.V.	—	—	—	122	0	0.06	0
7. Heated	9,887	12	0.12	39,105	24	19	0.06
8. Killed ether	8,933	0	0	35,244	1	17	0.003
9. Mixed (*a*)	1,758	0	0	20,339	10	10	0.05
(*b*)	1,509	1	0.07	2,388	1	1	0.04
(*c*)	3,209	0	0	3,209	0	2	0
10. Yatren	—	—	—	1,699	2	1	0.12
Total	42,846	21	0.05	167,400	80	81	0.05
					P = 0.002		
Non-Europeans							
1. Cords	9,672	16	0.17	21,136	40	58	0.19
2. Dilutions	—	—	—	597	0	2	0
3. Killed phenol	59,407	138	0.23	162,501	464	445	0.29
5. Fermi's vacc.	2	0	0	2	0	0	0
Total	69,081	154	0.22	184,236	504	505	0.27
					P = 0.018		

The values of P in the above tables give evidence of heterogeneity, except in the cases of arm and trunk bites, amongst non-Europeans. The following points of detail may be noted :

(1) *Head bites.*—Amongst Europeans, the disturbing factors are a defective mortality amongst those treated by killed-ether vaccine and an excessive mortality amongst those treated by Yatren vaccine. Amongst non-Europeans, disturbance is due to an excessive mortality amongst those treated by cords.

(2) *Arm bites.*—Amongst Europeans, the disturbing factors are defective mortalities amongst those treated by Fermi and mixed (*a*) methods, and excessive mortalities amongst those treated by heated and killed-ether vaccines.

(3) *Leg bites.*—The disturbance in the European table is almost entirely due to the anomalously low mortality amongst those treated by the killed-ether method (1 fatal case amongst 35,224 persons treated during the period covered by the nine reviews, as compared with an expected 17). This curious feature has been referred to in previous reviews, and no explanation is as yet forthcoming.

Amongst non-Europeans, the disturbance arises from a defective mortality amongst those treated by cords.

The mortalities for the two race types classified according to position are as follows :

	Europeans	Non-Europeans	Ratio : $\frac{\text{Non-European mortality}}{\text{European mortality}}$
Head	1.29	3.47	2.7
Arm	0.15	0.53	3.5
Trunk	0.02	0.22	11.0
Leg	0.05	0.22	4.4

Also the mortalities of those bitten on head, arm, trunk and leg are for Europeans in the ratios of 100 : 12 : 2 : 4, and for non-Europeans of 100 : 15 : 6 : 8.

XI. DELAY IN COMMENCING TREATMENT

The percentage proportions of those commencing treatment during the various periods are as follows :

	0 to 4 days	5 to 7 days	8 to 14 days	More than 14 days
1. Cords	58.1	23.7	12.7	5.9
2. Dilutions	56.4	19.9	16.9	6.7
3. Killed phenol	66.1	16.8	11.4	6.3
4. Live phenol	56.8	22.5	14.8	5.8
5. Fermi's vacc.	73.2	14.5	8.5	3.9
7. Heated	68.6	19.4	9.2	2.8
8. Killed ether	65.6	15.8	11.0	7.6
9. Mixed (*a*)	46.3	25.5	19.5	8.8
(*b*)	74.8	14.0	8.5	2.6
(*c*)	38.3	26.7	23.2	11.8
Total	64.0	18.3	11.8	6.0

The figures relating to mortality are :

	(a) 0 *to* 4 *days*			(b) 5 *to* 7 *days*		
	Number treated	Deaths	Mortality %	Number treated	Deaths	Mortality %
1. Cords	22,257	61	0.27	9,075	18	0.20
2. Dilutions	6,931	13	0.19	2,444	1	0.04
3. Killed phenol	86,799	306	0.35	22,111	66	0.30
4. Live phenol	2,013	0	0	798	0	0
5. Fermi's vacc.	15,150	32	0.21	2,993	4	0.13
7. Heated	20,422	65	0.32	5,774	12	0.21
8. Killed ether	18,004	30	0.17	4,342	2	0.05
9. Mixed (*a*)	3,504	4	0.12	1,927	1	0.05
(*b*)	3,469	6	0.16	651	1	0.15
(*c*)	2,491	4	0.16	1,739	3	0.17
Total	181,040	521	0.29	51,854	108	0.21

	(c) 8 *to* 14 *days*			(d) *More than* 14 *days*		
	Number treated	Deaths	Mortality %	Number treated	Deaths	Mortality %
1. Cords	4,884	10	0.20	2,256	11	0.49
2. Dilutions	2,077	2	0.10	820	1	0.12
3. Killed phenol	15,016	56	0.37	8,274	56	0.68
4. Live phenol	525	0	0	206	0	0
5. Fermi's vacc.	1,752	0	0	810	0	0
7. Heated	2,746	0	0	836	1	0.12
8. Killed ether	3,029	1	0.03	2,094	0	0
9. Mixed (*a*)	1,473	0	0	667	0	0
(*b*)	395	0	0	121	0	0
(*c*)	1,513	1	0.07	769	0	0
Total	33,410	70	0.21	16,853	69	0.41

Information was not available regarding 21,368 persons, of whom 29 died.

The figures subdivided according to race type are as under :

(a) 0 *to* 4 *days*

	Ninth review			All nine reviews			
					Deaths		
Europeans	Number treated	Deaths	Mortality %	Number treated	Observed	Calculated	Mortality %
1. Cords	2,246	2	0.09	18,048	28	34	0.16
2. Dilutions	6,931	13	0.19	41,089	94	78	0.23
3. Killed phenol	14,913	43	0.29	47,748	111	91	0.27
4. Live phenol	2,013	0	0	3,980	5	8	0.13
5. Fermi's vacc.	6,785	9	0.13	9,426	16	18	0.17
6. Fermi's S.V.	—	—	—	234	0	0.4	0
7. Heated	20,422	65	0.32	72,945	145	138	0.20
8. Killed ether	18,004	30	0.17	64,372	99	122	0.15
9. Mixed (*a*)	3,504	4	0.11	29,401	41	58	0.14
(*b*)	3,469	6	0.17	5,516	13	10	0.24
(*c*)	2,491	4	0.16	2,491	4	5	0.16
10. Yatren	—	—	—	2,080	8	4	0.38
Total	80,778	176	0.22	297,330	564	566	0.19
					P = 0.029		
Non-Europeans							
1. Cords	8,997	29	0.32	18,995	79	88	0.42
2. Dilutions	—	—	—	801	0	4	0
3. Killed phenol	63,506	249	0.39	185,629	877	864	0.47
5. Fermi's vacc.	6	0	0	6	0		0
Total	72,509	278	0.38	205,431	956	956	0.47
					P = 0.15		

(b) 5 *to* 7 *days.*

Europeans	Ninth review: Number treated	Deaths	Mortality %	All nine reviews: Number treated	Deaths: Observed	Deaths: Calculated	Mortality %
1. Cords	1,197	0	0	6,511	7	7	0.11
2. Dilutions	2,444	1	0.04	16,426	24	17	0.15
3. Killed phenol	5,272	1	0.02	14,778	9	15	0.06
4. Live phenol	798	0	0	1,424	1	1	0.07
5. Fermi's vacc.	2,083	1	0.05	3,041	2	3	0.07
6. Fermi's S.V.	—	—	—	80	0	0.08	0
7. Heated	5,774	12	0.21	23,669	29	25	0.12
8. Killed ether	4,342	2	0.04	13,321	6	14	0.05
9. Mixed (*a*)	1,927	1	0.05	13,383	15	14	0.11
(*b*)	651	1	0.15	986	1	1	0.10
(*c*)	1,739	3	0.17	1,739	3	2	0.17
10. Yatren	—	—	—	1,500	4	2	0.27
Total	26,227	22	0.08	96,858	101	101	0.10
					P = 0.2		
Non-Europeans							
1. Cords	3,420	7	0.20	7,857	34	37	0.43
2. Dilutions	—	—	—	129	0	0.6	0
3. Killed phenol	15.456	64	0.41	49,861	235	233	0.47
5. Fermi's vacc.	2	1	50.00	2	1	0	50.00
Total	18,878	72	0.38	57,849	270	270	0.47
					P = 0.6		

(c) 8 *to* 14 *days.*

Europeans	Ninth review: Number treated	Deaths	Mortality %	All nine reviews: Number treated	Deaths: Observed	Deaths: Calculated	Mortality %
1. Cords	929	1	0.11	5,761	4	4	0.07
2. Dilutions	2,077	2	0.10	11,384	10	8	0.09
3. Killed phenol	3,452	5	0.14	9,913	14	7	0.14
4. Live phenol	525	0	0	1,063	0	0.8	0
5. Fermi's vacc.	1,326	0	0	1,935	0	1.4	0
6. Fermi's S.V.	—	—	—	64	0	0.05	0
7. Heated	2,746	0	0	9,884	4	7	0.04
8. Killed ether	3,029	1	0.03	7,968	6	6	0.07
9. Mixed (*a*)	1,473	0	0	9,669	6	7	0.06
(*b*)	395	0	0	622	0	0.5	0
(*c*)	1,513	1	0.07	1,513	1	1.1	0.07
10. Yatren	—	—	—	1,189	0	0.9	0
Total	17,465	10	0.06	60,965	45	44	0.07
					P = 0.12		
Non-Europeans							
1. Cords	1,425	4	0.28	3,517	14	18	0.39
2. Dilutions	—	—	—	101	0	0.5	0
3. Killed phenol	10,599	49	0.46	33,422	172	168	0.51
5. Fermi's vacc.	1	0	0	1	0	0	0
Total	12,025	53	0.44	37,041	186	186	0.50
					P = 0.45		

(d) *More than 14 days.*

Europeans	Ninth review: Number treated	Deaths	Mortality %	All nine reviews: Number treated	Deaths: Observed	Deaths: Calculated	Mortality %
1. Cords	338	0	0	1,803	1	2	0.06
2. Dilutions	820	1	0.12	4,824	10	6	0.21
3. Killed phenol	1,382	4	0.29	4,234	11	5	0
4. Live phenol	206	0	0	502	0	0.6	0
5. Fermi's vacc.	509	0	0	795	0	0.9	0
6. Fermi's S.V.	—	—	—	12	0	0.02	0
7. Heated	836	1	0.12	3,051	4	4	0.13
8. Killed ether	2,094	0	0	5,252	1	6	0.02
9. Mixed (*a*)	667	0	0	4,326	3	5	0.07
(*b*)	121	0	0	183	0	0.2	0
(*c*)	769	0	0	769	0	0.9	0
10. Yatren	—	—	—	615	1	0.7	0.16
Total	7,742	6	0.08	26,366	31	31	0.12
				P = 0.009			
Non-Europeans							
1. Cords	694	11	1.60	1,799	28	20	1.56
2. Dilutions	—	—	—	123	2	1.3	1.63
3. Killed phenol	6,273	44	0.70	19,105	198	207	1.04
Total	6,967	55	0.79	21,027	228	228.3	1.08
				P = 0.14			

The only evidences of heterogeneity in the above tables are found in the groups : (*a*) Europeans arriving within 4 days, and (*b*) Europeans arriving later than 14 days. The main disturbing factors in both instances are excessive mortalities amongst those treated by killed-phenol vaccine, and defective mortalities amongst those treated by killed-ether vaccine.

The figures may be summed up as under :

Europeans	Number treated	Deaths: Observed	Deaths: Calculated	Mortality %
Less than 14 days	455,153	710	700	0.16
More than 14 days ...	26,366	31	41	0.12
Total	481,519	741	741	0.15
		P = 0.11		
Non-Europeans				
Less than 14 days	300,321	1,412	1,533	0.47
More than 14 days ...	21,027	228	107	1.08
Total	321,348	1,640	1,640	0.51
		P < 1 in a million.		

Thus, as was pointed out in former reviews, the handicaps of late arrival, though pronounced in the case of non-Europeans for some reason which does not appear, is not evident in the European statistics.

Again, it may be noted that in the successive periods 0-4, 5-7, 8-14 and more than 14 days, the ratios of non-European to European mortalities are 2.5, 4.7, 7.1, and 9 respectively, so that, as was noted in previous reports, the relative mortality increases with lateness of arrival.

XII. PARALYTIC ACCIDENTS

The figures relating to the occurrence of post-vaccinal paralyses are as follows :

	Number treated	Accidents	Proportion 1 in	Percentage	Deaths
1. Cords	38,659	14	2,633	0.038	2
2. Dilutions	12,284	0	—	—	0
3. Killed phenol	135,058	20	6,753	0.015	3
4. Live phenol	3,542	0	—	—	0
5. Fermi's vacc.	20,840	1	20,840	0.005	1
7. Heated	47,814	0	—	—	0
8. Killed ether	27,475	1	27,475	0.004	1
9. Mixed (*a*)	7,571	2	3,786	0.026	0
(*b*)	4,636	1	4,636	0.023	0
(*c*)	6,646	3	2,215	0.045	0
Total	304,525	42	7,250	0.014	7

The figures for the period covered by all nine reviews are :

	Number treated	Accidents	Proportion 1 in	Percentage	Deaths	Percentage fatal	Percentage not fatal
1. Cords	152,899	45	3,398	0.029	5	0.003	0.024
2. Dilutions	75,141	24	3,194	0.031	17	0.018	0.013
3. Killed phenol	488,795	55	8,887	0.011	14	0.003	0.008
4. Live phenol	7,006	0	—	0	0	0	0
5. Fermi's vacc.	28,159	4	7,040	0.014	3	0.011	0.003
6. Fermi's S.V.	390	0	—	0	0	0	0
7. Heated	140,959	8	17,620	0.006	2	0.001	0.005
8. Killed ether	90,919	9	10,102	0.010	4	0.004	0.006
9. Mixed (*a*)	57,227	28	2,044	0.049	3	0.005	0.044
(*b*)	7,307	2	3,654	0.027	0	0	0.027
(*c*)	6,646	3	2,215	0.045	0	0	0.045
10. Yatren	5,384	3	1,795	0.056	0	0	0.056
Total	1,060,832	181	5,861	0.017	48	0.005	0.012

These may be abbreviated as follows :

	Number treated	Accidents Observed	Accidents Calculated	Proportion 1 in	Percentage	Deaths	Percentage fatal	Percentage not fatal
(*a*) Killed	579,714	64	99	9,058	0.011	18	0.003	0.008
(*b*) Live	299,580	99	51	3,026	0.033	25	0.008	0.025
(*c*) Heated	140,959	8	24	17,620	0.006	2	0.001	0.005
(*d*) Other	40,579	10	7	4,058	0.025	3	0.007	0.018
Total	1,060,832	181	181	5,861	0.017	48	0.005	0.012

$P < 1$ in a million

It will be seen from the above that liability to accident is above average amongst those treated by cords, dilutions, the mixed methods 9(*a*), 9(*b*) and 9(*c*), and Yatren vaccine. When fatal and non-fatal accidents are considered separately, it appears that fatal accidents are above average amongst those treated by dilutions, Fermi's vaccine and possibly the mixed method 9(*a*), whilst non-fatal accidents are above average in the case of cords, the mixed methods 9(*a*), 9(*b*), 9(*c*), Yatren vaccine, and possibly dilutions.

XIII. SUMMARY AND CONCLUSIONS

In the following table, certain of the percentage mortalities are compared with those of previous reviews.

	First review	Second review	Third review	Fourth review	Fifth review	Sixth review	Seventh review	Eighth review	Ninth review
All patients	0.49	0.48	0.23	0.48	0.38	0.35	0.30	0.27	0.26
(*a*) Live vaccines	0.27	0.26	0.21	0.26	0.23	0.31	0.28	0.24	0.19
(*b*) Killed vaccines ...	0.65	0.80	0.28	0.66	0.51	0.40	0.35	0.31	0.32
Europeans	0.17	0.13	0.10	0.14	0.21	0.20	0.13	0.11	0.16
(*a*) Live vaccines	0.20	0.11	0.13	0.15	0.17	0.22	0.13	0.11	0.12
(*b*) Killed vaccines ...	0.10	0.14	0.10	0.12	0.19	0.16	0.14	0.09	0.16
Non-Europeans	0.84	0.88	0.78	0.83	0.62	0.54	0.48	0.42	0.39
(*a*) Live vaccines	1.02	0.82	0.71	0.74	0.46	0.65	0.67	0.44	0.33
(*b*) Killed vaccines ...	0.83	0.89	0.93	0.85	0.65	0.52	0.45	0.41	0.42
Biting animal :									
Dog	0.42	0.44	0.24	0.47	0.36	0.33	0.28	0.25	0.26
Cat	0	0.07	0.04	0.06	0.03	0.03	0.04	0.07	0.03
Wolf	18.31	0	5.52	7.3	10.2	11.2	5.41	4.04	6.78
Jackal	1.58	1.64	5.56	2.2	1.48	1.19	1.49	1.52	1.31

	First review	Second review	Third review	Fourth review	Fifth review	Sixth review	Seventh review	Eighth review	Ninth review
Category :									
A	0.29	0.34	0.47	0.44	0.49	0.37	0.32	0.33	0.30
B	0.18	0.38	0.31	0.34	0.28	0.29	0.23	0.19	0.18
C	0.60	0.57	0.25	0.62	0.41	0.37	0.35	0.29	0.29
D	0.10	0.05	0.02	0.01	0.14	0.06	0.12	0.06	0.009
Deep bites	1.18	1.06	0.74	1.10	0.85	0.75	0.69	0.58	0.60
Superficial bites	0.12	0.24	0.09	0.18	0.16	0.16	0.13	0.13	0.12
On bare skin	0.66	0.62	0.38	0.66	0.50	0.46	0.40	0.36	—
Through clothing	0.10	0.13	0.04	0.12	0.09	0.08	0.11	0.08	—
Locality :									
Head	2.85	2.42	1.24	3.17	2.84	2.64	2.22	1.83	1.59
Arm	0.40	0.49	0.25	0.35	0.29	0.29	0.26	0.29	0.26
Trunk	0.42	0.11	0.10	0.31	0.11	0.14	0.11	0.12	0.13
Leg	0.30	0.40	0.11	0.30	0.21	0.17	0.18	0.14	0.15
Delay in commencing treatment :									
0 to 4 days	0.35	0.46	0.18	0.53	0.40	0.35	0.28	0.27	0.29
5 to 7 days	0.59	0.46	0.16	0.38	0.31	0.29	0.27	0.20	0.21
8 to 14 days	0.64	0.49	0.28	0.40	0.35	0.27	0.23	0.25	0.21
More than 14 days	1.04	0.69	0.33	0.73	0.52	0.63	0.83	0.57	0.41
Post-vaccinal paralysis	0.025	0.009	0.027	0.021	0.019	0.018	0.023	0.009	0.014
(*a*) Cords	0.22	0.019	0.048	0.025	0.022	0.014	0.021	0.009	0.038
(*b*) Dilutions	0.03	0.03	0	0.105	0.058	0.036	0.045	0.013	0
(*c*) Killed phenol	0.006	0.003	0.026	0.008	0.014	0.010	0.012	0.008	0.015

The figures will now be discussed on similar lines to those followed in the eighth review. Race types will be separated as before, and attention will be concentrated on three tables in particular.

The first is that given in Section V, referring to all cases treated since the inception of these reviews. It will be noticed that, in contrast with the result arrived at in the eighth review, there is now evidence of differences between the groups of persons of each race type treated by the various methods. Amongst Europeans, there now comes to light an excessive mortality amongst those treated by heated vaccines ; and defective mortalities amongst those treated by the killed-ether method and the mixed method designated as 9(*a*). Amongst non-Europeans, a defective mortality appears in a new group (those treated by Fermi's vaccine), though it is to be observed that the number involved is small.

The second table is that given for categories A, B and C in Section VII—that is to say, the table includes all persons treated, with the exception of those bitten by dogs suspected *not* to be rabid.

The chief departures in the case of Europeans occur in the groups killed phenol, heated and Yatren, where deaths are above average, and the mixed method 9(*a*), where deaths are in defect. Amongst non-Europeans, there is no evidence of difference of efficacy.

The third table is given in Section VIII and relates to those severely or superficially bitten. It differs from the first in that those with " no visible lesion " have been excluded. In this table also, evidence of heterogeneity has been found. In the case of Europeans, an excessive mortality has occurred amongst those treated by killed-phenol vaccines and dilutions, whilst the mortality is below average amongst those treated by the mixed method 9(*a*). In the case of non-Europeans, however, there is no evidence of heterogeneity.

One may summarise these results as follows :

(1) There is no evidence of difference of efficacy between treatment by cords and killed phenol in the statistics of either race type. Little can be said with regard to Fermi's vaccine, as, though it shows an apparent significant deficiency of deaths amongst non-Europeans, the difference in the case of Europeans, though also in defect, is not significant.

(2) The remaining methods have been applied in the treatment of Europeans only. The outstanding feature is that all three tables—1, 2 and 3—agree in demonstrating a deficiency of deaths amongst those treated by the mixed method 9(*a*).

One may now apply the more powerful method of analysis introduced in the seventh review. It will be remembered that it was pointed out in that review that, employing the idea of " fiduciary probability " or " confidence coefficients ", it was possible to make statements as to the limits within which any presumed superiority of one treatment over another must lie. Such statements may have any assigned degree of reliability—95%, 99%, etc. Where the number of observations is small, the range between lower and upper limit will obviously be large ; and where the observations are many, the range will be contracted, and the statement will be " more precise ". (In the present analysis, it has been found more convenient to state the degree of superiority of any one treatment over the *mean* of the whole group of treatments.)

The approximate formula is

$$p_1 - p_m = p'_1 - p'_m \pm \frac{c}{N}\sqrt{\frac{n_2\, n_3\, n_4}{n_1\, N}}$$

It must be remembered that this formula can only be safely used when all the numbers occurring in it are reasonably large. No exact limit can be given, but when any number is less than 10, it is best not to apply the formula.

In the above formula, p_1 is the probability of death for the treatment in question, p_m is that for all methods of treatment. The dashed *p*s are the observed values, and the undashed the estimated values. n_1 is the number treated by the particular method in question, n_2 the number treated by the *remaining* methods, $N = n_1 + n_2$ is the total treated population. n_3 is the total number of deaths, n_4 the total number of escapes ($N = n_3 + n_4$). c is a constant which varies according to the degree of reliability required. For a 90% reliability, it is 1.64 ; for a 95%, it is 1.96 ; and for a 99% reliability, it is 2.57. In what follows, the value $c = 1.96$, corresponding to a 95% reliability, has been adopted. It should be again emphasised that the above formula is only approximate; it is applicable when $p'_1 - p'_m$ is not too large, and we believe that it may safely be applied to the figures with which we have to deal.

The values of $p_1 - p_m$ so calculated are tabulated below for Europeans and non-Europeans respectively :

(1) For all cases ;

(2) For all, excluding category D (*i.e.*, those bitten by dogs not suspected to be rabid) ;

(3) For all, excluding those in whom " no visible lesion " was observed.

In interpreting such a table as the following, it is to be noted that, when the upper limit is positive and the lower limit is negative, a zero value is allowable ; that is to say, it is possible that the treatment in question is neither better nor worse than the mean. When both limits are of like sign, then the treatment in question is significantly better, or worse as the case may be, than the mean. When one or other limit is zero, the difference becomes just significant. This corresponds to a value of $P = 0.05$ (for a 95% reliability), as obtained by ordinary methods.

	(1) All cases		(2) Excluding D		(3) Excluding " no visible lesion "	
Europeans	Upper	Lower	Upper	Lower	Upper	Lower
1. Cords	+0.0215	—0.0359	+0.0061	—0.0861	+0.0026	—0.0962
2. Dilutions	+0.0430	—0.0088	+0.0347	—0.2253	+0.0717	+0.0023
3. Killed phenol	+0.0345	—0.0141	+0.0585	+0.0035	+0.0897	+0.0239
4. Live phenol	+0.0210	—0.1584	+0.0166	—0.1766	+0.0044	—0.2042
5. Fermi's vacc.	+0.0313	—0.0803	+0.0132	—0.1204	+0.0725	—0.0999
6. Fermi's S.V.	+0.2280	—0.5366	+0.5204	—0.8566	+0.2592	—0.6406
7. Heated	+0.0460	—0.0114	+0.0433	+0.0063	+0.0281	—0.0163
8. Killed ether	—0.0131	—0.0493	+0.0019	—0.0505	+0.0023	—0.0571
9 Mixed (*a*)	—0.0109	—0.0705	—0.0054	—0.0750	—0.0107	—0.0859
(*b*)	+0.1251	—0.0505	+0.1167	—0.0697	+0.1401	—0.0839
(*c*)	+0.0582	—0.1260	+0.0505	—0.1455	+0.0480	—0.1686
10. Yatren	+0.1895	—0.0151	+0.2455	—0.0055	+0.3220	+0.0350
Non-Europeans						
1. Cords	+0.0107	—0.0819	+0.0759	—0.0755	+0.0737	—0.0839
2. Dilutions	+0.6800	—0.0042	+0.0658	—0.7654		
3. Killed phenol	+0.0235	+0.0027	+0.0099	—0.0077	+0.0085	—0.0081

It will be seen from the above table that, with the following exceptions, the zero point is included within the range :

Europeans :

Those treated :

(1) By dilutions in Table (3) ;
(2) By killed phenol in Tables (2) and (3) ;
(3) By heated vaccine in Table (2) ;
(4) By killed ether in Table (1) ;
(5) By the mixed method 9(*a*) in Tables (1), (2) and (3) ;
(6) By Yatren in Table (3).

Non-Europeans :

Those treated by killed phenol in Table (1).

As in the previous review, the outstanding feature is the significantly defective mortality amongst those treated by the mixed method 9(*a*). The killed-ether method shows some apparent superiority in Table (1) referring to " all cases ", but this suggestion of superiority disappears when either category D or " no visible lesion" cases is excluded.

Treatment by cords and killed-phenol vaccines may be contrasted by the application of a formula similar to that employed above. This formula applies to the case where only two methods of

treatment are contrasted—that is to say, it is applicable to a 2 × 2 table. The formula is

$$p_1 - p_2 = p'_1 - p'_2 \pm c \left(\frac{n_3 \, n_4}{n_1 \, n_2 \, \mathrm{N}} \right)^{\frac{1}{2}}$$

where the symbols have the same significance as before, except that the suffix 2 applies in this case to the second method of treatment. The limits for $p_1 - p_2$, where the p_1 refers to cords and p_2 to killed phenol, may be tabulated as follows :

	(1) All cases		(2) Excluding D		(3) Excluding "no visible lesion"	
Europeans	+ 0.0021	— 0.0237	— 0.0127	— 0.1293	— 0.0374	— 0.1698
Non-Europeans .	+ 0.0078	— 0.1056	+ 0.0835	— 0.0853	+ 0.0818	— 0.0924

The only significant differences are in the case of Europeans when either D cases or " no visible lesion " cases are excluded. As this apparent superiority of cord treatment is not found in the case of non-Europeans, it is unlikely that it depends upon the method of treatment, but rather to differences in the standards adopted by various institutes in the classification of individuals according to category, or according to the character of the lesion.

Thus, in spite of the large increase of the statistics which has occurred since the publication of the eighth review, there seems to be little to choose between the cord, dilution, killed-phenol, live-phenol, Fermi, heated, and killed-ether methods as regards their immunising properties, whilst the mixed (*a*) system (dilutions : plus Alivisatos in severe cases) is apparently associated with a reduction of mortality which does not exceed 45% and may be as low as 3%.

Two considerations must always be borne in mind in discussing these findings. The first is purely statistical. The above statements are made on a basis of reliability of 95%. This means that one of twenty statements is likely to be wrong. As only the Europeans treated by the mixed (*a*) method out of a considerable number of treatment groups has shown evidence of apparent superiority of method, it is necessary not to attach undue weight to this positive result. It may eventually prove to be important, but, on the other hand, it may merely be the emergence of the one-in-twenty chance.

The second consideration to be emphasised is that any statistical analysis such as the present assumes that there is no selection or unsuspected variation in the nature of the material associated with

the various types of treatment. Obviously, if one method of treatment was applied to a series of cases only lightly bitten, whilst the other was applied to much more severely bitten cases, marked differences in death rate might occur, independent of differences in efficiency of treatment. It does not seem possible by purely statistical methods to exclude, with any certainty, the possibility of such complications. When, however, a significant superiority is shown in (2) and (3) as well as in (1) (of the table on the preceding page), there would seem to be a *prima facie* case for giving the method in question special consideration.

Attention may be directed at this stage to the combined table relating to wolf bites in Section VI. It will be noted that in this case race types are not separated. It is unlikely that this difference of procedure is of importance, as persons bitten by wolves are at a very high degree of risk and in most circumstances are likely to come for treatment independently of their race type. The average mortality is 7.76% and the value of P given in the table is 0.65. The limits (calculated where the fiduciary method is applicable) are as follows :

2. Dilution	+ 8.30	— 1.82
3. Killed phenol	+ 2.40	— 0.92
5. Fermi	+ 3.17	— 6.31
7. Heated	+ 5.90	— 2.58
8. Killed ether	+ 1.66	— 4.20
9. Mixed (*a*)	+ 3.85	— 7.33

Thus no significant differences are as yet apparent. The statistics are still too small to bring out any differences between the efficacies of the various methods, if such indeed exist.

It thus appears that, from the examination of statistics relating to approximately a million persons treated by various methods, there is little evidence of superiority of one method over another, with the possible exception of a superiority in favour of the live-vaccine treatment described as mixed (*a*) (that is, a dilution treatment with, in severe cases, the addition of Alivisatos method). Evidence in favour of the superiority of this method of treatment continues to increase but, for reasons referred to above, it cannot yet be claimed that absolute proof has been attained.

It may seem remarkable that in such large statistics differences of apparent efficacy have not become evident, but it must be

emphasised, as was done in the last review, that the controlling factor is the mortality rate. The lower this may be, the greater the number of patients required to demonstrate significant variations. In the case of Europeans, the mortality rate is of the order of 0.15% ; and in the case of non-Europeans, of 0.56%.

It may be said in general terms that, with regard to the treatment methods—cords, dilutions, killed phenol, live phenol, Fermi's original method, heated vaccines and killed-ether vaccines—it is unlikely that a difference of efficiency of more than some 25% between any two of these methods exists. The possible superiority of the mixed (*a*) method has already been referred to.

Finally, having examined the statistics with regard to immunising efficiency, we turn to the risks of paralytic accident which are involved. The results arrived at in the last review continue in general to hold, and these are summarised in Section XII. The occurrence of accidents is less than 1 in 7,000 amongst those treated by killed phenol, Fermi's original, heated- and killed-ether vaccines, and is greater than 1 in 3,500 amongst those treated by cords, dilutions, the mixed (*a*), (*b*) and (*c*) methods, and by Yatren vaccines. The percentage of fatal accidents is relatively high amongst those treated by dilutions and by Fermi's original vaccine.

Anti-Rabies Treatment in the Union of Soviet Socialist Republics.

In the first, fourth, fifth and eighth reviews, the statistics from the Union of Soviet Socialist Republics from 1927-1934 were analysed. Figures are now available relating to the years 1935, 1936 and 1937.

The schedules submitted give detailed information regarding the numbers of treated persons. These are enumerated according to the headings of the schedule issued by the Health Section of the League of Nations. The characteristics of fatal cases are, however, not classified, the only available figures being the total numbers of deaths. Consequently, the detailed system of analysis employed in the main report could not be followed, and it was deemed best to treat the statistics separately.

Schedules have been received from only 19 institutes as compared with 72 in 1927. The general characteristics of these schedules are as follows :

	Year	Number of persons treated	Deaths	Mortality %	Accidents
(1) *Pasteur*					
1. Thlissi	1935	845	0	0	1
2. ”	1936	753	1	0.13	0
		1,598	1	0.06	1
(2) *Fermi*					
1. Vologda	1935	176	0	0	0
2. ,, ...	1936	124	0	0	0
3. ,, ...	1937	153	0	0	0
4. Koujbychev	1935	458	0	0	0
5. ,, ...	1936	353	1	0.28	0
6. ,, ...	1937	194	0	0	0
7. Leningrad	1935	756	0	0	0
8. ,, ...	1936	791	1	0.15	0
9. ,, ...	1937	886	0	0	0
10. Minsk	1935	219	0	0	
11. ,, ...	1936	194	0	0	1
12. ,, ...	1937	225	0	0	
13. Odessa	1935	801	0	0	0
14. ,, ...	1936	1,936	2	0.73	0
15. ,, ...	1937	1,942	0	0	0
16. Omsk	1936	719	0	0	0
17. Rostov	1935	876	0	0	
18. ,, ...	1936	3,263	0	0	1
19. ,, ...	1937	2,544	3	0.04	
20. Tomsk	1935	106	0	0	0
21. ,, ...	1936	130	0	0	0
22. ,, ...	1937	130	0	0	0
23. Tchkalovsk	1936	514	0	0	0
		17,490	7	0.04	2
(3) *Högyes-Philipps*					
1. Veroneij	1935	261	0	0	0
2. ,, ...	1936	186	0	0	0
3. ,, ...	1937	338	2	0.58	0
4. Kief 1935-1937		5,759	1	0.02	0
5. Smolensk	1935	114	0	0	0
6. ,, ...	1936	143	0	0	0
7. ,, ...	1937	160	0	0	0
8. Tambov	1935	270	0	0	0
9. ,, ...	1936	214	0	0	0
10. ,, ...	1937	327	0	0	0
11. Kharkov	1935	441	1	0.22	0
12. ,, ...	1936	602	0	0	0
13. ,, ...	1937	1,074	2	0.18	0
		9,889	6	0.06	0

	Year	Number of persons treated	Deaths	Mortality %	Accidents
(4) *Remlinger*					
1. Irkutsk	1935	165	0	0	0
2. ,, ...	1936	113	2	1.77	0
3. ,, ...	1937	160	0	0	0
		438	2	0.46	0
(5) *Pasteur + Fermi*					
1. Thilissi	1937	739	3	0	0
(6) *Philipps + Fermi*					
1. Gorki	1935	606	0		
2. ,, ...	1936	337	0		
3. ,, ...	1937	978	2	0.20	0
4. Perm	1935	342	0	0	0
5. ,, ...	1936	334	0	0	0
6. ,, ...	1937	295	0	0	0
		2,892	2	0.07	0
(7) *Remlinger + Högyes*					
1. Penza	1935	1,393	1	0.07	0
2. ,, ...	1936	828	0	0	0
3. ,, ...	1937	1,674	6	0.35	0
		3,895	7	0.18	0
Grand total		36,941	28	0.08	3

In the following table are given the number of persons treated at the institutes from which schedules have been submitted throughout the period covered by these reviews, and also the average numbers per institute.

	Number of institutes	Number of persons bitten and treated	Average number per institute
1927	72	74,430	1,034
1928	60	66,154	1,103
1929	53	53,518	1,010
1930	55	49,399	898
1931	52	38,961	749
1932	50	27,647	553
1933	46	17,410	378
1934	46	15,630	340
1935	16	7,829	489
1936	18	11,534	641
1937	16	11,819	739
		374,331	773

(The figures from Kief (from which, as shown above, one inclusive schedule relating to the period 1935-1937 was received) have not been included in this table.)

It will be noted that the number of institutes from which schedules were received diminished abruptly at the year 1935 ; the average number of patients per institute at the same date showed a tendency to rise. It would thus appear that, in certain districts at least, the tendency for the number treated to decline, as exhibited during the earlier years, has not been maintained.

The figures relating to mortality are shown opposite.

The value of P for the combined table is less than one in a million, which shows that the statistical groups of those who had received the various forms of treatment differ very markedly.

The efficacies of the various methods have been examined by the method of limits used in the earlier portion of this review, and the results are given in the following table.

	Upper limit	Lower limit
Pasteur	+ 0.0951	+ 0.0461
Fermi	+ 0.0131	— 0.0419
Högyes-Philipps	— 0.0188	— 0.0422
Remlinger	+ 0.3379	+ 0.0905
Pasteur + Fermi	+ 0.5285	+ 0.2219
Philipps + Fermi or Alivisatos	+ 0.0380	— 0.0890
Remlinger + Högyes	+ 0.1261	— 0.0887

In the eighth review, it was found that the method of Högyes-Philipps appeared to be significantly superior to the aggregate, that the methods of Remlinger and Pasteur + Fermi were inferior, whilst the efficacies of the methods of Pasteur, Fermi and Philipps + Fermi or Alivisatos were in agreement with the aggregate. The only difference this year is that the method of Pasteur has shifted to the group of those inferior in efficacy. The new method—Remlinger + Högyes—is of average efficacy.

Three accidents only are reported as having occurred during the triennium. No statements are made as to their morbidity. If these are added to those occurring during the earlier years treated by these reviews, the following table emerges :

	Number treated	Accidents	Proportion
Pasteur	115,075	22	1 in 5,231
Fermi	70,532	8	1 in 8,817
Högyes-Philipps	244,676	30	1 in 8,156
Remlinger	3,664	0	—
Pasteur + Fermi	3,239	0	—
Philipps + Fermi or Alivisatos	16,984	1	1 in 16,984
Remlinger + Högyes	3,895	0	—
	458,065	61	1 in 7,509

	1935			1936			1937			1927-1937			
	Number bitten	Deaths	Mortality %	Number bitten	Deaths	Mortality %	Number bitten	Deaths	Mortality %	Number bitten	Deaths Observed	Deaths Calculated	Mortality %
Pasteur	845	0	0	753	1	0.13	—	—	—	81,189	188	131	0.23
Fermi	3,392	0	0	8,024	4	0.05	6,074	3	0.05	67,509	99	109	0.15
Högyes-Philipps	1,086	1	0.09	1,145	0	0	1,899	4	0.21	206,127	269	332	0.23
Remlinger	165	0	0	113	2	1.77	160	0	0	3,998	15	6	0.38
Pasteur + Fermi	—	—	—	—	—	—	739	3	0.41	2,611	14	4	0.54
Philipps + Fermi or Alivisatos	948	0	0	671	0	0	1,273	2	0.16	14,761	20	24	0.14
Remlinger + Högyes	1,393	1	0.07	828	0	0	1,674	6	0.35	3,895	7	6	0.18
Total	7,829	2	0.03	11,534	7	0.06	11,819	18	0.15	380,090	612	612	0.16

$P < 1$ in a million.

It will be noted that the above proportions are in general considerably lower than those given in Section XII of the main review, and this is particularly marked in the case of those treated by dilutions (Högyes-Philipps).

CONCLUSIONS

1. The steady decline in the number of those presenting themselves for treatment during the years 1927-1934 does not appear to have been maintained in the areas served by the institutes from which statistical returns have now been received.

2. As regards immunising properties, the statistics according to the different methods of treatment fall into three groups :

(*a*) The live vaccine of Högyes-Philipps, being apparently superior to the aggregate ;

(*b*) The methods of Fermi, Philipps + Fermi or Alivisatos, and Remlinger + Högyes, being nearly equal to the aggregate ; and

(*c*) The methods of Pasteur, Remlinger and Pasteur + Fermi, being apparently inferior to the aggregate.

ANTI-RABIC IMMUNISATION : LIVING VACCINES AND KILLED VACCINES

By

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and

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"... Discoveries have been increasing and accumulating in regard to chemical vaccines. There can be no doubt that we shall soon have many others. That of rabies, for instance, must soon be known and used."

L. PASTEUR,
Œuvres, Vol. VI, page 550.

1. The first International Conference on Rabies, held in Paris in 1927, considered the whole range of problems connected with the disease : the nature of the rabies virus, treatment and accidents of treatment and the prevention of rabies in animals. It made a thorough examination of the problem of anti-rabic vaccination, and the debate, which was opened by the report of A. C. MARIE, led to two main conclusions :

(1) There is unanimity of opinion regarding the efficiency of Pasteur treatment by the original dried cord method and by the glycerinated cord method of immunisation.

(2) It is also agreed that living fixed virus injected under the skin is innocuous to man in the vast majority of cases ; in exceptional circumstances, however, it may be infective. For this reason, certain institutes have adopted the use of a dead

or attenuated vaccine. Such vaccines, killed or attenuated either by carbolic acid or ether, produce a satisfactory immunity.[1]

The latter conclusion appears, however, to have been adopted with some reservations, since the Conference recommends " that comparative tests on a large scale be carried out in certain selected institutes with vaccines killed by carbolic acid and ether ".

At the time when the Conference was being held, A. C. Marie noted that the authors of various methods of anti-rabic vaccination were definitely tending to abandon the use of avirulent cords and to determine the dose of fixed virus which is necessary for the active immunisation of man. It was generally thought that, " in order to obtain complete immunisation, it was essential to employ virulent cords ".[2]

For some years past, however, there has been a tendency to replace the living vaccines by avirulent, " chemical " (Pasteur) vaccines. Thus, in the year 1935, we find that the institutes using dried and glycerinated cords treated 21,851 persons, whilst 62,509 were treated with killed phenol or avirulent vaccines. So far as results are concerned, only very slight differences were observed : in the former group, the general mortality rate is 0.32% with 2 cured paralytic accidents and, in the latter, it is 0.35% with 5 paralytic accidents, 2 of which were mortal.[3]

During that same year (1935), some thirty institutes were using more or less attenuated virulent cords, including fresh cords (0) in the institutes of Hanoï, Vientiane, Bucharest and Cluj. Seven institutes went to the length of using 1 day cords, whilst eighteen were content with 2 day cords as their most virulent vaccine. At the same time, an almost equal number of anti-rabic institutes used the avirulent carbolised vaccines, and were able to record no less satisfactory results.

The problem which arises in these circumstances is to determine whether any special indications and contra-indications attach to the vaccines and whether the immunity which the avirulent " chemical " vaccines are able to confer is not established as speedily and as solidly as that obtained with live vaccines.

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[1] Report on the work of the International Rabies Conference.

[2] Reports to the International Rabies Conference, pages 61 and 52.

[3] McKendrick : " Eighth Analytical Review of Reports on the Results of Anti-Rabic Treatment ", *Bull. Health Org.*, 1938, **7**, 1.

* * *

2. The Pasteur treatment, as applied in the Paris Pasteur Institute, gives " results which approach perfection : there have been no paralytic accidents since 1911, and not a single case of death in the course of twelve consecutive years. . . . "[1]

At Bucharest, the Roumanian method (6- to 0-day dried cords and heated emulsions) still registers failures. Comparison, over a period of fifty years, of the results obtained in Paris and Bucharest discloses the differences shown by the following tables :

I. *Paris (Pasteur Institute).*[2]

Ten-year periods	Persons treated	Deaths	%
1886-1895	17,337	86	0.49
1896-1905	11,872	36	0.30
1906-1915	5,044	8	0.15
1916-1925	11,698	21	0.17
1926-1935	5,616	0	0

II. *Bucharest (Babeș Institute).*[3]

Then-year periods	Persons treated	Deaths	%
1888-1897	4,238	73	1.72
1898-1907	10,469	44	0.42
1908-1917	19,116	37	0.19
1918-1927	35,671	77	0.21
1928-1937	61,064	128	0.29

During the last ten-year period, the number of persons bitten in Paris fell by 50% and mortality was nil ; at Bucharest, a gradual increase in the number of bitten persons is observable from 1908 onwards, accompanied by a substantial rise of the mortality rate.

The question therefore arises whether this should be ascribed to inadequacy of the Roumanian method.

When the immunising power of anti-rabic treatments is considered, the total death rate is not necessarily a measure of their efficiency as general methods ; whatever the method of vaccination used, this is not the only determining factor in the results obtained.

In the paper already referred to, P. Lépine and V. Sautter judiciously observe that, " in most of the anti-rabic institutes of

[1] P. Lépine and V. Sautter : *Ann. Inst. Pasteur*, 1937, **59**, 39.

[2] *Ann. Inst. Pasteur*, Numéro commém. de la rage, October 25th, 1935.

[3] G. Proca : *România medicală*, 1935, **12**, and *Bull. du Service antirabique*, 1935-1937.

the principal countries of Europe, the majority of the bitten persons taking anti-rabic treatment as a measure of precaution come under category C, in which the real risk entailed is always difficult to gauge and, be it admitted, frequently very small ".

Among the factors likely to affect the efficiency of treatment one way or the other, the first to be considered is the rabies virus itself.

3. In countries where rabies is widely prevalent—and this is true of Roumania—category C of animals suspected of rabies is bound to include a higher proportion of actually infected individuals. Moreover, the epizootic outbreaks of rabies which recur almost uninterruptedly in these areas appear to strengthen the pathogenic action of the street virus. A. C. MARIE has found, in fact, by experiment, that passage through the brain of the dog, so far from weakening this virus, appears, on the contrary, to fix its virulence.[1]

With these considerations in mind, we have taken, as an index of virulence, the period of incubation of rabies among untreated subjects and have found that short incubation periods are, on the whole, frequent. The comparative statistics reproduced below will give a more accurate idea of that frequency.

I. *Dodero's Statistics (supplemented)*[2]*: Period of Incubation of Rabies.*

Statistics	Cases observed (absolute figures)	Up to 30 days %	31 to 60 days %	61 to 90 days %	More than 90 days %
Babeş Institute (1888-1938)	290	25.2	38.5	18	17.7
BAUER	423	21.5	39	19	20.5
HÖGYES	210	28	38	22	12
Health Commission (1862-1872)	170	22	43	21	14

II. *Statistics of the Babes Institute.*

Statistics	Cases observed (absolute figures)	Less than 21 days %	21-40 days %	41-60 days %	61-90 days %	More than 90 days %
NITSCH[3]	100	2.0	21.0	26.0	15.0	36.0
BUSSON[4]	35	5.7	34.2	28.5	25.7	5.7
DODERO[5]	54	15.0	46.0	26.0	13.0	—
Babeş Institute (1888-1938)	290	8.2	33.1	23.7	17.9	16.8

[1] *L'étude expérimentale de la rage*, page 28.
[2] *Ann. de l'Inst. Pasteur*, 1938, **61**, 193.
[3] *Zbl. f. Bakt. O.*, 1906, **42**, 647.
[4] *Ibid.*, 1930, **115**, 294.
[5] *Loc. cit.*

It follows from this that, among untreated subjects, *deaths from rabies having a short incubation period are more frequent at Bucharest* than at Vienna (Busson), or at Cracow (Nitsch), and are more infrequent among Europeans than among non-Europeans (Dodero).

Classification of the deaths from rabies recorded in our statistics according to the age of the untreated subjects and according to the position of the bite yields the following tables :

III. *Period of Incubation and Age (percentage).*

Days	Children from 0 to 15 years of age	Persons over 15 years of age
Less than 21	18.7	3.0
21 to 40	38.5	30.4
41 to 60	19.7	25.7
61 to 90	12.5	20.6
More than 90	10.2	20.1
Cases observed (absolute figures)	96	194

IV. *Incubation, Position of Bites and Age (percentage).*

	Children from 0-15 years of age			Persons over 15 years of age		
Days	Head	Upper limbs	Lower limbs	Head	Upper limbs	Lower limbs
Less than 21	26.3	13.3	15.3	10.3	2.1	—
21-40	52.6	31.1	23.0	44.8	29.1	21.4
41-60	10.5	24.4	30.7	24.1	27.0	21.4
61-90	7.9	17.7	7.6	10.3	20.4	32.1
Over	2.6	13.3	23.0	10.3	21.1	25.0
Cases observed	38	45	13	29	137	28
Per 100 deaths	39.5	46.8	13.4	14.9	70.6	14.4

Tables III and IV show that the frequency of cases of rabies having a short incubation period is greater in the case of head bites and in that of children of 0 to 15 years, and that it greatly exceeds the average for all other bites whatsoever (Tables I and II).

In the course of time, deaths from rabies having a longer incubation period become more and more frequent. Thus we have :

V. *Periods of Incubation (percentage).*

Years	11-30 days	31-60 days	61-90 days	Over 90 days	Cases observed
1888-1897	14.2	51.4	28.5	5.7	35
1898-1907	25.0	55.5	8.3	11.1	36
1908-1917	29.6	37.0	18.6	14.8	27
1918-1927	32.2	28.8	17.0	22.0	59
1928-1937	22.0	35.7	21.1	21.1	109

Judging by the duration of the period of incubation of rabies, it would appear that, despite fluctuations, the general tendency has been, since 1908, for the severity of the bites to diminish. It is interesting to note that, in the first two ten-year periods, wolf bites are much more frequent among treated subjects than in the last three periods.

* * *

The pathogenic power of autochthonous street virus has been experimentally studied in 389 cases of canine rabies.

Intra-cerebrally inoculated rabbits died from rabies after varying periods, viz :

Less than 10 days after inoculation, in 23 cases (5.9%) ;
From 10-15 days after inoculation, in 140 cases (35.9%) ;
From 16-25 days after inoculation, in 178 cases (45.7%) ;
More than 25 days after inoculation, in 48 cases (12.3%).

Among the cases having a short incubation period (less than 10 days), we note a period of :

5	days	in	2	cases
6	»	»	3	»
7	»	»	1	»
8	»	»	4	»
9	»	»	13	»

In a new series of 30 cases of paralytic rabies (wolf, horse, cat and 27 dogs) that were examined in 1934, incubation periods of 6 to 7 days were found in 3 cases (10%), periods of 10 to 15 days in 15 cases (50%), periods within the limits of 16 and 30 days in 10 cases (33%) and a period of 47 days in 2 cases.

From these results, it will be seen that strains of " reinforced " street virus are comparatively infrequent in Roumania and amount at most to 10% of all strains examined.

There is, moreover, no parallelism between the period of rabies incubation in untreated subjects and incubation in the rabbits intra-cerebrally inoculated with the brain of these subjects. Cases with a long incubation period among untreated subjects may correspond to incubation periods of medium duration (13 to 15 days) in trepanned rabbits ; more rarely, the reverse is observed (B. Busson [1]).

[1] " Experim. Studien über das Lyssavirus ", *Zbl. f. Bakt. O.*, 1930, **115**, 294.

The *capacity* of autochthonous street virus *to produce Negri bodies* cannot always be determined by the microscopical examination of animals ; if the dogs are killed before the corpuscles have had time to appear, the examination will be negative so far as the dogs' brains are concerned, whilst it will frequently be positive at the first passage (rabbit).

In dogs which have died as a result of the progressive course taken by the disease, an abundance of corpuscles is an almost constant and a characteristic feature.

The following table sums up a series of recent experiments on the capacity of Roumanian street virus to produce Negri bodies :

(a) *Canine Rabies with Negri Bodies.*

Trepanned rabbits	Corpuscles	Absence of corpuscles
50 cases	38 times	12 times

(b) *Dogs suspected of Rabies : Absence of Negri Bodies.*

41 cases	17 times	14 times

It will be observed that 24% of rabbits inoculated with brains having an abundance of Negri bodies showed no sign of these formations ; the explanation should perhaps be sought in the fact that, at Bucharest, use is made of small rabbits, which die of rabies more rapidly.

The 10 strains of street virus which we have studied in regard to their *plasticity*—that is to say, power of adaptation to the rabbit's brain—although selected at random—2 in man and 8 in dogs—display the same characteristic nine times out of ten. The duration of the period of incubation, whatever it might have been in the first passage, fell, in the second passage, to 6 days in one case, to 5 days in one case, and to 4 days in 7 cases. In subsequent passages, incubation lasted 4 or 5 days, as though the virus had become fixed after a single passage.

If we adopt the terminology proposed by Lévaditi,[1] among the 10 strains examined, we found 1 strain that had undergone " spontaneous mutation " (No. 1412, incubation 5 days in the first passage and from 4 to 5 days in subsequent passages), and 8 strains that were " easily mutable ", 2 of them from human cases.

[1] *Ann. Inst. Pasteur*, 1926, **40**, 973.

We believe that the frequency of this plasticity or ready adaptability of strains of street virus will require to be determined by examination of a larger number of cases, since the pathogenic power of street virus and its plasticity are probably associated characteristics.

So far as the *neuroprobasis* of our street virus is concerned, experiments on mice sometimes show that this virus, when inoculated under the skin, reaches the cerebro-spinal axis after an incubation period of 25 to 36 days, whilst the fixed virus, Bucharest strain, regularly produces paralysis in mice after 7 to 11 days.

After the intracaudal inoculation (Proca) of street virus into mice, the incubation period of rabies is usually short. Of a total of 56 white mice thus inoculated, 3 were paralysed in less than 7 days, 38 between the 8th and the 15th day and 10 between the 16th and the 28th day, whilst 5, or 8.9%, survived.[1]

The existence " in nature of reinforced or attenuated street viruses " (A. C. Marie) was known at the time of the first Rabies Conference, the Koritschoner virus representing the reinforced type and the virus of the so-called " mad-dog " sickness (ulu-fato of West Africa and Sudan), the naturally attenuated type, of lesser virulence than that of the commonly found street virus.

Since the Conference, several strains of reinforced virus have been described (Stuart and Krikorian, D. Jonnesco, Palawandow and Serebrennaja, and J. L. Pawan), which are noteworthy for their pathogenic action, and produce rabies in rabbits after an incubation period of 1 to 3 days.

Save for their extreme virulence, the reinforced viruses do not appear to present unusual properties. It is true that the antigenic power of the fixed virus derived from these strains of enhanced virulence has not been studied, and that there is no evidence, based on experiments in cross immunity, to show that any fixed virus gives protection against the reinforced viruses as effectively as a passage virus of the latter type. It would be well if the contention that all anti-rabic viruses are " antigenically identical " (Remlinger and Bailly[2]) could be studied more closely.

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[1] D. Jonnesco : " Diagnostic de la rage au moyen de l'inoculation intracaudale ", *C.R. Soc. Biol.*, 1934, **116**, 545.

[2] *Ann. Inst. Pasteur*, 1930, **45**, 376.

Attempts have been made to assign a value to street viruses in terms of the limiting brain dilutions which are virulent for the rabbit. Nicolau, Mathis and Mme. Constantinescu isolated, from a child which died of street rabies at Dakar, an autochthonous virus which was only virulent in a dilution of 1:500 at the 4th passage. Dilutions of 1:1,000, 1:2,000 and 1:5,000 had no pathogenic action. On the other hand, a reinforced virus, isolated at Odessa by Palawandow and Serebrennaja, killed rabbits in dilutions of 1:75,000 instead 1:25,000 (Odessa fixed virus). Another reinforced virus, found in a rat at Istanbul, by Zehoi Muammer, was still active in dilutions of 1:500,000. Remlinger, from whom we have borrowed these details, adds the findings of his personal research on various street viruses isolated in Morocco.[1]

Among 11 street viruses examined, the minimum active dilutions were : 1:2,000 once, 1:5,000 twice, 1:10,000 three times, 1:50,000 once, and 1:100,000 four times.

The author observes that, " if virulence is taken to mean the aggressive or pathogenic power, concordance between the figures of the dilutions at which the various rabies viruses are active and their harmfulness for man appears highly improbable ".

This concordance appears to us all the less probable, when it is sought to establish it as between the behaviour of the rabbit infected through the cerebral route and the behaviour of man infected by bite.

The method of dilutions might, however, yield valuable information of the pathogenic power of the rabies virus, even though it should be for the rabbit alone, if available technical processes made it possible to produce completely homogeneous emulsions, which is not the case.

Lépine, Cruveilhier and Sautter found that the 1:10,000 emulsion of fixed virus, Paris strain, was virulent in 1 case out of 2, and the 1:50,000 emulsion in 1 case out of 6, whilst the 1:100,000 emulsion was always inactive. Remlinger himself, experimenting with the " Podenko " street virus found 1:500 and 1:2,000 dilutions to be inactive, whilst the 1:1,000 and 1:5,000 emulsions were pathogenic. As this scientist points out,[2] " the regularity

[1] *Ann. Inst. Pasteur*, 1937, **58**, 377.
[2] *Ibid.*, 1931, **47**, 643.

of the results leaves something to be desired, and, in the tables recording the activity of the various dilutions, gaps, or " holes " as we call them, are seen to occur ".

If, however, the higher dilutions act no less vigorously than the strongest doses ; if, for instance, a rabbit infected with a 1:500,000 emulsion of fixed virus shows the first symptoms of rabies on the 6th day, at the same time as a rabbit which has been given 0.25 c.c. of the 1:50 emulsion, so paradoxical a result arouses doubts as to the homogeneity of the dilutions and the value of the method of titration employed.

* * *

4. Cultivated *in vivo*, in the rabbit's brain, the virus undergoes, in the long run, modifications which are not spontaneously reversible and are durable, and which are maintained by inoculations from brain to brain. The new type thus created is the passage virus or *fixed virus*.

The constancy of its essential characteristics is not absolute. The research of Remlinger, Lépine, Nicolau, etc., has shown that the virulence, in especial, of a strain of passage virus, as well as its resistance to the action of attenuating factors, may change in one particular direction in the course of successive passages.[1]

We ourselves have studied 2 strains of attenuated fixed virus, characterised by the prolongation of the period of incubation in rabbits, guinea-pigs and mice, and by the appearance of a capacity to produce Negri bodies, which our passage virus appeared to have finally lost. These 2 strains were isolated from the brains of mice which had been infected with the fixed virus, Bucharest strain. These animals,[2] which had been treated with anti-rabic serum, had died of rabies after an extended period of incubation (42 and 57 days). Although the modified virus studied lacked stability, its cultivation was kept up for more than 15 months, and even in the 27th passage (rabbit) incubation was twice as long as with our normal fixed virus.[2]

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[1] P. Lépine : *Rabies.* Rabies virus in the treatise of C. Lévaditi and Lépine : *Les ultravirus des maladies humaines*, 1938, pages 395-488.

[2] G. Proca and D. Jonnesco : " Sur une modification durable du virus rabique de passage ", *C.R. Soc. Biol.*, 1935, **120**, 1274.

The gradual modification of the passage virus, which is slow but appears to be " inescapable ", makes it necessary to apply a control of live vaccines in respect of the virulence of the fixed virus. For, unless that virulence is accurately known, there is some risk, and treatment may be either too weak or else too drastic and dangerous.

The fixed virus, Bucharest strain, that was isolated in 1887 from the brain of a rabid wolf, produced, before the 50th passage, paralysis in rabbits after an incubation period of 6 to 7 days. Now that the cultivation of this virus has been continued for 52 years by means of 3,204 passages, it causes paralysis after 4 days, and death 2 days later.

The *Jassy virus* (Paris strain, 1891), which has been cultivated for 38 years by means of 2,671 passages, causes rabies within the same period of incubation (4 days), and the duration of the disease (2 days) is the same.

It will be remembered that, in Paris, the rabbits intracerebrally inoculated with the fixed virus showed the first signs of infection on the 6th day ; paralysis appeared on the 7th or 8th day, and the animal died on the 9th or 10th.[1]

At Bucharest, the degree of virulence has been determined this year by a series of tests, the findings of which are summarised in the following table :[2]

Fixed Virus, Bucharest Strain.

(a) *Emulsions prepared by Hand-grinding.*

1.	*Brain*,	dilution	1 : 10,000 ;	Inoculated	dose	0.5 ;	rabies	10	times out of	10
2.	»	»	1 : 10,000 ;	»	»	0.2 ;	»	4	» » »	7
3.	»	»	1 : 20,000 ;	»	»	0.2 ;	»	6	» » »	9
4.	»	»	1 : 50,000 ;	»	»	0.2 ;	»	2	» » »	5
5.	*Fresh cord* (0)		1 : 10,000 ;	»	»	0.2 ;	»	3	» » »	4

(b) *Emulsions prepared with the Marble Mill.*

6.	*Brain*,	dilution	1 : 10,000 ;	Inoculated	dose	0.5 ;	rabies	5	times out of	5
7.	»	»	1 : 10,000 ;	»	»	0.2 ;	»	6	» » »	7

The period of incubation varies according to the dilution titre and the dose injected. Thus, paralysis occurs after 120 hours in all 10 cases under 1 ; after 108, 120 and 144 hours (twice) in the

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[1] P. LÉPINE : *Loc. cit.*, page 443.

[2] S. BOBES : Communication to the Bucharest Biological Society, meeting of July 6th, 1939.

4 cases of No. 2 ; in 96, 120 (4 times) and 144 hours in the 6 cases of No. 3 ; and after 144 and 168 hours in the cases of No. 4. With the fresh cord of No. 5, the period of incubation is 144 hours and 192 (twice).

The emulsions prepared by means of the marble emulsifier yield shorter incubation periods. In particular, in the 5 cases of No. 6, the incubation is 84 hours, 96 hours (3 times) and 120 hours. In the 6 cases of No. 7, the periods of incubation noted are 72 hours, 96 hours (twice), 120 hours (twice) and 144 hours. The rabbits sub-durally inoculated with 0.2 c.c. of 1:100 emulsion went down with paralysis after 72 and 96 hours.

Sheep (10), of 24 and 34 kilogrammes body-weight, intracerebrally inoculated with 0.5 c.c. of 1:100 emulsion were stricken with paralysis on the evening of the 4th day. Death ensued 24 hours later.

It is interesting to observe that the degree of virulence of the Paris strain (1,540 passages) is slightly less than that of the Bucharest strain (3,204 passages). As has already been seen, the 1:10,000 brain emulsion of the Paris strain is virulent in 1 case out of 2, whilst the 1:50,000 emulsion is virulent in 1 case out of 6.

The capacity of the virus to travel along the nerve from the periphery to the nerve centres (Lévaditi's[1] *neuroprobasis*) is not always on a par with its virulence. When the rabbit is chosen as an experimental animal, and the subcutaneous route is taken for the introduction of the virus, the absolute innocuity of certain strains of fixed virus is established. In the case of the Bucharest strain, the effect varies with the dose injected. Thus, the dose of 5 c.c. of 1:100 brain emulsion appears to be harmless for rabbits (10 out of 10 inoculated animals survived), whereas, of 8 rabbits inoculated with 5 c.c. of 1:20 thick emulsion, 2 were paralysed, 1 after 16 days and the other after 18 days.

In the case of mice, a comparatively heavy dose of 0.5 c.c. of a 1:100 brain emulsion injected subcutaneously paralyses 14 out of 20 animals after incubation periods of 7 days (6 cases), 8 days (6 cases), 10 and 11 days.

The *simultaneous* injection of 0.5 c.c. of a 1:100 fixed virus and an equally concentrated street virus produces rabies in 11 out of

[1] *C.R. Soc. Biol.*, 1928, **98**, 186.

20 cases ; 2 mice died without exhibiting characteristic symptoms. The incubation period was 7 days (4 cases), 8 days (1 case), 9 days (2 cases), 10 days (3 cases) and 15 days (1 case).

Lastly, when we made a subcutaneous injection, first, of street virus emulsion (0.5 c.c.) and, 5 to 7 days later, of fixed virus (0.5 c.c. of a 1:100 emulsion), 12 out of the 20 mice inoculated became paralysed after an incubation period of 6 days (6 cases), 7 days (3 cases), 9 days (2 cases) and 10 days (1 case). Clearly, therefore, the neuroprobasis of original street virus (wolf) subsists in the Bucharest strain after more than 3,000 passages from brain to brain (rabbit) as a constitutional characteristic which is attenuated, certainly, but irremovable ; adaptation to the rabbit's brain does not finally efface one of the essential and almost hereditary features of the old original strain.

With the subcutaneous inoculation test, two kinds of fixed virus can apparently be distinguished : (1) strains of positive neuroprobasis—Sassari, Odessa, Bucharest, etc.—and (2) strains having no neuroprobasis—Paris, Vienna, Budapest, Riga, etc. It should be observed, however, that this is an artificial distinction, as the position, as regards neuroprobasis, may change according to circumstances. In Paris, A. C. Marie found that, subcutaneously injected in small quantities, the fixed virus produced rabies in mice 20 or 30 times out of a hundred.[1] The same author later found that, of 15 mice similarly inoculated with Pasteur fixed virus in a 1 : 40 dilution, only one contracted rabies (6.6%) ; of 10 mice treated with Indian ink 48 hours before infection, none succumbed.[2]

The Bucharest strain, showing a clearly positive neuroprobasis with a dosage of 5 c.c. of a 1:20 emulsion, protects all the rabbits inoculated (10) if they are given a subcutaneous injection of 5 c.c. of 1:100 brain emulsion.

Consequently, the subcutaneous inoculation test, even if tried out on a large number of susceptible animals of a particular species (dog, rabbit), will not supply exact information as to the positivity or negativity and the extent of the neuroprobasis of a fixed virus utilised as a live vaccine. Only the effects ensuing on virulent injections in the subcutaneous cellular tissue of man will enable

[1] Reports to the Rabies Conference.
[2] *C.R. Soc. Biol.*, 1929, **101**, page 6.

an unequivocal answer to be given to a question, which is nowadays less harassing than in the past : Can a fixed virus used as a vaccine transmit rabies ?

* * *

5. At the first International Rabies Conference, A. C. Marie pointed out that it was difficult to say whether the fixed virus was innocuous or virulent for man. " The views of Pasteur himself, Pfeiffer and J. Koch have been generally accepted, and fixed virus has always been regarded as possessing no pathogenicity for man." A. C. Marie considered that " the injection of fixed virus into the cellular tissue of the abdominal region is unattended with risk ", but added " such considerations are only applicable to certain strains of fixed virus and not to others ".

Nevertheless, the clearly established cases of *laboratory rabies* —1 observed by França, the 8 cases of ascending paralysis, occurring during vaccinations and traced in the literature on the subject by Kozewalow, 1 case observed by Neufeld, and McKendrick's 2 cases—left no doubt as to the pathogenic effects of fixed virus isolated from the brain in these cases.

Similarly, the papers published as a result of Kozewalow's observations—Marie mentions those of Simon, Pfeiffer, Lubinski, Geiger, Levi, Cumming, Philips, Haris and Papamarku—all agree as to " the preponderating part played by fixed virus in the etiology of post-vaccinal accidents ".

Accordingly, at the close of the discussion on the pathogenicity for man of the fixed virus, the Conference, while agreeing that, in most cases, " living fixed virus injected under the skin is innocuous ", adds that, " in exceptional circumstances, . . . it may be infective ".

P. Bassoe and R. Grinker have since published (1930) a report on the case of a woman who, though she had not been bitten, underwent anti-rabies treatment for 14 days ; symptoms of the Landry type of paralysis appeared and she died a month later.

In 1935, Remlinger reported his observations of 3 cases of fatal ascending paralysis after anti-rabies treatment by the dilution method ; in 2 cases, fixed virus was isolated from the brain ; in the 3rd case, no autopsy was made, but the doctor in attendance had allowed himself to be prevailed upon to give treatment, although

no genuine infection had occurred. In this latter case, the symptoms, the course of the paralysis and the very close analogy between this case and that of a patient simultaneously following the same treatment, and affording a thoroughly reliable demonstration of the nature of the accidents, all pointed conclusively to a diagnosis of fixed-virus rabies.[1]

At the Babeș Institute, an opportunity offered of isolating fixed virus from the brain of 2 cases treated at Cernăuți in 1933 by the Högyes method. The Director of the Anti-rabies Station there had observed several cases of facial paralysis in the persons treated and attributed them to the fixed virus strain (Jassy) which he was using and which he then replaced by the Bucharest strain. Immediately after the change had been made, two cases of paralysis with a fatal issue occurred among the persons treated with the new vaccine. The investigations which the anti-rabies institutes of Bucharest and Jassy were asked to make proved the existence in these cases of a rabies virus having the essential characteristics of a fixed virus.

Few cases are known of paralytic rabies followed by death and positive inoculation in rabbits ; according to Remlinger, not more than 30 can be traced in medical literature.[2] The same authority, however, believes that the term " laboratory rabies " should be given a considerably wider extension, so as to include cases of fatal post-vaccinal paralysis in which the inoculation is, however, negative (auto-sterilisation), slight myelitis and mild neuritic paralysis usually affecting the facial and oculo-motor nerves.

Though, as Remlinger points out, there is a great tendency nowadays to regard the fixed virus as responsible for such accidents, it has to be admitted that their pathology is rather obscure. To explain even the pathogenesis of fatal paralysis, references are made to " incautiousness in handling the fixed virus, certain cosmic influences capable of transforming a passage virus attenuated for man into a pathogenic virus", and lastly more factors more easily grasped, such as the injection of virulent cords too early or in excessive quantities.

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[1] P. Remlinger : " La rage dite de laboratoire ", *Ann. Inst. Pasteur*, 1935, Numéro commém. de la rage.

[2] *Loc. cit.*, page 66.

Remlinger also draws attention to the " brutal effect of a subcutaneous injection which lacerates the peripheral nerve ramifications and exposes axis cylinders to complete immersion in a virulent emulsion". In experiments on the intracaudal inoculation of mice, the number of positive cases was found to increase when thicker needles were used, probably because they caused more extensive lesions and were calculated to injure or lacerate the fine nerve network of the surrounding tissue.

When the cause of the accidents has been traced—fixed virus in the cases yielding positive inoculations into animals—their pathogenesis must be studied. My own observations lead me to believe that the body-defence reactions in the subjects which have had a subcutaneous injection of fixed virus may be a factor of primary importance in the causation of post-vaccinal accidents.

The resistance of animals susceptible to rabies when infected through some other route than direct injection into the brain varies from one animal to another within limits which depend on the species concerned. What might be termed the resistance rate of mice, for instance, is 70 or 80% in the case of the Paris strain subcutaneously innoculated, but only 30% in that of the Bucharest strain. Using the same method of inoculation, a rabbit's resistance is 100% in Paris and 75% in Bucharest, if the test is made with strong doses of brain emulsion.

The resistance of the human organism to street virus is estimated at more than 80%, taking all kinds of bite together. Its resistance to fixed virus introduced into the subcutaneous cellular tissue must be much greater, judging by the very few post-vaccinal accidents occurring in persons treated with virulent cords.

Nevertheless, whatever its magnitude, the natural resistance of the organism may fail in some circumstances. A. C. Marie has shown that the almost complete immunity of a guinea-pig inoculated through the peritoneal route is overcome if the blockage of the reticulo-endothelial system is brought about.[1]

In human beings, it has often been found that " among the predisposing causes " of post-vaccinal paralytic accidents " alcoholism, syphilis, neuropathic constitution, cold, fatigue and overwork are important " (Marie).[2]

[1] *Ann. Inst. Pasteur*, 1934.
[2] Reports to the Conference, page 8.

Among the causes likely to weaken the resistance of the organism, there are certainly unknown factors whose operation at any particular moment cannot be foreseen. So long as we known nothing of these causes and are unable to counteract their depressing influence, fixed virus will remain dangerous, whatever precautions may have been taken regarding the doses injected and the way in which the virus is administered.

Fixed virus having been isolated from the human brain in cases of post-vaccinal paralytic accidents, experiments proving that this virus, when injected under the skin of animals, does not become generally disseminated in rabbits, dogs or baboons (Remlinger, Nicolau, Kopciowska, J. van Genderen) cease to have any valid bearing on the cases of inoculated persons. Moreover, in the experiments made by Quast, Stuart and Krikorian, R. Soldati and Busson, the brain of animals inoculated under the skin sometimes became virulent and was found to contain fixed virus. The differences in the neuroprobasis of the strains used might explain the discordance of the results, though other factors may also be of importance.

This would seem to settle the question, which used to be keenly contested, of the pathogenic effect of fixed virus and to suggest the following rule based on observation of the facts : To be quite sure of avoiding laboratory rabies, do not use virulent cords but replace them by " chemical " vaccines, the immunising power of which is certain.

Moreover, there is not necessarily any relation between the virulence of rabies emulsions and their vaccinating properties. The virulent components can be killed by antiseptics (carbolic acid, ether, formalin) or neutralised by anti-rabic serum (Marie), without the emulsions being thereby deprived of their immunising power. Rabies antigen is more complex : it is the *in vivo* culture of the parasite with its infected neurones, its products of the metabolism of the infective germs and of the parasitised cells and its products of autolysis or of cell-disintegration. When this complex antigen is replaced by a fixed virus, freed from most of the above impurities, the organism undergoing vaccination behaves as though, for a time, it permitted a slow dysgonic growth of the vaccine virus.

In the case of the Bucharest strain, the properties of the rabies antigen, whether natural or modified by drying, heating or

antiseptics, have been studied in detail by Babeș, and his pupils[1] both in the laboratory and in practical inoculations made on human beings. The rabies antigen of the Bucharest Institute confers complete immunity, and the blood of the animals inoculated quickly becomes rabicidal. The neutralising titre of the serum seldom exceeds 1:20, but its effect *in vivo* is undeniable.

Recent studies made of the immunising power of the carbolised antigen led us to utilise for the preparation of anti-rabic serum this avirulent vaccine, which is more easily tolerated by animals (asses). The Institute also supplies the carbolised vaccine to a number of departmental anti-rabic treatment stations, as well as for the inoculation of dogs and domestic animals.

* * *

6. The possible general dissemination of the fixed virus in the organisms of vaccinated persons is not the only risk inherent in immunisation by live vaccine.

It was denied that attenuated but still somewhat virulent cords could have a decisive influence on the course of the disease before 2 or 3 weeks, reckoning from the beginning of the treatment. At the first Rabies Conference, A. C. Marie declared that " all cases of death occurring within 15 or 20 days after the beginning of the treatment must be attributed exclusively to the bite—that is, to street virus ".[2] It would, however, be advancing a theory rather than a properly verified fact of observation to claim that, in such cases, street virus was the only cause.

As far back as 1906, Nitsch showed that, in Paris (1897-1904), Warsaw (1895-1904) and Cracow (1893-1905), the incubation periods for rabies were shorter in the case of persons treated than those not treated.[3]

The following table summarises Nitsch's statistics :

Institutes	Period of incubation Days	Deaths	Percentage
1. Cracow	23-47	22 out of 37	60 *treated*
2. Paris	16-45	27 out of 42	64 *treated*
3. Warsaw	21-45	29 out of 40	72 *treated*
4. Cracow, *not treated*	19-48	36 out of 100	36 *not treated*

[1] *Traité de la rage*, Paris, 1912.
[2] Report to the Conference, page 64.
[3] *Zbl. f. Bakt. O.*, 1906, **42**, 647.

The problem raised by NITSCH was of special interest to us ever since we had observed the exceptional frequency of deaths following short incubation periods among persons treated in the anti-rabies institutes of Cluj and Bucharest.

According to documents published by the Cluj Institute,[1] the rabies incubation period among patients in 1923-1927 was very often short—*e.g.* :

Less than 30 days in 37 out of 50 cases, for which the incubation period was known, or 74% ;

31-60 days in 9 out of 50 cases, for which the incubation period was known, or 18% ;

More than 200 days in 4 out of 50 cases, for which the incubation period was known, or 8%.

In the next five-year period (1928-1932), incubation lasted less than 40 days in 50 out of 58 cases, or *87*%.

The statistics of the Babeș Institute on the rabies incubation period were published in two notes compiled in 1935 and 1936,[2] and in a general survey published in connection with the Institute's fiftieth anniversary.[3] The chief data are summarised hereunder :

I. Between 1925 and 1933, *rabies in the persons treated* developed in the *first month*, 13-30 days after the bite—viz. :

In children (up to 15 years of age), in 34 out of 46 fatal cases, or 73.9% ;

In persons over 15 years of age, in 28 out of 53 fatal cases, or 52.9% ;

In persons of all ages, in 62 out of 99 fatal cases, or 62.6%.

II. Comparison of the incubation periods of treated and untreated persons in the age-groups 0-15 years and 16-25 years gives the data summarised in the following tables :

A. *Incubation Period from 11-30 Days (All Bites).*

	Persons not treated	Persons treated
Children (0-15 years)	33 out of 70 fatal cases, or 47%	34 out of 46, or 73.9%
Persons from 16-25 years	3 out of 31 fatal cases, or 9.7%	11 out of 22, or 50%
Total	36 out of 101 fatal cases, or 35.6%	45 out of 68, or 66.1%

[1] M. BOTEZ : *Vaccinarea antirabică la Cluj* and *Ann. Inst. Pasteur*, 1935, Numéro commém. de la rage.

[2] G. PROCA : *Buletinul Serviciului antirabic.*

[3] *România medicala*, April 1938, page 93.

B. *Incubation from 11-30 Days and Site of Bites.*

1. *Bites on the head.*

Age	Persons not treated	Persons treated
0-15 years	19 out of 70 fatal cases, or 27.1%	29 out of 46, or 63%
16-25 years	3 out of 31 fatal cases, or 9.6%	11 out of 22, or 50%

2. *Bites on the upper limbs.*

0-15 years	12 out of 70 fatal cases, or 17.1%	3 out of 46, or 6.5%
16-25 years	2 out of 31 fatal cases, or 6.4%	2 out of 22, or 9%

3. *Bites on the lower limbs.*

0-15 years	2 out of 70 fatal cases, or 2.5%	1 out of 46, or 2.1%
16-25 years	—	—

In cases of head bites, the great differences between persons treated and those not treated was due to the severity of the original injuries and the fact that the treatment is followed mainly by persons with very grave lesions.

The note summarised concluded as follows : " Our comparative statistics, which differ from those of NITSCH in that they refer to cases picked out of the same population, do not confirm the suspicion that anti-rabies treatment might artificially shorten the incubation of the disease . . . . Treatment has never brought the incubation period for rabies below the shortest period observed in untreated cases. The special frequency of short incubation cases and cases where rabies developed in the first month after the bite, whether among treated or untreated persons, merely reflects the severity of the infectious bites and the virulence of the specific autochthonous agent."

Notwithstanding this seemingly justifiable conclusion, it has to be admitted that the fixed virus might not impossibly accelerate the course of the rabies infection either by producing blockage phenomena or by changes in the humoral fluids or tissues of the neural axis in particular. The hypothesis that the fixed virus inhibits the defensive reactions of the organism is worth putting to the test of experience, especially since our knowledge of the etiology of post-vaccinal paralytic accidents no longer allows us to exonerate the virus *a priori*.

In the first place, it may be pointed out that, in Paris, deaths after a short period of incubation were very common between November 9th, 1885, and December 31st, 1886, a time when " *intensive*

treatment " (3 inoculations daily and administration of virulent cord on the 1st, 3rd, 6th and 10th days) was given. Of 18 fatal cases, there were 9 in which incubation lasted less than 3 weeks (2 to 19 days after the end of the treatment).[1] Later " we abandoned this somewhat drastic method of treatment, which was liable to entail certain dangers ".[2] Deaths of treated patients during treatment and generally after less than 30 days' incubation then became the exception, but the Pasteur Institute in Paris no longer uses 1-day and 0-day cords.

The Bucharest and Cluj Anti-Rabic Institutes have throughout applied intensive treatment in cases of head bites, always using the most virulent cords. At Cluj, 2-day cords are injected on the 3rd day of treatment, 1-day cord on the 4th day, and fresh (0) cord associated with an *unheated* brain emulsion on the 5th day. In the case of head bites, this treatment is repeated 4 times. Even in cases with no apparent lesions, fresh cord emulsion is injected on the 5th and 9th days.[3]

At Bucharest, in cases of dangerous bites, the authorities have long been accustomed to use fresh (0) cord emulsions on the 3rd or 4th day, and sometimes on the 2nd day, of treatment. Students of Babeș have loyally obeyed the master's teaching that " *the most virulent cords most promptly induce the condition of greatest resistance to the disease* " and that " *the organism may be prepared in a single day by a single series of 6- to 0-day cords* " (*Treatise on Rabies*, Paris, 1912).

The following statistics of the Babeș Institute for 4 ten-year periods (1904-1933) show that, as a result of the intensive treatment above described, cases of short-incubation rabies are indeed exceptional :

Deaths after Short Incubation.

10-year periods	Per 100 deaths (treated)
1894-1903	84.7
1904-1913	81.1
1914-1923	72.5
1924-1933	70.3

[1] Pasteur : *Œuvres*, Vol. VI, page 863.
[2] Lépine and Cruveilhier : *Ann. Inst. Pasteur*, 1935, Numéro commém. de la rage, page 20.
[3] M. Botez, *Vaccinarea antirabică la Cluj*, page 8.

Since 1933, one of us, as Director of the Anti-Rabies Service, has introduced a few modifications in the system of treatment and, following the precedent set by the Pasteur Institute, has excluded 1-day and 0-day cords ; these were no longer used in 1936 and 1937. The results will be seen from the following table :

Deaths after Short Incubation in the Case of Treated Persons.

Year	Per 100 deaths from rabies
1934	46.1
1935	42.8
1936	27.7
1937	10.0

The decline in recent years, connected as it is with the change in treatment, is so substantial and so abrupt that it cannot be attributed to any attenuation of the virulence of the pathogenic agent (street virus), or other fortuitous reasons.[1]

The following comparative table clearly shows the effect of the modified treatment on the incubation period for rabies :

Deaths of Treated Persons.

Incubation period	1933	1934	1935	1936	1937
13-30 days	15 deaths	6	6	5	2
31-60 ,,	4 ,,	3	3	3	6
61-90 ,,	3 ,,	1	1	4	7
More than 90 days	0 ,,	3	4	8	10[2]

It is true that, in cases of serious bites, we have, since 1934, applied anti-rabic serotherapy, but the serum was injected in sometimes inadequate quantities and the number of persons treated with serum was relatively small. The practice of using heated brain emulsions was continued, but with no effect on the period of incubation.

It follows from all the above observations that there is some danger that the intensive treatment applied at Cluj and, before 1936, at Bucharest may unpredictably accelerate the progress of the rabies infection. The age of the persons bitten (0-15 years) and the

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[1] G. Proca : " Revision of Anti-rabies Treatment Methods " (in Roumanian), *România medicală*, April 1st, 1938.

[2] The incubation period in these cases was : 101, 105, 108, 138, 131, 174, 205, 270, 330 and 357 days.

fact that the bites were situated in the head would seem to facilitate the general dissemination of the street virus under the action of live vaccines.

A later paper by J. DODERO[1] confirms my view of the influence of treatment on the incubation period. The death rate among treated natives after less than one month's incubation was 64%, while among untreated natives it was 40%. " Anti-rabies treatment would therefore seem to shorten the period of incubation."

J. GENEVRAY and J. DODERO concluded in 1934 that " the introduction of 1-day and 0-day cords into the treatment schemes and the more widespread application of drastic treatments greatly diminished the death rate in the case of persons who underwent anti-rabies treatment.[2] A study of the authors' tables B and C shows, however, that, before the modifications shown were made, " stubborn " cases—that is, cases with long periods of incubation—represented 25% of the deaths, whereas, after the introduction of 1-day and 0-day cords, the number of long incubation cases was more than halved (11.8%), which means that incubation was shortened after this modification in 88.2% of the cases (formerly 75%).

7. The possible action of a fixed virus on the development of rabies in persons infected by bites is not an isolated fact for which there is no precedent. LÉVADITI and NICOLAU " have shown that, if one and the same rabbit is given an injection of 2 neurotropic viruses, such as the herpes virus and neurovaccine, these viruses may live together for a certain time. Nay more, one of them may cause a latent infection induced by the other virus to develop or it may facilitate the growth of the latter in an organism unreceptive to it.[3]

Does not the evidence adduced indicate that, in some cases, the fixed virus, used as a vaccine, may facilitate the dissemination and growth of the street virus in the organisms of persons bitten ?

Apart from this, the same authors have seen that, " if the fixed virus is administered during the incubation period to rabbits which

[1] " Sur la durée d'incubation de la rage au Tonkin", *Ann. Inst. Pasteur*, 1938, **61**, 193.

[2] *Ann. Inst. Pasteur*, 1934, **52**, 352.

[3] LÉVADITI, NICOLAU and Mlle. SCHŒN : " Recherches sur la rage ", *Ann. Inst. Pasteur*, 1926, **40**, 937.

have first been inoculated with street virus . . . (Negri bodies) are less frequent in proportion as the injection of the fixed virus is nearer in time to the beginning of the incubation period ".

Though the interpretation of the phenomenon is not clear, it suggests that the fixed virus acts on the brain infected by the street virus and perhaps on the latter itself.

Dwijkoff and Bogolowski, Gerlach and Schweinburg, quoted by Busson, and the latter author himself,[1] agree that an anti-rabies injection will prevent or stop the formation of Negri bodies in the brains of bitten animals or persons who have been treated.

Some recent experiments by Lépine and Sautter[2] show that rabbits which have been vaccinated for 10 to 15 days with dried cords 4, 3 and 2 days old are sensitised towards street virus inoculated by the intra-cerebral method 10 days after the treatment is ended. The rabies incubation period is from 9 to 18 days in 7 cases out of 9 among such animals, whereas, among rabbits *treated only for 5 days* and given 4-day old cords and once a 3-day old cord, the incubation period was *longer—i.e., 20 to 44 days—in 4 cases out of 5.*

Whether it inhibits or weakens the defensive reactions of the organism, the influence of fixed virus on the development of rabies in vaccinated bitten patients is still hard to understand. It may be pointed out in this connection that what is most important in this connection is the actual fact of the phenomenon, and not its explanation.

8. A counter-proof of our demonstration was soon forthcoming. In 1938, the interim-management of the Babeş Institute reverted to 1-day and 0-day cords, but took the precaution, which the experience of the previous years had shown to be absolutely essential, of only using such cords after longer preparation. In the case of serious bites, 1-day and 0-day cords were only given on the 5th day and repeated on the 11th, 16th and 21st or 25th days. At the same time, the injection of anti-rabic serum from the beginning of the treatment was continued in accordance with our former practice (1934-1937).

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[1] "Über den Einfluss der Schutzimpfung auf die Ausbildung der Negri-Körperschen im menschlichen Gehirn", *Zbl. f. Bakt.*, I, 1929, **115**, 135.

[2] *C.R. Soc. Biol.*, 1939, **130**, 617.

If we take as a basis of comparison the year 1933, when serum was not used and 1-day and 0-day cords were abused, it will be seen what effect intensive treatment may have on the course of rabic infection. For head bites, formerly treated with the most virulent cords, we find the following figures in the records of the Babeș Institute :

Period of incubation	1933	1937	1938
12-30 days	11 deaths	1 death	2 deaths
31-60 ,, ...	0 ,,	1 ,,	2 ,,
61-90 ,, ...	2 ,,	1 ,,	2 ,,
More than 100 days ..	0 ,,	3 ,,	1 ,,
Total	13 out of 21	6 out of 25	7 out of 21 deaths

In 1933, 1-day and 0-day cords were administered 7 times from the 2nd day of treatment and 6 times from the 3rd day ; in 1937, the use of these cords was abandoned, and, in 1938, they were injected on the 5th and 6th day, anti-rabic serum being associated with the live vaccines during these two years. The conclusion which emerges is that intensive treatment shortens the period of incubation of rabies, while the treatment with serum and no 1-day or 0-day cords prevents this more effectively than the scheme of treatment used in 1938 (6-0 day cords and serum).

The statistics of the Babeș Institute for seven years (1932-1938) also show that the exclusion of 1-day and 0-day cords and the use of anti-rabic serum greatly decrease the number of deaths during treatment ; the figures are as follows :

Year	Live vaccine	Serum	Deaths during treatment
1932	6-0 day	—	6 out of 13 vaccinated persons died of rabies
1933	6-0 ,,	—	9 ,, ,, 21 ,, ,, ,,
1934	6-0 ,,	40 cases treated	6 ,, ,, 13 ,, ,, ,,
1935	6-0 ,,	179 ,, ,,	4 ,, ,, 14 ,, ,, ,,
1936	6-2 ,,	197 ,, ,,	1 ,, ,, 18 ,, ,, ,,
1937	6-2 ,,	314 ,, ,,	1 ,, ,, 25 ,, ,, ,,
1938	6-0 ,,	241 ,, ,,	1 ,, ,, 21 ,, ,, ,,

The decrease in the number of deaths occurring during treatment in 1936 and 1937 is particularly significant, as these deaths are usually due to head bites, and it was in these particular cases that we used anti-rabic serum.

Encouraged by the results of previous experiments on the *in vivo* action of the specific serum (V. Babeș, A. C. Marie, Remlinger, Fermi, Speranski, etc.), and by our own research,[1] we began, in 1934, to treat with anti-rabic serum persons bitten on the head and, in more exceptional cases, those seriously bitten on the upper limbs ; the serum being injected from the 1st day of treatment.[2]

The preliminary trials from 1934 to 1937 led us to conclude that, in cases of head bites, anti-rabic serum administered in sufficient doses from the beginning of treatment acts as an adjuvant of active Pasteur immunisation. Its *in vivo* action in human beings is reflected in the reduction of the death rate. The deaths which occur in spite of the serum treatment generally take place after an incubation of 3 to 4 months, even in the case of children bitten on the head.

The prolongation of the period of incubation makes it possible to complete the treatment and, in the most severe cases, to attempt to strengthen the acquired immunity, which is occasionally feeble, by a new series of vaccination injections.

We were compelled to introduce a method of mixed treatment—serotherapy associated with Pasteur vaccination—by the present prevalence of rabies in Roumania and by the frequently established fact that autochthonous street virus possesses enhanced pathogenic power.

To the conclusions which we reached in 1937, we may now add that anti-rabic serum often prevents the harmful action of live vaccines, even in the case of 1-day and 0-day cords.

In the course of five years, 971 persons who had been bitten were treated with serum at the Babeș Institute :

Year	Treated with serum	Deaths
1934	40	1
1935	179	4
1936	197	5
1937	314	13
1938	241	9
5 years	971	32—*i.e.*, 3.29%

[1] *C.R. Soc. Biol.*, 1934, **115**, 1001 and 1313.

[2] G. Proca, S. Bobeș and D. Jonnesco : " Serotherapy of Rabies ", *Bulletin of the Roumanian Academy of Medicine*, Vol. IV, No. 5.

In cases of head bites, the effect of serotherapy on mortality is obviously definite, though inconsiderable. For instance, with the intensive treatment *without serum*, as applied from 1932 to 1934, the Babeș Institute recorded 24 deaths among 1,182 persons bitten on the head—*i.e.*, 2.03% ; the introduction of serotherapy associated with less drastic treatment brought the death rate down to 1.41% (17 deaths among 1,203 persons bitten on the head in the period 1936-1938).[1]

Serotherapy exerts a far greater influence on the period of incubation than on the death rate, as is shown by the following table :

Head Bites.

Period of incubation	Persons treated without serum (1932-1934)	Persons treated with serum (1936-1938)
12-30 days	21 deaths	4 deaths
31-60 ,, ...	0 ,,	3 ,,
61-90 ,, ...	2 ,,	3 ,,
More than 100 days	1 ,,	7 ,,
Total	24 deaths	17 deaths

It will be seen that, *without serum*, the cases of rabies with a long period of incubation (above 60 days) represent 12.5% of the deaths ; the use of serotherapy increases this percentage to 58.82%.

This increase in the period of incubation makes it possible to strengthen the immunity by supplementary injections. In these cases, revaccination is greatly facilitated by the use of carbolised vaccine.

* * *

9. The ill effects produced by the fixed virus used as a vaccine may, in theory, be classified under three headings : (*a*) laboratory rabies with fixed virus ; (*b*) mixed rabies with atypical symptoms caused by the concurrent action of the fixed virus and the street virus ; and (*c*) short-incubation rabies due to the diffusion of street virus under the influence of the fixed virus.

(*a*) The distinguishing characteristics of fixed virus rabies lie in its etiology rather than in its symptoms or in the course taken by

[1] See Annexes : Rabies after Bites on the Head, I and II.

the disease, since street rabies sometimes assumes clinical forms which strangely resemble laboratory rabies.

The etiological diagnosis of rabic paralyses is essential in all cases, but is at times difficult (J. Koch, Remlinger, etc.). The case studied by G. Marinesco and St. Draganesco[1] may be mentioned as an example ; it relates to a person treated at the Babeş Institute for two scratches on the back of the thumb. After 13 days' treatment with 6-0 day cords and heated brain emulsions, the patient developed paraplegia, the subsequent course of which assumed the form of a serious syndrome, ending in death on the 6th day after the first ill effects and 19 days after the bite. The 1-0 day cords were used for the first time on the 4th day.

The virus isolated from the brain acted after a shorter period of incubation—*i.e.*, 5 to 6 days from the first subdural inoculation (instead of 7 days in the case of the Bucharest fixed virus) and 4 to 5 days in the passage experiments. " Accordingly, the two viruses showed a certain difference and are not identical." Since there are, in nature, strains of street virus which are similar in character, and particularly in period of incubation, to the fixed virus, the authors conclude that this fact " makes it highly probable that, even in the case in question, the virus was a reinforced street virus which the preventive treatment was unable to influence ".

The hypothesis has not been confirmed, but everything suggests that comparative tests on rabbits and dogs would have settled the question.

(*b*) Mixed rabies is not known or has not been recognised in human beings. The fixed virus isolated from the brains of vaccinated persons who died of paralytic rabies showed the characteristics of a pure virus ; it may be wondered whether it was really so from the beginning in the vaccinated persons' neural axes. This would seem to be doubtful. The inoculation of rabbits is not an infallible procedure for detecting the simultaneous presence of the two viruses. When a mixture of equal parts of the street virus and fixed virus is injected into rabbits, it is found, as in A. C. Marie's experiments, that, " although they are animals which are used to obtain the fixed virus, *6 times out of 15 they contract street rabies* after the injection of the mixture ".[2]

[1] *Ann. Inst. Pasteur*, 1935, **54**, 299.
[2] "Sensibilité du cobaye à la rage", *C.R. Soc. Biol.*, 1930, **103**, 868.

P. Remlinger and J. Bailly, who investigated the question whether it was " possible to detect the simultaneous existence of street virus and fixed virus in a rabic brain ", gave an affirmative answer,[1] but recommended the inoculation of the suspected bulbar material: (1) under the *dura mater* of a rabbit, (2) in the anterior chamber of the eye of another rabbit and (3) in the anterior chamber of the eyes and neck muscles of two dogs. The authors specify that the animals should be studied from the point of view of the period of incubation, the date of death, the symptoms of paralysis or excitation shown and the existence of Negri bodies. The authors point out that " success is not inevitable. It was achieved several times at Vienna by Busson."[2]

Other separation procedures must be tried, for, when live vaccines are used, the fixed virus is introduced into an organism already infected by street virus, and these viruses may not impossibly act together and cause mixed rabies. This double etiology, if confirmed by experience, may perhaps explain the atypical symptoms of rabies in certain cases.

(*c*) Rabies with a short, or shortened, period of incubation is said to be due to the activating action (*sensu* Lévaditi) of the fixed virus, which brings out the latent infection caused by the other virus.[3] But the question arises whether this malignant rabies is not in reality an unknown mixed rabies and whether the strains of reinforced virus isolated from the brain in such cases were pure and always belonged to the class of street virus.

The etiology of this malignant form of rabies is obscure : the possible action of reinforced strains does not explain all cases. Moreover, we have laid great stress on the causal connection between the intensity of the treatment and the frequency of short-incubation cases.

The explanation suggested by the investigations of Lévaditi and his collaborators appears to us very probable, as a working hypothesis may help to guide research.

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[1] *C.R. Soc. Biol.*, 1928, **98**, 519.
[2] *Studies on Rabies*, 1938, page 74.
[3] Lévaditi, Nicolau and Mlle. Schœn : *Ann. Inst. Pasteur*, 1926, **40**, 937.

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10. The fixed virus deposited under the skin is an immunising factor while at the same time a *contagium vivum* and acts differently according to the relation between its power to infect power and that of the organism to resist. When the local and general defensive reactions are comparatively weak, they are unable to stay the development of the virulent components or prevent their wide diffusion and the invasion of the neurones ; the disease then breaks out in the form of rabies or a post-vaccinal accident. This contingency is quite exceptional in view of the natural resistance of the organism to the rabic virus. In the vast majority of cases, the unreceptive state, or immunity, is established under the action of the vaccine virus. In the condition of definitive immunity, the two viruses become harmless to the host (total autosterilisation ?). Immunity is, however, at times incomplete, fragile and transient ; the virulent components, which are at first localised in one point of the organism, pass at a given moment from the latent state to active life and rabies breaks out after a period which may be very lengthy has elapsed since the end of the treatment. These cases are known as " treatment failures " or *real insuccesses*, the frequency of which would indicate the value of the immunisation method employed.

The cases of rabies with a prolonged period of incubation have become more numerous at Bucharest since we associated serotherapy with anti-rabic vaccination. According to the experience of 1936 and 1937, the bites on the upper limbs furnish the highest percentage of rabies with a long period of incubation ; this fact appears to indicate that we are dealing, in these cases, with a condition of incomplete immunity or of " failure " of immunity (Nicolau), since the period of treatment is always shorter than in the cases of bites on the head. A remedy which we have recommended and sometimes applied is *revaccination* at intervals not exceeding 2 months. This practice was suggested to us by the work of L. Cruveilhier and Ch. Viala, who had observed " that one or two injections of anti-rabic vaccine were sufficient to cause the reappearance in the blood of virus-neutralising properties which had disappeared ". The

same authors point out that they were able, by a single reactivating injection, to maintain the rabicidal power of the blood of experimental rabbits until their death (7 or 8 months).[1]

We consider that, from the practical point of view, the most suitable vaccine for reactivating or " supplementary " injections (Ramon) is killed carbolised vaccine.

11. The value of a method of anti-rabic vaccination as determined by the frequency of success can only be established by statistical means ; but this does not always give comparable results.

The statistics of the anti-rabies institutes are compiled from data that are frequently heterogeneous—for instance, in the group of persons treated, no distinction is made in respect of age, although children are more sensitive to street virus and may, in some cases, represent a comparatively large proportion of the bitten persons. Moreover, all the institutes have not equally accurate information on the after-history of their old patients ; lastly, a factor of greater heterogeneity may perhaps reside in the proportion of really infecting bites, since the number of these varies in relation to the number of persons treated from one year to another in the same institute and within even wider limits as between different institutes.

In spite of these causes of uncertainty, the results recorded in the 8 analytical reviews by McKendrick furnish highly valuable information regarding the comparative value of the different methods of anti-rabic vaccination.

The results obtained at Bucharest from 1934 to 1938 (five years) have been compared, according to McKendrick's analytical reviews, with the results obtained by foreign institutes from 1928 to 1935 (eight years). As a basis of comparison, we have taken, on the one hand, the group of deep bites (independently of their position), and, on the other, superficial and deep bites on the head, on the assumption that the statistical returns are probably less heterogeneous in these groups.

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[1] L. Cruveilhier and Ch. Viala : " Pouvoir rabicide et immunité antirabique", *Ann. Inst. Pasteur*, 1937, **59**, 207.

The following table shows the differences noted :

Method	Deep bites			Head bites		
(a) *Europeans*	Persons treated	Deaths	%	Persons treated	Deaths	%
1. Roumanian method ...	1,982	48	2.42	1,985	29	1.46
2. Dried cords	5,329	27	0.51	1,656	12	0.72
3. Dilutions	10,459	78	0.75	4,137	61	1.47
4. Killed carbolised vaccines	9,197	75	0.82	3,146	41	1.30
(b) *Non-Europeans*						
1. Dried cords	3,136	75	2.39	778	49	6.30
2. Dilutions	—	—	—	48	1	1.80
3. Killed carbolised vaccines	112,307	881	0.78	9,683	351	3.62

It will be seen that, at Bucharest, the death rate attendant upon the use of the Roumanian method is three to four times as large, in the group of deep bites, as that recorded by other methods applied to Europeans ; it will be seen in particular that the lowest rate is obtained with dried cords and that mortality among persons treated with killed carbolised vaccines is 0.82%.

Among non-Europeans, the mortality in the case of treatment with dried cords is almost the same as in that of treatment by the Roumanian method, while treatment with the killed carbolised vaccines is attended by a mortality rate one-third of that recorded with the treatment practised at the Babeș Institute (0.78 as against 2.42%).

The differences in the group of head bites are less marked among Europeans ; the death rate is the same for the Roumanian as for the HÖGYES method and twice as high as for dried cords. Among the non-Europeans treated with dried cords, the rate of mortality is very high (6.3%) and is markedly higher than among those treated with killed carbolised vaccines.

In the ten-year period from 1929 to 1938, the *total mortality* at the Bucharest Institute is higher than that observed for Europeans treated from 1928 to 1935 by various methods :

Method	Persons treated	Deaths	%
1. Roumanian method	67,148	141	0.21
2. Dried cords	53,773	77	0.14
3. Dilutions	61,080	108	0.18
4. Killed carbolised vaccines	55,644	82	0.15

The live vaccines used at Bucharest therefore do not improve the statistics of the Babeș Institute, and their alleged superiority over killed avirulent carbolised vaccines does not appear from the tables which we have prepared.

On adopting McKendrick's abridged classification, we find, by the statistical method, that the killed avirulent vaccines are, on the whole, at least as effective as the live vaccines. The eight analytical reviews from 1928 to 1935 give the following results in this respect :

	Europeans			*Non-Europeans*		
	Persons treated	Deaths	%	Persons treated	Deaths	%
(*a*) Killed vaccines	119,088	161	0.14	280,696	1,761	0.63
(*b*) Live vaccines	170,644	258	0.15	57,821	357	0.62

* * *

The power of immunisation of the killed vaccines would appear to be equal to that of the live vaccines. It should, however, be observed that the rate of mortality taken as a basis represents the numerical ratio of deaths from rabies to the number of vaccinated persons, *whether infected or not*, who form a heterogeneous mass, variable, in the proportions of its constituent parts, according to the institute or the country considered.

A more reliable indication of the value of anti-rabic treatment would be the fatality rate—that is to say, the ratio of deaths to really infected persons treated.[1] It is true that this ratio is but rarely defined with accuracy, but there is reason to believe that the fatality rate is equal to the death rate in the homogeneous group of bitten persons in category A, and probably in cases of head bites and deep bites.

This necessary distinction between the fatality and mortality rates will help us to understand why, in the case of dried cords, the death rate is 0.65% in category A and half that figure (0.33%) in category C ; in the latter category, there is a larger proportion of harmless bites and the rate is a death rate, whereas, in category A, we are dealing with the fatality rate.

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[1] The persons really infected are likened to patients whose *inapparent* disease develops towards cure or towards death.

At Bucharest, the fatality rate (A) usually exceeds the death rate of category C—for instance, we have the following figures :

	1933 %	1935 %	1936 %	1937 %
1. Category A.....	0.40	0.60	0.58	1.03
2. Category C.....	0.27	0.17	0.31	0.20

McKendrick's eight analytical reviews, taken as a whole, show a still greater difference between the groups "deep bite" and "superficial " bite. The seriousness of the infection considered in relation to the nature of the wounds does not alone explain this difference. It has also to be considered that the probability of real infection is not the same in the two groups, so that we know the fatality rate for the homogeneous " deep-bite " group, while in the case of the heterogeneous "superficial-bite " group we are taking the mortality rate as standard of measurement.

The figures, for the correct interpretation of which this distinction was required, are as follows for Europeans :

	Deep bite (%)	Superficial bite (%)
1. Dried cords	0.51	0.05
2. Killed carbolised vaccines	0.82	0.05

The efficacy of live and killed vaccines appears to be the same according to the mortality rate (0.05%) of the 2nd group, but it is considerably lower for the killed carbolised vaccines if we take as an indication the fatality rate of the group in which the cases of real infection constitute almost the whole.

At Bucharest, the average for five years (1934-1938) is 2.73% in one group and 0.13% in the other group.

The conclusion, if we take the most reliable indication that could be chosen (*i.e.*, the fatality rate), is that killed carbolised vaccines are more efficacious than the vaccines used at the Babeș Institute but less efficacious than the dried cords.

* * *

12. The dried-cord method is found to have great advantages : " efficacy, in spite of weak concentration due to the presence of live

virus ; harmlessness, since the virus has, through desiccation, practically lost its capacity to travel along the nerves (LÉPINE and CRUVEILHIER) ; mild vaccinal reactions, owing to the small quantity of foreign proteins injected during treatment ".[1]

The innocuity of the rabic cords would undoubtedly constitute a great advantage, but we have already seen what the position is; it may be pointed out that paralytic accidents are three to four times as frequent when live vaccines are used. According to MCKENDRICK, the figures were as follows for eight years :

	Persons treated	Paralytic accidents	%
(*a*) Killed vaccines	417,181	43	0.010
(*b*) Live vaccines	232,888	82	0.035

These figures relate only to known accidents, and it must not be forgotten that the victims of live vaccines were sometimes persons who had not been bitten or otherwise infected by mad animals.

From this point of view, the great advantage of innocuity attaches to killed vaccines, which completely lack infective power. But this undeniable superiority is not sufficient to determine the choice between killed and live vaccines ; effectiveness is the first consideration, harmlessness comes next.

P. LÉPINE and V. SAUTTER, preceded in this direction by STUART and KRIKORIAN, CUNNINGHAM and MALONE, SHORTT, MALONE and CRAIGHEAD (mentioned by LÉPINE and SAUTTER), observe that, in the conditions specified in their papers, " *the total figures obtained show a clear-cut superiority of carbolised vaccine over vaccine prepared from dried cords of 2, 3 and 4 days* ". Even carbolised vaccines containing less than 5% of brain matter which showed considerably reduced effectiveness *are usually superior to live vaccines* (dried cords).[2]

Practical routine vaccination with avirulent carbolised vaccines has not belied the conclusions of the laboratory experiments. We have already seen, however, that, in the case of deep bites among Europeans, the death rate was higher among persons treated with killed vaccines ; a similar difference in favour of dried cords appears

[1] P. LÉPINE and Mlle. SAUTTER : "Essais expérim. sur la valeur pratique des vaccins antirabiques phéniqués", *Ann. Inst. Pasteur*, 1937, **59**, 39.

[2] *Loc. cit.*, page 50.

in the case of head bites. Is it possible that live vaccines are specially indicated in these two categories of bites ?

There are other questions to be solved before a choice can be made between live vaccines and chemical vaccines. We think that the time necessary for immunisation by means of these latter vaccines, together with the duration of the immunity conferred, call for additional investigations. The slowness of immunisation might be gauged from the frequency of cases of short-incubation rabies and the shorter duration of the unreceptive state from the percentage of long-incubation cases. The statistics give us no information on these points.

13. The future belongs to the chemical vaccines, as PASTEUR foresaw when he wrote : " Since the work to which I refer, discoveries have been increasing and accumulating in regard to chemical vaccines. There can be no doubt that we shall soon have many others. That of rabies, for instance, must soon be known and used."

When relating the experiments which he had carried out in 1887 and 1888, with the assistance of E. VIALA, PASTEUR concluded that " heated cords rendered non-virulent were vaccinal by the operation of a chemical vaccine ".[1]

The best known and most widely used is FERMI's killed carbolised vaccine. It has been established by long practice and laboratory investigations have fully confirmed its efficacy.

In Roumania, carbolised vaccine has been used since 1933 by the anti-rabies station of Cernăuți and, since 1936, by that of Galaţz. The Cernăuți statistics record over a period of five years (1934-1938) 6,779 persons treated, with 17 deaths (0.25%). At Galaţz, the death rate in 1937 was 0.2% (3 deaths among 1,449 persons treated) ; at Cernăuți, the cases of short-incubation rabies (up to 30 days) accounted for 5 out of 17 deaths—*i.e.*, 29.4%.

We may point out that the Galaţz station uses a vaccine of higher concentration—*i.e.*, 5%—while the other station used a 1% vaccine during the first years, and, since 1936, a 3% vaccine associated with brain emulsions heated to 60-65°C. There have been no post-vaccinal accidents at Galaţz ; on the other hand, at Cernăuți a case of paraplegia with paralysis of the bladder, which was, however,

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[1] *Œuvres*, Vol. VI, page 550.

cured, was observed in 1938. From 1931 to 1933, when the HÖGYES process was used, *6 cases* of paralytic accidents were recorded at the Cernăuți station, 4 of which were fatal (including the 2 cases of laboratory rabies already mentioned).

At Budapest[1], the HÖGYES method was abandoned in 1936 in favour of vaccine carbolised with 1% of phenol associated, in serious cases, with live vaccine carbolised with 0.5% of phenol. The statistics for recent years (1936-1938) show that, among 9,222 persons treated, the mortality was 0.08% and that there were no post-vaccinal accidents.

In 1934, with the method of dilutions, 6 cases of acute transverse myelitis were observed, all of which were cured, and, in 1935, 1 case of inferior bulbar paralysis and 1 case of ascending paralysis of the Landry type, both of which ended fatally.

It is interesting to compare these figures with the rabies statistics in Bulgaria,[1] where the HÖGYES method is still used combined with the ALIVISATOS process (anti-rabies institutes of Sofia, Varna and Tirnova), or the HÖGYES-PHILIPS process modified by BOECKER (11 stations). Over a period of five years (1934-1938), the total mortality was 0.11% (30 deaths among 26,168 persons treated), with 7 paralytic accidents, which were cured.

The period of incubation in the 30 cases of rabies occurring among the vaccinated persons was as follows :

17-30	days	in	13	cases—*i.e.*,		43.3%
31-60	,,	,,	12	,,	,,	40.0%
61-90	,,	,,	2	,,	,,	6.6%
More than 90	,,	,,	3	,,	,,	10.0%

Rabies with a long period of incubation (over two months) is therefore rarely observed (16.6%), while rabies with a short period of incubation is much more frequent (43.3%).

The Jussy Anti-rabies Institute[1] uses 3% FERMI vaccine associated with heated emulsions (60-65°C.). During a period of five years (1934-1938), 20,914 patients were treated and 14 failures were recorded—*i.e.*, a reduced mortality rate of 0.06%. In 1938, the *total* mortality was 0.25% (14 deaths out of 5,535 persons treated), and the *reduced* mortality 0.09% ; the period of incubation

[1] See Annexes, reply to the questionnaire.

in the 5 cases where the treatment failed was 28, 29, 38, 60 and 145 days, while it was below 30 days in 11 out of 14 fatal cases (78.5%).

* * *

The question has arisen whether vaccines " killed " by phenol are really sterilised, avirulent and merely chemical. Fermi observes that carbolised vaccine immediately loses its virulence for the animal if it is injected subcutaneously, but still remains virulent in intracerebral injections for 6 to 10 days after its preparation.

P. Lépine and V. Sautter[1] show that, by inoculating rabbits intracerebrally with high doses of carbolised vaccine, rabies can often still be determined after a very long time—as much as 90 days ; according to these authors, " the carbolised vaccines contain *living fixed virus* (though practically harmless), so that the fixed virus treated with phenol immediately loses its neuroprobasis and, in a few days, its virulence for the intracerebral route, without the virus being really killed ".

What is certain is that Fermi's carbolised vaccine behaves like an avirulent chemical vaccine which is perfectly harmless when injected under the skin. The avirulence of the carbolised vaccine, on the one hand, and its undeniable efficacy, on the other, have caused it to be adopted by a large number of anti-rabies institutes.

The time has now come to ask, and to secure an answer to, the question whether there is any valid reason for having recourse to live vaccines and accepting the risks which they involve.

At the time when the Pasteur treatment with more or less attenuated cords constituted the only means of averting rabies, this treatment had to be advised and applied as such ; there was no alternative.

At present, the matter is one which requires discussion. The harmlessness of live vaccines is not a rule with no exceptions, and the existence of an ideal live vaccine without neuroprobasis in every case is very improbable, since the properties of vaccine viruses are contingent and dependent on circumstances ; it is impossible to be

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[1] " État du virus fixe dans les vaccins phéniqués ", *C.R. Soc. Biol.*, 1938, **127**, 192.

sure that certain individual conditions will not modify the resistance of the organism to the action of these viruses.

Moreover, it should be borne in mind that the anti-rabies institutes do not treat only patients who are in all cases necessarily doomed to develop rabies. Immunisation by means of live vaccines exposes indiscriminately the persons infected and those who, although bitten, are not infected to accidents which are at times fatal ; it involves serious risks both for patients who would die of rabies and those who would remain unaffected if they were not treated. The risks and dangers due to live vaccines would be avoided if they were replaced by chemical vaccines.

In these circumstances, should live vaccines be abandoned and chemical vaccines used exclusively ? Or would it be sufficient to define more exactly the indications and contra-indications of live vaccines ?

An International Rabies Conference could alone decide this point with the necessary authority and competence.

ANNEX 1

QUESTIONNAIRE ON RABIES AND ANTIRABIC VACCINATION IN THE COUNTRIES OF CENTRAL AND SOUTH-EASTERN EUROPE

I. *Present State of Rabies in the Country* (or the area covered by the Institute).

(*a*) Incidence of canine rabies, epizootic foci, during the past five years (1934-1938).

(*b*) Number of persons bitten and of deaths from rabies among human beings recorded for each of these years (including deaths of persons not treated).

(*c*) Antirabic vaccination of dogs : methods applied and results obtained.

II. *Street Virus, Strains isolated by the Institute.*

(*a*) Virulence of fresh virus in rabbits ; its capacity to produce Negri bodies.

(*b*) Frequency of non-Negri-body-producing strains and " reinforced " strains with a short period of incubation, not exceeding 10 days.

(*c*) Number of passages necessary to transform the street virus into fixed virus.

III. *Period of Incubation of Rabies.*

(*a*) Among bitten persons not treated and among persons who have undergone treatment.

(*b*) Frequency of cases with a short period of incubation, up to 30 days after the bite, for the two categories.

IV. *Fixed Virus used by the Institute.*

(*a*) Origin and number of passages up to January 1st, 1939 ; interval between passages.

(*b*) Effects of inoculation by trepanning in rabbits ; period of incubation and duration of the disease. Symptomatology and course taken by the disease in exceptional cases ; percentage of such cases.

(*c*) Degree of *neuroprobasis* according to the effect of subcutaneous and intra-ocular inoculations ; incidence of rabies and period of incubation in animals thus inoculated, especially rabbits and dogs.

(*d*) Limit of virulence for dilutions of the rabic cord (fresh fixed virus) ; concentration required to obtain rabies *constantly* in trepanned rabbits.

V. *Variations of Fixed Virus.*

(*a*) Nature of variations observed and conditions under which they occurred.

(*b*) Is the attenuation of the fixed virus under given conditions always identical ?

VI. *Antigenic Value of Live Fixed Virus.*

(*a*) Description of type of vaccination adopted and results obtained in rabbits or dogs.

(*b*) Use of very virulent 0- and 1-day cords ; indications and contra-indications.

VII. *Chemical Antirabic Vaccine (Killed).*

(*a*) Preparation ; control of its virulence.

(*b*) Antigenic power : time necessary to establish immunity ; degree and duration of specific resistance conferred by the vaccine on animals (dogs and rabbits).

VIII. *Antirabic Vaccination of Human Beings.*

(*a*) Treatment scheme explained in detail.

(*b*) Statistics for 1938 according to the Geneva form, supplemented by separate headings for children of 0-15 years.

(*c*) Total number of deaths due to rabies among vaccinated persons, including those which occurred during treatment, with summary of observations and information as to the period of incubation.

(*d*) Post-vaccinal accidents ; their nature and frequency.

IX. *Various Observations* and bibliography of the works published by the Institute from 1928 until the present time.

N.B.—Kindly reply before July 1st, 1939, to the following address : Institut Dr. J. Cantacuzino, Section de la Rage, Bucharest.

* * *

The questionnaire was sent to the anti-rabies institutes of Warsaw, Košice, Cracow, Prague, Vienna, Budapest, Zagreb, Sofia, Istanbul, Athens, Odessa, Kiev and Moscow, as well as to Rcumanian institutes.

ANNEX 2

REPLY FROM THE BUDAPEST PASTEUR INSTITUTE

I. (*a*) Rabid animals :

1934	362
1935	405
1936	369
1937	237
1938	90

(*b*) Persons receiving complete vaccine treatment :

		Deaths	%
1934	3,456	4	0.11
1935	3,897	1	0.02
1936	3,762	4	0.10
1937	3,508	3	0.08
1938	1,952	1	0.05

Persons not presenting themselves for vaccination :

		Deaths	%
1934-1938	5	5	100

(*c*) The prophylactic vaccination of dogs is carried out with sheep vaccine by the Kerbler method according to UMENO and DOI. In the period 1934-1938, the number of dogs vaccinated per year ranged from 38,000 to 400,000, making a total of 1,107,000.

The result is excellent in the centre and western part of the country ; in the eastern districts, the improvement will only make itself felt later, since the disease is imported from across the frontier.

II. (*a*) The street virus injected subdurally made rabbits ill at the end of about 21 days (rather less than more). The animals died after an illness of 3 to 5 days. The examination for Negri bodies gave a positive result in most cases.

(*b*) In recent years, we have not observed any virus with a period of incubation as short as 10 days.

(*c*) The street virus is transformed into fixed virus after a series of 24 to 26 passages through the rabbit.

III. (*a*) Appearance of rabies in vaccinated persons :

In 4 cases, within 20 days In 4 cases, within 40 days In 3 cases, within 70 days In 1 case, on the 237th day In 1 case, on the 363rd day	13 cases

(*b*) Among persons not vaccinated :

In 4 cases, within 20 days In 2 cases, within 70 days In 1 case, on the 114th day In 1 case, on the 130th day	8 cases

IV. (*a*) Högyes used a virus (February 1886) obtained from a dog's brain, and fixed it by means of 26 passages. The number of passages up to the present is 18,459. At present, a passage is effected every three days.

(*b*) The period of incubation is three days ; on the fourth day, fever appears ; on the fifth day, the animal is restless ; on the sixth day, paralysis sets in, and death usually occurs about the seventh day.

In exceptional cases, the incubation period is shorter and the disease begins with extreme apathy, but it has been proved that, in almost all cases, the cause was sought in concomitant diseases.

(*c*) Our fixed virus is not infectious when introduced subcutaneously ; when injected intra-ocularly the result is not certain ; no recent experiments have been made on this point.

(*d*) When injected intracerebrally, our fixed virus produces infection sometimes in a dilution of 1 : 500,000, and regularly in a dilution of 1 : 50,000 ; for vaccinations we use a dilution of 1 : 100.

V. (*a*) As a rule, we observed no variations, although the season, the breed of rabbit and the diet may all have their influence.

(*b*) It would seem that the attenuation is maintained continuously.

VI. (*a*) Vaccination of rabbits or dogs is no longer carried out for experimental purposes, the experience gained being regarded as sufficient.

(*b*) We do not use cords more virulent than the customary ones.

VII. (*a*) The vaccine is used after two days ; a complete brain is crushed and subjected to 1% and $\frac{1}{2}$% carbolisation. From time to time, it is bacteriologically examined, so far with an invariably negative result. The 1% carbolised emulsion never kills the rabbit ; the $\frac{1}{2}$% carbolised emulsion kills it 8 to 10 days later than the fixed virus.

(*b*) We have observed that, at the end of 21 to 25 days, a serum is obtained which neutralises the fixed virus *in vitro*. It may be reckoned that immunity begins to develop between the 18th and 21st days.

Högyes observed an immunity lasting two years in dogs.

VIII. (*a*) Slight and superficial cases are given the vaccine for 5 or 6 days ; for deeper bites affecting the dermis, vaccination lasts 8-9 days ; in the case of multiple bites affecting the tissues under the skin, vaccination is continued for 10 days. For all bites on the face, vaccination is extended to 10 days.

The dose depends on the physical condition and age of the patient. Small children are given a quarter of the adult dose and bigger children half the dose. Feeble adults and persons over 60 years of age are also given a half dose. Adolescents are given two-thirds of the total dose. Persons over 18 years of

age and robust adults are given an initial dose of fixed virus in the form of 3.5 c.c. of a 1% emulsion of brain, to which 1% of carbolic acid has been added.

(*b*) See appendix.

(*c*) One fatal case was recorded—that of a weakly, undernourished person who, however, had no organic defect according to the post-mortem findings. The period of incubation (22 days) was in accordance with the fact that the bites were on the face.

(*d*) Since carbolised brain emulsions have been used, no post-vaccinal complications have occurred.

In 1934, there were 6 cases of post-vaccinal paralysis—*i.e.*, 0.17% of the total number of vaccinated persons.

In 1935, there were 2 cases of post-vaccinal paralysis—*i.e.*, 0.05% of the total number of vaccinated persons.

Six cases of acute transversal myelitis developed and were cured after varying periods of time.

There was one case of acute inferior bulbar paralysis and one typical case of Landry's paralysis, both fatal.

Statistics regarding Rabbits used for the Preparation of Vaccine.

Number of animals : 882.

Number of animals infected with fresh brain : 377—*i.e.*, 42.74%.

Of these :	%
28 were killed at the end of the 5th day	7.43
284 were killed at the end of the 6th day	75.33
47 were killed at the end of the 7th-8th day	12.47
18 died—*i.e.*	4.77

Number infected with glycerinated brain substance : 505—*i.e.*, 57.26%.

Of these :	%
59 were killed at the end of the 5th day	11.69
356 were killed at the end of the 6th day	70.49
60 were killed at the end of the 7th-8th day	11.89
30 died	5.94

* * *

Appendix

RESULTS OF ANTI-RABIES TREATMENT
AT THE PASTEUR INSTITUTE, BUDAPEST, IN 1938

1. Method of treatment : Phenol dilution of vaccine, 1% and 0.5%.

(*a*) Dosage for cases :

Mild 5-6 days' treatment according to age
Ordinary 8 days' treatment according to age
Severe 10 days' treatment according to age.

(*b*) The dilution retains its efficacy for 2 months in the ice chest.

					Deaths			
	All ages	1-14 years	All ages	1-14 years	All ages	1-14 years	All ages	1-14 years
2. Total number of persons treated	1,952	*506*	100	*100*	1	*1*	0.05	*0.19*
4. Species of biting animal :								
Dog	1,494	*439*	76.50	*86.76*	1	*1*	0.06	*0.23*
Cat	246	*46*	12.60	*9.09*	—	—	—	—
Ruminants	64	*10*	3.32	*1.98*	—	—	—	—
Other animals	148	*11*	7.58	*2.17*	—	—	—	—
5. Evidence of rabies in the biting animal :								
Category A	200	*78*	10.25	*15.42*	1	*1*	0.50	*1.28*
Category B	182	*27*	9.32	*5.34*	—	—	—	—
Category C	1,570	*401*	80.43	*79.24*	—	—	—	—
6. Severity of bite :								
Deep	576	*134*	29.51	*26.28*	1	*1*	0.17	*0.75*
Superficial	971	*275*	49.74	*54.35*	—	—	—	—
No visible lesion	405	*97*	20.75	*19.37*	—	—	—	—
7. Intervention of clothing :								
On bare skin	1,312	*446*	67.21	*88.14*	1	*1*	0.07	*0.22*
Through clothing	640	*60*	32.79	*11.86*	—	—	—	—
8. Position of bite :								
Head	73	*48*	3.74	*9.49*	1	*1*	1.36	*2.08*
Arm	1,185	*265*	60.71	*52.37*	—	—	—	—
Trunk	25	*12*	1.28	*2.37*	—	—	—	—
Leg	669	*181*	34.27	*35.77*	—	—	—	—
9. Delay in commencement of treatment :								
0 to 4 days	1,362	*364*	69.78	*71.93*	1	*1*	0.07	*0.27*
5 to 7 days	247	*69*	12.65	*13.64*	—	—	—	—
8 to 14 days	280	*56*	14.34	*11.07*	—	—	—	—
15 to 21 days	33	*9*	1.69	*1.78*	—	—	—	—
More than 21 days	30	*8*	1.54	*1.58*	—	—	—	—

10. Measures taken to keep cases treated under observation for 6 or 12 months : Bitten persons sent for treatment by the authorities remain under medical supervision after they return home. After periods of 6 months and 1 year, the Institute receives a report by a doctor on the health of each person treated. A detailed medical report is also made on persons who die during this period.

12. Information concerning each death due to rabies.
Method : Phenol dilution of vaccine, 1% and 0.5%.
Dosage : Severe case.
Length of treatment : Ten days.
Biting animal : Dog.
Category : A.
Severity of bite : Deep.
Intervention of clothing : On the bare skin.
Position of bite : Head.
Number of days between bite and arrival : 2.
Number of days between bite and death : 22.
Post-mortem : Positive. Negri corpuscles.

ANNEX 3

REPLY BY THE ANTI-RABIES SECTION OF THE SOFIA INSTITUTE OF PUBLIC HEALTH

I. *Present State of Rabies in Bulgaria.*—(*a*) Rabid animals (dogs, cats, etc.) during the past 4 years in the whole country.

Year	Rabid animals
1935	1,513
1936	1,280
1937	1,479
1938	1,319
1935 to 1938 ...	5,591

(*b*) No information is available concerning deaths amongst persons not treated.

Year	Persons bitten	Deaths
1934	6,621	10
1935	4,665	9
1936	4,798	7
1937	5,196	2
1938	4,888	2
1934 to 1938	26,168	30

(*c*) The vaccination of dogs is effected in most cases by the Doi and Umeno method. It is performed by the veterinary surgeons, and they have information as to the results obtained.

II. For the moment, the Institute is not working on street virus.

III. (*a*) We have no information as to the period of incubation of rabies in the case of bitten persons not treated.

Incubation period in the case of vaccinated persons :

Year	Days
1934	17-72
1935	19-114
1936	19-116
1937	18-25
1938	20-201

(*b*) Cases with an incubation period not exceeding 30 days amongst persons vaccinated were as follows :

Year	Number of cases	Incubation period (days)
1934	5	17, 23, 24, 27, 29
1935	1	19
1936	3	19, 20, 29
1937	2	18 and 20
1938	1	20

IV. (*a*) The fixed virus used by the Institute comes from the Pasteur Institute in Paris. It has undergone about 1,500 passages at intervals of from 3 to 8 days.

(*b*) The first symptoms of rabies appear on the 4th day in rabbits inoculated with the fixed virus. The disease lasts from 2 to 3 days. Usually, the animal is quiet until the 4th or 5th day, when paralysis of the extremities appears, accompanied sometimes by convulsions and cries. On the 6th or 7th day, total paralysis supervenes, and is followed by death.

(*c*) There has been no research into this question.

(*d*) 1 : 5,000 to 1 : 10,000.

V. No variations have been observed.

VI. There has been no research into this point.

VII. None is prepared.

VIII. (*a*) In the Sofia, Varna and Tirnovo Institutes, the method used for the vaccination of human beings is that of Högyes-Alivisatos, whereas, in the 11 other anti-rabies stations, the Högyes-Philips method, as modified by Boecker, is practised. Severe bites (head, arm or deep and multiple lesions) by animals that are certainly rabid are treated exclusively at Sofia, Varna and Tirnovo by the combined Högyes-Alivisatos method.

(*b*) In 1938, 1,742 children from 0 to 15 years were vaccinated.

(*c*) Deaths amongst vaccinated persons :

Year	Persons vaccinated	Deaths	Number of days between beginning of treatment and death
1934	6,621	10	17, 23, 24, 27, 29, 32, 46, 53, 57, 72
1935	4,665	9	19, 33, 36, 45, 49, 49, 50, 77, 114
1936	4,798	7	19, 20, 29, 45, 52, 100, 116
1937	5,196	2	18, 25
1938	4,888	2	20, 20
1934 to 1938	26,168	30	

(*d*) The post-vaccinal accidents observed in the past 5 years were as follows :

Year	Number of cases	Nature of accidents
1934	5	3 cases of paresis (oculomotor nerve, facial nerve, bladder) and 2 cases of paralysis of the lower extremities.
1935	0	
1936	0	
1937	0	
1938	2	2 cases of paralysis of the lower extremities.
1934 to 1938	7	

ANNEX 4

REPLY BY THE JASSY ANTI-RABIES INSTITUTE

I. *Present State of Rabies in the District covered by the Institute.*

(*a*) In 1934, there were 3,567 cases of canine rabies of categories A, B and C.

,, 1935 ,, ,, 2,754 ,, ,, ,,
,, 1936 ,, ,, 1,668 ,, ,, ,,
,, 1937 ,, ,, 2,570 ,, ,, ,,
,, 1938 ,, ,, 4,021 ,, ,, ,,

(*b*) In 1934, the number of persons treated was 4,630, of whom *1* died and *2* arrived with symptoms of rabies (not treated).

,, 1935 ,, ,, ,, ,, 3,887, of whom *3* died and *3* arrived with symptoms of rabies (not treated).

In 1936, the number of persons treated was 3,079, of whom *1* died and *1* arrived with symptoms of rabies (not treated).

,, 1937 ,, ,, ,, ,, 3,783, of whom *4* died and *2* arrived with symptoms of rabies (not treated).

,, 1938 ,, ,, ,, ,, 5,553, of whom *5* died and *9* arrived with symptoms of rabies (not treated).

(*c*) The vaccination of dogs is carried out by the Jassy Laboratory of Veterinary Bacteriology.

II. *Autochthonous Street Virus.*

(*a*) For 67 inoculations (trepanations) carried out on rabbits with fresh street virus from rabid dogs, the incubation period varied between 18 and 25 days ; histopathological examination disclosed Negri corpuscles.

(*b*) No information.

(*c*) No information.

III. *Period of Incubation of Rabies amongst Human Beings.*

(*a*) The incubation period was short in 2 out of 17 cases that were not treated.

(*b*) The incubation period was short in 8 out of 14 cases that were treated.

IV. *Fixed Virus used by the Institute.*

(*a*) The fixed virus comes from Paris (1891) ; the number of passages is 2,671 and the interval between passages is 7 days.

(*b*) The period of incubation is 4 days and the duration of the disease is 2 days.

(*c*) No information.

(*d*) The limit of virulence for dilutions of rabic cord was 1 : 200.

V. *Variations in Fixed Virus.*

(*a*) Until 1931, the preservation in glycerine of the brain substance extracted from rabbits which died of rabies induced by fixed virus was effected in refrigerators at an inconstant temperature (above + 10°C.).

Under these conditions, the shortening of the incubation period was frequently noted, and the disease ran its course in from 8 to

10 days. It was accordingly necessary to make successive passages through guinea-pigs, so as to raise the incubation period once more to 6 days for guinea-pigs and 7 days for rabbits.

From 1931 onwards, the preservation was effected in electric refrigerators at a constant temperature of + 3°C., and the fixed virus retained its virulence intact. It would seem, however, that the incubation period and the duration of the disease were both stabilised at 6 days.

VI. *Antigenic Value of Live Fixed Virus amongst Animals.*

(*a*) No information.

VII. *Chemical Antirabic Vaccine (Killed).*

(*a*) 5% emulsion of fixed virus with phenol. Preparation : a 10% emulsion of brain and cord is made in distilled water with 1% of phenol. This is well shaken up and kept at 37°C. for 3 days, being shaken up 4 times a day. An equal quantity of distilled water is then added, this giving a 5% emulsion of nerve substance in a 0.5% solution of phenol. Bacteriological control is carried out and the control of the virulence is effected by intracerebral inoculations into a group of 6 rabbits. The vaccine is kept in the electric refrigerator, and is considered to remain effective for 3 months after its preparation.

(*b*) The following experiments were carried out :

(1) 12 rabbits, given a subcutaneous inoculation with 0.4 c.c. of street virus, received for 15 days 1 c.c. of 1% Semple vaccine. 9 rabbits died between the 3rd and 9th days after the termination of the vaccination, with symptoms of rabies. 3 survived. The same experiment was repeated, with the same result, using 1% Puscariu vaccine, attenuated by heating to 60° and 65°C.

(2) 12 rabbits, vaccinated for 15 days with 1 c.c. of 1% Semple vaccine, were inoculated after 5 injections of vaccine with 0.4 c.c. of 1 : 50 street virus. 9 rabbits survived and 3 died between the 3rd and 9th days after the termination of the vaccination. The same experiment was carried out with attenuated 1% Puscariu vaccine and all the rabbits survived.

(3) 12 rabbits, vaccinated for 15 days with 1 c.c. of Semple vaccine, and then given a subcutaneous inoculation with 0.4 c.c. of street virus 10 days after the vaccination, all survived. The same result was obtained with attenuated Puscariu vaccine.

VIII. *Antirabic Vaccination of Human Beings.*

(*a*) Mixed method : daily inoculations with Puscariu vaccine and Fermi's vaccine.

1. Puscariu vaccine : emulsion of 9 to 10 grammes of fixed virus (cerebrum, cerebellum and cord of rabbit) in 1,000 c.c. of distilled water ; attenuation by heating to 65° and 60°C.

2. Fermi's vaccine : 3% phenol vaccine.

Persons under treatment are given, on alternate days, 2 c.c. of Puscariu vaccine attenuated by heating to 65° and 60°C. in one side of the abdomen and 4 c.c. of Fermi's vaccine in the other side.

STATISTICS FOR 1938

	Number of cases	Number of deaths	Children 0-15 years: Number of cases	Children 0-15 years: Number of deaths
Total number of persons treated	5,535	0	1,679	0
Species of biting animal :				
Dog	4,373	5		
Cat	385			
Ruminants	531			
Other animals	7			
Human	39			
Evidence of rabies in the biting animal :				
Category A	1,462			
Category B	2,007	4		
Category C	947	1		
Category D	602			
Severity of bite :				
Deep	1,748	5		
Superficial	2,469			
No visible lesion	1,379			
Intervention of clothing :				
Through clothing	1,771	1		
On bare skin	3,764	4		
Position of bite :				
Head	199			
Upper limbs	1,933	5		
Body	153			
Lower limbs	1,871			
Delay in commencement of treatment :				
0-4 days	3,007	4		
5-7 days	1,536	1		
7-14 days	747			
15-21 days	245			
More than 21 days				

Length of treatment.

No visible lesion	:	6 to 8 days
Slight lesions	:	12 ,,
Medium ,,	:	14 to 16 ,,
Severe ,,	:	18 ,,

Information regarding fatal cases observed in 1938.

	Length of treatment	Biting animal	Category	Severity of bite	Position of bite	Number of days between bite and treatment	Number of days between bite and death
1.	16 days	dog	B.	deep	hand	1 day	60 days
2.	18 days	dog	B.	deep	hand	3 days	28 days
3.	16 days	dog	B.	deep	hand	4 days	38 days
4.	18 days	dog	B.	deep	forearm and hand	6 days	29 days
5.	16 days	dog	C.	deep	hand and thigh	3 days	145 days

LEAGUE OF NATIONS

BULLETIN
OF THE HEALTH ORGANISATION

VOL. IX, No. 2. Year 1940.

REPORT ON TERMINOLOGY IN MALARIA

CONTENTS

* For index to Commentary, see Part II. — Glossary. Besides an alphabetical list of terms with definitions, this gives references to the section and sub-section in which terms are dealt with in the Commentary.

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Report on Terminology in Malaria

INTRODUCTION

During its meeting in Geneva on October 27th-30th, 1937, the Reporting Committee of the Malaria Commission considered a communication from Dr. EJERCITO, the member of the Malaria Commission representing the Philippines, asking whether the Malaria Commission could usefully endeavour to standardise the terminology most commonly employed :

(a) In the epidemiology of malaria, and

(b) In questions relating to the species, sub-species, varieties and races of anopheles.

The Committee examined a memorandum drawn up on this subject by Dr. E. PAMPANA, the Secretary of the Commission, and decided to entrust the further work for the standardisation of the nomenclature employed in malariology to a Sub-Committee consisting of Sir Rickard CHRISTOPHERS, Dr. L. W. HACKETT, Professor Edmond SERGENT, Professor W. SCHÜFFNER and the Secretary of the Commission. With regard to the second question raised in Dr. Ejercito's proposal, the Committee considered that this was a matter of zoological systematics and is therefore not within its province.

The Health Committee, at its twenty-sixth session in November 1937, approved the constitution of the Sub-Committee for the preparation of a draft report on the subject.

The members of the Sub-Committee, with Professor M. CIUCA representing the Secretary of the Commission, met in London on June 20th-22nd, 1938, at the London School of Hygiene and Tropical Medicine and subsequently in Amsterdam at the Third International Congress on Malaria. The present report gives the results of their discussions as approved at the last-mentioned meeting and in subsequent correspondence.

Certain points regarding the form and content of the report require mention.

Language.

The official language in which the reports of the League are normally published are French and English. Terms in these languages will be found in as nearly as possible equivalent form in the respective French and English texts, a reference in one of the texts to terms in the other being made only when there is some special reason for this. Thus " paludisme " in the French text replaces " malaria " in the English text and, except in an explanatory paragraph, these names will be found only in the respective texts.

Zoological Systematics and Methods.

Questions relating to the purely zoological nomenclature of species and varieties of anopheles, as decided by the Reporting Committee, are not part of the Sub-Committee's task. The reasons on which this decision is based would appear to hold good also regarding the zoological nomenclature of the different forms of malaria parasites. Nevertheless, there are some points connected with zoological nomenclature which are distinctly relevant to terminology in malaria and such points have been dealt with in the report. Questions of technique and methods are dealt with only where this is considered necessary to explain or define terminology.

Principles underlying the Use of Index Values.

From the number of rates, indices, etc., which have been described in the literature and which it is desirable to mention and sometimes explain, it might appear that research in malaria epidemiology largely consists in determining a multiplicity of such rates, indices, etc. Actually, of course, this is not so. Information of very varied character is obtained by the worker, who may, however, wish to express some portion of this in a simple form for purposes of presentation or comparison. He therefore makes use of this or that rate or index as the case may be, often for a quite specific and restricted purpose — *e.g.*, in malaria control to obtain some definite measure of results achieved. The really important rates and indices, as should be apparent from this report, are relatively few and, in regard to these, the chief consideration is that their nature, limitations and applications should be generally

appreciated, rather than that terminology should be made so precise as to cover all possible contingencies in their use.

It is scarcely possible, for example, that the term "spleen rate" should be so defined that exactly the same significance attaches to values obtained by every observer in every country in the world. *So long as an author's methods in obtaining this rate are sufficiently indicated*, the term spleen rate should still apply whether taken by palpation standing or lying down or by whatever method it may have been found most desirable to employ. If it is desired to map with the accuracy of a physical constant the intensity of malaria as shown by the spleen rate all over the word, then very special methods to co-ordinate results obtained in different countries would be necessary and in this case a degree of precision would be introduced which it is neither practical nor even desirable at present to read into the term. Meanwhile, such uniformity as is really helpful is desirable and the Sub-Committee has concerned itself chiefly in clarifying the issues from this point of view.

Form and Scope of the Report.

The report has been drawn up in two parts : (1) a systematically arranged Commentary (Part I) in which terms will be found in proper perspective and context, with such description or explanation as seems necessary ; (2) an alphabetically arranged Glossary (Part II) giving brief definitions or descriptions of terms used.

Acknowledgments.

The writer of the draft report* is indebted not only to the members of the Sub-Committee, but also to those from whom help on certain lines has been asked and whose remarks are quoted in the text. Thanks are also due to Professor Swellengrebel for his collaboration with Professor Schüffner in drawing up a commentary on points raised in the provisional draft circulated to members of the Sub-Committee, as also to Colonel S. P. James and Colonel J. A. Sinton, whose help in reading over and commenting on the final text is gratefully acknowledged.

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* *Editor's note.* — Sir Rickard Christophers.

Part I

COMMENTARY [1]

[1] Terms given in the Commentary in italics are those considered as desirable for use or not unsuitable. Terms left in roman type are to a large extent unnecessary as specific terms or unsuitable for use. This does not apply to specific or generic names, etc., given in italics.

Section I

THE MALARIA PARASITES AND THE INFECTIONS TO WHICH THEY GIVE RISE

Sub-section 1. — The Name Malaria.

Two names only are now in general use in scientific writings to designate the condition of infection or disease in man [2] brought about by parasites of the genus *Plasmodium* — viz., *Malaria* [3] in

[2] The term malaria is also now commonly applied to infection by parasites of the genera *Plasmodium* or *Haemoproteus* in birds *(bird malaria, avian malaria)* and in monkeys and apes *(monkey malaria, ape malaria)*. Infections of dogs, cattle or other animals with *Babesia*, *Theileria*, etc., are usually termed piroplasmoses, tick fever, etc., but are occasionally referred to, though undesirably, as dog or cattle malaria.

[3] The conception of malaria as a single morbid process or disease, and certainly the use of the term " malaria ", is of relatively late origin. Torti (1658-1741), who first definitely distinguished malarial from other fevers through the former's property of being cured by cinchona bark, does not employ the term malaria, or indeed any single term synonymous with this, in his famous work " Therapeutice specialis ", of which the first edition was published in 1712. A reference to the word malaria in general literature is quoted by the Oxford Dictionary as made in regard to the fevers in Rome by Hugh Walpole in 1740 (information kindly supplied by the Librarian, Royal Society of Medicine).

Maculloch (1827) was the first English medical writer, in his book " Malaria ", to use this name, which he states he borrowed from Italy.

From remarks by Twining (1832) (" Clinical Illustrations of the More Important Diseases of Bengal ", etc., Baptist Mission Press, Calcutta) it would seem that the name malaria was first used more in the sense of the condition or conditions responsible for the causation of the miasmatic fevers than as indicating the disease as such (private communication from Colonel Sinton). Later, a clinical and eventually a parasitological designation seems to have been the natural course of evolution of the term. Epidemiologically, there is often some return to its use in a general sense as designating a condition not wholly confined to human infection — *i.e.*, as carrying the idea of prevalence of the parasite in man and mosquito in an area.

use by English, German and Italian writers, and *paludisme* [4] in use by French and (as *paludismo)* by Spanish writers.

Though these names are indicative of more or less mistaken conceptions of the true causation of the disease, no question of any more appropriate name appears to arise.[5]

It is usual to apply the word *malaria* for " malaria parasite ", " malaria mosquito ", but to use *malarial* for " malarial conditions ", " malarial infection ", " malarial fever ".

Certain derivations from the name are commonly used — *e.g.*, *malariology*, *malariologist*, *malariometry*, as also similar derivations from paludisme in the French.[6]

Sub-section 2. — The Malaria Parasites.

The organisms causing malaria are commonly referred to collectively as the *malaria parasite* or as *malaria parasites.*

The zoological nomenclature of the parasites is very confused and has been the subject of a great deal of discussion in the literature. In regard to the generic name, it is now most generally held that only one genus should be used to include all the human species and that, except for the parasite of birds formerly known as " Halteridium " now placed in the genus *Haemoproteus*, all the pigmented red cell parasites in man, bats and birds should be included in the same genus, which genus by priority of naming is *Plasmodium* Marchiafava and Celli, 1885. It is therefore undesirable in the face of such opinion to use the generic name *Laverania* as

[4] The name « paludisme » is referred to by Laveran in his « Traité des fièvres palustres, 1884 » as having been used by the French Surgeon Verneuil (1823-1895) to designate all the morbid processes produced by the microbes of paludal fevers and maladies derived therefrom.

[5] Malaria, even in fairly recent medical literature, is sometimes termed " intermittent fever ", " fièvre intermittente " and " Wechselfieber ". It was also popularly known as " ague ", " Kaltes Fieber ", etc., or by names indicating its supposed origin from swamps, such as " marsh fever ", " swamp fever ", " Sumpffieber ", " fièvre paludéenne ", " fièvre palustre ", or from regions where it occurred, as " coast fever ", " jungle fever ", etc. Such names are, however, now practically obsolete in scientific writings.

[6] See French text.

applied to the malignant tertian parasite or similar forms in the higher apes.[7]

In regard to the specific names, there is not even this degree of unanimity and the position, especially in respect of the naming of the malignant tertian parasite, is extremely complicated. Up to recently, controversy has been concerned chiefly with the question whether *praecox*, *immaculata* or *falciparum* was the correct name to use. Grassi and Feletti, since they believed that certain parasites (proteosoma) seen by them in birds from a malarious locality were identical with the parasite of " quotidian fever with short intermissions " — that is, with what is now known as the malignant tertian parasite — gave to both of these parasites the name *praecox*. Those who hold that — though at the time two species were given the same name — the authors, later, indicated the human parasite as *praecox* consider this name the correct one for the malignant tertian parasite. But others hold that the bird parasite was clearly indicated at the first naming so that *praecox* cannot be valid for the human parasite and that the correct name for this must be dependent on subsequent naming. Those who consider that, as a result of such subsequent naming, the first valid name was *immaculata* Grassi and Feletti, 1892, use this designation. But others, for various reasons, do not consider *immaculata* to be valid and employ *falciparum* Welch, 1897, which they consider to be the first name for which validity can be claimed.[8]

Actually, the position has been shown to be even more difficult than this, since Laveran's name *malariae* is undoubtedly valid as applied to the malignant tertian parasite, which he clearly described, and the name *malariae* given by Grassi and Feletti to the quartan parasite was not, as assumed by many later writers, intended to misplace Laveran's name, but was their own name given to a species in another genus (Haemamoeba). In other words, there were two species with the same specific name, but in two different genera

[7] Some authors, however, consider it desirable to use *Laverania* for the reason that the crescentic shape of the gametocytes is a good differentiating character. It seems, however, better to accept the more common opinion at least for the present. For discussion of validity of the name *Plasmodium*, see paper referred to in footnote [9].

[8] For a full account of the position in respect to these names, see Ed. and Et. Sergent and Catanei, Arch. Inst. Pasteur d'Algérie, 1929, 7, 233, as also remarks upon *immaculata* in references given in note [9].

— *i.e.*, *Laverania malariae* Laveran 1881, the malignant tertian parasite, and *Haemamoeba malariae* Grassi and Feletti 1890, the quartan parasite. Both names are perfectly valid so long as the two genera were maintained. The latter (not the former) specific name, however, becomes invalid when only one genus *(Plasmodium)* is employed, since *Laverania malariae* has priority of naming. Such a view, if accepted, makes *praecox*, *immaculatum* and *falciparum* as well as *malariae* applied to the quartan parasite all invalid.[9]

In regard, therefore, to the specific names of the parasites, the Sub-Committee, in view of the great difficulty in deciding upon the correct name by right, or of using such name if decided upon without now causing intolerable confusion, and for the sake of the practical advantages to be gained by uniformity, advises the adoption of the names as given in the decision (Opinion 104) of the International Commission on Zoological Nomenclature (1928).[10] Such procedure would in no sense be arbitrary, or

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[9] For a complete summary of the position, see Christophers and Sinton, *Brit. Med. Jour.*, December 3rd, 1938, page 1130 (with correction *ibid.*, January 21st, 1939, page 146). Also Sergent, Ed. and Et., Parrot and Catanei, *ibid.*, April 8th, 1939, page 747.

[10] The relevant parts of the Opinion are as follows :

" Opinion 104. — The following fifty-seven generic names with type species cited are hereby placed on the official list of generic names :

. . . . . .

" *Laverania* Grassi and Feletti, 1890a, 60, mt. malariae (homonym) so *falcipara* Welch, 1897, 36, 47, type host *Homo*. (For authors who consider the parasite of æstivo-autumnal malaria generically distinct from that of quartan malaria.) Not Laverania Labbé, 1899a, 82, type *ranarum*, type host *Rana esculenta*.

" *Plasmodium* Marchiafava and Celli, 1885d, 791, mt. tsd. *malariae* (as restricted to quartan fever), type host *Homo*.

. . . . . "

That *Laverania* is not employed as a genus (on zoological grounds) would not appear to alter at all the citation of the specific name of the malignant tertian parasite as *falciparum*, which, further, is the name at present most widely used. The only possible cause of uncertainty would appear to be whether the Opinion can be regarded as concerned only with the generic names, the correctness of the genotypes not being raised. The answer to such an objection would be to make application for a new decision of the Commission to fix names as at present in general use. The difficulty is not so much to determine the correct name by the laws of priority, which is a matter of careful research, as the impossibility of applying such when found without now causing confusion.

unscientific, since zoologists have agreed to abide by such decisions of the International Commission in cases of this kind.[10 a] This nomenclature is also that now in most common use.

Accepting the above considerations as to the use of generic and specific names and excluding all forms the status of which has not been fully established, the following are the zoological names of the known human malaria parasites :

Full zoological designation	Abbreviated form in text
Plasmodium malariae (Laveran), 1881	*P. malariae.*
,, *vivax* (Grassi and Feletti), 1890 . . .	*P. vivax.*
,, *falciparum* Welch, 1897	*P. falciparum.*
,, *ovale* Stephens, 1922	*P. ovale.*

P. tenue Stephens, 1914, is also accepted by many as very probably a distinct species. The name *tenue* may, however, be invalid as preoccupied by *P. tenuis*, a parasite of birds.[10 b]

Both the generic and specific names, in accordance with usual zoological procedure, should invariably be given in italics or, if the context demands, in some other distinctive type. The genus should be given with a capital letter, the species with a small initial letter, even though the name be one designating a person or place.[10 c] A specific name with a capital may, however, be used for a name designating a person or place in botanical nomenclature — *e.g.*, *Cinchona Ledgeriana.*

[10 a] See *Proc. of the Ninth Internat. Congr. of Zoology*, held at Monaco in 1913. For a useful reprinting of the rules, see Wenyon's Protozoology, Vol. 2, page 1336.

[10 b] See Wenyon, *Trop. Dis. Bull.*, 1916, 7, 25. *P. tenuis* was described by Laveran and Marullaz, *Bull. Soc. Path. Exot.*, 1914, 7, 20 (January) from a specimen of " rossignol du Japon " bought in Paris. The description of *P. tenue* by Stephens was published in *Ann. Trop. Med. and Parasit.*, 1914, 8, 119 (April). It is to be noted, however, that the terminations of the names *tenue* and *tenuis* do not actually come within the list of endings given in Article 35 of the Rules of Nomenclature as insufficiently distinguishing specific names.

[10 c] This is the usual, if not universal, procedure in zoology, but use of a capital for a specific name designating the name of a person does not seem actually to be forbidden in the rules (see Article 13 of Rules of Nomenclature).

The describer's name and year of description is also usually set out when giving the formal name of a species, to avoid all ambiguity due to possible synonymy. The describer's name should follow without comma after the specific name, and then, after a comma, the year of description. Brackets [11] around the describer's name are used only where zoological procedure requires this — *viz.*, to indicate that the species was originally described under another generic name. The brackets (parenthèses) are therefore correctly given in the case of *P. vivax* (Grassi and Feletti), since this species was originally described as *Haemamoeba vivax*, but not with *P. falciparum* Welch, originally named *Plasmodium falciparum*. The describer's name and year are not, however, usually repeated except where necessary to avoid ambiguity and the abbreviated name is all that is usually necessary when the full name has once been given in the context. The abbreviated name should not, however, be used in the title of a communication.

The use of *Pl.* for *Plasmodium* is unnecessary, as there is no zoological significance attached to the contracted form, which is merely a convenience. The only requirement is that there should be no ambiguity as to what genus is meant. *Pl.* might be used, therefore, if it was thought desirable to avoid confusion with another generic name beginning with P also dealt with in the communication, though such a consideration is not likely often to arise.

If the form is a named variety, the species name (without describer's name or date) is either followed directly (without comma) by the varietal name with its describer's name and date, or with the interpolation of sub-sp. or var. Varietal names (as distinct from specific names) are, however, little used at present in connection with the parasites and all that it is necessary to say in regard to customary procedure in dealing with varietal names will be found in the sub-section dealing with species and varieties of the vector anopheles (Section III, 2).

A shortened or colloquial name for the parasite may in many cases be useful for the sake of brevity, and it is also very necessary to have some suitable abbreviated or colloquial name to indicate infections caused by the different parasites. Names of this character,

[11] Actually " parentheses ". In typographical usage, [ ] are termed " square brackets " (formerly crotchets) and round brackets ( ) " parentheses ".

referring both to the parasite and its infection, as in common usage among English writers, are given below. In each case, the first name given is that most frequently employed.

Zoological name	Colloquial names
P. vivax	Benign tertian (B.T.).
	Simple tertian.
	Tertian.
P. malariae.	Quartan.
P. falciparum.	Malignant tertian (M.T.).
	Æstivo-autumnal.
	Subtertian.
	Malignant.
	Tropical.
	Pernicious.

The French names of this type are in general similar to the above (tierce bénigne, tierce maligne, etc.). For the malignant tertian parasite and its infections, *perniciosa* (Plehn, Ziemann, Maurer) and *tropica* (Koch) are names also often used by German writers.

Though benign tertian and malignant tertian are the names now used by most English writers and æstivo-autumnal by many American authors, such terms are not only inappropriate and archaic, but also very long and cumbrous. Simple tertian is objectionable for the same reason and also because the contraction (S.T.) would be liable to be confused with (S.T.) used as an abbreviated form of subtertian, a name still used by some. Ross, 1914, makes use of the names tertian, quartan and malignant for these parasites respectively, and some authors use tertian, quartan and tropical. Whilst such usage has the advantage of greater brevity, it is not free from objection. The names *perniciosa* and *tropica* imply zoological names for the parasite which do not exist as valid designations and are on this account undesirable. The name pernicious is apt to carry in English an erroneous implication and to be confused with pernicious manifestations of malaria.

In view of the above considerations and the fact that there are now other recognised forms of the parasite and infections than those mentioned, the Sub-Committee considers that it would be desirable, when an abbreviated name is used, to employ, in place

of such colloquial names as those given, the italicised specific name of the parasite referred to. Thus an infection by *P. vivax* would be a *vivax* infection, and one by *P. ovale* an *ovale* infection, or one respectively by the *vivax* or *ovale* parasite. The corresponding symbols *(e.g.*, (V) for *vivax*, (M) for *malariae*, etc.) might be a little difficult to use at first, but once they became familiar would be as convenient as the present (B.T.), (M.T.) and (Q) often employed.

Any such symbols, or abbreviated colloquial names, should invariably be explained on the first occasion of their use in any communication, as otherwise they may be meaningless or a source of error to a reader unfamiliar with the particular convention made use of.

Sub-section 3. — Terms relating to Forms of the Parasite seen in the Blood.

The forms of parasite seen in the blood include all known stages of the asexual cycle *(schizogony* [12]*)* and certain early stages of the sexual cycle *(sporogony)*, which is completed only in the mosquito.

In strict protozoological terminology, all multiplicative asexual forms are *schizonts*,[12] and such forms as are growing asexual parasites are *trophozoites*. In the case of the malaria parasites, however, where it is important to distinguish many of the stages in a detailed way, common usage has now given to these terms a somewhat specialised significance. Such usage is convenient in that it avoids the necessity for coining still other names, and the Sub-Committee suggests the following application of terms as reasonably devoid of objection.

Merozoite.

Young forms the immediate product of segmentation, as they appear still clustered around the residual pigment mass, or free, or attached to a red cell where they are as yet little changed and have not taken a definite ring form.

[12] Webster pronounces the root skĭzo-, but strangely enough prefers skīzont to skĭzont. Dorland (Medical Dictionary) gives skīzont, but skĭzogony. The adjectival form in most common colloquial use is schizogonous, but schizogonic is perhaps more correct.

Trophozoite.

Asexual forms in *(a)* ring stage, *(b)* later early amœboid or solid stages with chromatin mass as yet undivided in the process of schizogony.[13]

Schizont.

Large asexual forms in which the chromatin shows evidence of schizogonic division.[13] There may be such forms with two, four or more chromatin masses, but the merozoites have not yet been differentiated.

Mature schizont.

A fully developed schizont in the merozoite stage, commonly known as sporulation forms, segmentation forms, « rosettes » or « formes en rosaces ».

Since the process here concerned is not that of spore formation, the terms " sporulation forms " and " sporont " are objectionable. The term " segmenting bodies " formerly much used is also now less often employed, though the maturing of a generation and liberation of the merozoites is still commonly referred to as " segmentation " — *e.g.*, when indicating the relation of stage of the parasite to the fever attack. Probably the most suitable term in the last connection is " schizogony ".

Gametocytes.

Sexual forms in all stages as seen in the blood within the body. These are the male and female *gametocytes (microgametocyte* and *macrogametocyte* respectively). Further changes *(exflagellation* and *maturation* respectively) producing the *microgametes (flagella)* and *macrogametes* do not occur, so far as is known, except outside the body of the vertebrate host. The word gametocytes is therefore the correct name (not gametes) for such sexual forms in the body.

The gametocytes of *P. falciparum* from their characteristic appearance are commonly called *crescents.* It is not uncommon to find the term gametocytes *(e.g.*, in reference to the action of certain drugs) used as if the terms gametocytes and crescents were

[13] The presence in some young ring forms of two or more chromatin granules bears no relation to the schizogonic process. In practice, in a microscopical examination of malarial blood, the very young gametocytes which can hardly, if at all, be distinguished from true trophozoites are counted as trophozoites.

synonymous. Actually, where crescents are intended, it is better to use this term or *falciparum* gametocytes.

Trophozoites usually grow and mature so that the majority or large number are always at about the same stage of development. Such forms constitute *generations*, one or more of which in different stages of growth may be present in any given time in the host. Commonly, the stages of growth present represent generations maturing at approximately the same time on successive days. Such regular development of the generations with corresponding manifestations gives rise to the characteristic *periodicity* displayed by many plasmodial infections. The characteristic period which a given species of parasite takes for the completion of the growth of a generation — *i.e.*, from any given stage to the same stage in the succeeding generation — constitutes the duration of the schizogonic cycle or *schizogonic period* of the parasite.

Sub-section 4. — Terms relating to the Structure of the Parasite and the Changes produced in the Red Cell.

Terms relating to the morphology of the parasites are *cytoplasm*, *chromatin (granules* or *masses)* and *pigment (grains*, *granules* or *clumps)*. The nature of the " vacuole " seen in the ring forms is still uncertain *(i.e.*, whether nuclear or nutritive), so that provisionally the best term would appear to be *vacuole*. Malarial pigment is frequently referred to as *haemozoin*, the word melanin formerly used being incorrect, since the pigment is now known to be a form of, or related to, haematin.

Abnormal appearances brought about by the parasites in the host red cell are referred to collectively as *alterations* or *modifications* (of the red cell) or *cell changes*, and more specifically by names indicating the actual changes produced. Among such changes, the most important are *enlargement*, *decolorisation* and certain forms of *granulation* seen in the stained cell. Of such granulations the most important are the fine even granulations brought out by Leishman or Giemsa stain in *vivax*, *Schüffner's stippling* (Tüpfelung), and the coarse, more irregular markings seen in *falciparum* infected cells, *Stephens' and Christophers' spots* or *Maurer's spots* (Flecken). Stippling very similar to that in *vivax* is seen with *ovale*, and a form of stippling brought out only by special

staining in quartan is termed by James *Ziemann's stippling*. Maurer's spots are seen in a marked form in *tenue*.[14]

Among other changes described are the " brassy corpuscles " of early literature seen in fresh preparations with *falciparum*, darker staining of the cell in *malariae*, decrease in the size of the cell seen with the same parasite and an oval shape of the cell associated with a wavy edge at one or both ends *(crenulation* or *fimbriation)* in *ovale* infection.

Among hæmatological changes other than those connected with change in the red cell due to the presence of the parasite in this structure are a *reticulocyte increase* (seen in Leishman or Giemsa stained preparations as polychromasia, heterogeneity of the red cells, etc.), increase in the number of polymorphonuclear leucocytes, *leucocytosis*, increase in the relative proportion of large mononuclear cells, *mononuclear increase*, presence of large cells of endothelial type, *macrophages*, and of cells containing malarial pigment, *pigmented leucocytes*. A discussion of the nomenclature of the cells concerned is beyond the function of the Sub-Committee.

Sub-section 5. — Terms used in the Description of the Nature and Course of Infections.

Terms used to describe the nature and course of infection may relate to one or other of several different aspects of such infection, *viz.* : (1) the clinical *(e.g.*, malarial fever, malarial attack, malarial cachexia, latent malaria, etc.); (2) the parasitological or immunological *(e.g.*, malarial infection, primary attack, relapse, reinfection, superinfection, etc.); (3) that connected with treatment, prophylaxis or control.

Clinically, there is an *incubation period*, usually in *vivax* or *falciparum* infection, of about ten to fourteen days duration, followed by onset of the first attack due to such infection, *fresh infection*.[15]

[14] Gater has observed that " fleck " is a good English word and Maurer's flecking, or even *stippling* and *flecking* without the appended author's names might be a usage which would probably serve all that is required in practice to describe the respective conditions. It may be noted that, though it is usual to use the term Maurer's spots for the flecked type of granulation, both types of granulation were originally described by Schüffner and the second by Stephens and Christophers previous to Maurer.

[15] Primary attack of parasitological terminology. Initial fever (in part) of Korteweg.

Even in the absence of any further contraction of infection, this first attack is frequently followed at intervals by others due to the same original infection. Such subsequent attacks have been variously designated by different authors in relation to their time of occurrence as recrudescences, recurrences or relapses.[16] These terms are, however, so similar in meaning and have been employed with such different significance by different authorities that it requires a mental effort now to adhere to any agreed-upon convention in their usage and, in the Sub-Committee's opinion, all such attacks should be termed *relapses*. It is desirable, however, to reserve the name *long-term relapse* for the peculiar relapse occurring in *vivax* infection after a very long interval *(i.e.*, commonly about nine months subsequent to infection). Attacks not due to the original infection, but resulting from subsequent fresh infection are *reinfections*.[17] (See also parasitological and immunological application of terms.)

By attack in the above sense is meant a whole period of acute illness, which, however, frequently consists of a number of separate " attacks " in the sense of short manifestations of malaria (ague fits). It is very desirable that terminology should distinguish these two meanings of " attack " and, in default of a better term, it would seem advisable to use *paroxysm*[17a] where the second of these

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[16] James's classification of relapses in *vivax* infection, which has been very generally followed, as given by this author *(Trans. R. Soc. Trop. Med. and Hyg.*, 1931, 24, 501, is as follows :

Recrudescence. — A return of fever and parasites at any time within eight weeks after recovery from the primary attack.

Relapse. — A return of fever and parasites later than eight weeks, but earlier than twenty-four weeks after recovery from the primary attack.

Recurrence. — A return of fever and parasites later than twenty-four weeks after recovery from the primary attack. This means, as a rule, later than twenty-six weeks from date of primary infection.

It is desirable, however, to have a term signifying any return of " acute " infection after an interval from the primary attack greater than that due merely to periodicity and the term " relapse " seems best to serve this purpose.

[17] The French terms " rechute " and " récidive " correspond respectively to " relapse " and " reinfection " as distinguished above.

[17a] Corresponding to the French " accès ", whereas " atteinte " would be the equivalent French word for attack in the above sense.

meanings is intended. Thus an *attack* may consist of several or many paroxysms or of a single paroxysm only. But an attack may also consist of a period of irregular high temperature extending over a number of days where the paroxysms are confused or indistinguishable (as often in *falciparum* malaria or at the first onset of *vivax* infection [18]), or of such a period followed by a succession of paroxysms.

Where the paroxysms are distinct with an interval of normal temperature and follow regularly, the fever may be described as of *intermittent* type, and where the temperature remains high for some days without definite intermissions, it may be described as of *remittent* type. It is undesirable, however, to use the terms intermittent and remittent in such a way as might be taken to imply that these are distinct forms or types of malaria (as in the older literature).

The occurrence of a paroxysm normally depends upon the maturation (schizogony) of a generation of parasites. Thus the number of generations present at the time in the blood and the schizogonic period or cycle of the particular parasite give rise to clinical *periodicity*. Such periodicity may take the form of paroxysms on alternate days, *tertian periodicity*, or on every third day, *quartan periodicity*, or of paroxysms (due in man to interpolated generations) occurring daily, *quotidian periodicity*. Or paroxysms may occur on two successive days with one day interval *(double quartan periodicity)*.

Associated with infection may be effects due to localisation of the parasites (of *falciparum)* causing blocking of the capillaries of different organs, *pernicious manifestations*, the clinical characters of which are distinct from those of the normal malaria attack.[19] Such manifestations are variously named according to the organ affected or the symptomatology.

Following upon infection may be various *sequelæ*, such as anæmia, psychoses, etc. The term malaria cachexia has been much

[18] Primary attack (in part) of parasitological terminology; initial fever of Korteweg.

[19] The use of the word pernicious as applied to ordinary or severe malignant tertian infections or to the parasite causing these is undesirable, though *falciparum* is the only parasite in which pernicious manifestations are known.

used to indicate a condition where the patient exhibits intense anæmia and other consequences of prolonged infection. Much of the malaria cachexia of older clinical writings, especially in India, was, however, kala azar, and the term is less commonly used than formerly. Various minor manifestations in malarial subjects, assumed to be the result of latent infection, and especially when these exhibited periodicity or were modified by quinine administration, are sometimes described as latent or larval malaria. Latent is a term, however, best reserved for the parasitological conception of an infected person during a period of latency and its use in the clinical sense best avoided. The latter term is also now little used. Hæmoglobinuria occurring as a result of malarial infection, whether following quinine administration or not, constitutes the condition usually referred to in English writings as *blackwater fever* (hæmoglobinuric fever of authors).

Parasitologically, *infection* [20] is characterised by a period of invasion without clinical symptoms or without parasites being demonstrable in the blood (*incubation period*),[21] followed by more or less marked parasitic and clinical manifestations (*primary attack*).[22] This sequence is usually followed (except when parasites have been eradicated by a sterilising treatment) by a more or less prolonged period in which the evidences of infection depend upon the interacting processes of multiplication of the parasites and immunity reactions of the host (see infection immunity or pre-

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[20] " Infection " is used both in the sense of introduction of infective material — *i.e.*, inoculation (by natural or artificial means) — and as indicating the resulting condition of infection. The two usages are, however, only very occasionally likely to be confusing, Infection is used in connection with micro-organisms (bacteria or protozoa), infestation in connection with ecto- or endo-metazoan parasites.

[21] Incubation latency, procritical phase, or procritical latency of Sergent and Parrot, 1935 ; prepatent period of Hegner, 1926.

[22] *Crisis* of Sergent and Parrot, 1935. Crisis of Taliaferro *et al.* is used, however, in the usual clinical sense to indicate the sudden fall in numbers of parasites due to development of specific immunity in the host.

Korteweg uses the term *initial fever* for the irregular fever which may herald a fresh infection.

The term " primary attack " as used above appears to be the most generally suitable for the whole first attack of a fresh infection.

munition).[23] Infection may in this state at different times be unassociated with parasitological or clinical manifestations, *latent*,[23a] or displayed as attack conditions, *active*. Each such active period, other than the primary attack, constitutes a *relapse*. In this connection, a distinction may require to be made as between a relapse indicated by clinical symptoms (usually associated with parasites in the blood), *clinical relapse*, and one indicated only by the reappearance or increase in the number of parasites as shown by microscopic examination, *parasitic relapse*.

In certain cases, an immediate primary attack may not occur, or may have been so mild as to have escaped attention.[24] It frequently happens in such a case that the first attack occurs only after a long period (commonly about nine months in the case of *vivax* infection) — *i.e.*, it resembles a long-term relapse.

Certain terms are used in connection with the study of infection and immunity. Those forms of parasite which are capable of distinction on morphological characters constitute *species* or *varieties* (see remarks under Section III, 2). Parasites of the same species or variety may, however, show differences in immunological and other characters. Where parasites giving rise to infection have been derived from some single source and have been maintained without intermixture with parasites from other sources through a number of generations, they constitute *strains* (of a species). The word strain is also used in a slightly different sense as implying the occurrence of forms of the parasite which behave immunologically as though distinct from other forms of the same species — *i.e.*, the several species include an unknown number of strains which are immunologically distinct. When parasites of a given strain have established themselves in a host, the host is said to be *infected* with such strain. When such strain has been eliminated through natural process of recovery or through sterilising treatment and the host

[23] Hegner (1926) gives the name patent period to that in which parasites can be demonstrated, these undergoing a rise, peak and fall in numbers. Following this is the subpatent period when parasites cease to be demonstrable microscopically. There may be a second patent period (relapse).

[23a] The whole period following clinical recovery from the primary attack in bird malaria is sometimes termed the *chronic stage*. Also termed infection latency and metacritical latency.

[24] " Latence d'emblée " (Sergent); incubation latency continued into infection latency.

is later infected with the same or another strain, it is said to have been reinfected *(reinfection)*. A super-added infection brought about in the host whilst the original infection is still present constitutes a *superinfection.*

So long, however, as the original infection is present, inoculation of a host with the same strain usually leads only to a very slight and transient increase in the number of parasites or to no observable increase, the host being in a state of *infection immunity* or *premunition.*[25]

Any strain of parasite which fails to bring about a definite exacerbation of infection in a host in a state of infection immunity to a strain of parasite A, or which itself will bring about a state of infection immunity to A — *i.e.*, which behaves in a similar manner immunologically — is said to be an *homologous* strain (of a species) to A. When, however, a strain on inoculation brings about a definite superinfection [26] in an animal infected with the original strain, or fails to protect against such original strain — *i.e.*, which behaves in a dissimilar manner immunologically — it is said to be *heterologous* to the original strain. In the case of a superinfection, there may then be a primary attack due to such heterologous strain *(i.e.*, a new infection) in an already infected host.

Where microscopic examination fails to detect infection, the blood of an infected animal inoculated into another susceptible

[25] Concomitant immunity of Sinton ; also relative tolerance of Kelsch and Kiener, 1889, relative immunity of certain authors, immunity tolerance of Mesnil, immune infestation of Christophers, infection tolerance of Yorke, labile immunity of C. Schilling.

[26] Before the recognition of immunologically distinct strains of the same species, the term superinfection was used to indicate a super-added infection with another species or an infection resulting from reinoculation with the same species. The former case led to a *mixed infection of species* (recognisable often by microscopic examination), the latter to a *mixed infection of strains* (unrecognisable by microscopic examination).

A definite superinfection as defined in the text is obviously only possible in the case of a separate species or heterologous strain. A strain which on inoculation gave rise to a definite superinfection would by the definition be excluded from being considered an homologous strain.

It might be asked what a very small increase in the number of parasites following inoculation of a strain would be termed. Unless such an effect was sufficiently definite, the strain giving rise to it would be considered in practice as homologous and the operation in this case has been termed an *homologous superinfection* (Sinton and Mulligan).

animal may make manifest such infection, *isodiagnosis* (Sergent), or the refractoriness of the original host to superinfection may also indicate its state of infectedness. Latent infection may also be made manifest by removal of the spleen, *relapse following splenectomy*. Or latent infection not evident to microscopic examination may be demonstrated by utilising a vector in which development of the parasite is demonstrable, or through which subsequent infection of another host is brought about, *xenodiagnosis* (Brumpt).

Immunity in malaria which may persist for a varying period, even after parasites have disappeared *(i.e.*, which may be present when infection immunity in a strict sense has ended through disappearance of the parasite from the body), has been termed residual immunity (Sinton).[27]

So-called "hereditary", "inherited" or "congenital" immunity —*i.e.*, immunity from mother to child, better, *inherited* immunity— might conceivably be : (1) *transmitted innate immunity (i.e.*, merely hereditary insusceptibility) ; (2) passive immunity by transplacental passage of protective bodies produced by the mother, *transmitted passive immunity*[28] ; or (3) infection immunity (premunition) the result of infection transmitted through the placenta, *immunity through transplacental infection.*

A person infected with a species or strain of parasite may also be *infective*, but in nature only when gametocytes are present in

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[27] Immunity in a general sense involves a refractory state which prevents or hinders infection, reinfection or superinfection, or which reduces the number of parasites in, or their effect upon, the host. It may be *innate* (natural or species immunity, "résistance innée") where the host is simply not susceptible, or *acquired* as the result of infection, or *passive* due to protective substances produced by another host. In contradistinction to infection immunity is the so-called *specific* or *true immunity* of Sergent (residual immunity of Sinton) due to the formation of antibody substances and persisting, often for a long time, after disappearance of infection. It was formerly thought that immunity of the last-mentioned type did not occur in malaria, but recent work supports the view that this type of immunity may also be present (residual immunity of Sinton).

[28] Also there is suggested an active immunity through infection produced by the transplacental passage of the "toxins" of the maternal parasites (Sinton). Whilst the immunity of certain aboriginal races is clearly due to infection suffered in childhood, one or more of the above might modify the severity of infection in the early stages of such immunisation.

the blood capable of undergoing further development in the vector (see Section III, 6) and when the necessary circumstances are present leading to transmission. The blood of an infected person is, however, infective to a susceptible host by reason of the asexual stages of parenterally introduced parasites, *infective inoculation.*

Certain terms in connection with treatment and prophylaxis have special meanings or implications. *Treatment of the attack*, aims, clinically, at temporary or permanent cure of the clinical attack whether this be a primary attack or a relapse, and, parasitologically, at destruction of the asexual phases of the parasites to which the fever and other symptoms are due. *After-treatment* is *(a)* treatment to assure or hasten convalescence, *(b)* treatment specially directed to reduce liability to subsequent relapses, *treatment to prevent relapses* or *(c)* treatment aimed at destruction of the infective forms of the parasite, *gametocyte therapy*. *Clinical cure* indicates that immediate symptoms due to an attack have been relieved and the patient apparently recovered. *Radical cure* implies elimination of the parasite from the body due to natural recovery or to treatment.[29] *Spontaneous recovery* is clinical or radical cure brought about by nature.

By prophylaxis is commonly meant the use of drugs to prevent infection or minimise its effects. This term, however, has a more general meaning in that it includes such measures as the use of the mosquito net, personal precautions against infection, use of screened quarters, etc. It seems desirable, therefore, to employ *drug prophylaxis* when the first meaning is intended, reserving *prophylaxis* for its more extended meaning as covering any action of a precautionary character.[29 a] Such action by an individual constitutes *individual prophylaxis* and when applied to a community *collective prophylaxis.*

When the effect is merely to prevent occurrence of attacks during such time as the drug is being administered, it is *clinical*

[29] The French use of the term is somewhat different in that it implies only that recovery is complete in the clinical sense.

[29 a] The Greek meaning of the term is " a watching, guarding, after " and the general meaning " That defends from or tends to prevent disease ; also *transf.* preservative, precautionary " *(Oxford Dictionary)*. Dorland *(Amer. Illust. Med. Dict.)* gives " The prevention of disease ; preventive treatment ". It seems doubtful if it covers the whole idea of preventive medicine — *e.g.*, mosquito control measures — but it certainly seems to cover a personal precaution such as the use of a mosquito net.

(drug) prophylaxis. True causal prophylaxis (or, better, *true causative prophylaxis)* has been defined by James [29 b] as treatment which destroys the sporozoites (or any intermediate stage between sporozoites and trophozoites), thus actually preventing infection.

Measures directed to the prevention of malaria in communities are commonly spoken of as *control measures* [30] or *preventive measures.* Control measures carried out against the vector are *mosquito control measures* as dealt with in Section III. Control measures directed to protection of the community from the bites of anopheles independent of destruction or prevention of breeding of these insects — *e.g.*, by screening or other devices to this end — constitute *protection* or *protective control measures.* When directed to achieve this end by attracting anopheles to feed on cattle in place of man, such measures constitute *zooprophylaxis* (see Section III).

Measures directed to providing hospital and other facilities for cases of sickness independent of any further intention to control malaria constitute *treatment of the sick.* When treatment, however, is carried out with the intention of reducing infection in an area, it constitutes *preventive treatment* — *e.g.*, when patients, parasite carriers or cases known to have had malaria are treated so that they may not infect others. General drug administration to a community or population is *mass drug prophylaxis.* Where the aim is definitely destruction of gametocytes (usually by plasmoquin administration), it is *gametocidal prophylaxis.* Efforts to eliminate all infection in a community are described as *eradication measures.* When drugs are given by the mouth, the method is spoken of as *oral administration* and when by injection as *subcutaneous*, *intramuscular* or *intravenous* administration as the case may be, or as applying to all the latter-mentioned methods as *parenteral* administration.

[29 b] See Third and Fourth General Reports of the Malaria Commission, League of Nations, on Treatment and Prophylaxis of Malaria. The word " causative " is preferred by James (private communication) since it is less likely to be confused by the printer with " casual ".

[30] Such usage is to be distinguished from " control " used in the scientific sense as a check to deductions from experiments. In case of ambiguity, " control " groups or areas in the latter sense may be referred to as " comparison " groups or areas.

Section II

MALARIA IN THE HUMAN COMMUNITY

Sub-section 1. — Terms applied to Types of Prevalence and Intensity of Malaria in the Human Community.

Certain terms are used to describe various kinds of manifestations of malaria. Malaria is *autochtonous* when contracted locally, and *imported* when infection takes place outside the specified area. Autochtonous malaria natural to an area or country may be termed *indigenous*, and autochtonous malaria contracted from imported cases (as in post-war malaria in areas from which the disease had normally disappeared) as *introduced* malaria. Malaria resulting from infection artificially produced in malaria therapy is known as *induced* malaria. When malaria is the direct outcome of human operations, especially those giving rise to increase in breeding-places — *e.g.*, borrow-pits on railways, etc. — it is often referred to as *man-made malaria.*

Malaria may be described as *sporadic* when cases (infection) are too few and scattered to cause any appreciable effect on the community. It is described as *endemic* when constantly present in a degree to give some measurable amount of morbidity or splenomegaly. It is described as *hyperendemic* when few in the community escape infection, so that infection and immunity are in balance at a high level and the spleen rate is permanently over 50%. *Hypoendemic* is a term applied to those conditions where malaria is constantly present, but is scarcely measurable in the inter-epidemic period.

Epidemic malaria is a periodical or occasional sharp increase in the morbidity or mortality due to malaria. Epidemic conditions may be classed as *outbreaks* when occurring in small communities independent of conditions relative to the general area or community, as *seasonal epidemics* when due to the normal spring or autumn prevalence, as *regional epidemics* when large areas are affected with an unusually high degree of the normal seasonal epidemic.

Epidemics due to large-scale engineering or planting activities bringing about importation of non-immune labour into malarious conditions where mixture of susceptible and infected individuals and various other factors increase the malarial incidence constitute the *malaria of (tropical) aggregation of labour* (Christophers and Bentley). A condition of high endemicity following upon a regional epidemic is known as *post-epidemic hyperendemicity.*

Infection in a minority community dependent on the endemic malaria in a surrounding indigenous community has been termed *incidental* malaria (« paludisme accidentel »)[31]. The indigenous community in such circumstances forms the *reservoir of infection.*

Where conditions are such that large numbers of anopheles are present without malaria in man, there is said to be a state of *anophelism without malaria. Recession* is a term that has been applied to disappearance of malaria due to natural causes in temperate countries where it can be regarded as indicating a retreat from the normal limits of the disease.

Terms commonly used to designate the amount or degree of malaria are incidence, prevalence and intensity. Incidence is commonly used in statistical, epidemiological or medical usage as referring to the number of cases, or to the percentage of infected persons, in an area. Prevalence is a loosely applied term indicating the frequency of, or liability to, infection from malaria in an area, or the degree of splenomegaly, or the percentage of persons with parasites in the blood, etc. Intensity may be used in the sense of severity of infections — *i.e.*, parasite density — or of likelihood of an individual in the area to contract the disease. All these terms are preferably replaced by more precise conceptions indicating the particular manifestation of infection which is referred to. Thus —

[31] The word " fortuitous " has been suggested, but is in English perhaps less appropriate, as the meaning intended is not so much mere chance as unimportant in relation to the great body of indigenous infection.

Prevalence = amount of	Frequency	(General, clinical or statistical)	Number of cases — *e.g.*, morbidity = incidence (in usual medical sense).
		(Parasitological or epidemiological)	*(a)* Proportion infected — *e.g.* parasite rate, true infection rate.
			(b) Proportion with splenomegaly = spleen rate.
	Intensity	(General, clinical or statistical)	Severity of cases — *e.g.*, case mortality, incidence of blackwater fever.
		(Parasitological or epidemiological)	*(a)* Mean numerical value of infections = parasite density.
			(b) Mean size of enlarged spleens = average enlarged spleen.
	Likelihood	(General, clinical or statistical)	Reputation of unhealthiness.
		(Parasitological or epidemiological)	Number of infections inoculated = transmission rate, or infective density.

Even so, such different measures of malaria are not only unlike in kind and therefore often with difficulty comparable, but their results may be opposed. Thus, in the adult community in a hyperendemic area, the infection rate may be 100%, but the morbidity and even the parasite density may be very low. Under these conditions, some measure of malaria which will indicate what appears to be the intensity of the disease in some general fashion has a useful rôle, even though it may be difficult to assign a precise significance to it. Such a measure is given by the spleen rate as the measure of *endemicity*.[32]

The word endemicity is useful as giving a general measure of the endemic prevalence of malaria. It was in early researches commonly measured by the parasite rate (endemic index of Stephens

[32] In this connection, a useful analogy is found in an attempt to measure "fineness" of the weather. One may use rainfall, hours of sunshine, light intensity or other measures, but for practical purposes "hours of sunshine" gives a precisely determinable measure which coincides for the most part with what is meant as fineness of the day.

and Christophers). However, for various reasons, the degree of splenomegaly has more and more come to be used as the standard by which malaria in an indigenous community is measured. Hence the great importance of the spleen indices, such as the spleen rate, average enlarged spleen, splenometric index, etc.

Methods which aim at recording and measuring endemicity and the prevalence of malaria in communities in a numerical fashion form the subject of *malariometry*. From the point of view of malariometry, malaria is regarded as *static* when the conditions are such as to produce an equilibrium state in regard to malaria, and as *non-static* when they are such that malaria prevalence is unstable. The terms are in large degree the malariometric equivalents of endemic and epidemic malaria (or more correctly of hyperendemic and epidemic malaria, since communities in a state of moderate endemicity are most usually liable to seasonal fluctuations in the disease — *i.e.*, mildly epidemic rather than static in character). In non-tropical endemic areas, " stable " malaria is usually considered to include normal seasonal variations as part of the picture.

Sub-section 2. — Rate, Index and Ratio.

A good deal of uncertainty exists as to the use of *rate* and *index* in certain terms connected with the measurement of malaria. Thus some writers have replaced the old-established *spleen rate* by *spleen index* on the assumption that this usage is more correct. Others have either followed such a change in the belief that it was desirable as being more correct or have been left with the feeling of uncertainty as to what the correct usage should be. There is even the doubt whether both rate and index should not be used under circumstances demanding one or the other. It appears to the Sub-Committee that such feeling of uncertainty is sufficiently general to make a review of the situation desirable.

In the first place, it is desirable to make it clear that the use of *index* in French is not necessarily based on any decision as to whether on technological grounds the English usage should be rate or index, but to the fact that in the French language there is no term other than index which can be suitably used.[33]

[33] The word *taux* has not an identical significance with rate and is not suitable in the present connection.

In English there are two words, rate and index. The former, which usually carries the idea of some simple form of proportion, is that mostly used in medical statistics — *e.g.*, death rate, birth rate, still-birth rate, etc. The latter is mostly used where the proportion in question is not the direct measurement required but only indicates this — *e.g.*, the refractive index is the size of an angle, but it indicates measurement of a property, *viz.*, refractivity.

Ross, however, in his " Prevention of Malaria ", when seeking for terms to indicate new ideas in the mensuration of malaria, laid considerable emphasis on the use of the word rate only when some specified ratio *(a)* had been actually ascertained for the whole community *(i.e.*, was not merely derived from a sample), and *(b)* was the absolute value in nature *(i.e.*, was not merely an observed value subject to error). In the absence of such completeness or accuracy, the term, it was maintained, should be index. In practice, however, if both terms be used in the above senses, there would be a complete reduplication, depending on accuracy or completeness of the observations of every stated proportion. The death rate, if subject to inaccuracy, would become an index, and the still-birth rate would not be a rate, because it requires an arbitrary decision as to the time in pregnancy when the fœtus is considered as viable.

The Sub-Committee believes, therefore, that, whilst the distinction drawn by Ross is important, there is a more important distinction better worth preserving in the use of the terms rate and index — *viz.*, that the former implies a simple and direct observed proportion, the latter a measurement of one type of value used to indicate measurement of another kind of value. Thus the percentage of palpably enlarged spleens in a group would be the *spleen rate* of the group, but is an *index* of the endemicity of malaria in the population. The amount of quinine sold in a village might similarly be an index of the malaria morbidity.

There is, however, a further complication in that to biometricians (as kindly noted by Dr. Muench and Dr. Putman, of the Rockefeller Foundation, through Dr. Hackett) " rate " is a simple proportion " usually applied to events occurring within a given period of time ". Thus the marriage rate is the proportion married within a given time, not the proportion of married persons in the community. To express a continuing characteristic of a population

(such as splenomegaly) which is not an event, the term " frequency " is preferred. As, however, " frequency " has been widely used in a different connotation in malaria, it would be difficult now to apply it in the present case.

The choice, taking into account general usage and practical suitability, therefore appears to lie between spleen rate and spleen index. The Sub-Committee believes that, on the whole, " rate " is here most suitable, especially as it has the claim of long usage and custom. Where necessary, *index* has been used — *e.g.*, splenometric index or Macdonald's index, which are not rates. *Ratio* has been used where the proportion of one measured specified value to another is expressed as a figure, relating to unity, and not as a percentage. Whilst such definitions are naturally not always capable of precise application, they appear to constitute in general a useful and reasonable guide to procedure in malariometric terminology.

Sub-section 3. — Terms used in the Statistical Measurement of Malaria.

The *morbidity rate* (or *morbidity*) from malaria would be (theoretically) the number of persons sick from malaria in a given unit of time. Such a rate is rarely possible of measurement, and morbidity rates as actually employed are usually based on admissions to, or attendances at, hospitals or dispensaries. These are very dependent on the circumstances controlling admission or attendance and frequently, also, the actual population to which such figures relate is unknown.

The true *mortality rate* (or *death rate*) from malaria is in practice as unascertainable as the true morbidity rate, but for different reasons. In this case, the recording agency may be effective as regards the record of deaths, but may often give little information of real value because so many of the deaths recorded as due to malaria are in reality due to other conditions and, on the contrary, deaths directly or indirectly due to malaria are often not recorded as such. This is especially likely to happen since mortality from malaria in a general community is preponderantly infantile, and even with medical supervision diagnosis in such cases is difficult. Data of this type may, however, be of use in indicating seasonal and other variations in malaria even though, as absolute values, they cannot be relied on.

For the above reasons, the *total deaths* (*i.e.*, deaths from all causes) in an area may, in some circumstances, be even more valuable as material for statistical study than the returns of malarial mortality. This applies particularly to conditions where malaria occurs in epidemic form in native populations and there is good general registration of deaths, independently of any question of correct diagnosis of the cause of death. This is because the temporary effects of the disease here cause considerable or even enormous variation in the total deaths, such effects being evidenced by characteristic peaks in the graph of total (weekly) deaths, *epidemic rises* of Indian observers. The measure of an epidemic rise is the *epidemic figure*, or number of deaths in a selected epidemic month divided by the normal monthly deaths for the area. By plotting such epidemic figures for numerous small registration circles, the intensity and distribution of regional epidemics can be mapped.[34]

It is often required to use data, not for the whole community, but for a particular age group or groups. In specifying such age groups there is some difficulty in avoiding ambiguity as to the exact age group referred to, especially in the endeavour to do so briefly as in compiling tables.[35] Age is most commonly taken as that on the last birthday (method A). In this method of stating age, a child of 2 may be any age from 2 to just short of 3. In malaria field work, especially in the tropics, where the age of children has to be guessed or given approximately, the age figure is more nearly that of the nearest birthday — *i.e.*, a child of 2 may be any age from 1½ to 2½ (method B).[36] To describe a child as 2 and over but under 3 (as in method A) and, especially when defining age groups to avoid ambiguity in such fashion, is both troublesome and clumsy, and the best way to avoid such redundant description and to retain accuracy of expression would appear to be to adopt a convention by which it is understood that any given age figure includes children

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[34] For description of the method, see "How to do a Malaria Survey", Govt. of India, *Health Bulletin*, 1936, No. 14, 3rd Ed.

[35] Thus the age group 0-2 of some authors is identical with age group 0-1 of others employing a different method of statement.

[36] The accurate method would be to record ages in years with the necessary fractions (method C), but this in practice, unless very small groups are used, would come down to one of the above methods.

of that age to just under the next year of age.[37] If children in the first year of life are given as 0, then quinquennial age groups would work out as below:

0-1	Children under 2	Children in the first five years of life.
2-4	Children in third, fourth and fifth year of life	
5-9	Children in the second five years of life.	
10-14	Third quinquennium.	
15-19	Fourth quinquennium.	

Where ages are recorded by method B, these groups would not be strictly accurate, but, as method B is admittedly used in circumstances where the age given is approximate, such inaccuracy as is involved would usually not be important and the age groups given would indicate sufficiently well what is intended. It will be evident that in all cases it is desirable that a clear statement should be given as to any convention used and the method of determining age stated.

Terms used to describe age groups are liable to differ in different countries, but in tropical countries for the most part the periods may be distinguished as: *infants* (0-1), *children* (2-9), *adolescents* (10-14), *young adults* (15-19) and *adults* (20-). Strictly, in statistical work, " infant " applies only to age group 0 — *e.g.*, infantile mortality. There are some advantages in so restricting the term, since this group is especially important in relation to the frequency of infection and season of transmission. It has been suggested that

[37] According to Dr. H. Muench, Biometrician to the Rockefeller Foundation (private communication to Dr. Hackett), the above-noted convention has been very generally adopted by biometricians in the United States. Some other conventions are also in use to facilitate designation of age groups in adults, but, as detail in this respect is rarely required in malaria work, it is unnecessary to refer to them here. The above convention is that adopted in the present report, so that children in the first two years of life are given always as age group 0-1 and those in the third to the tenth years of life as age group 2-9. The importance of group 2-9 is that, as more fully explained in Section II, 4 and 5, both the parasite rate (though not the parasite density) and the spleen rate are very constant throughout this period, but may alter considerably in the next quinquennial group. Since, however, in malaria work, ages have often to be given very approximately, such groups as a rule in practice are to be regarded more as a general guide to procedure than as a matter of strict adherence.

age group 1 might if necessary be designated " babies " and that age group 2-9, on which most of the determinations in connection with malaria are made, might suitably be referred to as the " index group ". Group 10-14, whilst often showing well-developed individuals, may also contain many who might be described as children. In French usage (*e.g.*, Algeria) are *nourrissons* (0-1) and *enfants* (2-14, with sub-groups 2 to 5, 5 to 10 and 10 to 15), then, above 15, *adolescents* and *adultes*.

Sub-section 4. — Terms used in the Measurement of Malaria by Examination of the Blood.

(a) *General considerations on parasite rates and indices.*

A measure of the incidence or frequency of malaria — *i.e.*, the infection rate[38] — would be the percentage of persons in a given community who have the parasites of malaria in their blood. The difficulty in regard to such a value is due to the fact that not all the infected persons in the community are detected by a blood examination. Hence the number found positive — *i.e.*, the parasite rate — is not exactly equal to, and may be widely different from, the true infection rate. Before discussing the terminology and the definition of such terms as parasite rate, parasite density, infection rate, etc., it is therefore desirable to refer briefly to the question of the relation between results obtained by examination of the blood and the actual blood condition present. This will be most readily done by considering the results obtained from a more and more prolonged examination given to a series of blood films from an infected community, preferably a child community under more or less static malaria conditions.

In any such community, many of the infections are likely to be of a relatively high order, say 1,000 parasites per c.mm. or over.

[38] Infection rate has been used not only as the percentage of actually infected persons, but has also been applied (Swellengrebel, 1920, see under *species infection rate*) to indicate the number of " infections " —*i.e.*, counting infections by different species of parasites per 100 persons. Like " endemic index ", the term " infection rate " has now been used in so many senses that it has ceased to serve a useful function. It is better, therefore, to use parasite rate to indicate the number of infected persons found by examination, and *true infection rate* if the hypothetical number of actually infected persons is to be indicated (see also parasite asymptote and Macdonald's index).

All such infections will be detected by a moderate degree of examination, say one or two fields of a thick film. There would remain, however, undetected a number of infections of a smaller order, say 100 parasites per c.mm. or less. Further, more and more of such infections would be brought to light as more and more time is given to the examination — *i.e.*, as more and more blood is examined. On the average, it is clear that to find one parasite when these number 1,000 per c.mm. one-thousandth of a c.mm. of blood must be examined — *i.e.*, approximately 5,000 r.b.c. To detect an infection of 100 parasites per c.mm. on the average, it would, however, be necessary to examine 50,000 r.b.c., or about 0.01 c.mm. blood — *i.e.*, about 100 microscopic fields each containing 500 r.b.c. in a thin film, or an equivalent number of fields in a thick film.[39]

Two important points here arise: *viz.*, (1) that without some indication of the infection intensity *(i.e.*, the value of the infections which different positive results represent), a very imperfect idea of the real degree of parasitism is given by the parasite rate *(e.g.*, this may be made up entirely or mainly of very small infections, or of quite large infections, obviously indicating a different state of affairs in the two cases), and, (2) that the rate itself is actually largely dependent on the number of light or heavy infections present, since the larger the infection the greater the likelihood that this will be detected in any given degree of examination.

It will be clear, therefore, that it is desirable when specifying infection in a community to give, not only the *parasite rate*, but also an indication of the *parasite density*. These two values are required to cover the two separate conceptions of the frequency or incidence of infections and of the intensity or number of parasites which these infections represent. An even more complete specification of the characters of infection in a community is to be obtained from a study of the frequency distribution of the values of infections. The fact that infections may be those with different species of parasite has also to be considered (see *(e)* of this sub-section, species prevalence).

(b) *Parasite rate.*

The *parasite rate* [40] may be defined as the percentage of children (age group 2-9) showing parasites in a short examination at some

[39] See remarks on thick films in Section II, 4, *c)*.

[40] Blood index of some American authors.

specified time.[41] The percentage of adults similarly showing parasites is the *adult parasite rate*, and that of children 0-1 the *infantile parasite rate*. Similarly, the parasite rate at any age group may be specified as the parasite rate of that age group — *e.g.*, *parasite rate* 2-4.[42]

The expression " in a short examination " is intended to indicate that the value ascertained is to some extent dependent on the time devoted to the examination of each slide. With very little increase of labour, however, both the parasite rate and the approximative parasite density can be simultaneously obtained by using one of the simple routine counting methods of examining slides described in the section on parasite density. The parasite rate can then be given as relating to a definite minimum infection limit as there explained.[43]

The age group 2-9 is that selected to cover the period of maximum infection and reaction, and thus most suitably to indicate the degree of premunition in the community. It also has the advantage that, though the numerical value of the infections may show a reduction with increasing age in the group, the actual percentage of children infected remains usually very constant. The same age group is also that used for the spleen rate, which also remains very constant within these age limits. Babies (age group 0-1) are sometimes included for convenience, but children under 2, as in the definition, are best treated as a separate group, since they are liable to show different conditions in respect to infection depending upon whether

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[41] Schüffner prefers to apply the term parasite rate without reference to any age group. The parasite rate in children would then be *parasite rate 2-9*. Since, however, it is the rate in children which is almost entirely used to compare malaria in different communities and since it is this which is usually meant when the term parasite rate is used, it is perhaps simplest to adopt the convention as given above. The important thing to realise is that for comparison the child rate is in general use. If all in the community be included, the results would often depend very largely on the proportion of adults.

[42] See method of specifying age groups (Section II, 3).

[43] Thus, for example, if 100 fields of a thin film be always examined before returning a slide as negative, approximately 50,000 r.b.c., or about 0.01 c.mm. of blood, will have been passed in review. Hence, on the average, infections of 100 parasites per c.mm. and over will be detected (see Section II, 4 (*b*)). The parasite rate as determined will then be on this basis, *viz.*, percentage showing infections of 100 parasites per c.mm. or over.

they have passed through a fever season or have not been born sufficiently long to have acquired infection. As a group, however, these have an importance of their own (see average new-born infection period and infantile parasite rate). The adult parasite rate is also very important, especially in relation to the parasite rate in children.

The same considerations hold good for the parasite rate as are given under the spleen rate in regard to the source of the children examined and, if school-children are utilised, this should always be stated.[44] Any possibility that the children have been subject to quinine medication has especially to be carefully excluded or taken note of.

(c) *Parasite density.*

The parasite rate takes no cognisance of the fact that the infections encountered may vary very greatly in intensity — *i.e.*, in the number of parasites characterising them. The number of parasites per c.mm. in any given individual is the *parasite count.* The mean or average parasite count for a community or age group constitutes the *parasite density*, which thus is a measure of intensity of infection in a community as judged by the numerical value of infections. The parasite density can be expressed either as the *mean parasite count* (mean number of parasites per c.mm. taking negative observations into count in arriving at the mean), or as the *mean positive parasite count*[45] (where negative observations are excluded and only the value of actual infections is taken).

Unfortunately, the enormous variation in the numerical value of the infections usually encountered makes these means less satisfactory data than they might otherwise have been. Thus, if one infection is of very great magnitude, it may grossly swamp the effect of a majority of much smaller counts. Nevertheless, when infections are reasonably graded and where a sufficient number of children are utilised, the mean positive parasite count may be

[44] See remarks on the use of school-children given in Section II, 5 (*a*).

[45] The mean positive parasite count expressed as the mean of the number of parasites found in 200 fields of a thin film multiplied by 10 has been termed by Gill, 1928, the infestation index and used by this author to indicate intensity as contrasted with frequency of malarial infections in a community. The word infestation, though it expresses well the idea of intensity, has been generally applied to ecto- or endo-metazoan parasites.

a very valuable figure. In practice, it may be desirable to omit from the mean any grossly outlying values, or better, some other measure than the mean may be used — *e.g.* the mode or the quartiles obtained from an array (see parasite frequency distribution).[46] The geometric mean — *i.e.*, the square root of the product of the values — is another method of minimising extreme deviations in the upper range.

For accurate enumeration of parasites, a suitable technique must be used — *e.g.*, small measured quantities of blood spread over a known area, or Sinton's fowl-corpuscle method. The following, however, are methods in use to obtain approximate results:

(1) Counting the parasites seen in a thick or thin film respectively in a given time of examination.

(2) Counting the parasites in a given number of microscopic fields in a thin film or an equivalent number in a thick film[47],

[46] Enumerative studies, whilst almost essential to advance in the investigation of malaria, are beset with difficulties and much remains to be done before entirely satisfactory methods of dealing with the degree of parasitism can be put forward. Meantime it seems desirable to set out as far as possible the general principles which up to the present have guided enumerative work.

[47] In the case of thick films, the spread-out drop is not fixed but covered in the unfixed condition with diluted Giemsa stain (one drop of stain to a cc. of water or even more dilute). The drop should be spread so that the film is of such a thickness only that it does not flake off in parts when wetted and preferably so that it dries homogeneously — *i.e.*, before «rouleau» formation has had time to form and give it a markedly granular appearance. For enumerative work, a standard thickness should be aimed at. This is facilitated by taking drops as nearly as possible of a size and spreading to the same extent.

In a method recently described by Korteweg, a drop of blood placed on the slide towards one end is allowed to run along by sloping the slide. When the other end is reached, the slide is tilted in the opposite direction and the drop allowed to continue its flow as far as it will go. This gives an optimum thickness for staining and examination and one relatively constant for rough enumerative work.

Since identification may sometimes require a thin film, especially when parasites are very scanty, it is usual to make at the same time a thick and a thin film of each individual, which may conveniently be made on the same slide. In this case the thin film, but not the thick, is fixed.

Thin films should be of such a thickness that, in the part counted, cells should neither overlap nor be sparsely scattered.

using a square ocular aperture of known area relationship to the whole field when necessary.

(3) Counting the parasites in relation to the number of red cells or to leucocytes simultaneously encountered, using a square ocular aperture of suitable area. The first method is applicable only to thin films where alone the red cells are left intact and the second to thick films where alone the leucocytes are sufficiently uniformly distributed for such a method to be used.

Use of a direct time limit is generally agreed to be unsatisfactory.[48] Perhaps the method of counting the parasites in 100 fields of a thin film is the simplest routine method, the same objective and eyepiece being employed or any necessary adjustment in this respect made. As the rough average number of red cells in the field for a given objective and eyepiece can be ascertained, results can, if desired, be expressed as parasites per c.mm.[49] If thick films be made of a roughly standardised thickness, counts obtained by examining so many fields can also be roughly expressed in parasites per c.mm. Counting the leucocytes similarly enables a rough quantitative result to be given.

The question arises as to the value of such very approximate determinations. The natural reaction is to dismiss such methods as too unreliable to serve any useful purpose. Further consideration will show, however, that the enormous range in the value of the infections (lying between 100 or less and 100,000 or more parasites per c.mm.) is an asset in one's favour, since the order of infections becomes of greater significance than the exact accuracy of the results. The value of such rough methods is also evidenced by their obvious utility in practice. Thus a record of + or — gives no indication whatever of the numerical value of a recorded result,

[48] To avoid the monotony of counting fields as the examination goes on, some observers ascertain in the first place by trial the time they take to examine 100 fields and use this as the limit where a negative result is to be returned. In this case the fields should not be streamed past the eye, but critically examined for a brief period before they are passed on for the next. It is not necessary that the fields should be contiguous.

[49] Thus, if the microscopic field in use contains about 500 r.b.c., the number of parasites per 100 fields multiplied by 100 gives approximately the number of parasites per c.mm.

nor is it found by experience that such entries as scanty, numerous, etc., give much more. On the other hand, entries such as 100/3, 25/10, 12 per field, etc., roughly indicating respectively 3, 40 and 1,200 parasites per 100 fields, or 300, 4,000 and 120,000 parasites per c.mm. are at least a useful indication sufficient for many purposes.[50]

Of age groups in communities subject to static malaria, the parasite density may be considerably higher in age group 2-4 than in that of 5-9, and this may make separation of these two groups important.[51]

(d) *Parasite frequency distribution.*

From the data giving the numerical value of infections in a community it may be useful to study the *parasite frequency distribution.* The usual method of setting out the frequency distribution *(i.e.*, numbers in any given class as ordinates and the measurement specifying the classes as abscissæ) is often less useful in this case than an array (infections arranged in order of magnitude spaced at regular intervals along the base line). From an array can be ascertained at once by inspection the *median (i.e.*, the middle infection by count along the base line). Or it may be advantageous to utilise for certain purposes the upper, or upper and lower, quartile (the infection occurring halfway along the base line between the median and the upper and lower limits of the array respectively). Or a frequency distribution with density scale in logarithms may be the best form.

[50] It is perhaps desirable to note that the determination of density as the average value of the parasite count is itself only a means of comparing what is by its very nature a complex and mixed phenomenon. For example, a child may show a small infection, *(a)* because it is really suffering from a small infection, or *(b)* because at the height of a heavy infection parasites at the time may not be numerous in the peripheral blood. Nevertheless, such a figure may indicate average differences at different age periods or enable one community to be compared with another. The actual interpretation of the data elicited must remain with the research worker. See also remarks in the Introduction as to principles underlying use of index values.

[51] The rapidity with which the parasite density falls constitutes, according to Bagster Wilson, the best index we have of malaria intensity in the community.

An interesting and informative study of infection values can also be made by plotting the infections detected at successive numbers of fields examined. This can be done by noting in the case of each slide the number of fields before a parasite is first encountered and plotting a curve on the basis of the results obtained. Such a curve at first rises very rapidly, but after, say, 500 fields, much more slowly and is eventually seen to be approaching an asymptotic value which can be roughly gauged by inspection. Such a value is a form of expression of the true infection rate (absolute parasite rate) and may be termed the *parasite asymptote.* Its determination has the property of bringing more and more intensive examination to bear on the negative films and thus exploring the frequency of the lower-grade infections.

(e) *Species prevalence.*

So far, infections have been dealt with without reference to the fact that these are sometimes infections by one species of parasite and sometimes by another, or, as often happens, two or more species may be present in the same individual, so that the number of infected persons is not the same as the number of infections considering each species separately. Commonly, infections of one species are predominant in certain areas *(e.g., falciparum* in the tropics, *vivax* in Europe), so that in general the parasite rate and parasite density refer in practice more or less satisfactorily to such species. The question of a plurality of infections has, however, to be given consideration in relation to terms employed. Such terms are rather apt to be ambiguous and the following is perhaps the usage to be recommended where it is necessary to employ them.

The percentage of individuals found infected with any given species is the *species infection rate* for that species — *e.g.*, the *vivax*, *malariae* or *falciparum infection rate.* The percentage which infections with any given species forms of the total infections found is the *relative prevalence* of that species. A statement giving in some recognised order the relative prevalences of the species found is the *parasite formula* (the figures given totalling 100). The percentage of infections, counting each species separately, in a

community is the *all-species infection* rate,[52] which may be greater than the parasite rate and may exceed 100.

Sub-section 5. — Terms applied to the Measurement of Malaria by Enlargement of the Spleen.

(a) *General considerations relating to measurement of splenomegaly.*

Just as in the measurement of infection there are two distinct phenomena being measured — *viz.*, (1) the proportion of a community infected and (2) the number of parasites represented by such infections — so in the measurement of splenomegaly there is the proportion of a community showing splenomegaly and the degree of splenomegaly shown.

The first to use, as a measure of malaria, the proportion of persons in a community showing splenomegaly — *i.e.*, the spleen rate — appears to have been Dempster, 1846. Ross (in his report on Mauritius, 1908) was the first to make systematic use of the size of the enlarged spleen in a malarious community to measure malaria.[53] Very many observers have subsequently utilised these methods in studying malaria prevalence and mapping malaria, and the subject has become somewhat complex, largely due to the different ways of obtaining and studying the data. It is desirable as far as possible to bring these varying methods and usages under a common terminology and to simplify the issues by a clear statement of underlying principles.

Whilst the spleen rate would most simply be regarded as the percentage of persons in any community showing splenomegaly, it has been found that the rate is often very different in the children from what it is in the adults of a community and that the rate in children for various reasons is the more representative as a measure

[52] The term " infection rate " used by Swellengrebel, 1920, to indicate the percentage of infections as distinct from the percentage of infected individuals, whilst very suitable in one sense, is likely to be ambiguous from the number of ways in which this term has been used, or might be used as seen above. See also footnote [39].

[53] The measurement of the organ in a clinical case by fingerbreadths probably dates a good deal further back — *e.g.*, Twining, 1832, in some of his cases, records that the spleen could be felt two fingerbreadths below the cartilages of the left false ribs (Twining · " Clinical Illustrations of the More Important Diseases of Bengal ", Baptist Mission Press, Calcutta, 1832) (private communication from Colonel Sinton).

of malaria. The " spleen rate " as ordinarily understood and used by malariologists has therefore come to be the spleen rate in the children of a community, the spleen rate in adults being distinguished as the adult spleen rate (see definition of terms in Section II, 5 (*b*)).[54]

The age group usually selected is that which includes children in their third to tenth year of life — *i.e.*, age group 2-9, or the same group as was recommended for determination of the parasite rate. Roughly, this includes children from those who have begun to run about (" toddlers ") to any who are still obviously children and not adolescents.[55] Young babies (age group 0-1) are commonly carried in their mothers' arms and cannot be examined under the same conditions as children who have begun to run about. Also many babies, especially those who have not passed through a fever season, may not yet have had time to develop splenomegaly. In the later age group (10-14), the spleen rate has often become considerably reduced and, without knowledge on this point, cannot be assumed to be the same as in age group 2-9, in which period it is generally very constant.[56] In adults, the spleen rate may even

[54] As with the parasite rate, Schüffner prefers to use " spleen rate " as applicable to whatever age group has been examined. The same considerations apply here as have been noted under parasite rate, and it seems perhaps desirable that by spleen rate should be meant the child spleen rate, the spleen rate in any other group being referred to as the spleen rate for that group. Otherwise, when referring to the spleen rate as ordinarily understood, it would be necessary to term it " spleen rate 2-9 " or " child spleen rate ". This is not the same thing as recommending that the spleen rate be taken by including both children and adults in the result. If this be done, a much less useful value is obtained and it is not the " spleen rate " as understood by most malariologists.

[55] In some countries, a somewhat different grouping may be selected in accordance with conditions prevailing. In Algeria, for example, the age group 0-15 (enfants) is in common use. Actually in practice, the age group limits given are to be taken more as a general guide than as a matter of strict observance. Importance is chiefly to be given to excluding obvious disturbing causes, such as would result if babies or adolescents were included in the children group.

[56] Within the age group 2-9, the spleen rate usually remains very constant. There is not therefore the same desirability to split up this group into ages 2-4 and 5-9 as there is when dealing with parasite density. The indices relating to spleen size are also little liable to change during the age period in question.

be in reverse relation to that in children — *i.e.*, a very intense malaria with a high spleen rate in children is not infrequently associated with a greatly lowered adult spleen rate. In other circumstances, both the child spleen rate and the adult spleen rate may be high.

Splenomegaly may be regarded as giving an indication of the amount of infection immunity or premunition in a community. The value of the spleen rate as taken in children lies in the fact that, in this case, the incidence and degree of splenomegaly are always those in a community not previously affected by malaria, and so these conditions are more comparable from one community to another than would be the case in later age groups, where the incidence and degree of splenomegaly are largely influenced by previous infection in childhood. There are also other advantages in using the child spleen rate. Thus, in adults, development of the abdominal muscles, the likelihood of there being old standing adhesions and fibroid changes in the organ, as well as greater variation in the shape and position of the spleen, are all unfavourable to accurate observation. In children, on the other hand, the thin abdominal wall, the usually more recently turgid condition of the enlarged organ and the highly characteristic shape of the spleen in the different degrees of enlargement are all favourable to examination. A not unimportant point is that, in children of the age group used, probably but few spleens pathologically enlarged even in the smallest degree escape detection, since the organ, as well as being enlarged, has an increased consistency which favours detection of the pathologically enlarged as against the normal organ. Thus the spleen rate in children probably closely indicates the actual proportion of those in whom there is actual enlargement of this organ. It is not therefore merely an index, but probably very close to a true rate, using the terms index and rate in the sense of Ross (see Section II, 2). Again, the children of a village are much more likely to have been born and to have grown up in the same locality than are the adults, who also are more likely to have been exposed to contracting infection outside the area as a result of their occupations, etc.

Both in relation to parasite determinations and observations on splenomegaly, the question of the source of the children examined has some importance. The parasite rate and also the incidence of

splenomegaly is liable to be greater the lower the social status of the children examined. For this and other reasons,[56a] school-children are apt to show lower rates than children collected in villages or in the streets of a town. It is not always practicable or desirable to exclude the use of school-children, but it should always be stated that results relate to them if this be the case.

In general, as described in a later sub-section, there are two main methods of procedure among malariologists in regard to palpation of the spleen when determining the spleen rate, *viz.*: that employed in Algeria, India and some other countries in which the child is examined standing,[57] and that used notably by the Rockefeller Foundation in their recent work in Europe and elsewhere in which the child is examined lying down and in which stress is laid on the use of deep inspiration in this position to assist palpation.[58]

[56a] The following are reasons which may lead to a reduced rate in school-children, *viz.*: (1) social status is often relatively high, (2) children below the age of 5 are but little represented, (3) sick children are likely to be absent, (4) school quininisation may influence results or the taking of quinine may be more frequent due to education and social status. Also the children are often drawn from a wider area — *e.g.*, from villages to a school in town — and may not reflect the actual local conditions so well. (See " How to do a Malaria Survey ", *loc. cit.*, page 75.)

On the other hand, the much larger samples obtainable in schools, the possibility of examining the same group at different times, the greater information available about the child are distinct advantages.

Much would depend upon the circumstances in different countries, areas, etc., so that it is impossible to lay down a strict rule, but on the whole some allowance must be made for a lower rate in school-children where comparison is being made with rates obtained on village or street children, and it is therefore desirable, as noted in the text, that it should be so stated when school-children are being utilised in determining rates.

[57] A modification of this method consists in examination of the child sitting as employed by Schüffner. For determination of the spleen rate, it is not very material what exact technique is employed except as noted in the text for the question of so-called P. I. spleens.

[58] Schüffner does not agree that the deep inspiration is a special method relating to the " recumbent " method of palpating the spleen, but one to be ordinarily employed with the child sitting or standing also. Actually, personal experience of observers so largely determines their technique that it would be unjustifiable to assume that an examination in the standing or sitting position is necessarily less effective than one made in the recumbent position.

The chief difference in the results of these two procedures relates to the greater prominence given in the second of the methods to spleens so slightly enlarged that they are described as " palpable on deep inspiration only " or " P.I. spleens ". Until more comparison has been made of the two methods in the field by those familiar with both, it is not possible to give any exact indication of the extent to which the two methods actually differ in respect to the class of enlarged spleen noted. In the meantime, comparison of spleen rates as taken in different countries by different observers is greatly facilitated by a record of the size of the spleen as dealt with later, this being one of the reasons why it is important to indicate the proportion of different-sized spleens as well as merely to determine the spleen rate.

The methods employed by different malariologists in measuring and recording the size of the spleen are dealt with in subsection 2 *(c)*. In general, it may be said that measurement of the size of the enlarged spleen in a community involves the recognition of certain definable classes of size, the numerical prevalence of which in a given community can be ascertained and recorded. From such records, certain average values — *e.g.* the average spleen, average enlarged spleen, etc. — can be arrived at which are intended to serve as an indication of the degree of splenomegaly present. Since such information may be useful, not only as a matter of theoretical interest, but also as a means of comparing different observers' results, it is greatly to be recommended that data obtained regarding splenomegaly be recorded in some such form as below. By giving the data in this way, both the spleen rate and any other index desired can be readily calculated at any time.

Class of spleen	Age group				
	0–1	2–4	5–9	10–14	adults
0					
1					
2					
3					
4					

In practice, the age and class of spleen are recorded at the time the examination is made by an assistant. From such field notes, the data are classified at leisure and any necessary calculations made.

The actual classes used and the form of average figure employed are a matter for choice by the observer from those given in detail later. Here it is necessary only to refer to certain considerations which may influence such choice.

First, the object which the observer has in mind is important. If the object is scientific research upon the splenomegaly of indigenous communities, then a precise and accurate technique is not only desirable but necessary. But if the object is only one of practical utility in control or other malaria work, less elaborate methods may serve. Even then it should be remembered that, whilst simplification is very desirable, reducing the number of classes (for example) may carry the simplification to a point at which the results cease to have any real value. In particular, in this subject it is necessary to guard against a fictitious appearance of usefulness in results due to not clearly recognising the nature or implications of the processes to which the data have been submitted. This may be perhaps most clearly illustrated by an analogy, bearing in mind what has been demonstrated by observations in the field — *viz.*, that in conditions of static malaria *(i.e.*, in most malarious indigenous communities) the average amount of enlargement in those affected with splenomegaly is not greatly different whatever the intensity of the malaria may be. Let it be supposed that we wish to compare the average adult stature in two communities. If we class men as short, medium and tall, giving these as classes 1, 2 and 3, and take an average of our observations based on this numerical valuation, we should not expect to arrive at any useful result, the method being insufficiently delicate for the purpose intended — *i.e.*, the classes used, whilst very simply determined, are too few and approximate. We may expect the same comparative lack of value and certainty if we attempt to determine the average enlarged spleen by what may seem very easy, but are really not sufficiently careful and detailed, methods. Let us now suppose that there are, along with the adults, a number of children whose stature we class and value as 0. It is clear that, as regards the adult stature — *i.e.*, the information we really require — an average obtained from our

figures will be quite meaningless. Counting in the children in calculating the average — *i.e.*, doing what we do when we determine the average spleen — will not tell us at all what the average adult stature is, but will tell us only the average stature of the individuals who actually form the community and this will obviously depend upon how many children have been included, and this will clearly have nothing to do with adult stature. If we wish, then, to know what effect a certain malarial intensity has had upon the degree of splenomegaly in those showing splenomegaly, we must arrive at this by sufficiently accurate measurement of the enlarged spleens and take the average of these only — *i.e.*, the average enlarged spleen. If we use an average determined on the whole community — *i.e.*, the average spleen — then we do not arrive at a measure of the effect of the intensity of malaria on the spleen size, but at a datum which may be influenced by this, but which is to a probably much greater extent influenced by the proportion of negative persons (*i.e.*, children in the case of our analogy), which is really a separate effect produced by the malaria. Naturally, this second form of average shows much greater variation and, if we do not mind which special effect of malaria we are measuring, it may suit our purpose very well. But it is actually less informative than if we kept our effects separate and gave in our hypothetical community the average stature of the adults and also the number of children. This illustration, if not too tedious, may be helpful in appreciating some of the points in connection with indices of spleen size which are dealt with in Section II, 5 *(c)*.

(b) *Spleen rate.*

The *spleen rate* may be defined as the percentage of children aged 2-9 (or other age group suited to local conditions) showing palpable [59] enlargement of the spleen.[60] A similar proportion in adults is the *adult spleen rate.* The spleen rate may also be determined for any desired age group — *e.g.*, *spleen rate 10-14.* The spleen

[59] The term palpable, it should be noted, is here used in a different sense from " palpable " as a class of enlarged spleen referred to later — *i.e.*, in the latter case, a spleen which can be felt but which does not project beyond the costal margin.

[60] See footnote [54].

rate is an extremely valuable measure of endemic malaria, not being so liable as the parasite rate to rapid changes with season and particularly valuable because determinable with so little expenditure of labour. The adult spleen rate is also important, especially in relation to the spleen rate.

The need for certain conventions in taking the spleen rate to give as much uniformity as possible in the results has been generally recognised. It is doubtful, however, in view of the varied conditions in different countries and the variety of methods in use by different competent observers, whether a rigid standard in this respect can be usefully laid down. *The particular technique and conventions employed by an observer should, however, desirably always be stated.* The following are the two chief standard methods in use :

(1) Examination carried out with the child standing.[61] Spleens which can be felt, but which do not extend beyond the costal margin are recorded as " palpable " or as + (see under classes of spleen).

(2) Examination carried out with the child lying down and the abdomen exposed as in the clinical examination of a case. Spleens are recorded as " palpable " when they do not extend beyond the costal margin and as " palpable on deep inspiration " when they can be felt only under these conditions. The latter class of spleens are very generally referred to as P. I. spleens.

In both methods, various devices are used by observers to ensure relaxation of the abdomen (child told to look upwards, child gently pushed backwards against a wall, child made to draw the knees up in the recumbent position, etc.)

The results obtained in the two methods do not apparently differ greatly except that in certain circumstances there may be a large P. I. spleen class in the second method, which possibly

[61] Schüffner prefers a sitting position to a standing one, the muscles being at their greatest relaxation at the moment of sitting down. He does not consider " deep inspiration " special to the second method but one necessary for every palpation and one which makes it easier to find the spleen quickly. See also remarks in the text on other devices to ensure relaxation, etc.

might not all have been recorded by use of the first method.[62] Where malaria is transitory or treatment has been extensively carried out, the P. I. spleens may even form the majority of the enlarged spleens detected. In such cases, the spleen rate as determined by the two methods is not comparable without an accompanying statement of sizes of spleen.

It is generally agreed that percussion as a means of recording enlargement is undesirable. If used, the results should be recorded so that the relation to other methods is apparent.

Certain requirements should be met :

(1) Those examined should be of some agreed-upon age suitable to the conditions in the particular country (usually 2-9 age group).

(2) The number examined should be reasonably large. For moderate or high spleen rates, 50 children (often all that are available in a small village) or better 100 is such a reasonable number.

(3) If school-children are utilised, they should be recorded as school-children (see remarks on use of school-children in Section II, 5 *(a)*).

There has been some discussion as to whether the term should be " spleen rate " or " spleen index ". On the basis of definitions already given, it is clear that it would come under the definition of a rate.[63] The name " spleen rate " has been in use for a long time and to change it to " spleen index " on the grounds that it may not accurately record all enlargements would appear to be giving a refinement of meaning to the term, which is unnecessary.

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[62] Dr. Paul Russell and others (Russell, Menon and Rao, J. Mal. Inst. of India, 1938, 1, 293) note that, in their experience, enlarged spleens as a general rule do not come lower by gravity when the subject stands. They do not " sag " but tend to be lifted higher, presumably by their anatomical attachments. This has been confirmed by Dr. Sweet (private communication), who notes that there may be a difference of 0.5 cm. in the apex umbilicus measurement and in a few cases a spleen not felt when the child stands becomes P. I. (palpable on deep inspiration) when the child is lying down. Sinton, however, has observed that very large spleens in adults may descend considerably on standing (private communication). See, however, footnote [63 a].

[63] See Section II, 2, also remarks on splenomegaly in children in Section II, 5 *(a)*.

(c) *Size of spleen.*

For recording the size of the spleen, various methods are in use.[63 a]

(1) The degree of enlargement is given in *fingerbreadths* projection beyond the costal margin. In India, the usual notation has been to record sizes as " palpable " [64] (not given a value in determining the average enlarged spleen) and in seven further classes — *viz.*, 1, 2, 3, and 4 fingerbreadths, handsbreadth (5), to umbilicus (6) and beyond umbilicus (7). Sergent in Algeria uses the same notation in general, but includes " palpable " spleens under 1 and spleens of a greater size than a handsbreadth as 6. The figures in both cases indicate the weighting which is used in obtaining the mean (average). Spleens that cannot be felt are recorded as negative (0). The mean of such valuations is therefore some value in fingerbreadths between the respective extremes in either case. A fingerbreadth is roughly about 2 cms. If multiplied by 2, therefore, the mean in fingerbreadths very closely approximates to a mean obtained by measuring the projection in cms.

(2) The spleen projection is determined by subdividing the abdominal area to the left of the mid-line into a given number of equal intervals by lines roughly parallel to the costal margin and recording by number the space in which the apex of the spleen [65] is found to lie on palpation. Schüffner, who proposed this method, divided the area from the left costal margin to the right groin into eight divisions, one to four above, and five to eight below, the line through the umbilicus. Hackett and Missiroli record three sizes above and two below a line drawn horizontally through the umbilicus, P. or P.D.I. spleens being included in class 1 and the other classes

[63 a] It should not be forgotten that it is the child spleen here being considered. Measurements of the size of the adult spleen can be carried out as, for example, has been done by Covell, but this is a different proposition entirely and considerations based on conditions in the adult have no relation to those here dealt with.

[64] The class " palpable " includes any spleen which can be felt, but which does not project beyond the costal margin. This is equivalent to Schüffner's + class, which carries no weighting though included among positive spleens. See subsequent remarks on recommended classes.

[65] The apex is the most prominent part of the projecting spleen.

weighted according to their numbers. Boyd uses only two groups from the costal margin to the umbilicus and one beyond. The figures as given are used in weighting to obtain a mean or average value, which is one proportionate to the abdominal measurements used.

(3) The apex is located by a touch of the finger and marked with a spot from a grease pencil. Either the distance of this spot from the costal margin (C.M., or costal margin measurement) or its distance from the umbilicus (A.U., or apex-umbilicus measurement) is then measured to the nearest centimetre with a short length of tailor's tape. The mean of the measurements gives the mean projection in cms. Usually the A.U. measurement is adopted, the mean value obtained usually lying between 6-8 cms., but the C.M. measurement can be used if preferred. As the distance from the costal margin to the umbilicus in the average child is 13 cms.,[66] subtraction of the mean A.U. measurement from this figure gives the mean C.M. measurement.[66 a]

Since the distance from the costal margin to the umbilicus in the mean child is about 13 cms., it is possible to express the values obtained by proportionate measurements also approximately in cms. if desired. Thus values in the case where four equal divisions are used between the costal margin and the umbilicus, if multiplied by 13/4 or approximately 3, will be approximately in cms. It has previously been noted that fingerbreadths multiplied by 2 would give measurements (or a mean) in cms. Such considerations will show that, in spite of seemingly very different methods being in use, the fundamental process is the same in every case — *viz.*, the measurement of the projection of the spleen in various units — the units in the three methods described (fingerbreadths, proportionate units and cms.) being respectively of about the order of 2, 3 and 1,

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[66] An average obtained by Christophers and Khazan Chand for Indian children as examined in the field : average age 6.4 years ; average sitting height 60 cms. ; nipple umbilicus measurement 21 cms. : *costal margin to umbilicus* 13 cms. For these studies, see *Indian Jl. Med. Res.*, 1924, 11, 1065.

[66 a] A full description of the method, which has been extensively used in India in researches on splenomegaly, will be found in " How to do a Malaria Survey ", Govt. of India *Health Bulletin* No. 14, 3rd Ed., 1936.

and being roughly convertible on multiplication by these factors into cms.[67]

Method No. 1 has the advantage of extreme simplicity of working in the field and the results given are by no means to be despised.[68] Method No. 2, and especially Schüffner's method, is perhaps most widely used and has the advantage of giving a proportionate rather than an absolute value, thus allowing for variations in size of child. Method No. 3 has the advantage of providing very suitable data for accurate analysis, and, if used with a sufficient number of children, gives values independent of size of child, or can be used with a correction factor for this purpose.

A true average enlarged spleen — *i.e.*, the average weight, volume, or even length — cannot, except rather hypothetically, be arrived at with existing data. Ross's original estimates were that small, medium and large spleens were respectively three, six and nine times the volume of the normal, by which figures the different sizes were multiplied in arriving at the mean or average enlarged spleen. This, however, is an entirely arbitrary weighting and the result would be greatly dependent on the actual estimates made. Christophers studied the correlation of various degrees of enlargement as seen in children and, by means of studying models and *post-mortem* material, gave estimates which enabled a nearer approach to be made to the true value. Such efforts to arrive at the real value of the average enlarged spleen must be considered as awaiting further intensive research before they can be satisfactorily applied to the problem. For the present, what is employed is the mean of the different degrees of projection of the organ in a

[67] What is really being measured is the mean position in the abdomen of the apex of the enlarged spleen, this position being always in terms of the measurement units used. This is why the divisions in any scale used should at least be equal, otherwise any mean figure obtained would be almost meaningless. It is also desirable that the classes should be started at 0 (normal spleen, or spleens at the costal margin) and with further classes at 1, 2, 3, etc., of whatever units be employed. Some trial calculations will very quickly demonstrate the simple relationships which hold if these requirements be met.

[68] The method, for example, is that in use in Algeria, and the writer would regard it as the most expeditious and useful of all methods where results for practical purposes in the field were required.

community as measured by one or other of the methods already indicated.

Ross (1910) recognised two values, *viz.:* (1) the *average spleen* or the mean enlargement of the organ considering the whole community *(i.e.*, including those negative to examination), and (2) the *average enlarged spleen* or the mean size of only those spleens which are found enlarged. Though the means here were intended to apply to the volume of the spleen, the same terms are applicable to measurement of projection. The respective significance of these two forms of average has been already discussed in Section II, 5 *(a)*.

When the children of suitable age in a community have been examined and the sizes of the spleen recorded, either of the averages can be readily arrived at as shown in the illustrative calculation given below.

Class of spleen	Weightage for class	Number of children in class	Number of children multiplied by the weightage
0	0	40	0
1	1	14	14
2	2	34	68
3	3	10	30
4	4	2	8
	Total	100	120
	Total positive	60	

Average enlarged spleen (A) = 120/60 = 2.0

Average spleen (A′) = 120/100 = 1.2

It will be evident that any such figures given by a particular author as relating to the average spleen, average enlarged spleen or splenometric index, etc., are entirely dependent on and relative to the units of measurement employed by this author and are not to be taken as equivalent to figures obtained by another author which may relate to units of measurement of quite different value. This is unfortunate, but appears to be unavoidable. All that can be done is to appreciate that such figures do relate to a particular author's units of measurement and, as explained previously in the text, to make such allowance as is possible based on what these units may be. It will be clear how important it is to state precisely what units are used in arriving at such data.

As already noted, there are in relation to splenomegaly two fundamental values of quite different character concerned, *viz.*: the proportion of spleens enlarged, S, and the extent to which on the average they are enlarged, A. Any other indices than these can be regarded as variously compounded from S and A, thus —

Spleen rate	$= S$
Average enlarged spleen	$= A$
Average spleen	$= A \times \frac{S}{100}$
Splenometric index [69]	$= A \times S$

The two values, A and A′, are actually very simply related, since $A' = A \times \frac{S}{100}$ and $A = A' \times \frac{100}{S}$. A can therefore be readily deduced from A′ and *vice versa*, provided S (the spleen rate) is also given.[70]

In regard to the choice of classes, there are obvious advantages in recognising a class weighted as 0 to include negative spleens. Spleens that are palpable but do not project beyond the costal margin must obviously be recorded as a class, since they enter into the calculations by increasing the number of persons with enlarged spleen. It would appear desirable, however, for obvious reasons, that these also should be given a weightage value of 0. For such spleens, Schüffner suggests the designation + (counted as positive, but weighted 0), followed by classes 1, 2, 3 etc. of his subdivisions, which are weighted the same value as the classes. The Malaria Survey of India (using measurement in cms.) employs a class 0 for negative spleens and a class " palpable " (weighted as 0, but included in the number of enlarged spleens) where an enlarged spleen does not project beyond the costal border, the projection of other spleens being weighted according to their cm. measurement. Whatever may be the basis used in arriving at the classes, there would seem to be considerable advantages in using the following designation

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[69] See Section II, 6, Indices of Endemicity.

[70] Similarly, the splenometric index (A×S) as used by some authors is readily determined from A or A′, always provided the spleen rate, S, is given.

of classes and weightings, it being desirable that the measurement used as units be of equal length (see note [67]). [71]

Class	Weighting	Type of spleen
0	0	Negative.
+	0	Palpable but not extending beyond the costal border.
1	1	Enlarged 1 unit beyond costal border.
2	2	Enlarged 2 units beyond costal border.
etc.		

The brackets show the classes included respectively in the two means.

In regard to the choice of method of measurement employed and the average selected for use, remarks in Section II, 5 *(a)* and in Section II, 6 may with advantage be noted.

Provided the units used are of equal length, this fixes a point on the abdomen representing the actual average projection beyond the costal margin.

Some authors prefer, however, to weight class 1 spleens as 1 since otherwise this class does not influence the result. In tropical countries, the number of such spleens is usually relatively small and the weighting given to them usually has little effect on the result. Where, however, low spleen rates are being dealt with, the proportion of P. or P.D.I. spleens may be large and, if these are not weighted, anomalous results will be obtained.

(d) *Frequency distribution of classes of enlarged spleen.*

Data giving the number of enlarged spleens of different measurements can be studied from the point of view of *frequency distribu-*

[71] Some malariologists use a weighting of 1 for normal spleens and one of 2, 3, 4, etc., for the classes of positive spleen. This, however, introduces a complication in that the simple relationships referred to in the text no longer hold. In this case, however, a value for the average enlarged spleen so arrived at can be converted into A as described in the text by subtracting 1 from the final result. It would seem desirable that the simpler system of weighting be used. This has the additional advantage that A is then quite simply the average projection of the enlarged spleens and A plotted on the measurement line on the abdomen gives the position of the mean projection.

tion of the classes. Such frequency distribution, unlike the parasite infection frequency, is well displayed in frequency curves of the usual type. The curve given is a very valuable aid to the study of splenomegaly in malaria, since the frequency distribution of different classes of spleen is the fundamental phenomenon on which such values as the average enlarged spleen are dependent.

Sub-section 6. — Indices of Endemicity.

(a) *The parasite rate as index of endemicity.*

Under the term endemic index, the parasite rate for children 2-10 was used by Stephens and Christophers, 1902, for purposes of comparing and mapping endemic malaria. Though extensively used, it has been found more and more convenient in practice to use the spleen rate for this purpose. It is undesirable, therefore, to have a term " endemic index " which is distinct from the measure most usually adopted in measuring endemicity — *viz.*, the spleen rate. It is also liable to be confused with the " endemic index of Ross," which is of a different nature (Section II, 6 *(f)*). For these reasons, the use of the term endemic index is best discontinued.

(b) *The spleen rate as index of endemicity.*

In practice, the spleen rate has been more widely used in measuring and mapping malaria than any other method (see under endemicity, Section II, 1). Where malaria is static, the spleen rate obviously gives a remarkably good measure of the degree of infection immunity or premunition in any given community. It has the advantage over the parasite rate of being easily and rapidly ascertained. It has the further advantage of being less liable to violent fluctuations than the parasite rate. Even in epidemic conditions, the spleen rate affords an immediate indication of the prevalence of infection. Whilst no single figure can be expected to express all the detailed phenomena of malaria prevalence and intensity, the spleen rate appears to give, in a broad and general sense, the best measure of the " malariousness " of a place or community. It is from this point of view a priceless asset to the malariologist and worthy of the closest study, not only in relation to its use as a practical means of measurement, but also as a phenomenon regarding the exact nature and significance of which we

cannot have too detailed knowledge. The spleen rate might therefore well have been used as an agreed-upon index of endemicity and named the endemic index. This, however, would now cause confusion and it is on this account undesirable to give it this name, though in actual practice it is the usual datum line to which malaria phenomena in communities are referred. The definitions, methods of ascertaining and usage of the different values connected with the spleen rate and their terminology have been given in a previous sub-section and need not be further dealt with.

(c) *Splenometric index and average spleen.*

The *splenometric index* is a single-figure index (spleen rate × average enlarged spleen) designed to include degree of splenomegaly as well as the spleen rate. This term was proposed by Parrot and is in extensive use in Algeria. The splenometric index is the *average spleen* of Indian authors (except that this is expressed as the latter value multiplied by 100) (see under size of spleen, Section II, 5 *(c)*). The nature of these indices has already been referred to and no further remarks are necessary beyond saying that they have the advantages and disadvantages of any composite index and therefore that it is desirable always to give also the spleen rate to which they relate to enable their exact nature to be elucidated if necessary.

(d) *Average enlarged spleen.*

Where malaria is static *(i.e.*, the most usual condition in tropical communities) [72] it appears to produce in those infected on the average a certain relatively fixed degree of splenomegaly. This mean splenomegaly in children 2-9, *average enlarged spleen*, has been found by careful researches in India to be usually much the same where the malaria incidence is low — *i.e.*, where splenomegalic individuals form a small proportion only of a non-infected community — as in conditions where the whole community is heavily infected. This same mean splenomegaly in children is characteristic also of single infected families (small malarial foci) among a sur-

[72] By " static " malaria is not meant that there is no variation at all in the spleen rate, which commonly does vary to some degree even in the most intensely infected villages, but that a more or less constant endemicity is present, as against conditions where malaria is freshly introduced, or is dying out as the result of a long period of cessation of infection or effect of mass treatment.

rounding non-infected community. A moderate increase, however, occurs when the spleen rate of the community exceeds about 80%. Reduction below this more or less fixed value can, however, occur — *e.g.*, in Europe or elsewhere when different recovery conditions in the host, shortness of the malaria season, mass treatment or other factors bring about such a result. A reduction in the average enlarged spleen is therefore a significant fact, over and above the fact of reduction in the spleen rate, which may have value in judging of the malarial conditions relative to a community. This average value naturally falls with fall in the spleen rate when expressed as a mean for the whole community — *i.e.*, as the average spleen.

(e) *Malaria rate of Ross (1910).*

This is the number of actually infected persons (infantile, child or adult) expressed as a percentage. It is not directly ascertainable and can only be deduced or estimated from such information as the parasite rate and/or spleen rate, etc. The term is undesirable since it is ambiguous and, apart from this, suggests morbidity rate rather than the meaning actually intended, for which *true infection rate* (see note [39], Section II, 4 *(a)*) would seem to be preferable. Whilst it is not impossible that the actual number of infected persons might be ascertained (see parasite asymptote, Macdonald's index, as also possibly by a serum reaction), each method of arriving at the result is likely to have a somewhat different value. Hence it would usually be desirable to give the actual nature of the value ascertained.

(f) *Endemic index of Ross (1910).*[72 a]

This was defined by Ross as the number of children 2-10 showing evidence of malaria either by presence of parasites or by spleen enlargement or both. Its determination requires the recording of the condition for each child separately so that four groups can be formed, *viz.*, P, PS, S and 0, where P indicates presence of parasites, S enlargement of the spleen and 0 neither condition present. Whilst possibly giving a nearer approach to the true

[72 a] This is not the " index of Ross " of Brumpt, which is the average enlarged spleen — *i.e.*, a mean obtained by weighting the number of different categories of spleens by their category number (*e.g.*, I, II, III, IV, etc.). Also termed " indice splénique moyen " by Brumpt.

infection rate, the method is dependent on the same limitations as are inherent in the parasite rate or spleen rate, in that it does not necessarily indicate the number accurately. Apart from other considerations, "endemic index" cannot now be used without being liable to cause confusion.

(g) *Macdonald's index.*

Making the assumption that all children showing enlargement of the spleen are almost certainly infected, whether shown to be so by ordinary blood examination or not, Macdonald has determined the proportion of children under different conditions who show parasites when enlargement of the spleen is present. If now microscopic examination shows only about 60% of the actual infections, the number of infections found in children who do not show enlargement of the spleen would presumably also [73] be only this proportion of the real number of those infected. To arrive, therefore, at the number of children among those without enlarged spleen who are really infected, it would be necessary to multiply the number found infected by $\frac{100}{60}$. In general terms this becomes $\frac{100}{x}$ (Macdonald's factor) where x is the percentage of those with enlargement of the spleen showing parasites.

If the parasite rate (as relating to the whole community) in those with splenic enlargement be p_1 and in those without splenic enlargement be p_2, then P, the actual infected rate (true infection rate of Macdonald), will be given by

$$P = p_1 \times \frac{100}{x} + p_2 \times \frac{100}{x}$$

but p_1 and p_2 together make the actual rate, p, as found by microscopic examination, thus the true infection rate, P, is given by

$$P = (p_1 + p_2) \frac{100}{x} = p \frac{100}{x}$$

The value p_1 was found to be very constant in all communities, but p_2 varied. Further, the variation in p_2 bore a direct relation to the spleen rate.

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[73] Actually it is not certain that the factor would necessarily be the same in both cases, since, especially with the higher spleen sizes, there is a reduced likelihood for parasites to be found when splenomegaly is present.

It may be noted that these results tend to give additional support to the spleen rate as a measure of endemicity, for it can readily be seen that, if all those with enlargement of the spleen are in the first place infected — and there is a further additional value to be added representing children without splenic enlargement but yet infected and this additional amount is also directly proportionate to the spleen rate — then the true infection rate can be expressed as a functional derivation of the spleen rate.

(h) *Average new-born infection period.*

This index, suggested by Daniels, is the average time before infection becomes evident in infants, dating from the time of birth. An important objection to the index is that infants commonly remain largely uninfected until the onset of the fever season, when most of them may show infection. Conversely, the months in which the new-born to the greatest extent contract infection has been used (Strickland) to ascertain the period of greatest liability to infection in a community.

(i) *Infantile parasite rate.*

The parasite rate in children age group 0 determined at the end of the epidemic season has been shown to have value as an index of liability to contract infection in a given locality; it is termed *transmission index* by Barber, Rice and Mandekos, 1936.

Section III

TERMS APPLIED TO THE VECTOR

Sub-section 1. — The Name Anopheles.

Some hesitation is often felt as to whether to write *Anopheles*, " Anopheles ", Anopheles or anopheles. A good working rule adopted by some authors is to employ the first — *i.e.*, *Anopheles* — only when giving a definite zoological value to the name, and to use anopheles [74] whenever it is merely a more or less colloquial designation. This does away with the necessity of using Anophelines or Anopheles mosquitoes, anopheles being used in both cases.

Sub-section 2. — Species and Varieties of Anopheles.

About 200 species of anopheles are known forming the tribe *Anophelini*, one of three tribes into which the subfamily *Culicinae* (or true mosquitoes) of the family Culicidae is divided.[74a] All but a very few rare forms of the tribe are, however, included in the genus *Anopheles*, of which four subgenera are recognised *(Stethomyia, Anopheles, Nyssorhynchus* and *Myzomyia).*[75]

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[74] According to Senior White, *Nature* uses *Anopheles*, capitalised and italicised, only when the species is also given. If used alone it is capitalised only. Covell and some other authors use the uncapitalised form as recommended in the text. Being in this form, anopheles is used like mosquito as a name with which everyone is supposed to be familiar.

[74a] See Edwards : " Genera Insectorum, Diptera, Fam. Culicidae ", 1931. This work gives a complete systematic account and provides the simplest source from which current correct names of species and status of varieties, etc., up to 1931 may be obtained.

[75] The first-mentioned includes only a few rare and localised forms, leaving the great majority of species in the three remaining subgenera. In the Old World there are only two of the subgenera, *Anopheles* and *Myzomyia*, *Nyssorhynchus* being restricted to South and Central America.

For later works dealing with Indian and African anopheles respectively, see Christophers : " The Anopheline Fauna of British India ", 1933, Taylor and Francis, London ; Evans : "Anophelines of the Ethiopian Region ", British Museum, 1938 ; and G. Senevet : " Les Anophèles de la France et de ses colonies ", 1935, Lechevalier, Paris.

It is usual, at least once in any communication, to accord to any species dealt with its full zoological nomenclature (see also under malaria parasites, Section I, 2) and in such formal designation to give the subgenus in brackets — *e.g.*, *A. (Myzomyia) jeyporiensis* James, 1902.

In addition, named forms of subspecific rank are recognised in the case of certain species. These have the character of species in that they are identifiable by small but definable morphological differences (which may often be differences in ornamentation of a stable character) and possess distinctive zoogeographical distributions, but are either so nearly related to the species to which they are allocated as not to merit the status of species, or are for certain reasons conveniently maintained as a group of subspecies rather than so many distinct species. Such subspecific forms are accorded the rank of *subspecies* or *variety* and in nomenclature are distinguished by a third or trinomial name having the general facies of a specific name and placed (in italics with small initial letter) following the specific name, either consecutive to this or preceded by subsp. or var. as the case might be. Which particular designation is employed depends chiefly upon the custom in respect to the zoological group concerned. Since throughout the *Culicidae* systematists employ " variety " and add the varietal name following var. written in roman type without comma, this is the correct procedure in regard to the nomenclature of subspecific forms accorded a name in the Anophelini — *e.g.*, *A. jeyporiensis* var. *candidiensis* Koidzumi, 1924.

When such forms, as frequently happens, are eventually considered to be distinct species, they take the varietal name as their specific name — *e.g.*, *A. jeyporiensis* var. *moghulensis* becomes *A. moghulensis*. In this case the describer's name is that of the namer of the variety.

Where forms differ merely by what is suspected or known to be individual, seasonal or other variation not of a permanent evolutionary character, the name *variant* (Christophers) has been proposed. Most of such forms are covered by the terms *melanism* (general increase of areas of pigmentation), *hypomelanism* (decrease in areas of pigmentation with extension of pale markings), or *pigment anomalies* (irregular, often freakish, effects).

The term race has been very frequently used in recent malaria

literature in connection with the forms of *A. maculipennis*. Race is usually employed in genetic and other studies to indicate subspecific differentiation not amounting to an actual definable morphological distinction. It has something of the character of subspecies or variety, but, whereas the essence of the latter distinction is morphological, the distinctions upon which races are based are usually genetic.[76] It is generally agreed, however,[76] that the forms of *A. maculipennis*, though commonly referred to as " races " of *A. maculipennis*, have in general the character of species — *i.e.*, though the adult forms are with difficulty distinguished by morphological or ornamental characters, the egg, at least, is sufficiently distinct to differentiate the forms in this

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[76] The following notes, kindly furnished to the Sub-Committee by Dr. Marston Bates, are of interest in this connection :

Definitions of categories of natural populations.

" *Biotype*, du Rietz 1930 : ' A biotype is a population consisting of individuals with identical genotypical constitution.' This is the same concept to which Lotsy (1916) tried to restrict the name *species :* ' to designate a group of individuals of identical constitution, unable to form more than one kind of gametes ; all monogametic individuals of identical constitution consequently belong to one species.'

" The term *biotype* was apparently originated by Johannsen in 1909, but I do not seem to have the exact reference. It has been most used by the botanists. Du Rietz himself says that it seems unlikely that there is such a thing as a biotype in nature, but he uses the term in building up definitions of other categories, and seems to find the concept very useful. Thus Robson and Richards refer (page 64) to the ' differentation of populations into a large number of intercrossing biotypes.' The biotype is thus the natural equivalent of the laboratory pure line, and I find in Robson (page 27) the term used quite clearly in this sense — ' . . . the individuals representing which very often live together and interbreed, but are capable of being bred-out into distinct stocks (biotypes) '.

" *Subspecies*, du Rietz, 1930 : ' A subspecies is a population of several biotypes forming a more or less distinct regional facies of a species.'

" *Species*, du Rietz, 1930 : ' The smallest natural populations permanently separated from each other by a distinct discontinuity in the series of biotypes are called species. A species is thus a population consisting either of one strictly asexual and vital biotype, or of a group of practically indistinguishable, strictly asexual and vital biotypes, or of many sexually propagating biotypes forming a syngameon separated from all others by more or less complete sexual isolation or by comparatively small transitional populations.' "

stage and they possess distinctive zoogeographical distributions [77] and have been shown in a number of cases to be incapable of cross-breeding.[78] It has to be remembered also that, not infrequently, varieties or even species of anopheles are only to be differentiated because they show markings. *A. maculipennis*, being a dark, obscurely coloured form, has no such markings to give help in this direction. Also there are certain small differences, which though not very satisfactory for practical purposes, seem to indicate real differences — *e.g.*, the hypopygeal characters. In general, therefore, the status of the forms appears to be not unlike that of other (named) varieties of anopheles, some of which are possibly distinct species, and it is undesirable that the nomenclature of these forms of *A. maculipennis* should not conform to that in general use in the group. In this case they are to be regarded neither as " races " nor as " biotypes " but, at least provisionally, as " varieties " used in the accepted sense of subspecies. Such considerations, however, apply only where the usual criteria of varietal rank are reasonably employed. Most of the recognised varieties at present conform to such criteria, but it does not follow that every variation shown, for example, by eggs is necessarily an indication of good varietal status.

If this view be accepted, the forms of *A. maculipennis* would be designated, as are other varietal forms, by the name of the variety followed by the describer's name — *e.g.*, *A. maculipennis* var. *atroparvus* Van Thiel, 1927. It is usual in the case of anopheles varieties to retain the original species name without any varietal designation so far as the type form of the species is concerned. In the

[77] Some of the distributional areas are as distinct as those of many species — *e.g.*, var. *labranchiae*. In other cases, the distributional areas overlap or intermingle, but this is not in itself an objection, as the same phenomenon may be seen in the case of other quite well-accredited varieties.

[78] In the case of *A. maculipennis*, experiments by Swellengrebel and others have shown that some of the forms are sexually isolated in that cross fertilisation does not produce offspring. Certain habits which transcend mere details of behaviour, such as the onset of true hibernation under given conditions, the pairing or not in captivity, etc., also distinguish these forms and tend still further to establish a specific differentiation. It is desirable, however, in questions of nomenclature to adhere as far as possible to such morphological characters as can be used in the zoological treatment of the group. But further evidence based on genetic and other grounds naturally strengthens action taken in this respect.

case of *A. maculipennis*, it is, however, in practice very convenient to be able to designate the type form when this is intended and the use of *typicus* as a varietal name seems in these circumstances to be without objection.[79]

Sub-section 3. — Stages of Growth and Development.

(a) *Metamorphosis.*

The stages of metamorphosis are : the *egg* (not ovum [80]), *larva*, *pupa* (active pupa, not nymph) [81] and *imago* (often termed *adult)*. The liberation of the larva from the egg is most commonly referred to as *hatching*. The castings of the successive larval skins are *ecdyses*, from which the larval stages are named, the first larval stage being that from hatching to the first ecdysis. It is a point of practical importance in detection of the stages that, whilst the body of the larva grows during the stage so that size in the same stage may be very different, the head remains unchanged and therefore a ready means of determining the stage. The fourth ecdysis, which determines the final or completed larval stage *(fourth-stage larva)*, results in *pupation*, this being followed in due course by *emergence* of the imago. Deposition of eggs by the female constitutes *oviposition*. The site chosen for oviposition and subsequent development of the larvæ in nature is the *breeding-place*.

Some terms used in connection with artificial rearing require consideration. A species stated to have been reared in captivity may relate merely to the fact that larvæ or eggs have been collected and subsequent emergence of the adults obtained, or may indicate that fertilisation has been brought about under experimental conditions with subsequent development. It is desirable to make

[79] The decision as to which is the type form and which forms are actually valid varieties is a matter of zoological systematics. It may be held that, as it is not known for certain which form Meigen actually described, the name *basilei* given by Falleroni should be used in place of *typicus*. In present procedure, *typicus*, though used like the name of a variety, is presumed to be Meigen's form and to antedate *basilei*.

[80] The ovum is correctly the sexual cell ; the egg consists of all the elements of the follicle as well.

[81] Nymph is strictly applied to insect forms undergoing no marked changes in metamorphosis — *i.e.*, ametabolous insects.

use of distinctive phraseology in the two cases and, whilst *bred out* in captivity might serve for the first case, *reared* in captivity might be reserved for the second. The eggs deposited by a female at any one oviposition (which may be examined or subsequently bred out for examination) are referred to as a *batch*.

(b) *Gonotrophic cycle.*

Fertilisation is evidenced by packing of the spermatheca with spermatozoa, the proportion of fertilised females in nature being the *fertilisation rate*. A blood meal is evidenced by the presence of blood in the midgut, unless this has been digested. As the blood meal is digested, the ovaries in normal circumstances enlarge. A female containing blood in the gut is commonly referred to as a *fed* female, and one which has not fed on blood, or in which the blood had been completely digested, as an *empty* female. If the ovaries are perceptibly enlarged, the insect is designated a *gravid* female.

Development of the ovaries is indicated by various stages of the follicles as under : [81 a]

Stage 1 — Ovum without marked granules.
Stage 2 — Granules present to granules occupying half the follicle.
Stage 3 — Granules occupy over half the follicle, which, however, is not elongated.
Stage 4 — Follicle elongated or shape of mature egg, but superficial structures are not evident.
Stage 5 — Egg fully formed and floats visible.

Females which have not previously oviposited are said to be *nulliparous* (females) and those which have previously passed through one or more ovipositions *multiparous* (females). In the former, all five stages of development of the follicle are passed through, in the latter the first two stages are already passed through by the oncoming follicle before oviposition, the time of development required, as counted from one oviposition to another, being correspondingly shortened. In the case of multiparous females, an indication that they have previously oviposited is given by

[81 a] For a detailed description of these stages, see "How to do a Malaria Survey", *loc. cit.*, pages 59-73.

enlargement of the ovarian tube (Mer) and sometimes by a retained egg.

The complete cycle from time of feeding to oviposition is the *gonotrophic cycle*, which may extend over a period of two, three or more days. Where the ovaries remain in an undeveloped state whilst the insect continues to take blood meals, the condition is described as *gonotrophic dissociation* (Swellengrebel).

(c) *Age.*

Determination of the age of females, so far as this can be ascertained, is important. Stage 1 ovaries indicate a *newly emerged adult* (24 hours or under). All other ovarian stages indicate only some stage of a gonotrophic cycle, whether the first or a subsequent cycle being indeterminable, except as indicated by the condition of the oviducts or a retained egg undischarged at the last oviposition, these being evidence that at least one cycle has been passed through.

An indication of age may also be given by the general condition of the insect. Perry's classes of females indicating relation of age to wing appearance are :

Class 1 — Wing markings clear and fringe complete.
Class 2 — Wing markings fairly clear, but fringe worn.
Class 3 — Wings shabby and fringe much worn.
Class 4 — Wings threadbare.

Sub-section 4. — Life History and Behaviour.

(a) *Breeding-places.*

The collections of water from which anopheles are derived are the breeding-places (in relation to any specified species). These may be *temporary* or *permanent.* Temporary breeding-places which are dry at the moment of inspection and likely breeding-places not actually showing larvæ form *potential breeding-places.* The number of adults (per unit time, etc.) from breeding-places is the anopheles *output.*

(b) *Dispersion.*

The spread of anopheles from their breeding-places in search of food, etc., constitutes *dispersion. Distance of dispersion* is the

mean or maximum distance, as the case may be, to which individuals disperse from their breeding-places.[82] The distance which may be covered under some special conditions — *e.g.*, preceding hibernation — is *long-distance dispersion.* When anopheles reach their final destination by passing from house to house, or from shelter to shelter, in an unknown succession of steps, this is spoken of as *infiltration* (of an area). When anopheles are brought into an area by vehicles, etc., this is described as *passive dispersion* or *transportation.* When carried long distances by wind, the condition may be described as *wind dispersion.*

(c) *Feeding and resting habits.*

The source from which an anopheles obtains its blood meal is the *host.* The place where a blood meal is obtained is the *feeding-place*, and the places where anopheles are found resting during the day are the *day resting-places.*

The blood meal may be obtained from man or from cattle or other animals. The names anthropophilic (or androphilous[83]), zoophilous and even neutrophilous have been given to species or varieties of anopheles to indicate respectively a supposed preference to feed upon man, upon cattle, or upon both indiscriminately. Such names do not well represent the facts and are liable to give rise to misconception. Actually, the different varieties usually include among possible hosts a number of animal forms, which may include man, the order of preference for different hosts varying with the different varieties, *host preference.* The animal most preferred is the *host of predilection.*

The proportion (ratio) of freshly fed anopheles giving a positive precipitin reaction for human blood is the *human blood ratio* for the particular conditions in which the captures have been made. Such ratio gives some indication of the degree of host relationship to man under the particular circumstances and is closely bound

[82] Boyd uses " dispersion radius ", but also " flight " or " length of flight ". Swellengrebel uses " range of flight ". " Flight range " is also in common use. It seems desirable to distinguish between the distance a mosquito can fly at a single effort, say flight range, from the total distance it may cover after many such flights, here given as " distance of dispersion ".

[83] Androphilous means " loving a human being of the male sex " and is less suitable than anthropophilic.

up with the extent to which particular species under given conditions act as vector species. The human blood ratio has also been termed the *anthropophilic index.*[83 a]

Where, owing to host preference, there is reduction in the number of anopheles feeding on man, due to attraction to cattle (or other domestic animals), this is referred to as *deviation* (animal).

Day resting-places may be human habitations, *houses*, or places where animals are kept, *stables* (as a general covering term), or outlying unoccupied sheds, etc., *shelters*, or in banks, undergrowth, etc., *natural shelters.*

The conditions of temperature and humidity in the immediate surrounding space in which a mosquito lives when resting or otherwise, as distinct from the temperature and humidity of the general atmosphere, constitute *microclimates*, such microclimates often differing greatly from that of the outer air or even of the room occupied.

(d) *Other habits and behaviour.*

In the case of anopheles, as with most mosquitoes, oviposition is dependent on a blood meal. Rarely, as in a certain species of *Culex*, the necessary store of nutriment for the eggs is accumulated in the larval state, oviposition taking place in the absence of any blood meal by the imago, *autogenous* races (Roubaud).[84]

In some species or varieties, fertilisation of the female takes place without difficulty under experimental conditions in a limited space, in others it is necessary to have a large space or even facilities for swarming of the males. The two conditions have been termed by Roubaud, *stenogamous* and *eurygamous* forms respectively.

Certain species tide over the cold season or winter through resistance of the egg, larva or adult (*wintering*). When such resistance by the imago is associated with changes in the condition or behaviour of the imago, it is termed *hibernation.* When the female develops fat, adopts a characteristic attitude and ceases to feed and oviposit,

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[83 a] Brumpt has suggested that the degree of anthropophilism could be indicated by the ratio of natural infection rate of a given species with a given *Plasmodium* to the same rate under experimental conditions. To such a ratio he has applied the term " index casanier ".

[84] The ordinary condition in which a blood meal is necessary for oviposition is termed by Roubaud *anautogenous.*

hibernation is said to be *complete*. In certain cases, the female, while ceasing to oviposit, remains more or less active and continues to take blood meals, *partial hibernation*. Such a condition (gonotrophic dissociation) may occur before the actual onset of winter conditions.[84 a]

When in resistance to a dry hot season special adaptation takes place, this is referred to as *æstivation*.[85]

The terms *tropism* and *taxis* are applied to various reflex urges such as might cause a mosquito to be attracted or repelled, to fly in a given direction, etc., but such conditions have been little studied in the mosquito and the terminology as to the correct usage of tropism and taxis and other terms employed is complicated.

Sub-section 5. — The Anopheles Community.[86]

The total number of anopheles in a village and its immediate surroundings is the *anopheles population*. The number of anopheles per person, per room or per house is the *anopheles density*.

Enumeration of the proportion of males and females, of nulliparous and multiparous females, of females of different classes of ovarian development, of fed and empty females, of those unfertilised, etc., gives the *anopheles population composition*. The number of females entering the area nightly from outside is the *nightly influx*, and the number leaving for oviposition, etc., the *nightly efflux*. The number of newly hatched mosquitoes (stage 1 ovaries) entering the area is the *nightly newcomer influx*. The redistribution of the anopheles population following various movements of the individual insects at night is the *nightly turnover*.

[84 a] Roubaud uses the term *asthenobiose* for a condition of temporary biological abeyance resembling hibernation or æstivation, but occurring independently of temperature or humidity as an immediate cause. Insects showing such a state in any stage of their developments are also termed *heterodynamous*, as contrasted with *homodynamous* where no such state occurs.

[85] It has not been shown that, in the case of anopheles, actual modification of the life processes takes place in hot dry conditions, though some specialisation in habits may possibly occur under such conditions.

[86] All terms in this section should strictly be applicable to the conditions for a particular species only, since there is no guarantee that all species present are in the same biological state or environment.

Where different species enter habitations, etc., at different times of the night, this has been termed *crepuscular succession.*

Sub-section 6. — Relation to Infection.

(a) *Stages of the parasite in the mosquito.*

On entering the midgut, the gametocytes, male and female, undergo respectively *exflagellation* and *maturation* — *i.e.*, the setting-free of the *flagella* or *microgametes* and extrusion of polar bodies (?) and other changes by which the macrogametocyte becomes the *macrogamete.* On *fertilisation*, the macrogamete undergoes further changes, becoming the *ookinete (travelling vermicule).* The ookinete in turn, after passing through the gut wall, becomes the *oocyst.* Within the oocyst are developed the *sporozoites.*

The term zygote (strictly all the products of sexual union) was formerly in use to designate the oocyst, but it is not now used in this sense, except often in inadvertence. Certain more or less apparently distinct subdivisions of the contents of the oocyst were formerly thought to be the sporoblasts. It is doubtful if this term is correct and in any case the subdivision is largely, if not entirely, apparent. It is also possible that the so-called sporozoites may not actually represent this stage as understood in protozoological terminology, since they are suspected to undergo further subdivision in the human body and might thus represent sporoblasts.

(b) *Infection in the anopheles community.*

The percentage of female anopheles caught in nature showing sporozoites on dissection of the glands is the *sporozoite rate.* The percentage showing oocysts on dissection of the midgut is the *oocyst rate.* These rates should normally relate to some specified species. They should also be accompanied by information as to the source of capture — *i.e.*, whether caught in houses or stables. In the case of small villages, uniform admixture of anopheles may, however, usually be assumed.

The percentage of anopheles showing either sporozoites in the glands or oocysts in the midgut has been termed " index of natural infection ". It is doubtful if such an index is desirable owing to the ambiguous nature of the information which may be given by

it. Thus the sporozoite rate is an index of the natural transmissible infection in the anopheles of a locality and the oocyst rate may give an indication of the infectiveness of the human community, whereas the two taken together are less informative. The term " index of experimental infection " appears even less desirable, as experimental conditions differ so widely.

The term " infective density " is applied by Gordon and Davy to the average number of female anopheles found with sporozoites in the glands per room per diem. The term relates to systematic research carried out over a considerable period in which the anopheles density and infective density were determined by house-to-house and room captures. These authors also apply the term " anopheline infective ratio " to the sporozoite rate expressed as a ratio. The term *transmission rate* is sometimes employed and defined as the number of infections inoculated per unit time per 100 persons. All such terms, however, except sporozoite rate and oocyst rate (or the corresponding ratios), are at present more representative of efforts to clarify ideas regarding measurable aspects of malarial studies than of practicable applicability, and their satisfactory use still awaits the collection and study of the necessary data.

Sub-section 7. — Terms applied to Methods of Control directed against the Vector.

Certain terms have come into general use relating to *mosquito control* or *mosquito control measures*. These may be directed against (1) the aquatic stages, *larval control* or *anti-larval measures*, and (2) the adult mosquitoes, adult control or *anti-imaginal measures*.

When control measures are specifically directed against some one or more species of anopheles known to be the most important vectors concerned in transmission of malaria in an area, they constitute *species control* or *species sanitation*.

Mosquito control may further be of a direct mechanical or toxic character, *drainage*, *filling-in*, *paris-greening*, *screening*, use of *insecticides*, etc., or indirect, aimed at bringing about effects which destroy or hinder the insects through change in their biological environment, etc. Methods of the last-mentioned type are

most appropriately termed *naturalistic.*[87] Among naturalistic methods are those aimed at changing the conditions suitable for breeding by mechanical alteration in the nature of the breeding-places or the water in such breeding-places — *e.g.*, *draining*, *sluicing*, *shading* or *clearing*, etc. — by methods bringing about *pollution*, by *altering the salt contents* as by letting in the sea and in other ways. They also include introduction of *natural enemies* (predators or parasitic organisms), efforts to bring about deviation, *zooprophylaxis*, and efforts to reduce pullulation in the summer through operations directed against the insects in winter, *off-season attack*, *winter killing.*

The following is the classification of mosquito control measures recently proposed by Russell and Hackett (Third Intern. Congr. of Malaria at Amsterdam, 1938) :

NATURAL : Restriction of anopheline breeding or contact with man by natural environmental conditions.

ARTIFICIAL : Restriction of anopheline breeding or contact with man through conscious human intervention :

	Against aquatic stages	Against adults
I. *Mechanical* . . .	*e.g.* ditching.	*e.g.* screening.
II. *Toxic*	*e.g.* paris-greening.	*e.g.* insecticide sprays.

[87] The term " naturalistic " to cover such methods has been proposed by Williamson *(vide* Hackett, Russell, Scharff and Senior White, Third Intern. Congr. of Malaria, 1938). The term " biological " is also used to express the idea of control through artificial interference with the normal favourable environment, but more usually refers to control through introduction of natural enemies. The study of plants and animals as they occur in communities in nature constitutes the science of Œcology, but whilst the term œcological is that most usually applied to studies of this kind, the term naturalistic seems most appropriate as applied to methods of control working through changes in the biological environment. As pointed out by Professor Pittaluga, the word " mesological " also occurs in the Oxford Dictionary in this connection. Introduced by Bertillon in 1883, its meaning does not seem to differ essentially from that of œcological and it has never come into general use.

	Against aquatic stages	Against adults
III. *Naturalistic:*		
(a) Chemical . .	(1) pollution of water.	(1) creating repellent barriers of odorous plants.
	(2) changing salt content of water.	(2) administering drugs like sulphur which cause odorous perspiration.
(b) Physical . .	(1) natural fills, warping, silting.	(1) destruction of shelters, clearing.
	(2) sluicing.	(2) creation of plant barrier to flight.
	(3) flooding.	(3) rendering bedrooms or dwellings unattractive as resting-places.
	(4) fluctuating water level.	
	(5) intermittent drying.	
	(6) agitating the surface.	
	(7) stagnating (ponding) or setting water in motion.	
	(8) muddying.	
	(9) shading or clearing.	
	(10) drying or planting.	
(c) Biological. .	(1) natural enemies (predators).	(1) introduction of natural enemies.
	(2) changing of flora and fauna.	(2) deviation by animals.
	(3) off-season attack.	(3) winter killing.

Part II

GLOSSARY*

* *Note.* — Names in capitals are suitable for employment. Those in small letters are less suitable or best disused.

The *Glossary* may also be used as an index to the *Commentary*, the roman and arabic numerals and letters at the end of the definitions giving respectively the section, sub-section, and any further subdivision in which the term is dealt with in the *Commentary*.

Part II

GLOSSARY

GLOSSARY

ACQUIRED IMMUNITY
IMMUNITÉ ACQUISE
See immunity.

ACTIVE (infection)
ACTIVE (Infection)
As contrasted with " latent ". See infection. I, 5.

ADULT
ADULTE
See age group, schizont, parasite rate, spleen rate, imago.

Æstivo-autumnal
Estivo-automnale (Fièvre)
Colloquial name for *falciparum* parasite or infection. I, 2.

AGE GROUP
CATÉGORIES D'AGES
A group composed of those individuals in a community between stated age limits or of a stated age. The way in which age groups are noted differs according to the authors, *e.g.* :

Children in their 1st, 2nd, 3rd, 4th, 5th year . . 0-4 or 0-5
„ „ 6th, 7th, 8th, 9th, 10th year . 5-9 or 6-10

See II, 3.

AGGREGATION OF LABOUR (MALARIA OF)
PALUDISME DES CAMPS
A form of epidemic malaria commonly seen in large engineering works, plantation labour, etc., in the tropics, in which admixture of non-immunes and infected individuals and other favouring factors play a large role. Synonymous with so-called " malaria of opening-up of the soil ". Usually referred to under the name " tropical aggregation of labour ", but is not confined to the tropics. II, 1.

Ague
Fièvres
Obsolete name in England for malaria. I, 1.

ALL SPECIES INFECTION RATE
INDICE TOTAL DES INFECTIONS SPÉCIFIQUES (humaines)
Number of infections found by microscopic examination per 100 persons in a community counting infections by each species of parasite separately ; may be over 100. " Infection rate " of Swellengrebel. Equivalent to the sum

of the *species infection rates* for those species found. See species infection rate. II, 4, *(e)*.

Amount (of malaria)
Degré (de l'infection palustre)
An indeterminate term; see prevalence, incidence, intensity.

ANAUTOGENOUS
ANAUTOGÈNE
See autogenous.

Androphilic
Androphile
See anthropophilic.

ANOPHELES
ANOPHÈLE
Anopheles (Latin): genus of tribes Anophelini; anopheles: term applied to mosquitoes of the tribe Anophelini in a general way as the vector mosquito of malaria, etc. III, 1.

ANOPHELES COMPOSITION
COMPOSITION DE LA POPULATION ANOPHÉLIENNE
The proportion of males and females, fertilised and unfertilised, nulliparous and multiparous females and females in different stages of the gonotrophic cycle, etc. III, 5.

ANOPHELES DENSITY
DENSITÉ ANOPHÉLIENNE
The number of anopheles per person, per room or per house as specified; with less precision, the degree of prevalence of anopheles in a locality or region. III, 5.

ANOPHELES POPULATION
POPULATION ANOPHÉLIENNE
The total number of anopheles in a village or area. Those leaving nightly for oviposition form the *nightly efflux*, new arrivals the *nightly influx*, and the number of newly hatched entrants the *newcomer influx*. Changes of distribution of the individual anopheles occurring nightly within the area constitute the *nightly turnover*. See also: anopheles composition. III, 5.

ANTHROPOPHILIC
ANTHROPOPHILE
Applied to mosquitoes with a tendency to bite man. A relative term carrying often the erroneous implication that species exist with a special desire to feed on man. The true

condition is the existence of a scale of *host preference*, *q. v.*, which varies with the species and locality. The word androphilic sometimes used is objectionable as it signifies loving a human being of the male sex. See also: zoophilie, anthropophilic index. III, 4, *(c)*.

ANTHROPOPHILIC INDEX
INDICE D'ANTHROPOTROPISME
See human blood ratio.

ARRAY
SÉRIE STATISTIQUE
Statistieal arrangement of numerieal values in order of magnitude from which the median or the quartile values ean be readily aseertained on inspection. II, 3.

ASTHENOBIOSIS (Roubaud)
ASTHÉNOBIOSE
Condition of temporary biological abeyance in an arthropod resembling the usual effeets of hibernation or æstivation, but oeeurring independently of temperature or humidity as a direct eause. See also: heterodynamous. III, 4 *(d)*.

ATTACK (Malarial)
ATTEINTE (de paludisme)
The whole period of an aeute phase of malaria. To be distinguished from paroxysm, *q. v.* See also: infection. I, 5.

AUTOCHTONOUS (malaria)
AUTOCHTONE (Paludisme)
Malaria contracted loeally or in a eountry. It may be *indigenous* when normal to the country, or *introduced* when derived from imported gametoeyte earriers as in some parts of the United Kingdom after the war. II, 1.

AUTOGENOUS (mosquitoes)
AUTOGÈNE (Pouvoir)
Applied to species or "raees" of mosquito whieh have the power to reproduee without feeding upon blood in the adult stage (Roubaud). Anautogenous is the ordinary eondition where the mosquito has not this power but requires a blood meal. III, 4, *(d)*.

AVERAGE ENLARGED SPLEEN RATE
HYPERTROPHIÉE MOYENNE

Mean projection in a community of the apex of the spleen where this organ is palpable — *i.e.*, the mean is calculated with reference only to those individuals in whom the spleen is enlarged. To arrive at the value, the spleen is measured in projection beyond the costal border (C.M. or costal margin measurement) or alternatively by distance of the apex from the umbilicus (A.U. or apex umbilicus measurement), the values so obtained added and this figure divided by the number of persons showing enlargement. Synonym with " mean splenic index " of Brumpt, and also with "index of Ross" of Brumpt (not to be confused with the " endemic index " of Ross, *q. v.)*. Symbol : A. For further information, see under " size of spleen " and full account given in the Commentary. See also : average spleen, size of spleen, splenometric index". II, 5, *(a)* and *(c)*, also II, 6, *(d)*.

AVERAGE NEW-BORN INFECTION PERIOD
MOMENT D'INFECTION DES NOURRISSONS

Mean age (in months) at which parasites are first found in the blood of young infants. The period gives a measure of the liability to infection in an area. See also : transmission index. II, 6, *(h)*.

AVERAGE SPLEEN
RATE MOYENNE

Mean projection as determined under average enlarged spleen, but considering all individuals in the community — *i.e.*, the sum of measurements is divided by the total number of persons in the community whether the spleen is enlarged or not. Symbol : A′. See also average enlarged spleen, splenometric index. II, 5, *(a)* and *(c)*, also II, 6, *(c)*.

BATCH
PONTE

All the eggs laid at a single oviposition by a single female as used for examination or breeding-out. III, 3, *(a)*.

Benign tertian
Tierce bénigne

Colloquial name for *P. vivax* parasite or infection ; preferably *vivax* parasite or infection. See *vivax*. I, 2.

BIOTYPE
BIOTYPE
A population consisting of individuals unable to form more than one kind of gamete; natural equivalent of the laboratory pure line stock or strain. Unsuitable term to indicate the recognised varietal forms of *A. maculipennis*. III, 2.

Blood index
Indice parasitaire (humain)
Alternative term for parasite rate.

BREEDING-OUT
ÉLEVAGE (de moustiques)
Raising from eggs, larvæ or pupæ as distinct from "rearing", *q. v.* III, 3, *(a)*.

BREEDING-PLACES
GÎTES (larvaires)
Situations in which anopheles eggs, larvæ or pupæ are found in nature and from which adult mosquitoes are presumed to be derived. These may be *temporary*, when liable to dry up, or *permanent*, also *potential* when dry or not showing larvæ when examined. III, 4, *(a)*.

Cachexia (Malarial)
Cachexie paludéenne
Clinical term (somewhat obsolete) to describe the anæmic state following prolonged malarial infection; formerly liable to be applied to cases of visceral leishmaniasis diagnosed as chronic malaria. I, 5.

CASE (of malaria)
CAS (de paludisme)
Clinical term indicating an individual showing clinical manifestations of malaria. I, 5.

(TRUE) CAUSAL PROPHYLAXIS
PROPHYLAXIE CAUSALE
Elimination of the parasite by drug treatment in the incubation period by destroying the sporozoites or any intermediate stage between these and the trophozoite, so that neither primary attack nor subsequent infection follows; contrasted with *clinical prophylaxis* in which drug treatment merely inhibits clinical manifestations during its continuance, as also with *early treatment* which may destroy the schizogonous forms derived from the sporozoites. I, 5.

Also termed *causative prophylaxis* (See footnote [29 b]).

CHILD
ENFANT

See age groups.

CHROMATIN
CHROMATINE

Characteristically staining nuclear material of the parasite. I, 4.

CLASS
CLASSE

One of the statistical groups into which the individuals in a frequency distribution have been divided (usually on a basis of magnitude) for convenience of tabulation and analysis.

CLINICAL CURE or RECOVERY
GUÉRISON CLINIQUE

Abatement of active manifestations of infection without necessarily actual elimination of such infection. See radical cure. I, 5.

CLINICAL PROPHYLAXIS
PROPHYLAXIE CLINIQUE

See causal prophylaxis.

CLINICAL RELAPSE
RECHUTE CLINIQUE

Relapse with clinical manifestations as distinct from *parasitic relapse*, shown only by reappearance or increase in number of parasites. I, 5.

COLLECTIVE PROPHYLAXIS
PROPHYLAXIE COLLECTIVE

Prophylaxis applied to a population or community as contrasted with *individual prophylaxis*, where the individual himself adopts measures of prevention. I, 5.

CONCOMITANT IMMUNITY (Sinton)
PRÉMUNITION

See infection immunity.

Conveyance
Transport

Alternative term for transport, *q. v.*

CRESCENTS
CROISSANTS

Macro- and micro-gametocytes of *P. falciparum*, also seen in certain malaria parasites of anthropoid apes. I, 3.

Crisis
Crise
Name (Sergent and Parrot, 1935) applied to the invasive attack (primary attack) in an infectious disease. Used also (Taliaferro *et al.)* in the ordinary clinical sense — *i.e.*, fall in numbers of parasites due to acquisition of specific immunity. I, 5.

CYTOPLASM
CYTOPLASME
Characteristically staining "protoplasm" as distinct from the nuclear material of the parasite. I, 4.

DAY-TIME RESTING-PLACES
ABRIS DIURNES
Places in which anopheles rest during the day. See house, stable, shelter, natural resting-place. III, 4, *(c)*.

DECOLORISATION
DÉCOLORATION (des globules rouges)
See red cell changes.

DENSITY
DENSITÉ
See anopheles density, parasite density.

DEVIATION (Animal)
DÉVIATION (animale)
Reduction in the number of anopheles feeding on man due to the presence of attraction to cattle (or other domestic animals). See also : zoophylaxis. III, 4, *(c)*.

DISPERSION
DISPERSION
The spread of anopheles adults from a breeding-place, *active* when due to their own flight, *passive* when carried by vehicles, etc., *transportation* (conveyance), or wind, *wind dispersal.* III, 4, *(b)*.

DISPERSION, Distance of
DISPERSION, Distance de
The distance to which anopheles under normal conditions disperse from their breeding-places, that to which they may extend under special conditions — *e.g.*, prehibernation period — being *long-distance dispersion.* See also : infiltration. Flight range, distance of flight are also used, but are to be distinguished from distance an anopheles can fly — *e.g.*, at a single act of flight. III, 4, *(b)*.

ECDYSIS
MUE
The periodical casting of the cuticle which accompanies growth of the larva and marks the larval stages : see larval stages. III, 3, *(a)*.

EGG
ŒUF
Name of the first stage in the life history : includes the ovum with surrounding follicular structures. III, 3, *(a)*.

EMERGENCE
NAISSANCE
Passing-out of the imago from the pupa. III, 3, *(a)*.

EMPTY (females)
VIDE (Femelle d'anophèle)
Term to distinguish females which neither contain blood in the gut nor have enlarged ovaries, as distinct respectively from *fed* and *gravid* females. III, 3, *(b)*.

ENDEMIC (malaria)
ENDÉMIQUE (Paludisme)
When malaria is constantly present in degree to give a measurable amount of morbidity or splenomegaly. In malariometry, *static* as opposed to *epidemic* malaria conditions. See also : endemicity, endemy. II, 1.

Endemic index
Indice endémique
(of Stephens and Christophers) = parasite rate. (of Ross) = percentage of children showing either parasites or enlargement of the spleen. See section on indices of endemicity (II, 6). Distinct from " index of Ross " of Brumpt, *q.v.*

ENDEMICITY
ENDÉMICITÉ
The degree of prevalence (frequency and intensity) of malarial infection in the natural population of a country or area. See parasite rate, parasite density, spleen rate, average enlarged spleen, average spleen. See section on malaria in the human community. II, 1-6.

ENDEMY
ENDÉMIE
A disease which is constantly present in a region. More specifically in relation to malaria, a condition where infection ceases to be an occasional occurrence and becomes conditioned by regular epidemiological laws involving infection and immunity of the community. See endemicity.

ENLARGEMENT See red cell changes.
(of the red cell)
HYPERTROPHIE
(du globule rouge)

ENLARGEMENT See spleen (size of).
(of the spleen)
HYPERTROPHIE (de la rate)

EPIDEMIC A condition in which a disease attacks
ÉPIDÉMIQUE at the same time and place a large number of persons, such number being markedly above that normal to the area. See epidemic malaria.

EPIDEMIC (of malaria) A marked and relatively rapid increase
ÉPIDÉMIE in the morbidity or mortality due to
(de paludisme) malaria. May occur as an *outbreak* (in a small community), as exacerbation due to the season, *seasonal epidemic*, or as widespread *regional epidemics* or other condition. II, 1.

EPIDEMIC MALARIA The condition in which malaria occurs
PALUDISME in epidemics of this disease. Contrasted
ÉPIDÉMIQUE with endemic or static malaria, *q.v.* II, 1.

EPIDEMIC RISE Increase in total deaths (all causes)
EPIDEMIC FIGURE occurring during the epidemic period
ÉLÉVATION in relation to normal deaths in the same
ÉPIDÉMIQUE population ; shown as a rise in the graph
CHIFFRE of total deaths *(epidemic rise)* or as a
ÉPIDÉMIQUE figure indicating the number of times the epidemic deaths are greater than the normal *(epidemic figure)*. The latter is used in some countries in the mapping of epidemic malaria. II, 3.

EURYGAMOUS Applies to a species (mosquito) in
EURYGAME which fertilisation only takes place in a relatively large space *(e.g.*, several cubic metres) (Roubaud). The reverse condition — *i.e.*, where a species will couple and fertilise in a limited space — is *stenogamy* (Roubaud). III, 4, *(d)*.

FALCIPARUM
FALCIPARUM (Plasmodium)
Specific name (Welch, 1897) for the malignant tertian parasite *(P. falciparum)*. May suitably be used as *falciparum* parasite and *falciparum* infection in place of the colloquial names malignant tertian, subtertian, tropica, etc. I, 2.

FED (females)
GORGÉE (Femelle d'anophèle)
Female anopheles with blood in the gut : see empty females.

FEEDING-PLACE
LIEUX D'ALIMENTATION
The room or stable, etc., where female anopheles obtain their blood meal. III, 4, *(c)*.

FERTILISATION RATE
FERTILISATION RATIO
INDICE DE FÉCONDATION (anophèles)
The proportion of anopheles found in nature with spermatozoa, expressed as a percentage (rate) or as a proportion of unity (ratio). III, 3, *(b)*.

Flight (Distance of)
Flight range
Distance de vol
See dispersion.

FREQUENCY (of infection)
FRÉQUENCE (d'infection)
The proportion of infected persons in a community, as contrasted with the intensity of the infections : see prevalence, incidence.

FREQUENCY DISTRIBUTION
FRÉQUENCE (Tableau de)
A statistical arrangement in which the numbers of individuals or items in each class are given in the order of the numerical magnitude of the class and from which the mean and other statistical values may be computed.

GAMETE
GAMÈTE
Sexual form in its complete state of development : not occurring in the body of the vertebrate host where the sexual forms are the gametocytes, *q.v.* I, 3. Gamete is also used as a cytological or genetic term — *e.g.*, in footnote [76].

GAMETOCYTE
GAMÉTOCYTE
Male and female sexual forms (micro- and macro-gametocytes respectively) before these become the gametes outside the body of the vertebrate host by *exflagellation* or *maturation* respectively. I, 3.

GAMETOCYTE CARRIER
PORTEUR DE GAMÉTOCYTES
An individual in whose blood gametocytes are present and who is thus potentially infective to anopheles. I, 5.

GONOTROPHIC CYCLE
CYCLE GONOTROPHIQUE (des anophèles)
The changes taking place from ingestion of a blood meal to oviposition. III, 3, (*b*).

GONOTROPHIC DISSOCIATION
DISSOCIATION GONOTROPHIQUE (chez un moustique)
A condition seen in partial hibernation in which female anopheles under certain conditions may continue to take blood meals, but without any accompanying development of the ovaries. III, 3, *(b)*.

GRANULATIONS (of the red cell)
GRAINS (de Schüffner)
See stippling, Maurer's spots.

GRAVID (females)
GRAVIDE (Femelle d'anophèle)
See empty (females).

Haemamoeba
Haemamoeba
Generic name (Grassi and Feletti, 1890) for certain parasites: now sunk under *Plasmodium*, *q.v.*

HAEMOPROTEUS
HAEMOPROTEUS
Generic name (Kruse, 1890) given to certain parasites of birds: "Halteridium" of older writers. I, 1.

HAEMOZOIN
HÉMOZOÏNE
See malarial pigment. I, 4.

Halteridium
Halteridium
See Haemoproteus.

HATCHING
ÉCLOSION
(d'un moustique)
Passing-out of the larva from the egg. See also emergence. III, 3, *(a)*.

HENRY'S REACTION
RÉACTION DE HENRY
A sero-flocculation test for malaria. I, 5.

HEREDITARY IMMUNITY
IMMUNITÉ HÉRITÉE
See inherited immunity.

HETERODYNAMOUS
HÉTÉRODYNAME
Species of which one of the generations of the life-history cycle exhibits asthenobiosis, *q.v.* Species not showing such a condition are *homodynamous* (Roubaud).

HETEROLOGOUS (strain)
HÉTÉROLOGUE (souche)
A strain A is heterologous to another strain B when it possesses different immunological properties. That is, strain B can superinfect animals already infected with strain A and *vice versa*. Strain B in this case may be either another species of parasite or another strain of the same species. See also homologous. I, 5.

HOMODYNAMOUS
HOMODYNAME
See heterodynamous.

HOMOLOGOUS
HOMOLOGUE
Strains which are immunologically similar — *i.e.*, which produce immunity against each other and so fail to superinfect. See superinfection, also heterologous. I, 5.

HOST
HÔTE
A parasitised or fed-upon organism; parasitised red cell; mammalian species from which anopheles obtain blood. III, 4, *(c)*.

HOST PREFERENCE
HOST OF PREDILECTION
PRÉDILECTION (pour un hôte)
The degree of preference which any species, variety or strain of anopheles may exhibit for different vertebrate hosts. The animal most preferred is the *host of predilection*. III, 4, *(c)*.

HOUSE
HABITATION
Any human habitation as distinct from stable or shelter, *q.v.* III, 4, *(c)*.

HUMAN BLOOD RATIO
INDICE D'ANTHROPOPHILIE
The proportion of any species or variety of anopheles which under particular circumstances gives a positive precipitin reaction for human blood, the blood meal being that taken the previous night. Also termed *anthropophilic index.*

HYPERENDEMICITY
HYPERENDÉMICITÉ
An epidemiological term applied to the condition when few in the community escape malarial infection so that the spleen rate is permanently over 50 %. See also : post-epidemic hyperendemicity. II, 1.

IMAGO
IMAGO
Correct entomological name for the completely developed stage of the mosquito ; often termed adults by malariologists.

Immaculata
Immaculata (Haemamoeba)
Specific name (Grassi and Feletti, 1892). Synonym of *P. falciparum.* I, 2.

IMMUNITY
IMMUNITÉ
A refractory state which prevents infection, reinfection or superinfection, or which maintains the number of parasites at a reduced level, or which reduces the clinical effects of infection. Such refractory state may be due to " natural ", " species " or *innate* immunity (résistance innée of Sergent and Parrot) independent or previous or existing infection, or the result of previous or existing infection, *acquired* immunity. Acquired immunity may be due to the presence of antibodies or other mechanism independent of continued infection as a cause and often persisting for a long time following such infection, *true immunity* of Sergent, *residual immunity* of Sinton, or may be conditioned by the continued presence of the infecting organism, *infection immunity (premunition* of Sergent and Parrot). Acquired immunity may also be due to the presence of antibodies produced by another animal, as after injection of an immune serum, *passive* immunity. See also infection immunity, residual immunity, transmitted immunity, also infection. I, 5.

IMPORTED (malaria)
IMPORTÉ (Paludisme)
Malaria occurring in an area in persons infected outside such area. See also autochtonous malaria. II, 1.

INCIDENCE
FRÉQUENCE
A general term usually indicating frequency rather than intensity of infection. With more precision, *morbidity rate* (clinical), *infection rate* (epidemiological). II, 3.

INCUBATION PERIOD
INCUBATION (Période d')
Clinical term designating the period of latency between the initial infection and the first clinical effects or appearance of parasites as the case may be.

See latency. I, 5.

INDEX
INDICE
Term applied to a measurement of one type of value used to measure another type of value; thus the spleen rate is a measure of the prevalence of splenomegaly, but might be an index of malaria prevalence (endemicity). See splenometric index, Macdonald's index, transmission index. See also : rate, ratio. II, 2.

Index (of natural infection), (of experimental infection)
Indice d'infection (naturelle ou expérimentale) (anophèles)
Terms applied respectively to the proportion of anopheles in nature showing sporozoites in the glands or oocysts on the gut, and to the proportion of anopheles showing evidence of infection under experimental conditions. III, 6, *(b)*.

Index casanier (Brumpt)
Indice casanier
A suggested index giving ratio of natural to experimental infection rate. See footnote [83a]. III, 6, *(b)*.

Index of Ross (Brumpt)
Indice de Ross
A weighted mean of spleen size — *viz.*, average enlarged spleen. Not " endemic index of Ross ", *q.v.* Also termed " indice splénique moyen " (Brumpt).

INDIGENOUS (malaria) *INDIGÈNE (Paludisme)* Autochtonous malaria natural to an area or country, as distinct from autochtonous malaria contracted from imported gametocyte carriers. See autochtonous malaria, imported malaria. II, 1.

INDIVIDUAL PROPHYLAXIS *PROPHYLAXIE INDIVIDUELLE* See collective prophylaxis.

INDUCED MALARIA *PALUDISME PROVOQUÉ* Malaria artificially brought about for the purpose of malariotherapy (or experimentation).

INFANT *NOURRISSON* Children of age group 0-1. See Commentary for method of stating age groups, and terms used in this connection. II, 3.

INFECTION *INFECTION (humaine)* The condition of having parasites in the body, whether indicated by clinical effects, or the finding of parasites in the blood, *active* infection or, in the absence of such indications, *latent* infection. See latent, isodiagnosis, splenectomy, xenodiagnosis. I, 5.

INFECTION IMMUNITY *PRÉMUNITION* A refractory condition dependent on an immunity arising from existing infection which prevents further infection as a result of inoculation of an homologous strain. Commonly known as *premunition.* A condition in which the infection and immunity processes are in balance so that infection may be *latent* (unaccompanied by clinical or parasitological evidence) at one time and *active* (during attacks, relapses) at others. Also called *concomitant immunity* (Sinton). See also immunity. I, 5.

INFECTION RATE (human community) *INDICE D'INFECTION (humaine)* The actual number of persons infected. In this sense, better as true infection rate, *q.v.* Also used for the number of separate infections in 100 individuals — *i.e.*, all species infection rate.

INFECTION RATE (human community)
INDICE D'INFECTION SPÉCIFIQUE (humaine)
Percentage of total infections which are due to a given species of parasite — *e.g.*, *vivax* infection rate.

INFECTIVE
INFECTANT
A host in a state capable of giving rise to infection of a vector through possessing gametoeytes in the blood, or a vector possessing sporozoites in the glands or blood capable of giving rise to infection when parenterally introduced. I, 5.

INFECTIVE DENSITY (Gordon and Davy)
Number of female anopheles found with sporozoites in the glands per room per diem. III, 6, *(b)*.

Infective ratio
Indice sporozoïtique
See sporozoite ratio.

INFESTATION
INFESTATION (humaine)
Term applied to the presence of non-microbie parasites in contradistinction to infection applied to microbic (protista or bacteria) parasites. I, 5.

Infestation index (Gill)
Indice d'infestation
Mean positive parasite eount of 200 fields of a thin film multiplied by 10. II, 4, *(c)*.

INFILTRATION
INFILTRATION
Passage of anopheles into an area by an unknown succession of steps.

INHERITED IMMUNITY
IMMUNITÉ HÉRITÉE
Term applied to immunity passed from mother to child. This may be (1) hereditary innate immunity, or (2) transmitted passive immunity from the mother, or (3) infeetion immunity (premunition) from intra-uterine infection aequired *via* the placenta. See innate immunity under immunity, also transmitted immunity. I, 5.

INJECTION
INJECTION
Introduction by a parenteral route (subcutaneous, intramuscular or intravenous) of therapeutic material (drugs, vaccines, sera) as contrasted with *inoculation* in which living infective organisms are so introduced.

INNATE IMMUNITY See immunity.
RÉSISTANCE INNÉE

INOCULATION See injection.
INOCULATION

INTENSITY (of malaria) As applied to an individual, (clinically) severity of symptoms, (parasitologically) the numerical value of the infection — *i.e.*, number of parasites per cmm., *parasite count*. As applied to a community commonly morbidity rate, but with more precision *parasite density*, *q.v.*, as against frequency of infection, which are, however, usually correlated values. Applied to liability to contract infection — *i.e.*, malaria outside man, *infective density* (potential of contagion), see also : transmission rate, transmission index. In general, *endemicity*, *q.v.*
INTENSITÉ (du paludisme)

INTERMITTENT With regular intermissions of fever — *i.e.*, tertian, quartan or quotidian periodicity of paroxysms. See intermittent stage. I, 5.
INTERMITTENT

Intermittent fever Obsolete name for malaria. I, 1.
Intermittente (Fièvre)

INTERMITTENT STAGE Later stage of the primary attack in *vivax* infection when the initial fever gives place to regular periodicity of paroxysms ; terminates at first infection latency. I, 5.
STADE INTERMITTENT

INTRODUCED MALARIA See autochtonous.
PALUDISME D'IMPORTATION

INVASION Stage of appearance of first clinical or microscopical signs of establishment of a species or strain in the body following introduction of the parasite. I, 5.
INVASION

ISODIAGNOSIS (Et. Sergent, 1920)
ISODIAGNOSTIC

Inoculation of a known susceptible animal with the blood of another animal to ascertain if the latter is infected. A positive result indicates presence of latent infection. See also splenectomy, xenodiagnosis. I, 5.

Larval (malaria)
Larvé (Paludisme)

Clinical term indicating obscure symptoms other than an attack believed to be manifestations of malaria. Somewhat obsolete.

LATENCY
LATENCE

A condition in which malarial infection is not evidenced clinically by active manifestations or from the parasitological point of view when parasites cannot be detected (or are scanty) by microscopical examination. There is normally a latent period preceding the primary attack, *incubation latency* (procritical phase of Sergent and Parrot), and also a period or periods of latency following upon the primary attack, *infection latency* (metacritical latency of Sergent and Parrot). Incubation latency in absence of the primary attack may continue into infection latency (latence d'emblée of Edm. and Et. Sergent, 1910). I, 5.

LATENT MALARIA
PALUDISME LATENT

See latency.

Laverania
Laverania

Generic name (Grassi and Feletti, 1890) given to the crescentic parasites of man (and birds ?). Now not differentiated from *Plasmodium* in opinion of most authorities. I, 2.

LONG-DISTANCE DISPERSION
DISPERSION À GRANDE DISTANCE

Dispersion which may occur preceding hibernation or otherwise in which distances of several kilometres may be traversed : see dispersion. III, 4, *(b)*.

LONG-TERM RELAPSE
RECHUTE TARDIVE

A relapse occurring after a period of latency lasting several months, and very commonly about nine months, after infection with *P. vivax* and the chief cause of the spring epidemic in Europe. I, 5.

MACDONALD'S INDEX
INDICE DE MACDONALD
The proportion of those children with enlarged spleen that show parasites by microscopical examination. By means of this index an estimation can be made of the *true infection rate* or actual proportion of infected. II, 6, *(g)*.

MACROGAMETE
MACROGAMETOCYTE
MACROGAMÈTE
MACROGAMÉTOCYTE
See gamete, gametocyte.

MALARIA
PALUDISME
Infection of man by various species of the genus *Plasmodium*. Also commonly applied to similar infections in certain animals. I, 1.

MALARIA (LATENT)
PALUDISME (LATENT)
See latency.

MALARIA PARASITES
PARASITES DU PALUDISME
Species of *Plasmodium* infecting vertebrates. The species in man are *P. vivax*, *P. malariae*, *P. falciparum* and *P. ovale*, as also possibly a further species, *P. tenue*. These are known under various colloquial names — *e.g.*, benign tertian, malignant tertian, etc. — but would be more suitable given by the informal use of the specific name — *i.e.*, *vivax*, *malariae*, *falciparum* and *ovale* parasites or infection, as the case may be. I, 2.

MALARIA PIGMENT
PIGMENT PALUDÉEN
Pigment related to, or identical with, a form of hæmatin occurring in the cytoplasm of the different forms of malaria parasites and also in the organs and tissues following destruction of these parasites. Sometimes termed *haemozoin*. I, 4.

Malaria rate (Ross)
Indice paludéen (humain)
True infection rate, *q.v.* II, 6, *(e)*.

MALARIAE
MALARIAE

Specific name (Grassi and Feletti, 1890) for the quartan parasite. May suitably be used as *malariae* parasite or *malariae* infection for the colloquial name of the parasite or its infection. I, 2.

Malarial fever
Palustres, paludéennes (Fièvres)

See malaria.

MALARIOLOGIST
MALARIOLOGY
MALARIOMETRY
PALUDOLOGUE
PALUDOLOGIE
PALUDOMÉTRIE

Terms in general use to indicate respectively those engaged in studying malaria, the study of malaria and quantitative methods applied to such study. I, 1.

Malignant tertian
Tierce maligne

Colloquial name for *P. falciparum* and the infection it gives rise to. Preferably *falciparum* parasite or infection. See falciparum. I, 2.

MATURE SCHIZONT
SCHIZONTE MÛR

See schizont.

MAURER'S SPOTS
TACHES DE MAURER

A form of alteration of the red cell consisting of irregular spotting or flecking produced by *P. falciparum* (and *P. tenue*). To be contrasted with the fine even granulations seen with *vivax* and *ovale*, Schüffner's stippling. The term *Maurer's flecks* has been suggested and the condition might be described as *flecking* as contrasted with *stippling*. I, 4.

MAXILLARY INDEX
INDICE MAXILLAIRE

An entomological character (number of maxillary teeth in any given species, variety, biotype, etc., of anopheles), stated as a mean. III, 2.

MEAN PARASITE COUNT
MOYENNE D'INFECTION (humaine)

The mean of the numerical value of infections in a community expressed as parasites per c.mm. or by other agreed convention and obtained by dividing the sum of all the parasite counts, *q.v.*, by the total number of persons examined. Symbol: P'. Given by $P \times \frac{E}{100}$ where P is the mean positive parasite count and E the parasite rate. II, 4, *(c)*.

MEAN POSITIVE PARASITE COUNT
MOYENNE D'INFECTION DES PARASITÉS (hommes)

The mean obtained by dividing the sum of the parasite counts in a community by the number of those found infected only. Symbol: P. See also mean parasite count. II, 4, *(c)*.

MEROZOITE
MÉROZOÏTE

The young parasites produced by division of a schizont before these become ring forms. I, 3.

Metacritical latency
Métacritique (Latence)

See latency.

MICROCLIMATE
MICROCLIMAT

Temperature and humidity in some small specified locus — *e.g.*, that in the immediate surroundings of an anopheles at rest, as distinct from that of the general atmosphere or even of the room or stable as a whole. III, 4, *(c)*.

MICROGAMETE
MICROGAMETOCYTE
MICROGAMÈTE
MICROGAMÉTOCYTE

See gamete, gametocyte.

MORBIDITY RATE
TAUX DE MORBIDITÉ

Recorded rate of sickness from malaria, usually given as per cent or per mille per annum. Usually compiled from admission rates, attendance rates, etc. II, 3.

MORTALITY RATE
TAUX DE MORTALITÉ

Recorded death rate from malaria, usually recorded per mille per annum. II, 3.

MULTIPAROUS (females)
MULTIPARES
(Femelles d'anophèles)
See nulliparous.

Natural immunity
Immunité naturelle
See immunity.

NATURAL RESTING-PLACES
ABRIS NATURELS
Where anopheles may be found resting in the day in places other than houses, stables or shelters. III, 4, *(c)*.

NIGHTLY TURNOVER
MOUVEMENT NOCTURNE (anophèles)
Redistribution of anopheles which takes place each night due to the efflux and influx of gravid ovipositing and newly emerged adults respectively and other movements within the area. III, 4, *(c)*.

Nuclear vacuole
Vacuole nucléaire
See vacuole.

NULLIPAROUS (females)
NULLIPARES
(Femelles d'anophèles)
Female anopheles which have not passed through an oviposition, as contrasted with *multiparous* females who have oviposited one or more times. III, 3, *(b)*.

OOCYST
OOCYSTE
The stage of the parasite as seen in the gut wall of anopheles. Sometimes termed zygote. III, 6, *(a)*.

OOCYST RATE
INDICE OOCYSTIQUE (anophèles)
Percentage of female anopheles caught in nature showing oocysts in the gut wall. III, 6, *(b)*.

OOKINETE
OOKINÈTE
The mobile vermicule stage following fertilisation of the macrogamete and preceding oocyst formation. III, 6, *(a)*.

OUTBREAK
ÉPIDÉMIE LOCALISÉE
A small explosive epidemic in a community — *e.g.*, among a ship's crew, or in an institution, etc. See epidemic of malaria. II, 1.

OUTPUT (of anopheles)
PRODUCTION ANOPHÉLIENNE
The number of anopheles produced from a breeding-place or area per unit of time. III, 4, *(b)*.

OVALE
OVALE (Plasmodium)
Specific name (Stephens, 1922) for *P. ovale*. I, 2.

P. I. (spleens)
P. I. (Rates)
See spleen, size of.

PALPABLE (spleens)
PALPABLE (Rate)
See spleen, size of.

PARASITE ASYMPTOTE
ASYMPTOTE PARASITAIRE
The level at which a curve plotted from recording the number of fields examined before reaching a positive result becomes horizontal. A form of indication of the true infection rate. II, 4, *(d)*.

PARASITE CARRIER
PORTEUR DE GERMES
An infected individual where the emphasis is on his potential power to become a gametocyte carrier and to be infective. I, 5.

PARASITE DENSITY
DENSITÉ PARASITAIRE
Mean value in a community of parasite infections as shown by the *mean parasite count*, or *mean positive parasite count*, or by the median or quartiles of an array, etc. as opposed to the number in the community infected or frequency of infection. II, 4, *(c)*.

PARASITE FORMULA
FORMULE PARASITAIRE
Statement giving the percentage prevalence of the species of plasmodium found in a blood survey of a community. Synonymous with *relative prevalence* (of parasite species). II, 4, *(e)*.

PARASITE FREQUENCY DISTRIBUTION
TABLEAU DE FRÉQUENCE DES PARASITES
See frequency distribution, array.

PARASITE RATE
INDICE PARASITAIRE (humain)

Percentage of children in age group 2-9 (or other age group selected on local considerations — *e.g.*, 2-15 in Algeria) showing parasites in the blood in a short examination. Also when so designated the rate for any particular age group — *e.g.*, *adult parasite rate*, *infantile parasite rate*, *parasite rate 2-4*, *5-9*, etc. II, 4, *(b)*.

PARASITIC RELAPSE
RECHUTE PARASITAIRE

See relapse.

PARENTERAL
PARENTÉRAL

See injection.

PAROXYSM
ACCÈS

A single "bout" of malaria characterised by a rise of temperature with accompanying symptoms, usually the result of schizogony of one generation of parasites. A schizogonic multiplication unaccompanied by fever may be described as a parasite paroxysm (accès parasitaire). I, 5.

PASSIVE DISPERSION
DISPERSION PASSIVE

See dispersion.

PASSIVE IMMUNITY
IMMUNITÉ PASSIVE

See immunity.

PERIODICITY
PÉRIODICITÉ

The periods (quartan, tertian or quotidian) at which paroxysms occur. Depending on the length of cycle, *schizogonic period*, of the parasite and the number and arrangement of the *generations* of the parasite present. I, 3 and 5.

PLASMODIAL INDEX
INDICE PLASMODIQUE (humain)

See parasite rate.

PLASMODIUM
PLASMODIUM

Generic name (Marchiafava and Celli, 1885) now used to include all the pigmented malaria-like parasites of man, apes, monkeys, bats and birds, with the exception of forms

in birds coming under the genus *Haemoproteus*. Family Plasmodidae, Mesnil, 1903, Sub-order Haemosporididae, Order Coccididae, Class Sporozoa. I, 2.

POPULATION
POPULATION

(1) The human community under examination, (2) the total number of anopheles in a village or area *(anopheles population)*. See age groups, anopheles population, anopheles population composition. III, 5.

POST-EPIDEMIC HYPERENDEMICITY
HYPERENDÉMICITÉ POST-ÉPIDÉMIQUE

A condition of hyperendemicity following upon an epidemic. II, 1.

Praecox
Praecox (Plasmodium)

Specific name (Grassi and Feletti, 1890) given to a parasite of birds and also to the malignant tertian parasite in man. The question of its validity or not for the parasite of man has been the subject of much discussion, see Commentary. For the sake of uniformity, it seems desirable to abandon the name whatever its claims both for the human and bird parasites for which the names *P. falciparum* and *P. relictum* respectively are now in most common use. *Praecox* will thus become a *nomen nudum*. I, 2.

PREMUNITION
PRÉMUNITION

See infection immunity. Used in the passive sense to indicate the condition and in the active sense for the act of conferring such resistance artificially. I, 5.

PREVALENCE (of malaria)
IMPORTANCE (du paludisme)

A general term including *(a)* frequency of infection or its clinical effects and *(b)* intensity — *i.e.*, parasite density (in the human community) or infection potential (in anopheles). II, 1.

PREVENTIVE TREATMENT
TRAITEMENT PRÉVENTIF

Treatment given to an individual to diminish the risk of his infecting others or to a community with a view to reducing the incidence of malaria. Also termed *mass drug prophylaxis*. I, 5.

PRIMARY ATTACK
PREMIÈRE ATTEINTE
Clinical or parasitological manifestations following the usual incubation period after infection with a species or strain of parasite. I, 5.

Procritical phase
Procritique (Stade)
See latency.

PROPHYLAXIS
PROPHYLAXIE
Preventive measures taken to protect a community, *collective prophylaxis*, or measures undertaken by an individual to protect himself, *individual prophylaxis*. See also : *clinical* and *causal prophylaxis*. I, 5.

Proteosoma
Proteosoma
Generic name (Labbé, 1894), given to a *Plasmodium* in birds : *P. relictum* Grassi and Feletti, 1892. I, 2.

PUPA
PUPE
The correct name in mosquitoes for the pre-imaginal stage — *i.e.*, a mosquito in this stage is an active obtected pupa, not a nymph which is not obtected and is a term applied to ametabolous insects — *i.e.*, insects not undergoing complete metamorphosis. III, 3, *(a)*.

Quartan
Quarte
Colloquial name for *P. malariae* or infection by this parasite. See also : *malariae*. I, 2.

RADICAL CURE
GUÉRISON COMPLÈTE
The complete eradication of the infecting parasites from the body of the host by natural or therapeutic means or both combined. See also : clinical cure, spontaneous recovery. I, 5.

RATE
TAUX
A simple and direct proportion expressed as a percentage, or per mille, etc., but usually applies to a proportion during a given lapse of time — *e.g.*, death rate *per annum*. See also : index, ratio. II, 2.

RATIO
PROPORTION

A fraction (not a percentage) used to show the magnitude of one quantity in relation to the magnitude of another. Also called a proportion.

REARED
ÉLEVAGE (de moustiques)

Used in contrasts to " bred-out " to indicate that fertilisation of females was also obtained in the laboratory. III, 3, *(a)*.

RECESSION
RÉGRESSION

Disappearance of malaria in certain temporate regions due to natural causes. II, 1.

Recrudescence
Recrudescence

In a general epidemiological sense, applied to renewed or increased incidence of malaria in a region. Employed also by certain authors to designate a relapse occurring at a certain period. See relapse.

Recurrence
Récurrence

See relapse.

RED CELL CHANGES
GLOBULE ROUGE (ALTÉRATIONS DU)

Any effect upon the host cell due to the presence in this of the parasite. Includes *enlargement*, *decolorisation* and certain forms of granulation in the cell brought out by staining — *viz.*, *Schüffner's stippling (stippling)* and *Maurer's dots (flecking)*, *q.v.*; or apparent reduction in size of the cell may occur with *P. malariae* infection and an oval shape and *crenulation* of the cell with *P. ovale*. Some changes are also described in the unstained cell — *e.g.*, the *brassy corpuscles* of some older writers. I, 3.

REGIONAL EPIDEMICS
ÉPIDÉMIES RÉGIONALES

Epidemics of a pandemic character affecting large tracts due to exaggeration of the normal seasonal epidemic.

REINFECTION (RECIDIVE)
RÉINFECTION (RÉCIDIVE)

To reinfect after the original infection has died out or has been eliminated by treatment. Strictly, might be used in the sense of a reinfection with the same strain, but commonly means any

fresh infection in contradistinction to a *relapse* — *i.e.*, renewed activity of the original infection. French authors use récidive (reinfection) and rechute (relapse) in the same sense. I, 5.

RELAPSE
RECHUTE

An attack other than the primary attack occurring during the course of an infection — *i.e.*, a period of renewed active infection occurring during infection latency, as distinguished from a further attack due to a subsequently acquired reinfection. May be accompanied by rise of temperature and other clinical signs *(clinical relapse)* or shown only by reappearance of, or increase in the number of, parasites *(parasitic relapse)*. May be *spontaneous* or *provoked* (see splenectomy). Relapses have been variously distinguished by different authors as recurrence, recrudescence or relapse in relation to the time at which such attacks occur. According to James, a relapse which occurs in the first six weeks following recovery from the primary attack is designated a recrudescence, one later than eight weeks and earlier than twenty-four weeks a relapse, and one later than twenty-six weeks a recurrence *(i.e.*, a long-term relapse as here used). See also : long-term relapse. I, 5.

RELATIVE PREVALENCE
FRÉQUENCE RELATIVE (d'une espèce de parasites)

See parasite formula. Or, when applying only to one species, the percentage which infections by this species forms of the total infections found by microscopic examination. I, 2.

REMITTENT
RÉMITTENT

Without definite intermissions — *e.g.*, the remittent stage (initial fever) in *vivax* infection and some attacks of *falciparum* infection. I, 5.

RESERVOIR OF INFECTION
RÉSERVOIR DE VIRUS

Parasite carriers, taken as a whole, constituting a source of contamination in a given place. II, 1.

RESIDUAL IMMUNITY
IMMUNITÉ RÉSIDUELLE

Immunity which may be present for a variable time even after disappearance of parasites from the body. See immunity. I, 5.

RING FORMS
FORMES ANNULAIRES (des Plasmodium)
Early stage of the trophozoite. See trophozoite.

SCHIZOGONIC PERIOD
SCHIZOGONIQUE (Période)
The time taken by any species of parasite to complete its schizogonic cycle. I, 3.

SCHIZOGONY
SCHIZOGONIE
Cycle of asexual reproduction. Also applicable to indicate period of breaking-up of schizonts initiating a paroxysm. I, 3.

SCHIZONT
SCHIZONTE
Intracellular asexual form in any stage (strict usage). Restricted in practice to designate asexual forms other than ring forms. There may be distinguished *young* schizonts with undivided chromatin, *medium* forms with two or more chromatin masses the result of schizogonic division, and *mature* (or *adult)* schizonts with the chromatin fully divided (segmenting forms, rosettes, daisy forms or schizonts in the merozoite stage). When chromatin has fully divided with formation of merozoites, the schizont is known as an adult or *mature schizont* (sporulation forms, segmenting forms, rosettes). For pronunciation of the word, see footnote [12]. I, 3.

SCHÜFFNER'S STIPPLING
GRAINS DE SCHÜFFNER
Fine evenly distributed granulations brought out by suitable staining in red cells infected with *vivax*. See also red cell changes, Ziemann's stippling.

SEASONAL EPIDEMIC
ÉPIDÉMIE SAISONNIÈRE
Epidemic due to the normal spring or autumn prevalence. II, 1.

SEQUELÆ
SÉQUELLES
Clinical term. Conditions following upon malarial infection — *e.g.*, anæmia, blackwater fever. I, 5.

SHELTERS
ABRIS ARTIFICIELS

Artificial structures, but neither habitations nor stables *(q.v.)*, in which anopheles adults are found resting. III, 4, *(c)*.

Simple tertian
Tierce bénigne

Colloquial name sometimes used for *vivax* parasite or infection. Preferably *vivax* parasite and *vivax* infection. See *vivax*. I, 2.

SPECIES
ESPÈCE

A concept variously expressed. In practice, zoological or botanical forms which can be differentiated by some constant morphological character or characters. III, 2.

Species immunity
Résistance innée

See immunity.

SPECIES INFECTION RATE
INDICE D'INFECTION SPÉCIFIQUE (humaine)

The percentage of individuals found infected with any given species of parasite — *i.e.*, *vivax infection rate*, *malariae infection rate*, etc. See also : all species infection rate, infection rate. II, 4, *(e)*.

SPECIES SANITATION
SPECIES CONTROL
ASSAINISSEMENT SÉLECTIF

The application of mosquito control measures to one or more species of anopheles known to be the most important vector or vectors in an area, the measures taken depending upon knowledge of its habits and especially of the nature of its breeding-places. III, 7.

SPLEEN (Size of)
TAILLE (de la rate)

Degree of spleen enlargement measured by the projection of the organ on the abdominal wall in relation to the costal margin or umbilicus. The method of measurement employed varies with different authors and will be found fully described in the Commentary. See also: spleen rate, average enlarged spleen, average spleen. II, 5, *(c)*.

SPLEEN RATE
INDICE SPLÉNIQUE

The percentage of children (age group 2-9) showing palpable enlargement of the spleen, or that for other age groups when so specified — *e.g.*, *adult spleen rate*, *spleen rate 2-5*, etc. The term applies in Algeria to age group 0-15 (enfants) as suitable under local conditions to cover immunising period (see Commentary). Taken *(a)* with child standing or *(b)* child recumbent, but accompanied always by statement as to which method is adopted and desirably also by statement of classes of spleen size. Spleens felt only on deep inspiration in the recumbent position are termed P. I. (palpable on deep inspiration) spleens. If an important class (as under some conditions in Europe or the United States of America), comparison of results by the two methods may be misleading unless a statement of spleen classes is also given. (For full account, see Commentary.) II, 5, *(b)*.

SPLENECTOMY
SPLÉNECTOMIE

Removal of the spleen which, in a latent infection, may bring about patent infection otherwise not to be detected. I, 5.

SPLENOMETRIC INDEX (Parrot 1923).
INDICE SPLÉNOMÉTRIQUE

Spleen rate multiplied by average enlarged spleen. Employed as a single figure index to indicate both frequency (S) and also degree (A) of splenomegaly =average spleen $\times$100. Symbol : S$\times$A.

See also : average spleen. II, 6, *(c)*.

SPONTANEOUS RECOVERY
GUÉRISON SPONTANÉE

Recovery without treatment. Sometimes used for natural abatement of clinical effects, sometimes as implying elimination of infection. I, 5.

SPORADIC (malaria)
SPORADIQUE (Paludisme)

Where cases of malaria are too few and scattered to cause any appreciable effect on the community. II, 1.

Sporoblast
Sporoblaste

See sporozoite.

SPOROGONY
SPOROGONIE
Stage of sexual reproduction or development and including that in the mosquito host. I, 3 and III, 6, *(a)*.

SPOROZOITE
SPOROZOÏTE
The final fully developed infecting form of the malaria parasite as seen in the salivary glands of the mosquito (or before or after rupture of the oocyst in this structure or in the hæmocœle). Possibly these are strictly sporoblasts, since they may perhaps undergo further division after inoculation into the mammalian host. The so-called sporoblasts seen at a certain stage in the oocyst are merely appearances simulating such bodies. III, 6, *(a)*.

SPOROZOITE RATE
INDICE SPOROZOÏTIQUE
The percentage of female anopheles showing sporozoites in the glands. If given as a ratio, the term would be *sporozoite ratio*. III, 6, *(b)*.

Sporulation form
See schizont.

STABLE
ÉCURIE
Any form of artificial structure in which animals are kept overnight, as distinct from habitation or shelter *(q.v.)* — *e.g.*, horse stables, cattle sheds, pigsties, etc. III, 4, *(c)*.

STATIC (malaria)
STATIQUE (Paludisme)
Malariometric term indicating a condition where malarial infection in a community is at equilibrium. More or less synonymous with endemic as contrasted with epidemic.

STENOGAMY
STÉNOGAMIE
Characterising a type of mosquito in which coupling takes place in a limited space (Roubaud). See also eurygamous. III, 4, *(d)*.

STEPHENS' AND CHRISTOPHERS' SPOTS
TACHES DE STEPHENS ET CHRISTOPHERS
Called also Maurer's spots *(q.v.)*.

STIPPLING
TACHE

See Schüffner's stippling. Stippling in *P. malariae* brought out by special methods of staining has been termed by James *Ziemann's stippling.* See also : Maurer's spots, red cell changes. I, 4.

SUBSPECIES
SOUS-ESPÈCE
VARIÉTÉ

Status given to a form which has some of the characters of a species — *e.g.*, definitive morphological characters and characteristic area of distribution, but in which the definitive characters are so little apparent that it is thought undesirable to consider it a distinct species and to which a third or trinomial name is given following upon the specific name of the species of which it is a subspecies. Synonymous with *variety*, which is the term used in the *Culicidae* for such a status. See also: variant. III, 2.

Subtertian
Subtierce

Colloquial name (now little used) for *P. falciparum.* See *falciparum.*

SUPERINFECTION
SURINFECTION

A fresh infection produced in an animal already infected with the same organism. Strictly, a superinfection can only occur if the second strain used is heterologous to that causing the original infection, but a small (parasitic) relapse may occur as a result of a subsequent *homologous superinfection* (Sinton and Mulligan). See footnote[26]. I, 5.

TAXIS
TACTISMES

Impulses of a reflex character which follow certain stimuli in insects. The term *tropism* is also applied when this designation is applicable. III, 4, *(d).*

Tenue
Tenue (Plasmodium)

Specific name (Stephens, 1914) given to a form of parasite resembling *P. falciparum*, but believed by some authors to be distinct. I, 2.

TRANSMISSION INDEX
INDICE DE TRANSMISSION

Parasite rate of age group 0-1 (Barber, Rice and Mandekos, 1936). Also termed *infantile parasite rate.* II, 6, *(i).*

TRANSMISSION RATE
INDICE DE TRANSMISSION
Number of infective bites per 100 persons per unit of time : see also infective density, transmission index. III, 6, *(b)*.

Transmitted immunity
Immunité transmise
See inherited immunity, passive immunity under immunity. I, 5.

TRANSPORTATION
TRANSPORT
Passive dispersion of anopheles by vehicles, trains, steamers, etc. Sometimes termed conveyance. III, 4, *(b)*.

TROPHOZOITE
TROPHOZOÏTE
Strictly any asexual growing form. Used in practice in restricted sense as indicating intracellular forms in their early stages of development (ring forms) of which the asexual or sexual nature cannot be recognised with certainty. I, 3.

Tropica
Tropicale (Fièvre)
Name applied to infection with *falciparum* ; preferably *falciparum* infection. I, 2.

Tropical aggregation of labour
Paludisme des camps
See aggregation of labour (malaria of). II, 1.

Tropism
Tropisme
See taxis.

TRUE CAUSAL PROPHYLAXIS
See causal (causative) prophylaxis.

TRUE IMMUNITY
IMMUNITÉ VRAIE
Term employed by Sergent, Parrot and Donatien to designate immunity which remains for a long time after recovery from infection, as seen in many infectious diseases, as opposed to premunition where immunity is dependent on continued infection. See also : residual immunity.

TRUE INFECTION RATE
INDICE D'INFECTION VRAIE (humaine)
The actual number of infected persons in a given community. Deducable only from information of various indirect data — *e.g.*, Macdonald's index, *q.v.* II, 6, *(e)-(g)*.

VACUOLE
VACUOLE
The clear space seen in ring forms and sometimes in larger parasites; nuclear vacuole of authors. I, 4.

VARIANT
VARIANTE
A merely individual, seasonal or freak departure from the usual species characters. See under variety. III, 2.

VARIETY
VARIÉTÉ
See subspecies.

VECTOR
VECTEUR (Anophèle)
A host transmitting infection after developmental stages of the life history of the parasite have been passed in it, as distinct from a merely mechanical carrier of infection. III.

VIVAX
VIVAX (Plasmodium)
Specific name (Grassi and Feletti, 1890) for *P. vivax*. *Vivax* parasite and *vivax* infection may suitably be used in place of the colloquial names benign tertian, simple tertian. I, 2.

Wechselfieber
Obsolete name (German) for malaria. Synonymous with intermittent fever. I, 1.

Wind dispersion
Dispersion par le vent
See dispersion.

XENODIAGNOSIS
(Brumpt, 1914)
XÉNODIAGNOSTIC
Use of a suitable intermediate host to demonstrate the presence of an infection when parasites are too scanty to be detected in the blood by microscopical methods. I, 5.

ZIEMANN'S STIPPLING
ZIEMANN (Taches de)
See stippling.

ZOOPHILIC
ZOOPHILE
Strictly speaking, should only be applied to a mosquito which bites animals to the exclusion of man, but often employed for mosquitoes which merely have a relative preference to bite animals. III, 4, *(c)*.

ZOOPROPHYLAXIS
ZOOPROPHYLAXIE

Attempts to bring about deviation, *q.v.*, as a means of combating malaria. III, 4, *(c)*.

Zygote
Zygote

Name formerly used for the oocyst.

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THE LEAGUE OF NATIONS ANTI-EPIDEMIC WORK IN CHINA IN 1939

INTRODUCTION

It is now over ten years since the League of Nations first received from the Government of the Republic of China a request for expert assistance in connection with the health services of that country. During this period, a close technical collaboration has been continuously maintained in regard to quarantine services, cholera control, general public health services, medical education, hospital administration, etc. In addition, through the Health Organisation of the League, over fifty Chinese medical officers have been given the opportunity of travelling to Europe and America for the comparative study of special branches of medicine and hygiene in a large number of countries.

Shortly after the outbreak of hostilities with Japan, the Chinese Government sent to the League a memorandum emphasising the grave danger of widespread epidemics amongst the civilian population as a result of the fighting, and requested the League to consider what assistance it could send to China for the purpose of meeting the emergency.

The Chinese request was considered by the Assembly of the League in September 1937. The Assembly decided to vote the sum of 2 million Swiss francs for anti-epidemic work in China ; as a result, three mobile units left for China before the end of the year. The work of these units was to reinforce the existing Chinese Health

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Organisation — that is to say, they were to advise and support the competent Chinese technical services, the Chinese authorities being themselves responsible for the work carried out. (1)

In addition to the essential equipment for transport (heavy and light lorries and touring-cars), each unit was provided with the necessary medical and anti-epidemic supplies, including one stationary and three mobile bacteriological laboratories, material for water disinfection, delousing appliances, sera, vaccines, etc.

On arrival in China, No. 1 Unit proceeded to Sian, capital of Shensi, No. 2 Unit to Changsha, capital of Hunan, and No. 3 Unit to Nanning, capital of Kwangsi, and to Canton. Early in February 1938, all three units had reached their stations, had established headquarters and were engaged in making the necessary official contacts. The League units, with the requisite Chinese medical and auxiliary staff, were at once incorporated in the local health

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(1) The personnel of these units was as follows :

No. 1 Unit :

Dr. H. Mooser (Swiss), Professor of Hygiene and Bacteriology at the University of Zurich ;

Dr. H. M. Jettmar (Austrian), Assistant Professor at the University of Vienna ;

Dr. H. Winzeler (Swiss), formerly Medical Officer to the International Himalaya Expedition ;

M. E. Etter (Swiss), engineer, formerly in charge of the construction of railway lines in Persia ;

M. E. O. Landauer (Austrian), sanitary engineer.

No. 2 Unit :

Dr. R. C. Robertson (British), Director of the Division of Pathology, Henry Lester Institute, Shanghai ;

Dr. R. Pollitzer (Austrian), late bacteriologist, Manchurian Plague Prevention Service ;

Dr. E. I. B. Hawes (British), former Assistant at the Aldershot Military Hospital for Infectious Diseases.

No. 3 Unit :

Surgeon-General A. Lasnet (French), late Director of French Colonial Medical Services ;

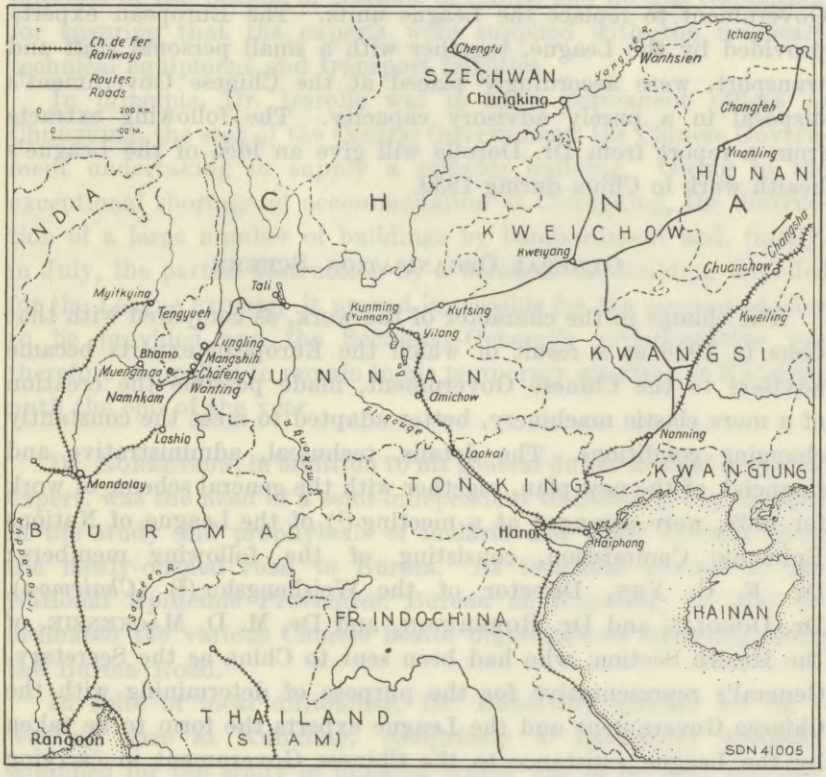
Dr. J. Laigret (French), of the Pasteur Institute, Tunis ;

Dr. P. Dorolle (French), Director of the Municipal Health Service, Hanoi ;

Dr. J. Mauclaire (French).

administration, and, at the same time, they kept in close touch with the Central Government through a special liaison officer.

Meanwhile, large quantities of medical stores, to the value of 500,000 Swiss francs, were purchased by the League in Europe and forwarded to the base in Hongkong, upon which each of the units could draw. The stores included, in addition to large quantities



of drugs, considerable supplies of sera, vaecines, laboratory equipment and disinfectants.

The activities of the units, though varying somewhat according to locality, were devoted chiefly to the establishment of laboratories for vaccine production, the creation of sanitary and health services in the towns and villages, general medical relief work, provision of hospital accommodation, health propaganda, and, in particular, the control of typhus fever, plague, cholera and malaria.

The activities of the units continued steadily throughout 1938, and, in September of that year, a further grant of 1,500,000 Swiss francs was made by the League Assembly for the continuation of the work.

Early in 1939, at the request of the Chinese Government, it was decided that the character of the League assistance should undergo a change. Chinese units had been gradually created by the Chinese Government to replace the League units. The European experts provided by the League, together with a small personal staff and transport, were accordingly placed at the Chinese Government's disposal in a purely advisory capacity. The following extracts from a report from Dr. Dorolle will give an idea of the League's health work in China during 1939.

General Organisation Scheme

The change in the character of the work, as compared with that done in 1938 as a result of which the European experts became advisers to the Chinese Government, made possible the creation of a more elastic machinery, better adapted to meet the constantly changing conditions. The details, technical, administrative and financial, of the new plan, together with the general scheme of work for 1939, were arranged at a meeting [1] of the League of Nations Epidemic Commission, consisting of the following members: Dr. F. C. Yen, Director of the Weishengshu [2] (*Chairman*), Dr. Dorolle and Dr. Robertson, and Dr. M. D. Mackenzie, of the Health Section, who had been sent to China as the Secretary-General's representative for the purpose of determining with the Chinese Government and the League experts the form to be taken by the League assistance to the Chinese Government in carrying out the anti-epidemic campaign during 1939.

In addition to the preparation of the general plan of work for 1939, the execution of which is dealt with in the present report, the Commission defined the duties of the League personnel and also laid down a programme for purchases of medical supplies, mainly in the light of the requirements stated by the Weishengshu.

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[1] Held at Chungking in March 1939.

[2] Department of Health of the Ministry of the Interior.

Generally speaking, and apart from a few modifications in matters of detail due to circumstances, the work of the Mission has proceeded on the lines laid down at the Commission's first meeting.

Dr. DOROLLE, who, from the time of Dr. Mackenzie's departure in April 1939, represented the Secretary-General, had, in addition to his technical duties, to administer the financial and material interests of the League of Nations in China, and he was responsible for ensuring that the experts were supplied with the necessary technical equipment and transport facilities.

In principle, Dr. Dorolle was to have permanent offices at Chungking, the seat of the Central Government, the Chinese Government undertaking to supply a suitable building. Owing to the exceptional shortage of accommodation at Chungking, the destruction of a large number of buildings by bombardment and, finally, in July, the partial destruction by a bomb of the building intended for the League experts, it proved impossible for the accommodation to be provided and the Secretary-General's representative was therefore compelled to remain in his temporary quarters at Kunming until the end of the year.

Dr. ROBERTSON, in addition to his general duties as chief technical expert, was the head of a semi-independent technical group engaged in the study and prophylaxis of malaria and other diseases along the newly-opened road to Burma. As technical adviser to the National Epidemic Prevention Bureau at Kunming, he also co-ordinated the various Chinese health organisations operating along the Burma Road.

In spite of local difficulties, Dr. Robertson quickly set up a working base at Kunming, comprising a laboratory specially equipped for the study of malaria, which was to be used not only for research work, but also for the practical and theoretical instruction of a number of technical workers in the service of the Chinese health organisations. From this base, Dr. Robertson was able to conduct the preliminary work on the Burma Road through a sub-station set up at Lungling and also to make two full and detailed inspections along the road in July and November.

Dr. JETTMAR was appointed technical health adviser to the North-West Epidemic Bureau and to the North-Western Provinces

in the matter of laboratory work and general epidemiology, with regard more particularly to problems connected with typhus and relapsing fever. Furthermore, Dr. Jettmar's veterinary knowledge was to be utilised in connection with the prevention of diseases amongst cattle generally, with particular reference to cattle-plague.

Dr. Jettmar stayed at Sian until the end of May, when the conditions created by the increasing difficulties of communication became a serious handicap to his work. As epidemics were at this time seriously threatening Chungking and the Yangtze Valley, it was decided to recall Dr. Jettmar to Chungking, where he has continued to act as technical adviser for epidemiological and laboratory questions and has carried out research work, chiefly on cholera and malaria.

By mid-November, the epidemic situation at Chungking had considerably improved, and the Weishengshu accordingly asked Dr. Jettmar to remove temporarily to Kunming and there give a series of lectures and practical demonstrations, chiefly on elementary malariology and parasitology, to qualified doctors and sanitary engineers. This practical instruction lasted till the end of the year and, at the beginning of January 1940, Dr. Jettmar left Kunming for Kweiyang, to give a similar series of training lectures.

In view of the wide experience he had gained in 1938 in dealing with epidemiological problems in the provinces of Hunan and Kweichow, Dr. POLLITZER was to occupy the special post of adviser on epidemiological questions to the sanitary administrations, and to the health laboratories of these provinces.

Dr. Pollitzer, whose base remained at Kweiyang until the beginning of September, travelled in the provinces of Hunan, Kweichow and Yünnan, devoting his time chiefly to the control of the annual cholera epidemic.

Early in September, in agreement with the Chinese authorities and with the French railway administration, Dr. Pollitzer made a study of cholera along the Yünnan Railway, in view of the danger of the epidemic spreading along this line. He helped to organise the campaign against cholera in Kunming and, as a member of the Plague Commission, at the end of December he made a difficult tour in a remote area of Yünnan, where it was feared plague existed.

Dr. Pollitzer had barely returned from this tour at the beginning of January 1940, when he was asked to remain provisionally at Kunming and, after Dr. Dorolle's departure on mobilisation, to take temporary charge of the financial and material responsibilities which previously devolved on the latter.

M. LANDAUER acted as adviser to the Weishengshu Practical Instruction Centre at Kweiyang (Public Health Training Institute), for both theoretical instruction and field work. In addition, he visited, from his base at Kweiyang, the various stations of the sanitary engineering units set up in Hunan, Kweichow, Kwangsi and Kiangsi.

The services of Dr. MAUCLAIRE were to be available for the purposes of consultation on sanitary engineering problems, but, as his primary duty, he was placed in charge of transport arrangements. From Kunming, he carried out cholera surveys in various villages along the road to Chungking before being recalled in September 1939 for military duties.

At the meeting of the League Epidemic Commission, it was also agreed to finance a number of Chinese laboratory and engineering units, and to contribute to the maintenance of the Weishengshu isolation hospital at Sian. In addition, five units were financed by League funds : viz., two laboratory units (one for the provinces of Kwangsi, Hunan, and Kweichow, the other in the North-West), a sanitary engineering unit, with headquarters at Kweilin (Kwangsi) and branches chiefly in the provinces of Hunan and Kweichow, and two anti-epidemic units on the Burma Road, designed chiefly to combat malaria.

The personnel of these units included a head (doctor, bacteriologist, or sanitary engineer) with a technical staff (doctors, engineers, sanitary inspectors, nurses) and auxiliary personnel (assistants, coolies, etc.). Each unit was supplied with the material required for its particular work, and sub-units were organised in important centres.

The League also contributed 6,000 dollars towards re-equipping the Kiangsi isolation hospital, which had been badly damaged by air bombardment.

Finally, out of an amount of 30,000 dollars earmarked for sanitary engineering expenditure, a delousing station was operated at Sian

and the cost was met of improvement works (wells, latrines, etc.) under schemes carried out either by the sanitary engineering unit itself or by various organisations controlled by the Weishengshu Anti-epidemic Corps.

The transport material supplied by the League for use in China during 1939 consisted of four heavy lorries, seven light lorries and four touring-cars. The heavy lorries were used on the Kunming-Kweiyang-Chungking line, first for conveying supplies of petrol and spare parts and later for assisting the Weishengshu in transporting medical supplies from the railway terminus to various districts in China. Notwithstanding the increasing difficulties due to the state of the roads, the shortage of petrol and the administrative formalities connected with the establishment of transport monopolies and the liquid-fuel monopoly, this heavy transport service did exceedingly good work and the weight of the medical material transported free of charge for the Weishengshu was about 45,000 kg.

The total petrol consumption amounted to about 8,000 American gallons. This is a large figure, but account must be taken of the state of the roads and the configuration of the territory. Chungking is barely 300 ft. above sea level, but Kweiyang lies at an altitude of more than 3,000 ft. and Kunming at 6,000 ft. The frontier between China and Burma lies at an altitude of about 1,300 ft. and, on the Burma Road and the road from Kunming to Kweiyang, there are passes of between 8,000 ft. and 8,500 ft. which the lorries had to cross regularly.

The League medical supplies in China in 1939 consisted partly of stocks left over at the dissolution of the League units at the end of 1938 and partly of supplies purchased out of the 1939 budget, either in Europe or in China.

The stocks which remained from 1938, and of which it had been possible to redistribute the larger part during the transition period, were used partly for the equipment of the experts, but the greater proportion was handed over to the Weishengshu. These stores, which included large quantities of disinfectants, vaccines, quinine and laboratory equipment, proved of great assistance to the Weishengshu pending the arrival of the supplies purchased by the League.

These supplies, which arrived at intervals throughout the year 1939, in addition to large quantities of other drugs, included

quinine (nearly 2½ tons — *i.e.*, over 7,000,000 tablets of 5 grs. each), powdered quinine, plasmoquine (100,000 tablets), atebrin (1,000 tablets), emetine (700,000 ampoules), and bleaching powder (11 tons).

The laboratory material included the equipment of thirty complete mobile laboratories for the use of the health stations of the Weishengshu, comprising in each case a microscope suitable for all ordinary bacteriological examinations, the requisite glassware, apparatus and instruments for bacteriological, serological, and chemical examinations, and a full supply of chemical products, reagents and stains.

The material sent in 1939 also included 5,400 syringes for anti-cholera vaccination together with 18,000 needles, and the necessary material for saline injections, supplies of heavy oil, paris green, liquid insecticide and sprays, together with large supplies of anti-mosquito netting.

In addition to the stores sent from Europe, the League also purchased a considerable amount of medical material in China itself, including 3 million doses of cholera vaccine, 240,000 doses of smallpox vaccine, and 76,000 cc. of anti-dysentery and anti-meningococcus sera. Through the generosity of the Government of French Indo-China, a gift of a further 1,000,000 doses of cholera vaccine was received.

Anti-epidemic Activities

The epidemic outbreaks with which the Chinese Health Administration had to cope in 1939 were chiefly of malaria, cholera, typhus, relapsing fever, and smallpox. Moreover, preventive measures were required during the year against the introduction of plague into Yünnan. The question of endemic goitre was also studied in Yünnan, together with a number of local problems, such as areas of schistosomiasis infection, etc.

In addition to their work in these fields, the services of the League experts were also utilised by the Government in connection with questions of rural hygiene, refugees, child nutrition, and the reorganisation of such large scientific institutes as the National Epidemic Prevention Bureau.

(a) *Malaria.*

The opening of the new road from Kunming to Burma, some 600 miles in length and running through regions that were formerly almost inaccessible, at once gave rise, as might be expected, to a very serious malaria problem.

More particularly in the western part of its course, the road passes through regions inhabited by non-Chinese tribes which constitute a reservoir of the virus, though they themselves do not apparently suffer severely from the effects of the disease. The importation of Chinese labour in the form of a large number of coolies who were already in a state of diminished resistance and who were, in general, unsatisfactorily housed and badly nourished, led to the development of epidemics of malaria which at one time jeopardised the whole future of the road construction. Later, when the road was opened to traffic, the very high percentage of cases of malaria amongst the drivers and staff of the transport organisations seriously affected their efficiency. There seems no doubt that accidents are frequently due to the weakened condition of the staff as a result of repeated attacks of malaria.

It is not possible here to do more than outline the work which was done by Dr. Robertson and which has been described in his special reports. Briefly, his work was along two lines : in the first place, after a tour of inspection, he drew up recommendations of a general character which were approved by the National Health Administration, and which constituted a basis for the anti-malaria programme ; secondly, thanks to the installation of sub-stations at Lungling and Mangshih, Dr. Robertson was able to undertake a scientific study of local conditions.

The recommendations made by Dr. Robertson may be summarised as follows :

(1) The urgent necessity for the creation of a co-ordinating organisation covering agencies doing medical work along the Burma Road (Government and provincial health organisations and those of the different transport services) ;

(2) The desirability of establishing a control of quinine, in order to avoid the waste entailed by badly organised and unmethodical distributions, which serve only to constitute a financial drain without any corresponding benefit ;

(3) The establishment of a plan of work including :
  (*a*) Surveys and research,
  (*b*) Treatment of cases,
  (*c*) The training of specialised workers,
  (*d*) General sanitary improvements ;

(4) The adoption, as a basis for work to be undertaken, of the recommendations contained in the Fourth Report of the League of Nations Malaria Commission (1) ; and the publication of a summary of that report in Chinese ;

(5) The establishment of four new special hospitals and eight dispensaries, four of the latter being mobile ;

(6) The establishment of four new field laboratories with standardised equipment ;

(7) The establishment of a station for breeding larva-eating fish ;

(8) Drainage and other anti-larval work in the neighbourhood of stations of the transport services, inns, and health centres ;

(9) The erection of all new buildings in non-dangerous zones ;

(10) The establishment of model hutments, protected by wire netting, for workers, and the supply of mosquito-nets to lorry drivers ;

(11) The improvement of the conditions of life and of nutrition generally both for coolies and for the staff of transport undertakings.

The primitive state of the native population (Shan tribes) along the most highly malarial sections of the Burma Road caused considerable difficulties in the investigations, but it was possible, nevertheless, to establish that, of 480 clinical cases observed, 333, or 69%, were due to *P. falciparum* ; 126, or 26%, to *P. vivax* ; and 21, or 4%, to *P. malariae*.

The percentage of infection by *P. falciparum*, moreover, reaches 93% in marshy valleys, such as those of Mangshih and Chafeng.

The endemicity rates were ascertained for children belonging to the local tribes. Of 627 children, 105 were found to be parasite-carriers, and 67 were found to have enlarged spleens.

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(1) *Bull. Health Org.* 1937, 6, 895.

As a result of entomological studies, fourteen species of anopheles were identified, including six which are known to be vectors: *A. minimus*, *A. maculatus*, *A. jeyporiensis*, *A. culicifacies*, *A. hyrcanus sinensis*, *A. annularis*.

The study of the breeding-places of these various species was carried out with the greatest care, and 1,172 dissections of anopheles were made, with the following aggregate results:

	Percentage of infection
A. minimus	9.81
A. maculatus	7.14
A. jeyporiensis	5.94
A. culicifacies	3.84
A. hyrcanus sinensis	3.45
A. annularis	1.57

Of the two Chinese units financed by the League which were working along the Burma Road, one (under Dr. C. H. Tai) had headquarters at Muengmao, a village in Chinese territory to the north of the Burmese village of Namhkam, and was so situated that it was necessary to pass through Burmese territory to reach it from Yünnan. Communications with this unit were therefore very long and difficult. During July, the headquarters of the unit were transferred nearer to the Burmese frontier at Lueng-Tao, in connection with plague prevention work.

Though the main aim of this unit was to combat malaria, it had, in order to gain the confidence of the primitive population unaccustomed to Western ideas of medicine, to undertake a large amount of minor medical work and to attend to all who asked for its help. Treatment was given to the following numbers of malaria patients:

June	33	October	691
July	1,081	November	844
August	2,903		
September	622		6,174

The second unit, under Dr. C. Jung Sun, was first installed at Chafeng, on the Burma Road, some distance from the frontier. Its aim was more particularly to prevent malaria among the workers

and Government transport staff operating the lorry services along the road. The number of individuals who received prophylactic doses of quinine from June to December 1939 was 5,901, and the number of patients treated for malaria 8,168.

Later, in view of the plague situation, the unit was moved to the frontier station of Wanting, where, while continuing to deal with malaria, its principal work was the establishment of a plague quarantine service. As in the case of the first unit, a large amount of ordinary therapeutic work had to be done for reasons of expediency.

A systematic study of malaria was also carried out in an entirely different region by Dr. Jettmar, whom the Weishengshu requested to undertake a malariological investigation in the Chungking district. This investigation was conducted in collaboration with the staff of six health stations, and included clinical studies, systematic examinations of the blood of patients, the determination of the spleen index in school-children, and entomological studies.

Of 921 patients examined, 750 cases were found to be positive, with 643 cases of *P. vivax*, or 86% ; 71 cases of *P. falciparum*, or 9% ; 33 doubtful cases, or 4% ; and 3 cases of mixed infection.

The examination of 359 school-children showed 169 palpable spleens — *i.e.*, 47%. This examination was carried out in twelve different schools, the minimum observed being 37% and the maximum 57%.

Of 4,816 mosquitoes caught, 4,623 were culex and 193 were anopheles. Amongst the latter there were found 53 *A. minimus* and 139 *A. hyrcanus sinensis* — *i.e.*, 28% and 72% respectively. Dr. Jettmar undertook a detailed study of the breeding-places and made laboratory experiments on the destruction of larvæ by their natural enemies (the larvæ of dragonflies, water-spiders, etc.), by means of a colloidal suspension of powdered *pyrethri radix* and by motor-spirit. Details of these experiments are given in his special reports.

(*b*) *Cholera.*

It would appear that the centre of origin of most of the cholera epidemics in Central and South-West China is to be found in Hunan, where an outbreak was studied in 1938 by Dr. Robertson and Dr. Pollitzer. In 1939, as in previous years, it was from this Hunan focus that cholera spread to the province of Kweichow and, from

there, owing to the new and rapid means of communication, to the province of Yünnan. Cholera centres were also reported in the north of Kwangtung. The valley of the Yangtze from Chungking to Ichang was also the seat of a somewhat serious epidemic.

Dr. Pollitzer, early in 1939, studied the cholera epidemic at Yuanling, a town situated on the River Yuan, whose valley appears to constitute the reservoir of cholera viruses in Hunan. When Dr. Pollitzer arrived there, 101 confirmed cases had been treated at the isolation hospital, whilst, at Changteh, some 50 cases had been observed amongst the population. In these two centres, and in various other towns which he visited, his work included the organisation of stations for the purification of river-water (often by the simplest means : viz., the addition of chloride of lime direct to the water-carriers' pails) and the establishment of wide-scale vaccination.

Circumstances were by no means favourable for the collection of exact statistical information, but the number of cases officially reported in Hunan was 1,000.

In June, cholera reached Kweiyang, having been carried there by a traveller who came from Yuanling. The number of known cases at Kweiyang exceeded 800. From Kweiyang, the disease reached most of the places situated on the Kweichow Province roads, and from there spread into the province of Yünnan.

In July, Dr. Pollitzer made a study of the river water at Kweiyang. Twenty specimens examined showed somewhat alkaline pH varying from 7.9 to 8.3. In eight of the specimens, Dr. Pollitzer demonstrated the presence of non-agglutinable vibrios of the cholera type, the vibrio being found in pure culture in one of the samples. In a ninth sample, he demonstrated the presence of a vibrio showing slight agglutinability disappearing after a few passages.

During the month of August, Dr. Pollitzer studied the cholera situation on the road from Kunming to Kweiyang, and proved the existence of foci in six intermediate stations and, at the same time, he gave every assistance to the local health services in stamping out these foci, which were threatening Kunming. Cholera had, indeed, made its appearance at Kunming about the middle of July, the case being that of a lorry-driver who came from Kweiyang ; but the epidemic was to spread chiefly during August, September, and October, with a total of about 500 known cases and a death-rate varying from 35% to 40% at different periods of the epidemic.

There is no doubt that the real number of cases was considerably greater.

A detailed study of the topography of the epidemic was made, and an investigation was carried out concerning the source of the drinking-water used by the patients. A campaign for the disinfection of wells was undertaken, and also a door-to-door vaccination campaign, which covered 57,000 persons out of a total population of about 200,000.

Apart from Kunming, cholera was reported in some twenty centres in the province. The situation became particularly disquieting when the epidemic reached an important centre situated on the railway (Yilang). At that time (late August and early September), Dr. Pollitzer made a study of the situation all along the line and, with the collaboration of the Chinese authorities and the railway staff, he carried out epidemiological investigations and made bacteriological examinations of the drinking-water in the chief stations.

Subsequently, with the object of constituting a basis for study that would be available should cholera make a fresh appearance the following year, Dr. Pollitzer made a study of the drinking-water at Kunming. He examined thirty samples taken from different sources. On the whole, the water from the wells showed a relatively low pH, dropping as far as 6.9, whereas water from rivers and lakes and from piped supplies constantly showed a pH of over 8. Bacteriological examination revealed the presence of *B. coli* in all cases. In no case was the presence of the cholera vibrio demonstrated. In this connection, it should be noted that the examinations were carried out during a very cold season.

Dr. Jettmar dealt with the question of cholera in the Yangtze valley from Chungking to Ichang. In several places on the river bank, particularly Ichang and Wanhsien, he was able to demonstrate the existence of epidemic foci and to furnish bacteriological proof of the nature of the disease. At the request of the Weishengshu, he was called upon to give the requisite advice for the organisation or reorganisation of isolation hospitals in different localities. His laboratory, which at that time was installed in the Chungking Municipal Hospital, devoted part of its activities to the bacteriological diagnosis of cholera in samples sent by the different public and private hospitals of the region.

Statistical information concerning the epidemic in Central China was furnished by M. Landauer. He estimated that, in 1939, 1,087 cases were observed in Hunan, with 251 deaths. The epidemic would appear to have been more widespread but less intense than in the previous year. In that province alone, vaccination would seem to have covered nearly 4 million persons.

In Kwangsi, also according to information collected by M. Landauer, the epidemic appeared about the middle of June, simultaneously in different towns, and increased in intensity until about the end of July, afterwards waning. Aggregate figures are not available, owing to the peculiarly difficult conditions prevailing in this province. All that is known is that about 500,000 anti-cholera vaccinations were effected. The epidemic made its appearance about the middle of July at Kweilin, where some 100 cases were observed. Energetic measures for the disinfection of drinking-water were taken at that time by the sanitary engineering unit maintained by the League of Nations. An extremely severe epidemic was observed at Chuenchow, a small town on the frontier between Hunan and Kwangsi, where, out of 30,000 inhabitants, as many as 50 deaths a day were recorded. There, too, the sanitary engineering unit devoted its efforts to the disinfection of drinking-water and other anti-cholera measures.

(c) *Plague.*

Plague is endemic in the north of Burma among the Shan tribes. During 1939, the movements of population in connection with the opening of the new road to Burma and the importation of labour into the frontier area resulted in a severe outbreak of plague at a point in Burma close to the Chinese frontier (Namhkam). From May to September, 45 cases were observed in Namhkam, with 33 deaths.

Dr. Robertson and Dr. Dorolle were consulted by the Weishengshu as to the measures to be taken and, after a local study, expressed the view that at the time there was relatively little danger of plague being introduced along the Burma Road, the goods imported into China not being of a character to harbour rats or fleas. The position would, however, change if and when the road was used to transport rice, textiles, etc. Danger appears to lie along the old caravan routes and, while the League experts urged the advisability of establishing a quarantine service at the beginning of the Burma Road, they

emphasised the necessity for specially careful anti-plague supervision over the district of Teng-Yueh, which constitutes the end-point of an important caravan route from Bha-Mo in Burma. Further, as a precaution, it was recommended that mobile laboratories specially equipped for the diagnosis and control of plague should be created.

The Chinese unit maintained by the League at Lueng Tao carried out 2,054 vaccinations against plague from August to November 1939. In addition, the unit made an active propaganda among the Burmese and Shans by means of illustrated leaflets drawn up in several languages, explaining the nature of plague and the part played by rats and fleas. This action was supplemented by visits from village to village (about twenty villages being visited each month) and by demonstrations at markets.

The League unit stationed at Wanting carried out quarantine work, consisting mainly in the examination of vehicles crossing the frontier and the vaccination of passengers, particularly during the period when the epidemic was most severe in Burma.

An idea of the extent of the traffic passing over the frontier may be obtained from the following figures :

	Number of vehicles entering	Number of passengers	Number of vaccinations
August	789	1,283	1,581
September . . .	1,006	1,514	1,681
October.	1,113	1,740	1,204

(d) *Smallpox.*

Throughout the year, large quantities of smallpox vaccine were regularly supplied to the Weishengshu.

During the latter part of the year, Dr. Pollitzer was engaged in the control of a widespread epidemic in Kweichow.

(e) *Typhus.*

During 1938, Professor Mooser did a large amount of work in connection with typhus in the North-West, particularly amongst refugees, and his work was continued in the first half of 1939 by Dr. Jettmar.

(f) *Relapsing fever.*

In addition to field work, Dr. Jettmar did a considerable amount of research investigation in connection with relapsing fever and in the serology of this disease.

M. Landauer also, during his tours, especially in the refugee camps, was engaged in the control of relapsing fever and in delousing work.

Supplies for the treatment of relapsing fever were furnished by the League, including 15,000 doses of novarsenobenzol.

(g) *Goitre.*

Goitre is prevalent in all the mountainous districts of Southern China, and more particularly in the province of Yünnan, where it constitutes a serious social problem. In certain remote valleys, percentages as high as 80 or more are not infrequent. The endemic incidence is particularly high in the district through which the Burma Road runs, and Dr. Robertson had occasion to study it while carrying out his investigations in connection with malaria. Dr. Robertson's observations are recorded in detail in his special reports.

As a preliminary step, a Yünnan " Anti-goitre Association " was formed, comprising representatives of the Governor of the Province, the Salt Monopoly Administration, the Provincial Health Director, the Chinese Red Cross Committee, and the Epidemic Commission of the League of Nations. Subsequently, a programme of enquiry was drawn up for seven selected districts in the province, and action was undertaken in two directions.

In the first place, the Salt Monopoly Administration, urged on by a particularly enlightened Director, undertook the difficult task of incorporating sufficient quantities of potassium iodide in the native rock-salt which constitutes the sole supply of sodium chloride in Yünnan. After many difficulties, due to the primitive methods of preparation, it was found possible, through the ingenuity of the Chinese engineers, to establish a method by which, after slight alkalisation of the mother-liquors by sodium carbonate, a solution of iodide is introduced at the moment when the saline solution is on the point of solidifying in the primitive pans used for making blocks of salt. This method is not perfect, but, when 48 mg. of potassium iodide were added per kg. of salt, it was possible to recover 18-25 mg. from the blocks. This process is now adopted in all the salt-mines worked by the Monopoly. In addition, considerable changes in the method of manufacture are contemplated, and these will make the incorporation of the iodine very much easier.

The second part of the programme of work provided for the prophylactic distribution of potassium iodide to school-children and university students.

Dr. Robertson, in co-operation with the Provincial Health Administration, devised a simple method of distribution, which was described in a leaflet published in the Chinese language.

A 25-cg. tablet is dissolved in the quantity of warm water contained in an ordinary rice-bowl (about 500 cc.). The children are given one Chinese spoonful — *i.e.*, about 10 cc. — of this solution once a month. In this manner, they absorb about 5 mg. per month, a quantity well in excess of the daily need of iodine, which is estimated at only 0.04 mg. This simple method is now applied in a great number of schools and universities in Yünnan. Amongst the other medical stores, the League supplied 140 kg. of potassium iodide, which represents 28 million monthly prophylactic doses for children.

The Salt Monopoly Administration has also devoted a part of its profits to the purchasing of large quantities of potassium iodide for iodising the salt which it places on the market. It further contributed to the general cost of the investigation into goitre and the propaganda campaign for goitre prophylaxis.

Nutrition

As a result of the rise in prices and the overcrowding in Kunming caused by the influx of refugees, the nutrition of the children suffered severely. A Committee was therefore formed with the aid of funds from abroad, and Dr. Dorolle was appointed Chairman of the Medical Sub-Committee.

On the lines of the action taken at Shanghai, the Committee established a number of centres for manufacturing and distributing soya milk, prepared according to the usual methods and provided with the necessary mineral ingredients.

National Epidemic Prevention Bureau

Dr. Robertson, a member of the Scientific Training Council of the National Epidemic Prevention Bureau, placed a bacteriological assistant at the disposal of the Director of the Bureau for work connected with the standardisation of sera and vaccines, the preservation of cultures, and the training of the technical staff. During

the period in which Dr. Robertson's assistant was responsible for the standardisation of production, the laboratory produced more than 8,200,000 cc. of vaccine and over 157,000 cc. of various sera. In addition, about 70,000 cc. of emulsions for agglutinations and 6,000 cc. of various antigens were prepared, as well as large quantities of diphtheria and tetanus formol toxoïd.

Sian Isolation Hospital

As stated above, the Epidemic Commission, at its meeting at Chungking, agreed to maintain the Sian Isolation Hospital in view of the danger of typhus and relapsing fever amongst the refugees in this area. The staff consisted of a medical director, three medical officers, a matron, twelve nurses, seven male nurses, six secretaries, and one pharmacist.

The average number of consultations was 651 per month, that of beds occupied daily 136 and the average number of persons admitted 15 per day.

During the first half-year, most of the persons admitted (nearly 80%) were suffering from relapsing fever. Typhus accounted for 10% of the admissions ; next came smallpox (6%) and influenza. There were also a few cases of diphtheria, scarlet fever, meningitis and typhoid fever. During the second half-year, typhus underwent the usual seasonal decline, and relapsing fever fell to 20% ; 40% of the cases treated in hospital were due to typhoid fever, and 30% to acute dysentery. No confirmed cases of cholera were admitted.

As a result of the very intense air bombardments suffered by the city of Sian, the Isolation Hospital had to be moved in June to a distance of about 10 km. from the town, leaving only a consultation and clearing station in the city.

Collaboration with Various Agencies

At the end of November, a mission directed by Dr. L. L. Williams, Senior Surgeon in the United States Public Health Service, was sent to China to co-operate with the National Health Administration in the study of malaria on the Burma Road.

In the course of a number of meetings held at Kunming, Dr. Dorolle and Dr. Robertson supplied this mission with all the

documentary material in their possession regarding the epidemiological situation. In addition, the necessary arrangements were made to supplement the equipment of the mission and to organise accommodation for it at various points along the Burma Road.

Friendly and close co-operation was constantly maintained between the League experts and the Chinese Red Cross Committee, particularly in regard to transport. Thus, all facilities were given to the convoys of that organisation in Kunming, and the League convoys received similar assistance in Kweiyang.

On several occasions, the League mission served as an intermediary between the Chinese medical training establishments on the one hand and the university authorities of Indo-China and the Pasteur Institutes on the other hand. A number of schools of medicine were thus enabled to reconstitute their collections, to acquire educational material, etc.

Conclusions

It may be useful to consider how far the organisation created early in 1939 met the needs for which its help was sought and how far it was able to render the services required of it.

The system of independent units, adopted on the Chinese Government's own proposal in the autumn of 1937, met the requirements of a period of disorganisation resulting from military operations which had deprived China of her capital city. These units undoubtedly rendered excellent service with the greatest possible efficiency, since their efforts were concentrated, under the authority of a responsible head, in a limited area where all the means available were adapted to the object in view.

Though, as an emergency measure, the system of units proved satisfactory, when conditions became more stabilised, certain defects of the system could then be noted.

In the first place, the sphere of action of the units was too restricted. Secondly, the work tended of necessity to be carried out too independently and not always in the direction desired by the Central Government owing to the extreme difficulty of maintaining close contact between the League units and the Central Administration. Lastly, " health expeditions " of this kind, though justifiable in a period of disorder, lost their justification when, after

the first shock, the National Government had resumed control over its various organisations and the latter had again begun to function fairly normally.

The system adopted by the Epidemic Commission in 1939 is, to some extent, a compromise between the formula of purely advisory technical collaboration and the formula of individual units of 1938. In theory, the League of Nations placed technicians in an advisory capacity at the disposal of the Chinese Government. In fact, and in entire agreement with the responsible organs of the Government, those technicians were given certain means of action of their own, a departure made necessary by the existing conditions. The disturbance caused by the war in a country undergoing rapid growth and intensive development, as is the case in China at the present time, made it impossible for the Government organisations to provide the League experts at all times and in all places with the means of transport and action required.

Lastly, through the Epidemic Commission and through the maintenance of a representative of the Secretary-General in China, an organ was set up which was responsible for administrative and financial matters and could, in certain urgent cases, take the necessary authoritative measures on behalf of the Secretary-General. This successful formula was entirely in accordance with requirements, since it relieved the other experts of details of administration, enabled questions to be settled rapidly on the spot, and simplified correspondence.

Thus, the organisation as conceived enabled the technical experts to carry on their advisory duties in full liberty and made it possible for them at all times to render the widely varying services which the Chinese Health Administration expected of them.

In short, it may be said that the organisation which the Epidemic Commission set up, in agreement with the Chinese authorities, proved technically and administratively satisfactory and, at the same time, was found to be sufficiently elastic to adapt itself to changing and difficult circumstances. The Chinese Government has, moreover, expressed its appreciation of the work of the League experts.

THE PREVENTIVE VACCINATION OF DOGS AGAINST RABIES

A CRITICAL REVIEW

by

R. GAUTIER

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The present study was undertaken, despite the difficulties of the moment, in response to the desire expressed by the Health Committee of the League of Nations in November 1939. At the outset, we decided not to deal with the vaccination of dogs *after* they have been bitten — this being an exceptional curative measure which is held in anathema by veterinary surgeons and for which there is no place in the sphere of public health — and to consider only vaccination *before* a bite. The latter is a really preventive measure which is of importance to humanity since its object is to lessen and, ultimately, to remove the risk of contamination of man from dog bite.

This critical review, which is, in fact, much more a review than a criticism, is based, on the one hand, on a bibliographical study of the subject and, on the other hand, on the information kindly furnished by the directors of a series of institutes where canine vaccination is applied.

We have approached the question from its experimental and practical angles, and this has provided the material for the two chapters which make up this study.

I. EXPERIMENTAL WORK

The experimental basis on which the preventive vaccination of dogs rests is very slender. One has only to consult the papers which have appeared since 1920 to realise this. The results they

embody carry little weight because they are drawn from too small a number of experiments. They are, moreover, often discordant and even contradictory and they admit of no comparison since experimental conditions were not similar. These results are, for the most part, already out of date, and they have also frequently been quoted and commented on (1). Accordingly, we need not analyse them again, for this would afford no argument, either for or against canine vaccination, which has not already been advanced. Let us rather seek to ascertain the reasons that might serve to explain the divergences and the lack of comparability.

The vaccines used in experiments on preventive immunisation of dogs are of very varying types: dried cords, dilutions, killed viruses, viruses attenuated to varying degrees by the action of phenol, ether, formol, chloroform or yatren. It is therefore not surprising that differences should have been noted in the antigenic value of these vaccines. But, even if account is taken only of data relating to one single type of vaccine, the results are still divergent. This remark applies, in particular, to the glycero-phenolised vaccine of the Japanese type which is still under discussion, the point at issue being whether an animal can be immunised or not by means of one single injection of vaccine. Japanese scientific workers answer this question in the affirmative. This opinion prevailed at first, but subsequently proved untenable in the light of further experiments. In a recent analysis of the attempts made to immunise dogs by a single injection, WEBSTER (2), who took into account only those experiments which complied with certain criteria, found them inconclusive. Some results are frankly negative, others are doubtful. As for those which are positive, they are in direct contradiction to a whole series of earlier findings. Nevertheless, the Japanese glycero-phenolised vaccine, the prototype of vaccines requiring only one inoculation, has been used on millions of dogs,

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(1) See: LUBINSKI and PRAUSNITZ, *Ergebn. der Hyg.*, 1926, 8, 127; VALLÉE, 1927, doc. L.o.N. C.H. 531; HUTYRA, *Bull. Office intern. des Epizooties*, 1927/28, 1, 475; KRAUS and SCHWEINBURG, *Handb. der Path. Mikroorg.*, 1930, 8, 756; SCHNÜRER and DAVID, *Ergebn. der Hyg.*, 1930, 1, 556; BISANTI, *Bull. Office intern. des Epizooties*, 1931/32, 5, 121; KONDO, *ibid.*, page 131.

(2) *American Jl. of Hyg.*, 1939, 30, 113.

and with results that are sometimes very striking ! There is thus, between the data furnished by the laboratory, on the one hand, and practical application, on the other, a contrast which is, to say the least of it, disturbing.

It seems obvious that a relationship can be established between the degree of attenuation and the differences noted in the immunising power of glycero-phenolised vaccine. During the first experimental period, this vaccine was prepared according to Umeno's method — that is to say, it retained the power of infecting the rabbit by subdural injection. Later, in view of the risk that might attach to the use of a vaccine still possessing a certain degree of virulence — the repugnance shown in some countries with regard to anything that might resemble a live vaccine is well known — a start was made at experimenting with a phenolised vaccine, rendered avirulent, which was still called " Japanese vaccine " although it no longer bore anything more than a distant resemblance to Umeno's original vaccine. The experimental results have, indeed, been different according to whether the vaccine used was virulent or killed. For example, Umeno and Doi (1) in 1921 — soon followed by Eichhorn and Lyon (2) — had succeeded, with a single injection of phenolised vaccine, attenuated but still retaining a certain virulence, in protecting the dog against the intraocular introduction of street virus, whereas Barnes and his collaborators (3), using in 1934 an avirulent phenolised vaccine and the subdural or intraocular route for the introduction of the test virus (street virus), met with nothing but failures. Similarly, Webster and Casals (4), in quite recent experiments, did not succeed in immunising either the mouse or the dog against intramuscular injection of a passage virus by using commercial phenolised vaccines administered in one injection and no longer containing any live virus.

For canine vaccination, as for the preventive treatment of man, one has to face the following dilemma : should one use live or killed vaccine ? This question is important ; it is one that exercises the minds of all rabiologists and, at the request of the Health

(1) *Kitasato Arch. Exper. Med.*, 1921, **4**, 89.
(2) *Jl. Amer. Vet. Assoc.*, 1924, **64**, 690.
(3) *Ibid.*, **84**, 740.
(4) *Jl. of Exper. Med.*, 1940, **71**, 719.

Committee of the League of Nations, Dr. Proca and Dr. Bobes [1] have once more resumed the study of it.

Whatever view may prevail, it cannot be denied that the modifications introduced in the original glycero-phenolised vaccine have not made comparison any easier. It may even be that these modifications are responsible for the divergences that have been noted.

There is, however, a further factor of uncertainty, viz. : the diversity of the tests which have been employed for estimating the resistance conferred on the animal by the vaccine. The severity of these tests has varied greatly according to the nature and the dose of the test virus used (fixed or street virus), and according to the method of its introduction (subcutaneous, intramuscular, intraocular, intralingual or intracerebral). The failure of certain vaccines has been explained by blaming the excessive severity of the tests to which they were subjected. How, then, does the matter actually stand ?

If the test virus is introduced subdurally, the results, taken as a whole, are negative : the animal dies. There are, indeed, a few exceptions, such as Pasteur's [2] classical experiment — in which 5 dogs treated with attenuated cords all resisted a subdural injection of street virus — and the survivals observed by Kelser [3] with the use of chloroform-treated vaccine, by Stuart and Krikorian [4] with phenolised vaccine, and by Okuwada [5] with vaccines of various types. But these are all isolated results which it has not been possible to reproduce.

It has, furthermore, not been proved that the dog can survive the intraocular injection of virus, Cunningham and Malone's [6] experiments not being conclusive.

If, on the other hand, the introduction of the test virus is effected peripherically, the vaccinated animal may resist, as was

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(1) *Bull. Health Org.*, 1940, 9, 79.

(2) *Jl. offic. de la Rép. Fr.*, 1884, 8 août, page 4228.

(3) *Jl. Americ. Vet. Assoc.*, 1930, 77, 595.

(4) *Jl. of Hyg.*, 1929, 29, 1.

(5) *Kitasato Arch. Exper. Med.*, 1933, 10, 279.

(6) *Med. Res. Memoirs*, Calcutta, 1933, No. 26.

indisputably shown by a number of experimental workers [FERMI (1), REMLINGER (2), HARVEY and ACTON (3), KELSER (4) and SHORTT (5)]. Hence, it has been proposed that, for the evaluation of the immunising power of a vaccine, the criterion adopted should be equivalent to the normal risk of infection as the result of a bite — *i.e.*, the intramuscular route. The question therefore arises whether, for canine vaccines, all determinations of their efficacy which are not based on a peripherical test should not be rejected.

In estimating the immunity conferred by a vaccine, it is not sufficient to take account only of the route by which the test virus is introduced. Its toxicity must also be considered, and this is a notion on which the information available is scarce. Whereas in serology attempts have long been made — as is shown by the work of the League's Permanent Commission on Biological Standardisation — to measure the activity of a toxin before bringing it into contact with the anti-serum, in rabiology, where the test virus may, to a certain extent, be assimilated to a test toxin, the question of determining the former's toxicity does not, so far, seem to have been considered. It would nevertheless be possible to conceive of a method by which a titre expressing its toxic power could be assigned to a test virus. If this were done, it would mean that a notion which is exact, because it is quantitative, would for the first time be introduced into the sphere of canine vaccination and valid comparisons would at last be made possible. It is therefore interesting to note that the research work in the field of anti-rabies vaccination which has been carried on for four years by WEBSTER and his co-workers at the Rockefeller Institute for Medical Research in New York is in fact directed towards measuring biological properties, since its object is to devise a method by which it may be possible to arrive at a quantitative determination of the immunising value of vaccines.

To achieve this object, the first step was to find an experimental animal which would be highly receptive to rabies and would react

(1) *Zeitschr. f. Hyg. u. Infektionskr.*, 1908, 58, 233.
(2) *Ann. Inst. Pasteur*, 1919, 33, 616.
(3) *Indian Jl. Med. Res.*, 1923, 10, 1020.
(4) *Jl. Americ. Vet. Assoc.*, 1930, 77, 595.
(5) *Indian Jl. Med. Res.*, 1935, 22, 537.

to infection in a sufficiently uniform manner. The choice fell on a strain of mice of Swiss origin whose sensitiveness to the rabic virus is ten times greater than that of the guinea-pig, the rabbit or the dog. It was then possible to define the minimum lethal dose (M.L.D.) of a given virus by injecting into the brain or the gastrocnemius muscle of these mice successively weaker dilutions of this virus. When the stage had been reached beyond which less than 50% of the animals succumbed, the dilution which gave that result was regarded as containing one M.L.D. [WEBSTER (1)]. Then it was proved that the mice in question could be strongly vaccinated since a single intraperitoneal injection of 10,000 intracerebral M.L.D. enabled them to resist the introduction into the brain of 1,000 M.L.D.

All the necessary elements are now available for a quantitative assay of the immunising properties of a canine vaccine. The technique will be to inject into mice by the peritoneal route one-eighth of the dose of vaccine which is prescribed for the dog and to test the resistance of these mice after three weeks — when the immunity will have attained its maximum — by injecting into the muscles of the neck dilutions of virus containing an increasing number of intramuscular M.L.D. The immunity conferred will be expressed by the number of M.L.D. contained in the dilution of virus which causes the death of less than 50% of the mice.

It was necessary, however, to ascertain whether there was any parallelism between the results of the assay carried out on the mouse and those that would be obtained by using the dog as an experimental animal. WEBSTER (2) was able to show that these results were identical and that, consequently, the mouse test could be used for measuring the immunising power of a vaccine in regard to a dog. This process, moreover, has already been employed by WYCKOFF and BECK (3) for the titration of commercial vaccines. In this connection, HABEL (4) enquired what must be the number of M.L.D. by the mouse test against which an antirabic vaccine should be able to furnish protection. In his view, it is not yet possible to give a final answer to this question. It may, however,

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(1) *Jl. of Exper. Med.*, 1939, **70**, 87.

(2) *Ibid.*, 1940, **71**, 719.

(3) *Jl. of Immunol.*, 1940, **39**, 17.

(4) *Publ. Health Rep.*, 1940, **55**, 1473.

be agreed that, to be of value in clinical use, a canine vaccine should at least protect against 1,000 M.L.D. in the mouse test. There are, in fact, certain vaccines which give protection against 50,000 M.L.D.

There still remained one factor capable of affecting the comparability of the results of vaccination — namely, the diversity of the fixed viruses used for producing vaccines. These viruses are not all derived from the same strain of street virus. They may consequently possess different properties. If they have a common origin, they may have evolved in different directions, since fixity is not an absolute characteristic. For want of anything better, recourse has hitherto been had to emulsions of virus containing nervous substance, whether homologous or heterologous, and this is not merely useless but harmful, since certain post-vaccinal accidents are attributed to the presence of nervous tissue. In the autumn of 1936, however, the culture of the rabic virus was obtained by KANAZAWA (1) in Japan, and by WEBSTER and CLOW (2) in New York. The last-named authors use Tyrode solution to which has been added monkey serum and brain emulsion of mouse embryo. This medium is inoculated with a dilution of mouse brain removed one week after intracerebral virus inoculation. The number of passages now exceeds 100, sub-cultures being made at intervals of three days.

This culture virus offers the following advantages : it contains very little nervous substance, keeps for two months in the refrigerator or in a dry state and, what is still more important in our view, its virulence is uniform. Furthermore, it possesses a very marked immunising power since from 5,000 to 20,000 lethal doses in the mouse test, administered in a single injection, protected 14 young dogs out of 14 against an intracerebral M.L.D. of virus, whereas of the 11 control animals 9 died.

There is still a further method of quantitative evaluation of the antigenic power of a vaccine, the possibilities of applying which have also been explored by WEBSTER (3). This is the sero-protection test on the mouse. Nevertheless, certain reservations must be made

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(1) *Japan Jl. of Exper. Med.*, 1937, 15, 17.

(2) *Science*, 1936, 84, 487.

(3) *Amer. Jl. of Publ. Health*, 1936, 26, 1207.

concerning the value of this method, for, as LÉPINE (1) points out, the rabicide power of the serum, although it accompanies the state of immunity, cannot be regarded as a reliable test of the degree of immunisation. It may be that the rabicide antibodies play a contributory part in the defence of the organism. They are, however, merely an epiphenomenon in the establishment of immunity, which can exist without their presence and which persists long after their disappearance.

It has seemed necessary to dwell somewhat on these recent researches because of the new horizons they open up : possibility of giving a numerical expression to the antigenic power of a vaccine and to the toxicity of a virus ; production of a uniform and stable virus possessing marked immunising properties and which, acting to some extent as a standard virus, may one day perhaps replace the fixed virus-brain emulsions with which we have had to be satisfied hitherto. As HABEL (2) emphasises, the need is felt for a standard virus, possessing a high antigenic value, which can be used for the manufacture of commercial vaccines, both human and canine. There would then no longer be any excuse for putting on the market vaccines which do not possess the required immunising power. But, even if such a virus were distributed to all producing institutes, there would be no certainty that its properties would not change after a certain lapse of time. Hence the need for periodically checking it by ascertaining its specificity, its resistance to chemical agents, its virulence when introduced subdurally and, above all, its immunising power as shown by the mouse test.

* * *

This chapter, which has been kept intentionally brief, reveals an impression of uncertainty. The ground is not solid ; it slips away at each step. Since the experiments are neither coherent nor concordant, they should be taken up once more, if possible with quantitative techniques permitting of the comparison of results and based on the employment of better-defined biological agents.

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(1) *Les ultra-virus des maladies humaines*, 1938, page 458.

(2) *Publ. Health Rep.*, 1940, **55**, 1619.

A start has already been made in this direction. It must be persevered with and confidence must be placed in the laboratory, for the problem does not seem to offer insurmountable difficulties. With skill and method it should be possible to overcome them.

It seems to have been demonstrated that, under certain experimental conditions, the dog may be preventively vaccinated against rabies. But this is not enough. We want to know definitely whether or not it is possible to confer a sufficient degree of immunity on the dog by means of a single injection of vaccine, no matter of what kind, provided its innocuity is certain ! It is on this that the whole problem centres, for, if it is to rank amongst sanitary police measures, the preventive vaccination of dogs must be easy to carry out. Preferably, therefore, it must be possible for it to be effected in a single operation.

II. PRACTICAL APPLICATION

The holding-up of postal communications owing to the present crisis has somewhat handicapped the collection of data intended for inclusion in this chapter. Certain information, such as that relating to the enforcement of the compulsory vaccination of dogs in Venezuela and in Uruguay, could not be verified and has therefore been disregarded. Hence, no claim is made to have dealt exhaustively with the subject of the present study, which has proved to be far-reaching.

The information collected is administrative, legal, statistical and technical in character. In most cases, no comments have been thought necessary, and we have preferred to leave the facts to speak for themselves.

1. Africa

British East Africa.

In 1936, at a conference attended by representatives of the veterinary services of Kenya, Uganda and Tanganyika, the view was taken that, in the present state of affairs, the introduction of compulsory canine vaccination was not desirable. Nevertheless, the conference recommended the continuation of the research work

undertaken in the laboratory at Kabete (Kenya) on the efficacy of an ether-treated vaccine obtained from sheep brain.

Algeria.

The preventive vaccination of dogs was officially authorised in Algeria by the Presidential Decree of December 14th, 1929 (1).

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(1) The following is a translation of the articles of this Decree which relate to the preventive vaccination of dogs :

Article 1. — In order to permit of more effective action against rabies, the preventive vaccination of dogs against this disease before a bite shall be authorised throughout the territory of Algeria.

Article 2. — For the purpose of such vaccination, the formolised anti-rabic vaccine prepared, according to the Plantureux process, by the Pasteur Institute of Algeria shall be used.

Article 3. — None but veterinary surgeons shall be authorised to carry out this vaccination.

Article 4. — The whole cost of vaccination shall be borne by the owners of the animals. The communes shall, however, be authorised to bear such cost for the purpose of enabling vaccination to be carried out on a wider scale in highly contaminated regions.

Article 5. — After each vaccination, or revaccination, the veterinary surgeon shall issue a certificate taken from a counterfoil book. This certificate shall specify the name and address of the owner, and shall give a very full description of the dog. It shall be presented by the owner to the mayor of the commune, who shall affix his visa thereon and shall enter it in a special register. The registration number shall be recorded by the mayor on the certificate. The certificate and the counterfoil thereof, on which the chief provisions of the present decree shall be reproduced, must also bear the signature of the owner of the dog.

Article 6. — All sanitary police measures at present in force shall remain so. The following modifications only shall be made in the regulations concerning the slaughtering of carnivorous animals after a bite. All carnivorous animals which have been bitten or mauled by a rabid animal, or which may have been in contact with such animal, shall be slaughtered immediately, on the order of the mayor, with the exception of dogs that have been vaccinated for the first time more than twenty days and less than one year previously or have been revaccinated less than one year previously. These dogs may be kept by their owners, at their own risk, provided, however, that the animals are revaccinated within a period of seven days after the bite, failing which they shall be slaughtered in the same way as non-vaccinated animals. A certificate

The vaccine, prepared at the Pasteur Institute, Algiers, consisted at first of cerebral matter taken from dogs which had succumbed to inoculation with fixed virus, emulsified at one-tenth and then subjected for two weeks to the action of 4 per mille formol. This vaccine was considered to be avirulent ; it was administered at the initial vaccination in two injections separated by an interval of three weeks, and subsequently once a year for safety. The dose administered varied between 10 and 30 cc. per injection, according to the size of the dog.

As some paralytic accidents were observed in 1931, the degree of dilution of the formol was brought to 6 per mille and the period of contact increased from seven to fourteen days. Furthermore, before being used for the preparation of the vaccine, the brains were kept for a few days in a mixture consisting of equal parts of saline and glycerine. Lastly, the Algiers virus, inoculation with which had on various occasions produced in dogs encephalitic symptoms much more nearly resembling rabies from street virus than rabies from fixed virus, was replaced as from 1932 by the virus with an exclusively paralysing action which is used by Remlinger at Tangiers.

Since then, vaccination has been continued without accidents, the total number of dogs inoculated between December 1929 and July 1940 being 33,500.

Three failures were recorded : these were cases of dogs which had been contaminated without the knowledge of their owners and which had not been revaccinated after the bite, as required by the terms of the decree mentioned above.

From the point of view of collective prophylaxis, mention should be made of the decision taken, in 1932, by a commune (Hussein Dey) in the suburbs of Algiers to bear the cost of the immunisation of dogs. Nearly all the dogs in the commune were then vaccinated or revaccinated, and this led to a rapid decrease in the number

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of revaccination, valid for a year, shall be issued by the veterinary surgeon carrying out the inoculation, and a declaration shall be made to the mayor of the commune as specified in Article 5.

Furthermore, such dogs shall remain for four months under the supervision of the sanitary service and, during that period, they shall not be allowed to go out on the public highway unless they are both held on a leash and muzzled.

of persons obliged to undergo the Pasteur treatment, as is shown in the following table :

Commune of Hussein Dey.

Year	Number of persons treated	
1931	72	
1932 (introduction of canine vaccination)	43	
1933	4	
1934	1	
1935	0	
1936	0	
1937	2	(bitten by a non-vaccinated dog)
1938	0	

Three neighbouring communes very soon followed this example, after calculating that it would be cheaper to vaccinate the dogs than to have to bear the cost of preventive treatment of such natives as might be bitten. In 1939, three other communes also made arrangements for free canine vaccination.

PLANTUREUX (1) considers that the experience of Hussein Dey shows that, even in Algeria, where rabies is frequent and where stray dogs abound, it is possible, through a widespread application of preventive vaccination, in conjunction with sanitary police measures, if not to bring about the complete disappearance of rabies, at any rate to reduce its frequency in a very great degree. The measure was very well received by the owners of dogs and has never given rise to the slightest objection.

As the number of persons treated at the Pasteur Institute at Algiers has continually decreased during the past three years, the question could be raised whether this phenomenon should not be regarded as being connected with the widespread application of canine vaccination. In order to answer this question, Professor Ed. SERGENT, Director of the Pasteur Institute, has been good enough to ascertain, on the one hand, the number of cc. of vaccine issued each year since 1930 for the preventive immunisation of

(1) *Acta Conv. Tert. de Tropicis Morbis*, 1938, I, 612.

dogs, and, on the other hand, the number of persons who have undergone the Pasteur treatment. In his opinion, it is impossible, for the moment, to draw any conclusion from a comparative study of these figures.

Gold Coast.

Owing to the frequency of rabies in this colony, and in order to supplement sanitary police measures, the preparation of a canine vaccine was undertaken in 1936 by the Pong-Tamale Veterinary Laboratory.

At the outset, difficulties arose in connection with the strains of virus, one of which was obtained locally and the other two from Nigeria ; these could not be fixed on the guinea-pig or the sheep, the incubation period growing continually longer. By using a strain that came from Dakar, it was possible to overcome this difficulty.

The vaccine is a 5% fixed virus-sheep brain emulsion in saline phenolised at 6 per mille. It is kept for a month in the refrigerator before use, so that the virus shall be rendered completely inactive. The immunity it confers lasts for about a year, as has been shown by tests effected by the intraocular or intralingual route.

Since 1938, at Accra and in townships of any size, discs for dogs are issued only on presentation of a certificate of vaccination, the price charged for the latter being 2*s.* 6*d.*

Only one failure has been reported, and that was in the case of a dog treated during the incubation period.

In 1938, 1,476 doses of canine vaccine were prepared by the laboratory of the Department of Animal Health. No more vaccine is produced than is strictly necessary, as the manufacture is considered to be " dangerous and unpleasant " !

Morocco.

As has been shown by the Chief of the Stock-breeding Service, Morocco, sanitary police measures, such as compulsory notification, slaughter, muzzling and discs, were almost useless against the " douar dog " [1], which is a definite reservoir of rabies virus. On the other hand, the statistics of the Pasteur Institute at Casablanca showed that, in a high proportion of cases, human beings were contaminated by dogs that were known — that is to

[1] Dog kept by the nomadic Bedouins for the protection of their flocks.

say, dogs that had masters. If, therefore, the owners had their dogs vaccinated, the latter could no longer serve as intermediaries for the transmission of virus as between the douar dog and man.

But the legislator had to be convinced of the advantages of canine vaccination, for this measure would have remained a dead letter if vaccinated dogs had not been exempted from the rigorous measures in force in case of contamination. Following on experiments carried out in Morocco itself, the value of canine vaccination was officially recognised in a Vizirial Order of July 17th, 1928 (1), which prescribes the conditions on which dogs that have been vaccinated and are suspected of contamination may be exempted from slaughter.

The vaccine officially used was, until 1937, the ether-treated virus described in 1918 by Remlinger, and prepared at the Pasteur Institute, Tangiers. The brains of two rabbits which succumbed to the fixed virus — brains which the laboratories usually discard, the cords only being used for the treatment of man — are immersed in ether, remaining so immersed for twenty hours for the " first vaccine " and fifteen hours for the " second vaccine ". The nervous substance thus treated not only retains its immunising power but also becomes friable and mixes very readily with water. Each brain is then emulsified in 40 cc. of normal saline, or of saline formolised at 1/1,800. The vaccine keeps for seven days in summer and for from eight to ten days in winter.

For the preventive treatment, the first vaccine is administered by means of two injections of 20 cc. ; the second vaccine is injected

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(1) The following is a translation of the article in this Order which relates to the vaccination of dogs :

Article 1. — To supplement the provisions of Article 5 of the Vizirial Order of July 29th, 1927, any dogs suspected of contamination with rabies, which, less than a year previously, have undergone preventive vaccination by a method approved by the Director-General of Agriculture, Trade and Colonisation, on the advice of the Chief of the Stock-breeding Service, may, on the written application of their owners, be exempted from slaughter. Owners are required to present their animals for a fresh immunisation within a period of ten days after contamination. They must undertake not to part with them and not to remove them from their usual residence during a period of six months.

The preventive vaccination of dogs against rabies may be carried out only by a veterinary surgeon approved by the Chief of the Stock-breeding Service.

twenty-four hours after, in a similar way. For revaccination, 40 cc. of the second vaccine are inoculated.

The large number of applications for vaccine made it necessary to substitute the dog for the rabbit as the source of supply of virus. The brain of a rabbit weighs from 6 to 8 grammes, that of the dog from 60 to 90 grammes — that is to say, the dog takes the place of ten rabbits. This substitution is, moreover, entirely advantageous ; it facilitates the brain extraction and, if the homologous nervous substance produces reactions, which is rarely the case, they are always much less marked than those which are produced by the nervous tissue of the rabbit.

There is, however, one disadvantage of a practical character connected with the use of the etherised vaccine — namely, the fact that it keeps for such a short time. Accordingly, from 1937 onwards, Morocco adopted a phenolised vaccine perfected by Remlinger. This vaccine, which is completely killed, since it has no effects on a rabbit when introduced subdurally, consists of a 5% fixed virus-dog-brain emulsion in 1% phenolised water, inactivated at 37° C. These phenolised emulsions are used in much the same way as the ether-treated virus, — *i.e.*, four 20-cc. ampoules for vaccination, and two ampoules for revaccination. Veterinary surgeons can obtain the quantities of vaccine necessary for these two types of treatment on payment of 4 and 2 gold francs respectively.

The following table shows how the number of vaccinations and revaccinations effected annually increased between 1928 and 1938 :

Year	Number of dogs and cats vaccinated	Number of dogs revaccinated	Type of vaccine
1928	234	—	Ether-treated virus
1929	519	64	,,
1930	496	163	,,
1931	856	204	,,
1932	879	417	,,
1933	669	503	,,
1934	671	436	,,
1935	715	525	,,
1936	827	555	,,
1937	1,251	727	Phenolised vaccine
1938	1,058	863	,,

Reviewing the results obtained from 1928 up to the end of 1936 with the use of ether-treated virus, REMLINGER and BAILLY [1] state that the failures with this method and the paralytic accidents to which it gave rise " may be counted on the fingers of one hand ", — that is to say, they are practically nil. As regards the phenolised vaccine used from 1937 onwards, it has not produced a single paralytic accident, but there is one failure to be recorded against this method. This was the case of a sheep-dog vaccinated in May 1938 which developed typical rabies in November of the same year without its being possible to ascertain how it had been contaminated. The virus isolated was " reinforced " as compared with the street viruses usually observed in Morocco.

Vaccination has not been made compulsory in Morocco except in the case of the watch-dogs kept by the various military units of the Protectorate, in regard to which it is carried out in accordance with a circular, dated December 22nd, 1930, issued by the General Officer commanding the troops in Morocco.

In the Spanish Zone, on the other hand, vaccination is compulsory and dogs are not allowed to enter the territory unless their owners can present a certificate showing that the dogs have been vaccinated within the year.

We have tried to ascertain whether the number of persons treated at the Pasteur Institute of Morocco (Rabat, and subsequently Casablanca) had decreased since the introduction of the optional vaccination of dogs.

Number of persons who underwent the Pasteur treatment.

Rabat		Casablanca	
1929	443	1934	999
1930	990	1935	1,031
1931	1,007	1936	1,217
1932	754	1937	880
		1938	673

Though a reduction is observed after 1937, it would be premature to regard it as significant.

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(1) *Maroc Médical*, 1938, 18, 243.

Nigeria.

The widespread development of rabies, more particularly in the Plateau Province, from 1930 onwards, led the health authorities to resort to the vaccination of dogs. In 1934, the Vom Veterinary Laboratory tried to fix a local virus for the purpose of preparing a vaccine, but this virus lost its infective power after eleven passages in the case of dogs, and after four passages in the case of rabbits, whereas it remained virulent for goats and sheep.

A vaccine — the type of which we have not been able to ascertain — was nevertheless prepared. It is administered in two injections with an interval of one week.

At Lagos, 94 dogs were vaccinated in 1937. Although the charge made for inoculation is only 6*d.*, the Municipal Health Service considers that even this sum is too high and has taken steps with a view to providing free inoculation.

Tunis.

The practice of the preventive vaccination of dogs is governed by the Beylical Decree of June 23rd, 1931 (1), which lessens the severity of the provisions concerning slaughter in the case of dogs previously vaccinated and which are suspected of having been contaminated.

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(1) *The following is a translation of the text of the Decree :*

Article 1. — Further to the provisions of Article 2 of the Decree of August 4th, 1924, any dogs suspected of being contaminated with rabies which have been preventively vaccinated less than one year previously by a method approved by the Administration, on the recommendation of the Pasteur Institute, Tunis, may be exempted from slaughter. The owner shall be required, for that purpose, to present his animal as soon as possible to a veterinary surgeon who shall subject it to a new immunisation within a period of not more than ten days after contamination. The owner must undertake to keep the dog under observation for a period of six months under the supervision of a veterinary surgeon, and, during that time, he must neither dispose of the dog nor remove it from his habitual place of residence. He must furthermore undertake to report to the veterinary surgeon any noteworthy change in the animal's state of health and present it to him finally at the end of the six-months period of observation.

Article 2. — The preventive vaccination of dogs against rabies may be carried out only by a veterinary surgeon. After this, a certificate shall

The vaccine is prepared at the Pasteur Institute, Tunis, and corresponds to Remlinger's ether-treated virus.

With a view to preventing any possibility of one animal being substituted for another, the "Remarks and advice concerning anti-rabies vaccination" which accompany the above-mentioned Decree contain a recommendation to the effect that the certificates should be very carefully drawn up. For the purpose of establishing the identity of dogs whose coats offer very few distinguishing features — *e.g.*, Alsatians — an imprint of the muzzle might be taken. There is also a reminder to the effect that the ether-treated virus cannot convey rabies by subcutaneous or intramuscular inoculation, that it is not eliminated with the saliva, and that it does not prevent the development of the disease if it is employed more than ten days after contamination.

The following table gives the aggregate results of the preventive vaccination of dogs in Tunis from October 1st, 1931, to December 31st, 1938:

Number of first vaccinations	3,073
Number of revaccinations	1,039
Number of vaccinations after contamination (dogs already preventively vaccinated)	51
Number of rabies cases observed amongst vaccinated dogs in the year following vaccination or revaccination	0

The efficacity of the measure is thus shown to be total. None of the dogs vaccinated contracted rabies, even in cases, which must certainly have been frequent, where revaccination was not carried out, the contamination having passed unnoticed.

Effective encouragement in respect of canine vaccination was given in the city of Tunis by the Municipal Council's decision that vaccinated animals should be exempt from tax as from January 1st, 1938.

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be drawn up giving a very detailed description of the animal and specifying the dates of the preventive inoculations.

This certificate shall be drawn up on a detachable leaf from a counterfoil book, and on the counterfoil shall be reproduced an exact copy of the particulars recorded on the leaf handed to the owner.

Canine vaccination employed on so small a scale (about 400 dogs treated each year) could not, and in fact did not, have any effect on the number of persons who had to undergo preventive treatment, as is shown by the following figures :

Number of Persons treated at the Pasteur Institute, Tunis.

Year		Year	
1930	1,811	1935	1,949
1931	1,686	1936	1,872
1932	1,788	1937	1,800
1933	1,849	1938	1,656
1934	1,930	1939	1,178

2. North and South America

Brazil.

Preventive vaccination of dogs has been practised since 1927 in the State of Espirito Santo. The vaccine, which is of the phenolised type, is prepared by the Rio de Janeiro Experimental Veterinary Station. The number of dogs vaccinated was 1,219 in 1927, 1,352 in 1928 and 1,691 in 1929. According to Torres (1), rabies decreased as a result of vaccination by 63 % in 1927 and 82 % in 1928, as compared with 1925.

In the plan for the reorganisation of the Rabies Prophylaxis Service at Rio, which he submitted in 1937, Penna (2) advocates the compulsory preventive vaccination of all dogs registered, urges that they should be placed under observation if they have run the risk of contamination, and recommends the use of a vaccine of the Semple type — *i. e.*, an avirulent phenolised vaccine. Before advancing this view, Penna asked fourteen Brazilian medical men and veterinary surgeons for their opinions concerning the value of canine vaccination before a bite, and the type of vaccine that should be used. Twelve replies were in favour of compulsory immunisation and seven of the use of an avirulent vaccine.

At Sao Paulo, the Pinheiros Institute began in 1935 to prepare a phenolised vaccine of the Umeno type, but obtained from horse-

(1) *Rev. de zootech.*, 1931, **17**, 129.

(2) *Bol. de Secret. Geral de Saude et d'Assist.*, 1937, **3**, 13.

brain fixed virus. As cases of paralytic rabies occurred as the result of vaccination, Kondo's modification of Umeno's technique was adopted. This modification consists in keeping the vaccine for three days at 37° C., so as to render its attenuation more complete. 1,218 doses of the last-mentioned vaccine were issued in 1939.

Cuba.

As the population of Havana had become alarmed at the frequency of rabies, the health authorities recommended, about 1925, optional canine vaccination as a prophylactic measure. This recommendation, however, was not carried into effect, the number of vaccinations having been very small by comparison with the total dog population.

The cases of rabies that occurred in other municipalities led the Government to take more rigorous and far-reaching measures. The Decree of May 18th, 1926 (No. 690), made canine vaccination compulsory throughout the country. We have not been able to find any information either as to the extent to which this Decree was applied or as to the results obtained through its enforcement. All we know is that the vaccine used was of the phenolised type.

Haiti.

During the fiscal year 1926/27, 367 dogs were preventively vaccinated at Port-au-Prince ; two cases of rabies developed amongst them.

In 1927/28, the number of dogs vaccinated was 347, and all of them remained free from rabies. BROUGHTON [1], who gave these figures, considered that the result obtained was encouraging and that compulsory vaccination should be introduced.

Mexico.

Since 1923, the Anti-rabies Institute of Mexico has directed its attention to the vaccination of dogs by the Japanese method and prepared a phenolised vaccine from a local strain of virus. As the results obtained were satisfactory, a provision making vaccination compulsory was included in the " Regulations on the ownership of dogs in the Federal District ", which were approved by a

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[1] *Jl. Amer. Vet. Assoc.*, 1929, **76**, 921.

Presidential Decree on January 14th, 1926 (1). These regulations provide that every dog-owner must have his dog vaccinated at the time when he registers it, and that the vaccination must be repeated annually. If a vaccinated dog happens to be bitten by a rabid dog, it will be slaughtered unless its owner has it " preventively vaccinated " immediately after the bite.

As, in the Mexican Health Code, canine vaccination is regarded as a federal service, it was incorporated in the duties of the provincial health services and thereby extended to the whole country. As certain professional and commercial interests claimed that they were prejudiced by the fact that the immunisation of dogs had to be carried out in an official institution, an attempt was made to remove this restriction. The Department of Health, however, did not agree with this proposal.

Canine vaccination started officially on July 1st, 1926, the vaccine then being available in sufficient quantity. 37,000 dogs were vaccinated in 1930 at the Anti-rabies Institute of Mexico. In 1932, there were 35,000 vaccinations in the country as a whole.

For reasons of economy, the Anti-rabies Institute of Mexico was attached in 1937 to the Institute of Health, which then made an analysis of the results obtained through the preventive vaccination of dogs. This investigation led to the conclusion that, in the form in which it was previously applied, this measure was " not only completely useless, but dangerous, for it may transform the vaccinated animals into virus-carriers ". This opinion was reported

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(1) The following is a translation of the articles in these regulations which deal with vaccination :

Article 6. — With a view to ensuring that dogs shall remain immune from rabies, their owners shall be required to present them for vaccination at the Anti-rabies Institute at the time of their registration and annually thereafter. For this purpose, the said owners must pay the cost of the vaccines.

The Department of Health shall determine the method of vaccination and shall fix the charge to be made for the vaccines.

Article 10. — Dogs which have been bitten by other dogs suffering from rabies shall be slaughtered, unless their proprietors wish to keep them, in which event they must have them preventively vaccinated, immediately after the bite, at the Anti-rabies Institute, and must pay the cost of the vaccine used.

to the Higher Health Council, which considered it to be well founded. Accordingly, canine vaccination was thenceforth prohibited.

United States of America.

Connecticut, basing itself on the Japanese experience, was the first State to introduce preventive vaccination of dogs, though it did so, indeed, in the form of an optional measure (1922). For each animal inoculated, a special disc was issued — which was often stolen and fixed to the collar of an animal that had not been immunised. In the event of a suspicious bite, the dog vaccinated was not slaughtered, but was placed in quarantine until the Commissioner on Domestic Animals issued a certificate to the effect that it might be released. In 1924, six vaccinated dogs that had been bitten by rabid animals survived.

The example of Connecticut was followed by Massachusetts, where the annual figure for rabid animals had increased from an average of 65 to 465 in 1923. A suggestion was then made that the issue of a dog licence should be made dependent upon the presentation of a vaccination certificate, but this measure was not adopted lest the cost of this certificate, in addition to that of the licence, should prove to be an obstacle to the registration of dogs, which was already very incompletely carried out. In 1925, the city of Milton offered to arrange for dog-owners to have their animals vaccinated in official clinics at a charge of one dollar — the charge made by private veterinary surgeons being from 3 to 5 dollars. If they did this, they would be exempted from the obligation to keep their dogs on a leash. Of 571 dogs registered, 239 were inoculated. One of them died of rabies twenty days after inoculation.

In the county of Los Angeles, 808 cases of canine rabies had occurred in 1923. An order was accordingly issued making the vaccination of dogs compulsory, the dog population being estimated at 50,000. The cost of inoculation was fixed at $2.50. Later, the Public Health Department of California extended this measure to the whole of the State, but did not provide for any exceptional measures in favour of vaccinated dogs in the case of a suspicious bite.

In Chicago, where 7 persons died of rabies in 1927, the Department of Health organised a vast prophylaxis campaign based partly on the optional vaccination of dogs.

In the State of New Jersey, the municipality of Orange issued an order making vaccination compulsory in 1924 ; 900 dogs were treated, and one case of rabies occurred amongst them nine months after inoculation.

In Illinois, 125,000 dogs were vaccinated between 1924 and 1928. Of the 18 cases of rabies which occurred amongst them, 10 developed less than a month after inoculation.

In 1935, South Carolina adopted a law requiring all dogs to be vaccinated and annually revaccinated. The charge made for inoculation was 50 cents, and half of this amount went to the Department of Agriculture to cover the cost of purchasing the vaccine and the other half to the inspector of rabies who carried out the inoculation.

The municipality of Detroit (Michigan) made the presentation of a vaccination certificate a condition for the issue of dog licences as from 1928. Amongst the 28,000 dogs vaccinated that year, 10 cases of rabies (0.36%) were observed, whereas 447 cases (4.48%) occurred amongst 98,000 non-vaccinated dogs. The latter would thus appear to be twelve times more liable to contract rabies than the former.

In 1928, the Surgeon-General of the Army ordered the compulsory vaccination of dogs in all military posts. The results were as follows :

Year	Number of dogs vaccinated	Cases of rabies amongst dogs vaccinated
1929	1,420	2
1930	4,012	3
1931	4,673	0
1932	4,474	9
1933	4,255	1 (?)
1934	4,482	0
1935	4,586	4

Rather than give more such examples — which in themselves are of no great demonstrative value — it is preferable to mention a few general opinions, coming from authoritative sources, as to the prophylactic value of canine vaccination.

In 1928, the Chief of the Bureau of Animal Industry, Washington, basing himself on the experience gained from 1923 to 1927, expressed

the opinion that it was difficult to pass judgment on the efficacity of the measure. This efficacity was certainly not absolute, since failures had been recorded. Such failures were, however, rare by comparison with the number of animals inoculated. In contaminated districts, veterinary surgeons had declared their satisfaction with vaccination, but it was difficult to determine exactly what part of the success was really due to vaccination since other means — the destruction of stray dogs, the issue of discs, muzzles — had been used at the same time.

In the view of OLESEN (1), of the New York City Health Service, canine vaccination had not yet emerged from the experimental stage. Notwithstanding the merits attributed to it by a few enthusiastic advocates, it was impossible at the time (1935) to rely on this measure.

Describing to the " Office international d'Hygiène publique " (1935) the administrative measures applied in the United States in the matter of prophylaxis against rabies, Surgeon-General CUMMING spoke as follows :

> " In a few places, the vaccination of dogs is insisted upon, but, in practice, so far as we are aware, this method has not been the subject of tests sufficiently well controlled to be of any scientific significance. It may be presumed that possibly a fairly high proportion of the dogs thus vaccinated acquired at least a certain resistance to infection, but the frequency, degree and duration of this resistance are not known."

To the question whether it was possible to base action against rabies exclusively on canine vaccination, SCHOENING (2), of the Bureau of Animal Industry, replied in the negative. It would really be necessary to inoculate 100% of the dogs and to have a vaccine that was efficacious in 100% of cases, and these were two conditions which could not, for the moment (1936), be fulfilled. (This requirement is, however, so rigorous that it does not harmonise with the laws of epidemiology. In point of fact, an epidemic comes to an end spontaneously as soon as the proportion of receptive subjects falls below a certain level ; this level varies, moreover, according

(1) *Publ. Health Rep.*, 1935, **50**, 1087.

(2) *Amer. Jl. of Publ. Health*, 1936, **26**, 265.

to the contagiousness of the infection and the conditions of the environment.)

At the forty-first session of the United States Live-stock Sanitary Association (Chicago 1937), its Rabies Committee expressed the opinion that vaccination had not, of itself, given satisfactory results except in districts where the majority of the dog population had been inoculated. The Committee nevertheless decided to encourage preventive immunisation, though it considered that vaccinated dogs should not benefit by any special privileges.

It will be noted that the opinions expressed are, to say the least of it, lacking in enthusiasm. It is true that the results obtained, so far as they can be judged from the documents published, are scarcely convincing. REMLINGER (1) explains this state of affairs by the fact that, in the United States, vaccination, both human and canine, has taken the wrong course, for, at the outset, it was commercialised. In his view, " the most powerful firm is not to be compared with a small laboratory under the direction of a real scientist ". This is a somewhat summary criticism of the producing firms, especially when one takes into account that the anti-rabies vaccines which they issue for the immunisation of dogs are, like all other biological products intended for veterinary use, tested and approved by a special branch of the Bureau of Animal Industry. It must be admitted, however, that some of the vaccines placed on the American market possessed only very slight immunising power, as was shown by BARNES (2) and by WEBSTER (3).

There are, in our opinion, other reasons which might be advanced in an endeavour to explain the somewhat reserved attitude generally adopted in the United States in recent years with regard to canine vaccination. This measure was definitely held in favour when it was first applied, as is shown by the fact that it was made compulsory in a series of States. At that time, however, the vaccines used were almost exclusively of the Japanese phenolised type — 3,700,000 doses of which were prepared between 1923 and 1934 — and it is known that these vaccines were not attenuated to the point of losing their virulence for small laboratory animals when adminis-

(1) *Maroc Médical*, 1938, 18, 243.

(2) *Jl. Amer. Vet. Assoc.*, 1934, 84, 740.

(3) *Amer. Jl. of Publ. Health*, 1938, 28, 44.

tered subdurally. Following on experiments carried out by SCHOENING from 1925 to 1928 concerning the infecting power of commercial canine vaccines, the Bureau of Animal Industry decided to require from producers that the virus contained in vaccines intended for sale should be completely killed. Federal regulations were issued, moreover, to the effect that, before issue, every vaccine must be proved to be avirulent for the rabbit when introduced subdurally — *i.e.*, it must satisfy the most severe of the tests by which its innocuity can be demonstrated.

Later experiments by SCHOENING (1) showed that, when administered by means of a single injection, vaccines which had been rendered avirulent possessed only a very relative immunising power, whether they were phenolised vaccines or the chloroform-treated vaccine of KELSER (2) (33% emulsion of rabbit-brain fixed virus in a 0.75% chloroform solution). The question therefore arises whether the change of opinion which appears to have occurred concerning the value to be placed on the vaccination of dogs by a single injection is in any way connected with the prohibition of the use of vaccines which still contain live virus. The attempt to render the vaccine definitely harmless has apparently at the same time robbed it of some or all of its immunising power. This view is put forward with every reservation, but it would seem to be more plausible than the hypotheses based on the exceptional virulence of the strains employed or even on the existence of "para-rabic" strains as an explanation of the reason why, in American circles, so little faith is placed nowadays in the preventive immunisation of dogs.

The matter is far from being settled, however, for there is a new factor which may have very important consequences : the International Health Division of the Rockefeller Foundation has decided to interest itself, with the powerful means at its disposal, in the question of rabies in general and in that of the vaccination of dogs in particular. It is pointed out, in its annual report for 1936, that it is generally agreed that rabies cannot be held in check by canine vaccination alone. Before a plan of campaign can be drawn

(1) *Jl. Amer. Vet. Assoc.*, 1930, 77, 25, and 1931, 78, 703.
(2) *Ibid.*, 1930, 77, 595.

up, better information will be necessary concerning the value of the immunisation of dogs, the epidemiology of rabies, and the means by which the degree of immunity, whether natural or acquired, may be judged. The State of Alabama, with a dog population estimated at 450,000, has been chosen as the field of action. Compulsory vaccination of dogs was introduced there in 1937 ; 220,000 animals were inoculated that year, and 134,000 in 1938. All that remains is to await the results of this investigation and to express the hope that they will be sufficiently conclusive to put an end to the controversy.

3. Asia

British India.

In 1927, the Kasauli Institute, in its reply to the questionnaire that had been sent to it in connection with the preparations for the International Rabies Conference, pointed out that canine vaccination was hardly practised at all at that time, notwithstanding the research work done by Harvey and McKendrick with attenuated cords (1907) and by Semple with phenolised vaccine (1911), with a view to perfecting the process.

Since 1933, this Institute has been immunising dogs, on a limited scale, it is true — the number of animals vaccinated having been only 515 in 1936, and 453 in 1937. The preventive treatment comprises seven daily injections of a phenolised emulsion of fixed virus from sheep-brain. Of 1,838 dogs thus treated between 1936 and 1938, only one is said to have developed rabies.

In the instructions which it issued in 1936 to the anti-rabies treatment centres in its district, the Pasteur Institute, Coonoor, emphasises the fact that vaccinated animals acquire an immunity which persists for at least six months. The Institute accordingly recommends that, when rabies breaks out in any district, the owners of dogs should have them vaccinated.

Indo-China.

Sanitary police action in Indo-China with regard to rabid dogs was governed by regulations directly based on those in force in France. Owing to local conditions, however — such as the large number of stray dogs, the lack of experienced sanitary agents and

the difficulties encountered in the strict application of the measures prescribed — no appreciable results had been achieved.

In 1932, the Grand Council of Economic and Financial Interests of Indo-China contemplated the advisability of undertaking the preventive vaccination of dogs throughout the territory. By an Order dated January 5th, 1933, the Governor-General instructed a " Rabies Committee " to determine the details of administrative procedure relating to such vaccination, whilst the Pasteur Institutes were asked to select the immunisation method best adapted to local conditions.

At the beginning of 1934, the Pasteur Institute, Nhatrang, placed at the disposal of the Veterinary Services a formolised vaccine, of the same type as the one used in Algeria, which might be considered as no longer containing any live virus. It was recommended that vaccination should, if possible, be carried out by means of two injections, at three weeks interval, but the total dose of vaccine might also be administered in a single injection.

Following on a report by the French Minister of Agriculture, drawing attention to the rate of increase of the number of persons who had to undergo Pasteur treatment in the anti-rabies institutes of Indo-China, the President of the Republic signed a Decree [1]

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[1] The terms of the Decree are as follows :

Article 1. — Preventive vaccination against rabies with the formolised vaccine prepared by the Pasteur Institute, Nhatrang, shall be authorised throughout the territory of Indo-China. None but veterinary surgeons shall be entitled to practise it.

Article 2. — After each vaccination or revaccination, the veterinary surgeon shall issue a certificate taken from a counterfoil-book. This certificate and also the counterfoil retained by the veterinary surgeon shall specify the name and address of the owner of the dog, and shall bear his signature. The chief provisions embodied in Article 3 of the present Decree shall also be reproduced thereon.

Article 3. — The following shall be exempted from the sanitary measures in force prescribing the slaughter of dogs and cats which have been bitten or mauled by a rabid animal or which may have been in contact with such an animal : namely, dogs that have been vaccinated for the first time more than twenty days and less than one year previously, or revaccinated less than one year previously, the identity of which can be certified by a veterinary inspector of the Animal Diseases Service. They may be kept by their owners, at the risk of the latter, provided,

on April 13th, 1935, authorising the preventive vaccination of dogs throughout the territory of the colony. The application of this Decree was made the subject of an Order dated July 4th, 1935, by the Governor-General of Indo-China, amending, to the advantage of vaccinated dogs, the laws previously in force.

From the beginning of 1934 to December 31st, 1937, 10,500 dogs were vaccinated or revaccinated, and this necessitated the use of 402,000 cc. of vaccine, the greater part (94%) of which was used by veterinary officers of the Animal Diseases Service, and the remainder by private veterinary surgeons. Cochin-China took 40% of the total supply of vaccine, Laos 19%, Tongking 16%, Cambodia 14% and Annam 10%.

In the opinion of the Director of the Pasteur Institute, Nhatrang, " it would be difficult and premature to formulate any conclusions

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however, that they are revaccinated within a period of seven days after the bite, failing which they shall be slaughtered. A certificate of revaccination valid for one year shall then be delivered by the veterinary surgeon performing the revaccination, and a declaration shall be made by the owner to the Chief of the Province or to the mayor of the municipality.

Furthermore, these dogs shall remain for four months under the supervision of the Veterinary Service, and must be presented for inspection by a veterinary surgeon every fifteen days, failing which they shall be slaughtered. During this period, they shall not be allowed to stray, nor shall they be taken on the public highway unless they are both held on a leash and muzzled.

No modification is made in the previous regulations concerning cats and other carnivorous animals.

Article 4. — The cost of vaccination shall be borne by the owners of the animals. Provinces and municipalities shall, however, be authorised to defray such cost themselves in order to permit of a wider application of vaccination in highly contaminated regions.

Article 5. — Each year, the veterinary inspectors shall, before February 1st, transmit to the Chief of the Veterinary Service a statement showing the number of dogs vaccinated during the previous year.

They shall indicate the results obtained and the observations made in respect of such vaccinations.

Article 6. — The Governor-General shall issue orders determining the model of the certificate of vaccination or revaccination, the charge thereof and, in general, all details concerning the application of the present Decree.

as to the effects of canine vaccination in Indo-Chinese practice, because the observations made in regard thereto by veterinary surgeons are too few in number. On several occasions, it has been reported that vaccinated dogs, which were thought to have been in contact with rabid animals, had remained immune, but there was no evidence that they had actually been contaminated ".

An experiment in optional vaccination was made in Cambodia. Between May 1st and June 29th, 1934, 385 dogs were immunised in six villages of the province of Kampot. The inhabitants showed an unexpected eagerness to present their dogs for vaccination ; it is true that the Administration undertook to defray the cost of the operation. In order to permit of the rapid identification of vaccinated dogs, each of them was provided with a collar, made of india-rubber, bearing the stamp of the Veterinary Service. These collars were produced by cutting-up worn-out inner tubes of tyres.

The obligation to have the injection of vaccine repeated after an interval of three weeks proved to be a serious obstacle. In one village, for instance, only half the animals that were to be revaccinated were presented at the appointed time, and a further date for inoculation had to be arranged.

One fatal case of post-vaccinal paralysis was observed. In addition, one dog, which was in the incubation stage, died from rabies two days after the first injection of vaccine. Further, one failure must be recorded against the method : a female dog which, through an error of judgment on the part of the vaccinator, received doses of emulsion that were insufficient to produce immunity developed rabies four months after the second injection, in all probability as the result of a bite that passed unnoticed.

This experiment in vaccination furnished the opportunity for recording one definite case of the efficacity of the vaccine. A stray rabid dog bit 4 other dogs before being slaughtered. Of the 4 animals bitten, 3 had been vaccinated by means of two injections, thirty-three and fifty-four days previously. After being given a third dose of vaccine, they were placed under observation and showed no abnormal symptoms. The fourth dog, which had not been vaccinated, died of paralytic rabies forty days after being bitten. Yet, the bites of the 3 dogs that had been vaccinated were more numerous and more severe than those of the unvaccinated animal.

Japan.

Though FERMI must be given the credit for recognising, as long ago as 1907, that, amongst the antiseptics which exercise a destructive action on the rabies virus, phenol occupied a special place because of its power to attenuate or kill that virus whilst preserving its antigenic properties, it was the Japanese scientists —UMENO, DOI, HATA, KONDO — who applied phenolised vaccine, from 1916 onwards, to the preventive immunisation of dogs. The large extent to which their example was followed is so clearly shown in the present paper that it would be superfluous to recall here the numerous practical applications that have been made of the Japanese method in the most varied countries.

UMENO and DOI (1) prepare their vaccine in the following way : the brain and cord of a rabbit which has succumbed to inoculation with fixed virus are emulsified in four times their volume of glycero-phenolised solution (60 parts of glycerine and 40 parts of saline, phenolised at 1.25%) ; the emulsion is kept for fourteen days at a temperature of 18-22° C. or for thirty days in the ice chest. HATA (2) advocates the straining of Umeno's vaccine through a cloth before attenuation, which is carried out at the temperature of the laboratory. If kept away from the light, such a vaccine is said to remain active for two months. KONDO (3) uses an emulsion of one-fifth fixed virus of canine origin in distilled water, 50% glycerinated and 0.5% phenolised; attenuation is effected by keeping it for seventy-two hours at 37° C.

These various vaccines thus differ only in regard to their concentration in nervous substance and the method adopted for attenuation. Notwithstanding the presence of phenol, they retain the power of conferring rabies on small laboratory animals. This is, indeed, one of the conditions required to show that these vaccines are active. Kondo's vaccine, for instance, must prove pathogenic for the guinea-pig when inoculated subdurally in a dose of 1/20,000 cc. However, when injected subcutaneously into a dog, it must prove to be inoffensive in a 5-cc. dose.

Administered at first in two injections and later in a single injection, the glycero-phenolised vaccine confers an immunity the

(1) *Kitasato Arch. Exper. Med.*, 1921, 4, 89.

(2) *Jl. of Immunol.*, 1924, 9, 89.

(3) *Bull. Office intern. des Epizooties*, 1931/32, 5, 131.

duration of which is estimated at twelve months ; hence the necessity for annual revaccination. It retains its activity for about two months and this enables it to be used far afield.

Mention has already been made of the reservations which had been put forward, from the experimental standpoint, in regard to the efficacy and innocuity of Japanese vaccines. Let us now try to ascertain what results followed their use in practice, taking published statistics as a basis. As it is impossible to reproduce them all here — whether they relate to cities (Tokyo, Yokohama, Kyoto, Osaka) or to prefectures — we shall give only one table which groups the vaccinations that were carried out in the country as a whole between 1918 and 1930 :

Year	Total number of vaccinations	Cases of rabies amongst vaccinated dogs	Cases of rabies amongst non-vaccinated dogs
1918	10,951	6	1,041
1919	41,789	16	860
1920	26,103	4	500
1921	53,744	18	892
1922	58,141	33	983
1923	116,050	31	2,613
1924	194,177	44	3,172
1925	254,067	45	3,075
1926	234,680	55	1,770
1927	209,032	20	966
1928	225,636	13	421
1929	145,953	2	165
1930	114,892	4	64
	1,685,215	291	16,522

The figure given for the number of animals treated is certainly impressive, but to be able to compare the morbidity amonst vaccinated and non-vaccinated dogs, we ought to know how many of the latter there were. An indication on this point is given by HATA,[1] who estimates the number of dogs vaccinated at Tokyo and Yokohama from 1919 to 1922 at 104,000 and that of non-vaccinated dogs at 52,000 — *i.e.*, a ratio of 2 to 1. It is doubtful whether this

(1) *Loc. cit.*

proportion could be applied to Japan as a whole, notwithstanding the fact that the annual vaccination of registered dogs was, by stages, made compulsory throughout the country between 1919 and 1926. The owners of dogs are required to make a declaration to the town hall of their commune. Animals thus registered must wear a disc with a registration number. Once a year, the authorities organise the " anti-rabies prophylaxis week ". On this occasion, all owners of registered dogs receive instructions to present their animals for vaccination and to keep them on the leash throughout the week. The sanitary police regulations provide for the payment of compensation to any owner whose dog happens to succumb as the result of vaccination. Article 7 of Law No. 29 on infectious diseases in animals specifies that " the Government of the prefecture may, when it deems it necessary, for purposes of control or as a preventive measure, instruct the police or the veterinary service to carry out the inspection, vaccination, inoculation or dipping of any animal."

It would have been interesting to have more recent information concerning the vast campaign for the immunisation of dogs conducted in Japan and as to any effect it may have had on the number of persons obliged to undergo Pasteur treatment. Circumstances have prevented us from securing information on the former point. As regards the latter, the Kitasato Institute has given, for the prefectures of Tokyo and Kanagawa, the number of dogs vaccinated and of persons " exposed to rabies " from 1917 to 1926 :

Prefecture of Tokyo.

Year	Number of dogs vaccinated	Number of persons exposed to rabies
1917	—	456
1918	—	511
1919	16,165	449
1920	15,928	262
1921	22,385	358
1922	19,943	192
1923	19,959	119
1924	24,150	690
1925	41,877	456
1926	44,903	354

Prefecture of Kanagawa.

Year	Number of dogs vaccinated	Number of persons exposed to rabies
1917	—	138
1918	6,644	318
1919	9,150	73
1920	2,927	62
1921	11,338	85
1922	8,300	Documents destroyed
1923	1,700	during the earthquake
1924	3,234	334
1925	10,137	587
1926	4,114	181

The figures for the prefecture of Kanagawa show the marked recrudescence of rabies that occurred after the earthquake in 1923 more clearly than a direct effect of canine vaccination on the number of " persons exposed to rabies ". It is true that the opposite argument is possible, as it might be urged that it was, in fact, because the vaccination of dogs was hampered owing to the earthquake that rabies became more widespread. But, in that case, what explanation could be given of the results for the prefecture of Tokyo, where the number of canine inoculations was increasing throughout the period under consideration ?

The question arises whether, granting that the immunisation of dogs continued at the same rate as formerly, any diminution could be observed in the number of persons who underwent Pasteur treatment during recent years. As the figures supplied by the Tokyo anti-rabies institutes are, in this connection, too small to have any significance, we give hereunder those of the Keijo Anti-rabies Service :

Year	Number of persons treated	Year	Number of persons treated
1929	1,220	1934	2,280
1930	1,363	1935	2,609
1931	1,399	1936	2,476
1932	1,619	1937	2,989
1933	2,000		

In view of the fact that, in Chosen, according to the opinion of the Local Government itself, the majority of the dogs had been vaccinated as long ago as 1927, this progressive increase in the number of persons treated is somewhat surprising. All the explanations that might be suggested — a slackening-off in vaccination, the presence of stray dogs in large numbers, the introduction from time to time of rabid animals from neighbouring countries — can be nothing more than mere hypotheses.

It is unnecessary to carry any further this study of the Japanese statistics, for SCHNÜRER and DAVID [1] have submitted them to a very close examination, to which we would refer the reader. An opinion expressed in 1927 by the Kitasato Institute may, however, be quoted : " Umeno's method is perhaps not ideal, but it is, at all events, the one best suited for practical application, for it is simple and its cost is reasonable. Further, it confers immunity for a relatively long period."

Lebanon.

Preventive vaccination of dogs was introduced into the Lebanese and Syrian Republics in 1935. It is practised as an optional measure in conditions similar to those obtaining in Morocco. The Beirut Anti-rabies Institute prepares ether-treated vaccine and supplies it to all veterinary surgeons who apply for it.

Malaya.

Since 1924, an Anti-rabies Service, attached to the Kuala Lumpur Institute for Medical Research, has prepared a canine vaccine of the Umeno type, the source of the fixed virus being generally sheep-brain instead of rabbit-brain.

An outbreak of rabies having occurred in the State of Selangor, 650 dogs were vaccinated in a district where there were at least twelve times as many dogs. All the animals vaccinated remained immune, whereas 8 non-vaccinated developed rabies. In one village, where 18 dogs out of 20 had been immunised, the only case of rabies observed was a dog that had not been treated.

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[1] *Ergebn. der Hyg.*, 1930, **11**, 556.

In 1927, the number of dogs vaccinated reached a thousand and only one failure had been recorded. In the following years, as a result of mass vaccinations, rabies outbreaks were stamped out, in particular at Kuala Lumpur, Malacca and Penang.

Singapore, which had been free of rabies since 1907, was reinfected in 1937, the following being the conditions in which this occurred. On April 21st, a dog that came from Zamboanga (Philippine Islands) was brought ashore on presentation of the following documents : a veterinary certificate of good health dated April 15th, an anti-rabies vaccination certificate of the same date, an export permit issued by the Bureau of Animal Industry, Manila, and a certificate by the captain of the vessel to the effect that the dog in question had not been ashore during the voyage. As one document was still lacking — viz., a certificate to the effect that the animal came from a district that was free from rabies — the dog was placed in quarantine until June 21st, on which date it was released, as the necessary certificate, signed by a veterinary surgeon at Manila, had by that time been produced.

On August 25th, notwithstanding all these safeguards, the dog died of furious rabies. Its brain gave a positive result. As the animal had been at large for two days, very severe sanitary police measures were at once ordered ; 1,043 stray dogs were slaughtered and an order was issued for the compulsory vaccination of registered dogs. For the supply of the vaccine, application was made to the Kuala Lumpur Institute, which delivered the 13,000 doses requested by using, as it has done since 1929, buffalo-brain as the source of fixed virus, a single brain furnishing from 250 to 300 doses of vaccine.

In September, four teams of vaccinators were at work in Singapore. Of 9,274 dogs registered, 8,373 were inoculated (90.5%). During the following months, vaccination was extended to the rural districts where 3,549 dogs out of 5,918 registered were treated (59.9%).

The outbreak died out in November, after 12 cases of rabies, duly verified by the laboratory, had occurred amongst non-vaccinated dogs. Furthermore, 4 cases developed amongst vaccinated dogs, respectively one, seven, eleven and twenty-five days after inoculation, from which it may be assumed that 3 animals at least were in the incubation stage at the time when they were

treated. Thirteen cases of post-vaccinal paralysis, 2 of them fatal, were also observed.

The expenditure occasioned by this canine vaccination campaign amounted to 12,000 Straits dollars, and the amount recovered from the owners of the dogs vaccinated was 5,989 dollars.

The number of persons who had to undergo preventive treatment was 132 ; they all remained immune.

Since then, the situation has remained normal. In 1938, the Kuala Lumpur Institute issued only 654 cc. of canine vaccine, as against 74,500 cc. in 1937.

Netherlands East Indies.

In 1927, the preventive vaccination of dogs was not practised in this colony, as the Director of the Pasteur Institute, Bandoeng, considered that there was not yet sufficient evidence of the efficacity of this measure. VRIJBURG (1) broke a lance in favour of canine vaccination in 1928. He wished to see it applied to all registered dogs, together with an attenuation of the sanitary police regulations in favour of vaccinated animals.

Palestine.

The Bacteriological Laboratory of the Department of Health began, in 1927, to prepare a canine vaccine of the Japanese type ; but, from 1931 onwards, in order to obviate any risk of infection by the vaccine itself, the concentration of the phenolised emulsion was reduced from 20% to 5%, the treatment comprising one 2-cc. injection on each of three consecutive days.

In the absence of any legal obligation, dog-owners showed very little eagerness to have their animals vaccinated. Thus, in 1934, only 10 dogs were inoculated, in 1936 76 and in 1937 109 ; they belonged, for the most part, to a pack of hounds used for hunting jackals. One of these dogs contracted rabies in 1936.

(1) *Nederl. Ind. Bladen v. Diergeneesk.*, 1928, April, 169.

4. Europe

Austria.

Under an Order of April 22nd, 1922, by the Ministry of Agriculture and Forests, the Vienna Veterinary School was authorised to carry out the immunisation of dogs suspected of being contaminated and also of dogs placed in the pound and of healthy dogs. Before having his dog vaccinated, the owner had to sign a statement to the effect that he was fully aware of the fact that the veterinary police measures remained in force notwithstanding the vaccination. It may be wondered what attraction vaccination could offer in such circumstances and it is not surprising that it did not become generalised. Moreover, as the delegate of Austria to the *Office international des Epizooties* stated in 1935, the results of vaccination were not conclusive and the sanitary police measures accordingly retained their full value.

The technique of vaccination was at first that of Schnürer — *i.e.*, five subcutaneous injections or four intraperitoneal injections of 0.6 gramme of diluted fixed virus from rabbit-brain. This made it necessary for the animal to remain at the Veterinary School for at least five days. In 1926, this School adopted Umeno's method and vaccinated 238 dogs, the charge made for the operation being two schillings.

Bulgaria.

Between 1928 and 1930, 218 dogs were vaccinated by Umeno's method. One animal died of paralysis twenty days after vaccination, but the laboratory examination of its brain gave a negative result.

Czecho-Slovakia.

The first attempts at vaccinating dogs preventively were made in 1924 at the Prague Institute of Serology. The results having been encouraging, compulsory vaccination was, on the recommendation of the Veterinary Services, introduced by decree in regions where rabies was prevalent, the vaccine used being of the phenolised type. In 1928, 869 dogs were inoculated in twenty-four communes of the Trous district, where 61 rabid dogs had been found within two months ; no further case of rabies occurred thereafter.

The following figures show the increasing favour in which canine vaccination was held :

In 1926	300	dogs were	vaccinated.
„ 1927	1,400	„ „	„
„ 1928	11,000	„ „	„
„ 1929	26,000	„ „	„
„ 1930	32,000	„ „	„

Of 70,000 dogs treated and kept under the supervision of State veterinary surgeons, 30 died of rabies, but, in every case, the animal had been bitten prior to vaccination.

Estonia.

In the regions of this country where sanitary police measures had not been operative, preventive vaccination was applied in 1927 under the supervision of the veterinary services. The vaccines employed were of various types — Kondo, Högyes, 10% emulsion of fresh fixed virus. Their use made it possible to stamp out in a very short time a serious epizootic.

Finland.

During the winter of 1929/30, 1,175 dogs were preventively immunised along the Russian frontier, the vaccine used being that of Schnürer. Five vaccinated dogs that were bitten by rabid animals remained immune.

In a more recent report (1937) by the Finnish Veterinary Service, no reference is made to canine vaccination.

France.

The vaccination of dogs is not admitted by the public authorities in European France. A circular which the Minister of Agriculture sent to the Prefects (September 13th, 1929) recalled the fact that rabies prophylaxis may be summed up in the single phrase : the capture and destruction of stray dogs.

Nevertheless, the advocates of canine vaccination have, on various occasions, urged that the mother-country should follow the example set by North Africa. In 1930, in an article entitled " The preventive vaccination of dogs against rabies must be

authorised in France ", REMLINGER (1) asks why it is that this measure has not yet been adopted there. In his view, " this regrettable abstention is due almost entirely to other than scientific reasons " ; on the one hand, " man is the only animal vaccinated by the Pasteur Institutes " and, on the other hand, " the sanitary laws enact that any animal that has been bitten or mauled by a rabid or suspected dog must be slaughtered immediately, and the application of this principle, which is that of the least effort, is thought to be sufficient ".

In 1935, at Remlinger's request, a Committee appointed by the Veterinary Academy of France was instructed to study the question. It reached the following conclusions :

> " The Committee, whilst not failing to recognise the high scientific value of the methods of vaccination against rabies in carnivorous animals and whilst paying a tribute, in particular, to the work of Dr. Remlinger, is of opinion that it would be imprudent to make vaccination the basis of a systematic method of prophylaxis and to abandon the present sanitary police measures which have resulted in the almost complete eradication of rabies in France.
>
> " It recognises, however, that veterinary surgeons remain free to practise preventive vaccination in order to protect animals from an infection resulting from a bite which has passed unnoticed. But it could not approve the vaccination of a carnivorous animal after a bite by a rabid animal."

A different view was adopted by the National Union of Veterinary Surgeons of France when, in 1935, it decided " to undertake action with a view to obtaining, through the vaccination of animals, a relaxation in the rigour of the sanitary laws concerning rabies ". In 1936, the same Union asked the Minister of Agriculture to arrange for experiments to be carried out on the efficacity of canine vaccination in the various veterinary schools and under the supervision of scientists of recognised standing · if the results proved to be conclusive, a relaxation in the rigour of sanitary police measures might be considered in favour of vaccinated dogs.

The Union's proposals were, however, definitely rejected in 1937, " the qualified services and the scientific societies which advise

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(1) *Recueil de méd. vét.*, 1930, page 526.

the Minister of Agriculture being opposed to any modification of the regulations in force ".

Germany.

When outlining, in 1935, before the Committee of the *Office international des Epizooties* the " present possibilities of prophylaxis against rabies ", GIESE and ZUNKER (1) defended the view — which was that of the German Reich — that prevention should be based exclusively on sanitary police measures. In explanation of this attitude, it should be pointed out, on the one hand, that such measures, rigorously applied, have brought about the disappearance of rabies throughout the interior of the country and, on the other hand, that the research work done by German rabiologists has not entirely confirmed the innocuity of canine vaccination. Thus, in the course of experiments carried out by GIESE (2) for the purpose of testing the efficacity of the Japanese method, the phenolised vaccine caused several cases of dumb or even furious rabies. Giese deduced that, if a particularly active fixed virus — such as the Breslau virus employed in his experiments — was used, the dose of vaccine prescribed was too large.

The preventive vaccination of dogs is not, however, forbidden in Germany, for this measure was made compulsory in March 1927 in certain infected districts of the Saar. Further, in virtue of a convention concluded with Czecho-Slovakia, all dogs in the frontier districts of Saxony were vaccinated in 1927. In both these cases, the vaccine used was the " Lyssin " of MIESSNER and BAARS,(3) this being a fixed virus subjected to desiccation, mixed with chalk and ground to a fine powder, whilst retaining a certain virulence.

The present attitude of the Government of the Reich is clearly expressed in the notes which it transmitted to the *Office international des Epizooties* on the state of rabies in Germany in 1937 and 1938. These notes state that the application of the measures prescribed in the Law of June 26th, 1909, concerning animal diseases has proved to be sufficient to stamp out rabies and that, accordingly, the vaccination of animals has not been practised.

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(1) *Bull. Office intern. des Epizooties*, 1935, **10**, 53.

(2) *Arb. a.d. Reichsgesundheitsamt*, 1926, **75**, 410.

(3) *Zbl. f. Bakt. Orig.*, 1927, **101**, 79.

Great Britain.

The Ministry of Agriculture and Fisheries, in its reply to the questionnaire drawn up in preparation for the Rabies Conference of 1927, considered the question whether, in the case of a dog that had been vaccinated a short time before its entry, the regulation period of six months quarantine might be reduced. It reached a negative conclusion, taking the view that no method of vaccination can be altogether effective. In the case of a dog that had been vaccinated with an attenuated virus, it might even be desirable to prolong the period of quarantine in order to obviate the risk of introducing rabies into a country which is free from it.

Hungary.

Although a first attempt at immunising dogs had been made in 1913, when 16,749 animals had been treated in the city of Budapest, it was only in 1929 that preventive vaccination was officially introduced in Hungary on a voluntary basis without, however, there being any resulting diminution in the number of cases of rabies. It was therefore decided in 1933 to make the immunisation of dogs compulsory in a district comprising thirty-one communes, situated on the north-east of the capital.

The results of this experiment having proved very encouraging, the vaccination of all sheep-dogs was ordered in 1934. These dogs, which were considered as the most dangerous vectors of rabies, numbered 32,000. No case of the disease was subsequently observed amongst them.

In 1935, canine vaccination was made compulsory in Budapest and around the city for a radius of 6 miles. The results were striking. Whilst previously the annual number of rabid dogs varied between 18 and 70, it fell to 0 in 1938. Post-vaccinal paralysis, fatal in about 1 case out of 3, occurred in 0.25% of the dogs treated in 1935, which were some 50,000 in number.

The Minister of Agriculture decided in that year to extend compulsory vaccination progressively to the dogs of the whole country. The measure was extended in 1936 to the province of Transdanubia, which occupies a third of the area of Hungary, in 1937 to the county of Pest, in 1938 to the northern counties, in 1939 to the counties of the south-east and in 1940 to Sub-Carpathia.

The following table shows the number of cases of canine rabies occurring in these different regions before and after the vaccination of dogs was made compulsory, and also the number of persons who received Pasteur treatment during the same periods :

Region	Date of introduction of compulsory vaccination	Number of cases of canine rabies				Number of persons treated		
		1933	1934	1937	1938	1933	1937	1938
City of Budapest . .	Spring 1935	70	27	2	0	1529	423	413
Transdanubia	Autumn 1936	505	248	13	2	1714	306	195
County of Pest . . .	Spring 1937	231	152	65	0	1996	620	407
Northern Counties .	Spring 1938	266	117	225	42	966	1363	620
South-eastern Counties	Spring 1939	166	114	95	80	640	480	439

Although a spontaneous decline of canine rabies occurred in 1938, both in the northern counties before the full effect of vaccination could be felt and also in the south-eastern counties where it had not yet been introduced, this diminution is not such as to deprive the results observed elsewhere of their demonstrative value. As to the 2 rabid dogs reported in Transdanubia in 1938, they were captured on the outskirts of the province and came probably from farther afield.

The magnitude of this vaccination campaign can be seen from the following figures :

Number of dogs vaccinated in Hungary.

1930	4,278	1935	85,721
1931	4,245	1936	269,424
1932	3,848	1937	384,137
1933	14,242	1938	481,406
1934	35,673	1939	752,500

The vaccine is prepared by " Phylaxia Ltd. ", a commercial undertaking placed under recognised scientific direction and purveyors to the Hungarian Government of sera and vaccines for medical and veterinary use. Their vaccine is a glycero-phenolised emulsion of fixed virus from sheep-brain and is subjected to official control. To be passed, it must still retain a degree of virulence

sufficient to kill at least one rabbit out of three when inoculated subdurally at a dose of 0,15 g.

Vaccination — which in no way excludes the strict enforcing of the usual sanitary police measures — is carried out in a single injection and must be repeated annually. Owners of dogs over 3 months old are obliged to have them vaccinated or revaccinated at their own expense before a given date. If the inoculation is performed by an unofficial veterinary surgeon, the vaccination certificate must be presented to the authorities within three days. If the owner of a dog has neglected to have it treated within the prescribed time-limit, he must present it to the district veterinary surgeon at the time and place specified. The animal will be vaccinated for the official small charge unless it belongs to an indigent person, in which case the inoculation is given free of charge. The certificate of vaccination has to be produced before the dog tax can be paid.

Whilst the reduction of rabies cases in dogs has been impressive where vaccination has had time to produce its full effect, the number of persons obliged to undergo Pasteur treatment, although showing a marked decrease, has not diminished in the same proportion. This might be attributed to the fact that it is necessary to treat not only the persons actually bitten, but also those who may have run a risk of infection, however remote, thereby increasing the ratio between the number of treated persons and that of rabid dogs.

Italy.

Whilst a certain number of dogs had been preventively immunised with phenolised vaccines at the Rome and Sassari Anti-rabies Institutes, and with dried cords at the Bologna and Palermo Institutes, the vaccine which was most largely used was Finzi's special glycero-phenolised vaccine, prepared by the Veterinary Institute, Milan, and administered to some 15,000 dogs between 1922 and 1926.

The city of Novi di Modena was the first to make vaccination compulsory (1924) ; Turin, Genoa, Alessandria, Mondovi, Pignerol, Bolzano and Merano followed suit soon afterwards, as well as the prefectures of Pavia, Piacenza and Treviso (1925-1926).

The only information we possess on these vaccinations is that " they were carried out under very strict sanitary supervision and

that no failure was observed " (FINZI (1)). On the other hand, when referring to the 2,114 dogs vaccinated at Bolzano, BISANTI (2) points out that 44 of them were tested after two, four and six months by the introduction of street virus into the anterior chamber of the eye. The infecting dose " had been kept within the most restricted limits, as was shown by the control animals which had been infected in the same way. The results yielded by vaccination proved insufficient ".

We have in vain attempted to collect data concerning the later development of canine vaccination in Italy. It does not appear, however, that this measure has been extended to the whole of the country, at least officially, since a circular, dated October 9th, 1935, issued by the Director-General of Public Health, which referred to the prophylaxis of canine rabies, did not mention vaccination, but insisted only on the necessity for capturing stray dogs and of keeping under observation for six months the animals likely to have been infected.

Lithuania.

The State Veterinary Institute delivered 18,447 preventive doses of canine vaccine during 1933.

Poland.

In 1934, a Committee of the Polish Association of Veterinary Surgeons expressed the view that the preventive vaccination of dogs should not be taken as the basis of rabies control in Poland, but that, applied individually, this measure could be a useful complement to it. The Committee therefore asked that the existing prohibition of the immunisation of dogs should be lifted.

It is no wonder that, in these circumstances, the number of dogs vaccinated between 1925 and 1935 did not exceed 652, of which 128 were revaccinated after a year. The vaccine, prepared for experimental purposes at the State Institute of Hygiene, Warsaw, was of the phenolised type. None of the treated dogs developed

(1) *La Clinica veterinaria*, 1927, page 274.

(2) *Bull. Office intern. des Epizooties*, 1931/32, 5, 121.

rabies, although several of them were bitten by certainly rabid animals.

Portugal.

As early as 1925, the preventive vaccination of dogs was made compulsory by a decree of the Minister of Agriculture (1). The carrying-out of this measure was entrusted to the municipalities which, according to the decree, were to organise vaccination centres and to establish kennels where imported dogs would remain until vaccinated. In point of fact, sixteen municipalities only were in a position to meet these requirements. Nevertheless, as the result of an active propaganda campaign, 72,731 dogs, out of the 350,000 in the country, were immunised between 1925 and 1929, 56,614 of them with Umeno's vaccine and the others with killed vaccines of various types.

Fifty-two cases of post-vaccinal paralysis, several of which were fatal, were observed among the dogs treated with Japanese vaccine — *i.e.*, a percentage of 0.09. In four cases, the presence of fixed virus was detected in the brains of animals which succumbed after inoculation.

In many instances it was definitely proved that vaccinated dogs, subsequently infected, had remained immune ; thus, in the district of Braganza, a rabid dog had bitten numerous others, one of which had not been vaccinated ; the latter alone contracted rabies. In Serpa, the opposite case was observed : the dogs bitten had not been vaccinated except one, which was the only one to survive.

As from 1935, the annual number of vaccinations showed a definite tendency to decline ; by a Decree of August 15th,

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(1) The following is a translation of Article 1 of this Decree, which was promulgated on November 16th, 1925, under No. 11242 :

Anti-rabies vaccination of dogs over 4 months old is declared compulsory throughout the territory of the Republic. The importation of dogs remains prohibited except in cases where it is proved that they were vaccinated within the year preceding the date of importation.

The importation of non-vaccinated dogs may be authorised, provided they are declared and placed in municipal kennels until they have been duly vaccinated.

1939 (1), therefore, the Minister of Agriculture relieved the municipalities of the duty of providing for the immunisation of dogs and entrusted the Department of the Director-General of Stock-breeding with this task, the expense involved being chargeable to the budget of the latter.

In the Instructions relating to the enforcement of this decree, it is specified that vaccination shall be made compulsory in communes on application being made to the Department of the Director-General of Stock-breeding by the municipal veterinary surgeons or, failing this, by the Inspectors of Stock-breeding. After vaccination, a numbered certificate will be delivered to the owner to enable him to obtain the municipal dog licence.

The question of the vaccines to be used was settled by a decision, dated April 8th, 1940, of the Director-General of Stock-breeding. The active glycero-phenolised vaccines of the Umeno type are

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(1) The following is a translation of some of the Articles of this Decree (No. 29441) :

Article 1. — The Department of the Director-General of Stock-breeding shall be empowered to declare compulsory the vaccination of dogs against rabies by communes or by regions, according to requirements, and having regard to the quantities of vaccine available.

The notification rendering vaccination compulsory shall be communicated by means of posters displayed in the usual public places.

Article 2. — In communes where anti-rabies vaccination has been declared compulsory, dog-owners shall be obliged to present their animals for vaccination on the day and at the time and place appointed by the veterinary authorities.

Dogs introduced in these communes shall be presented for vaccination within ten days of their arrival.

Article 3. — Municipal licences for the possession and the circulation of dogs . . . shall be granted only on presentation of a vaccination certificate, or of a duplicate thereof should the original be lost.

If the vaccination has been carried out by a veterinary surgeon chosen by the owner, the certificate issued by the veterinary surgeon shall be presented to the competent official authority and endorsed by the latter.

Article 4. — The expense involved, including the cost of vaccines, shall be borne by the Department of the Director-General of Stock-breeding.

The use of vaccines chosen by the owners and approved by the Department of the Director-General of Stock-breeding shall be authorised.

authorised if they are so attenuated as to have lost their pathogenicity by the subcutaneous or intramuscular route whilst remaining virulent for the rabbit or guinea-pig when introduced subdurally ; the same applies to vaccines derived from phenol- or formol-killed virus, provided that the amount of nervous substance administered to the animal is at least 1 gramme. In order to ensure that locally produced or imported vaccines meet this requirement, the percentage of nervous substance present in the vaccine must be indicated on the container.

In 1939, 18,365 ampoules containing 5 cc. of a vaccine constituted by a 5 or 10% fixed virus emulsion in saline, glycerinated at 30% and phenolised at 0,75%, were used in Portugal. For the first vaccination, the equivalent of 4 grammes of cerebral matter is administered in a single inoculation ; for revaccination, the quantity can be reduced to 1 gramme.

Of 1,400 dogs treated in 1939 in the district of Arganil, 1 died of rabies (confirmed by laboratory examination), but it is not certain that the vaccine dose administered was sufficiently large.

The graph which may be constructed by plotting the number of persons treated annually in the three anti-rabies institutes of Portugal (1) rises progressively from 1,094 in 1910 until it reaches 5,196 in 1919. When preventive vaccination was enforced (1925), the curve stood at 4,793. In subsequent years, the decline was at first very rapid, then less so, the annual figures being :

Year	Number of persons treated	Year	Number of persons treated
1925	4,793	1930	1,543
1926	4,409	1931	1,337
1927	3,650	1932	971
1928	2,736	1933	766
1929	1,655	1934	659

Taking only these figures into consideration, one would be inclined to attribute their diminution to canine vaccination. Although the rôle of this factor cannot be entirely disregarded, account must be taken of the general trend of the curve and this seems to indicate the decline of an epidemic wave. A similar conclusion is reached,

(1) *Bull. Office intern. des Epizooties*, 1935, 10, 393.

moreover, by setting out, as hereunder, the figures showing the number of persons treated annually at the Camara Pestana Institute, Lisbon :

Year	Number of persons treated	Year	Number of persons treated
1928	1,329	1934	374
1929	726	1935	945
1930	685	1936	637
1931	631	1937	933
1932	408	1938	2,028
1933	265		

The reduction is certainly marked between 1928 and 1933, but the subsequent rise is no less striking and indicates, perhaps, the beginning or the peak of a new wave of infection.

As regards the effect of canine vaccination, Professor MARQUES DOS SANTOS, Director of the Anti-rabies Department, Coimbra, considers that, although this measure may have contributed to reduce the frequency of rabies in Portugal, the lack of precise information from veterinary sources precludes any pronouncement as to its real value.

Roumania.

A vaccine obtained by the action of formol on street virus — and not on fixed virus as required by the method of Plantureux — was prepared in 1928 at the Bucharest Veterinary School. To confer immunity, three injections at four days interval, or two injections at twelve days interval, were considered necessary. Thirty-one dogs thus treated all resisted the subdural, intraocular or intramuscular administration of street virus.

Although attempts to vaccinate dogs preventively were subsequently made in various anti-rabies and veterinary institutes throughout the country (Bucharest, Chisinau, Cluj, Jassy), the introduction of compulsory canine vaccination is not contemplated at the present time.

Spain.

In a circular, dated August 6th, 1927, issued by the Department of Agriculture, it was stated that no reference to preventive

vaccination had been made in an earlier order concerning action against rabies because that measure seemed to be of slight efficacity and even dangerous. Civil Governors were accordingly recommended not to issue orders making vaccination compulsory but, on the other hand, to allow optional vaccination, provided that statistics were kept showing the numbers of dogs treated and a report drawn up concerning the results observed.

It was on the recommendation of LOPEZ (1), who had visited the United States for the purpose of studying the results obtained by the use of the Japanese method, that canine vaccination was introduced into Spain, where it was afterwards made compulsory. The Regulations of September 26th, 1933, concerning Animal Diseases (2) provide that, when a district is declared infected with rabies, all the dogs therein must be vaccinated.

Between 1933 and 1935, more than 5,000 dogs were treated and the results obtained were considered satisfactory.

Yugoslavia.

Research work on canine vaccination was first undertaken in 1935 at the Zagreb Veterinary School with a view to perfecting a vaccine obtained from non-attenuated fixed virus ; this vaccine, which proved innocuous, was mainly used for cattle.

Nowadays, the vaccine utilised for the immunisation of dogs is prepared by three Government institutes : the Belgrade and Zagreb Institutes deliver phenol-attenuated and killed vaccines, whilst the Novi Sad Institute prepares ether-killed vaccine, according to Hempt's method. From 1934 to 1939, 8,757 dogs were preven-

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(1) *Bull. Office intern. des Epizooties*, 1935, **10**, 389.

(2) The following is a translation of Article 218 of these Regulations :

When a case of canine rabies is confirmed in any district, the Civil Governor shall declare that district to be in a state of infection and if, from the facts ascertained, there is shown to be any probability that the rabid dog has bitten other animals outside the infected locality, the measures entailed by the declaration shall be extended to such other places as may be considered to be contaminated.

The official declaration of rabies shall entail the compulsory vaccination of all dogs in the district or districts declared infected and the curative treatment, if such treatment is possible, of larger animals that have been bitten.

tively treated with phenolised vaccines and 1,025 with etherised vaccine. The 3 cases of post-vaccinal paralysis which occurred all recovered. In a region where 113 cases of canine rabies had been observed during the two years preceding the introduction of vaccination, only 22 cases were reported during the following two years, and they occurred in non-vaccinated animals.

These results led the authorities to apply vaccination on a larger scale. In 1940, 12,419 dogs were immunised free of charge, by means of a single inoculation, in the most-infected districts of Croatia ; it was hoped to reach the figure of 22,000 vaccinations before the end of the year. It had, furthermore, been planned that all dogs in the region limited by the Rivers Drava and Sava should be immunised, so as to create a zone of defence against the westward spread of rabies. This plan, which involved the vaccination of some 50,000 dogs, could not be carried out in view of the general situation and for financial reasons.

Canine vaccination remains optional in Yugoslavia ; in 1938, the Ministry of Agriculture had, it is true, convened a meeting of experts to discuss the possibility of making the measure compulsory, but no conclusion in favour of doing so was arrived at.

Reporting in 1939 to the Congress of the National Veterinary Association, KODRNJA [1] suggested adding to the sanitary police measures in force :

(1) The optional vaccination of all dogs ;

(2) The compulsory vaccination of sheep-dogs, police-dogs and hunting-dogs in contaminated districts ;

(3) The compulsory vaccination of all dogs in the event of a marked epizootic.

5. INTERNATIONAL AGENCIES

The International Rabies Conference of 1927 passed the following resolution on canine vaccination :

> " In spite of the importance of the results already acquired in the study of anti-rabies vaccination of the various animal species, and the large number of animals already successfully

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[1] *Yugosl. Veterin. Glasnik*, 1939, 19, 73.

vaccinated, the Conference does not consider that it is in a position to propose important changes in sanitary legislation. Nevertheless, the Conference considers it desirable that :

" (1) Dogs be given prophylactic vaccination against rabies ; such vaccination, as far as possible, should consist of a single inoculation of a dead virus (still capable of conferring immunity), or of a fixed virus, modified or not, which is not pathogenic to the dog when inoculated subcutaneously or intramuscularly ;

" (2) This prophylactic vaccination should be repeated each year ;

" (3) Such vaccination — at least at the beginning — should be applied only in anti-rabies institutes and schools of veterinary medicine or by the local veterinary authority concerned ;

" (4) The administrative supervision should be such as to provide for the enumeration of vaccinated dogs and their inspection by a veterinary authority at the end of the fourth month following vaccination ".

At the International Conference which was held at Bucharest in 1938 to honour the memory of Professor Babes, the use of the various methods of preventive canine vaccination was advocated as a complement to sanitary police measures.

The point of view of the veterinary surgeons has been stated on three different occasions by the Committee of the *Office international des Epizooties*. In 1928 this body expressed the following opinion :

" The control of rabies can be facilitated by preventive vaccination, but the latter should be applied exclusively to healthy dogs placed under veterinary supervision, and this under the following conditions only :

" (1) The vaccination must be carried out by means of a killed virus, or of a virus so attenuated that it can no longer convey rabies by subcutaneous or intramuscular injection ;

" (2) The vaccination of dogs which have been bitten by rabid or suspect dogs, or by cats, is strictly forbidden."

Further, this Committee notes in 1931 :

" that its previous resolutions are still valid. Conclusions as to the prophylactic value of vaccination cannot be framed until further observations are available.

" It is indispensable that, prior to vaccination, even if carried out by means of an admittedly killed vaccine, a notification be made to the authorities concerned, and that the vaccines should be subjected to a sanitary control.

" The collection of information relating to anti-rabies vaccination in various animal species should be pursued."

Lastly, in 1935, the Committee expressed the view that canine vaccination should not :

" constitute a general method of prophylaxis. It may be used, with the required precautions, in countries where for some reason sanitary measures cannot be fully applied ".

It is worth noting how carefully the opinions just quoted are formulated. No allusion is made to the possibility of not killing at once vaccinated dogs bitten by a suspect animal. In other words, in the view of the Committee, vaccination cannot be accepted as justification for an exceptionally favourable attitude towards vaccinated dogs.

* * *

Any endeavour to formulate an opinion on the efficacy of a vaccination process is always a delicate task. It will, however, be facilitated if certain numerical data are available, and these, in the present case, should be :

(1) *The proportion of vaccinated dogs to the total canine population.* Whilst the number of animals vaccinated annually is usually known, the size of the canine population is seldom indicated and, if given, the figures are only approximate. The ratio between vaccinated and non-vaccinated dogs cannot therefore be computed exactly.

(2) *The percentage of rabies cases in vaccinated and non-vaccinated dogs.* Infections, developing among vaccinated dogs — which are mostly domestic — are relatively easy to trace. But this is not the case with non-vaccinated dogs, which are mostly stray dogs and, as such, are particularly exposed to infection. As, further, exact information as to the proportion of vaccinated dogs is lacking, any attempt to compare the morbidity in the two groups must yield very imperfect results.

It would be different if, instead of considering non-vaccinated dogs as a whole, a choice was made amongst them of a group of

dogs which could be kept under supervision. In this way, comparison would be facilitated, but the chances of infection would no longer be the same as in the case of dogs free to wander at large. No such experiment seems, moreover, to have been attempted.

(3) *The number of persons applying for Pasteur treatment before and after the introduction of preventive canine vaccination.* At the risk of hurting the feelings of invetcrate dog-lovers, the following remark seems in place : in the last resort, it is not the life of the dog that matters, but the life of the man. There has been a tendency to overlook this when arguments of a moral, sentimental or even pecuniary character have been advanced in favour of canine vaccination. For the appraisal of this measure, the decisive factor must be a reduction in the number of persons exposed to rabies infection. In civilised countries — and they are countries where canine vaccination has been introduced — this number is practically equal to that of persons applying for Pasteur treatment shown in the statistical reviews which the Health Organisation has published during the last ten years. The fluctuations in this figure may, accordingly, be easily followed, but it will have to be established whether any reduction that may have occurred is, in fact, attributable to canine vaccination or whether it might have been brought about by other factors, such as the spontaneous decline of an epizootic, the enforcement of more drastic sanitary police measures, etc.

The factor of " risk " must also be taken into consideration so as to emphasise the difference which exists, from this angle, between human vaccination and canine vaccination. This renders it a still more delicate matter to pass judgment on the results furnished by the latter.

All persons applying for Pasteur treatment — which is a vaccination after a bite, hence curative — have run a risk of infection the gravity of which depends on the site and type of the bite, and also on the class (A, B, C) to which the animal responsible for the bite belongs. If, for example, one vaccinated person in a thousand has contracted rabies, this proportion might serve as a criterion for appraising the Pasteur treatment, since it is admitted that all who have undergone this treatment have been exposed to infection, though in varying degrees.

Nothing similar applies to preventively vaccinated dogs, since the risk they may have incurred cannot be evaluated. If one vaccinated dog in a hundred develops rabies, this does not prove that the other 99 have been protected by vaccination, since it is impossible to determine whether or not they have been exposed to infection.

With such uncertain premises, it is no wonder that a statistical study of preventive canine vaccination has never been attempted. Such a study would, moreover, be hampered by a basic difficulty as long as the proportion of actually infective bites from certainly rabid dogs remains unknown.

Should one, therefore, in seeking to pass judgment on canine vaccination, regard with suspicion the figures given because they do not lend themselves to an exact statistical analysis ? On the contrary, they are the expression of actual facts and, as such, are the only objective data we can rely on.

Of the results referred to above, the most conclusive, in our view, are those relating to Hungary, where rabies, both in vaccinated and in non-vaccinated dogs, has entirely disappeared from the regions in which compulsory immunisation was enforced before 1938, and where a significant decline was observed in the number of persons having recourse to Pasteur treatment. It is worth recalling that, in Hungary, dogs receive one inoculation only. This is an instance of that flagrant contradiction between practical application and the experimental data to which we alluded at the beginning of this study. In this case, however, we do not hesitate to put our faith in the practical results.

When a country has thus succeeded in stamping out rabies, it must reckon on periodical reinfections conveyed by rabid stray dogs crossing the frontiers. Theoretically, this should not give rise to epizootics since the great majority of the dogs should be immune, because they have been vaccinated. The future will show whether this assumption is correct as regards Hungary, which is surrounded by countries where rabies is still prevalent.

Immunologically speaking, multiple injections of vaccine should confer a more durable and more solid immunity than a single injection. It is not possible, in the absence of comparable data, to confirm this statement in the case of canine vaccination, this measure being optional where immunisation is carried out by means of more

than one injection (Algeria, Indo-China, Lebanon, Morocco, Tunis), whereas it is, for the most part, compulsory where it is applied in a single injection (Hungary, Japan, Portugal).

Nor does the material collected demonstrate the advantage which any one type of vaccine might offer over another. In face of this inability to draw any conclusion, it is a consolation to realise that McKENDRICK, in his " Ninth Analytical Review " of annual reports from institutes concerned with the preventive treatment of rabies in man (1), does not find it possible to make a choice — as to their immunising power — between dried cords, dilutions, killed or live phenolised vaccines and ether-treated vaccine. He had, however, at his disposal precise statistics covering more than a million treated cases ! This shows the complexity of the problem, and also the large extent to which the vaccines utilised for the immunisation of man are equivalent.

Can this apply also to the preventive vaccination of dogs ? We are inclined to reply in the negative, for the following reasons : human vaccination seeks to create as rapidly as possible a state of immunity which does not need to be of long duration, as the risk of infection has already been run and will, in all probability, not recur. The experience of the Pastorian era is there to show that this result can be achieved by administering repeated, and indeed rather massive, doses of a live, attenuated or even killed virus, as long as the antigenic properties of the latter are retained. In preventive canine vaccination, on the contrary, the aim is to confer a prolonged state of immunity, sufficient to protect against a possible risk of infection. To attain this end, the use of a vaccine still possessing a certain virulence seems *a priori* preferable, since a pronounced and lasting immunity can be obtained only by the use of a vaccine of high antigenic power, especially when the vaccination has to be carried out in one injection, or two at the most. Experimental data show that this is a property which a live or an incompletely attenuated vaccine possesses to a higher degree than a killed vaccine. What remains to be ascertained — and this is the question we raised at the beginning of this study — is whether a certain virulence is compatible with innocuity, notwithstanding the fact that these two words are the antithesis the one of the other.

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(1) *Bull. Health Org.*, 1940, 9, 31.

To appreciate the value of a vaccination process, the rapid and total extinction of an epidemic focus can, to some extent, be taken as a criterion. From this standpoint, the example of Singapore is worth recalling, especially as the vaccination campaign was based on single inoculations. The fact that a drastic suppression of stray dogs took place before compulsory vaccination was introduced might indicate, however, that the latter was not the only factor to which the decline of the outbreak could be attributed.

A final word on the character, compulsory or optional, which canine vaccination may assume. Only when compulsory can it be considered as a collective measure of public health prophylaxis. To attain any success with it, countries must already possess a sound sanitary and administrative organisation ; otherwise, compulsion would be in vain. Differences are noticeable, moreover, in the method of application : in Malaya, Portugal and Spain, the vaccination of dogs is imposed only in the case of a definite epizootic and on a provincial or municipal basis, whereas in Cuba, Hungary and Japan it is a general measure, periodically applied, the object of which is to immunise the greatest possible number of dogs, irrespective of the degree of infection amongst them. The aims thus differ. In the former case, the goal is merely the extinction of a local focus, whilst, in the latter case, the object is a more ambitious one, since it is nothing less than the complete eradication of rabies. The Hungarian attempt, which seems to be nearing success, might be taken to show that there is nothing utopian about this.

Optional vaccination, on the other hand, is merely an individual and exceptional measure of prevention : an owner who cares for his dog is desirous to save its life in the event of a suspect bite and, at the same time, to insure himself against the risk he would run should his dog be bitten by a rabid animal. If the dog is to be kept alive in such an eventuality, however, vaccination must carry with it, in favour of treated dogs, an attenuation of the rigour of sanitary police measures, as is the case in Algeria, Indo-China, Morocco and Tunis. Elsewhere, it is difficult to see what attraction vaccination could offer to dog-owners, who are obliged to defray the cost of the treatment and to comply with certain administrative formalities, whilst being aware that their dog will not escape being killed should a risk of infection occur ! It is worth pointing out that, so far, a significant diminution in the number of persons applying for Pasteur treatment

is observed only where canine vaccination, although remaining optional, is given free of charge, as is the case at Hussein Dey near Algiers ; as a result of this measure, the proportion of vaccinated dogs is very high and has reached a level which probably equals that arrived at in countries where canine vaccination is said to be compulsory.

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At a time when supporters and opponents of preventive canine vaccination still confront one another, any conclusion would be out of the question. Let us be content with a few remarks :

First, the experimental study of the process seems to lag behind its practical application, since the latter is not founded on the former, as should logically be the case. The laboratory will therefore have to regain the ground lost and to solve the fundamental problems which still remain open, thereby enabling practical application to free itself from empiricism.

Further, the full information required to form a judgment is only very rarely given in the sources available. Too often one has to be satisfied with vague, unconvincing statements which seem to be dictated less by a careful study of the situation than by the desire to make it appear in a favourable light. More precision is required, and even, let us make bold to say, more frankness.

Lastly, even if enforced with the most rigorous compulsion, preventive vaccination will not reach those dangerous rabies-carriers, the stray dogs. It will therefore still be necessary to capture and kill them, not in the interest of the canine population itself — which, being vaccinated, can be taken as immune — but for the safeguarding of the human community. This implies that, amongst sanitary police measures, the preventive vaccination of dogs should be considered merely as an auxiliary.

December 1940.

NUTRITIONAL RESEARCH IN THE UNION OF SOUTH AFRICA

by

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The population of the Union of South Africa is slightly over ten millions, divided into two million Europeans and eight million non-Europeans. The non-Europeans may be further subdivided into seven million aboriginal Bantus, just under one million Coloureds (*i.e.* Eurafricans and Eurasians) and a quarter of a million Asiatics. The Europeans, as the ruling class, are the highest socio-economic section, but even among them malnutrition is prevalent among the so-called " poor whites," who probably constitute about a fifth of the European population. With isolated exceptions, the whole of the non-European population is socio-economically depressed and one may therefore assume extensive incidence of malnutrition among them.

No complete nutrition survey of the whole population has yet been undertaken. In 1929, the Carnegie Commission investigated the " poor white " question in South Africa, Dr. W. A. MURRAY reporting on the health aspect (1). Murray and his co-workers collected data regarding the dietaries and body measurements of over 1,700 children from this group of the population. The somatometric examination consisted of measurements of trunk length (sitting height), chest circumference and weight. These were related to age in a composite nutrition index. Adverse factors other than shortage of food constituents were considered, such as malaria, bilharziosis and ankylostomiasis.

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(1) MURRAY, W. A. : " Report of the Carnegie Commission on the Poor-White Problem in South Africa ". 1932, 4 : *Health Factors.*

Le Riche's Study of European School-children in Pretoria

A valuable study of European school-children in Pretoria was undertaken last year by Mr. Harding Le Riche working under the auspices of the South-African Council for Educational and Social Research (1). He examined children in twenty-one schools. The schools were classified as *well-to-do*, *average* and *poor*, according to the average economic condition of the homes from which the children were drawn. Of the total of 2,716 white children examined, 513 were in well-to-do schools, 1,158 in average and 1,045 in poor schools.

A physician carried out clinical examinations of the children in six of the schools, and found a very high incidence of malnutrition among them. The A.C.H. index ascertained by Le Riche selected fewer children as being ill-nourished than did the clinical estimate. But quite apart from this there was no agreement between the A.C.H. index and the clinical findings. The A.C.H. findings on boys and girls in the twenty-one schools showed that malnutrition increased with age for both boys and girls, but there was significantly more malnutrition among the boys. It would seem, as other observers have found, that here, as elsewhere, boys are more sensitive to an adverse environment than girls. Apart from intrinsic differences in metabolism in the two sexes, it is suggested that inadequacy of food intake and fatigue are more pronounced among boys than girls in view of the greater activity of boys and their consequent greater energy requirements. Comparison of the nutritional state of children from poor and well-to-do schools gave inconclusive results.

In addition to the arm, chest and hip measurements taken to arrive at the A.C.H. index, Le Riche made a number of other measurements such as weight, standing height, sitting height, bi-acromial width, bi-trochanteric width and skin and subcutaneous tissue measurements. Girls were found to be heavier than boys in

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(1) Le Riche, H.: "A Study of European School-children in Pretoria." South-African Council for Educational and Social Research. 1940. Series No. 13: *Physique and Nutrition.*

all the age groups studied (8 to 14 years) except at 9½ years, where the weights of the two sexes are virtually identical. The boys were taller than the girls from 8½ to 9¾ years and after 13¼ years, the girls exceeding the boys in height from 9¾ to 13¼ years. Except at 10½ years, where the measurements were the same, the boys were found to have larger chests than the girls. The girls exceeded the boys in bi-iliac width at all ages. In sitting height the boys were taller than the girls up to 10 years, after which age the girls were the taller. In upper arm girth the girls had thicker arms than the boys at all ages. As regards economic conditions, "poor" girls and boys were found to be lighter than those financially better off; "poor" children were shorter than those more favoured, the discrepancy being more pronounced among the girls, who were significantly shorter in more age groups than in the case of the boys; "poor" boys had shorter trunk lengths in only two age groups, while "poor" girls were shorter in four age groups than the more privileged children; the normal thoracic circumference was smaller in both sexes of "poor" children in most of the age groups; the bi-iliac width was smaller among "poor" boys in only two age groups, among "poor" girls in six age groups; both "poor" boys and girls had smaller upper arm girths (relaxed) than the more favoured children.

Finally, Le Riche examined the food consumed in 68 of the families from which his school-children were drawn. The median family income was found to be £20.63 per month; of this 40.68% was spent on food. In the case of 55 of the families, the caloric intake was below 4,000 per man unit per day. Assuming that the protein standard should be 80 grams per man unit per day, it was found that little more than half of the families studied had a protein intake of less than this figure. Assuming a standard requirement of 100 grams of fat per man unit per day, 45 of the budgets (66%) fell below this standard. Calcium intakes were calculated by assuming that two-thirds of the dietary calcium came from animal sources (mainly milk and milk products); on the basis of 0.68 grams calcium per man unit per day as a standard, 49 of the dietaries (72%) were defective in this respect, indicating an acute need for a higher consumption of milk and its products.

Preliminary Government Survey of the Nutritional Condition of European School-children in the Union

National awareness of the evils of malnutrition in the Union came to a head in 1937, when a woman member of Parliament, Mrs. Malherbe, raised the matter in the House of Assembly. Government accepted her proposal that a nutrition survey be conducted and voted the necessary funds (1). The Union Health Department called a conference of representatives of the medical inspectorates of the Education Departments of the four provinces — Cape, Transvaal, Orange Free State and Natal. This conference decided to accept in general the recommendations of the League of Nations group of experts as to the manner of conducting the survey and to use the general programme laid down in their report of April 1937.

During the second half of the year 1938, the four Chief Medical Inspectors of Schools and their staffs collected clinical, somatometric and dietetic data regarding 140,928 school-children selected at random. This number constitutes roughly a third of the European school population of the Union. The preliminary conference had given the workers an opportunity at arriving at identical methods of examination.

The *clinical examination*, which is generally admitted to be the most difficult to standardise, was, as the result of rehearsal, reduced to such uniformity that error due to personal factor was largely eliminated. A routine clinical assessment of nutritional state of the children was already in use throughout the four provinces, viz. :

Class	1	— General condition	excellent.
»	2	— »	» good.
»	3	— »	» requiring supervision.
»	4	— »	» requiring treatment.

(1) Union Department of Public Health : " Report on the Nutritional Condition of European School-children in the Union of South Africa, 1940."

Table I shows the distribution of the boys examined in the four provinces according to this assessment (Dunfermline scale).

Table I.

Clinical Examination of Union European School-boys : Percentage in each Nutritional Class in each Province

Province	Class 1	Class 2	Class 3	Class 4	Number of boys
Cape	9.6	58.8	27.3	4.2	19,914
Natal	22.5	61.0	13.3	3.1	2,747
Orange Free State . .	15.3	41.0	35.7	7.9	4,546
Transvaal	5.3	47.1	39.6	8.0	30,958
Union	8.4	51.3	33.8	6.5	58,165

From this table it appears that 6.5% of the European boys of the random sample examined were obviously ill-nourished, and as an approximate figure the sample reveals that malnutrition in varying degrees exists in 40% of the European school-boy population.

The nutrition condition of European school-girls in the Union, as revealed by the clinical examination, is set out in Table II.

Table II.

Clinical Examination of Union European School-girls : Percentage in each Nutritional Class in each Province

Province	Class 1	Class 2	Class 3	Class 4	Number of girls
Cape	6.8	61.4	26.7	5.1	14,482
Natal	27.7	60.9	9.5	1.8	1,956
Orange Free State . .	22.3	45.7	26.9	5.2	2,859
Transvaal	8.4	54.9	31.4	5.3	16,385
Union	9.9	57.1	27.9	5.0	35,682

Comparing the boys and girls for the whole of the Union, the latter are found to be much more satisfactory. While 40.3% of the boys showed some degree of malnutrition, this was true of only 32.9% of the girls. The improved showing of the girls is due to the larger proportion in the second nutritional class — boys 51.3%, girls 57.1%. Further, fewer girls than boys fall into the fourth

nutritional class: marked malnutrition was found among 6.5% of the boys and 5% of the girls.

The boys appear to be more seriously affected by malnutrition both in the Union as a whole and in each province, with the exception of the Cape. Natal is the best and the Transvaal the worst province for both boys and girls. Though the personal factor in clinical assessment may admittedly lead to wide variation in results, the findings of this survey are nevertheless of considerable value, particularly as no similar national studies have hitherto been conducted.

The *somatometric examination* consisted of measuring the weight, height and trunk length (sitting height) of each child. In the following tables, the average measurements for each sex for each province are compared with the average measurements for the total Union boys and girls included in the survey. The comparison is therefore, not with the optimum height, weight and sitting height possible in the Union, but with an average influenced by the inclusion of all children, whether nutritionally normal or not.

Table III gives the *average weight* in pounds for each sex by age groups.

Table III.

AVERAGE WEIGHT OF EUROPEAN SCHOOL-CHILDREN IN THE UNION OF SOUTH AFRICA

Age	Union		Cape		Natal		Orange Free State		Transvaal	
	M	F	M	F	M	F	M	F	M	F
7	51.40	50.37	51.33	50.17	52.19	51.29	50.69	49.99	51.32	50.32
8	56.63	55.43	56.51	55.35	58.03	55.98	56.81	55.47	56.46	55.46
9	62.08	61.42	61.67	61.43	64.24	63.05	62.03	61.84	62.00	61.15
10	67.81	67.92	67.76	67.79	69.51	68.69	67.03	68.24	67.66	67.95
11	74.21	76.51	74.00	76.38	76.09	79.72	74.08	77.04	74.02	76.23
12	81.22	86.72	81.30	86.31	82.70	89.30	80.80	87.52	80.93	86.87

Table IV gives the number of districts in each province with an average weight per boy and per girl below the Union average by 1 lb and more.

Table IV.

NUMBER OF DISTRICTS GIVING AN AVERAGE WEIGHT PER BOY AND PER GIRL OF 1 LB OR MORE BELOW THE UNION AVERAGE

Province	Number of magisterial districts	Number of districts included in the survey	Number with average below Union standard by 1 lb	
			Boys	*Girls*
Cape	131	88	20	33
Natal	44	19	0	3
Orange Free State . .	33	27	8	9
Transvaal	41	41	9	3
Union	249	175	37	48

Table V gives the *average height* in inches for each sex by age groups.

Table V.

AVERAGE HEIGHT IN INCHES FOR EACH SEX BY AGE GROUPS

Age	Union		Cape		Natal		Orange Free State		Transvaal	
	M	F	M	F	M	F	M	F	M	F
7	48.31	48.00	48.08	47.84	48.71	48.21	48.23	47.82	48.41	48.18
8	50.39	50.03	50.18	49.91	50.70	50.32	50.51	49.92	49.92	50.18
9	52.34	52.08	52.06	51.95	52.69	52.34	52.44	52.23	52.45	52.17
10	54.16	54.17	53.97	53.96	54.58	54.44	54.09	54.26	54.23	54.39
11	56.05	56.48	55.84	56.35	56.31	56.91	56.18	56.48	56.15	56.60
12	57.93	58.89	57.75	58.73	58.04	59.25	57.95	59.12	58.04	59.03

In Table VI is shown the number of districts in each province with an average height per boy and per girl below the Union average by half-an-inch or more.

Table VI.

NUMBER OF DISTRICTS GIVING AN AVERAGE HEIGHT PER BOY AND PER GIRL OF HALF-AN-INCH OR MORE BELOW THE AVERAGE UNION HEIGHT

Province	Number of magisterial districts	Number of districts included in the survey	Number with average below Union standard by half-an-inch	
			Boys	*Girls*
Cape	131	88	19	33
Natal	44	19	3	2
Orange Free State . .	33	27	2	5
Transvaal	41	41	0	0
Union	249	175	24	40

Table VII gives the *average sitting height* in inches for each sex by age group.

Table VII.

AVERAGE SITTING HEIGHT IN INCHES FOR EACH SEX BY AGE GROUPS

Age	Union		Cape		Natal		Orange Free State		Transvaal	
	M	F	M	F	M	F	M	F	M	F
7	25.65	25.34	25.33	25.10	26.24	26.06	25.13	24.79	25.84	25.59
8	26.44	26.16	26.10	25.91	27.03	26.71	26.02	25.62	26.63	26.50
9	27.18	26.90	26.81	26.70	27.94	27.53	26.77	26.43	27.36	27.17
10	27.87	27.74	27.58	27.50	28.51	28.32	27.27	27.16	28.06	28.07
11	28.59	28.74	28.26	28.51	29.10	29.35	28.18	28.34	28.80	29.01
12	29.31	29.86	29.00	29.65	29.84	30.46	28.76	29.35	29.53	30.17

Table VIII gives the number of districts in each province with an average sitting height per boy and per girl below the Union average by half-an-inch or more.

Table VIII.

NUMBER OF DISTRICTS GIVING AN AVERAGE SITTING HEIGHT PER BOY AND PER GIRL OF HALF-AN-INCH OR MORE BELOW THE UNION AVERAGE

Province	Number of magisterial districts	Number of districts included in the survey	Number with average sitting height below Union standard by half-an-inch	
			Boys	*Girls*
Cape	131	88	32	27
Natal	44	19	0	0
Orange Free State . .	33	27	11	12
Transvaal	41	41	1	0
Union	249	175	44	39

The physical examination results are of considerable interest. The tables giving the average weight, height and sitting height by sex and age groups indicate that girls in the age groups 7-12 years have an average weight and height greater than boys of the corresponding age groups. In sitting heights the boys have the greater measurements. The superiority of the girls in weight and height is not general: only in the later age groups do the measurements for girls surpass those for boys.

What is true of the Union as a whole is seen in Tables III, V and VII to be true of the individual provinces. In the later age groups of the period 7-12 years, the girls are uniformly heavier and taller than the boys. The average weights and heights of Natal boys and girls are seen to be considerably above those of the total Union returns and of the other provinces. This satisfactory condition of Natal school-children is borne out further by the district returns, where almost without exception the district averages for weight and height are above the Union standards. In the other provinces there are deficiencies which, from the various tables, are seen to be least prominent in the Transvaal.

Dietetic Data.

The difficulties associated with the study of diets are well known. In the present preliminary survey, enquiries into individual diets were therefore deliberately limited to certain general factors. Even so, the data are to be accepted with reservations. They must only be interpreted as giving certain very broad indications as to dietetic deficiencies. Adequate dietetic studies can only be accomplished by more intensive investigation than was possible in the present general survey. The facts collected in this preliminary survey were set out in a number of tables showing the percentage of boys and girls taking certain meals and articles of diet. These tables revealed that, in general, there is no mass lack of food among European school-children in the Union, the great majority of them having three meals a day. Most of them also have meat and vegetables as regular articles of diet. A very serious defect revealed by these tables is that only a third of the European children have milk as a regular article of diet, while even more serious is the fact that a third of the children receive no milk at all.

A much larger proportion of Natal children receive milk, vegetables and fruit regularly than of the Union as a whole or Cape Province children. This is in keeping with the clinical and somatometric findings that the incidence of malnutrition and departures from the physical standards were least in Natal. Even in Natal the consumption of milk by children is not satisfactory, as 20.4% of the boys and 20.1% of the girls do not receive this article in their diet.

Conclusions.

The Governmental report is of value as it provides the first general assessment of the incidence of malnutrition, the variation of physical measurements of weight, height and sitting height, and the broad dietetic intake of European children in the Union. Malnutrition as revealed by clinical examination and physical measurement is shown to be present in minor forms in large numbers of European children. The survey records indicate, however, that, in this section of the population at any rate, gross starvation and gross deficiency are not prevalent.

The survey records are confirmatory of scientific studies in other countries that the assessment of malnutrition is a difficult subject and that the use of the clinical method is subject to much variation. In the present study this is borne out by the results of the clinical and physical tests not being correlated in all cases. Accepting the summation of evidence provided by the three sets of data — clinical, physical and dietetic — the broad deductions possible from the survey material are as follows :

(*a*) Gross starvation in Europeans on a mass scale has not been revealed ;

(*b*) Malnutrition of varying degree, but mostly in minor forms, affects approximately a third of both European boys and girls ;

(*c*) Deficiencies in height, weight and sitting height affect chiefly the Cape and the Orange Free State. Natal children are on the average above the Union standards in all three features.

(*d*) Dietetic deficiencies are chiefly those of the important protective foodstuffs — milk, meat, vegetables and fruit.

Bantu Survey

The preliminary general survey of school-children left untouched the large Bantu population. Considerable evidence had accumulated of malnutrition often to a marked degree among these people. Extensive outbreaks of pellagra (1) and other deficiency diseases

(1) Cluver, E. H. : " Pellagra among the Maize-eating Natives of the Union of South Africa. " *Brit. Med. Jl.*, 1929, October 26th, page 751.

have been described. The Union Health Department therefore considered it imperative in carrying out the wishes of Parliament to collect some reliable information of this group. The available funds allowed of only a small investigation. A small itinerant team examined selected sample groups. The work was undertaken by Dr. S. Kark and Mr. H. Le Riche, both of whom had had previous experience in field nutrition investigation (1). They spent one month in collecting information regarding 800 school-children in each of the following Bantu communities : Pretoria City Location, Witzieshoek, Bloemfontein Location, Qumbu and Kentani in the Transkei, Maritzburg Location and Nqutu in Natal, Tzaneen and Bochem in the Northern Transvaal. These nine communities represent fairly typical urban and rural Bantu groups in the Transvaal, the Orange Free State, the Transkeian Territories and Natal, the chief native areas of the Union. The limited information regarding 7,000 Bantu children, which the Bantu survey aims to gain, is admittedly an imperfect foundation upon which to establish the degree, nature and types of malnutrition in the 7,000,000 people composing the total Bantu population of the Union. School groups were chosen for examination, as they alone were easily accessible and offered roughly comparable social and economic Bantu classes in the different areas of the Union.

Before commencing their work, the two members of the team tested the record forms and the technique of examination on a group of Bantu children in Pretoria. Besides testing the procedure, this trial enabled the observers to arrive at standards of physique and methods of classification which would reduce the variability in assessment on passing from group to group. The Director of the South-African Institute for Medical Research arranged for the laboratory work associated with the Bantu survey to be undertaken at the Institute. The following facts were secured for each child : age, appearance, weight, standing and sitting heights, chest circumference, upper arm girth, chest depth and width, bi-iliac and bi-trochanteric width, and the thickness of the subcutaneous tissues at various sites. A full clinical examination of each child, and a collection of blood, fæces, urine and throat swabs from a sample was also made.

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(1) Gear, H. S., and Cluver, E. H.: " The South-African Programme of Nutritional Studies." *South-African Med. Jl.*, 1939, September 23rd.

Through the active interest and co-operation of City Councils and Medical Officers of Health of Pretoria, Bloemfontein and Maritzburg, the Government Native Affairs Department, the various Provincial Educational Departments and the local officials, the investigation has succeeded beyond expectation. In no case has there been any difficulty in getting the children to submit to examination.

Study of an Indian Community

The preliminary school survey included records of a small number of Indians in Natal. The number scarcely sufficed to allow of critical analysis ; so it is fortunate that a Government investigation in Durban will contribute something more tangible to understanding malnutrition in the Indian community, whose social and dietetic customs differ so greatly from those of other social groups (1). Dr. B. A. Dormer, Medical Superintendent of the King George V Jubilee Hospital for Tuberculosis, has examined 1,000 Indian school-children in Durban and has also collected very full data concerning the existence of food deficiency conditions among them. In addition, dietetic facts of the families included in the survey were gathered. A comprehensive report on the various factors possibly influencing nutrition in the Indian community will thus become available.

Other Nutritional Research in Progress

The studies mentioned above required only a proportion of the appropriation voted by Parliament for the nutritional survey programme. The sum available was entirely insufficient to equip the Union Health Department for the highly technical research involved in proceeding to the next stage recommended by the League of Nations experts, which required intensive studies of dietetic, social, economic and agricultural factors on the one hand, and detailed medical, bio-chemical and physiological findings on the other. The Department was unable to release its routine medical officers, even if they had had the prerequisite experience and

(1) See footnote on page 337.

knowledge of techniques to undertake such highly specialised work. Further, there were no departmental facilities available in the nature of fully equipped laboratories. The possibility of further stages in the work being undertaken by extra-departmental agencies was therefore examined. Such agencies would require personnel experienced in the special techniques and a basic organisation capable of handling the mass of work involved. Fortunately, the Department's approach to the three institutions obviously meeting the above requirements — the South-African Institute for Medical Research and the Medical Schools of the Universities of Cape Town and the Witwatersrand — met with a more than willing response. Relatively small grants given to each of these institutions has promoted the work involved in the next stage of the nutritional research programme. By delegating certain aspects of the nutritional programme, the Government has secured some of the best workers in the field and the co-operation of the leading medical institutions in the country. Delegation has also resulted in extending and stimulating interest in nutritional problems.

Europeans on the Witwatersrand : Effect of Social and Economic Factors.

The preliminary general school survey merely revealed the incidence of malnutrition. For causes and associated influences in social and economic fields, the assistance available in the medical and social research departments of the University of the Witwatersrand was solicited. Professor J. L. GRAY, head of the University Department of Social Studies, undertook an investigation into the social and economic factors operating in the causation of malnutrition in Europeans on the Reef. The underlying assumptions of this investigation are, first, that, in so far as insufficient or improper food is a cause of malnutrition, the reasons must be closely analysed by a study of the total social condition of families ; secondly, that living conditions, other than food habits, may play a larger part in producing clinical signs of malnutrition than is commonly recognised. The objects of the investigation are :

(1) To ascertain the incidence and distribution of good and bad nutrition, as determined by clinical examination, among children of different family and socio-economic condition. Data are being collected concerning the size and structure of families

and households, occupation and incomes of members, distribution of household expenditure, place of origin, duration of urban residence and education of members, family health and mortality, housing and sleeping conditions and food habits.

(2) To discover the relations between differences in nutritional status and (*a*) genetic differences, *e.g.* by comparisons between sibs, twins and unrelated individuals ; (*b*) fertility ; (*c*) social and educational background of parents ; (*d*) income ; (*e*) dietetic habits ; and (*f*) other non-dietetic family conditions.

(3) To conduct experiments into the effects of supplementary feeding — *e.g.*, with milk — with groups consisting of institution children, twins and sibs, and children in foster-homes.

(4) To construct a typical minimum human needs budget for the Witwatersrand which will serve as a standard by which to determine the poverty line.

(5) To assess the respective importance, if possible quantitatively, of alternative procedures to obtain nutritional improvements in the population — *e.g.*, better allocation of existing expenditure, dietetic education, children's allowances, increased family income and better housing.

Correlation of Agricultural Factors with Malnutrition.

An effort to elucidate some of the factors operating in rural European communities has been made possible through the co-operation of the South-African Institute for Medical Research, Johannesburg. Dr. William Fox undertook the investigation of problems of production and distribution of foodstuffs, the importance of prices and purchasing power, and also the influence of personal and family characteristics. This study differs from that of the Witwatersrand in that an effort is being made to correlate agricultural factors with malnutrition. The team will visit as many localities as possible, since rural communities differ greatly in local agriculture and in the availability of food, especially the " protective " foodstuffs. As far as possible, areas included in the itinerary are representative of different climatic and geographical regions, types of community — *e.g.*, large-scale and peasant farming — and finally of different types of farming.

Intensive Study of 1,000 Cape Coloureds.

Though the Union Health Department was able to make good to a small extent the unavoidable omission of the Bantu and Indian races from the preliminary general survey, it was unable itself to investigate the Coloured community. Not to have some knowledge of this section would have been a serious omission, as evidence obtained during other enquiries had indicated serious malnutrition among them. Professor J. F. Brock, of the University of Cape Town, has started on this investigation. He has undertaken to apply all the available methods of testing the state of nutrition in respect of individual components of the diet — *e.g.*, vitamin C saturation test, hæmoglobin and blood studies, X-ray of bones and study of calcium and phosphorus metabolism. These investigations are being collated with the results of clinical examination and somatometric measurements in a group of approximately 1,000 Coloured school-children. The University Department of Social Science is studying the families of the children examined, in order (*a*) to place their economic status in relation to that of the rest of the population of Cape Town, and (*b*) to study intensively the dietary intake of selected families by the family budget method.

Investigation into the Effect of Malnutrition on Functional Efficiency.

An interesting approach to the assessment of malnutrition is the investigation being carried out by Dr. E. Jokl, of the Witwatersrand Technical Institute. He has examined the physical performances of different racial groups and is co-ordinating these findings with other nutritional assessments of the same groups. His records include tests of short-, middle- and long-distance running races, the short putt and long and high jumps. His team is also examining various groups of children in areas other than the Witwatersrånd, which have already been examined by other workers — *e.g.*, the Indian children of Dr. Dormer's investigation in Natal and the Coloured children of Professor Brock's survey in Cape Town. It will therefore be possible to compare Dr. Jokl's athletic data obtained from various racial groups in different parts of the country with the clinical and physical findings of the other nutrition workers obtained from the same groups.

THE POOR RICE-EATER'S DIET

by

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The cereals which are most widely cultivated in India are rice, wheat, great millet *(Sorghum vulgare)*, spiked millet *(Pennisetum typhoideum)* and the common millet *(Eleusine coracana)* known in India as " ragi " or " marua ". Barley and maize *(Zea mays)* are also grown in various areas. But taking the country as a whole, the area under rice exceeds that under all other cereals put together ; in the great provinces of Bengal, Bihar, Orissa and Madras, rice is the staple food of the majority of the population.

In Ceylon, Thailand, Malaya, Indo-China, the Dutch East Indies, the Philippine Islands and South China, the diet is based on rice ; it is said, indeed, that rice is the staple food of about half the human race. It is thus natural that nutrition workers in India and other Far-Eastern countries should pay great attention to the problem of the nutritive value of rice and the deficiencies of the poor rice-eater's diet ; there is no more important problem for them to study. A great deal of work on this subject has been carried out in research laboratories in India and other Eastern countries. In Coonoor, in the last few years, the problem has been investigated from various angles.

The research programme of the laboratories includes the carrying out of dietary surveys in sample areas throughout India. A careful quantitative study is made of all the foods consumed by a group of families — usually from twenty to forty — the survey lasting about three weeks. Surveys have been repeated in the same area at different seasons. Intake per consumption unit of calories, protein, various mineral elements and vitamins is worked out, and, in addition, the average composition of the diets as regards actual foodstuffs is determined. The table shows the foodstuff composition of the diet in various rice-eating groups in different parts of India :

MEAN INTAKE OF VARIOUS FOODSTUFFS

(Ounces per consumption unit per day)

	Madras Presidency			*Bengal :*	*Assam :*	*Orissa :*	*Central Provinces :*	*Kashmir* [1]	*Tehri-Garhwal* [2]
	Rural area	Tea Plantation labourers	Families with leprosy : Madras City	rural area	tea plantation labourers	rural area	rural area		
Rice	15.0 (millet 5 oz.)	18.0	14.0	25.0	19.0	19.0	26.0	26.0	16.0 (millets 3.0)
Pulses	1.3	1.0	0.8	0.4	0.9	1.0	1.1	0.6	1.8
Leafy vegetables	0.3	none	negligible	0.2	0.8	1.4	1.5	5.2	0.5
Non-leafy vegetables . .	1.5	3.0	3.0	7.0	4.0	6.0	3.0	2.0	4.7
Vegetable fats and oils .	0.5	0.5	0.5	0.3	0.3	0.3	0.2	0.9	0.2
Fish, meat, and eggs . .	negligible	1.5	1.4	0.7	0.7	0.6	negligible	0.2	negligible

Condiments and sugar in small quantities were also included in the diets.

[1] The families included in this survey consumed a little milk (2.2 ozs. per consumption unit per day).

[2] One of the Punjab States in the Himalayan foothills.

It seems that the diets of poor rice-eaters are very similar in composition throughout India. The figures given in the table are based on surveys carried out in widely separated parts of the country — areas some of which are several thousand miles distant from each other. The available data justifies the following statements : the poor rice-eater in India consumes, in addition to his staple cereal, only very small quantities of other foods such as pulses, vegetables, and meat. Milk is taken in negligible amounts or not at all. While foods other than those listed, such as fruits, may occasionally be consumed, the table gives a fairly accurate picture of the composition of ordinary daily diets.

If the diets shown are worked out in terms of protein, minerals, and vitamins, and the results compared with the standards suggested by the Technical Commission on Nutrition and other standards drawn up by physiologists, it is found that the rice-eater's diet falls short of such standards in almost every important constituent. The deficiency of calcium is particularly striking ; average diets based on rice may supply not more than 0.2 gramme calcium daily. The importance of this and other deficiencies will be discussed in a later section.

The Milling of Rice

Within recent years there has been considerable discussion in Europe and America about the desirability of consuming wholemeal bread in place of bread made from refined wheat flour. In general, nutrition workers have urged the wider use of the former, which is richer in various food factors, and notably in vitamin B_1. Others have maintained that the ordinary mixed diets of Western civilisation contain sufficient vitamin B_1 derived from foods other than cereals, and that the substitution of the under-milled for the highly milled cereal is not a matter of great moment. As regards the poor rice-eater's diet, illustrated in the table, there can be no controversy of this nature ; it is quite clear that the state in which the staple cereal, which supplies 80-90% of total calories, is consumed, must be of great importance. The League of Nations Inter-Governmental Conference of Far-Eastern Countries on Rural Hygiene, which met in Java in 1937, expressed itself on this point

as follows :

> " The Conference emphasises the fact that the degree of milling to which rice is subjected is of vital importance in connection with the problem of nutrition throughout the East. In many countries, the poorer classes consume foods other than rice in small quantities, and it is very difficult, for economic reasons, to increase the amount of supplementary foods in the diet ; in such circumstances, the nutritive value of the main article of food, which is influenced by the degree of milling, becomes of great significance."

The Conference, in its resolution about rice, made no specific reference to the beriberi problem. The most important difference between highly milled and under-milled *raw* rice is in respect of vitamin B_1 content. (This is not so in the case of parboiled rice; reference will be made to this point later.) But the outer layers of the grain are richer than the starchy endosperm in other factors — protein, and certain vitamins and minerals besides vitamin B_1 — and the removal of these layers on milling leaves the grain impoverished in other respects besides vitamin B_1 content.

Throughout the East the habit of consuming rice pounded by domestic methods — rice which retains a considerable proportion of its integuments — seems to be disappearing. A lengthy discussion of " the rice problem " took place at the Java Conference, which made it clear that, while all were agreed that the widespread use of highly milled rice in Eastern countries was to be deplored, the factors underlying the change have been imperfectly analysed and understood. Accordingly, the Conference recommended further investigation of " the nutritive, commercial, economic, and psychological aspects of the problem ". The Nutrition Advisory Committee of the Indian Research Fund Association (now the National Advisory Nutrition Committee for India) took note of the recommendation at its meeting in November 1937 and itself recommended suitable investigations along the above lines in India.

The enquiry, which is being undertaken by the Coonoor Laboratories, is designed to throw light on the following questions :

> *(a)* To what extent has the habit of consuming milled rice spread in India ?

(b) If it is actually the case that more and more people are eating milled rice in preference to under-milled varieties, what are the factors underlying this change in habit ?

(c) Is it possible or desirable to reverse the present tendency ? If so, what steps can be taken ?

The investigation, which has been confined to the Madras Presidency (population in round numbers 50 millions), has been carried out by the questionnaires and field investigations. While it is not yet complete, certain points of interest have emerged, to which brief reference will be made.

It can be reckoned that about 70% of the rice-eating population of Madras consumes machine-milled rice (in other parts of India — *e.g.*, Bengal — the percentage is smaller). In Madras, the custom of pounding rice persists only in certain rural areas, and there seems no reason to suppose that such areas will long escape the general tendency. If a campaign to encourage the use of home-pounded rice is contemplated, it is important that the extent to which the substitution of home-pounded by milled rice has proceeded should be realised. It is not a question of checking a process which has just begun, but of changing a habit which is already widely established and appears to be becoming universal.

The rice mill has spread everywhere, even into remote rural areas. Its extended use is bound up with other social and environmental changes. The development of transport and the improvement of roads enables the rice-grower to bring his grain to the mill. The ubiquitous motor-bus has loosened the bonds which attach the villager to his own plot of ground and traditional manner of life. Cheap electric power is obtainable over wide areas and other sources of power — the steam and internal combustion engine — are familiar and obtainable.

The mill saves trouble and labour. About six hours work per week are required to pound sufficient rice to cover the requirements of an average family. The mill will deal with a similar quantity in a few minutes. To take the paddy (unhusked grain) to the mill may involve little effort. In certain areas the mill-owners collect the paddy in their own carts and mill it for a small charge. A frequent arrangement is that the paddy is milled for nothing on the condition that the miller keeps the husk and bran.

If rice is to be pounded, there must be paddy stored in the home. Often the small land-holder may find it necessary to dispose of his grain to merchants immediately after harvesting to obtain some ready money or credit. His rice thus becomes an article of commerce and is not retained as food for his own use. With the money or credit accruing from the sale he obtains machine-milled rice — the only kind of rice purchasable in the bazaar — which may originate in another part of India or be imported from Burma or Thailand.

Such large economic groups as industrial and plantation labourers and village labourers with small or negligible holdings, employed in daily labour in return for wages, are occupied with their daily task and have less time and energy to spare for work in the home than independent cultivators. Women are employed as wage-labourers as frequently as men. Even if the poorer classes generally were anxious to pound grain for their own use, there are various other obstacles in the way. Under existing commercial conditions, paddy cannot easily be bought in small quantities for daily use, as can milled rice, and poor families cannot afford to purchase grain in bulk. Even if they could, they would have no place to store it in. Home-pounding requires accommodation which is not available when families are huddled together in huts. Three or four instruments are required : two pounders costing about Rs. 1-4-0 (about 1*s.* 9*d.*), a sieve costing about 4 annas (about 4*d.*) and a stone mortar. In certain districts the villagers no longer possess the necessary pounding instruments.

In various places, attempts have been made to produce hand-pounded rice as an article of commerce — *i.e.*, groups of labourers perform the task of husking more usually carried out by the mill. Rice so produced is invariably a little dearer than milled rice, because of the cost of the labour involved, just as any hand-made product tends to be dearer than a machine-made product. Even the smallest difference in price is all-important to the poor, who are forced to buy the cheapest varieties of rice. About a fifth of the rice consumed in the Madras Presidency is imported, mainly from Burma ; most of this is milled parboiled rice. As a rule, the imported varieties are the cheapest in the market. Elsewhere in India imports are of relatively minor importance.

At the present time, in South India, a small proportion of the well-to-do, influenced by the propaganda of nutrition workers, are taking to home-pounded rice and it is largely for the benefit of this minority that such rice is now reaching the market in small quantities in certain centres.

In general, then, the economic and social environment is inimical to the practice of home-pounding. Economic factors are largely responsible for bringing about the present situation. Psychological motives — the desire of the poorer classes to imitate the well-to-do in their general preference for white, highly-milled, rice — do not appear to be of major importance. There is reason to believe that the poorer classes in South India would have no objection to under-milled rice if it were easily available and cheap and that, indeed, such rice would actually be preferred by a considerable proportion of the population. At present under-milled or home-pounded rice can only be purchased in a few centres. The mills themselves do not turn out any under-milled rice.

As far as South India is concerned, problems of storage and transport are not highly significant in this connection. Under-milled rice, stored under ordinary conditions, deteriorates rather more rapidly than milled rice, but, as far as can be ascertained, prolonged storage of rice is rare under the present system of distribution. The province lives, as it were, from hand to mouth as regards its rice supply. Stocks of home-grown and imported rice are rapidly consumed and replaced by the next harvest crop or by fresh imports.

The views expressed above may require some modification as more data are collected, and further investigation of certain points is necessary. Nevertheless, the preceding paragraphs outline roughly certain aspects of the problem in South India. It is probable that very similar factors are operative elsewhere in the East. Anderson [1] describes the introduction of small mechanical mills into a rural area in Malaya and the readiness with which local rice-growers make use of them. In the district in Malaya in which his investigation was carried out, the miller, in return for the service of milling, keeps the husk and bran and a small proportion of the grain itself, and demands no cash payment.

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[1] *Jl. Malay Branch Brit. Med. Assoc.*, 1938, 2, No. 1.

Parboiled Rice

In most parts of India parboiled rice — *i.e.*, rice which is steamed before milling — is consumed in preference to raw rice. Early in the century, Braddon (1) showed that beriberi rarely occurs when parboiled rice is consumed; this was ascribed by other workers to the fact that parboiled rice (at that time) was usually taken in the hand-pounded or under-milled state. Some years ago, the present writer (2) showed that parboiled rice retains a considerable proportion of its vitamin B_1 content even when highly milled; during the process of steaming, some of the vitamin contained in the germ and pericarp diffuses through the grain and cannot be removed when milling subsequently takes place. Much of the rice consumed in India is parboiled rice milled to a fairly high degree. The superiority of such rice over raw milled rice is shown with great clearness by the epidemiology of beriberi in South India. About 40,000 cases of the disease are reported annually in the Madras Presidency. Of these about 95% occur in a circumscribed area — the Northern Circars — in the north-east of the province. In this area, the population consumes raw rice, whereas elsewhere in South India, and throughout most of the country, the poorer classes prefer parboiled rice. In other Eastern countries, parboiled rice is rarely or never consumed, except by emigrants from India.

Methods of dealing with the Problem

The so-called rice problem has been discussed again and again at international conferences and many proposals designed to check the use of milled rice have been put forward. No very striking practical results have emerged, and many regard the problem as discouraging and impossible of solution. There is some basis for this view. Nevertheless, it should be remembered that Governments, and the educated classes in general, are to-day much better informed about nutrition than they were a few years ago. In India, for example, considerable progress has been made in the education of

(1) *The Cause and Prevention of Beriberi.* 1907. London, Rabman Ltd.
(2) *Jl. of Hygiene.* 1932, **32**, 184.

the people, and the nutrition worker can exert a genuine influence on dietary habits. National nutrition committees and organisations have been established in many countries. There is no reason why a new and vigorous attack on the rice problem should not be made.

One of the recommendations of the Java Conference — that only under-milled rice should be supplied in Government institutions — is already the practice in various countries. In Madras, there is a Government regulation that only hand-pounded rice must be used in hospitals and prisons. In the Philippines, an executive order forbidding the use of highly milled rice in any public institution was issued as long ago as 1909.

At present there is a tendency in South India, on the part of municipalities, etc., to refuse to license new rice mills, or to extend the licences of existing mills, the object being to encourage the use of hand-pounded rice and provide employment. It is questionable whether this policy can achieve results under present economic conditions ; the hand-produced product tends to be dearer than the milled varieties, and, if in a given area the local supply of the former is increased at the expense of that of the latter, the result will probably be that the poor will buy milled rice produced elsewhere in India or cheap imported varieties. A policy which aims at the general revival of hand-pounding throughout the country is doomed to failure. People will not walk if the motor-bus is available, even though it may be more healthy to walk. The rice mill is a convenience, saving time and labour, and, historically, attempts to encourage handicrafts at the expense of machinery have rarely been successful.

In areas into which the mill has not yet penetrated — in which at the present time the population is still economically adjusted to the production and consumption of hand-pounded rice — the position is different. Here there may be a strong case for discouraging the introduction of the rice mill. In the Madras Presidency, the change-over from home-pounded to machine-milled rice has proceeded much further than in most provinces ; in Bengal, Bombay, Assam, Orissa, Bihar, the United Provinces, the Central Provinces, and elsewhere in India, the majority of the rice-eating population still consumes home-pounded rice. Throughout the country, however, the same trend towards the abandonment of home-pounding exists. It is therefore of great importance to

decide what attitude should be adopted towards the penetration of the mill into areas in which domestic methods of preparing rice for consumption are still largely followed.

Where the habit of home-pounding has been generally lost, and the people are economically adjusted to the consumption of machine-milled rice, the only universal solution is the production of under-milled rice by the mills themselves and the simultaneous education of the people to consume such rice. The example of the well-to-do may be of assistance in education. In this case, supply must create demand and demand supply. Whether legislation prohibiting the production of rice milled beyond a certain degree should be introduced is a matter for further consideration. Such legislation involves the problem of suitable milling standards. In the case of raw rice, it is probable that the establishment of standards which are sufficiently satisfactory for use in practice does not present insuperable difficulties. With parboiled rice, the problem of standards may be less easy to solve, but where such rice is used the ill-effects of milling are less serious. These various questions are being studied in India.

The superiority of milled parboiled rice over raw milled rice must again be emphasised. In South India, the Brahmin community prefers raw rice and such rice is quite generally regarded as more " civilised ". There appears to be a slight tendency for the general use of raw rice to increase in areas in which the mass of the population consumes parboiled rice, possibly because of the influence of this idea. This trend should be checked by education and propaganda. In the districts in which raw rice is widely consumed and in which a serious beriberi problem exists, the use of parboiled rice should be encouraged. Whether parboiled rice can be introduced into other Eastern countries in which beriberi is prevalent, such as Thailand and the Philippine Islands, is a matter which should receive the attention of nutrition workers in these countries.

When rice is washed before use it loses a proportion of its vitamin B_1, the loss depending on the amount of washing, the variety of rice, etc. In India, rice is often washed three times previously to cooking ; on a rough average, this results in a loss of about 25% of the vitamin originally present. Further losses occur on cooking — amounting to another 25% if excess of cooking water is used

and the water is discarded. PLATT (1) has suggested that one method of attacking the beriberi problem may be to discourage excessive washing. In India, it would be very difficult to bring about any change in the usual custom.

VARIOUS DEFECTS OF THE POOR RICE-EATER'S DIET

A series of experiments on human beings and laboratory animals has within recent years been carried out in India with the object of obtaining precise information about the various defects of typical diets largely based on rice. It is a tradition of the Coonoor Laboratories, since Sir Robert McCarrison carried out his well-known experiments in which he fed groups of rats on diets resembling those consumed by various Indian peoples, to devise experiments in which diets composed of natural foodstuffs, rather than highly purified synthetic diets, are employed. In one series of experiments with rats, the following diet was used :

	Ounces	Grammes
Raw milled rice	21.00	596.4
Dhal arhar *(Cajanus indicus)*	0.70	19.9
Black gram *(Phaseolus mungo)*	0.70	19.9
Brinjal (egg plant) *(Solanum melongena)*	1.00	28.4
Amaranth leaves *(Amaranthus gangeticus)*	0.50	14.2
Raw plantain	0.50	14.2
Gingelly oil	0.10	2.8
Coconut	0.05	1.4
Meat (mutton)	0.06	1.7

This is called the " poor South-Indian diet " in the Laboratories. The quantities as shown correspond roughly to the intake of an adult man per day (2,600 calories). It will be seen that the composition of this diet, used in animal feeding experiments, corresponds closely with that of diets actually consumed by poor rice-eaters in various parts of India, as determined by surveys (see table on page 343).

On this diet, fed in the proportions indicated, young rats show very poor growth — an average weekly increase in weight of 3-4 grammes is the best that can be obtained. In a long series of experiments, which have been published in the *Indian Journal*

(1) *The Nutrition Problem in the Colonial Empire*, Part I, 1939.

of Medical Research, the diet has been supplemented in various ways and the effect on the growth rate of rats noted. The addition of 28 grammes of dried whole or separated milk powder — corresponding to about 220 cc. of reconstituted liquid milk in terms of human daily intake — greatly improved the nutritive value of the diet for rats. Average weekly increase in weight for ten weeks was 7-9 grammes, twice to three times that occurring on the unsupplemented diet, and the general condition of the animals was improved. Another effective supplement was 14 grammes of dried yeast. Meat and eggs have some supplementary value but less than that of milk. Soya bean and other pulses in varying quantities do not form an effective supplement. Rat growth experiments of this nature unquestionably provide an *indication* of the foods which can most usefully be recommended as additions to human dietaries.

The effect of the additional milk was remarkable. Milk supplies proteins of high biological value and various vitamins and mineral salts. It seemed important to investigate the relative significance of these factors. As regards protein, we found that the addition of pure protein (casein) to the basal diet produced little improvement. Contrary to generally accepted views, typical rice diets are not markedly deficient in protein ; the proteins of rice are of high biological value. Other experimcnts suggested that various factors in the vitamin B_2 group were insufficiently present in the poor rice-eater's diet. But the most striking observation was that much of the supplementary effect of milk could be produced by a calcium salt. The addition of calcium lactate or calcium phosphate to the basal diet improved the growth rate very considerably, and in certain experiments the response to the calcium-supplemented diet did not fall far short of thc response to the milk-supplemented diet.

Parallel experiments have been carried out on ill-nourished children in day schools and boarding-schools. The consumption of 28 to 48 grammes of skimmed milk powder daily, in the form of reconstituted milk, produced an acceleration in growth and an improvement in well-being. Similarly, a daily intake of 0.5 to 1 gramme of calcium lactate accelerated the growth of children and appeared to improve their gencral condition. The observation that the poor rice-eater's diet is deficient in calcium and that the

addition of a calcium salt is in itself of value may be of great importance. In densely populated and poverty-stricken Eastern and tropical countries, the supply of milk is deficient and its cost far beyond the reach of the poor. But 1 gramme of calcium lactate — a partial substitute for milk — could be supplied daily to a child at the cost of 1-2 pence a month. It is not unlikely that the provision of calcium salts on a wide scale to children and adults would effect a striking change in standards of health and physique. From the biological standpoint, it is very interesting that the nutritive value of a diet of natural foodstuffs, consumed by a large percentage of the human population of the globe, can be raised by the addition of inorganic substances of which an unlimited supply is available.

Brief mention must be made of other deficiences of the poor rice-eater's diet. Keratomalacia due to vitamin A deficiency is a fairly common disease in South India and various forms of xerophthalmia are observed in a high percentage of school-children. Night-blindness is very common. Follicular hyper-keratosis of the skin, which has been called toad-skin or phrynoderma, is also frequently observed ; vitamin A is in all probability a factor in the causation of this condition. The prevalence of such conditions shows that *one* of the common defects of the poor rice-eater's diet is deficiency of vitamin A. In the animal experiments described above, the animals were well stored with vitamin A at the outset, and any improvement in growth rate and general condition brought about by the various supplements cannot be ascribed to additional supplies of this vitamin.

The basal diet used contains enough vitamin B_1 to fulfil the requirements of rats under the particular experimental conditions. The animals had some store of the vitamin at the outset and the addition of pure vitamin B_1 did not improve the growth rate. Signs of vitamin B_1 deficiency were not observed in animals fed on the basal diet alone. The experiments were thus designed to throw light on defects of the poor rice-eater's diet other than deficiency of vitamin A and B_1. They show clearly that, while the vitamin B_1 content of rice diets is of great importance, such diets are also grossly defective in other respects.

Recently, we have obtained evidence suggesting that the poor rice diet may be partially deficient in nicotinic acid. The rice-eater

rarely gets true pellagra, probably because the nicotinic acid content of his staple cereal is somewhat higher than that of maize. But certain pellagra-like conditions — *e.g.*, stomatitis — which are very common in various parts of India, are probably due to deficiency of nicotinic acid and other factors in the vitamin B_2 complex.

Scurvy in sevcre form is rarc in the rice-eating areas of the tropics, though intake of fruit and vegetables is often low. Detailed clinical investigation might reveal that mild " sub-clinical " forms of scurvy are fairly common, but so far no worker in the East has succeeded in demonstrating the existence of such conditions. In India, rachitic diseases are extremely prevalent in certain districts in the foothills of the Himalayas, but in general they are rare in rice-eating areas throughout the world, which as a rule enjoy abundant strong sunshine.

Methods of improving the poor rice-eater's diet have been frequently referred to in the preceding paragraphs. With regard to vitamin A, green leafy and other vegetables, and certain fruits, contain this vitamin in abundance. Vegetables and fruits will also supply other food factors besides vitamin A. To increase the production and consumption of such foods in rice-eating countries presents no insuperable difficulty. Red palm oil, obtained from the fruit of the tree *Elaeis guineensis*, is extremely rich in carotene ; this oil is consumed in large quantities in Africa and could be popularised in other countries.

In India, the partial substitution of rice by wheat or one of the millets has been recommended. This improves the nutritive value of rice diets, but is feasible only in certain circumstances. Pulses are rich in vitamin B_1 and an increased intake helps to obviate the danger of beriberi. A greater consumption of fish is also desirable for a number of reasons.

In general, it should be the aim of nutrition policy to rely on natural foodstuffs and, as far as possible, on locally produced foodstuffs, for the improvement of diet. There may, however, be circumstances in which certain groups may benefit from the distribution of vitamin concentrates and mineral salts as such. A preparation containing calcium salts, iron, and nicotinic acid might be cheaply manufactured on a large scalc and distributed to children as a sort of milk substitute. The identification and synthesis of vitamins, which is proceeding apace, may enable some at least

of the important vitamins to be produced in bulk so cheaply that their distribution in the parts of the world in which there is greatest need of them would be economically feasible. Those concerned with nutrition problems in the tropics and the East should bear this possibility in mind.

THE RICE PROBLEM

by

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In a paper published in 1933 in the *Geneeskundig Tijdschrift voor Nederlandsch-Indie*, Vol. 73, page 945, [1] we had shown the effects produced on rice by different degrees of milling, by washing and steaming and the combination of these processes. The number of rice samples examined was subsequently increased to about one hundred. The table given in this previous publication is reproduced hereunder :

	Vitamin B_1 content expressed in standard international units per 500 gr. of rice		
	Rice husked not milled	Undermilled rice	Polished rice
In the original condition	400 — 500	250 — 400	100 — 200
After washing	250 — 300	125 — 200	100
After steaming	—	250 — 400	100 — 200
After washing and steaming	about 250	75 — 125	100
After soaking and steaming.	—	200 — 250	—
After soaking, washing and steaming	—	125 — 200	—
After boiling and evaporating . . .	—	250 — 400	100 — 125
After prolonged washing	—	well below 100	well below 100

The combination of milling and washing very greatly reduces the vitamin B_1 content : steaming in itself is less detrimental to

[1] This paper was published before we had made a study of the vitamin B_1 requirements of the Javanese. In a later study in the *Geneesk. Tijdsch. v. Ned.-Indie*, 1935, 75, 2050, we came to the conclusion that an adult Javanese of an average weight of 50 kg. requires 200–250 I.U. of vitamin B_1 daily.

the rice. The washing of husked rice has comparatively little influence. The value of rice considered as a foodstuff containing vitamin B_1 is therefore, in the first place, determined by the *degree of milling*, since washing is, after all, a normal procedure which is applied to very many foodstuffs. The rice problem is therefore reduced to the question whether the rice should or should not be milled. Although other valuable substances besides vitamin B_1 are lost in milling and washing, the rice problem in the Netherlands Indies is nevertheless closely bound up with the beriberi problem.

Simple though it may seem to be at first sight, this is really a very difficult problem to solve. It was thoroughly discussed, as early as in 1924, at the Far-Eastern Association of Tropical Medicinc Congress at Tokyo, and subsequently, in 1937, at the Inter-Governmental Conference on Rural Hygiene held under the auspices of the League of Nations at Bandoeng. Although we cannot claim to have made very much progress towards the solution of the problem during the last fifteen years, the information now available nevertheless constitutes a more solid foundation on which to base an opinion.

The practical solution of this problem does not, however, lie in the hands of the nutrition experts. If milling were merely a bad habit from the nutritional standpoint, the matter would be simple enough, but in reality the question is more intricate. Milled rice keeps better than unmilled rice. Moreover, its white colour gives it a much more pleasing appearance than rice which has been merely husked, which is usually yellowish or brown or red, and its use denotes a kind of social standing, [1] since the more prosperous natives, the Chinese and the Europeans as a rule eat only white rice which has been thoroughly milled. White husked rice is also found, but it is rarer and not so purely white as thoroughly milled rice ; moreover, it does not keep any better than coloured rice of the same type.

The difficulties to be faced are therefore very great. Action must be directed against the consequences of a faulty habit of

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[1] This is why, in the Netherlands Indies, more beriberi has occurred in times of prosperity than in times of depression, when cases are very rare.

feeding and also (which is more serious) against a method of preserving which in itself is efficient, but which is very harmful to the nutritive value of the product.

In the Netherlands East Indies, conditions are fairly favourable.[1] Java and some other islands produce quantities of rice which are stored as "gabah" (paddy in India) and are "home-pounded" or milled immediately before use. Only 15% of the total rice output of Java passes through the mills (1936). About a third of this is partly milled, and the rest, part of which is exported, is completely milled. Unfortunately, in the large towns, and in some other parts of the country, the natives consume increasing quantities of completely milled rice.

In regions where rice is not produced in sufficient quantities, the situation is more difficult, for the rice has to be imported and stored. In principle, the types of rice that might prove to be suitable are gabah, steamed ("parboiled") rice and milled rice. The first keeps well, but is expensive to transport owing to its bulk and it could be used only if there was a mill on the spot. The process of steaming rice is unknown in the Netherlands Indies and hitherto no one seems to have taken an interest in it. The only alternative, therefore, is milled rice, but, if it is to constitute the chief food, it requires a vitamin B_1 supplement, which would not be necessary if husked rice or home-pounded rice were used. If the diet includes maize as the second principal cereal (which is often the case), or if a large quantity of leguminous matter is eaten (which also frequently occurs), or much meat or fresh fish (which rarely happens in the Netherlands Indies, even along the coasts), the vitamin B_1 supply may yet be sufficient. There are, in fact, no other possibilities and, if those just mentioned cannot be realised, the vitamin B_1 intake will be very low, and this will be followed by the usual consequences.

This is why, in the Netherlands Indies, in regions where rice has to be imported, beriberi occurs when the maize crop or the fishing has been poor. In such regions, husked rice (which keeps

[1] The studies of beriberi by Eykman, Grijns and those who came after them related to soldiers and prisoners and not to the general mass of the population. Nowadays native soldiers, prisoners, coolies on estates, etc., are all supplied with rice that has been merely husked.

badly and entails loss for the dealer) is usually more expensive than milled rice. The opposite is the case in Java, to that island's advantage.

It is therefore clear that the rice problem necessarily differs according to the district, the number of large towns, the type of agriculture, the habits of the population, the degree of industrialisation, etc. It is therefore to be recommended that the regulation of the prices of the different kinds of rice, the determination of the number of mills and the prescribing of the degree of milling appropriate to certain seasons and certain parts of the country should be left to the initiative of the Government. Milled rice, being easy to transport and to keep, continues to be indispensable.

In principle, the cultivation of plants rich in vitamin B_1 (millet, legumes, maize) should be encouraged. In many tropical countries, however, there is no arable land or the soil is not fertile enough. Much can, nevertheless, be done by means of intensive and well-directed propaganda.

Professor B. C. P. Jansen's proposal to vitaminise the rice-eating population was thoroughly investigated in Java, but kitchen salt was found to be the only foodstuff which was suitable for serving as a vehicle for vitamin B_1. Our tests proved, however, that, in the conditions prevailing in the tropics, vitamin B_1 added to salt is not stable. Moreover, the financial difficulties encountered would be insuperable. Lastly, beriberi is now so rare in the Netherlands Indies that such a measure would seem to be unnecessary.

THE RICE PROBLEM IN THAILAND

by the

CHIEF OF THE NUTRITIONAL ORGANISATION, DEPARTMENT OF PUBLIC HEALTH, BANGKOK

Rice is the life-blood of the nation. It is the staple food for the people as well as the backbone of national economics. Thailand exists as an economically modern nation by using her huge surplus of rice as the chief export in foreign trade. Traditionally, everybody is taught that rice is the only true and healthy food, while other auxiliary comestibles are more or less " toxic " and are to be consumed only in minute or insignificant quantities as appetisers in order to enable more rice to be swallowed. Every mother is delighted when her child consumes an immense quantity of rice. With such a dietetic doctrine in vogue among the masses, it is easy to understand their low general physical health and energy.

During the days of antiquity, rice was hand-pounded and therefore could not have been highly polished. With the arrival of modern Western civilisation together with machinery towards the end of the 19th century, rice-milling was also established as a major and highly profitable industry operated by Europeans and Chinese millionaires. In those days, only the city of Bangkok and some few accessible regions possessed rice-mills ; but, of late, this profitable industry had penetrated even into remote backward areas. Small rice-mills may now be seen everywhere in rural regions, the machinery being transported in pieces by bullock carts or mule packs up hill and down dale into rice-producing villages. The unfortunate result is that white highly polished rice is now universally consumed except in a few very backward regions where paddy is still being pounded by hand.

As long as the masses still persist in idolising rice — now white rice— without increasing other food auxiliaries such as eggs, meat, fish and vegetables in balanced proportion, the problem of mineral

and vitamin inadequacy becomes of paramount importance in public health. To urge people now accustomed to palatable and pleasant-looking white rice to revert to crude hand-pounded red rice would be very difficult. The industry of rice milling is too advanced in our national economy to be radically altered unless worldwide export markets demand " non-polished " rice. Therefore, our guiding policy now is large-scale propaganda to the people to balance their one-sided and vitamin-starved diet by consuming more and more of the " auxiliaries " such as meat, fish, eggs, beans, vegetables and fruit, all of which are produced locally in abundance with even a surplus for export to neighbouring countries.

Recent information from Japan, moreover, indicates that a great deal of crude unpolished rice is not absorbed by the intestinal tract, and thus a considerable part of the " red " rice so consumed becomes economic and physiological waste in addition to causing gastro-intestinal disturbance for many persons. We have observed this in many of our prisons, where some prisoners absolutely refuse to eat crude hand-pounded rice, claiming that this gives them indigestion. It is reported that the Japanese authorities have experimented with various grades of milled rice in order to arrive at the correct grade of milling for the purpose of good physiological absorption, least economic waste, and sufficient supply of the vitamins and minerals. According to T. KIMURA [1], " Haigamai " rice is now being advertised as the properly milled rice for human consumption. Such rice is so milled that the indigestible and unpalatable bran is removed while the embryo-bud which contains the vitamin remains intact. It is also easier to cook and more appetising than the ordinary unpolished rice recommended by medical authorities in the Far East. It is claimed that " Haigamai " rice is even more tasty than white polished rice, while as to the digestibility and absorbability there is not much difference between the two. Half-milled rice is inferior, while unpolished rice is the worst of all as regards digestibility. Thus both the Japanese Army and Navy have now adopted " Haigamai " rice as the principal staple of diet instead of ordinary unpolished rice. There is " Haigamai " rice of varying grades of milling, and, if such rice is to be adopted as the staple of Asia, it would be timely

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[1] *Jl. Publ. Health Assoc. of Japan*, 1938, August.

to have an international standard for defining and regulating the degrees of milling and to determine the percentage of embryo-bud to be retained.

For many years past, the Government of Thailand has been advocating ordinary unpolished rice with no good result except in some prisons, where the inmates are under strict compulsion as regards what they eat. People once used to white polished rice almost always rebel against crude hand-pounded rice. Perhaps the new scientific way of retaining the embryo-bud together with a certain percentage of milling as practised in Japan may ultimately be adopted as the proper way of milling rice for mass consumption in Asia. If so, it is hoped that some committee of experts, such as the Technical Commission on Nutrition of the League of Nations, will deal with the colossal problem of rice and determine what grade of milled rice, 50 or 70%, is the ideal staple food and whether to retain the embryo-bud or not. This is a difficult question and will take much time for investigation and further research before suitable conclusions can be arrived at.

Besides full-blown beriberi averaging about 2,000 deaths annually in Thailand, there are many borderline conditions of ill-health and physical weakness due to under-nutrition or malnutrition. Such cases were formerly neglected by orthodox medical men who looked only for text-book symptoms and signs of full-blown beriberi and other diseases. If, however, one were to take into account all borderline cases of nutritional deficiency, the number would run into millions. As rice is the staple food of nearly half of the human race, it becomes of paramount importance for every Government in the Far East to make a great endeavour to secure that this rice be consumed in the most nutritive form possible. It is to be hoped that the research now being carried on in Japan, India, Java and Malaya may soon bring forth satisfactory conclusions as regards the proper grade of milled rice suitable for the nutritional requirements of the people of Asia.

The Imperial Government Institute for Nutrition at Tokyo, after a study of rice extending over many years, decided that rice *70% polished* (without the use of powder for polishing) and boiled or otherwise cooked *without washing* is the most healthful and economical for human consumption. The washing of rice before cooking is both unhealthful and wasteful; such practice is universal in

Thailand. The time may soon arrive for us to promulgate a law for the control of rice-milling and the standardisation of the technique of rice-polishing. As regards washing rice before cooking, this is of course a matter of public propaganda.

It is expected that a national food council will soon be established and a special technical committee on rice-milling may also be formed and attached to this council. The problem of rice in Thailand is primarily that of " vested economic interests ". When the grade of milling is universally standardised by international conference, legal measures may be enacted in order to enhance the nutritive qualities of rice.

DIETARY STANDARDS FOR FILIPINOS

approved on June 23rd, 1939,
by the Section of Nutrition, National Research Council of the Philippines.

In view of the pressing need for dietary standards applicable to Filipinos, this Section has deemed it proper to lay down tentative standards pending further investigations into the quantitative measurement of the minimum requirements of Filipinos for the different nutritionally essential factors. These standards are based largely on the " Report on the Physiological Bases of Nutrition " which was issued by the Health Organisation of the League of Nations. Due consideration was given to the smaller body-weight and stature of Filipinos (1) as compared with Europeans and Americans for whom the League of Nations' standards were intended, and to the results of dietary surveys conducted in the Philippines.

I. Caloric Requirements

(a) An allowance of 2,000 net calories (2) is considered adequate for an adult, male or female, " living an ordinary everyday life and not engaged in manual work."

The basic requirement of 2,400 calories recommended by the League of Nations was reduced in order to conform to the smaller body-weight and stature of adult Filipinos. Hence, an average adult Filipino of sedentary habits will require only 2,000 calories per day. But if he or she is engaged in some occupation

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(1) The average weight of Filipinos is 53 kg. ; average height, 163 cm. The League of Nations' standards are based on a " mean man " of 70 kg.

(2) The term " net calories " refers to the amount of energy available from the food actually assimilated.

involving muscular activity, additions must be made to this basic requirement.

(b) In the absence of data for Filipinos, the following supplements proposed by the League of Nations have been adopted *in toto* :

Light work	up to 75	calories per hour of work.
Moderate work . .	75–150	,, ,, ,, ,,
Hard work	150–300	,, ,, ,, ,,
Very hard work .	300 calories and upwards	per hour of work.

Only domestic work, taking the average of a mixed housekeeping day, clerical work, book-binding and tailoring can be classed as " light work ". All physical exercises should be rated from " moderate work " upwards, while all sports pursued as such should fall under " very hard work ". An allowance of 600 calories (75×8 hours) per day may be taken as an average supplement for muscular activity. Thus, an average adult Filipino, male or female, engaged in some occupation involving manual work will require daily an average of 2,600 calories.

(c) The basic figure of 2,000 calories for the requirements of adults of both sexes is taken as unity, and from this value the energy requirements for children of various ages may be calculated, using the following coefficients :

Age (years)	Coefficient	Calories
1–2	0.35	700
2–3	0.42	840
3–5	0.50	1,000
5–7	0.60	1,200
7–9	0.70	1,400
9–11	0.80	1,600
11–12	0.90	1,800
12 and upwards	1.00	2,000

Allowance for muscular activity characteristic of every healthy child and adolescent must be made. This allowance should be added to the basic requirements tabulated above to obtain the total daily calorie requirement. Thus, the activities of children of both sexes

from 5–11 years may be considered as equivalent to light work, of boys from 11–15 years as moderate work, and of girls from 11–15 upwards as light work.

Mothers, pregnant and nursing, are given the following allowances :

Women	Coefficient	Calories
Pregnant	1.00	2,000
Nursing.	1.25	2,500

In addition to the above basic requirements for mothers, allowance for mothers engaged in household duties, whether pregnant or not, must also be made. The extra energy required for the performance of household duties may be reckoned as equivalent to light work for eight hours daily.

II. Protein Requirements

The League of Nations' proposed standard of 1 gramme per kg. of body-weight may not be sufficient to meet fully the physiological needs of an adult Filipino, for the protein content of the average Filipino diet is preponderantly of plant origin. Since proteins of plant origin are not as nutritionally efficient as proteins of animal origin, it is recommended that the League of Nations' standard be raised to 1.5 grammes per kg. of body-weight. An average adult of 53 kg. body-weight would therefore require 80 g. of proteins per day.

The proteins in the diet must come from a variety of sources, so that a deficiency of one protein may be made up by another. Animal proteins being superior in biological value to plant proteins, it is recommended that at least 50% of the total proteins should be derived from animal sources.

The protein intake per kg. of body-weight should be increased in children and in pregnant and nursing mothers. An extra allowance is needed in pregnant women for the growth of the fœtus, in nursing mothers for successful lactation, and in children for growth. To secure a high quality of protein which is essential in these cases, it is suggested that the total proteins should be made up largely of animal proteins.

The following allowances of total proteins are recommended:

Age (years)	Grammes per kg. of body-weight
1–3	3.5
3–5	3.5
5–12	3.0
12–15	3.0
15–17	2.5
17–21	2.0
21 and upwards	1.5
Women	
Pregnant 0–3 months	1.5
4–9 „	2.0
Nursing	2.5

III. Mineral Requirements

The most important mineral elements in the diet, because the most likely to be deficient, are calcium, phosphorus and iron. Very little information of an experimental nature with regard to the dietary requirements of Filipinos for these different mineral elements is available. For this reason, the standards proposed by the Technical Commission on Nutrition [1] have been adopted after due allowance for the smaller body-weight of the average adult Filipino.

It is believed that the following allowances are adequate to meet the maintenance requirements of adults, and to supply the increased demands during growth, pregnancy and lactation:

	Calcium (gramme)	Phosphorous (gramme)	Iron (milligrammes)
Adult men	0.60	0.80	8
Adult women	0.60	0.80	10
Pregnant women	1.00	1.20	12
Nursing „	1.00	1.20	12
Children	0.80	1.00	10

[1] *Bull. Health Org.*, 1938, 7, 460.

These values refer not to total but to available calcium, phosphorus and iron.

IV. Vitamin Requirements

To date, no work on the determination of the vitamin requirements of Filipinos has as yet been done. Since the Technical Commission on Nutrition has pointed out that figures for vitamin requirements are only approximate, it was thought advisable for the present to avoid even a tentative statement of the quantity of each vitamin required.

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Authors are alone responsible for views expressed in signed articles.

THE ADOPTION OF CRYSTALLINE VITAMIN B_1 HYDROCHLORIDE AS THE NEW INTERNATIONAL STANDARD OF VITAMIN B_1 AND COMPARISON OF ITS POTENCY WITH THAT OF THE FORMER STANDARD

A Summary of Co-operative Experiments organised by the Accessory Food Factors Committee (Lister Institute and Medical Research Council)

and edited by

T. F. MACRAE

(Lister Institute, London), Secretary of the Vitamin B_1 Sub-Committee.

TABLE OF CONTENTS

4473. S.d.N. 1030 (F.). 1525 (A.). 11/41. — Imp. de la T. d. G.

1. Introduction and General Summary

In a previous number of this *Bulletin* (1938, volume VII, page 874), the circumstances were described and the results of the investigations given which led to the adoption of synthetic crystalline vitamin B_1 hydrochloride as the International Standard. The memorandum issued with the New Standard was also included (page 882). The present report includes a detailed description of many biological tests carried out in seventeen different laboratories in Europe, America and Japan, in order to compare the potency of the old and the new standard of vitamin B_1.

The International Conference on Vitamin Standardisation, at its meeting in 1931, adopted as standard for vitamin B_1 the adsorption product of vitamin B_1 prepared by the method of Seidell, as described by Jansen and Donath. The unit was defined as the vitamin B_1 activity of 10 mg. of this product, which was designated the " Standard adsorption product ".

At the second Conference, in 1934, it was recommended that " the potency of the Standard adsorption product should be tested relatively to that of crystalline vitamin B_1 preparations at present available, with the aim of ultimately adopting pure crystalline vitamin B_1 as the International Standard ".

At that time, natural crystalline vitamin B_1 had already been isolated by the following investigators : Professor B. C. P. JANSEN, Amsterdam ; Professor R. A. PETERS, Oxford ; Dr. A. SEIDELL, Washington ; Professor U. SUZUKI, Tokio ; Dr. R. R. WILLIAMS, New York ; Professor A. WINDAUS, Göttingen.

The nearly simultaneous discovery during 1936 in America, Britain and Germany, of methods for synthesis of the vitamin by relatively inexpensive processes greatly increased the amounts available of the pure vitamin.

Through the good offices of Sir Henry DALE, a large supply of synthetic vitamin B_1 hydrochloride was contributed by the following four firms : Messrs. Merck & Company, Rahway, N.J. ; Messrs. E. Merck, Darmstadt ; Messrs. F. Hoffmann-La Roche, Basle ; and I. G. Farbenindustrie, Elberfeld. The samples were mixed, recrystallised, and the final material examined for criteria of purity by Dr. A. R. TODD at the Lister Institute.

Co-operative tests of the biological potency of this material compared with that of the Standard adsorption product were organised by the Vitamin B_1 Sub-Committee of the Accessory Food Factors Committee (appointed jointly by the British Medical Research Council and the Lister Institute). For this purpose, small amounts of the recrystallised vitamin, suitably dried, together with the necessary amounts of the Standard adsorption product, were distributed from the National Institute for Medical Research to workers in Europe, America and Japan. Seventeen reports were received, which contained the results of thirty-one experiments of comparison, in which many different methods were employed. A summary of the results obtained is given in Table I.

The results obtained showed a satisfactory degree of concordance, when account is taken of the great diversity of the eight different methods employed. For the present purpose, it was obvious that

Table I.

Method	Observer[1]	Amount of synthetic vitamin B_1 hydrochloride found equivalent to 10 mg. of the adsorbate International Standard —*i.e.*, to possess the potency of one international unit	Average for group
		Microgrammes	Microgrammes
1. Rat-growth	S. Ohdake, Tokio	2.0	
	L. S. Fridericia, Copenhagen	2.5	
	C. A. Elvehjem, Madison, Wis.	3.0	
	L. J. Harris, Cambridge	3.0	
	T. F. Macrae, London	3.0	
	H. von Euler, Stockholm	3.1	
	I. G. Farbenindustrie, Elberfeld	3.45	
	H. E. Munsell, Washington	3.7	
	K. H. Coward, London	3.85	
	R. R. Williams, New York W. L. Sampson, Rahway, N. J.	4.0	3.2
2. Rat-curative	Messrs. E. Merck, Darmstadt	2.0	
	E. M. Nelson, Washington	2.6	
	R. R. Williams, New York W. L. Sampson, Rahway, N. J.	2.5	
	L. J. Harris, Cambridge	2.7	
	N. B. Guerrant, Pennsylvania	2.7	
	B. C. P. Jansen, Amsterdam	3.0	
	P. di Mattei, Pavia	3.5	2.7
3. Rat-bradycardia ..	R. R. Williams, New York W. L. Sampson, Rahway, N. J.	2.5	
	L. J. Harris, Cambridge	2.5	
	Messrs. Hoffmann-La Roche, Basle	2.63	
	I. G. Farbenindustrie, Elberfeld	3.33 (subcut.) 3.61 (orally)	2.8
4. Pigeon-curative ...	Messrs. E. Merck, Darmstadt	1.9	
	S. Ohdake, Tokio	2.4	
	K. H. Coward, London	3.3	
	P. di Mattei, Pavia	3.4-3.8	2.8

[1] The name of only one investigator in each laboratory is given. For names of collaborators see the individual reports.

Method	Observer[1]	Amount of synthetic vitamin B_1 hydrochloride found equivalent to 10 mg. of the adsorbate International Standard —*i.e.*, to possess the potency of one international unit	Average for group
		Microgrammes	Microgrammes
5. Chicken-prophylactic	C. A. Elvehjem, Madison	3.0	3.0
6. Catatorulin[2]	R. A. Peters, Oxford	2.2	2.2
7. Thiochrome	B. C. P. Jansen, Amsterdam	2.5	
	Messrs. Hoffmann-La Roche, Basle	2.56	
	H. von Euler, Stockholm	2.7	2.6
8. Colorimetric (method of Kinnersley & Peters)	H. von Euler, Stockholm	2.6	2.6

[1] See footnote on preceding page.
[2] After elution of the acid clay adsorbate with $Ba(OH)_2$.

less weight should be attached to results obtained by chemical methods than to those of biological tests. Of the latter, higher values for the potency of standard adsorption product in terms of the synthetic material were generally obtained with prophylactic than with curative methods. It is suggested that with sick animals there may be incomplete elution in the alimentary tract of the vitamin B_1 from the acid clay adsorbate, so that curative methods tend to give too low a measure of the vitamin B_1 potency of the adsorption product and relatively too high a value for the pure material. With growth tests, on the other hand, a higher value for the adsorption standard in terms of the pure material is obtained because the animals, being healthy, are better able to elute the vitamin from the acid clay. The results obtained by the many investigators support this theory, for the rat-growth method gave the highest average value for the amount of the pure material possessing a potency equivalent to that of the present unit.

There is also the possibility that the presence in the acid clay adsorbate of other growth factors for rats, in addition to vitamin B_1, may account for the relatively high potency of the acid clay adsorbate shown in rat-growth experiments. While the basal diets used in these experiments seemed mostly to be well supplied with the other vitamins of the B group, it is still possible that a deficiency of some essential dietary factors other than vitamin B_1 may have influenced the results.

There are therefore two possible reasons for the differences found by the various methods :

(*a*) Difference in availability to the animals of the vitamin B_1 adsorbed on the acid clay ;

(*b*) Possible complications due to the presence in the acid clay standard of factors other than vitamin B_1, which may be adsorbed from the rice-polishings extract in the process of its preparation.

Amounts varying from 2 to 3.5 μg. were suggested by the various participants, as the quantity of the pure synthetic vitamin B_1 hydrochloride of which the potency is equivalent to that of 10 mg. of the standard adsorption product—*i.e.*, the potency of one international unit. A good average value appeared to be 3 μg. The Vitamin B_1 Sub-Committee therefore proposed "*that pure synthetic vitamin B_1 be adopted as International vitamin B_1 Standard and that the international unit be defined as the vitamin B_1 activity of 3 μg. of the pure material*".

In dealing with all international standards, it has been regarded as a fundamental principle that the unit should be defined as the specific activity of a definite weight of the standard substance, without reference to any particular method, or methods, of biological comparison. This makes the unit independent of future improvements in testing methods. The adoption of the value as defined above, rather than the fractionally lower one indicated by some forms of test, would satisfy another fundamental principle by avoidance of any suggestion that, with a change of standard, the value of the unit had been reduced.

It was urged by some participants that, with the isolation and synthesis of the pure vitamin B_1, the need for a unit had disappeared,

and that dosage and activity should henceforward be indicated in actual weights of the pure vitamin. The Vitamin B_1 Sub-Committee agreed that this may be desirable as an ultimate objective. It remembered, however, that the action originally taken, in adopting the old standard and defining an international unit, had as its object the removal of a prevalent confusion, due to the use of a multiplicity of units based on different animal reactions. It believed that abandonment of the use of an international unit then, before those concerned with the dosage and measurement of vitamin B_1 had become familiar with the pure substance and the degree of its activity, would inevitably produce a relapse into the former confusion. It was hoped that a new definition of the value of the existing unit in terms of a pure standard would in itself familiarise investigators and practitioners with the activity of the pure substance and make easier an ultimate transition to a notation more logically based on exact weights.

The results of the biological tests, together with the recommendations of the Vitamin B_1 Sub-Committee of the Accessory Food Factors Committee, were submitted in April 1938 to the members of the Conference on Vitamin Standardisation, who unanimously agreed to the recommendation "*that the specimen of pure synthetic vitamin B_1 hydrochloride which had been thus investigated should be adopted as the International Standard of vitamin B_1 and that the unit should be defined as the potency of* 3 *microgrammes of the pure material*". This recommendation was later adopted by the Permanent Commission on Biological Standardisation of the League of Nations Health Organisation at its meeting in Geneva in 1938, and, in consequence of this decision, the former International Standard, known as the Standard adsorption product of vitamin B_1 was replaced by the preparation of crystalline vitamin B_1 hydrochloride described above.

In the following sections are given detailed accounts of the experiments of comparison which were carried out prior to the adoption of the new standard. The results obtained have been statistically examined by Dr. K. H. Coward and Dr. E. A. G. Shrimpton, and a short summary of their findings is given as an appendix to this report.

Since the publication of the explanatory memorandum on the second International Standard of vitamin B_1, which has been issued

with the New Standard, it has been discovered that the standard does not, as therein stated, contain approximately 1 molecule of water of crystallisation, but is practically in the anhydrous condition. This error has therefore been put right and the corrected memorandum is appended ; this also contains additional criteria of purity, including the extinction coefficients of the standard at various wave-lengths, determined by Dr. E. R. Holiday.

2. Comparison between the Original International Standard of Vitamin B_1 (Standard Adsorption Product) and the New Standard (Vitamin B_1 Hydrochloride) by Rat-growth Methods

A. *Report of K. H. Coward (London).*

Method. — Full details of the method employed have been published elsewhere. [1] As the rats ceased to grow, they were put into separate cages and divided into 5 groups, which received the following supplements : (1) no dose, (2) 0.01 g., (3) 0.02 g. Standard adsorption product daily, (4) 2.5 μg., (5) 5μg. crystalline vitamin B_1 daily. The litters were divided as evenly as possible amongst the groups. The growth rate of the animals was observed for a period of three weeks.

All doses were given twice weekly, three doses on Monday and four doses on Thursday. This method gives results equal to daily dosing (unpublished results from this laboratory). The standard adsorption product was mixed with finely powdered destrin, and the doses were weighed on separate dishes and moistened with a little water to prevent scattering.

The solution of the crystalline vitamin given to the animals was prepared daily by dilution with N/100 HCl of a stock solution of the vitamin containing 3 mg. per ml. The solution was fed directly into the animal's mouth.

The results are given in Table II.

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[1] Coward, Burn, Ling and Morgan : *Biochem. Jl.*, 1933, 27, 1719.

Table II.

COMPARISON OF THE POTENCY OF THE STANDARD ADSORPTION PRODUCT OF VITAMIN B^1 AND THE CRYSTALLINE STANDARD BY A RAT-GROWTH METHOD (K. H. COWARD)

Group 1 No supplement of vitamin B_1		Group 2 Dose 0.01 g. adsorbate daily		Group 3 Dose 0.02 g. adsorbate daily		Group 4 Dose 2.5 μg. crystals daily		Group 5 Dose 5.0 μg. crystals daily	
Rat No.	Animal died during	Rat No.	Increase in weight during 3 weeks, g.	Rat No.	Increase in weight during 3 weeks, g.	Rat No.	Increase in weight during 3 weeks, g.	Rat No.	Increase in weight during 3 weeks, g.
9247♂	2nd wk.	9323♂	1	9249♂	26	9251♂	—11	9248♂	13
9362♂	1st ,,	9372♂	1	9344♂	19	9321♂	—10	9250♂	—1
9417♂	2nd ,,	9443♂	—3	9364♂	19	9343♂	— 7	9322♂	6
9465♂	2nd ,,	9467♂	1	9365♂	12	9418♂	—11	9445♂	2
9446♀	3rd ,,	9252♀	0	9416♂	5	9444♂	— 9	9466♂	9
9345♀	1st ,,	9253♀	4	9468♂	13	9368♀	died	9346♀	10
		9366♀	—7	9324♀	18	9373♀	died	9367♀	—2
Average for group....			—0.4		16.0		— 9.6		5.3

A curve of response to the standard adsorption product was constructed from the mean growth response to that material. By application of this curve, the activity of the crystalline material was calculated. The results are given in Table III.

Table III.

Substance	Daily dose	Mean weight increase g.	Equivalent of adsorbate as read from response curve I.U.	Potency I.U. per g. crystalline vitamine B_1	
				For each dose	Mean
Standard adsorption product	(1 I. U.) 0.01 gm.	— 0.4			
	(2 I. U.) 0.02 gm.	+ 16.0			
Crystalline vitamin B_1	2.5 μg.	—9.6	0.69	276,000	
	5 μg.	+5.3	1.27	254,000	260,000

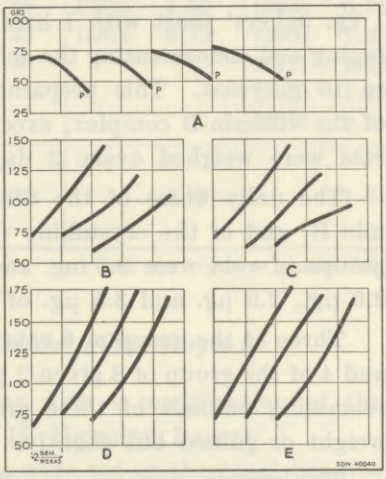
10 mg. of the Standard adsorption product is equivalent in vitamin potency to 3.85 μg. of the crystalline vitamin.

B. *Report of C. A. Elvehjem (Madison, Wis.).*

The rats were depleted of the major part of their vitamin B_1 reserves by restriction to the basal Ration 112 used in the laboratory of the Department of Physiological Chemistry, University of Wisconsin, for a two-week period. Male rats were used. The results of the assay are shown in Figure 1.

The four rats which continued on the basal ration died after exhibiting polyneuritis in 26 to 31 days after the start of the experiment. The growth of the rats fed 200 mg. (20 I.U. vitamin B_1) of the Standard adsorbate per 100 g. of Ration 112 was somewhat irregular. However, the results closely parallelled those obtained by feeding a second group of rats 60 μg. of the sample of crystalline vitamin B_1 hydrochloride per 100 g. of Ration 112. An increase of the crystalline vitamin B_1 supplement to 80 μg. of vitamin B_1 hydrochloride per 100 g. of Ration 112 resulted in an average weight increase of 3.0 g. daily during the five weeks experimental period, which was not inferior to that shown by rats fed 100 μg. of vitamin B_1 hydrochloride per 100 g. of Ration 112.

Figure 1.



P = Polyneuritis.
A : Ration 112 only.
B : 200 mg. of Standard adsorption product per 100 g. of Ration 112.
C : 60 μg. crystalline vitamin per 100 g. of Ration 112.
D : 80 μg. crystalline vitamin per 100 g. of Ration 112.
E : 100 μg. crystalline vitamin per 100 g. of Ration 112.

These results indicate that *10 mg. of the Standard adsorption product has approximately the same vitamin potency as* 3 μg. *of the crystalline vitamin* B_1 *hydrochloride.*

C. *Report of H. and B. von Euler (Stockholm).*

Method. — Immediately after weaning, the rats were fed on a basal diet free from vitamins of the B-group. They gained slightly in body-weight for 10 days and, after remaining constant in weight for a few days, loss occurred ; this preliminary period lasted 16-21 days. The animals were then given the test samples of vitamin B_1, together with the other vitamin-B supplements. Growth of the rats was observed for 4 weeks.

The following basal diet was employed : Vitamin-free caseinogen 100 g., rice starch 300 g., hardened arachis oil 65 g., salt mixture 25 g., lemon juice 10 ml. and cod-liver oil 10 ml. In addition, each rat received daily, as sources of B-factors other than vitamin B_1, 10 μg. riboflavin and 0.5 ml. of a solution prepared by autoclaving 1 kg. bakers' yeast with 1 litre water at pH 9 for 5 hours, centrifuging and concentrating the centrifugate until 1 ml. was equivalent to 10 g. yeast. This preparation contained all essential factors of the vitamin-B complex, except vitamin B_1 and riboflavin. The rats were weighed every 2 days.

The daily doses of the Standard adsorption product of vitamin B_1 and of the crystalline vitamin administered to the various groups of rats were 9.0 mg. and 10.0 mg. of the former and 1.5 μg., 2.0 μg., 2.5 μg. and 3.5 μg. of the latter respectively.

Three of the group of 6 rats given 1.5 μg. of crystalline vitamin and 4 of the group of 8 given 2 μg. died within a short period. The remaining animals of these groups either did not gain in body-weight or gained but slightly.

The growth responses recorded in the other groups of animals are given in Table IV.

From these results, by constructing a curve of response to the crystalline vitamin and applying this curve, the *biological potency of 10 mg. of the Standard adsorption product is calculated to be equal to that of 3.1 μg. of crystalline vitamin* B_1 *hydrochloride.*

Table IV.

COMPARISON OF THE POTENCY OF THE STANDARD ADSORPTION PRODUCT OF VITAMIN B_1 AND THE CRYSTALLINE STANDARD BY A RAT-GROWTH METHOD (H. AND B. VON EULER)

Animals given crystalline vitamin						Animals given adsorbate			
2.5 μg. daily[1]		3.0 μg. daily[2]		3.5 μg. daily		9 mg. daily		10 mg. daily	
Rat No.	Average daily weight increase, g.	Rat No.	Average daily weight increase, g.	Rat No.	Average daily weight increase, g.	Rat No.	Average daily weight increase, g.	Rat No.	Average daily weight increase, g.
16135	0.54	16255	1.00	16352	1.22	15858	0.74	16259	0.92
16136	0.61	16256	0.83	16353	1.33	15838	0.63	16264	1.00
16139	0.29	16257	1.21	16354	1.33	15835	0.82	16287	0.81
16143	0.40	16258	1.04	16355	1.22	15572	0.55	15917	1.04
16155	0.21	16260	0.95	16357	1.22	15575	1.02	15908	0.85
16156	0.40	16265	0.79	16358	1.22	15573	0.75	15909	1.20
		16266	0.83	16359	1.00			15911	1.26
								15910	1.12
								15912	0.91
Average daily weight increase for group, g.	0,41		0.95		1.22		0.75		1.01

[1] Rat No. 16138 = sick, killed on 12th day.
16157 = pneumonia.

[2] Rat No. 16283 = pneumonia.

D. *Report of L. S. Fridericia (Copenhagen).*

The estimations were carried out with a modification of the rat-growth method worked out by H. KRIEGER LASSEN. [1]

Three groups of young male rats were fed on the basal diet free from vitamin B_1 but containing surplus of the vitamin B_2 complex (autoclaved yeast extract); the three groups received respectively 2 μg. and 3 μg. of the crystalline vitamin, and 10 mg. of the Standard adsorption product per rat daily. The growth rate of the animals was observed for 5 weeks. The results are given in Table V.

From the growth rates observed in groups 1 and 3 and by application of the method of calculation of H. KRIEGER LASSEN, 10 mg. of the acid clay standard is found to be equivalent in activity

[1] *Collected Papers*, University Institute of Hygiene and Budde Laboratory, Copenhagen, 1936, 7, Paper No. 6.

Table V.

COMPARISON OF THE POTENCY OF THE STANDARD ADSORPTION PRODUCT OF VITAMIN B_1 AND THE CRYSTALLINE STANDARD BY A RAT-GROWTH METHOD (L. S. FRIDERICIA)

Group 1 Animals given 2 μg. crystalline vitamin daily		Group 2 Animals given 3 μg. crystalline vitamin daily		Group 3 Animals given 10 mg. Standard adsorption product daily	
Rat No.	Weight increase in 5 weeks, g.	Rat No.	Weight increase in 5 weeks, g.	Rat No.	Weight increase in 5 weeks, g.
2758	30	2754	61	2723	48
2786	45	2760	58	2729	40
2773	43	2762	52	2731	48
2791	27	2763	40	2753	53
2800	40	2772	53	2757	43
2801	44				
Average weight increase for group	38.2		52.8		46.4

to 2.35 μg. of the crystalline vitamin and, from the growth rates observed in groups 2 and 3, 10 mg. of the acid clay is equivalent to 2.66 μg. of the crystalline vitamin. The average value therefore gives *2.5 μg. of the crystalline vitamin as the equivalent in vitamin B_1 activity of 10 mg. of the Standard adsorption product.*

E. *Report of L. J. Harris (Cambridge).*

Method. — For the tests by the growth method, young male piebald rats (standard strain), weighing 50 to 60 g., were placed on a basal diet consisting of cane-sugar 60, " light white " casein 20, arachis oil 15 and salt mixture 5, supplemented with 15% of autoclaved marmite solution. [1] Each rat received daily 2 drops of cod-liver oil. The animals continued to gain weight on this diet for about 18 to 21 days, and then the weight dropped fairly sharply. Dosing of each rat was started on day on which it had lost 7 g. or more from its maximum weight at the peak of the growth

[1] Prepared by diluting " laboratory " marmite with water, adding $Ba(OH)_2$ to pH8-10, autoclaving for 1 hour at a pressure of 1.5 atmospheres, adjusting to pH 4 with H_2SO_4, filtering and adding water to make 1 c.c. of solution $\equiv$ 0.5 g. marmite.

curve. The following daily doses were given to groups of six rats :

International Standard adsorption product: 10 mg., 20 mg., 30 mg.

Crystalline vitamin B_1—HCl : 2.5 μg., 5.0 μg., 7.5 μg.

The crystalline vitamin B_1 was given as an aqueous solution. Once a week, a stock solution was prepared and kept in an evacuated Thunberg tube in a refrigerator. Dilutions were made from this daily, 1 ml. containing 10 g. of the crystalline material.

Calculation of Results. — Results were calculated on two separate bases — namely, the weight gained in 14 days and in 21 days ; the same value was obtained on either basis. Table VI contains the numerical results. From the dose-response curves which were constructed, *10 mg. of the Standard adsorption product as the equivalent of 3.03 μg. crystalline vitamin B_1 gave the nearest coincidence between the curves for the two materials.*

Table VI.

Comparison of the Potency of the Standard Adsorption Product of Vitamin B_1 and the Crystalline Standard by a Rat-growth Method (L. J. Harris)

Material tested	Acid clay, mg.			Crystalline B_1, μg.		
Dose given	10	20	30	2.5	5.0	7.5
	g.	g.	g.	g.	g.	g.
Gain in weight after 14 days: individual rats	0	21	40	1	15	31
	8	23	42	2	22	36
	14	26	42	3	23	36
	14	32	44	5	28	38
	19	37	47	14	29	38
	24	40	53	15	36	43
Mean	13.2	29.8	44.7	6.7	25.5	37.0
Gain in weight after 21 days: individual rats	1	31	53	1	24	47
	2	37	57	3	30	49
	21	43	57	3	34	50
	22	49	60	3	40	54
	28	50	65	15	41	55
	28	57	70	18	53	60
Mean	17.0	44.5	60.3	7.2	37.0	52.5

If the responses are plotted against the *logarithms* of the doses, a linear curve is obtained. [1] Such logarithmic curves, constructed for both the 14-day period and the 21-day period gave values in good agreement with that already recorded above. (See Table VII.)

Table VII.

ACTIVITY OF CRYSTALLINE VITAMIN-B_1 HCl AS COMPUTED FROM LOGARITHMIC CURVES (L. J. HARRIS)

	Dose of crystals, μg.	Equivalent of adsorbate, as read from curve (I.U.)	Calculated weight (in μg.) of crystals equivalent to 1 I.U. (10 mg. adsorbate)
14-day test	2.5	0.813	3.08
	5.0	1.58	3.16
	7.5	2.40	3.13
			Average 3.12
21-day test	2.5	0.773	3.23
	5.0	1.65	3.03
	7.5	2.44	3.08
			Average 3.11

F. *Report of the I. G. Farbenindustrie (Elberfeld).*

Method. — The rat-growth method employed was similar to that of CHICK and ROSCOE.[2] The young animals (40-50 g. body-weight) were given a diet consisting of extracted casein 20, extracted rice starch 30, cane-sugar 30, pea-nut oil 15 and McCollum's salt mixture 5. Each rat received daily the following supplements: 2 drops of cod-liver oil, 10 μg. riboflavin and a preparation from yeast equivalent to 2 g. dry weight to supply the additional vitamin-B factors. The animals increased in body-weight for about 10 days, after which they began to lose weight. When the body-weight had either decreased or remained constant for about one week, the animals were given their test samples either of the Standard adsorption product or of the crystalline sample

[1] See COWARD: *The Biological Standardisation of Vitamins.* 1938: London.

[2] *Biochem. Jl.*, 1929, **23**, 498.

of vitamin B_1 : 3 groups of animals received 10 mg., 16 mg. and 25 mg. each daily of the former preparation and 3 groups 2.5 μg., 4.0 μg. and 6.25 μg. of the latter, respectively. The growth rate was observed for a period of 5 weeks. The total body-weight increase of the individual rats is given in Table VIII.

Table VIII.

Comparison of the Vitamin B_1 Potency of the Standard Adsorption Product and of the Crystalline Standard by a Rat-growth Method (I. G. Farbenindustrie)

Group 1 Total weight increase in g. in 5 weeks of animals receiving the adsorbate (daily)			Group 2 Total weight increase in g. in 5 weeks of animals receiving crystalline vitamin B_1 (daily)		
10 mg.	16 mg.	25 mg.	2.5 μg.	4.0 μg.	6.25 μg.
23	32	40	9	26	23
19	28	31	12	28	24
15	32	39	16	22	39
13	38	30	14	23	27
20	40	42	17	14	34
14	22	38	9	24	39
13	23	43	5	19	28
15	19			26	28
14				12	34
23				26	
Average for group 16.9	29.2	37.5	11.7	22.0	30.6

When the average weight increases were plotted against the logarithms of the doses, straight lines were obtained for both groups of animals. Those lines were parallel and showed that *10 mg. of the Standard adsorption product (1 I.U.) was equivalent to 3.45 μg. of the crystalline vitamin.*

G. *Report of T. F. Macrae, C. E. Work and M. M. El-Sadr (London).*

Method. — The growth tests were carried out by the method described by Chick and Roscoe.[1] Young rats received at weaning

[1] *Biochem. Jl.*, 1929, **23**, 498.

a basal diet consisting of extracted casein 20, rice starch 60, lard 3, hardened cotton-seed oil 12, McCollum's salt mixture (No. 185) 5, and water 100; the diet was cooked by steaming. Each animal received 0.08 ml. cod-liver oil daily. After 1 week, each animal was given the additional supplement of 1.0 ml. of an aqueous extract of yeast which had been autoclaved at pH 5 and 120° C. for 5 hours and was equivalent to 0.5 g. dried brewers' yeast. For about 10 days after receiving the autoclaved yeast extract, rats increased in body-weight and thereafter the body-weight began to decrease. After the body-weight had remained constant or decreased for a period of 5-7 days, the animals were given the materials to be tested for vitamin-B_1 activity, and the growth rates were observed for a period of 4 weeks. The doses of standard adsorption product or of crystalline vitamin administered and the increases in body-weight observed are given in Table IX.

Table IX.

COMPARISON OF THE POTENCY OF THE STANDARD ADSORPTION PRODUCT OF VITAMIN B_1 AND THE CRYSTALLINE STANDARD BY A RAT-GROWTH METHOD (T. F. MACRAE *et al.*)

Animals given the crystalline vitamin								Animals given the adsorbate 10 mg. daily	
2 μg. daily		2.5 μg. daily		3 μg. daily		3.5 μg. daily			
Rat No.	Total weight increase in 4 weeks, g.	Rat No.	Total weight increase in 4 weeks, g.	Rat No.	Total weight increase in 4 weeks, g.	Rat No.	Total weight increase in 4 weeks, g.	Rat No.	Total weight increase in 4 weeks, g.
								787♂	29
		778♂	24	779♂	28			788♂	27
919♂	9	786♂	15	785♂	34	926♂	54	918♂	44
781♀	2	917♂	41	916♂	41	923♀	47	924♂	61
790♀	12	927♂	30	925♂	27	929♀	46	931♂	42
		784♀	19	782♀	29			780♀	36
		792♀	25	791♀	34			783♀	15
		922♀	32	921♀	32			789♀	21
		930♀	20	928♀	37			920♀	36
Average for group, g.	7.7		25.8		32.8		49.0		34.6

By comparison of the weight increases observed on the various doses of the crystalline vitamin and the adsorbate and by application

of the curve of response to the crystalline vitamin, it was calculated that *the vitamin potency of 10 mg. of the adsorbate standard was approximately equal to that of 3μg. of the crystalline vitamin B_1.*

H. *Report of H. E. Munsell (Washington, D.C.).*

The rat-growth method for the estimation of vitamin B_1 used in the investigators' laboratory was employed. Four groups of rats were given varying amounts of the crystalline vitamin and the Standard adsorption product. The rats of the litters employed were divided as evenly as possible amongst the groups. The results are given in Table X.

Table X.

COMPARISON OF THE POTENCY OF THE STANDARD ADSORPTION PRODUCT OF VITAMIN B_1 AND THE CRYSTALLINE STANDARD BY A RAT-GROWTH METHOD (H. E. MUNSELL)

Daily amount of crystalline vitamin B_1						Daily amount of adsorbate	
2 μg.		3 μg.		4 μg.		10 mg.	
Rat No.	Gain in weight in 5 weeks, g.	Rat No.	Gain in weight in 5 weeks, g.	Rat No.	Gain in weight in 5 weeks, g.	Rat No.	Gain in weight in 5 weeks, g.
21477♀	—3	21480♀	8	21479♀	13	21478♀	21
21482♀	1	21488♀	11	21481♀	12	21483♂	2
21487♀	—5	21530♂	15	21484♂	16	21531♂	13
21528♂	—3	21536♀	1	21486♀	16	21535♀	14
21534♀	—2	21543♂	12	21529♂	13	21545♂	9
21544♂	—6	21549♀	9	21537♀	13	21548♀	18
21557♂	—7	21560♂	6	21546♂	12	21558♂	15
21564♀	1	21561♀	3	21559♂	19	21562♀	6
21565♂	—6	21566♂	—4	21563♀	16	21567♂	1
21571♀	—10	21572♀	0	21569♂	14	21574♀	3
21577♀	—12	21580♀	—2	21570♂	11	21579♀	8
21578♀	—12	21584♀	2	21573♀	2	21586♀	10
21587♀	—10	21592♂	13	21581♀	7	21589♀	11
21590♂	4	21598♀	9	21585♀	14	21591♂	17
21595♀	0	21599♂	8	21594♂	24	21596♀	14
21600♂	—2	21603♀	6	21598♀	13	21601♂	16
				21602♂	11	21605♀	16
				21604♀	16		
Average for group	—4.5		6.0		13.5		11.4

From the curve of response to the crystalline vitamin, it was calculated that *the vitamin potency of 10 mg. of the adsorbate standard was approximately equal to that of 3.7 μg. of the crystalline vitamin.*

I. *Report by S. Ohdake and T. Yamagishi (Tokio).*

Young albino rats of about 40 g. body-weight were fed a diet free from vitamin B_1, composed of the following ingredients : purified starch 60, purified casein 20, arachis oil 15 and McCollum salt mixture (No. 185) 5. Each rat received daily supplements of 3 drops of cod-liver oil and 0.4 g. of autoclaved yeast. After a preliminary period of 1 week, the animals were given the various supplements of the Standard adsorption product and the crystalline standard. Other animals were given a crystalline specimen of vitamin B_1 prepared from rice polishings (Oryzanin). The growth rates of these rats were observed for a period of 5 weeks. Negative control animals received no vitamin B_1, and they developed acute symptoms of vitamin B_1 deficiency in 4-5 weeks.

The results are given in Table XI.

Table XI.

Comparison of the Potency of the Standard Adsorption Product of Vitamin B_1, the Crystalline Standard and Natural Vitamin B_1 from Rice Polishings (Oryzanin) by a Rat-growth Method (S. Ohdake and T. Yamagishi)

Material	Daily dose	Rat No.	Average weekly weight increase for period of 5 weeks	Average for group
			g.	g.
Standard adsorption product	5 mg.	720	4.8	4.0
		722	2.6	
		733	4.6	
	10 mg.	717	8.3	9.0
		718	8.5	
		721	9.4	
		732	9.6	
		735	8.5	
		773	9.5	
	15 mg.	714	9.4	9.8
		734	10.1	

Material	Daily dose	Rat No.	Average weekly weight increase for period of 5 weeks	Average for group
			g.	g.
Crystalline vitamin B_1 standard	1.0 μg.	761	4.2	6.0
		764	6.0	
		763	7.8	
	1.5 μg.	757	5.6	7.8
		759	9.2	
		760	8.9	
	2.0 μg.	739	8.2	8.6
		740	7.3	
		787	9.2	
		819	9.8	
	2.5 μg.	729	8.7	9.5
		736	9.0	
		783	12.1	
		784	8.1	
		785	9.4	
	3.0 μg.	820	13.2	13.2
Crystalline vitamin B_1 from rice polishings	1.0 μg.	744	4.8	5.0
		746	4.7	
		745	5.5	
	1.5 μg.	766	6.1	6.9
		767	7.2	
		768	7.5	
	2.0 μg.	769	7.6	8.8
		770	8.0	
		771	9.8	
		772	9.6	
	2.5 μg.	775	9.1	9.2
		777	8.4	
		780	8.9	
		782	10.2	
	3.0 μg.	778	10.1	10.5
		781	10.9	

The above growth rates indicate that *the potency of 10 mg. of the adsorbate is equivalent to that of 2 to 2.5 μg. of the crystalline standard;* the natural crystalline vitamin had the same potency as the crystalline standard.

J. *Report of R. R. Williams (New York) and W. L. Sampson (Rahway, N.J.).*

No direct comparison of the adsorbate and crystalline standards was made by rat-growth methods, but the potency of the adsorbate and Merck synthetic vitamin B_1 was compared. This particular preparation of crystalline vitamin B_1 was one of the four which were employed in the making of the standard. The authors found that the Merck synthetic material had the same biological potency as the crystalline standard by a rat-curative method (page 401) and therefore the inclusion of this report is desirable.

The basal diet of CHASE and SHERMAN was employed. Two distinct experiments were carried out. The growth rates which followed administration of the various supplements are given in Table XII.

Table XII.

COMPARISON OF THE POTENCY OF THE STANDARD ADSORPTION PRODUCT OF VITAMIN B_1 AND MERCK SYNTHETIC VITAMIN B_1 BY A RAT-GROWTH METHOD (R. R. WILLIAMS AND W. L. SAMPSON)

Experiment 1.	Daily amount of Merck crystalline vitamin B_1			Daily amount of adsorbate standard
	5 μg.	4 μg.	3 μg.	10 mg.
Weight increase of individual rats for period of 28 days, g.	41	19		42
	45	49	31	14
	8	30	54	50
	56	16	28	20
	46	43	—1	48
	46	27	16	22
	37	39	6	37
			10	
Average for group	39.8	31.8	20.6	33.3

Table XII *(continued).*

Experiment 2.	Daily amount of Merck crystalline vitamin B_1				Daily amount of adsorbate standard
	4.5 μg.	4.0 μg.	3.5 μg.	3.0 μg.	10 mg.
Weight increase of individual rats for period of 28 days, g.		21	17		
	32	38	17	4	28
	16	35	27	7	37
	18	22	41	12	14
	35	18	16	8	30
	29	29	19	17	29
	58	38	23		14
	36	14	17		11
		50	23		16
		24	37		37
		17	38		40
		15	23		38
					31
Average for group	32.0	26.7	24.8	9.6	27.0

In both experiments, *the vitamin potency of 10 mg. of the adsorbate Standard was approximately equivalent to that of 4 μg. of the Merck synthetic vitamin* B_1.

3. Comparison between the Original International Standard of Vitamin B_1 (Standard Adsorption Product) and the New Standard (Vitamin B_1, Hydrochloride) by Rat-curative Methods

A. *Report of N. B. Guerrant (Pennsylvania State College, Pa.).*

Rats which had developed paralytic signs due to deficiency of vitamin B_1 were given single doses either of the adsorbate standard of vitamin B_1 or of the crystalline material. The duration of the cures effected and the maximum growth responses observed are given in Table XIII.

From the experimental data, it was calculated that *10 mg. of the Standard adsorption product had approximately the same vitamin potency as 2.7 μg. of the crystalline vitamin* B_1.

Table XIII.

Comparison of the Potency of the Standard Adsorption Product of Vitamin B_1 and the Crystalline Standard by a Rat-curative Method (N. N. Guerrant)

Amount of standard adsorption product									Amount of crystalline vitamin			
15 mg.				20 mg.					3 μg.			
Weight of rat, g.	Degree of paralysis	Days cured	Maximum growth response, g.	Weight of rat, g.	Degree of paralysis	Days cured	Maximum growth response, g.		Weight of rat, g.	Degree of paralysis	Days cured	Maximum growth response, g.
71	+	10	9	61	+	16	5		69	+	7	5
75	+	10	11	70	++	13	5		73	++	6	2
67	+++	3	1	72	+	16	9		74	++	7	6
75	+	13	10	65	++	7	8		68	+	7	2
60	++	5	4	62	+	9	9		70	+	6	5
72	++	4	8	62	++	10	10		66	+	4	7
88	++	8	3	76	++	7	11		63	++	7	5
58	+++	3	1	65	++	8	10		63	+	4	4
59	+	8	6	69	+	13	6		61	+	5	3
63	++	2	7	63	+	12	7		68	++	7	3
65	+	13	4	79	++	14	7		57	+	10	7
63	++	5	3	82	++	15	10		74	+	7	2
65	++	5	6	68	+	8	4		75	++	7	4
64	+	9	7		+				76	++	7	6
									84	+	3	1
									66	+	3	2
									77	++	4	4
									61	+	4	3
									58	++	7	7
									73	+	5	2
Average for group: 68	1.7 (+)	7.0	5.7	69	1.5 (+)	11.4	7.8		69	1.4 (+)	5.8	4.0

Amount of crystalline vitamin												
4 μg.				5 μg.					6 μg.			
60	+	10	5	78	+	13	7		87	++	10	8
70	+	11	4	70	++	15	3		72	++	15	7
79	++	6	2	62	++	15	7		54	+	10	6
60	+	7	3	80	+	12	4		56	++	11	2
80	+	7	4	67	+	12	6		56	++	21	6
63	+	7	4	65	++	7	9		60	++	10	6
68	++	9	6	74	+	12	4		62	+	14	8
62	++	5	4	58	++	8	7		66	+	10	8
75	++	6	7	57	++	13	7		62	++	8	10
68	+	9	5	64	++	9	5		60	+++	6	8
62	+	12	8	69	++	10	4		67	++	11	7
78	+	4	4	67	+	9	3		65	+	17	12
60	++	4	5	76	+	12	8		71	++	12	6
79	+	5	3	73	++	8	3		68	+	13	7
70	+++	12	5	74	+	8	5		62	++	9	2
64	++	6	6	65	++	5	3		61	+	15	7
55	+	9	3	66	++	7	8					
68	++	9	7	73	++	12	8					
57	+	11	6	72	++	10	7					
69	++	10	3	63	++	9	6					
79	+	9	4									
74	+	13	3									
56	+	6	1									
62	+	8	5									
67	++	5	3									
60	+	11	2									
61	+	5	5									
70	++	5	4									
64	+	6	4									
58	++	10	6									
65	++	9	7									
66	++	11	4									
71	+	8	2									
60	++	6	3									
67	+	5	4									
70	+	6	5									
Average for group: 67	1.5 (+)	7.7	4.7	69	1.6 (+)	10.3	5.7		6.9	1.8 (+)	12.8	7.3

\+ = slightly paralytic.
++ = definitely paralytic, thrown into spasm by rotation.
+++ = severely paralytic, unable to stand.

B. *Report of L. J. Harris (Cambridge).*

The technique used was that described by Birch and Harris.[1] Young male piebald rats (standard strain) weighing between 70 and 90 g. were placed on the basal diet specified on page 384, with the following supplements : (1) autoclaved dried brewers' yeast, heated for 3 hours under 15 lb. pressure, 10% by weight (as source of vitamin-B_1 complex) ; (2) fresh (unheated) dried brewers' yeast, 0.4% by weight (to supply the small amount of vitamin B_1 required to induce the condition of chronic hypovitaminosis favourable to the development of convulsions) ; (3) cod-liver oil, 2 drops per rat daily.

After about 15 to 20 days on this diet, rats began to give evidence of polyneuritic symptoms. If convulsions supervened when the animals were suspended by the tail and rotated, a test dose was given and the number of days which elapsed before convulsions reappeared was determined, the animals being rotated daily to ascertain freedom from convulsions or otherwise. Table XIV gives the details of the tests carried out.

Table XIV.

Comparison of the Potency of the Standard Adsorption Product of Vitamin B_1 and the Crystalline Standard by a Rat-curative Method (L. J. Harris)

Material tested	Single dose given	Days cured, individual animals	Mean
Standard adsorption product, mg. ...	20	5, 6, 8, 8, 8, 9, 11, 11	8.25
	40	9, 10, 10, 11, 12, 12, 15, 15, 16	12.22
	60	14, 14, 15, 15, 15, 16, 17, 18	15.50
Crystalline vitamin B_1, µg....	6	7, 8, 8, 8, 10, 10, 11, 11	9.13
	12	9, 11, 12, 13, 14, 14, 14, 17	13.00
	18	14, 14, 14, 15, 15, 16, 17, 18	15.38

From the average values, the dose-response curves were constructed. Best agreement between the separate curves for adsorbate and crystals was reached when the scales chosen bore the relation

10 mg. adsorbate (1 I.U.) = 2.70 µg. crystalline vitamin B_1.

[1] *Biochem. Jl.*, 1934, 28, 602.

It is important to note that some falling-off from the linear relation occurs with large doses of the vitamin, and that this is more pronounced with the pure crystalline material than with the less active adsorbate. It is obvious, therefore, that, in deciding on the scales of equivalence, principal importance should be attached to the central points in the curve.

C. *Report of B. C. P. Jansen (Amsterdam).*

A modification of the method of estimation described by SMITH [1] was employed.[2] Young rats received a diet consisting of washed polished rice 60, purified casein 3, salts 3, col-liver oil 1 and autoclaved yeast 10. The animals increased in body-weight for some weeks, after which a fairly rapid fall in body-weight occurred. When the body-weight had decreased by 20-30 g., the animals were given test doses either of the adsorbate standard or of the crystalline material, and temporary increases in the body-weight of the animals occurred. The product of (1) the maximum weight increase observed in g. and (2) the period in days which elapsed before the body-weight of the animal was the same as when the test dose was administered, was taken as the measure of vitamin potency of the test material. Each rat could be used for many tests.

The results obtained are given in Table XV opposite.

Thus the potency of 50 mg. of the adsorbate standard is approximately equal to that of 15 μg. of the crystalline standard ; *10 mg. of the adsorbate is therefore approximately equivalent to 3 μg. of the crystals.* In previous experiments, it was found that crystalline vitamin B_1 from yeast or rice bran has the same potency as the synthetic vitamin.

D. *Report of P. di Mattei (Pavia).*

The JANSEN modification [2] of the method of SMITH [3] was employed.

Young rats were given the following diet : washed powdered rice 90, casein 3, salt mixture (Harden and Zilva) 3, halibut-liver oil 1, yeast (autoclaved 5 hours at 120°) 10. After about 4 weeks,

[1] *U.S. Public Health Rep.*, 1930, **45**, 116.

[2] *Zeitschr. f. Vitaminforsch.*, 1936, **5**, 254.

[3] *U.S. Pub. Health Rep.*, 1930, **45**, 116.

Table XV.

COMPARISON OF THE POTENCY OF THE STANDARD ADSORPTION PRODUCT OF VITAMIN B_1 AND THE CRYSTALLINE VITAMIN BY THE SMITH-JANSEN METHOD (B. C. P. JANSEN)

Administering of 50 mg. (= 5 I.U.) of the original standard adsorbate gave			Administering of 15 μg. of the synthetic product gave		
1. Maximum growth, g.	2. Period, days	Product of 1 × 2	1. Maximum growth, g.	2. Period, days	Product of 1 × 2
12	7	84	15	5	75
13	9	117	16	8	128
13	6	78	12	7	84
14	6	84	12	9	108
12	5	60	14	10	140
7	7	49	16	7	112
15	8	120	13	6	78
11	8	88	12	6	72
13	9	117	15	7	105
8	8	64	14	7	98
11	7	77	15	7	105
9	6	54	16	4	64
11	8	88	12	5	60
10	14	140	18	7	126
13	8	104	10	6	60
10	7	70	13	7	91
9	5	45	14	7	98
16	7	112	13	7	91
10	5	50	17	7	119
15	6	90	15	9	135
14	6	84	14	6	84
15	8	120	14	8	112
13	5	65	13	5	65
8	6	48	8	5	40
15	6	90	15	10	150
13	8	104	18	8	144
13	7	91	15	7	105
15	7	105	10	5	50
			15	7	105
			13	9	117
			12	7	84
			13	7	91
Mean		86	Mean		97

when the animals' body-weight had decreased by 15-20 g., the animals were given orally test doses of the adsorbate or the crystalline vitamin. The maximum increase in body-weight in g. which resulted and the time in days for the animal to return to the same body-weight as when the test dose was given were observed. The product of these was taken as the index of vitamin activity. The results of the various tests are given in Table XVI.

Table XVI.

COMPARISON OF THE VITAMIN POTENCY OF THE STANDARD ADSORPTION PRODUCT OF VITAMIN B_1 AND THE CRYSTALLINE STANDARD BY THE SMITH-JANSEN METHOD (P. DI MATTEI)

Rats given adsorbate standard			Rats given crystalline vitamin		
1. Maximum weight increase, g.	2. Duration of increase, days	Product, 1 × 2	1. Maximum weight increase, g.	2. Duration of increase, days	Product, 1 × 2
	Dose 100 mg.			Dose 35 μg.	
13.0	20.0	260.0	14.5	15.0	217.5
6.0	11.5	69.0	12.0	12.0	144.0
15.0	13.0	195.0	12.5	14.0	175.0
18.0	15.0	270.0	17.0	15.5	263.5
	Average....	198.5		Average....	202.5
	Dose 86 mg.			Dose 30 μg.	
11.5	10.0	115.0	10.0	11.0	110.00
12.0	18.0	216.0	10.5	23.5	246.75
9.0	12.5	112.5	12.0	17.5	110.00
10.0	12.0	120.0	9.5	9.5	90.25
	Average....	140.9		Average....	139.50
	Dose 75 mg.			Dose 26 μg.	
10.5	8.5	89.25	10.0	10.0	100.0
10.5	9.0	94.50	6.0	7.5	45.0
10.0	9.0	90.00	11.0	9.0	99.0
6.0	7.0	42.00	9.0	10.0	90.0
	Average....	78.9		Average....	83.5

The results indicate that *the potency of 100 mg. of the adsorbate standard is approximately equal to that of* 35 μ*g. of the crystalline vitamin or that 10 mg. of the adsorbate (1 I.U.) is equal to 3.5* μ*g. of crystalline vitamin* B_1.

E. *Report of Messrs. E. Merck (Darmstadt).*

The method employed [1], although differing in detail, resembles that of SMITH.[2]

Rats whose body-weights were decreasing in consequence of deprivation of vitamin B_1 were given single test doses of the vitamin, either as the standard adsorbate or crystalline vitamin. The magnitude and duration of the resulting increase of body-weight due to administration of the vitamin B_1 were taken into consideration in calculating what is called the " activity number ", which is a measure of the vitamin potency of the test dose.

The results obtained are given in Table XVII.

Table XVII.

COMPARISON OF THE POTENCY OF THE STANDARD ADSORPTION PRODUCT OF VITAMIN B_1 AND THE CRYSTALLINE STANDARD BY A METHOD SIMILAR TO THE SMITH-JANSEN METHOD (E. MERCK)

Dose administered	Number of animals	Activity number of individual animals	Average activity number of group
50 mg. adsorbate standard	15	320, 420, 470, 270, 410 340, 330, 260, 300, 360 400, 270, 300, 240, 330	335
10 μg. crystalline standard	15	470, 320, 310, 350, 330 320, 260, 370, 360, 340 320, 440, 330, 400, 270	346

It is thus taken that 50 mg. of the adsorbate standard is equivalent in vitamin potency to 10 μg. of the crystalline standard or that 10 mg. (1 I.U.) $\equiv$ 2 μg.

The potency of a sample of synthetic crystalline vitamin B_1 which was included in the making of the crystalline standard has been compared with that of the adsorbate standard ; in all, 216 tests have been made.

Again, 10 mg. of the adsorbate had the same potency as 2 μg. of the crystals.

[1] See MOLL, *E. Merck's Jahresbericht*, 1935, **49**, 57.

[2] *U.S. Pub. Health Rep.*, 1930, **45**, 116.

F. *Report of E. M. Nelson and O. L. Kline (Washington).*

When the litters of young rats were 12-13 days of age, the mothers were given a diet deficient in vitamin B_1, consisting of sucrose 61.25, casein 18.0, salts 4.0, autoclaved yeast 4.0, autoclaved peanuts 10.0, purified liver extract 0.75 and cod-liver oil 2.0. The young animals were weaned at 25-30 days and were maintained on the above deficient diet until acute polyneuritic symptoms were observed. The rats were then given a single dose of the standard adsorbate of vitamin B_1 and were observed daily ; the period from the time the dose was administered until recurrence of the acute polyneuritic symptoms was noted and was named the curative period. The animals were then given successive doses of 5 μg. and 6 μg. of the crystalline standard and the curative periods were again determined.

The curative period was used as a measure of vitamin B_1 potency.

The results are given in Table XVIII.

Table XVIII.

Comparison of the Potency of the Standard Adsorption Product of Vitamin B_1 and the Crystalline Standard by a Rat-curative Method (E. M. Nelson and O. L. Kline)

Curative period in days.

Rat No.	Crystalline vitamin 5 μg.	Adsorbate standard 20 mg.	Crystalline vitamin 6 μg.
399	7	9	12
400	9	8	12
403	8	6	9
404	8	12	14
406	7	9	9
407	10	8	13
416	8	9	10
417	11	12	12
418	7	8	8
420	7	6	9
425	6	7	10
426	6	7	12
429	9	8	12
430	8	7	11
432	8	10	9
434	8	8	11
436	8	10	14
437	8	9	10
443	9	10	14
Average	8.0	8.5	11.1

It is concluded from the above *10 mg. of the adsorbate standard (1 I.U.) and 2.6 μg. of the crystalline standard are equivalent in potency.*

G. *Report of R. R. Williams (New York) and W. L. Sampson (Rahway, N.J.).*

Young rats were maintained on a diet deficient in vitamin B_1,[1] and after 4-5 weeks, when the animals had lost 30-40% of their maximum weights, they were examined twice daily for " polyneuritic " symptoms. Animals which showed distinct convulsive seizures when rotated by the tail were then given single doses either of the standard adsorbate or of the crystalline vitamin. The animals were considered cured when complete freedom from convulsions or inco-ordination was maintained for a period of at least 4 days. Rats which died within 24 hours of administration of the test dose were not included in the results. Animals which showed no cure after 48-96 hours were conserved for further testing by administration of an amount of vitamin B_1 sufficient to effect a cure. When rats so treated again showed " polyneuritic " symptoms, they were again ready for further tests.

A curative dose is defined as the minimum amount of substance that will effect a cure in 70% ± 10% of the test animals. The results are given in Table XIX.

Table XIX.

Comparison of the Potency of the Standard Adsorption Product of Vitamin B_1 and the Crystalline Standard by a Rat-curative Method (R. R. Williams and W. L. Sampson)

Substance	Dose	Number of animals used	Number of animals cured	% cured	Average weight gain of cured animals, g.	Average duration of cures, days
Crystalline standard	4 μg.	17	3	18	6.3	9.0
	5 ,,	23	18	78	8.0	7.7
	6 ,,	10	10	100	6.9	7.8
Standard adsorbate	16 mg.	27	10	55.5	7.0	6.6
	18 ,,	10	7	70	6.0	6.5
	20 ,,	17	13	76	7.7	7.5
	24 ,,	8	8	100	12.3	12.2

[1] Ammerman and Waterman : *Jl. Nutrition*, 1935, **10**, 25.

Thus 5 μg. of the crystalline vitamin, which cured 78% of the test animals, and 20 mg. of the acid clay adsorbate, which cured 76%, were equivalent doses.

10 mg. of the adsorbate standard is therefore equivalent in vitamin B_1 potency to 2.5 μg. of the crystalline standard.

* * *

Additional Data. — In other experiments, in which many rats were used, Merck crystalline vitamin B_1 was found by the above method to be equal in potency to the standard crystals.

Quinine sulphate eluates, prepared from 12 mg. portions of the standard adsorbate,[1] cured 72% of "polyneuritic" rats, while 5 μg. of Merck's crystals cured 73.8%. Thus quinine sulphate eluates of the adsorbate standard are more potent than the adsorbate itself when administered to "polyneuritic" rats. The eluate from 10 mg. of the adsorbate standard is equivalent in potency to 4.2 μg. of the crystalline vitamin.

4. Comparison between the Original International Standard of Vitamin B_1 (Standard Adsorption Product) and the New Standard (Vitamin B_1 Hydrochloride) by the Rat-bradycardia Method

A. *Report of L. J. Harris (Cambridge).*

The procedure followed that previously described.[2] Young piebald rats (standard strain) of body-weight 45-50 g. were given the basal diet described previously (page 384). After 21-25 days, the animals began to lose weight, and, as soon as the body-weight was within 10 g. of the original body-weight at the start of the experiment, daily measurements were taken of the heart rates. Dosing was begun as soon as the heart rate had fallen to between 375 and 400 beats per minute, compared with the normal rate of 500-550. Test doses, either of the crystalline vitamin or the standard adsorbate, were then given, and the duration of the cure

[1] Williams : *Jl. Amer. Chem. Soc.*, 1937, **56**, 1187.

[2] Birch and Harris : *Biochem. Jl.*, 1934, **28**, 602 ; Leong and Harris : *Biochem. Jl.*, 1937, **31**, 672 ; Drury and Harris : *Chem. Ind.*, 1930, **49**, 851.

was determined by daily measurement of the heart rate. In interpreting the " duration of the cure " to the nearest day, the following criterion was used : the cure was not regarded as at an end so long as the rate remained at any value greater than 5 beats per minute above the minimal level.

Rats were generally used several times over for consecutive tests. Any animal whose heart rate had fallen below 375 was given a dose of vitamin B_1 to bring it well over 400 ; the rate was then allowed to fall slowly to 375-400 before another test was made.

The results of the tests are given in Table XX.

Table XX.

Comparison of the Potency of the Standard Adsorption Product of Vitamin B_1 and the Crystalline Standard by the Rat-bradycardia Method (L. J. Harris)

Material tested	Dose given	Number of tests performed	Duration of cure, days	
			Individual animals	Mean
Adsorbate standard, mg.	10	12	1, 2, 2, 2, 3, 3, 3, 3, 3, 3, 4, 4	2.75
	20	12	3, 3, 3, 4, 4, 4, 4, 4, 4, 4, 4, 5	3.83
	30	11	4, 4, 4, 4, 4, 5, 5, 6, 6, 6, 7,	5.00
	40	12	4, 5, 5, 6, 6, 6, 6, 8, 8, 8, 8, 8	6.50
Crystalline vitamin B_1, µg.	2.5	12	1, 2, 2, 2, 3, 3, 3, 3, 4, 4, 4, 4	2.92
	5.0	12	2, 2, 3, 3, 3, 3, 4, 4, 4, 4, 5, 7	3.67
	7.5	11	4, 4, 4, 5, 5, 5, 5, 6, 6, 7, 8,	5.36
	10.0	11	5, 5, 5, 5, 6, 6, 7, 7, 8, 8, 9,	6.45

If the doses are plotted against the days cured, the resulting dose-response curves lie virtually along straight lines both for the crystalline and the adsorbate standards. The relative slopes of the two straight lines give the relative activities of the two materials. The relation thus found agrees almost exactly with the figures : *adsorbate 10 mg. = 2.50 µg. crystalline vitamin* B_1.

B. *Report of Messrs. F. Hoffmann-La Roche (Basle).*

The results are based on 150 tests on rats in which the activity of the adsorbate was compared with that of the crystalline vitamin B_1,

Table XXI.

Comparison of the Potency of the Standard Adsorption Product of Vitamin B_1 and the Crystalline Standard by the Rat-bradycardia Method (F. Hoffmann-La Roche)

Adsorbate standard

10 mg.			20 mg.			30 mg.		
Number of animals	Duration of effect, days	Total days for group	Number of animals	Duration of effect, days	Total days for group	Number of animals	Duration of effect, days	Total days for group
18	0.0	0.0	6	0.0	0.0	1	1.8	1.8
1	1.4	1.4	7	2.0	14.0	1	2.5	2.5
3	1.5	4.5	1	2.4	2.4	2	3.0	6.0
1	1.7	1.7	4	2.5	10.0	3	3.5	10.5
1	1.8	1.8	1	2.8	2.8	5	4.0	20.0
9	2.0	18.0	11	3.0	33.0	1	4.3	4.3
1	2.3	2.3	1	3.2	3.2	3	4.5	13.5
4	2.5	10.0	3	3.5	10.5	1	5.0	5.0
2	2.7	5.4	13	4.0	52.0	2	6.0	17.0
1	2.8	2.8	1	4.2	4.2	1	6.5	6.5
3	3.0	9.0	1	4.3	4.3			
1	3.3	3.3	4	4.5	18.0			
1	3.4	3.4	1	4.7	4.7			
1	3.5	3.5	5	5.0	25.0			
2	4.0	8.0	1	6.0	6.0			
1	4.5	4.5						
1	5.0	5.0						
51		84.6	60		190.1	20		82.1
Average : 1.7 jours			3.1 jours			4.1 jours		

Crystalline standard

2.5 μg.			5 μg.			6.7 μg.		
4	0.0	0.0	3	0.0	0.0	1	2.0	2.0
2	1.5	3.0	1	1.5	1.5	1	3.0	3.0
2	2.0	4.0	1	1.8	1.8	3	4.2	12.6
1	2.7	2.7	6	2.0	12.0	2	5.0	10.0
3	3.0	9.0	2	2.3	4.6			
			1	2.5	2.5			
			15	3.0	45.0			
			1	3.2	3.2			
			2	3.4	6.8			
			1	3.5	3.5			
			7	4.0	28.0			
			3	4.5	13.5			
			1	5.0	5.0			
12		18.7	44		127.4	7		27.6
Average : 1.55 jours			2.9 jours			3.9 jours		

using the method of BIRCH and HARRIS.[1] The standard curve was ascertained using 10, 20 and 30 mg. doses of the standard adsorbate. The crystalline standard was given in doses of 2.5, 5, and 6.7 μg. The results of the test are given in Table XXI.

By application of the curve of response to the standard adsorbate, it was found that 19 mg. of the adsorbate corresponded to 5 μg. of the crystalline standard. *10 mg. of the original standard adsorbate is therefore equivalent in potency to 2.63 μg. of the crystalline standard.*

C. *Report of the I. G. Farbenindustrie (Elberfeld).*

The young rats (body-weight 50-55 g.) were given the diet and supplements described on page 384. In some of the later experiments, the carbohydrate supplied was changed from 30% cane-sugar + 30% rice starch to 48% cane-sugar + 12% rice starch; this did not influence the results. The body-weight of the animals increased for 8-10 days and then, after remaining constant for a few days, it decreased. When the body-weight had fallen to 55 g., the measurement of the pulse rate was taken. The adsorbate standard was given orally, and the crystalline vitamin both orally and subcutaneously. The same animals were employed for several tests, as recommended by BIRCH and HARRIS.[1]

Normal rats have a pulse rate of 500-600 beats per minute. When that of the deficient animals had fallen to 400 per minute or less for 2 consecutive days, the animals were given single doses either of the adsorbate or the crystalline vitamin. The pulse rate was then taken at least once daily to determine the duration of effect of the dose. When the pulse rate had again fallen to the level at which the vitamin was administered, the curative effect was considered to be at an end. The results are given in Table XXII.

The curves of response to the acid clay adsorbate standard and the crystallin vitamin did not run parallel and therefore the comparisons at the various dose levels did not agree. However, by taking the average values, it was considered that *10 mg. of the adsorbate standard was equivalent in potency to 3.61 μg. of the crystalline vitamin administered orally and 3.33 μg. administered subcutaneously.*

[1] *Biochem. Jl.*, 1934, 28, 602.

Table XXII.

COMPARISON OF THE STANDARD ADSORPTION PRODUCT OF VITAMIN B_1 AND THE CRYSTALLINE STANDARD BY THE RAT-BRADYCARDIA METHOD (I. G. FARBENINDUSTRIE)

Adsorbate by mouth

10 mg.			10 mg.			50 mg.		
Number of experiments	Duration of effect on individual rats, days	Average duration, days	Number of experiments	Duration of effect on individual rats, days	Average duration, days	Number of experiments	Duration of effect on individual rats, days	Average duration, days
19	0, 0 2, 2, 2, 2 3, 3, 3 4, 4, 4, 4, 4 5, 5, 5 6 7	3.42	34	0, 0 2, 2, 2 3, 3, 3, 3, 3 4, 4, 4, 4, 4, 4, 4, 4, 4 5, 5, 5, 5, 5, 5, 5, 5 6, 6, 6, 6, 6, 6 8	4.15	12	3, 3 4, 4, 4, 4 5, 5, 5 6 7 11	5.08

Crystalline vitamin by mouth

2 μg.			4 μg.			8 μg.		
13	0, 0 2, 2, 2, 2, 2 3, 3, 3, 3, 3 4	2.23	12	0 2, 2 3, 3, 3, 3, 3 4, 4 5 6	3.17	19	3, 3, 3 4, 4, 4, 4, 4, 4, 4, 4, 4 5, 5, 5, 5, 5 6 8	4.42

Crystalline vitamin subcutaneously

2 μg.			4 μg.			8 μg.		
13	0, 0, 0 2, 2, 2, 2, 2 3 4, 4, 4 5	2.31	24	0, 0 2, 2, 2, 2, 2, 2, 2 3, 3, 3, 3 4, 4, 4, 4 5, 5, 5, 5 6, 6 7	3.37	18	2 3, 3, 3 4, 4, 4, 4 5, 5, 5, 5, 5, 5, 5, 5 6 8	4.50

D. *Report of R. R. Williams (New York) and W. L. Sampson (Rahway, N. J.).*

It was observed in using the rat-bradycardia method that there was a very marked day-to-day variation in the heart rates of the test animals, as well as a great difference in the duration of the cures following administration of a given dose.

The results obtained are given in Table XXIII.

Table XXIII.

Comparison of the Potency of the Standard Adsorption Product of Vitamin B_1 and the Crystalline Standard by the Rat-bradycardia Method [1] (R. R. Williams and W. L. Sampson)

Substance	Dose	Duration of cure in days (individual)	Average
Crystalline vitamine B_1	5 μg.	4, 5, 6, 2, 6, 2, 8, 2	4.4
	7,5 μg.	3, 8, 4, 3, 5, 4, 4	4.4
	10,0 μg.	3, 9, 5, 4, 3, 2, 8, 5, 12, 6, 3, 6, 3, 4	5.2
Adsorbate	20 mg.	2, 3, 6, 3, 2, 5, 3, 2, 4, 7, 4, 3	3.7

Dose levels of 2.5 μg. of the crystalline vitamin and 10 mg. of the adsorbate failed to produce an appreciable effect on the heart rate.

The above indicates that *10 mg. of the adsorbate standard is equivalent to somewhat less than 2.5 μg. of the crystalline vitamin.*

5. Comparison between the Original International Standard of Vitamin B_1 (Standard Adsorption Product) and the New Standard (Vitamin B_1 Hydrochloride) by Pigeon-curative Methods

A. *Report of K. H. Coward (Londres).*

Details of the method employed have been published elsewhere.[2]

The pigeons were kept on wire grids and fed exclusively on a diet of polished rice until they developed neck retraction. Tap-water was also supplied. Only about half of the birds developed neck retraction within 28 days and could be used for the test. The remainder, which either died before showing the symptoms or failed to show any, were discarded after 28 days.

[1] The crystalline vitamin administered in these experiments was Merck synthetic vitamin B_1 hydrochloride.

[2] Coward *et al.* : *Biochem. Jl.*, 1933, **27**, 1719.

As the birds became ready for dose, they were given single doses either of the crystalline vitamin or the adsorbate standard. Absenee of any symptoms of neck retraetion and good museular tone 24 hours after dosing was taken as the eriterion of eure. The birds were examined daily after the dose was administered, until neck retraction developed again or death oeeurred. The duration of the eure, as well as the proportion of birds eured, was reeorded for eaeh group. The results are given in Table XXIV.

Table XXIV.

Comparison of the Potency of the Standard Adsorption Product of Vitamin B_1 and the Crystalline Standard by a Pigeon-curative Method (K. H. Coward)

Material given	5 μg. crystals	10 μg. crystals	30 mg. adsorbate
Number of birds dosed	15	15	15
Number of birds cured	5	10	10
Percentage cured	33.3	66.7	66.7
Average duration of cure, days.....	3.6	4.3	4.8

Since the doses of 10 μg. of the crystalline material and 30 mg. of the adsorbate eured the same percentage of birds, it is evident that these doses are equal in potency and therefore *10 mg. of the adsorbate standard is equivalent to 3.3 μg. of the crystalline standard.*

B. *Report of P. di Mattei (Pavia).*

Pigeons were fed on a diet of polished riee. Each bird received 25 g. of the diet and, if this was refused, forced feeding was resorted to. After 13-17 days, the first polyneuritie symptoms appeared. and when these beeame suffieiently pronouneed, groups of birds having symptoms of similar severity were given varying amounts of the crystalline standard and of the adsorbate standard. The birds were then observed daily ; in determining the efficacy of the dose administered, the effects on the survival period, body temperature, weight and polyneuritic symptoms were taken into consideration.

All the birds whieh reeeived 40 mg. of adsorbate or 10 μg. of the erystals died within 10 days ; those given 50 mg. of the adsorbate or 18 μg. of the crystalline vitamin died within 25 days ; those given 60-70 mg. of the standard adsorbate or 24 μg. of the erystalline material were alive after 35 days, but were not in quite perfeet condition ; and those given 80 mg. of the adsorbate or 27-30 μg. of the erystalline standard were in perfeet condition after 45 days.

From these results, it is calculated that *10 mg. of the adsorbate standard (1 I.U.) has the vitamin potency of 3.4-3.8 μg. of the crystalline material.*

C. *Report of Messrs. E. Merck (Darmstadt).*

The pigeon day-dose method of KINNERSLEY and PETERS [1] was employed.

Pigeons suffering from neck retraction developed on a diet of polished rice were given single doses of either 50 mg. of the adsorbate standard or 10 μg. of the crystalline standard, and the number of days which elapsed before the polyneuritic symptoms developed were observed. The " day doses " of the preparations of vitamin B_1 were obtained by dividing the amount of the preparation administered by the number of days cured. The results obtained are given in Table XXV.

Table XXV.

COMPARISON OF THE POTENCY OF THE STANDARD ADSORPTION PRODUCT OF VITAMIN B_1 AND THE CRYSTALLINE STANDARD BY THE PIGEON DAY-DOSE METHOD (E. MERCK)

Adsorbate standard, 50 mg.			Crystalline standard, 10 μg.		
Pigeon No.	Number of days cured	Pigeon day-dose, mg.	Pigeon No.	Number of days cured	Pigeon day-dose, μg.
488	5	10.0	646*	5	2.0
508	5	10.0	647	5	2.0
507	6	8.3	649	6	1.7
507	4.5	11.0	650	5	2.0
508	5	10.0	651	5.5	1.8
545	5	10.0	645	6	1.7
555	6	8.3	655	5	2.0
610	5	10.0	648	5	2.0
627	5.5	9.0	659*	5	2.0
626	6	8.3	660*	6	1.7
630	4	12.5	662	5	2.0
			661	7	1.5
	Average	10.0		Average	1.9

* These pigeons received their doses by subcutaneous injection ; other birds received the vitamin orally.

Using this method of comparison, therefore, 10 mg. of the adsorbate standard is equivalent to 1.9 μg. of the crystalline material ; the crystalline vitamin was equally potent, whether administered orally or subcutaneously.

[1] *Biochem. Jl.*, 1925, **19**, 820.

In many tests of comparison, *10 mg. of the adsorbate standard has also been found to have the same vitamin potency as 1.9 μg. of synthetic vitamin B_1* (E. Merck). These experiments substantiate the results obtained with the crystalline standard preparation.

D. *Report of S. Ohdake and T. Yamagishi (Tokio).*

The vitamin potency of the adsorbate and standard crystalline vitamin was compared by two pigeon-curative methods.

(a) *Day-dose Method.* — The curative "day-doses", not only of the original adsorbate standard and the new synthetic standard, but also of the crystalline vitamin B_1 isolated from rice polishings (Oryzanin) were determined by the method of KINNERSLEY and Peters.[1] The single doses of acid clay adsorbate were given orally and of the crystalline vitamin B_1 by injection ; the results are given in Table XXVI.

Table XXVI.

COMPARISON OF THE POTENCY OF THE STANDARD ADSORPTION PRODUCT OF VITAMIN B_1 AND THE CRYSTALLINE STANDARD BY THE PIGEON DAY-DOSE METHOD (S. OHDAKE AND T. YAMAGISHI)

Standard adsorption product				Synthetic vitamin B_1 standard				Crystalline vitamin B_1 from rice polishings (Oryzanin)			
Pigeon No.	Single dose, mg.	Days cured	Day-dose, mg.	Pigeon No.	Single dose, μg.	Days cured	Day-dose, μg.	Pigeon No.	Single dose, μg.	Days cured	Day-dose, μg.
904	75	6	12.5	901	10	2.5	4.0	911	10	5	2.0
918	,,	6	12.5	916	,,	6	1.6	922	,,	4	2.5
955	,,	7	10.7	908	,,	3	3.3	948	,,	3	3.3
956	,,	6	12.5	909	,,	5	2.0	953	,,	3	3.3
906	50	7	7.2	909	,,	3	3.3	935	9	5	1.8
908	,,	4	12.5	915	,,	4	2.5	935	8	4	2.0
919	,,	5	10.0	920	,,	5	2.0	945	,,	4	2.0
956	,,	5	10.0	922	,,	3	3.3	945	,,	3	2.6
				936	,,	4	2.5	946	,,	3.5	2.3
				940	,,	4	2.5	948	,,	4	2.0
				933	8	3	2.6				
				933	,,	3	2.6				
				907	,,	3	2.6				
				927	,,	5	1.6				
				927	,,	5	1.6				
				929	,,	2	4.0				
				934	,,	4	2.0				
				936	,,	4	2.0				
Average 10.98				Average 2.6				Average 2.4			

[1] *Biochem. Jl.*, 1925, **19**, 820.

Consideration of the above results indicates that *10 mg. of the adsorbate standard (1 I.U.) has the vitamin potency of 2.4 μg. of the crystalline standard; the natural crystalline vitamin B_1 has apparently the same potency as the synthetic vitamin.*

(b) *Curative Test for Pigeons.* — The birds were given a diet consisting of purified starch 60, purified casein 20, arachis oil 15, and McCollum's salt mixture (No. 185) 5 ; each bird received the additional daily supplements of 3 drops of cod-liver oil and 0.4 g. of autoclaved yeast. After 4-5 weeks, acute symptoms of vitamin B_1 deficiency appeared, and the pigeons were then given daily, for 7 days, doses either of the adsorbate standard, the synthetic vitamin-B_1 standard or the natural crystalline vitamin from rice polishings. In determining the comparative potencies of the doses administered, the time taken to cure the acute symptoms and the increase in body-weight observed during the seven days were taken into consideration. The results are summarised in Table XXVII.

Table XXVII.

Comparison of the Potency of the Standard Adsorption Product of Vitamin B_1 and the Crystalline Standard by a Pigeon-curative Method (S. Ohdake and T. Yamagishi)

Daily dose	Number of pigeons	Average time taken to effect cure, hours	Average weight increase during 7 days, g.
mg.	*Standard Adsorption Product.*		
10	3	Not cured	— 15
15	2	72	— 5
20	1	24	20
30	1	12	41
	Synthetic Crystalline Standard (by Injection).		
μg.			
2.0	3	Not cured	Died
2.5	3	Not cured	6
3.0	3	24	11
3.5	3	4	16
4.0	3	3	22
	Natural Crystalline Vitamin B_1 from Rice Polishings (Oryzanin) (by Injection).		
μg.			
2.5	3	Not cured	7
3.5	3	12	28
4.0	2	3	18
5.0	3	3	17

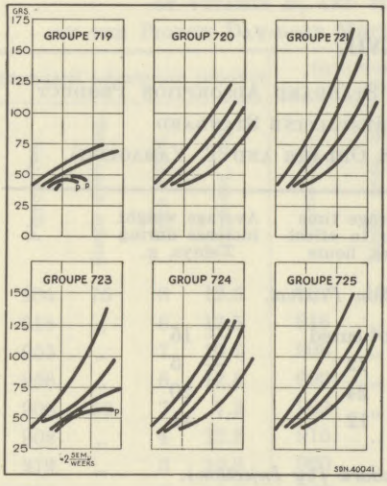
The above results indicate that equivalent vitamin potencies are possessed by 20 mg. of the adsorbate and by 4 μg. of the crystalline vitamin, synthetic or natural. *The international unit of the adsorbate (10 mg.) therefore has the potency of 2 μg. of the crystalline standard or of the crystalline natural vitamin.*

6. Comparison between the Original International Standard of Vitamin B_1 (Standard Adsorption Product) and the New Standard (Vitamin B_1 Hydrochloride) by a Chicken Prophylactic Method)

Report of C. A. Elvehjem (Madison, Wis.).

The antineuritic potencies of the preparations were compared by the chicken prophylactic assay method. The basal ration (Ration 242A) has been described elsewhere.[1]

Figure 2.



P = Polyneuritis.

Group 719 : 50 μg. crystalline vitamin per 100 g. of Ration 242A.

Group 720 : 60 μg. crystalline vitamin per 100 g. of Ration 242A.

Group 721 : 70 μg. crystalline vitamin per 100 g. of Ration 242A.

Group 723 : 175 mg. Standard adsorption product per 100 g. of Ration 242A.

Group 724 : 200 mg. Standard adsorption product per 100 g. of Ration 242A.

Group 725 : 225 mg. Standard adsorption product per 100 g. of Ration 242A.

The results are shown in Figure 2. Two of a group of four chicks, fed 50 μg. of the synthetic vitamin-B_1 sample per 100 g. of ration (Group 719), died after exhibiting the polyneuritic syndrome in 14-17 days. All the chicks fed 60 μg. of vitamin B_1 per 100 g. of ration (Group 720) were protected from polyneuritis during the 5 weeks experimental period. Somewhat better growth resulted when the level of vitamin B_1 was increased to 70 μg. per 100 g. of ration (Groupe 721).

Since several previous trials have shown that chicks fed on

[1] *Jl. Assoc. Off. Agric. Chem.*, 1935, 18, 353.

Ration 242A were protected from polyneuritis when the ration was supplemented with 200 mg. (20 I.U.) of the adsorbate standard per 100 g., this was fed at this level. A sample of the adsorbate standard was also fed at 175 mg. and 225 mg. per 100 g. of ration as confirmatory checks. All the chicks fed 200 mg. (20 I.U.) of the adsorbate standard per 100 g. ration (Group 724) were protected from polyneuritis during the experimental period. One of a group of four chicks fed 175 mg. (17.5 I.U.) of the adsorbate per 100 g. of ration (Group 723) died after showing polyneuritis. The growth of the remaining chicks in this group was not good. Chicks fed 225 mg. (22.5 I.U.) of the adsorbate standard per 100 g. of ration (Group 725) were protected from polyneuritis during the experimental period.

The results obtained with the adsorbate standard and the synthetic vitamin B_1 are in accord with previous results obtained by the chick assay method.

These results show that *10 mg. of the adsorbate standard of vitamin B_1 (1 I.U.) is approximately equivalent to 3 μg. of the synthetic standard.*

7. Comparison between the Original International Standard of Vitamin B_1 (Standard Adsorption Product) and the New Standard (Vitamin B_1 Hydrochloride) by the Catatorulin Method

Report of H. W. Kinnersley and R. A. Peters (Oxford).

The crystalline standard of vitamin B_1 was compared by the catatorulin method with a baryta extract [1] of the adsorbate standard, made as previously described.[2]

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[1] Attention is drawn to the work of Sampson and Keresztesy (*Proc. Soc. Exp. Biol. Med.*, 1937, **36**, 30), who found that baryta extracts only about half of the vitamin B_1 from the acid clay. (See also page 402 of this volume.) The previous finding of Kinnersley and Peters (*Biochem. Jl.*, 1936, **30**, 985) that baryta extracts of the standard acid clay adsorbate give the same curative response in pigeons as the clay itself may be explained also by the conclusion of Sampson and Keresztesy that only 50% of the vitamin present on the clay is available to a severely depleted animal.

[2] Kinnersley and Peters : *Biochem. Jl.*, 1936, **30**, 985.

Table XXVIII gives the results of the comparison.

Table XXVIII.

Comparison between the Potency of the Standard Adsorption Product of Vitamin B_1 and the Crystalline Standard by the Catatorulin Method (H. W. Kinnersley and R. A. Peters)

		"Estimated value" μg.
Experiment 1355.	10 mg. adsorbate standard less than 3.1 μg. crystals....	2.5
„ 1356.	10 „ „ „ much less than 3.9 μg. crystals	1.95
„ 1357.	10 „ „ „ „ „ „ 3.6 μg. „	2.3
„ 1363.	10 „ „ „ equivalent to 1.95 μg. crystals	1.95
	Average	2.2

The " estimated value " was obtained by use of a comparison curve.

In previous experiments with this method, *10 mg. of the adsorbate standard was found equivalent to 2 μg. of natural crystalline vitamin B_1 from yeast.*

8. Comparison between the Original International Standard of Vitamin B_1 (Standard Adsorption Product) and the New Standard (Vitamin B_1 Hydrochloride) by the Thiochrome Fluorimetric Method [1]

A. *Report of H. von Euler, H. Heiwinkel and H. Willstaedt (Stockholm).*

(*a*) To a weighed amount of the adsorbate standard, 3 ml. of a 10% solution of NaOH and 0.1 ml. of a 10% solution of potassium ferricyanide were added. After shaking for 10 minutes, the thiochrome formed was extracted with 15 ml. of isobutyl alcohol. Samples of this extract were diluted with known amounts of isobutyl alcohol until the fluorescence of the solution in ultra-violet light matched that of a similar solution prepared from a weighed amount of the crystalline vitamin. The matching was made by eye.

Four experiments of comparison were carried out, and the amounts of the crystalline vitamin found equivalent to 10 mg. of the adsorbate (1 I.U.) were 3.0 μg., 2.7 μg., 3.4 μg., and 4.6 μg., respectively. In the last experiment, the amount of adsorbate

[1] Jansen : *Rec. Trav. Chim.*, 1936, **55**, 1046.

employed was probably too small, and therefore the result was disregarded. Taking the average of the remaining experiments, 10 mg. of the adsorbate standard was equivalent to 3.0 μg. of the crystalline specimen.

(*b*) Further experiments were carried out using modified methods described by KARRER and KUBLI [1] and WESTENBRINK and GOUDSMIT.[2] In these experiments, the solutions were compared photometrically. With the method of KARRER and KUBLI, 10 mg. of the adsorbate in 2 experiments was found equivalent to 2,06 and 2.6 μg. respectively of the crystalline vitamin. In the experiment with the method of WESTENBRINK and GOUDSMIT, 10 mg. of the adsorbate was equivalent to 2.6 μg. of the crystals. The average result for these 3 determinations was therefore that 10 mg. adsorbate was equivalent to 2.4 μg. of the crystalline vitamin.

By taking the average of the different estimations described in (*a*) and (*b*) above, *10 mg. of adsorbate standard was found equivalent to 2.7 μg. of the crystalline standard.*

B. *Report of Messrs. F. Hoffmann-La Roche (Basle).*

The comparison was made by the method of JANSEN [3] as modified by KARRER and KUBLI. [2] Four estimations were carried out, and in these experiments 1 mg. of the crystalline standard was found equivalent to 4,100 mg., 3,800 mg., 3,800 mg. and 4,000 mg. respectively, of the adsorbate standard, the average value being 3,900 mg. *The amount of the adsorbate standard (10 mg.) representing 1 international unit was therefore equivalent to 2.56 μg. of the crystalline vitamin.*

C. *Report of B. C. P. Jansen (Amsterdam).*

The method of comparison employed was that described by the author.[3] Many experiments were carried out, of which the following are examples. (See Table XXIX.)

From the following results, it is apparent that 20 mg. of the adsorbate is equivalent to 5 μg. of the crystals, and thus *10 mg. of the adsorbate standard (1 I.U.) is equivalent to 2.5 μg. of the crystalline vitamin.*

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[1] *Helv. Chim. Acta*, 1937, 20, 369.

[2] *Rec. Trav. Chim. Pays-Bas*, 1937, 56, 803.

[3] *Ibid.*, 1936, 55, 1046.

Table XXIX.

COMPARISON OF THE STANDARD ADSORPTION PRODUCT OF VITAMIN B_1 AND THE CRYSTALLINE STANDARD BY THE THIOCHROME FLUORIMETRIC METHOD (B. C. P. JANSEN)

5 µg. of the synthetic vitamin				20 mg. of the adsorbate standard			
Amount of 0.1 % $K_3Fe(CN)_6$ added, ml.	Galvanometer deflection, mm.			Amount of 0.1 % $K_3Fe(CN)_6$ added, ml.	Galvanometer deflection, mm.		
	1st estimation	2nd estimation	3rd estimation		1st estimation	2nd estimation	3rd estimation
0.1	29	35	30	0.05	31	32	35
0.2	32	36	32	0.10	33	35	32
0.4	33	36	32	0.15	31	37	34
0.7	34	37	30	0.20	31	36	32
1.0	33	35	30	0.30	31	33	29
Quinine : 0.3 mg. in 100 ml.	75	76	71	Quinine : 0.3 mg. in 100 ml.	75	76	71

9. COMPARISON BETWEEN THE ORIGINAL INTERNATIONAL STANDARD OF VITAMIN B_1 (STANDARD ADSORPTION PRODUCT) AND THE NEW STANDARD (VITAMIN B_1 HYDROCHLORIDE) BY THE COLORIMETRIC METHOD OF KINNERSLEY AND PETERS [1]

Report of H. von Euler (Stockholm).

The modified method of WILLSTAEDT and BARANY was employed.[2] Elution of the vitamin B_1 from the adsorbate standard was carried out either by treatment with 1% sodium carbonate solution or with warm aqueous pyridine solution.[3] These methods of elution gave similar results. The colour which resulted when these eluates were treated with 2 : 4-dichlorobenzene diazonium chloride was photometrically compared with the colour given with weighed amounts of the crystalline vitamin.

The amounts of crystalline vitamin found equivalent to 10 mg. of absorbate in 5 experiments using sodium carbonate for the elution were 2.6 µg., 2.6 µg., 2.2 µg., 2.7 µg., and 3.0 µg. respectively. The corresponding values using pyridine for the elution in 2 experiments were 3.0 µg. and 2.0 µg. respectively.

The average amount of crystalline vitamin found equivalent to 10 mg. of the adsorbate standard by this colorimetric method was 2.6 µg.

[1] *Biochem. Jl.*, 1934, **28**, 667.

[2] *Enzymologia*, 1938, **2**, 316.

[3] See LOHMANN and SCHUSTER : *Biochem. Z.*, 1937, **294**, 188.

APPENDIX I

THE ACCURACY OF THE RESULTS OBTAINED IN THE COMPARISON BETWEEN THE ORIGINAL INTERNATIONAL STANDARD OF VITAMIN B_1 (STANDARD ADSORPTION PRODUCT) AND THE NEW STANDARD (VITAMIN B_1 HYDROCHLORIDE) [1]

Report of K. H. Coward and E. A. G. Shrimpton.

A short account only of the methods used in calculating the results and the accuracy obtained in these comparisons is given here. A fuller account will be published later elsewhere.

(*a*) Calculation of the Weight of Crystalline B_1 equivalent to 10 mg. of the Adsorbate Standard (1 I.U.) of Vitamin B_1

For each test, two curves relating response to the logarithm (to the base 10) of the dose given were constructed for adsorbate standard and crystalline B_1 respectively, whenever possible. When the curve of the response was not a linear function of the log. of the dose, a suitable transformation of the response was used. For the rat convulsions method, the log. of the response was a linear function of the log. dose.

The average slope of the two lines, relating response (or its transformed value) y to x (the log. of the dose) was determined. The mean responses $\overline{y}_1$ and $\overline{y}_2$ for animals given the standard and test preparations respectively, and the mean log. doses $\overline{x}_1$, $\overline{x}_2$ given to these animals were calculated, weighted in each case by the number of animals used on each dose. Two parallel lines were drawn passing through the points $\overline{x}_1$, $\overline{y}_1$ and $\overline{x}_2$, $\overline{y}_2$ respectively, having the average slope b. The horizontal distance between these two lines is the log.

[1] See also Report of the Sub-Committee on the Accuracy of Biological Assays, British Pharmacopœia Commission. General Medical Council, 1936.

of the ratio of the potencies of the crystalline B_1 and the adsorption standard.

From this ratio, the weight of crystalline B_1 (expressed in μg, or micrograms) equivalent to 10 mg. (1 I.U.) of the adsorbate standard of vitamin B_1 was calculated from the data supplied by individual workers. This resulting equivalent is given for each worker separately, grouped according to the method of assay in Tables XXX to XXXIII.

(*b*) Calculation of the Accuracy of Each Result

If M is the log. of the equivalent weight, and σ_M^2 the error variance of M, then

$$\sigma_M^2 = \frac{\sigma^2}{b^2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right) + \frac{\sigma_b^2}{b^4}(\bar{y}_1 - \bar{y}_2)^2$$

In this expression, σ^2 is the variance of response of individual animals given the same dose and n_1 and n_2 are the total numbers of animals used for testing the old and new standards respectively. The second half of the expression involves σ_b^2, the error variance of the slope. If the slope were known exactly or the mean responses $\bar{y}_1$ and $\bar{y}_2$ were equal, this factor would vanish.

The percentage limits of error of the results, the equivalent weights, are given by

100 antilog. + 2.576 σ_M and 100 antilog. − 2.576 σ_M for the upper and lower limits corresponding to odds of 1 in 100

and

100 antilog. + 1.96 σ_M and 100 antilog. − 1.96 σ_M for the upper and lower limits corresponding to odds of 1 in 20.

Suppose the limits of error of the result corresponding to odds of 1 in 100 are 67-150%, then the observed result, the equivalent weight, will, in the long run of similar experience, 99 times in every 100 lie within 67-150% of the true value. The limits of error corresponding to P = 0.99 and P = 0.95—namely, to odds of 1 in 100 and 1 in 20 respectively—aregiven in Tables XXX to XXXIII for each calculated equivalent.

(*c*) The Combination of the Results

The separate equivalents may be combined by weighting the log. of the equivalent inversely to its accuracy. Thus if

M = log. equivalent weight
σ_M^2 = error variance of M
$W = \frac{1}{\sigma_M^2}$

then $\overline{M}_W$, the weighted mean of M, will be $\frac{\Sigma(WM)}{\Sigma(W)}$ the summation extending over all the results combined, and the mean equivalent will be the antilog. of this.

The error of $\overline{M}_W$ is given by $\frac{\Sigma[W(M-\overline{M}_W)^2]}{(r-1)\Sigma(W)}$, where r is the number of results combined, and is the overall estimate of error including any significant differences between individual results, if such exist. The mean equivalent weight and the corresponding limits of error are given in Table XXXIV for each method of assay separately and for all four methods combined.

(*d*) Estimate of Equivalent Weight from all the Material examined

Table XXXIV shows that the mean value of the weight of crystalline B_1 equivalent to 1 I.U. of adsorption standard (10 mg.) is 3.056 μg. In the long run of similar experience, this result lies, 99 times in every 100, within 93-108% of the true result. It may be noted that the value 3, which has been adopted for the new unit of activity, lies well within the limits of accuracy of this estimate.

The limits of error in Table XXXIV were, of course, determined partly by the numbers of animals used in the tests and, since these were different in the different tests, these limits of error are not an indication of the comparative accuracy obtainable by the different methods. Further calculations were therefore made assuming that each worker had used only 10 animals for the adsorption standard and 10 for the crystalline B_1. (Table XXXV.) The limits of error of each method are thereby made comparable, but, as every worker realises, the limits of error obtained in a test are themselves subject to variation, and a single experiment is not necessarily a reliable

indication of the average variation obtainable by a particular method. It is hoped to publish an analysis of these results and a comparison of the accuracy obtainable in the different methods in a later paper.

Table XXX.[1]

INCREASE IN WEIGHT OF RATS

Observer	Length of test, weeks	Result: Weight of $B_1 \equiv 1$ I.U. adsorbate standard	Number of rats: Adsorbate standard	Number of rats: B_1	Limits of error: P=0.99 %	Limits of error: P=0.95 %
Ohdake	5	1.77	11	16	77–130	81–123
Fridericia	5	2.50	5	11	76–132	81–124
I. G. Farbenind.	5	3.36	25	26	82–121	86–116
Munsell	5	3.69	17	50	85–117	88–113
Elvehjem	5	2.69	3	9	66–151	73–137
Macrae	4	2.96	9	22	86–116	89–112
von Euler	4	3.16	15	20	95–106	96–104
Harris	3	3.20	18	18	83–121	86–116
Coward	3	3.90	14	12	77–129	82–122
Harris	2	3.51	18	18	81–123	85–118

[1] In this table and in Tables XXXI, XXXIII and XXXV, only the name of one investigator from each laboratory is given. The names of the other collaborators are given in the individual reports.

Weight of crystalline B_1 equivalent to 10 mg. of adsorption standard (1 I.U.) is 3.15 μg.

Table XXXI.

CURE OF CONVULSIONS IN RATS

Observer	Result: Weight of $B_1 \equiv 1$ I.U. adsorbate standard	Number of rats: Adsorbate standard	Number of rats: B_1	Limits of error: P = 0.99 %	Limits of error: P = 0.95 %
Merck (Darmstadt)	1,88	15	15	70–144	76–132
Nelson	2,84	19	38	76–132	81–124
Williams and Sampson	2,83	50	62	85–117	88–113
Harris	2,72	25	24	76–131	81–124
Guerrant	2,48	27	92	84–119	87–114
Mattei	3,40	12	12	86–117	89–113

Weight of crystalline B_1 equivalent to 10 mg. of the adsorbate standard (1 I.U.) is 2.82 μg.

Table XXXII.

BRADYCARDIA IN RATS

Observer	Result	Number of rats		Limits of error	
	Weight of $B_1 \equiv 1$ I.U. adsorbate standard	Adsorbate standard	B_1	P = 0.99 %	P = 0.95 %
Williams and Sampson ..	1.58	12	29	4–2,383	9–1,173
Harris	2.44	47	46	78–128	83–121
Hoffmann-La Roche	2.75	131	63	79–127	83–120
I. G. Farbenind. *(a)*	3.93	65	44	51–198	59–170
(b)	3.60	65	55	51–196	59–168

Weight of crystalline vitamin B_1 equivalent to 10 mg. of the adsorbate standard (1 I.U.) is 2.70 μg.

Table XXXIII.

PERCENTAGE CURE OF RETRACTED NECK IN PIGEONS

Observer	Result	Number of pigeons		Limits of error	
	Weight of $B_1 \equiv 1$ I.U. adsorbate standard	Adsorbate standard	B_1	P = 0.99 %	P = 0.95 %
Ohdake	3.39	8	18	10–1,007	17–601
Coward	3.33	15	30	14–706	22–456

Weight of crystalline vitamin B_1 equivalent to 10 mg. of the adsorbate standard (1 I.U.) is 3.36 μg.

Table XXXIV.

RESULTS ACCORDING TO METHOD OF ASSAY

Method	Number of results	Mean result	Limits of error	
		Weight in μg. of $B_1 \equiv 1$ I.U. adsorbate standard	P = 0.99 %	P = 0.95 %
Increase in weight of rats	10	3.151	90–111	92–108
Cure of convulsions in rats	6	2.824	84–119	88–114
Bradycardia	5	2.700	84–119	88–114
Cure of retracted neck in pigeons	2	3.355	18–825	20–498
All methods combined ...	23	3.056	93–108	94–106

Table XXXV.

THE ACCURACY OF THESE METHODS CALCULATED FOR AN EXPERIMENT IN WHICH 10 ANIMALS WERE GIVEN THE ADSORBATE STANDARD AND 10 ANIMALS THE CRYSTALLINE B_1, FOR COMPARISON WITH FIGURES ALREADY PUBLISHED (see footnote, page 417)

Method	Observer	Length of test, weeks	Limits of error	
			P = 0.99 %	P = 0.95 %
Increase in weight of rats	Ohdake	5	74–135	79–126
	Fridericia	5	80–126	84–120
	I. G. Farbenindustrie	5	74–135	79–126
	Munsell	5	79–127	83–121
	Elvehjem	5	71–140	77–130
	Macrae	4	85–118	88–114
	von Euler	4	93–107	95–106
	Harris	3	78–129	82–122
	Coward	3	75–133	80–125
	Harris	2	76–132	81–124
Cure of convulsions in rats	Merck		65–155	71–141
	Nelson		65–153	72–140
	Williams and Sampson		71–140	77–130
	Harris		66–151	73–138
	Guerrant		70–142	76–132
	Mattei		84–118	88–114
Bradycardia	Williams and Sampson		3–3,149	7–1,456
	Harris		59–169	67–150
	Hoffmann-La Roche		50–200	58–171
	I. G. Farbenindustrie (*a*)		24–423	33–306
	(*b*)		22–454	31–324
Cure of retracted neck in pigeon	Ohdake		10–1,018	17–606
	Coward		12–848	19–526

APPENDIX II

ALTERATIONS AND ADDITIONS TO THE MEMORANDUM ON THE NEW STANDARD (VITAMIN B_1 HYDROCHLORIDE) ISSUED WITH THE STANDARD

In the memorandum [1] which has been issued with the present international vitamin B_1 standard, it is stated that the standard contains approximately 1 molecule of water of crystallisation. Shortly after publication of this memorandum, BASTEDO, TRENNER and WEBB [2] carried out a comprehensive study of the hydration of vitamin B_1 hydrochloride, and from their work it was apparent that the standard could not have retained 1 molecule of water of crystallisation during the drying procedure to which it was subjected, and indeed must be anhydrous vitamin B_1 hydrochloride. This has been verified by analysis of the standard in the laboratories of Roche Products Ltd., and of the Lister Institute. Alterations to the memorandum are accordingly necessary, in order to describe the standard preparation correctly and fully. The absorption spectrum of the standard preparation has recently been investigated and added as an additional criterion of purity ; the relevant data are set forth at the end of this appendix.

The following changes are therefore made in the memorandum :

Page 884. — Description of the Standard.

The paragraph beginning : " The preparation contains . . . " has been deleted and the following substituted : " The preparation contains no water of crystallisation and is stable when protected from access of moisture ".

Page 886. — Suggestions regarding the Use of the Standard Preparation.

3. Precautions to be taken in the preparation of solutions :

The first two sentences : " The pure vitamin B_1 hydrochloride. . .if exposed to ordinary moist air " have been deleted and the following sentence substituted : " The pure vitamin B_1 hydrochloride gains weight if exposed to ordinary moist air ".

[1] *Bull. Health Org.*, 1938, 7, 882-886.

[2] *J. Amer. Chem. Soc.* 1938, 60, 2303.

Criteria of Purity (page 884).

Immediately after the description of the picrolonate, the following has been inserted :

Absorption Spectrum.[1] — Measurements were made on a solution of the standard in aqueous alcohol (approximately 50%) of density 0.9351 at 20°C. which contained N/100 HCl. The solution employed was made up by weight and contained 0.00735 g. of standard per 100 g. solution. The absorption measurements were made with a Hilger E 316 spectrograph and Spekker photometer with alternatively spark between tungsten steel electrodes and hydrogen tube as light sources. The plates were matched with a photo-electric split beam micro-photometer. Observations were made to test whether the vitamin was stable under the conditions of irradiation likely to be used in absorption measurements. No change of the absorption was detected after ¼-hour exposure to the light of a 15.000 volt condensed spark. Concentrations have been converted to a volume basis and the results are expressed as Extinction Coefficients (K) for each wave-length recorded. K is defined as equal to $\frac{1}{cd}$ log. $\frac{Io}{I}$, where c is the concentration of the vitamin in g. per litre and d is the length of the absorbing layer in cm. Io is the intensity of the incident light at the stated wave-lengths and I that of the transmitted light at the same wave-length.

The values of K given in the following table are the means of seven measurements and have a standard error of 0.282.

Extinction Coefficients of the Standard in Aqueous Alcohol (Density 0.9351 at 20°C.) containing $\frac{N}{100}$ HCl.

λ	K	λ	K	λ	K
2300 Å	23.65	2525 Å	39.56	2750 Å	20.69
2325	27.03	2550	38.57	2775	17.46
2350	28.15	2575	37.45	2800	13.52
2375	35.47	2600	36.04	2825	9.85
2400	39.41	2625	34.91	2850	6.62
2425	42.09	2650	33.22	2875	4.65
2450	43.36	2675	30.97	2900	3.52
2475	42.52	2700	27.87	2925	2.96
2500	40.83	2725	24.36		

[1] Determined by E. R. Holiday, London.

THE RELATIVE ANTIRACHITIC POTENCIES OF VITAMIN D_2 (CALCIFEROL FROM IRRADIATED ERGOSTEROL) AND OF VITAMIN D_3 (FROM IRRADIATED 7-DEHYDROCHOLESTEROL)

A Summary of Experiments organised for the Accessory Food Factors Committee (Lister Institute and Medical Research Council)

Edited by K. H. COWARD
(Pharmaceutical Society, London),
Secretary of the Vitamin D Sub-Committee.

Prior to September 1939, it was intended to hold the third meeting of the International Vitamin Standardisation Conference before the end of the year. This plan had to be abandoned, owing to the outbreak of war, but much experimental work was organised in Great Britain in preparation for the Conference, and there seems no reason to delay the publication of such results as are already complete.

One item of the agenda for the proposed Conference would have been a discussion of the desirability of retaining vitamin D_2 from irradiated ergosterol as international standard for vitamin D, or of substituting for it vitamin D_3 from irradiated 7-dehydrocholesterol. At the Conference of 1934, much time was spent in considering the difficult and anomalous position created by the discovery that the relative antirachitic potency of vitamin D as cod liver oil and as irradiated ergosterol was not the same for rats and fowls. At that time, no completely satisfactory solution of the difficulty could be reached, owing to the insufficiency of the existing knowledge. Since that time, much progress has been made, and the situation is certainly ripe to be reviewed again. In preparation, therefore, for such a review, the Accessory Food Factors Committee desired that British

workers should make certain relevant experiments. They were fortunate in having the necessary material placed at their disposal through two generous gifts to Sir Henry DALE, one of vitamin D_3 from the I. G. Farbenindustrie A.G., Wuppertal-Elberfeld, through Professor HÖRLEIN, and one of vitamin D_2 from the Glaxo Laboratories.

It has long been known that cod liver oil and irradiated ergosterol have not the same relative vitamin D potency for rats and fowls. A standardised solution of irradiated ergosterol was adopted as international standard for vitamin D at the first International Conference in 1931.[1] At the second Conference in 1934,[2] the above discrepancy was fully realised, but no change could be made, although it was pointed out that, if comparative tests were made against the international standard to determine vitamin D potency with any species other than the rat, the result could not safely be expressed in international units. Obviously, it would be more satisfactory to have as standard some material possessing maximum antirachitic activity for all species, if such could be found and was not subject to other drawbacks of any kind.

In 1935, vitamin D_3 was obtained by the irradiation of 7-dehydrocholesterol (WINDAUS, LETTRÉ and SCHENCK, 1935) and was shown to be identical with the vitamin D of tunny liver oil (BROCKMANN, 1936 ; BROCKMANN and BUSSE, 1937, 1938) and halibut liver oil (BROCKMANN, 1937). Il was also reported to have the same antirachitic potency for rats — 40,000 I.U. per mg. — as calciferol (SCHENCK, 1937). Vitamin D_3 could thus be obtained as a stable crystalline substance with well-defined physical constants and fulfilled the requirement of having maximum antirachitic activity for both rats and fowls.

Should it be thought desirable to change the international standard for vitamin D from vitamin D_2 to vitamin D_3, it would be necessary to ascertain the exact value of crystalline vitamin D_3 in international units per mg. As stated above, this value has already been reported as 40,000 I.U. per mg., but, before this value could be

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[1] See Report of the Conference on Vitamin Standards, London, 1931 (document C.H.1055(1)).

[2] See Report of the Second Conference on Vitamin Standards, *Bull. Health Org.*, 1934, **3**, 428.

adopted officially, it would be necessary to have confirmation from as many independent laboratories as possible. For that reason, the Vitamin D Sub-Committee organised the experiments which are the subject of this communication and obtained the collaboration of workers in nine separate laboratories in Great Britain. The tests on rats were carried out by the following experts :

A. L. Bacharach, Glaxo Laboratories Ltd., Greenford, Middlesex ;
R. G. Booth, British Cod Liver Oil Producers, Hull ;
W. A. Broom, Boots Pure Drug Co., Nottingham ;
K. H. Coward and E. W. Kassner, Pharmaceutical Society, London ;
S. K. Kon and K. M. Henry, National Institute for Research in Dairying, Shinfield, Reading ;
S. W. F. Underhill, The British Drug Houses Ltd., London ;
T. A. Webster, National Institute for Medical Research, London ;
H. Wilkinson, Lever Bros. and Unilever Ltd., Port Sunlight, Cheshire ;
S. S. Zilva, A. E. Kellie and G. A. Snow, Lister Institute, London.

The other problem connected with a possible change of international standard from vitamin D_2 to vitamin D_3, which it was thought ought to be studied, was that of the relative antirachitic potency of the two vitamins for the human species. The difference in potency for the rat and the fowl is great and easily demonstrated, but for the rat and the human being it is small or non-existent. A very large number of attempts, far too many to summarise here, have been made to ascertain whether the two vitamins from various sources have the same relative potency to heal rickets in infants as they have to heal rickets in rats, but none of them has succeeded in giving an absolutely definite answer. To provide convincing proof, it is necessary to ascertain the minimum dose of each vitamin which will produced the same response in the same conditions, but, obviously, the variability of the human material available makes the establishment of identical conditions impossible. The error in the result is therefore bound to be very great and may well exceed the 100% or 200% which represents the maximum difference in relative potency which it is thought may exist between these two forms of the vitamin.

With a generous supply of the two vitamins available for experiment, it seemed worth while, in spite of the difficulties outlined above, to make a further attempt to compare their action on human subjects. Clinical trials were therefore inaugurated to test the

relative action of vitamins D_2 and D_3 on children with rickets, on adult and adolescent human beings with osteomalacia and late rickets, and on a case with parathyroid tetany. The tests on infants with rickets were made in Glasgow by MORRIS and STEPHENSON (1939). The tests on cases of osteomalacia and late rickets were made by D. C. WILSON (1940) in the Kangra Valley in India, from which region she has already reported studies of this disease (1930-1931); the test with a case of parathyroid tetany was made at University College Hospital, London, by H. P. HIMSWORTH and M. MAIZELS (1940). These three clinical studies agree in their failure to detect any difference in the relative potency of the two vitamins. Owing to the difficulties inherent in the study of human material, the workers were unable to fulfil completely the requirements stated above as indispensable for securing a definite answer, but Dr. WILSON was successful in administering a dose of both vitamins which produced improvement so slow that the patients refused to continue treatment unless the dose could be increased. When this dose was doubled, healing with both vitamins was accelerated. The principle of establishing the minimum dose may therefore in this case be said to have been, at least to some extent, fulfilled, and yet no difference in potency of the two vitamins was detected. From these clinical researches, it would appear that the probability is still further heightened that the relative potency of vitamins D_2 and D_3 is the same for human beings as for rats.

Experimental.

Chemical and Physical Constants of the Preparations.

Vitamin D_2, calciferol : Data supplied by A. L. BACHARACH, Glaxo Laboratories Ltd. :

Batch 239 A. Lab. Ref. AR8/515.

Appearance : white ; small crystals.
Melting-point : 116° to 118° C.

$\left[\alpha\right]_{546.1}^{20}$ (4 % w/v solution in ethanol) = + 124.4°.

$E_{1\ cm.}^{1\%}$ 265 mμ (0.04 % w/v solution in ethanol) = 465.

Vitamin D_3 : Data supplied by R. K. Callow, National Institute for Medical Research, London.

Melting-point : 78° to 82°C. (Block, short stem, evacuated capillary).

$$\left[\alpha\right]^{25}_{546.1} = +125°.$$

$$\left[\alpha\right]^{25}_{D} = +105° \text{ (C = 1\% in ethanol).}$$

$$E^{1\%}_{1\text{ cm.}} \quad 266\ m\mu \text{ (0.0025\% in alcohol)} = 424.$$

$$t^{max.} = 1.06.$$

Biological Activity. — The biological activity of the specimen of vitamin D_2 had previously been determined (in a test for which the limits of error were 79.5% to 125.5% for P = 0.99 and 84% to 119% for P = 0.95), and was found to be 41.4×10^6 I.U. per g.

The Biological Comparison carried out on Rats.

The solutions of vitamin D_2 and D_3 were prepared by T. A. Webster (National Institute for Medical Research, London) and were made of the same strength — viz., 0.0025% in olive oil w/v. Assuming that the crystalline vitamin D_2 contained 40,000,000 I.U. vitamin D potency per g., a solution of the above strength would contain 1 I.U. per mg. About 5 g. of each solution and 50 g. of the olive oil used for their preparation were sent to each of the nine participants in the test. Workers were asked to make their comparisons by the technique in general use in their laboratories, so that the methods used might vary. The relevant details of the methods employed, together with the results obtained, are summarised in Table I on the following page.

Table I.

Observer	Method	Diet No.	Ca : P ratio of diet	Dose μg.		Total number of rats used	Ratio of potency Vitamin D_3 : Vitamin D_2
				Vitamin D_2	Vitamin D_3		
Bacharach	Line test (curative)	1	about 8 : 1	Daily (*a*) 0.03 0.05 (*b*) 0.01125 0.01875	Daily (*a*) 0.03 0.05 (*b*) 0.01125 0.01875	(*a*) 72 (*b*) 147	(*a*) 0.954* (*b*) 0.985
Booth.....	Line test with camera lucida drawings for measurement (curative)	2	4 : 1 up to 4.25 : 1	Daily (*a*) 0.0125 0.025 Single dose (*b*) 0.125 0.25	Daily (*a*) 0.0125 0.025 Single dose (*b*) 0.125 0.25	(*a*) 32 (*b*) 32	(*a*) 1.132 (*b*) 1.350
Broom	Combination of X-ray and line test (curative)	3	4.5 : 1	Daily 0.0125 0.0375	Daily 0.0125 0.0375	133	0.953
Coward and Kassner	Line test (curative)	4	4 : 1	Single dose 0.25	Single dose 0.25	40	0.931
Coward and Kassner	Ash content (prophylactic)	4	4 : 1	Daily 0.000625 0.00125 0.0025	Daily 0.000625 0.00125 0.0025	36	1.223
Kon and Henry ...	Ash content (prophylactic)	5	4 : 1	Daily 0.0025 0.005	Daily 0.0025 0.005	60	1.003
Underhill	Line test (curative)	4	4 : 1	Daily 0.02	Daily 0.015 0.02 0.03 0.04 0.05	75	Slightly less than 1.0*
Webster	X-ray (curative)	6	8.8 : 1	Daily 0.025 0.05 0.1	Daily 0.025 0.05 0.1	192	0.932
Wilkinson	Line test with camera lucida drawings for measurement (curative)	7	4 : 1	Daily 0.00625 0.0125	Daily 0.00625 0.0125	18	1.025
Zilva, Kellie ..... and Snow	Ash content A/R ratio (prophylactic)	8	4 : 1	Daily 0.00025 0.00125 0.0025	Daily 0.00025 0.00125 0.0025	60	1.0 approx.*

Weighted mean, omitting the three results marked* : 1.000.

Rachitogenic Diets used.

	%
1. White flour	85
Egg albumin	10
Calcium lactate	2.8
Sodium chloride	2
Ferric citrate	0.2
with 2-3 g. fresh spinach leaves per animal per day.	
2. Ground yellow maize	72
Wheat gluten	18.5
Whale meat meal	5
Calcium carbonate	3.5
Sodium chloride	1
3. White flour	36
Flaked yellow maize	35
Wheat gluten	20
Meat meal (fat extracted)	5
Salt mixture	4

	%
Calcium carbonate	75.0
Sodium chloride	15.0
Magnesium sulphate	7.5
Ferric citrate	2.4
Potassium iodide	0.06

4. Ground yellow maize	76
Wheat gluten	20
Calcium carbonate	3
Sodium chloride	1
5. Ground yellow maize	76
Wheat gluten	20
Calcium carbonate	3.35
Sodium chloride	1
6. White flour	48
Ground yellow maize	27
Wheat gluten	20
Calcium carbonate	4
Sodium chloride	1
7. Yellow maize (ground flaked)	84
Meat meal (extracted)	12
Salt mixture	4

	%
Calcium carbonate	75.0
Sodium chloride	15.0
Magnesium sulphate	7.5
Ferric citrate	2.4
Potassium iodide	0.1

8. Wheat	33
Maize	33
Wheat gluten	15
Gelatin	15
Calcium carbonate	3
Sodium chloride	1

Method of calculating the Results.

When, as in the present case, the curve relating the biological response to the dose is logarithmic, the potency of the test substance is calculated in terms of another substance in the following way by:

(*a*) Calculating the best straight lines (curves of response) between the points determined experimentally, y being the average response for the group and x the $\log._{10}$ of the dose given. If there are more than 2 points for a curve, it is necessary to weight each observation according to the number of animals in the group;

(*b*) Finding the average slope of the two curves of response, and weighting, if necessary, for the number of animals from which each curve is calculated;

(*c*) Finding the mean dose and mean response in each curve and weighting, if necessary, for the number of animals in each group;

(*d*) Finding the equations of the lines with average slope passing through the middle point of each curve of response;

(*e*) Finding the horizontal distance between these parallel lines.

This is the log. to the base 10 of the ratio of the potencies of the two substances.

The following calculation of one of the results in the present series shows how the procedure outlined above may be simplified (*a*) when all groups contain the same number of comparable animals and (*b*) when the middle points of the two curves of response have the same abscissa.

	Numerical expression for average degree of healing	Dose μg.		Log. dose	Number of rats used
Vitamin D_2	2.167	0.03		$\bar{2}$.477	18
	2.583	0.05		$\bar{2}$.699	18
Diff. =	0.416		Diff. =	0.222	
Vitamin D_3	2.083	0.03		$\bar{2}$.477	18
	2.583	0.05		$\bar{2}$.699	18
Diff. =	0.500		Diff. =	0.222	

The average slope of the two curves of response is :

$$\frac{0.416 + 0.500}{2 \times 0.222} = 2.063$$

The co-ordinates of the "mid points" of the curve for vitamin D_2 are $\bar{x} = 2.588$, $\bar{y} = 2.375$

The co-ordinates of the "mid points" of the curve for vitamin D_3 are $\bar{x} = 2.588$, $\bar{y} = 2.333$

$$\text{Since } \frac{y}{x} = 2.063, \quad \frac{-2.333\ 2.375}{x} = 2.063.$$

$$\therefore \quad x = \frac{-\ 0.042}{2.063} = -\ 0.02036 = \bar{1}.97964$$

$$= \text{log. } 0.95419.$$

Therefore the ratio vitamin D_3 : vitamin D_2 = 0.95419
= 0.954 (say).

Most of the participants sent in reports of their comparisons in a form from which the value of vitamin D_3 in relation to vitamin D_2 could be calculated mathematically. One worker, however, had not assigned any numerical value to the healing of each rat. He had divided each litter of rats into two groups ; to one group he had given a particular dose of vitamin D_3 and to the other group a dose of 0.02 g. vitamin D_2. Direct comparison was made between the healing of the rats given the dose of vitamin D_3 and that of the rats given vitamin D_2. His result is summarised in Table II.

Table II.

Litter No.	Number of rats	Dose of vitamin D_3 µg.	Result : Healing >, < or = that produced by the dose of 0.2 µg. vitamin D_2	Ratio of response of vitamin D_3 : D_2
1	7	0.015	slightly <	< 1.333
2	7	0.015	<	
3	5	0.02	>	slightly < 1.0
4	6	0.02	=	
5	6	0.02	<	
6	9	0.02	slightly <	
7	4	0.03	<	slightly > 0.667
8	5	0.03	slightly >	
9	6	0.03	slightly >	
10	5	0.03	>	
11	7	0.04	>	> 0.5
12	8	0.04 & 0.05	both doses >	> 0.4

Conclusion : The ratio of the potency of vitamin D_3 : vitamin D_2 is slightly less than 1.

Another worker had compared three graded doses of vitamin D_3 with three graded doses of vitamin D_2. The averages of the A/R ratio (calculated from the values for the ash content of the fat extracted bones to the organic matter in the bones) were, for the lowest doses of vitamines D_3 and D_2, 0.528 and 0.546 respectively, values not significantly different, while the corresponding values for the two highest doses were 0.628 and 0.629, the same result. From these two doses, he concluded that vitamin D_3 had the same potency as vitamin D_2. The average values for the middle doses were, however, 0.509 for vitamin D_3 and 0.567 for vitamin D_2 ; the middle dose for vitamin D_3 thus gave a lower result than the lowest dose. This difference was, however, found to be statistically insignificant, though the worker concerned declared that, since his three doses of vitamin D_3 had not given a graded series of responses, he would have preferred to repeat the whole experiment in order to satisfy himself. In view of the results obtained by other workers, however, this was considered unnecessary.

A third worker carried out his tests in two separate stages ; in each, he compared two different weights of vitamin D_3 with the same

two weights of vitamin D_2. In the first stage, however, the doses selected were rather high and fell in the region of the dosage-response curve where the relationship between response and dose is no longer logarithmic. He has therefore suggested that the results of that test should be omitted in calculating the value of the weighted mean from all the tests.

It is concluded that, for the rat, vitamin D_2 and vitamin D_3 have equal antirachitic potency. For the specimen of vitamin D_2 used, the antirachitic potency had previously been ascertained as 41.4×10^6 I.U. per g. The result is therefore in agreement with that of SCHENCK *(1937), who reported the potency of crystalline vitamin D_3 to be 40,000 I.U. per mg.*

SUMMARY

Separate solutions of vitamins D_2 and D_3 in olive oil were prepared, each being 0.0025% by weight.

These were distributed to workers in nine different laboratories, together with additional olive oil of the same batch for dilution. Each worker determined the relative antirachitic potency of the two substances by experiments on rats according to the method of comparison generally used in his laboratory.

Of the methods used two were curative, including the X-ray method and the " line " test, two workers using Morgan's method of measuring healing, and one was prophylactic by measuring the ash content of the bones, one worker using the A/R ratio for expressing his results.

In ten experiments, from which the relative values for vitamins D_2 and D_3 could be calculated mathematically, the value for the ratio of the potency for rats of vitamin D_3 to that of vitamin D_2 varied from 0.9313 to 1.350. The mean of the values weighted according to the number of animals used in each estimation was 0.995. Two other experiments whose results were calculated in a less orthodox manner gave the value for the ratio as approximately 1.0. From inspection of the range of the twelve values obtained it is safe to conclude that *vitamin D_2 and vitamin D_3 have for the rat the same antirachitic potency i.e., 40,000 expressed in I.U. per g.*

* * *

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INTERNATIONAL STANDARD FOR VITAMIN E

Introduction

Plans had been made for the holding of a third International Conference on the Standardisation of Vitamins in the autumn of 1939, but the outbreak of war prevented their realisation. Among the subjects on which experimental work had been organised, with a view to action by the proposed Conference in the light of the results obtained, was the possibility of adopting an International Standard for Vitamin E and the definition of an International Unit in terms of it. The investigation of this possibility, organised by the Vitamin E Sub-Committee of the (British) Accessory Food Factors Committee (Lister Institute and Medical Research Council), had been directed to the examination of the suitability, as a Standard for Vitamin E, of the synthetic, racemic α–tocopheryl acetate, a supply of which had been generously offered for this purpose by the Hoffmann-La Roche Company of Basle, through Dr. Guggenheim. The investigation had been carried out by the co-operative activity of fourteen laboratories in Europe and America, and the results had been assembled in readiness for an international decision. A brief report on these results, drawn up by the aforesaid Sub-Committee, is printed below, and it is intended that a much fuller account of the investigation and analysis of its results shall be published later.

The results of these co-operative trials, in any case, proved to be so uniformly favourable to synthetic, racemic α–tocopheryl acetate, as a suitable substance to constitute the projected International Standard for Vitamin E that the Committee has found itself prepared to make a clear recommendation for its adoption. There is now no near prospect of the meeting, at any date which can be foreseen, of a truly international conference to which such a recommendation would normally be presented. Even consultation by correspondence, with the experts of other countries who would normally be concerned with such a decision, has become increasingly

difficult. If the uniformly favourable results of the international trials, and the arrangements made provisionally for the issue of the proposed Standard when adopted, had to wait for the restoration of normal facilities for international meeting or consultation, they would remain ineffective for an indefinite period.

In these circumstances, it was decided to bring the Committee's report and recommendations to the notice of those members and officers available in Britain of the League of Nations Permanent Commission on Biological Standardisation and of the International Conference on Vitamin Standardisation. Sir Edward Mellanby, Chairman, Dr. J. C. Drummond and Dr. K. H. Coward, members, Dr. H. Chick and Miss E. M. Hume, secretaries of the International Vitamin Standardisation Conference, in consultation with Sir Henry Dale, a member of the Permanent Commission, consented to accept the responsibility of taking such decisions, in the present emergency, as would normally be taken by a properly constituted international conference and by a subsequent meeting of the Permanent Commission. They have accordingly adopted the proposed Standard for Vitamin E, accepted the recommendation defining the International Unit in terms of it, and authorised the National Institute for Medical Research, Hampstead, acting on behalf of the League of Nations Health Organisation, to proceed with the distribution of the Standard. This emergency action has been taken with the knowledge and approval of the League of Nations Health Organisation.

The Standard will thus be available to those who need it, and a uniformity in the notation of Vitamin E activity will be effected among those who use it. It is earnestly to be hoped that the day will not be far distant when a full and properly constituted international conference will have the opportunity of considering the effects of this action, and of endorsing or revising it in the light of experience.

INVESTIGATION OF RACEMIC TOCOPHERYL ACETATE AS INTERNATIONAL STANDARD FOR VITAMIN E

In preparation for the proposed third meeting of the International Conference on Vitamin Standardisation, the Vitamin E Sub-Committee of the Accessory Food Factors Committee, at the request of the Health Organisation, set on foot a co-operative study of *dl*-α-tocopheryl acetate as a possible International Standard

for Vitamin E. It has in the interval been decided that it would be more accurate to use the name " synthetic racemic tocopheryl acetate ", and this will be done in future.

A supply of the substance sufficient for extensive biological and stability tests, and to provide a standard should the substance ultimately be adopted, was very kindly provided by Messrs. Hoffmann-La Roche, of Basle, through the British associated company, Messrs. Roche Products, Ltd., Welwyn Garden City.

Workers in Europe and America experienced in vitamin E tests were invited to participate, and solutions were prepared by Dr. P. Hartley and issued to seventeen laboratories. The workers were asked to test four solutions of the tocopheryl acetate of graded strengths, the proportion in which the series was graded being stated, but no indication being given of the identities of the numbered solutions corresponding with the different strengths.

The object of the test was to obtain the relation between dosage and response, the response used being the fertility rate defined as the percentage of positively mated female rats which produced a litter. Vitamin E deficiency is a condition which in an individual animal is not cured in a smoothly graduated series of stages ; for statistical purposes, the response is treated as of an all-or-none, not of a graded, type. However, the dosage-response relation can, as is usual in such cases, be transferred into a linear one by plotting the normal equivalent deviation (or probit) of the percentage response against the logarithm of the dose.

Arrangements were made whereby the stability of the feeding solutions after the tests and of the original material after keeping was tested spectrophotometrically by Dr. R. A. Morton, who reported that the stability of all the materials was entirely satisfactory.

Thirteen of the seventeen laboratories invited completed the biological tests and sent in reports which were submitted to Dr. J. O. Irwin and Dr. E. J. Williams, then at Cambridge, for statistical analysis. In four of the laboratories, the slope of the dosage-response curve proved not to differ significantly from zero ; in other words, the responses to the graded doses were not themselves significantly graded. No determinations of the median fertility dose could therefore be made, and the results did not lend themselves to further statistical analysis. For the remaining nine laboratories, such a

study could be made and the results are summarised in the accompanying table.

Laboratory	Number of rats used	Slope of probit/log. dose line	Standard error of slope	Median fertility dose (mg.)	Limits of error	
					95%	99%
1	83	5.17	1.09	0.56	86–117	82–122
2 (*a*)	40	5.34	1.42	0.55	82–123	77–131
(*b*)	42	7.00	1.73	0.66	85–118	80–125
3	91	3.60	0.99	0.66	72–139	65–155
4	68	2.63	0.96	0.72	58–172	49–204
5	48	9.23	3.35	0.84	85–117	81–123
6 (*a*)	79	6.83	1.32	1.13	88–114	85–118
(*b*)	50	5.07	1.30	1.14	82–123	77–131
7	78	5.52	1.03	1.36	87–116	83–121
8 (*a*)	52	5.89	1.48	1.50	85–117	81–123
(*b*)	52	11.55	3.17	1.05	90–112	86–116
9	58	6.53	1.62	1.71	84–119	80–125
Means and errors		4.989	0.383	0.986	78–128 *	72–139 *
Total	689					

Notes. 2 (*a*) and (*b*). Ratio of 4 doses the same in each case, but bigger absolute dose given in 2 (*b*).

6 (*a*) and (*b*). Virgins used in 6 (*a*); rats which had resorbed in 6 (*b*).

8 (*a*) and (*b*). Criterion in 8 (*a*) birth of at least one living young one; criterion in 8 (*b*) birth of at least one young one, living or dead. Rats used in 8 (*a*) same as in 8 (*b*).

* This error includes error due to inter-laboratory difference.

The table shows the number of rats used by each worker, the slope of the probit/log. dose line, the median fertility dose and the limits of error for each worker's result. The median fertility dose is that dose which enables 50% of the rats used to bear a litter. The results have been arranged in the table to show the variation in size of the median fertility dose, from 0.56 mg. synthetic racemic tocopheryl acetate in the first laboratory, to 1.71 in the last laboratory, the average value being almost exactly 1 mg. The reasons for the variation will be discussed when a fuller report is made, but it is interesting to note that the size of the median fertility dose varied in laboratory 2 in two separate tests, and in laboratory 8 when the definition of a litter was varied so as to require the

inclusion of at least one living young one in the litter. These observations of the great variation in the size of the median fertility dose add further evidence, if that were needed, of the necessity for establishing an international standard for vitamin E so long as biological tests are needed.

The accuracy of the biological technique, as evidenced by the limits of error, seems to be about the same as that usually found with vitamins for a biological method which has been fully elaborated and in use for some time, and the whole co-operative study affords a satisfactory basis for recommending that synthetic racemic tocopheryl acetate should be adopted as International Standard for Vitamin E.

The workers who took part were :

A. L. Bacharach, Glaxo Laboratories Ltd., Greenford, Middlesex.
A. Z. Baker and M. D. Wright, Vitamins Ltd., Hammersmith, London.
F. Bergel, Roche Products Ltd., Welwyn Garden City, Herts.
A. M. Copping, Lister Institute, London.
K. H. Coward and B. G. E. Morgan, Pharmaceutical Society, London.
V. Demole and H. M. Wuest, F. Hoffmann-La Roche & Co., Basle.
H. von Euler, Biokemiska Institutet, Stockholm.
H. M. Evans, University of California, Berkeley, California.
P. Hartley, National Institute for Medical Research, Hampstead, London.
J. O. Irwin, Queens' College, Cambridge.
B. C. P. Jansen, University of Amsterdam, Laboratory of Physiological Chemistry, Amsterdam.
C. Kennedy and L. S. Palmer, University of Minnesota, Department of Agriculture, St. Paul, Minn.
K. E. Mason and W. L. Bryan, Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tenn.
H. A. Mattill, Department of Chemistry, State University of Iowa, Iowa City.
T. Moore, Dunn Nutritional Laboratory, Cambridge.
R. A. Morton, Department of Physical and Inorganic Chemistry, The University, Liverpool.
A. R. Todd, Department of Chemistry, The University, Manchester.
S. W. F. Underhill, The British Drug Houses Ltd., London.
E. J. Williams, Forest Products Research Laboratory, Melbourne.

E. M. Hume
(Secretary Vitamin E Sub-Committee),
Lister Institute, Division of Nutrition,
London, S.W.1.

DESCRIPTION OF THE VITAMIN E INTERNATIONAL STANDARD AND DEFINITION OF THE UNIT

(a) *International Standard.*

The adoption of synthetic racemic α–tocopheryl acetate as International Standard is recommended. The Standard Preparation shall conform to the requirements stated in Note 1 in regard to its chemical and physical constants.

(b) *Definition of Unit.*

The unit recommended for adoption is the Vitamin E activity of 1.0 mg. of the standard preparation.

This amount represents about the average value for the total median fertility dose which prevents resorption gestation in rats deprived of vitamin E, when the substance is administered orally; if it is administered parenterally its activity is less.

(c) *Mode of Preparation.*

It is recommended that the National Institute for Medical Research, London, *acting for the purpose as central laboratory on behalf of the Health Organisation of the League of Nations*, shall undertake the care, storage and distribution of the International Vitamin E Standard of which 100 g. have been given by Messrs. Hoffmann-La Roche, of Basle.

(d) *Mode of Distribution.*

It is recommended that the International Standard Preparation shall be issued in the form of a standard solution in oil, the strength of the solution being such that 1 gramme contains 10 International Units or 10 mg. synthetic racemic α–tocopheryl acetate (see Note 2).

Note 1.

Properties of synthetic racemic α-tocopheryl acetate (vitamin E) $C_{31}\ H_{52}\ O_3$.

(i) Appearance : a pale golden yellow, clear, viscous oil ; almost odourless.

(ii) Specific gravity : $d_4^{20^\circ}$ 0.9545–0.9665

(correction for 1° temperature variation, 0.0007).

(iii) Refractive index : $n_D^{20^\circ}$ 1.4958–1.4972

(correction for 1° temperature variation, 0.0004).

(iv) Absorption spectrum : in absolute alcohol shows a broad band λ max. 2855 Å

$$E\ \frac{1\%}{1\ \text{cm.}}\ 42.5\ \pm 1.$$

(v) Contains not more than 2% of free tocopherol and 96-100% (±2%) of tocopheryl acetate.

Note 2.

Nature of the oil to be used as diluent.

The oil used for the preparation of the standard solution is olive oil having the following properties :

(i) Specific gravity : 0.915–0.918 (15.5°/15.5°).

(ii) Refractive index (at 40°C.) : 1.4605–1.4635.

(iii) Acid value : not more than 2.0.

(iv) Saponification value : 188–195.

(v) Iodine value : 79–85.

(vi) Unsaponifiable matter : 0.8–1.3% (iodine value of unsaponifiable matter not less than 185).

(vii) (*a*) After passage of a current of air at 15–20°C. for three days, the oil, on addition of an ethereal solution of phloroglucinol with a few drops of concentrated hydrochloric acid, gives not more than a faint pink colouration (Kreis test for rancidity). The fresh oil should not give the faintest colouration in this test.

(*b*) The peroxide value (Lea test) of the fresh oil should not exceed 1 and, after passage of a current of air at 15–20°C. for three days, should not have risen to more than 10.

M. 44.

MEMORANDUM ON THE INTERNATIONAL STANDARD FOR VITAMIN E

from the Department of Biological Standards, National Institute for Medical Research, Hampstead, London, N.W.3.

The members of the International Vitamin Standardisation Conference available in Great Britain have recommended that synthetic racemic α–tocopheryl acetate shall be adopted as the International Standard for Vitamin E. It is further recommended that, as in the case of the other International Vitamin Standards, the National Institute for Medical Research, Hampstead, N.W.3, *acting for this purpose on behalf of the Health Organisation of the League of Nations*, shall undertake the care, storage, dispensing and distribution of the International Standard for Vitamin E.

Description of the Standard

Through the generosity of Messrs. Hoffmann-La Roche, Basle, 10 sealed ampoules of pure synthetic racemic α–tocopheryl acetate, each containing 10 g. of material, were placed at the disposal of the International Vitamin Standardisation Conference for investigation, particularly in regard to the suitability of the substance for adoption as the International Standard for Vitamin E.

The material was received at the National Institute for Medical Research, Hampstead, in February 1939, and, on arrival, it was placed in cold storage (–2°C. to –4°C.) where it has since been constantly maintained. Small quantities of the α–tocopheryl acetate have been supplied from time to time to the Vitamin E Sub-Committee of the Accessory Food Factors Committee for the purpose of chemical, physical and biological investigations. Eight sealed ampoules (80 g.) remain available for use and issue as the International Standard for vitamin E.

The International Standard preparation conforms to the following requirements, in regard to chemical and physical

constants, which the British members of the Conference have prescribed :

(i) Appearance : a pale, gold yellow, clear, viscous oil ; almost without odour.

(ii) Specific gravity d: $\overset{20^\circ}{4}$ 0.9545–0.9665
(correction for 1° temperature variation, 0.0007).

(iii) Refractive index : n $\overset{20^\circ}{D}$ 1.4958–1.4972
(correction for 1° temperature variation, 0.0004).

(iv) Absorption spectrum : in absolute alcohol shows a broad band λ max. 2855 Å

$$E \underset{1\ cm.}{1\%} \quad 42.5 \pm 1$$

(v) Contains not more than 2% of free tocopherol and 96–100% (±2%) of tocopheryl acetate.

1 mg. of α–tocopheryl acetate ($C_{31}\ H_{52}\ O_3$) is equivalent to 0.911 mg. of free α–tocopherol ($C_{29}\ H_{50}\ O_2$).

Definition of the Unit

The International Unit has been defined as the vitamin E activity of 1.0 mg. of the Standard Preparation.

This quantity represents the average amount which prevents resorption gestation in rats deprived of vitamin E when the substance is administered orally ; if it is administered parenterally, its activity is less.

Form of Issue of the Standard

The Standard Preparation is issued as a standard solution in olive oil, the strength of the solution being such that

1 gramme contains 10 International Units of Vitamin E
(or 10 mg. of synthetic racemic α–tocopheryl acetate).

In order to ensure the stability of the standard solution which will be issued for use in laboratories, the sample of olive oil used had been carefully selected and conforms, in its chemical and physical

properties, to the following requirements which have been prescribed by the British members of the Conference :

(i) Specific gravity : 0.915 to 0.918 (15.5°/15.5°).

(ii) Refractive index (at 40°C.) : 1.4605 to 1.4635.

(iii) Acid value : not more than 2.0.

(iv) Saponification value : 188 to 195.

(v) Iodine value : 79 to 85.

(vi) Unsaponifiable matter : 0.8 to 1.3% (iodine value of unsaponifiable matter not less than 185).

(vii) (*a*) After passage of a current of air at 15–20°C. for three days, the oil, on addition of an ethereal solution of phloroglucinol with a few drops of concentrated hydrochloric acid, gives not more than a faint pink colouration (Kreis test for rancidity). The fresh oil should not give the slightest colouration in this test.

(*b*) The peroxide value (Lea test) of the fresh oil should not exceed 1 and, after passage of a current of air at 15-20°C. for three days, should not have risen to more than 10.

(Department of Scientific and Industrial Research : Food Investigation Special Report N° 46, 1938 ; pages 98-102, 106-110).

The standard solution is issued in ground-glass-stoppered bottles of brown glass, closed with a viscose cap. Each bottle contains approximately 10 g. of the standard solution. It will be supplied, on application, to Directors of National Control Centres in those countries in which these have been established, for local distribution in their respective countries ; and, on application, directly from the National Institute for Medical Research, Hampstead, to laboratories, institutes and research workers in those countries in which National Control Centres have not yet been established.

While the investigations so far carried out indicate that the solution of tocopheryl acetate in olive oil is very stable, it is nevertheless recommended that, on receipt by national control centres and by individual laboratories, the standard should be placed in cold storage, at 0°C. or lower temperature, and should be maintained in cold storage when not in use in the laboratory.

Purpose of the Standard and Indications for its Use

The Standard Preparation is intended to be used for the assay of vitamin E activity. By means of comparative tests in parallel series of animals, a determination can be made of the amount of the preparation under test which is equivalent in activity to a known quantity of the standard, and the vitamin E activity of the preparation under test can then be expressed as the number of International Units present in a given weight. The Standard and the material under test should be administered orally, since vitamin E is not fully effective when given parenterally.

It may be stated that the median effective dose has been found to range from 0.5 to 1.5 mg. (0.5 to 1.5 International Units) in tests in which the prevention of resorption gestation in rats deprived of vitamin E is the criterion of activity.

It is recommended that not less than 10 rats should be used for each of the two doses of the material being tested and not less than 10 rats for each of the two doses of the Standard. These doses should not have a ratio to one another greater than 2 : 1. Ten rats should be used as negative controls.

PROGRESS REPORT ON PRODUCTION OF TETANUS TEST TOXIN

by Johs. IPSEN
(State Serum Institute, Copenhagen.)

I. The Methods employed in Twelve Laboratories for the Production of Tetanus Test Toxins

At their meeting in Paris in October 1938, the serologist members of the Permanent Commission on Biological Standardisation recommended that further investigations should be undertaken into the qualitative differences of tetanus toxins produced in various laboratories. The Department of Biological Standards of the Statens Serum Institute, Copenhagen, was to ask various laboratories to prepare a toxin *from a strain supplied by it and according to a specified method, this toxin to be used to assay tetanus antitoxins which previous tests have shown to possess different qualities.*

As a preliminary step in this investigation, the laboratories invited to participate in the enquiry were asked to supply the Copenhagen Institute with information on certain details regarding the technique of routine production of their tetanus test toxin. This information was intended to facilitate a decision as to whether it was necessary to propose a particular method, and, if so, what indications should be given.

Twelve Institutes kindly gave their assistance by answering a questionnaire sent out by the Department of Biological Standards. The answers are recorded in the appended table, which gives a conspectus of the details of the procedure followed in preparing test toxins in various laboratories.

It may be assumed that each of these laboratories, which are all highly experienced in such matters, has chosen the method which, in its hands, gives the maximum yield, and that any one of the

Name of Laboratory	Name of Strain	Composition of				
		Base medium	Concentration of			
			Peptone	Salts	Dextrose	Other components
The Wellcome Physiological Research Laboratories, BECKENHAM.	T. 11, 12, 19, 34, 43	Horse-muscle broth	Witte 1%	NaCl 0.7%	0,5%	No
Instituto de Bacteriologia, BUENOS-AIRES.	Japanese	Martin, veal and beef	Peptic digest of veal or beef	NaCl. of peptone	1%	No
Statens Serum Institut, COPENHAGEN.	Tullock Type II	Veal-meat	Riedel 1%	NaCl 0.5%	No	No
Lister Institute for Preventive Medicine, ELSTREE.	Tullock Type I	Horse-meat infusion	Witte 1%	No	1%	No
Staatliches Institut für exp. Therapie, FRANKFURT.	A 471, A 46 A 66, A 71	Veal-meat	Witte 1%	NaCl 0.5%	No	No
Institut Pasteur, GARCHES.	American	Veal-meat infusion	Peptic digest of veal-meat	No	0.4%	No
National Institute of Medical Research, HAMPSTEAD.	Lab. strain P.	Horse-muscle infusion	Difko 1%	NaCl 0.5%	No	No
Istituto Sieroterapico, MILANO.	Tullock Type III	—	Difko 2%	NaCl 0.5%	No	No
Statens Bakteriologiska Laboratorium, STOCKHOLM.	American (Ramon)	Martin, (according to Loiseau & Phillippe)	Peptic digest of veal or beef meat	No	0.4%	No
Rijks Institut voor de Volksgezondheid, UTRECHT.	(Wellcome) No. 12	Veal-meat infusion	Witte 1%	NaCl 0.5%	1.2%	No
National Institute of Health, WASHINGTON.	Tullock Type III	Beef-infusion	P.D.C. 1%	NaCl 0.5%	1%	No
State Epidemiological Institute, WARSAW.	French (Institut Pasteur)		Witte 1%	NaCl 0.5%	0,2%	0.6% KH_2PO_4

the Medium			Period of incubation	Temperature of incubator	Conditions used whether anærobic or not (vacuum)	Manner in which the Test Toxin was prepared: filtration, precipitation, glycerol
Pieces of meat at bottom	P_H					
	Initial	Final				
No	5.6	6.9-7.1	5-14 days	37° C	No	Filter-candle + $(NH_4)_2SO_4$
No	7.2	ca. 7.2	4 days (3)	37° C.	Yes $+H_2$	Berkefeld + $(NH_4)_2SO_4$
Yes	6.4-6.6	7.2-7.5	8 days	37° C.	Cooked paraffin oil	Seitz Filter + $(NH_4)_2SO_4$
No	7.4	ca. 6.8	11 days	35.5° C.	Filled to neck	Berkefeld: equal volume of glycerol
No	8.0	7.9-8.6	10-12 days	37° C.	—	Berkefeld N15 + Na_2SO_4
No	6.4	7.2-7.3	12 days	36° C.	No	Infusorial earth. Chamberland H_3. Equal parts of glycerol
No	7.0	6.8-7.0	10 days	37° C.	Filled to neck. Freshly steamed	Filter + $(NH_4)_2SO_4$
No	6.7	6.6	12 days	37° C.	Paraffin oil	$(NH_4)_2SO_4$
No	6.4	7.6	10 days	37° C.	Heating at 95°-99° C. for 5-10 minutes	$(NH_4)_2SO_4$
Yes ¼ chopped veal	7.2-7.3		9 days	37° C.	Paraffin oil	Seitz E.K. + $(NH_4)_2SO_4$
No	7.6		12 days	37° C.	No, but flasks filled to neck and heated to 100°	Paper + Berkefeld + $(NH_4)_2SO_4$ dried over P_2O_5
Yes	7.0	6.9	12-15 days	36-37° C.	40-50 mm. vacuum	Berkefeld 2× +50% glycerin

twelve procedures could be chosen to serve as a kind of standard method for the production of test toxin. The circumstance, however, that some institutes have disregarded certain factors that are included by others suggests a possibility of simplifying the procedure, on the assumption that only the factors used by all laboratories are essential. Thus, where three institutes prefer to keep the pieces of meat at the bottom of the containers, whilst the other nine omit this detail, it may be inferred that this factor is not strictly necessary to obtain a good tetanus toxin.

Another point considered in working out the proposed method described hereunder was the importance of ensuring that the components of the medium should, so far as possible, be reproducible everywhere. For this reason, it would be preferable to choose a peptone from a single factory for the peptic digests. Expense is not a consideration in this connection, since this method is only to be used in making a comparatively small amount of test toxin, and not for the production of toxins for immunisation and preparation of formol-toxoids.

The veal extract will, admittedly, be impossible to " standardise ", but if it were proved that this factor exerts an important influence on the qualities of the toxin, this would mean that the problem of preparing uniform tetanus toxins is very far from solved.

The above considerations have led us to propose the method which is set forth below. In this connection, we have received valuable advice and criticism from Dr. L. E. Walbum, Dr. G. Reymann and Dr. Per Holm, to whom we tender our acknowledgments. Needless to say, our intention has not been to prescribe a method which would give as potent a toxin as possible, but rather to find a simple procedure for the preparation of a suitable toxin which can be reproduced in all laboratories. We would, however, emphasise the need for strict observance in detail of all the directions given.

By way of preliminary, we have prepared a quantity of tetanus test toxin by this method in our laboratory. It has given the following constants :

L +/5 (mice intraveneously) = about 0.10 mg.
Minimum lethal dose = about 0.00006 mg.

II. A Proposed Method for Producing Tetanus Test Toxin

Preparation of the Medium.

Mince 10 kg. of fresh veal without fat and add 20 litres of distilled water ; allow to stand overnight at 2-4°C.

Next day boil for 10 minutes and decant the fluid. Adjust the volume of fluid to 20 litres by adding distilled water ; thereupon, add :

(1) 0.5% sodium chloride,
(2) 1% Witte peptone.

Adjust the p_H to 8.0 by means of NaOH. Filter the preparation through ordinary filter paper. Deliver 1 litre of the broth into each of twenty Erlenmeyer flasks of 1½ litres capacity. Autoclave the flasks at 124°C. for 20 minutes and immediately redistribute the contents of the flasks in such a way that the height of the fluid in each flask (about thirteen) is 15 cm. These thirteen flasks, which are ready for inoculation, are placed in the incubator at 37°C.

Inoculation of the Broth.

Preliminary inoculation. — Inoculate one of the flasks with an ample amount of strain 3 [1] furnished by the Copenhagen Institute in glucose-broth. Replace the flask in the incubator and observe during the ensuring 24 to 48 hours.

Final inoculation. — When the growth of the bacillus is perfectly spread throughout the volume of broth, inoculate the remaining twelve flasks with 2 cc. of the infected broth from the first flask.

Place the twelve flasks in the incubator for 6 × 24 hours after the final inoculation. Then filter the broth through a Berkefeld or Seitz filter and pour the contents of all flasks together in a large container. Thereupon saturate the fluid with $(NH_4)_2SO_4$. One hour after, collect the precipitate on the surface and remove the excess fluid by means of filter-paper.

Place the lot in a large desiccator and powder after drying. Allow to remain in the desiccator until dried to constant weight.

May 1939.

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[1] This strain (Nat. Coll. No. 67, Tullock Type II) has been micromanipulated by Professor I. Zeissler, Altona, and found perfectly pure.

COMPARISON OF TETANUS TEST TOXINS PREPARED BY SEVEN INSTITUTES FROM THE SAME STRAIN AND BY THE SAME METHOD

by

Johs. IPSEN

(State Serum Institute, Copenhagen).

The plan of producing a test toxin from the same strain of *Bac. tetani* (as outlined in the foregoing progress report (4)) was successfully carried out in the course of 1939 through the kind co-operation of the following investigators :

Professor A. SORDELLI, Instituto de Bacteriologia, Buenos-Aires ;

Professor R. PRIGGE, Staatliches Institut für experimentelle Therapie, Frankfort-on-Main ;

Professor F. PEPEU, Istituto Sieroterapico Milanese, Milano ;

Dr. G. LÖFSTRÖM, Statens Bakteriologiska Laboratorium, Stockholm ;

Dr. W. Aeg. TIMMERMAN, Rijks Instituut voor de Volksgezondheid, Utrecht ;

Dr. W. T. HARRISON, National Institute of Health, Washington.

In addition, a toxin was prepared in 1939 by the author of the present paper, following the technique described, and this toxin, together with the dried ammonium-precipitated preparations furnished by the above institutes, formed the basis for a series of investigations carried out in Copenhagen in the course of 1940.

The main object of these investigations was to determine whether the seven toxins showed any differences in qualitative respect.

The first question was to examine the extent to which the *potency of various sera* (in terms of the international standard tetanus antitoxin) would vary when using each of the seven toxins in question as test-toxin. The variation found should then be compared with the variation which might be expected from the error of assay of tetanus antitoxins with one test-toxin.

A further description of the quality of a toxin is possible, however. Margaret Llewellyn Smith (7) has demonstrated that the slope of the curve which is obtained when the reaction is plotted against the logarithm of the dose of antitoxin is different when two tetanus toxins are used for the assay of the same sera. Slope deviations indicate some qualitative differences in the toxins, and we shall therefore include an investigation of the slopes of the reaction curve in the treatment of the results.

Qualitative differences of toxins can be detected by comparison of the *ratio of the direct and indirect toxicity* — *i.e.*, the ratio between m.l.d. and the test-dose against a certain dose of antitoxin. This has been used to advantage by the author in a study of the qualitative differences among toxins of *Bac. perfringens* (5). Sordelli (8) has demonstrated that tetanus toxins with widely deviating " toxicity " ratios give abnormally deviating titers against different antitoxins. Hence we shall also use the number of m.l.d. per test-dose in characterising the toxins of this investigation.

The above-mentioned study of perfringens toxins shows other means of demonstrating the complexity of toxins — viz., by assays of the direct toxicity in various test animals, injecting the agent intracutaneously as well as intravenously, examining the hemolytic capacity, etc.

The possible hemolytic action is of no interest as far as the present problem is concerned, since it is generally known that tetanolysin takes no part in the course of a tetanic intoxication. Moreover, since the tetanus filtrates contain no skin-reacting substance, a comparison of the direct toxicity in mice, guinea-pigs and rabbits remains the only possibility of revealing the presence of any multiple toxins in the tetanus filtrates. Such experiments were carried out with the seven toxins in question, and comparison was made with experiments employing tetanus toxins of other origin.

* * *

In order to secure a sound basis for a qualitative comparison of the toxins, it was necessary to revise the methods of estimating, from the experimental data, such values as the minimal lethal dose of the toxin, the slope of the reaction curve, etc. The author believes that the death time of the animals injected with toxin or toxin-antitoxin mixtures is more suitable for this purpose than the mortality rate of a group of animals, now commonly used.

An attempt to utilise the death time is found in an earlier paper on the titration of tetanus antitoxin (1938) (1), but in later publications the author has developed a method which is better founded, theoretically and experimentally ; this method is used in the treatment of the results of the present investigation.

For the purpose of clarity, it seems reasonable first to deal with the assays of direct toxicity and later present the method of computing the titer of the antitoxins. The arrangement will be be as follows :

1. Calculation of the minimal lethal dose of tetanus toxins.
2. The ratio of m.l.d. for mice, guinea-pigs and rabbits.
3. The reaction curve of toxin-antitoxin mixtures.
4. Calculation of the potency of the antitoxin.
5. Comparison of the potencies of antitoxins assayed with the seven tetanus toxins.
6. The ratio of direct and indirect toxicity.

1. Calculation of the Minimal Lethal Dose of Tetanus Toxins

The minimal lethal dose of a tetanus toxin can be evaluated in mice as well as in guinea-pigs and rabbits. The m.l.d. is defined as the dose of toxin which kills 50% of a group of *mice in 120 hours*, of *guinea-pigs in 100 hours*, and of *rabbits in 160 hours*.

In previous papers (2, 3) of which this chapter is a short summary, the author has shown that a constant relation exists between the dose of toxin (expressed in m.l.d.) and the time in which 50% of a group is killed. The slope of the corresponding curve is specific to a toxin and a species of animals. In case of tetanus toxin and the three kinds of animals concerned, a curve was drawn once for all from which it was possible to determine the number of m.l.d. which

on the average would kill in a certain length of time. Accordingly, the death time can be tabulated as a function of the number of m.l.d. If $f(T)$ is the logarithm of this time function, D the actual dose, and d the dose which kills 50% in infinite time, we have

$$f(T) = \log \frac{D}{d} = \log D - \log d \qquad (1)$$

Separate tables of $f(T)$ have been prepared for each of the three kinds of animals, and the $f(T)$ table for mice is quoted in the Appendix to the present paper. The table is used as follows in the calculation of the m.l.d. of an unknown tetanus toxin :

The death time is observed for each animal receiving the dose D. $f(T)$ is found in the particular table for the species of animal used, and the quantity

$$\log d = \log D - f(T) \qquad (2)$$

is calculated for each observation, and the mean value is determined. This mean is the best estimate of the dose which kills 50% in infinite time. M.l.d. may now be found by adding 0.19 to log d, 0.19 being the value of $f(T)$ for the time contained in the above-mentioned definition of m.l.d. for all species of animals :

$$\log (\text{m.l.d.}) = \log d + 0.19 \qquad (3)$$

Since it can be proved that log d is normally distributed, it follows that the standard error of the determination of log d is

$$s_{\log (\text{m.l.d.})} = \frac{s}{\sqrt{N}} \qquad (4)$$

where s is the standard deviation of log d from the mean and N the number of animals killed in the whole experiment.

It is possible to demonstrate that s is identical with the standard deviation in the normal distribution which is formed by the logarithm of the minimal lethal dose for each individual. s^2 may be calculated directly in each experiment as the mean of the square of the deviation of the values of log d from their mean, and s is fairly constant in experiments with the same lot of animals, independently of the length of the death time.[1]

Hence the advantage of using the $f(T)$ table is that the death time is transformed into a normally distributed quantity which is equivalent to the toxicity of the toxin.

[1] s thus expresses the degree of uniformity in resistance of the lot of animals.

It may be established statistically by an analysis of variance that we are justified in using the $f(T)$ table, prepared from experiments with other toxins, in the experiments with the seven toxins in question.

The variance of $f(T)$ for one dose ($s^2_{f(T)}$) was compared with the variance of values calculated as log D—f(T50) ($s_{\log d}$) for each dose by means of R. A. Fisher's z-test, f(T50) being the mean of $f(T)$ for each dose.

Table 1 (page 457) records a detailed protocol with the computation of log (m.l.d.) and the variances.

Since the standard deviation of $f(T)$ ($s_{f(T)}$) in groups receiving the same dose is not significantly different from the standard deviation ($s_{\log d}$) of log D — f(T50), it follows that the standard deviation (s) of the mean log d can be calculated as mentioned above, and from s we obtain the error of the mean according to equation (4).

Table 2 (page 457) records a survey of this analysis of variance for all seven toxins.

The logarithmic standard deviation of the potency (s) varied somewhat according to the lot of mice used in each experiment, but our mice showed, as an average, a standard deviation of 0.194. This means that we may reckon with the following accuracy in the assay if the indicated number of mice are used :

Number of mice killed	Percentage standard error of assay %
1	56.3
2	37.3
5	22.4
10	15.2
20	10.5
50	6.5
100	4.6

The most economical number of mice will be 20, this number giving the acceptable error of about 10%. It is believed that mice can be obtained having a greater uniformity of resistance than those used in these experiments. In that case, the error of m.l.d. will be even smaller than that indicated above.

The method of determining m.l.d. in case of rabbits and guinea-pigs is quite similar. $f(T)$ will naturally be found in the proper table. A graphical method, applicable to experiments with only one animal per dose and with large intervals between the readings of the death times of the animals, was described in an earlier paper (2).

Table 1.

CALCULATION OF M.L.D. FOR TOXIN W AND THE STATISTICAL TEST FOR FITTING TO THE $f(T)$ CURVE

Dose mg.	log D	Number of mice injected	Death time (hours)	$f(T)$	Mean $f(T)$ ($f(T50)$)	$s^2_{f(T)}$	Degrees of freedom	log D — $f(T50)$
0.00025	—3.6	5	73 97 97 97 97	0.42 0.27 0.27 0.27 0.27	0.300	0.00450	4	—3.900
0.00032	—3.5	5	73 97 97 97 97	0.42 0.27 0.27 0.27 0.27	0.300	0.00450	4	—3.800
0.0005	—3.3	4	73 78 106 121	0.42 0.38 0.23 0.19	0.305	0.01257	3	—3.605
0.001	—3.0	4	32 51 73 73	1.11 0.67 0.42 0.42	0.655	0.10590	3	—3.655
0.0025	—2.6	3	25 32 32	1.39 1.11 1.11	1.203	0.02614	2	—3.803

log d = — 3.759

$s_{\log d}$ = 0.251

4

Difference not significant

mean $s_{f(T)}$ = 0.167

Degrees of freedom 16

log m.l.d. = — 3.759+19 = — 3.569

m.l.d. = 0.00027 mg

Table 2.

ANALYSIS OF VARIANCE FOR SEVEN TOXINS

Toxin*	N	$s\,f(T)$	Degrees of freedom	$s_{\log d}$	Degrees of freedom	Probability of identity of $s_{f(T)}$ and $s_{\log d}$	s	Degrees of freedom	$\frac{s}{\sqrt{N}}$	Percentage error of m.l.d.
B	21	0.228	13	0.228	4	>5%	0.228	17	0.051	12%
C	29	0.235	19	0.303	9	>5%	0.258	28	0.049	12%
F	44	0.139	32	0.150	8	>5%	0.142	40	0.021	5%
M	19	0.206	9	0.180	4	>5%	0.201	13	0.046	11%
S	20	0.076	11	0.146	4	<5% and >1%	0.098	15	0.022	5%
U	20	0.183	13	0.277	4	>5%	0.210	17	0.047	11%
W	21	0.167	16	0.251	4	>5%	0.186	20	0.041	10%
							mean s 0.194	150		

* Toxins are designated with the first letter in the name of the city of origin.

It is apparent that the observation of the death time and the transformation into the function $f(T)$ form an exact basis for the computation of m.l.d. and the error involved.

2. The Ratio of M.L.D. for Mice, Guinea-pigs and Rabbits.

The direct toxicity of the seven toxins (in mg. of the dry precipitated material) varied, as it was to be expected, but a considerable agreement was found in the ratio of the toxicity values obtained in the three kinds of animals as expressed by the m.l.d. ratio : rabbit/mouse and guinea-pig/mouse.

The doses prepared for rabbits were in dilutions of 3 cc. each ; rabbits weighing 3,000 g. received 3 cc. i.v., and the volume was adjusted for animals of other weights so that 1 cc. was injected per 1,000 g. of weight.

The average weight of the guinea-pigs was 250 g., and here the dose was adjusted in a similar way. Animals weighing, *e.g.*, 280 g. received a volume of 2.8 cc. of the dilution containing the dose in 2.5 cc.

No correction was made for the variation in weight of the mice.

Table 3 gives the data obtained with the number of animals recorded.

Table 3.

Determination of M.L.D.

Toxin	Mouse		Rabbit		Guinea-pig		Ratio of m.l.d.	
	m.l.d. mg.	Number of animals	m.l.d. mg.	Number of animals	m.l.d. mg.	Number of animals	Rabbit/ mouse	Guinea-pig/mouse
B	0.00023	21	0.98	3	0.00026	3	4260	1.13
C	0.000069	29	0.37	3	0.00013	3	5360	1.88
F	0.0076	44	43.50	6	0.007	3	5720	0.92
M	0.00029	19	1.44	3	0.00068	3	4970	2.32
S	0.00032	20	1.59	3	0.00033	3	4970	1.03
U	0.00038	20	2.34	3	0.00087	3	6160	2.29
W	0.00027	21	1.29	3	0.00034	3	4780	1.26
					Geometric mean		5140	1.45

The m.l.d. ratio shows good agreement for all seven toxins, notwithstanding the small number of rabbits and guinea-pigs used. The variation of the ratio is even smaller than we should expect from the error of the assay.

The information — that the m.l.d. for rabbits is 5140 times the m.l.d. for mice, and that the ratio guinea-pigs/mice is 1.45 — appears to be of no interest unless a comparison is made of the ratios when toxins of other origin are used. We have therefore investigated seven other toxins from various institutes, prepared with different strains and by different methods. The same number of animals was used in these experiments ; the results are given in Table 4.

Table 4.

RATIOS OF M.L.D. FOR TOXINS OF VARIOUS ORIGIN

Toxin	Place of origin	Strain	Method [2]	m.l.d. Mouse mg.	Ratio of m.l.d.	
					Rabbit/ mouse	Guinea-pig/mouse
C. 35	Copenhagen	Strain 3 [1]	Copenhagen	0.00086	4270	3.78
B. II	Buenos-Aires	,,	Buenos-Aires	0.00026	5690	2.13
B. III	,, ,,	"Japan Strain"	,,	0.00025	*1790*	3.10
B. IV	,, ,,	,,	Standard	0.00031	*1480*	5.06
Tg. 135	Frankfort	Frankfort	Frankfurt	0.00021	*1390*	2.37
K.	Washington	Tullock No. 3	Washington	0.00038	*2070*	
K. 21 [3]	Copenhagen	Inst. Pasteur	Inst. Pasteur	0.00008	*17600*	2.04

The values in italics deviate significantly from the mean in Table 3.

[1] Strain 3 was used for the production of the seven toxins of the enquiry.

[2] Methods described in the Progress Report (4). "Standard" method designates the method proposed herein for the production of the toxins of the enquiry.

[3] Prepared by Miss E. Kirchheiner ; method and strain from the laboratory of Dr. Prévot, Paris.

In the case of these toxins, we find no significantly deviating values of the m.l.d. ratio guinea-pig/mouse, although all values are higher than the corresponding mean in the last column of Table 3.

In five cases out of seven, however, the m.l.d. ratio rabbit/mouse is significantly different from the mean value of 5140 of Table 3, four of them being lower and the last one considerably higher. Two of the toxins show a " rabbit/mouse " ratio of the same order of magnitude as the ratios found for the seven toxins in Table 3 ; these two toxins are particularly interesting, since they are the only

toxins of Table 4 which have been produced from strain 3. This fact suggests that the m.l.d. ratio for rabbit/mouse is *a quality to be ascribed to the strain of Bac. tetani* and not to the medium or the method of producing the toxin filtrate.

In order to put this hypothesis to a test, we re-examined a series of toxins which had been prepared in our laboratory two years before. Two strains had been used — viz., strain 3 and the Washington strain Tullock No. 3, which was also used for the production of toxin K of Table 4. Certain details have been changed in the preparation of these toxins so that they are especially suited to show that the quality in question is independent of the method of preparation. The results are given in Table 5.

Table 5.

Toxin	Strain	Medium	Incubation period	Precipitated by saturation with	m.l.d. Mouse mg.	Ratio of m.l.d. rabbit/mouse
1 C	Strain 3	Copenhagen	8 days	$(NH_4)_2SO_4$	0.00013	4790
2 C	,,	,,	4 days	»	0.00015	12900
3 C	,,	,,	8 days	Na_2SO_4	0.00030	5250
4 C	,,	,,	8 days	$(NH_4)_2SO_4$	0.00029	10700
						Geom. mean 7670
1 W	Tullock No. 3	Copenhagen	4 days	$(NH_4)_2SO_4$	0.000029	2690
2 W	,,	,,	8 days	Na_2SO_4 $+(NH_4)_2SO_4$	0.000055	1410
3 W 1	,,	,,	4 days	$(NH_4)_2SO_4$	0.000037	1450
3 W 2	,,	,,	4 days	Residual fluid of 3 W 1 + Na_2SO_4	0.00013	980
4 W	,,	,,	4 days	Na_2SO_4 + $(NH_4)_2SO_4$	0.000017	780
						Geom. mean 1330

The mean values in the last column of Table 5 seem to confirm our hypothesis. The toxins produced with strain 3 gave a mean m.l.d. ratio " rabbit/mouse " which does not differ significantly from the ratios of the toxins in Table 3 or from the first two toxins in Table 4. And the five toxins produced with the same strain as toxin K gave a mean value of the same ordre of magnitude as the ratio found for toxin K in Table 4.

Since the two mean values of Table 5 are significantly different, we have been able to show *a difference in the biological quality of toxins produced with different strains of Bac. tetani.*

3. The Reaction Curve of Toxin-Antitoxin Mixtures

(a) *Material and Method of Experiment.*

Three antitoxic tetanus sera were chosen for the comparison of the " test toxin " properties of the seven toxins in question. All of these sera had been used in similar investigations published in 1938 in this *Bulletin* (1), viz. :

Serum 3111, which is the present British Standard Antitoxin ;

Serum 4101 selected at Hampstead to replace the current British Standard.

Both sera were compared with the international standard, using several different toxins, the work being done by M. Llewellyn Smith (7) ; significant deviations of the titers were found when different toxins were used. Dilutions of these sera were placed at our disposal through the courtesy of M. Llewellyn Smith. 1 cc. of the dilution of 3111 was supposed to contain 100 AU, and 1 cc. of 4101 should contain about 60 AU.

Serum III (potency approximately 5,000 AU) was one of five sera which were investigated in several institutes (1). The titers of this serum showed the highest variation when tested with different toxins and by different methods.

The potency of these sera was in each case compared with that of the International Standard for Tetanus Antitoxin (TE).

First, the test dose L+/5 was determined for each toxin — *i.e.*, the dose which, mixed with 1/5 international unit and allowed to combine for one hour at room temperature, causes death of 50% in a group of animals 5 × 24 hours after intravenous injection of the toxin-antitoxin mixture.

This test dose was then added to 5 doses of the assayed antitoxin and 5 doses of the standard antitoxin. The dose interval was 12.5% (the difference between the logarithms of the doses was 0.05). After being allowed to combine for one hour, each mixture was injected into 5 mice taken from the same lot. Generally, all

three antitoxins were thus compared with the standard on the same day.

(b) *Reaction Curves.*

The potency of the antitoxin was computed as the difference between log dose of the antitoxin in cc. which showed 50% death on the fifth day (120 hours) and log dose of the standard serum (in AU) which gave identical results.

In order to obtain a reliable estimate of this *end-point* dose, curves were constructed by plotting the reaction against log dose of the serum.

Two forms of such reaction curves are possible :

(1) The quantal reaction curve, where the reaction is expressed as percentage of the animals surviving after 120 hours. This type of curve was used in the work of M. Llewellyn Smith and is the type most commonly employed.

(2) The quantitative reaction curve, in which the reaction is expressed as the *time* when 50% of the group is killed (T50). An attempt to use this reaction curve has previously been made by the author.

When the first curve is used, the dose giving 50% survival is found by interpolation ; from the second curve we find the dose showing T50 in 120 hours.

The quantitative reaction curve seemed preferable. The problem was that of finding a suitable method for the calculation of T50. This was possible by transforming the death time of each animal into the dose/time equivalent $f(T)$ and then calculating $f(T50)$ as a simple mean of these values.[1]

T50 can now be found in the $f(T)$ table, but it is of more interest to consider the curve which is formed when $f(T50)$ is plotted against the log dose of antitoxin ; it can be proved, by graphical and arithmetical tests, that *this curve is rectilinear.*

The curve : $f(T50)$/log (serum dose) can accordingly be subjected to simple arithmetical treatment, and the end-point dose may be accurately computed.

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[1] In case of groups where only part of the animals died, the calculation of $f(T50)$ followed a special method which is explained in detail in previous papers (2, 3).

This form of curve is, in several respects, more accurate than the dose/mortality curve :

(*a*) The slope of the curve is independent of the variations in resistance of the population of animals ;

(*b*) The curve can be followed through a wider range of doses ;

(*c*) The error of the reaction *f*(T50) can be calculated directly from the data, while the error of a percentage reaction is estimated theoretically ;

(*d*) The same degree of accuracy is obtained with a considerably smaller number of animals.

(c) *The Slope of the Curve : f(T50)/log (serum dose).*

The reaction curves for each combination of the four sera and the seven toxins were obtained by plotting *f*(T50) against log dose of antitoxin. These graphical representations showed that curves from experiments with the same serum had the same slope whichever of the seven toxins was used as test toxin. The reaction curve was only followed through a range of doses from 0.225 AU to 0.16 AU. Lower values of antitoxin were not examined, since the reaction was too uncertain (death time less than 36 hours). Thus the rectilinear course was not proved for the full length of the curve, but only for the part which is of practical significance. The apparent parallelism of the curves was confirmed in statistical tests where the variance of the reaction from the smoothed reaction curve was compared with the variance of the slope of the curve obtained with each toxin. Since these variances were not significantly deviating in assays of the same serum, the following mean slope could be attributed to the reaction curve for each serum and the seven toxins (Table 6) :

Table 6.

Serum	Mean slope of (β) reaction curve *f*(T50)/log serum	Mean error of slope (s_β)
TE	— 6.72	0.558
3111	— 3.40	0.151
4101	— 6.99	0.754
III	— 6.91	1.000

It is apparent that three of the four sera — viz., TE, 4101 and III — gave the same slope of reaction curve with the seven toxins, while 3111 developed a much flatter curve. Figure 1 (see pages 474 and 475) illustrates the difference. All reaction curves are translated so that the point of intersection of the reaction line with the abscissæ is the same for all toxins.

The evaluation of the potency of serum 3111 in terms of the standard cannot be made independently of the method of reading, since the slope of this serum (3111) is significantly different from that of the standard serum (and the sera 4101 and III).

The ratio of the potencies of the two sera will depend upon the end-point reaction chosen. If, for example, we use 50% mortality on the fifth day as end-point (f(T50) = 0.19), the titer of 3111 will appear 2.6% lower than the titer calculated at the end-point 50% mortality on the fourth day (f(T50) = 0.27).

No adequate theoretical explanation is available for this lack of parallelism between the reaction curves of certain tetanus antitoxins. It leads to the assumption that the antitoxic serum is composed of molecules of different affinity to the antigen. Since the value f(T) is equivalent to the amount of free tetanus toxin, the reaction curve illustrates the relation between free toxin and antitoxin in the mixture containing a constant amount of toxin and varying amounts of antitoxin. An analogy to these findings is to be found in the recent experiments of Heidelberger *et al.*[1] who studied the precipitation reaction curve of egg albumin and its homologous antibodies in rabbits and horses. A difference in the slope of this curve was found when various sera were tested against the antigen.

In the future, it is probable that the f(T) reaction curve will be the means of a more exact study of the properties of the tetanus antitoxin.

4. Calculation of the Potency of the Antitoxin and the Error of Assay

In order to find a practical solution of our problem — viz., the comparison of the properties of toxins — we must create a term of

[1] *E.g.*, M. Heidelberger, H. P. Treffers and M. Mayer, *Jl. Exp. Med.* 1940, 71, 271.

comparison for the titers of the antitoxic sera obtained for each of the toxins. It is therefore necessary to define a certain end-point reaction and to calculate the dose of each antitoxin which gives this reaction.

Here as before, 50% mortality at 120 hours has been selected as end-point. This corresponds to a value of $f(T)$ equal to 0.19. Calling the logarithm of the dose giving this reaction D_5, we may calculate this quantity from each of the doses giving death of tetanus.

The equation of our reaction curve is :

$$f(T50) - 0.19 = \beta (\log D - D_5) \qquad (5)$$

where D is the serum dose and β the slope in Table 6.

Hence we find

$$D_5 = \log D - \frac{1}{\beta} (f(T50) - 0.19) \qquad (6)$$

A value of D_5 is calculated for each D, and the mean D_5 is used as an expression of the potency of the antitoxic serum. The titer is calculated as the antilogarithm of the difference between D_5 for the standard and D_5 for the serum assayed.

Table 7 (page 466) gives a detailed protocol with the consecutive steps : transformation into $f(T)$, calculation of $f(T50)$, of D_5, and of the titer.

The Error of the Determination of D_5.

The error of the determination of D_5 can be determined directly from the reaction curve. The regression diagram, Figure 1, shows that the mean reactions $f(T50)$ deviate to a certain degree from the theoretical line. This deviation, expressed by the mean square of the deviations of the points from the line $MS_{y/x}$ * determines the error of D_5 (s_{D_5}) as follows :

$$s^2_{D_5} = \frac{MS_{y/x}}{\beta^2 \, n_D} \qquad (7)$$

where n_D is the number of doses giving response.

The quantity $MS_{y/x}$ is determined from the combined sets of reaction curves for each serum, and the following values were found (Table 8) :

* For the computation, see Ipsen (3, page 37).

Table 7.

Evaluation of Serum 3111 with Toxin F

Five mice per dose. To each serum dose is added 3.3 mg. toxin.

Serum	Dose of serum	log D	Death time in hours					f(T)					f(T50)	D_s
Stand.	0.25 UA	—0.602	S	S	S	S	S	—					—	—
	0.225 UA	—0.648	S	S	S	S	S	—					—	—
	0.2 UA	—0.699	72	88	112	141	S	0.43	0.32	0.21	0.14	S	0.243 *	—0.691
	0.18 UA	—0.745	49	49	64	64	88	0.70	0.70	0.50	0.50	0.32	0.544	—0.692
	0.16 UA	—0.796	all died 25 to 40 h.					1.39 to 0.89					1.140	—0.654
														Mean: —0.679
3111	0.0032 cc.	—2.504	S	S	S	S	S	—					—	—
	0.0028 cc.	—2.553	S	S	S	S	S	—					—	—
	0.0025 cc.	—2.502	88	141	141	S	S	0.32	0.14	0.14	S	S	0.140 *	—2.616
	0.00225 cc.	—2.648	88	112	112	112	112	0.32	0.21	0.21	0.21	0.21	0.232	—2.636
	0.0020 cc.	—2.699	72	72	72	88	88	0.43	0.43	0.43	0.32	0.32	0.386	—2.641
														Mean: —2.631

Titer of Serum 3111 = antilog (2.631–0.679) = antilog 1.952 = *89.5 A U*

S = survived.

* As these groups did not show full mortality, f (T50) was calculated as the *median* of the f(T). The method is dealt with by Ipsen (2, 3).

Table 8.

Variance of T50 from the Reaction Line

Serum	Number of observations	$MS_{y/x}$	$MS_{y/x/\beta^2}$
TE	52	0.02893	0.0252
3111	28	0.00565	0.0226
4101	26	0.03502	0.0268
III	20	0.03800	0.0282

It is striking that the reaction is determined with a significantly higher degree of accuracy for serum 3111 than for the other sera (see column 3). This means that the error of assay is practically of the same order of magnitude for all the sera (see column 4), notwithstanding the fact that the slope of the curve of serum 3111 is only half of the slope of the curves of the other sera.

Some interesting conclusions are reached when trying to explain this deviation. It might be presumed that the deviation of $f(T)$ is less for doses containing serum 3111, but an analysis of variance shows that the standard deviation of $f(T)$ is the same for assays with all sera and, moreover, equal to that (s) determined from experiments with toxin alone, as in section 1. Table 2 gives this standard error, 0.194. Since five mice are used per test dose, the variance of the mean reaction $f(T50)$ should be about $\frac{0.194^2}{5} = 0.007527$.

This expected value is only approximated in case of serum 3111 ($MS_{y/x} = 0.00565$). For the rest of the sera, we must reckon with a source of error not controlled by the number of mice injected per dose. It may be put as

$$MS_{y/x} = \mu^2 + \frac{s^2}{n} \qquad (8)$$

where s is the standard deviation of $f(T)$ (standard deviation in resistance), n the number of mice per dose, and μ the unexplained error (in our case about 0.15).

This error (μ) may perhaps be explained by assuming that the same number of toxin and antitoxin molecules have the possibility of combining in several different proportions, leaving more or less toxin free in the mixture. Or we may assume that the phenomenon of Danysz is interfering in different degrees during the mixing of

toxin and antitoxin. In the case of serum 3111, it may be assumed that this serum is composed of antitoxin molecules which are able to combine with the toxin molecules in only one way, so that a mixture of given amounts always will leave a certain amount of toxin free. It is impossible, from the experiments on hand, to conclude whether this phenomenon may be related to the flatter course of the curve: free toxin/antitoxin.

An important practical result of these considerations is that the *error of mixing toxin and antitoxin* must be taken into consideration when estimating the error of antitoxin assays. One conclusion is that, for certain sera, greater accuracy is obtained when injecting each animal with a separately prepared mixture than when a group of animals is injected with the same mixture. In the first case, the variance is $\frac{\mu^2 + s^2}{n}$, in the latter case it is $\mu^2 + \frac{s^2}{n}$. Thus in certain cases the error of the assay cannot be less than the quantity $\frac{\mu}{\beta \sqrt{n_D}}$, even if thousands of animals were to be injected with the same mixture of toxin and antitoxin.

The error of the titer (s_m) is determined by the error of D_5 for both the standard (s'_{D_5}) and the serum (s''_{D_5}) to be assayed, or

$$s^2_m = (s'_{D_5})^2 + (s''_{D_5})^2 \qquad (9)$$

On the average, three doses out of five gave response, so that n_D in equation (7) may be put equal to 3. Table 9 gives this error of the titer together with the values of s_m to be expected when two doses give response.

A standard error of 5.5% was obtained in the large investigation (1) in which five institutes, using practically the same experimental procedure, evaluated a number of sera with the same standard toxin. This value lies very close to our computed value.

Table 9.

EXPECTED STANDARD ERROR OF TITER TESTED WITH ONE TOXIN

Serum	Three doses responding		Two doses responding	
	Log error	Percentage error	Log error	Percentage error
3111	0.0195	4.6%	0.0238	5.6%
4101	0.0211	5.0%	0.0260	6.2%
III	0.0219	5.2%	0.0268	6.4%

5. Comparison of the Potencies of Antitoxins assayed with Seven Tetanus Toxins

Having established an exact method of computing the titers, and having estimated the possible error of the titers, we can now return to the main objective of the investigation — viz., a comparison of the potencies of the three sera determined with each of the seven toxins prepared by the same method.

Table 10 gives these titers in international AU per 1 cc. The standard deviation of the titer is calculated from the logarithmic values for each of the sera and, finally, a mean standard deviation is recorded.

Table 10.

Titer of Three Tetanus Sera determined with Seven Toxins and Their Standard Deviation

Toxin	Serum 3111	Serum 4101	Serum III
B	75.5	62.5	5110
C	78.0	51.5	5280
F	89.5	59.5	4670
M	76.5	60.0	5140
S	80.0	63.0	5130
U	80.0	60.5	4900
W	75.0	63.0	5220
Geometric mean	78.9	59.8	5060
Log. standard deviation	0.0269	0.0314	0.0186
Percentage standard deviation	6.4%	7.5%	4.3%

Log mean standard deviation 0.0262

Percentage mean standard deviation 6.2%

Table 10 leads to the conclusion that the seven toxins are of the same antigenic quality since it is possible to obtain titers of the three sera which do not differ more than titers obtained with the same toxin.

A comparison of these results with data on the accuracy of the assays of tetanus antitoxin obtained in previous investigations makes it possible to establish the following limits of error (P 0.99) of assay under various conditions:

	Limits of error
Assay with one and the same toxin	87%—115%
Assay with toxins prepared by the same strain	86%—117%
Assay with toxins of various origin	73%—137%

6. The Ratio of Direct and Indirect Toxicity

The seven toxins in question have so far shown identical qualities in all tests to which they have been subjected. A considerable difference was found, however, in the purity of these toxins expressed in the direct toxicity per mg. of dried preparation (mice). It should therefore be of interest, as mentioned in the introduction, to include a comparison of the ratio of direct and indirect toxicity, a quantity which is often referred to as the degree of " toxoiding ".

We shall have to compute this ratio from the m.l.d. for mice and the test dose L+/5. Both values were determined on the same lot of mice, m.l.d. with the accuracy of about 10% and L+/5 with the accuracy of 4%.

Table 11

Ratios of Direct and Indirect Toxicity of the Seven Toxins

Toxin	m.l.d. mg.	L + /5 mg.	Toxicity ratio
B	0.00023	0.41	1780
C	0.000069	0.12	1740
F	0.0076	3.16	415
M	0.00029	0.47	1620
S	0.00032	0.63	1970
U	0.00038	0.47	1240
W	0.00027	0.52	1930

It appears from Table 11 that the toxins, besides being different in toxicity per unit of weight, also are different in their toxicity per unit of antitoxin-combining power. Although the toxicity ratios of the toxins B, C, M, S and W show good agreement, toxins U

and, particularly, F show significantly deviating values — *i.e.*, the toxicity ratios of these two toxins exhibit a greater difference from the mean of the other five toxins than we should expect from the error of assay. Since it was impossible to demonstrate any difference of the toxins in the antitoxin assay, it may be concluded that the difference found in the toxicity ratio has no bearing upon the quality of the toxin in its use as a test toxin in antitoxin assays.

Discussion and Conclusions

In 1938, the Permanent Commission on Biological Standardisation (6) reached the following conclusions on the basis of several investigations :

(1) That the discrepancies observed in the titration of tetanus antitoxin in different laboratories are intrinsic to the reagents and are in no way due to lack of precision of the methods employed ;

(2) That the factor which exerts the greatest influence on the precision of the assay is the toxin used ;

(3) That the variation of the results obtained in different laboratories is due to qualitative differences in tetanus toxins and antitoxins.

The problem of the origin of these qualitative differences in toxins, and the question of how to describe these properties, are the main objects of the present paper. It seems probable that the difference between the toxins is not entirely capricious, since it was possible in seven laboratories to produce toxins of the same quality when the same strain was used. It might be anticipated that the modification in the technique — *e.g.*, in the composition of the medium — would influence the quality of the toxin, but it is of interest to note that a method has been proposed which should make it possible to furnish test toxins of the same quality to all laboratories concerned with the assay and control of tetanus antitoxin.

The heremention experiments show that toxins produced with the same strain are alike, not only in the qualities revealed by the antitoxin assay, but also in other respects — *e.g.*, in their

toxicity to rabbits and mice. This fact supports the assumption, not entirely proven, that the strain is of predominating importance for the quality of a tetanus toxin. The observation should lead to some sort of typing of the strains of *Bac. tetani* with respect to the toxin, and it is possible that the rather simple method of a comparison of the direct toxicity in rabbits, mice or other animals might furnish the basis of such a typing.

This form of typing *Bac. tetani* cannot be expected to follow the typing created by TULLOCK (9), who used, exclusively, the agglutination method and thus referred to a somatic property of the bacillus.

Still, the problem of assaying tetanus antitoxin is not entirely solved by typing the toxins. The manifest difference in the quality of the antitoxins which is revealed, for example, by a determination of the slope of the toxin-combining curve, makes the result of the assay to a certain degree dependent on the method of reading, particularly of the end-point reaction used for comparison with the standard. The question arises, therefore, whether it is at all possible to express the properties of a tetanus antitoxin by a simple indication of units per cubic centimetre.

* * *

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Appendix

TABLE OF $f(T)$

FOR MICE INJECTED INTRAVENOUSLY WITH TETANUS TOXIN

T = death time in hours.

$$f(T) = \log\left[\left(\frac{10.3}{\frac{T}{8}-1}\right)^2 + 1\right]$$

T	$f(T)$	T	$f(T)$	T	$f(T)$	T	$f(T)$	T	$f(T)$	T	$f(T)$	T	$f(T)$	T	$f(T)$
10	3.21	29	1.21	48	0.72	67	0.47	86	0.33	105	0.24	124	0.18	143	0.13
11	2.88	30	1.18	49	0.70	68	0.46	87	0.32	106	0.23	125	0.18	144	0.13
12	2.64	31	1.14	50	0.69	69	0.45	88	0.32	107	0.23	126	0.17	145	0.12
13	2.44	32	1.11	51	0.67	70	0.44	89	0.31	108	0.23	127	0.17	146	0.12
14	2.28	33	1.08	52	0.65	71	0.43	90	0.31	109	0.22	128	0.16	147	0.12
15	2.15	34	1.05	53	0.64	72	0.43	91	0.30	110	0.22	129	0.16	148	0.12
16	2.03	35	1.02	54	0.63	73	0.42	92	0.29	111	0.22	130	0.16	149	0.12
17	1.93	36	0.99	55	0.61	74	0.41	93	0.29	112	0.21	131	0.16	150	0.12
18	1.84	37	0.96	56	0.60	75	0.40	94	0.28	113	0.21	132	0.16	151	0.11
19	1.76	38	0.93	57	0.58	76	0.39	95	0.28	114	0.21	133	0.16	152	0.11
20	1.68	39	0.91	58	0.57	77	0.39	96	0.27	115	0.20	134	0.15	153	0.11
21	1.63	40	0.89	59	0.56	78	0.38	97	0.27	116	0.20	135	0.15	154	0.11
22	1.55	41	0.86	60	0.55	79	0.37	98	0.27	117	0.20	136	0.15	155	0.11
23	1.50	42	0.84	61	0.53	80	0.37	99	0.26	118	0.20	137	0.15	156	0.11
24	1.44	43	0.82	62	0.52	81	0.36	100	0.26	119	0.19	138	0.15	157	0.11
25	1.39	44	0.79	63	0.51	82	0.35	101	0.25	120	0.19	139	0.15	162	0.11
26	1.34	45	0.77	64	0.50	83	0.35	102	0.25	121	0.19	140	0.14		
27	1.30	46	0.76	65	0.49	84	0.34	103	0.24	122	0.18	141	0.14		
28	1.26	47	0.74	66	0.48	85	0.33	104	0.24	123	0.18	142	0.14		

Figure 1.

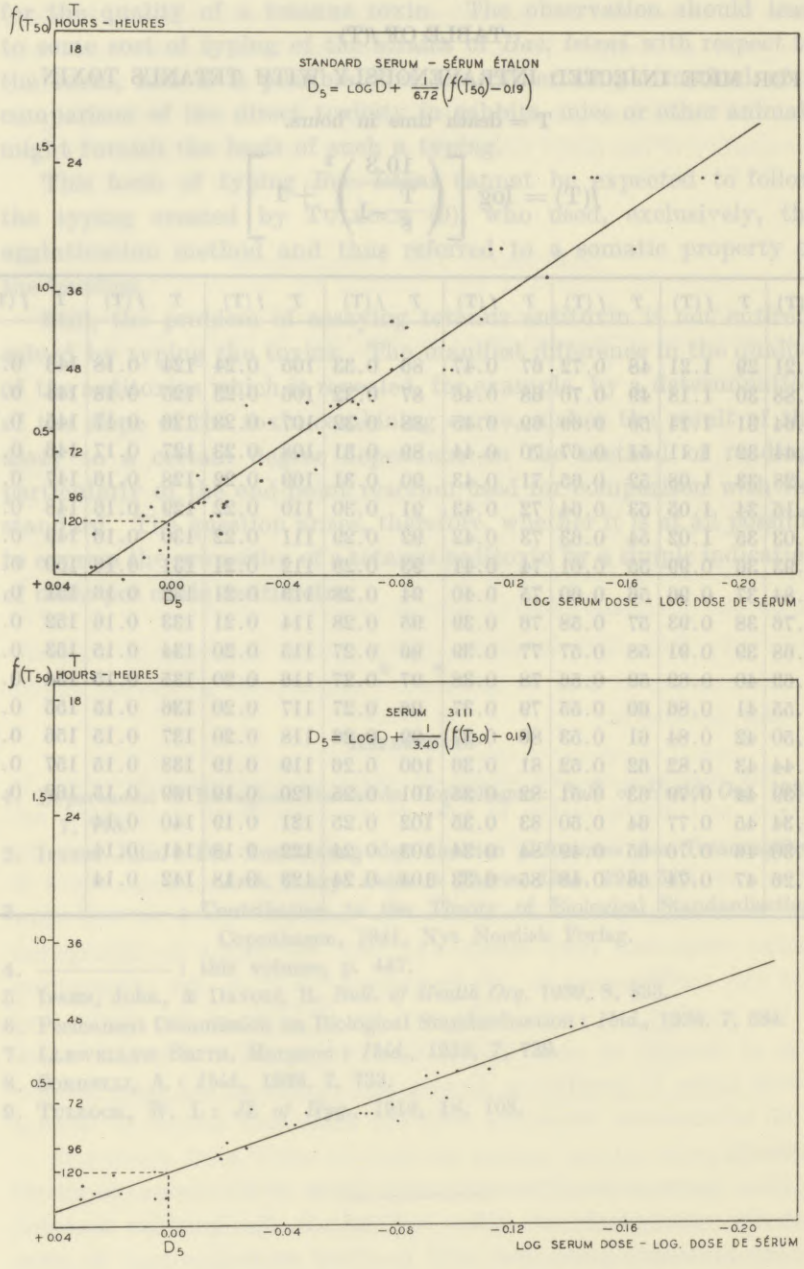
f(T50) T HOURS - HEURES
STANDARD SERUM - SÉRUM ÉTALON
D5 = LOG D + 1/6.72 (f(T50) − 0.19)
18
1.5
24
1.0
36
48
0.5
72
96
120
+ 0.04
0.00
− 0.04
− 0.08
− 0.12
− 0.16
− 0.20
D5
LOG SERUM DOSE - LOG. DOSE DE SÉRUM
f(T50) T HOURS - HEURES
SERUM 3111
D5 = LOG D + 1/3.40 (f(T50) − 0.19)
18
1.5
24
1.0
36
48
0.5
72
96
120
+ 0.04
0.00
− 0.04
− 0.08
− 0.12
− 0.16
− 0.20
D5
LOG SERUM DOSE - LOG. DOSE DE SÉRUM

Figure 1.

Reaction Curves of Standard Serum and Serum 3111

Mixtures of the same dose of toxin and varying doses of antitoxin are injected into groups of mice. The mean death time (T50) of each group is transformed into the function f (T50) which is proportional to the logarithm of free toxin. f (T50) is plotted against log (serum dose) and the curves obtained from the experiments with different toxins are parallel-displaced along the axis of abscissæ in such a way that the logarithm of the serum dose giving a mean death time of 120 hours (D_5) is represented by the same abscissa for all curves.

It should be noted that the slopes of the reaction curves of the two sera are different, and that the reaction points (T50) deviate more from the reaction line in the case of the standard serum than they do in the case of serum 3111.

ON THE STANDARDISATION OF AFRICAN VIPER ("BITIS ARIETANS") AND CAPE COBRA ("NAIA FLAVA") ANTIVENENES

by

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In October 1938, the Permanent Commission on Biological Standardisation suggested that the possibilities of standardising antivenenes other than the anti-European viper serum should be investigated.[1] Accordingly, our research work has been directed towards the standardisation of the serum against the venom of the African viper, *Bitis arietans* (puff adder), our assays being based on the method of BANIC and LJUBETIC,[2] as modified by IPSEN.[3] Satisfactory results having been obtained, we have applied the same method to the assay of Cape cobra antiserum and of the polyvalent viper-cobra antivenene which is prepared at the South-African Institute for Medical Research.

1. ASSAY OF THE MONOVALENT "BITIS ARIETANS" ANTISERUM

The serum submitted to titration originated from a horse immunised early in 1935 by means of *Bitis arietans* anavenom, the bleeding being carried out in April of the same year. One part

[1] Report of the Permanent Commission on Biological Standardisation. *Bull. Health. Org.*, 1938, 7, 683.

[2] M. BANIC and T. LJUBETIC : " Die Titration eines Serums gegen Schlangengift an Maüsen (Serum antivipera ammodytes). " *Zeitschr. f. Hyg.*, 1938, 120, 390.

[3] J. IPSEN : Progress Report on the Possibility of standardising Anti-snake-venom Sera. *Bull. Health Org.*, 1938, 7, 785.

of this serum was desiccated whilst the other was kept liquid, without addition of any antiseptic, and in the dark at 2° to 4° C. In the assays we performed in the course of recent years, this same serum has been regularly used as standard.

In order to avoid possible variations of toxicity between samples of venom from various animals of the same species and to enable us to work for several years under conditions remaining as similar as possible, we have made use of a *Bitis arietans* standard venom. At a previous meeting of the Commission, we had already emphasised the importance which attaches, in the standardisation of antivenenes, to the use of a standard venom. This view was shared by Ipsen in the report he submitted to the Commission on the possibility of standardising anti-snake-venom sera.[1]

This standard venom, the weight of which amounted to about 20 g., was obtained by mixing various samples of dried venom from some 130 specimens of *Bitis arietans* captured in the Cape Province, the Transvaal, the Orange Free State and Natal. After powdering, it was distributed into ampoules and kept in the dark.

The quantity of standard venom necessary for the assays was weighed and dissolved in 9 per mille saline, so as to obtain a mother solution containing 10 mg. venom per cc.

This mother solution was kept for an hour at room temperature during which time the main part of the insoluble substances, which are always present in *Bitis arietans* venom, settled at the bottom of the container. The venom solution was then centrifugalised. Thanks to this procedure, we succeeded in practically eliminating the deaths due to shock which occur when venom still containing insoluble particles is introduced by the intravenous route. The dilutions needed for the assays were obtained from the mother solution. In all our experiments, the venom was utilised within a few hours after dissolving.

The certainly lethal dose was established by injecting intravenously into mice weighing 20 g., and originating from the same batch, increasing quantities of standard venom contained in a constant volume of 0.5 cc., the difference between the doses amounting to 0.01 mg. of venom.

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[1] J. Ipsen : *Loc. cit.*

Repeated assays, in which up to 10 mice were sometimes used for the same dose, showed that, for the standard venom of *Bitis arietans*, the dose killing all mice within 5 to 7 hours was 0,02 mg. The animals succumb with asphyctic symptoms, sometimes accompanied by unco-ordinated movements and convulsions. Shortly before death, the expulsion of a sanguinolent liquid through the respiratory tract may be observed. Smaller doses of venom, such as 0.018 or 0.016 mg., may bring about the death of a certain proportion of mice in from 4 to 48 hours; with 0.012 mg. all survive.

According to Ipsen's modification of the method of Banic and Ljubetic, we assayed the *Bitis arietans* antiserum at various levels and plotted the result of each observation in a graph, the ordinates of which represent the volumes of serum (in cc.) and the abscissæ the quantities of venom (in mg.) contained in the mixtures. The latter are brought to a volume of 0.5 cc. by addition of 9 per mille saline. After one hour's contact at 37° C., they are injected intravenously into mice weighing 20 g. Comparative tests in which the mixtures were administered either at once or after one hour's contact either at room temperature or at 37° C. showed that it is the latter technique which gives the most regular results.

The period during which the results — death or survival — were noted was 48 hours; a shorter observation period (30 minutes) had been adopted for the assays pertaining to the standardisation of the anti-European-viper serum, but, in the case of the *Bitis arietans* antiserum, our experiments showed that much advantage could be derived from the adoption of a considerably longer period. The deaths occurring after 48 hours would not appear to influence the results to any considerable extent.

The procedure followed was:

1. Establishment of a graph in which the result for each mouse is plotted in a system of co-ordinates, the abscissæ of which represent the quantity of venom and the ordinates the volume of serum contained in the injected dose.

2. Determination of the certainly lethal dose of venom. This value is obtained as indicated above, the quantities of venom used varying between 0.016 and 0.022 mg. We have already seen that,

for the *Bitis arietans* standard venom used, the c.l.d. was 0.02 mg.

3. Injection into mice of mixtures containing a fixed dose of serum (0.4 cc. which corresponds to the highest level included in the graph) and of increasing quantities of venom : 0.1 mg., 0.15 mg., 0.2 mg., 0.25 mg., 0.3 mg., 0.35 mg., 0.4 mg., 0.5 mg. ; each mixture is injected into two mice. The amount of 0.4 cc. of serum is the largest quantity which can be used if the constant volume of 0.5 cc. of the mixture which is taken as the maximum dose of liquid to be injected intravenously into mice is not to be exceeded after the venom dilutions have been added.

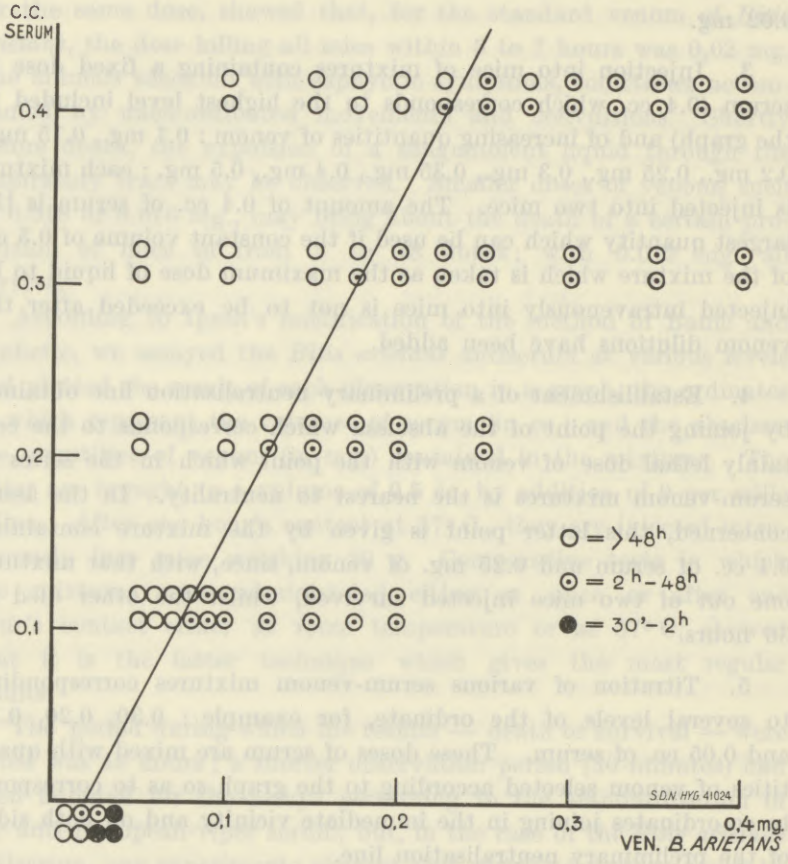
4. Establishment of a preliminary neutralisation line obtained by joining the point of the abscissa which corresponds to the certainly lethal dose of venom with the point which in the series of serum-venom mixtures is the nearest to neutrality. In the assay concerned, this latter point is given by the mixture containing 0.4 cc. of serum and 0.25 mg. of venom, since, with that mixture, one out of two mice injected survived, whilst the other died in 36 hours.

5. Titration of various serum-venom mixtures corresponding to several levels of the ordinate, for example : 0.30, 0.20, 0.10 and 0.05 cc. of serum. These doses of serum are mixed with quantities of venom selected according to the graph so as to correspond to co-ordinates joining in the immediate vicinity and on both sides of the preliminary neutralisation line.

6. Plotting on the graph the results of these assays ; it is thus possible to draw the final neutralisation line which separates at the various levels the animals which survived from those which died.

For the *Bitis arietans* antiserum assayed, the joining of the points nearest to neutralisation (one mouse having died out of two) at various levels gives a straight line which practically coincides with the preliminary neutralisation line.

Graph No 1.



7. Computation of the serum titre according to the formula of Banic as adapted by Ipsen. The use of this formula excludes the natural resistance of the test animal, a factor which can be responsible for the neutralisation of the main portion (about four-fifths of 1 c.l.d.) of the venom introduced. The formula is as follows :

$$\text{Quantity of venom neutralised by 1 cc. of antivenene} = \frac{\text{Amount of venom} - 1 \text{ c.l.d.}}{\text{Amount of serum} - \text{dose S 1}}$$

S 1 representing the dose of serum which protects the mouse against 1 c.l.d. of venom.

For the calculation of the neutralising power of an antivenene, assayed at various levels, two points situated on the neutralisation line are selected. The difference between the corresponding quantities of venom is divided by the difference between the corresponding doses of serum. The figure thus obtained gives the neutralising titre of the serum expressed in mg. of venom per cc. of serum.

Thus, for the *Bitis arietans* monovalent antiserum, by introducing in the formula the results corresponding to the 0.2 and 0.4 cc. serum levels, the titre will be equal to :

$$\frac{0.225 - 0.125}{0.4 - 0.2} = \frac{0.1}{0.2} = 0.5 \text{ mg.}$$

1 cc. of serum accordingly neutralises 0.5 mg. of venom.

By introducing in the formula the figures corresponding to the various titration levels, according to the possible combinations, the following results are obtained :

$$\frac{0.225 - 0.175}{0.4 - 0.3} = \frac{0.05}{0.1} = 0.5 \text{ mg.}$$

$$\frac{0.225 - 0.08}{0.4 - 0.1} = \frac{0.145}{0.3} = 0.483$$

$$\frac{0.175 - 0.125}{0.3 - 0.2} = \frac{0.05}{0.1} = 0.5$$

$$\frac{0.175 - 0.08}{0.3 - 0.1} = \frac{0.095}{0.2} = 0.475$$

$$\frac{0.125 - 0.08}{0.2 - 0.1} = \frac{0.045}{0.1} = 0.45$$

The extreme values being 0.45 mg. and 0.5 mg., the difference between them is 10%. The mean titre calculated from the complete set of results is 0.481 mg.

An analysis of these computations shows that a similar result — viz., 0.5 mg. — is arrived at when the neutralisation points corresponding to the levels 0.4, 0.3 and 0.2 cc. of serum are considered. Divergences appear, however, if the neutralisation point corresponding to the lowest level — namely, 0.1 cc. of serum — is embodied in the calculation. The theoretical neutralisation point for this level, obtained by prolonging the neutralisation line traced for the higher levels, corresponds to 0.075 mg. of venom — *i.e.*, a point

situated at equal distance between the result obtained experimentally (0.08 mg.) and that given by the mixture containing 0.07 mg. of venom.

By substituting in the above calculations the theoretical figure of 0.075 mg. for the experimental value of 0.08 mg., a uniform titre of 0.5 mg. is obtained, irrespective of the points on the neutralisation line which are taken into account.

Thus, for example:

$$\frac{0.175 - 0.075}{0.3 - 0.1} = \frac{0.1}{0.2} = 0.5 \text{ mg.}$$

Thus, the difference between the experimental and the theoretical result does not exceed 0.005 mg.

Having regard to the experimental factor — which is particularly important when dealing with antivenenes — and the small number of mice (2) receiving each mixture, the divergence of 10% observed at one level out of four does not appear excessive. It is even reduced to 4.8% if the mean of the various assays (0.481 mg.) is taken as the final titre.

As the scale of mixtures selected for the assay at the lowest level (0.1 cc. of serum) is already very narrow — the increase in the quantity of venom from one mixture to the other being only 0.01 mg. — the divergence observed could be eliminated only by the use of a still narrower scale of mixtures, differing between themselves by 0.005 mg. of venom. From the practical viewpoint, it is questionable whether the appreciable increase in laboratory animals and reagents that such a procedure would entail could be justified.

These experiments show that the Banic and Ljubetic method, as modified by Ipsen, which was proposed for the assay of the European viper antiserum, is applicable to the titration of the African viper *(Bitis arietans)* antiserum. When effected by means of the formula established by these authors, the computation of the titre of the serum furnishes an exact determination of its neutralising properties expressed in mg. of venom per cc. of serum.

2. Assay of the "Bitis arietans" Antibody contained in Polyvalent "Bitis arietans–Naia flava" Antivenene

The satisfactory results described above induced us to apply the same method to the assay of the viper antibody contained in the concentrated and purified *Bitis arietans–Naia flava* antivenene prepared at the South-African Institute for Medical Research.

This serum is obtained from horses immunised and re-immunised by means of formolised *Bitis arietans* and *Naia flava* anavenenes, according to the technique we described in 1931 [1] and have followed since then for the preparation of this serum [2].

The concentration of the serum is effected by fractional precipitation, the proportion of viper and cobra antibodies thus obtained being from three to four times greater than in the original serum, as we have been able to show [3]. 0.2% of chinosol is added to the serum.

For the titration of the viper antibody contained in the polyvalent serum S. 2732, prepared on March 3rd, 1939, we used the same technique as that followed for the assay of monovalent *Bitis arietans* antiserum.

In order to obtain an idea of the regularity and accuracy of the results in the case of a concentrated polyvalent serum, we made use of more extended scales of mixtures than would be necessary in practice. The mixtures were kept for one hour at 37° C. and then injected intravenously into mice weighing 20 g., the observation period being 48 hours.

---

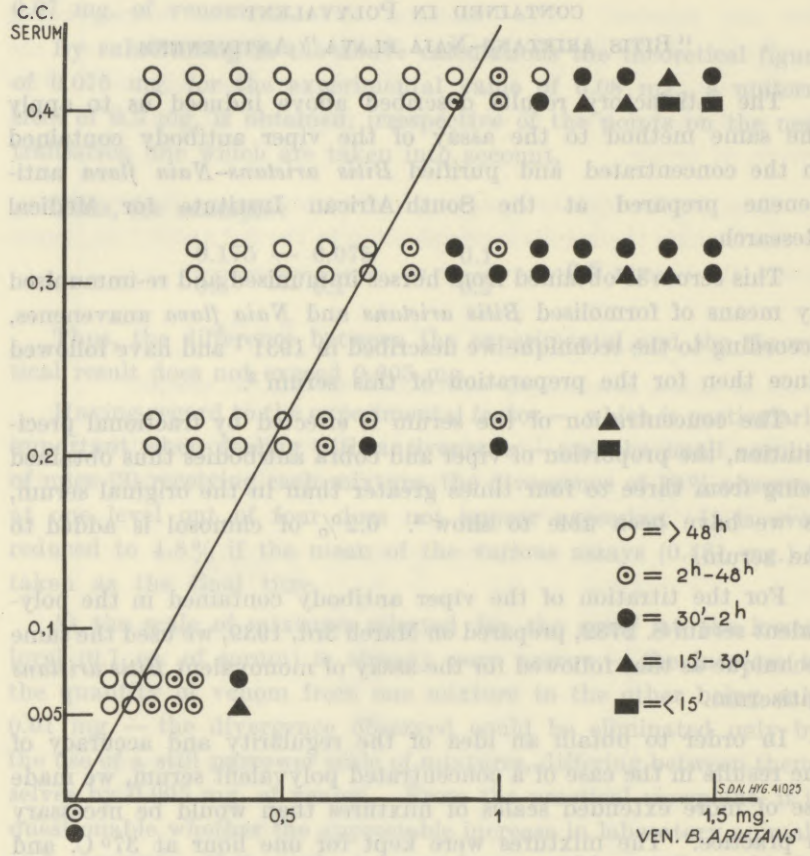
[1] E. Grasset and A. Zoutendyk : « Méthode rapide de préparation de sérums antivenimeux polyvalents — antivipéridés et cobras — au moyen des anavenins formolés. » *C.R. Soc. de Biol.*, 1932, 111, 432.

[2] E. Grasset and A. Zoutendyk : "Detoxication of Snake Venoms and the Application of Resulting Antigens to Rapid Methods of Antivenomous Vaccination and Serum Production." *Brit. Jl. Exp. Path.*, 1933, 14, 308.

[3] E. Grasset : "Concentration of Polyvalent African Antivenom Serum." *Trans. R. Soc. Trop. Med. and Hyg.*, 1932, 26, 267.

The results of this assay are plotted in the following graph :

Graph No 2.



From this graph it appears that, for the concentrated polyvalent serum, as in the case of the monovalent serum, the method at various levels applied to the titration of the pseudoglobulin makes it possible to establish a neutralisation line. Actually, for the serum-venom mixtures corresponding to the four levels considered, the points closest to neutrality are arranged on both sides of that neutralisation line.

The titre of the serum obtained by introducing in the above-mentioned formula the neutralisation points determined for two different titration levels (0.4 and 0.2 cc. of serum) is :

$$\frac{0.9 - 0.5}{0.4 - 0.2} = \frac{0.4}{0.2} = 2 \text{ mg.}$$

Further, the absolute titre can be calculated as follows by selecting any two points on the neutralisation line :

$$\frac{0.7 - 0.15}{0.3 - 0.05} = \frac{0.55}{0.25} = 2.2 \text{ mg.}$$

There is therefore a divergence of 10% between the calculated titre and the experimentally determined titre.

This experiment shows that the modified Banic method is applicable, with a satisfactory degree of accuracy, to the assay of the *Bitis* antibody contained in the purified pseudoglobulin of a concentrated polyvalent viper-cobra antivenene.

In the assay, extended scales, both of serum and venom (up to 45 c.l.d.), were used ; this tends to reduce to a minimum the probabilities of errors, such as those linked with the use of small doses of venom or serum ; further, the method applied eliminates the role played by the natural and individual resistance of the test animals.

In order to reduce to a minimum the number of animals required, we carried out titrations by the same technique, but using one mouse only for each mixture. In this way, from 20 to 25 animals were required instead of 50.

A first assay at a high serum level, such as 0.3 or 0.4 cc., enables the preliminary neutralisation line to be drawn. The second assay, performed 48 hours later, is made at four or five levels (including that selected for the preliminary assay) with short scales of mixtures chosen so as to correspond to points situated in the immediate vicinity of the preliminary neutralisation line.

By means of these two successive assays, it is possible to titrate within four days the neutralising power of a commercial concentrated polyvalent *Bitis arietans - Naia flava* antiserum, the degree of accuracy reached being practically the same as in the case of a monovalent serum.

3. Assay of the Monovalent "Naia flava" Antiserum

We further endeavoured to ascertain whether the modified Banic method was applicable to the titration of a monovalent *Naia flava* antiserum. Our experiments were made with the serum of horse 322, bled on December 12th, 1938. This serum was to

replace our former standard serum, the stock of which was nearing exhaustion. The horse had been immunised with *Naia flava* anavenom, formolised at 0.8% and detoxicated in Martin's broth, according to the procedure we described [1].

The venom used in this assay was a standard *Naia flava* venom prepared in 1936 ; the stock, amounting to some 20 g., had been obtained from over 150 specimens captured during 1934 and 1935 in Southern Africa. This venom, after powdering, was distributed into ampoules and kept in the dark. It served in all the standardisation experiments we carried out during the last five years. Its activity, assessed by the minimum lethal dose for a mouse or in terms of the standard serum, remained unchanged throughout the period (1939-1940) during which our research work was carried out.

Our experiments were made along the same lines as those followed for the titration of the *Bitis arietans* antiserum : determination of the c.l.d. of cobra venom — viz., the assay of the serum at various levels and the drawing of a neutralisation line ; one hour's contact at 37° C., intravenous injection of 0.5 cc. of each mixture into two mice weighing 20 g. ; an observation period of 48 hours.

Using venom doses, differing from one another by 0.001 mg., and inoculating 10 mice per dose, we ascertained that the c.l.d. was 0.013 mg. (death within 5-7 hours).

In the preliminary assay, the mixtures contained 0.3 cc. of serum and venom doses increasing from 0.04 mg. to 0.12 mg. The neutralisation point was approximately reached with the mixture containing 0.09 mg. The preliminary neutralisation line was obtained by joining this point with the point on the abscissa corresponding to 1 c.l.d. of venom — *i.e.*, 0.013 mg.

The subsequent assays were made at various levels — viz., 0.05, 0.1, 0.2, 0.3 cc. of serum — the mixtures being so selected as to yield results on both sides of the neutralisation line.

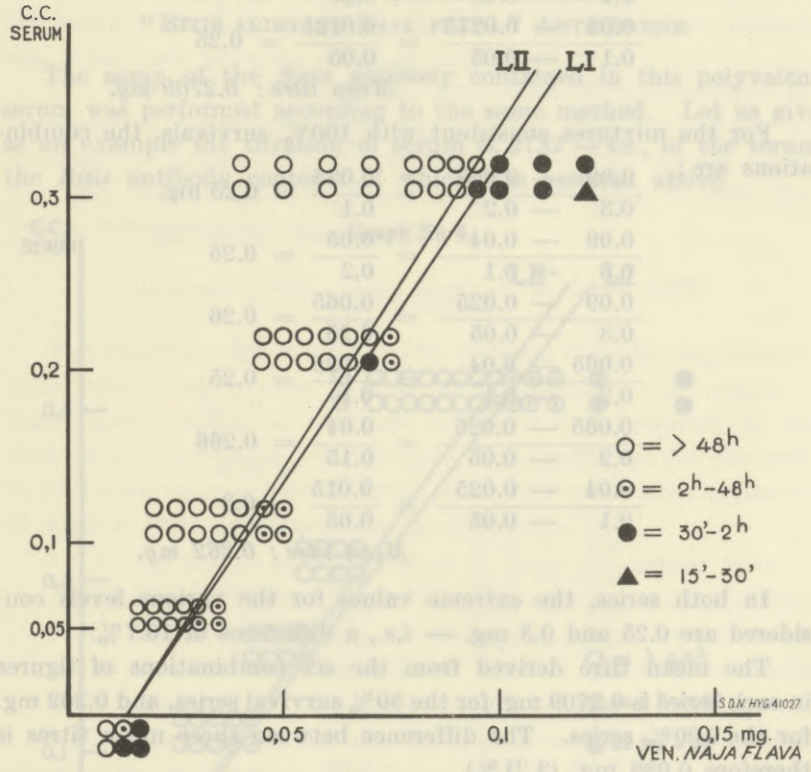
---

[1] E. Grasset and A. Zoutendyk: "Méthode rapide de préparation de sérums antivenimeux polyvalents — antivipéridés et cobras — au moyen des anavenins formolés." *C.R. Soc. de Biol.*, 1932, 111, 432.

For the assays performed at the levels of 0.3, 0.2 and 0.1 cc. of serum, the amount of venom increased by 0.005 mg. from one mixture to another ; for the assays at the 0.05 cc. serum level, the venom increase was only 0.0025 mg.

The following graph shows that, for the *Naia flava* antiserum, as was the case for the *Bitis arietans* antiserum, the neutralisation line obtained separates the surviving animals, shown on the left of the line, from those which died, shown on the right ; and this holds good whatever be the level of the assay.

Graph No 3.



In order to judge of the accuracy of the method, we made a double series of calculations : one based on the mixtures with which 50% of the test animals survived and the other based on the mixtures containing the largest dose of venom consistent with 100% survivals.

If the formula mentioned above is applied to the mixtures with which 50 % of the animals survive, the following combinations of figures are possible :

$$\frac{0.095 - 0.07}{0.3 - 0.2} = \frac{0.025}{0.1} = 0.25 \text{ mg.}$$

$$\frac{0.095 - 0.04}{0.3 - 0.1} = \frac{0.055}{0.2} = 0.275$$

$$\frac{0.095 - 0.0275}{0.3 - 0.05} = \frac{0.0675}{0.25} = 0.27$$

$$\frac{0.07 - 0.04}{0.2 - 0.1} = \frac{0.03}{0.1} = 0.3$$

$$\frac{0.07 - 0.0275}{0.2 - 0.05} = \frac{0.0425}{0.15} = 0.28$$

$$\frac{0.04 - 0.0275}{0.1 - 0.05} = \frac{0.0125}{0.05} = 0.25$$

Mean titre : 0.2709 mg.

For the mixtures consistent with 100 % survivals, the combinations are :

$$\frac{0.09 - 0.065}{0.3 - 0.2} = \frac{0.025}{0.1} = 0.25 \text{ mg.}$$

$$\frac{0.09 - 0.04}{0.3 - 0.1} = \frac{0.05}{0.2} = 0.25$$

$$\frac{0.09 - 0.025}{0.3 - 0.05} = \frac{0.065}{0.25} = 0.26$$

$$\frac{0.065 - 0.04}{0.2 - 0.1} = \frac{0.025}{0.1} = 0.25$$

$$\frac{0.065 - 0.025}{0.2 - 0.05} = \frac{0.04}{0.15} = 0.266$$

$$\frac{0.04 - 0.025}{0.1 - 0.05} = \frac{0.015}{0.05} = 0.3$$

Mean titre : 0.262 mg.

In both series, the extreme values for the various levels considered are 0.25 and 0.3 mg. — *i.e.*, a difference of 16.7 %.

The mean titre derived from the six combinations of figures in each series is 0.2709 mg. for the 50 % survival series, and 0.262 mg. for the 100 % series. The difference between these mean titres is therefore 0.089 mg. (3.21 %).

If we calculate the theoretical value representing the absolute titre, irrespective of the point considered on the neutralisation line, we obtain 0.275 mg. for the line I corresponding to 50 % survivals. For this same series, the mean titre obtained from the various assays is 0.2709 mg. This titre is therefore practically identical with the absolute value deduced from the neutralisation line.

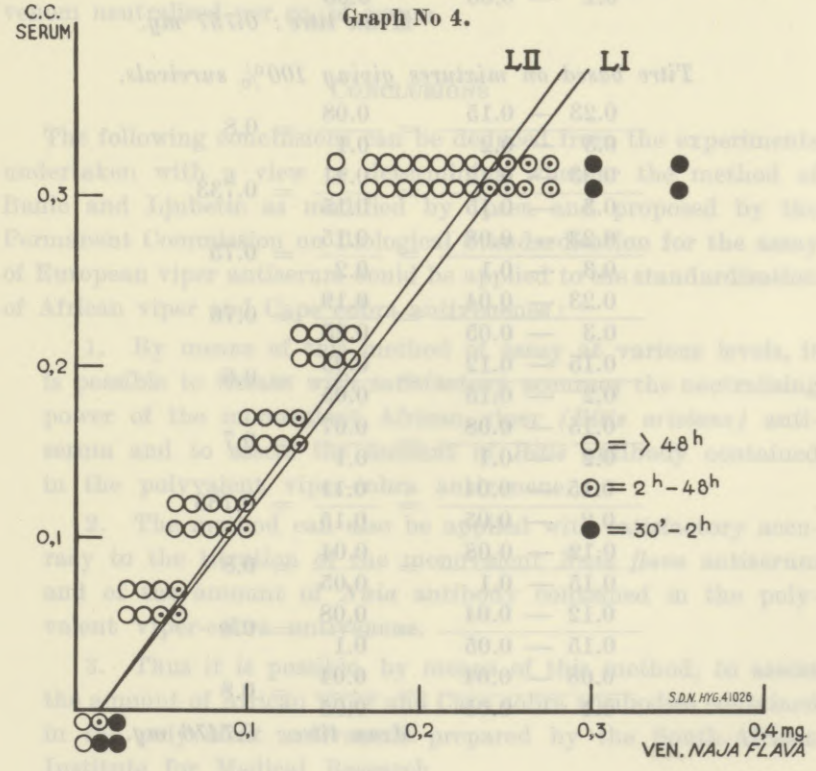
For the series with 100% survivals, the absolute titre is 0.26 mg. and the mean titre deduced from assays carried out at four levels is 0.262 mg. (line II). Here again, the titre obtained experimentally tallies for all practical purposes with the absolute titre.

These experiments show that the modified Banic method is applicable to the assay of anticobra serum *(Naia flava)* as it is to that of antiviper serum. The neutralising power of the serum is expressed in mg. of venom per cc. of serum.

4. Assay of the "Naia flava" Antibody contained in the Concentrated Polyvalent "Bitis arietans-Naia flava" Antivenene

The assay of the *Naia* antibody contained in this polyvalent serum was performed according to the same method. Let us give as an example the titration of serum S. 2732 — *i.e.*, of the serum the *Bitis* antibody contents of which was assessed above.

Graph No 4.



The titration was made at five different levels (0.05, 0.1, 0.15, 0.2 and 0.3 cc. of serum) and the amount of venom increased from one mixture to another by 0.01 mg.

As in the case of the monovalent serum, we effected a double calculation, one based on 50%, the other on 100% survivals.

Titre based on mixtures giving 50% survivals.

$$\frac{0.24 - 0.16}{0.3 - 0.2} = \frac{0.08}{0.1} = 0.8$$

$$\frac{0.24 - 0.09}{0.3 - 0.1} = \frac{0.15}{0.2} = 0.75$$

$$\frac{0.24 - 0.05}{0.3 - 0.05} = \frac{0.19}{0.25} = 0.76$$

$$\frac{0.16 - 0.09}{0.2 - 0.1} = \frac{0.07}{0.1} = 0.7$$

$$\frac{0.16 - 0.05}{0.2 - 0.05} = \frac{0.11}{0.15} = 0.733$$

$$\frac{0.09 - 0.05}{0.1 - 0.05} = \frac{0.04}{0.05} = 0.8$$

Mean titre : 0.757 mg.

Titre based on mixtures giving 100% survivals.

$$\frac{0.23 - 0.15}{0.3 - 0.2} = \frac{0.08}{0.1} = 0.8$$

$$\frac{0.23 - 0.12}{0.3 - 0.15} = \frac{0.11}{0.15} = 0.733$$

$$\frac{0.23 - 0.08}{0.3 - 0.1} = \frac{0.15}{0.2} = 0.75$$

$$\frac{0.23 - 0.04}{0.3 - 0.05} = \frac{0.19}{0.25} = 0.76$$

$$\frac{0.15 - 0.12}{0.2 - 0.15} = \frac{0.03}{0.05} = 0.6$$

$$\frac{0.15 - 0.08}{0.2 - 0.1} = \frac{0.07}{0.1} = 0.7$$

$$\frac{0.15 - 0.04}{0.2 - 0.05} = \frac{0.11}{0.15} = 0.733$$

$$\frac{0.12 - 0.08}{0.15 - 0.1} = \frac{0.04}{0.05} = 0.8$$

$$\frac{0.12 - 0.04}{0.15 - 0.05} = \frac{0.08}{0.1} = 0.8$$

$$\frac{0.08 - 0.04}{0.1 - 0.05} = \frac{0.04}{0.05} = 0.8$$

Mean titre : 0.7476 mg.

For the 50% survivals series, the mean titre is 0.757 mg. and the absolute titre, calculated from the points corresponding to the levels 0.3 and 0.05 cc. of serum, is 0.76 mg. The difference of 0.003 mg. (0.4%) between these two results is practically negligible.

For the 100% survivals series, the mean titre, deduced from the ten experimental results, is 0.7476 mg., whilst the absolute titre calculated from the points on the neutralisation line corresponding to the levels 0.3 and 0.15 cc. of serum is 0.733 mg. — *i.e.*, a difference of 0.0146 mg. (2.4%).

These computations show that the titre offering the highest degree of accuracy is, as might logically be expected, that given by the series with 50% survivals, for which the divergence between the mean and absolute titres is only 0.4%.

The neutralising power of the *Naia* antibody contained in the concentrated polyvalent *Bitis arietans-Naia flava* antivenene can therefore be assessed by the method of assay at various levels. The titre thus obtained expresses the number of mg. of *Naia flava* venom neutralised per cc. of serum.

Conclusions

The following conclusions can be deduced from the experiments undertaken with a view to determining whether the method of Banic and Ljubetic as modified by Ipsen and proposed by the Permanent Commission on Biological Standardisation for the assay of European viper antiserum could be applied to the standardisation of African viper and Cape cobra antivenenes :

1. By means of this method of assay at various levels, it is possible to titrate with satisfactory accuracy the neutralising power of the monovalent African viper *(Bitis arietans)* antiserum and to assess the amount of *Bitis* antibody contained in the polyvalent viper-cobra antivenene.

2. The method can also be applied with satisfactory accuracy to the titration of the monovalent *Naia flava* antiserum and of the amount of *Naia* antibody contained in the polyvalent viper-cobra antivenene.

3. Thus it is possible, by means of this method, to assess the amount of African viper and Cape cobra antibodies contained in the polyvalent antivenene prepared by the South-African Institute for Medical Research.

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Volume IX, No. 1 Year 1940

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