Annals of Tropical Medicine and Parasitology

THE UNIVERSITY OF LIVERPOOL

ANNALS

OF

TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by

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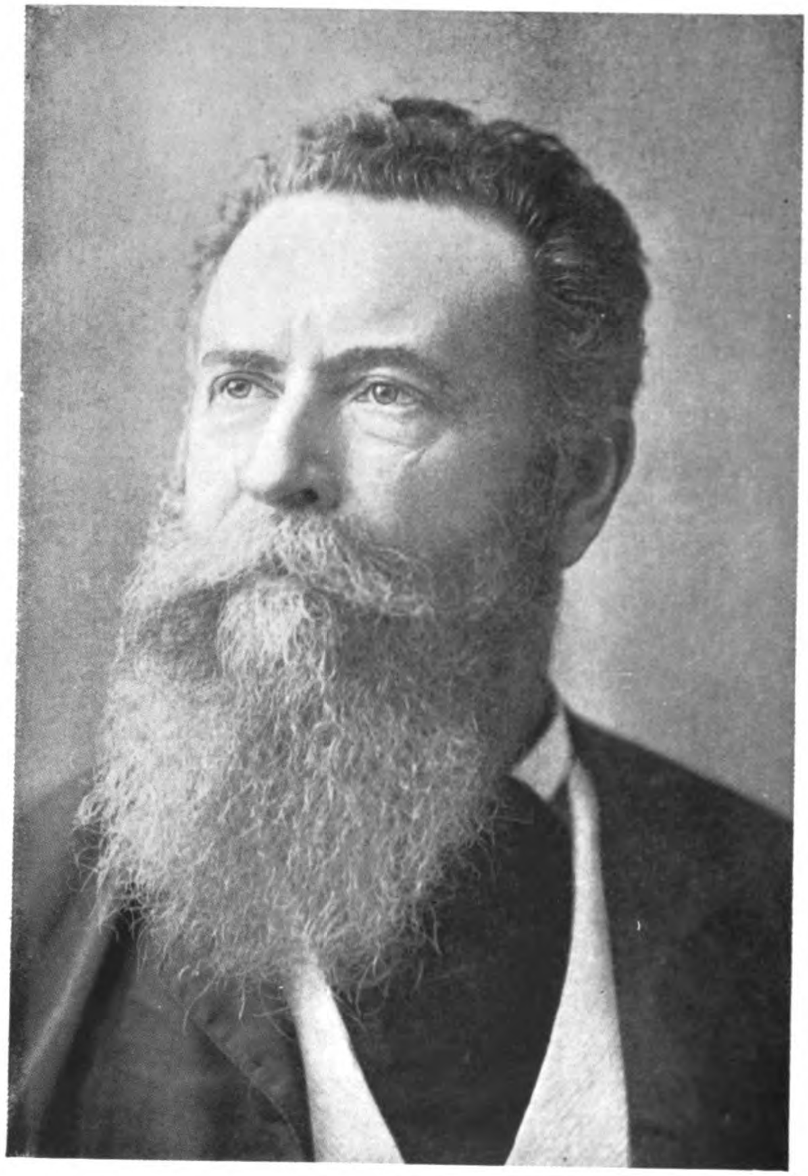

Joseph Bancroft

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All lettering, names or legends on text-figures, charts or maps should be printed *sufficiently large* to allow of *clear legibility* on reduction if necessary.

Plates and illustrations should be accompanied by short explanations.

References to authors in the text must be made in the following way:—'According to Smith (1900) the spleen is enlarged, but Robinson (1914) says the reverse.' The references should be collected in alphabetical order of authors' surnames at the end of the paper, and arranged in the following way:—

ROBINSON, S. (1914). 'The spleen in malaria.' *Annals of Nosology*, Vol. XX, pp. 20-25.

SMITH, J. (1900). 'Enlargement of the spleen in malaria.' *Journal of Pathometry*, Vol. I, pp. 1-20.

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A CONTRIBUTION TO THE QUESTION OF THE NUMBER OF RACES IN THE SPECIES *ENTAMOEBA HISTOLYTICA*

BY

A. MALINS SMITH, M.A., CANTAB.

From the Liverpool School of Tropical Medicine

(*Received for publication January* 31, 1919)

In the course of a recent paper (Smith, 1918) I discussed tentatively the idea put forward by Wenyon and O'Connor (1917) and by Dobell and Jepps (1918) that the species *Entamoeba histolytica* is composed of races distinguishable from each other by the dimensions of their cysts. I gave reasons for the conclusion that two races, the 'small' (average diameter $7{\cdot}7\mu$) and the 'ordinary' (average diameter $12{\cdot}6\mu$) certainly exist, but that the evidence for further races was insufficient. It was shown that the proof of the existence of such further races depended upon the measurement of large samples of a considerable number of infections. In my measurements the samples taken were too small, being usually fewer than fifty cysts, whereas Dobell and Jepps (1918) measured very large samples (five hundred cysts), but the number of infections they examined (seven) was small.

In order to obtain more complete data for the solution of this question I have measured fairly large samples (one hundred at least) of a considerable number of infections, twenty in all, and the results are set forth in the present paper. In addition to these samples of 100 or more, samples of fifty to one hundred were measured in thirteen cases, and some evidence is also drawn from these measurements. All the measurements are of cysts belonging to the 'ordinary' strain, no 'small' cysts having been measured. The methods of measurement were the same as those used in my previous paper (Smith, 1918).

Before the results of these measurements can be accurately considered, a preliminary question must be dealt with, namely whether the average size of the cysts of an infection remains constant from one day to another. Dobell and Jepps (1918) say 'For the complete demonstration of this fact (the existence of strains), however, it is necessary to prove that the mean diameter of the cysts from any patient is not subject to any considerable variation from day to day, but remains constant.' They state that this constancy is a fact which is confirmed daily in the course of routine examinations. Measurements in support of it are brought forward only in one case, their E42, in which five hundred cysts measured on May 21st, 1917, gave a mean diameter of 12·99μ, five hundred cysts measured on June 23rd, 1917, had a mean diameter of 13·24μ, giving a difference of 0·25μ. Some evidence will be brought forward later as to whether this is really a negligible difference in so large a sample. At present it may be remarked that the evidence by actual measurement is only given for one infection, and can scarcely therefore sufficiently establish the theory of general constancy in the average size of the cysts of infections. It is necessary to measure the cysts of a larger number of infections for this purpose. In Table I are shown the results of the measurement of samples of one hundred cysts (with two exceptions, viz., seventy-five and seventy-eight respectively) on different dates in eight different infections.

It will be seen that while the agreement between the results on different dates is in some cases close, in others it is by no means so, and it is not possible to tell by mere inspection what conclusion should be drawn from the results. It is first of all necessary to know the error of sampling, i.e. what variations are to be expected in measurements of random samples of one hundred from the same stool. The following data refer chiefly to the results of measurements of successive samples of fifty cysts from the same stool, but it will be seen later that deductions can be drawn from them as to samples of one hundred cysts. In every case where one hundred cysts were measured the difference between the average size of the first fifty and the second fifty was recorded. In cases where two hundred cysts were measured from the same stool there were, of course, four lots of fifty, and this gave six different numbers for the differences between fifties. Where three hundred were examined containing six fifties, there were fifteen differences to be obtained. In this way from

TABLE I.

Case	Date of stool	Dates of emetine treatment	No. measured	Average diam. (μ) of each 100 cysts	Differences (μ)
9	27.7.18	1.6.18 to 12.6.18 (inclusive)	300	12.83, 12·56, 12·53	0·33, 0·06, 0·03
	15.8.18		100	12·45	0·38, 0·11, 0·08
	10.10.18		100	12·50	0·05
11	3.5.18	3.5.18 to 14.5.18 (inclusive)	200	12·24, 12·06	0·29, 0·11, 0·10 0·08, 0·19
	14.5.18		100	11·95	***1·19, 1·08*** ***1·00, 0·90***
	21.5.18		100	12·14	***1·40, 1·59*** ***1·30, 1·07***
	30.7.18		300	13·31, 13·54, 13·14	***1·48, 1·17*** ***1·36, 1·25***
16	19.4.18	19.4.18 to 30.4.18 (inclusive)	100	13·13	0·02
	7.5.18		100	13·15	
10	3.4.18	7.4.18 to 18.4.18 (inclusive)	100	13·12	0·00
	19.4.18		100	13·12	***0·85***
	30·4·18		100	12·27	***0·76***
	4.5.18		100	12·36	0·09
17	4.4.18	25.3.18 to 5.4.18 (inclusive)	100	13·31	0·07
	9.4.18		100	12·91	0·40
	25.4.18		100	13·24	0·33
13	5.4.18	25.3.18 to 5.4.18 (inclusive)	100	12·80	
	6.4.18		100	13·27	***0·47***
12	4.3.18	23.1.18 to 1.2.18 (inclusive)	75	12·60	***0·57***
	30.4.18		100	13·17	
15	26.3.18	25.3.18 to 5.4.18 (inclusive)	100	13·03	0·11
	27.3.18		78	13·14	

eighteen infections sixty-eight different numbers were obtained showing the differences found between the average sizes of random samples of fifty cysts from the same stool. The figures are set out in Table II, and are placed in order of magnitude in Table III.

TABLE II.

Case	Date of stool	Differences between 50's from same stool (μ)	Differences between 100's from same stool (μ)
1	22.5.18	0·34	
3	23.10.18	0·03	
4	22.7.18	0·10	
5	15.4.18	0·02	
6	7.9.18	0·20	
7	7.5.18	0·07	
8	29.8.18	0·24	
9	27.7.18	0·00, 0·02, 0·05, 0·05, 0·05, 0·10, 0·10, 0·10, 0·12, 0·12, 0·17, 0·20, 0·20, 0·22, 0·22	0·02, 0·15, 0·17
	15.8.18	0·03	
	10.10.18	0·37	
10	3.4.18	0·05	
	19.4.18	0·03	
	30.4.18	0·15	
	4.5.18	0·41	
11	3.5.18	0·03, 0·08, 0·12, 0·20, 0·24, 0·32	0·18
	14.5.18	0·10	
	21.5.18	0·24	
	30.7.18	0·01, 0·02, 0·15, 0·17, 0·17, 0·18, 0·19, 0·20, 0·27, 0·28, 0·34, 0·35, 0·45, 0·47, 0·62	0·40, 0·17, 0·23
12	30.4.18	0·10	
13	5.4.18	0·22	
	6.4.18	0·34	
14	28.11.18	0·41	
15	26.3.18	0·17	
16	19.4.18	0·17	
	7.5.18	0·05	
17	4.4.18	0·03	
	9.4.18	0·20	
	25.4.18	0·41	
18	11.12.18	0·00, 0·03, 0·27, 0·27, 0·31, 0·31	0·03
19	16.5.18	0·32	

TABLE III.

	Differences between 50's in μ in order of magnitude	Differences between 100's in μ in order of magnitude
0·10 and below	0·00, 0·00, 0·01, 0·02, 0·02, 0·02, 0·03, 0·03, 0·03, 0·03, 0·03, 0·03, 0·05, 0·05, 0·05, 0·05, 0·05, 0·07, 0·08, 0·10, 0·10, 0·10, 0·10, 0·10, 0·10	0·02, 0·03
0·11 to 0·20	0·12, 0·12, 0·12, 0·15, 0·15, 0·17, 0·17, 0·17, 0·17, 0·17, 0·18, 0·19, 0·20, 0·20, 0·20, 0·20, 0·20, 0·20	0·15, 0·17, 0·17, 0·18
0·21 to 0·30	0·22, 0·22, 0·22, 0·24, 0·24, 0·24, 0·27, 0·27, 0·27, 0·28	0·23
0·31 to 0·40	0·31, 0·31, 0·32, 0·32, 034, 0·34, 0·34, 0·35, 0·37	0·40
0·41 to 0·50	0·41, 0·41, 0·41, 0·45, 0·47	—
0·51 to 0·60	—	—
0·61 to 0·70	0·62	—

From this it may be seen that only one of the sixty-eight differences exceeds 0·60μ, only six of the sixty-eight exceed 0·40μ, and half are below 0·18μ. Taking ten chances out of eleven as high probability, there is high probability that the differences between two random samples of fifty will not exceed 0·40μ. Now for samples of one hundred cysts this probability will be greater still. Table III shows also the differences between random samples of one hundred. Of these there are eight, and it will be seen that not one of them exceeds 0·40μ and seven are below 0·30μ. We may therefore quite safely say that random samples of one hundred cysts from the same stool will differ from each other by more than 0·40μ very rarely. If we now examine Table I we find that there are thirty-five different magnitudes representing the differences between samples of one hundred cysts from different stools of the same case passed on different days. Of these magnitudes sixteen exceed 0·40μ. Of the eight cases measured on different days, four show variations greater than 0·40μ. It is clear therefore that samples of one hundred cysts taken on different days often differ much more in average size than samples from the same stool, and that therefore their differences cannot be accounted for by errors of sampling. There is thus high probability of accuracy for the conclusion that some infections at any rate are not constant, but change in average size from one day to another.

It is probably worth while to add to the evidence in Case 11, the one showing the largest variations, by giving the results for samples of fifty also. The full record is then as follows:—

TABLE IV.

Case	Date	No. measured	Average diameter (μ)
11	27.4.18	50	12·46
	3.5.18	200	12·16
	14.5.18	100	11·95
	21.5.18	100	12·14
	30·7·18	300	13·33
	31.7.18	50	12·92
	2.8.18	50	13·20

The full record brings out the variability in size more clearly.

In Table I are given the dates of the periods of treatment with emetine. It has been suggested by Dobell and Jepps (1918) that emetine treatment may affect the size of the cysts in that, if the cysts reappear after treatment, they are liable on their reappearance to be larger than on normal occasions. There is only one of my results at all susceptible of this explanation, namely the record for Case 13 on the first day after treatment, April 6th, 1918 (see Table I). In this case, however, the cysts did not disappear during treatment. It will be noticed, too, that other records show similar and even greater variations which are not susceptible of this explanation. It would seem therefore that there is no evidence that the variation in Case 13 is due to the emetine treatment.

There remains the question of the genuineness of the samples. If substitution of one stool for another occurred in the hospital, then the samples would be worthless for the purpose of this investigation. It can only be said that the necessity of strict care in this respect was recognised from the outset, and that in the routine examinations in this laboratory extending over three years there has been almost no evidence that stools were sent up under the wrong name. The occasional possibility of this cannot be entirely excluded, but for the series in question at the present time the accuracy of the naming of the stools was several times investigated and no reason was found to consider it doubtful.

It may be remarked in passing that samples of five hundred cysts, such as were measured by Dobell and Jepps, ought to have an error of sampling considerably smaller than samples of one hundred cysts. Owing to the fact that the measurements are by different workers, my results cannot be applied in detail to their measurements. Since, however, my random samples of one hundred cysts from the same stool rarely differ by more than 0·30μ, it becomes a question whether 0·25μ (the difference obtained between the first and second samples of Case 42 by Dobell and Jepps) is not a signifrcant difference. There is not much direct evidence as to the amount of variation to be expected in the average size of samples of five hundred cysts from the same stool. Dobell and Jepps (1918) measured five hundred cysts from the stool of Case E42 in saline, and five hundred cysts from the same stool in iodine. The average in saline was 12·99μ, in iodine 12·86μ, a difference of 0·13μ. Since iodine may be taken to have no effect on cyst size, this difference is probably due to sampling error. Matthews (1919) measured one thousand cysts of *E. coli* from the same stool and obtained 15·33μ as the average for the first five hundred and 15·27μ for the second five hundred, a difference of 0·06μ. So far as they go, these numbers indicate that the sampling error for samples of five hundred cysts is about 0·10μ, and therefore that a difference of 0·25μ is of some significance.

The occurrence of change in the average size of the cysts of an infection obviously renders the existence of races more difficult to establish, but such change is in itself quite compatible with the existence of races, and in fact is to be expected on that theory. Dobell and Jepps (1918) have themselves pointed out that, in mixtures of the 'small' with the 'ordinary' race, the cysts of the two component races appear in varying proportions on different days, and thus differences in the average size of the cysts of the sample must necessarily be produced. In the case instanced by them the two component races were so far apart in size, 8μ to 9μ and 12μ to 14μ respectively, that the two races could easily be distinguished. But if races of $11{\cdot}6\mu$ and $13{\cdot}3\mu$ exist, then no doubt mixtures of these will exist also. In such a mixture the two races might appear in varying proportions on different days, producing differences in the average size of the whole infection without it being possible to disentangle the two components. The curve of such mixtures would usually be unimodal and the proof of the existence of the two races in the mixture would be difficult, if not impossible.

There remain, however, further tests of the validity of the theory of the existence of such closely neighbouring races. If the average sizes of all the infections measured are placed in order of magnitude, then if pure races are common, the averages will tend to group themselves around two or three nodal points corresponding to the two or three pure races supposed to exist. If mixtures of races are frequent this might not be the case, but it might, even in this event, be possible by constructing curves of the infections to find out whether the races were pure or mixed and to fix the pure races, and from these to disentangle the component parts of the mixtures. Table V shows how far this has been possible in my material.

TABLE V.

Case	No. of cysts measured	Average size (μ)	Modes of the curve
1	100	11·6	11·9
2	100	11·6	11·0
3	100	11.7	11·0 and 12·7
4	100	12·2	11·9
5	100	12·3	11·9
6	100	12·4	11·0 and 12·7
7	100	12·4	12·7
8	100	12·5	12·7
9	500	12·6	11·9 and 12·7
10	400	12·7	12·7
11	850	12·7	11·9 (and 12·7)
12	175	12·9	12·7 and 13·6
13	200	13·0	12·7
14	100	13·1	13·6
15	178	13·1	12·7 and 13·6
16	200	13·1	12·7
17	300	13·2	13·6
18	200	13·5	12·7 and 14·5
19	100	13·7	13·6
20	140	13·8	13·6

In the first place it is noticed that the average sizes of the twenty infections increase from 11·6μ to 13·8μ by very gradual steps. There are no marked gaps in the series. The largest gap is between Infection 3 and Infection 4, and this may indicate the separation out of a smaller race averaging 11·6μ. Consideration of those infections in which fifty cysts have been measured goes, however, against this view. Of the thirteen infections in which samples of fifty to one hundred cysts have been measured, no less than seven show averages between 11·7μ and 12·2μ, as is seen in Table VI.

TABLE VI.

Case	No. of cysts measured	Average diameter (μ)
21	50	11·7
22	50	11·7
23	50	11·8
24	50	11·9
25	50	11·9
26	50	11·9
27	50	12·0
28	87	12·1
29	51	12·1
30	87	12·2
31	92	12·4
32	66	12·7
33	50	12·8

Though these figures are not so reliable as those from larger samples, yet it seems highly improbable that the measurement of larger samples would have removed all these infections into the lower or the higher of the two groups indicated in Table V. It seems likely, therefore, that the gap in the gradual series of averages in Table V is not significant. There is thus no obvious indication that the averages group themselves round nodal points. We must, therefore, pass on to consider the curves of the individual infections. It will be clear from what has been said that, on the hypothesis of the existence of further strains within the 'ordinary' strain, (1) those infections which show considerable variation in size from one day to another should be mixtures of races, and (2) those infections whose average size when well-established lies intermediate between the sizes of supposed pure races should be mixtures. Let us test these hypotheses by reference to the curves of individual infections. Firstly with regard to variable infections. Reference to Table I will show that Cases 10 and 11 had infections showing considerable variability. In these sufficient cysts were measured to make the averages and the curves very reliable. The curve for Case 10 is given in fig. 1, for Case 11 in fig. 2. There is in neither of them any clear evidence that the infection is a mixture. The curve in fig. 1, on the contrary, is unimodal and one of the most nearly symmetrical of all, showing every sign of the curve of a pure race. Cases 9 and 16 show very small variations such as might be due to errors of sampling. Their curves (Case 9 in fig. 3, Case 16 in fig. 4) are very similar to those in figs. 1 and 2, showing if anything rather less sign of purity of race than the latter. It may be concluded that there is no proof of the variable infections being mixtures. We will next consider whether those infections intermediate in size between supposed pure races are mixtures, and will examine first whether the results themselves as seen in Table V suggest any particular sizes as belonging to pure races. In Table V, column 4, are given the modes of the curves for each infection. In some cases the curves are bimodal, in some unimodal. The bimodal curves are, however, scattered quite irregularly through the series. The bimodal curves are more likely to represent a mixture of races than the unimodal, but on account of the irregularity of distribution of both, the table gives no evidence of the position of the hypothetical pure races. It remains to be seen how far the races suggested by Dobell and Jepps can be fitted into the facts of Table V. These authors have given 11·6μ and 13·3μ as the average diameter of two races within what has been called here the 'ordinary' strain. The existence of the 11·6μ strain is not incompatible with the figures of Table V. Also there are sufficient infections with an average diameter about 13·1μ to form some

FIG. 1. CASE 10. 400 cysts. Average diam. 12·7μ.

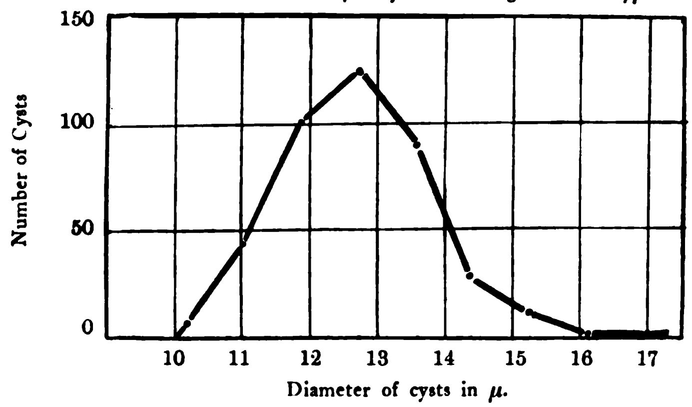


FIG. 2. CASE 11. 850 cysts. Average diam. 12·7μ.

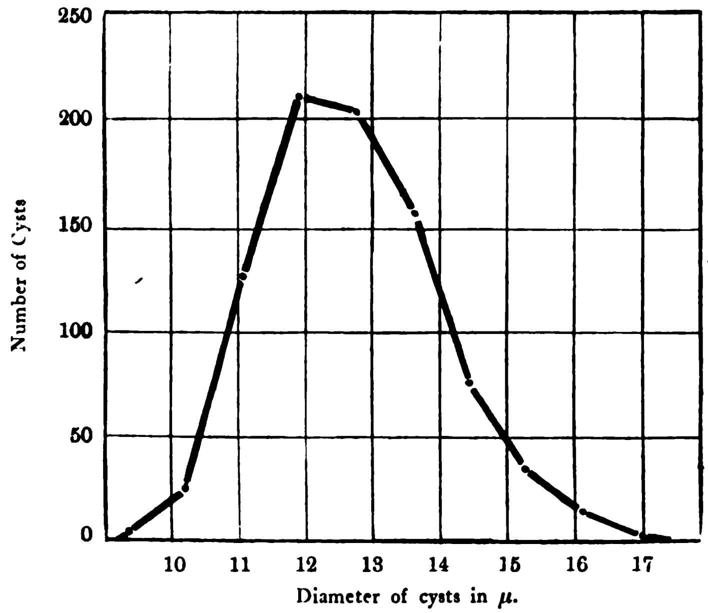


FIG. 3. CASE 9. 500 cysts. Average diam. 12·6μ.

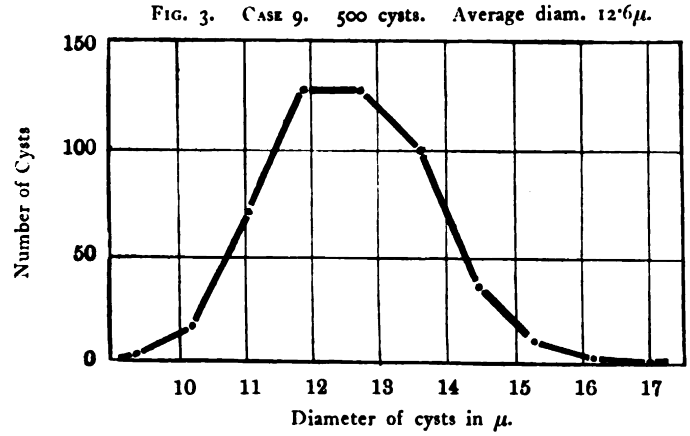
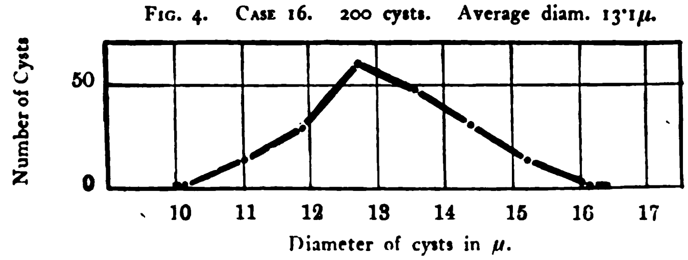


FIG. 4. CASE 16. 200 cysts. Average diam. 13·1μ.

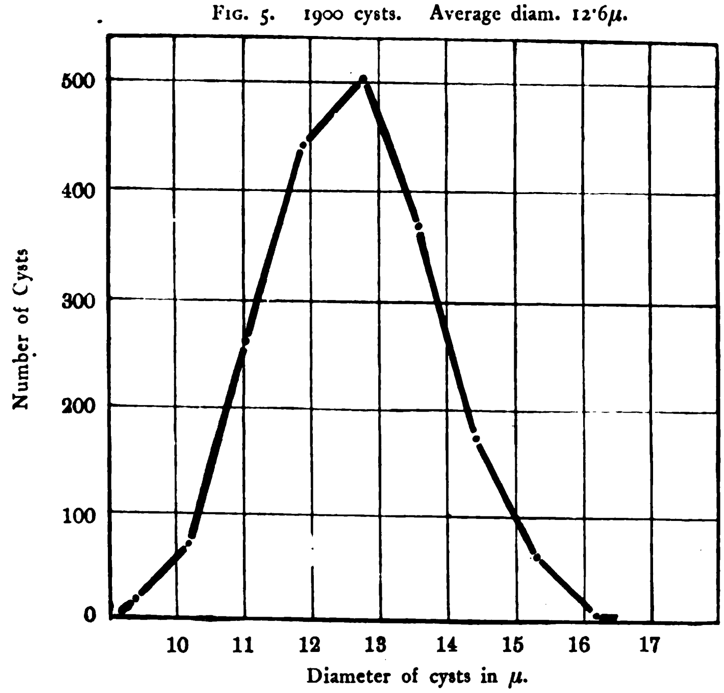


confirmation of a race of about this cyst diameter. My material, however, provided many cases with average diameter about 12·5μ, i.e. about midway between 11·6μ and 13·3μ, and in three of these cases, viz., Cases 9, 10 and 11, I measured large numbers of cysts in order to get a reliable curve for each infection so that it might be ascertained whether these infections showed any signs of being mixtures of the hypothetical 11·6μ and 13·3μ races of Dobell and Jepps. As the curves of figs. 1, 2 and 3 show, there is no definite evidence in either direction. Case 10, fig. 1 (average 12·7μ), has a very symmetrical curve just such as would be expected from a pure race. Case 9, fig. 3, shows a rather less regular curve with a flat top, and Case 11, fig. 2, is very similar. One would suppose, since cases of this average diameter occur so commonly and since they form fairly symmetrical curves, that a race of about 12·5μ existed. There is, however, similar evidence for the existence of races with average diameters of, say, 12·2μ and 12·9μ, and to hypothecate the existence of so many races so close to each other in average diameter is to render the whole theory incapable of proof.* Matthews (1919) has shown that in *Entamoeba coli* there is evidence of a pure race of average diameter 15·3μ and another of average diameter 18·5μ, while the infections (which are common), having an intermediate diameter, show signs of being mixtures and give in almost every case a bimodal curve. The curve for the whole species when equal samples are taken from each infection is itself bimodal. The case is very different for *E. histolytica.* Fig. 5 shows the curve for the 'ordinary' strain of *E. histolytica.* It is a curve of the measurements of one thousand nine hundred cysts in which each of nineteen cases makes an equal contribution of one hundred cysts. It is unimodal and fairly symmetrical, and gives no evidence of any sub-division of this strain into two or more components. It resembles very much the curve given in my previous paper for the same strain, a curve which was composed of a larger number of smaller samples taken at random in the course of the routine work in this laboratory.

* Cf. on this point Dobell and Jepps (1918), p. 340. 'It must be expressly noted that the strains of *E. histolytica* just described constitute merely a selection from those which we have studied. We have no doubt that, had it been possible for us to make an equally detailed study of all the cases in our series, we should have found other races possessing cysts with other mean diameters.'

It seems, therefore, that by all the tests so far applied the existence of further strains beyond the 'small' and the 'ordinary' is not confirmed. In fact, the interesting curve for Case 10 shown in fig. 1 is rather strong evidence to the contrary. Here is an infection which shows considerable variability from one day to another, and moreover lies intermediately between the 11·6μ and 13·3μ races of Dobell and Jepps. On both counts it should be a mixture of races, yet its curve is unimodal and almost exactly symmetrical, a curve such as would be given by a pure race. The

FIG. 5. 1900 cysts. Average diam. 12·6μ.



strongest argument for the existence of further races is the undoubted fact that two races, the 'small' and the 'ordinary', do exist. Their existence is susceptible of proof, because their mean sizes are so far apart that a curve representing a mixed infection is certain to be bimodal. Any daily variations in size so far measured or any possible errors due to sampling are small as compared with the difference between the two races. The existence of the finer races is not suceptible of proof either because they do not exist—in which case variations between individual infections must receive some other explanation—or because they are so numerous and so close together in average size that curves cannot show which infections are mixtures and which pure races. Variation from day to day and errors of sampling are large compared with the differences between the average size of these finer races.*

Finally in Table VII and fig. 6 are given some results as to the prevailing size of the cysts of infections of *E. histolytica* among people from various localities. It will be seen that there is a good deal of evidence that in convalescent dysenterics returned to this country from various theatres of war, about one-third of all the infections of *E. histolytica* are those with the 'small' race. The figures from Liverpool and those given by Dobell and Jepps (1917) agree in this, as do figures kindly supplied to me by Dr. D. L.

TABLE VII.

Size of Infections.

ABROAD (Dysenterics)

Cases	Infections	'Ordinary'	%	'Small'	%	Authority
56	59	38	64	21	36	Mackinnon, 'Chronic' Dysenterics
209	225	106	47	119	53	Mackinnon (1918)
200	215	140	65	75	35	Dobell and Jepps (1917)
306	325	215	66	110	34	Matthews and Smith, convalescent dysenterics examined at Liverpool in 1917 and 1918
			Majority		Minority	Wenyon and O'Connor (1917)

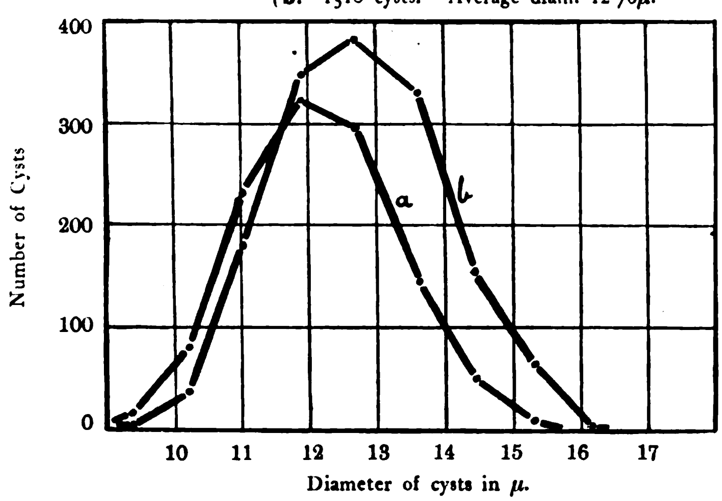
NEVER ABROAD (Healthy Carriers)

Cases	Infections	'Ordinary'	%	'Small'	%	Authority
98	99	84	85	15	15	Matthews and Smith, various samples of the home population examined in 1917 and 1918

* My measurements have not revealed a single example of the 15μ strain of Dobell and Jepps, of which they appear to have had one case, and of which Wenyon and O'Connor record one case. It is evidently very rare. I cannot, therefore, discuss the evidence for the existence of this strain. This fact, however, does not appreciably affect my main conclusions.

Mackinnon as to the incidence of such 'small' infections among her 'chronic' dysenterics.* It will be seen that, compared with this, the 'small' race is comparatively rare in those who have never left England, being present in only 15 per cent. of the ninety-nine *E. histolytica* infections found in this group. It will be noticed on the other hand that the 'small' race is remarkably prevalent among the two hundred and nine cases discussed by Mackinnon (1918). The significance of these facts is not at all clear. A further characteristic of the size of infections of the 'ordinary' strain, in people never out of England is illustrated in fig. 6, where it is shown that the average size of one thousand one hundred and fifty-three

FIG. 6. {*a.* 1153 cysts. Average diam. 12·15μ. *b.* 1510 cysts. Average diam. 12·76μ.}



cysts from fifteen† such infections in people who have never been abroad is 12·15μ, that of one thousand five hundred and ten cysts from eighteen† infections of the 'ordinary' strain from those who have been abroad is 12·76μ. The difference in the average of such large numbers seems significant, and thus the infections of those who have never been out of this country appear to be characterised not only by a very small proportion of the 'small' race, but also by a reduced proportion of the larger sizes of the 'ordinary' race. It may be that further measurements would tend to abolish these

* 'Chronic' dysenterics were arbitrarily defined as cases that had suffered from dysentery, with little or no real intermission, for at least a year previous to the examination made by Dr. Mackinnon.

† All infections in which 50 or more cysts were measured have been included, but no case contributes more than 100 cysts to the curves.

characteristics, though both the number of infections and the number of cysts in both categories is large. So far as the present evidence goes these characteristics exist, and they tend in the direction of the supposition that size of cyst depends to some undefinable extent upon locality.

It will be seen, too, that those who have never been out of this country are healthy carriers, as opposed to the convalescent dysenterics from abroad. Thus the occurrence of a reduced proportion of the very small and of the very large cysts is characteristic of these healthy carriers. Whether there is any significance in this fact it is impossible on the present evidence to say.

SUMMARY

1. Evidence is given that not all infections of *Entamoeba histolytica* remain constant from one day to another in the average size of their cysts.

2. While the species *E. histolytica* can undoubtedly be divided into two races characterised by smaller and larger cysts respectively, the existence of further races is not confirmed.

3 Infections with *E. histolytica* in healthy carriers who have not been out of this country are characterised by a smaller proportion of the 'small' race, and also by a reduced proportion of the larger cysts of the 'ordinary' race, as compared with infections from convalescent dysenterics from abroad.

REFERENCES

DOBELL, C. and JEPPS, M. W. (1917). On the three common intestinal entamoebae of man, and their differential diagnosis. *Brit. Med. Journ.* Vol. II, p. 607.

——— (1918). A study of the diverse races of *Entamoeba histolytica* distinguishable from one another by the dimensions of their cysts. *Parasitology.* Vol. X, p. 320.

MACKINNON, D. L. (1918). Notes on the intestinal protozoal infections of 1,680 men examined at the University War Hospital, Southampton. *Lancet*, Vol. CXCV, p. 386.

MATTHEWS, J. R. (1919). A mensurative study of the cysts of *Entamoeba coli. Ann. Trop. Med. & Parasitol.* Vol. XII, p. 259.

SMITH, A. MALINS (1918). Measurements of and observations upon the cysts of *Entamoeba histolytica* and of *Entamoeba coli. Ann. Trop. Med. & Parasitol.* Vol. XII, p. 27.

WENYON, C. M. and O'CONNOR, F. W. (1917). Human intestinal protozoa in the Near East. *Wellcome Bureau of Scientific Research*, also *Journ. R.A.M.C.* Vol. XXVIII., p. 151.

THE COURSE AND DURATION OF AN INFECTION WITH *ENTAMOEBA COLI*

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(*Received for publication* 17 *February*, 1919)

Little is known at present as to the length of time that infections with intestinal protozoa may persist, and details of the course of such infections have been published in only a few instances. Data of this kind can be obtained only by daily observation of infected cases over long periods of time, and opportunities for such investigations seldom present themselves. Published records relate chiefly to infections with *Entamoeba histolytica*, though infections with *E. coli* and with *Giardia intestinalis* have also been followed for considerable periods. The evidence, so far as it goes, favours the idea that an infection, once acquired, persists for a very long time, if not throughout the lifetime of the host.

Some records of examinations conducted in this laboratory show that several cases, infected with *E. histolytica* and intractable to treatment with emetine, have remained infected for seven to ten months—the time during which they were under our observation. It is pretty certain that these cases were infected for a considerable time before they reached us, and we know that some of them remained infected for some time after their departure, until we ultimately lost trace of the cases. But infections of one or two years' duration are short compared with those given for three cases described by Dobell and Stevenson (1918). These authors, from the evidence at their disposal, conclude 'that an infection with *E. histolytica* may persist certainly for sixteen, and probably for as long as thirty-four years.'

It is of interest, therefore, to enquire if the harmless intestinal protozoa behave similarly in this respect. It has been shown in a recent paper (Matthews and Smith (1919)) that infections with *Giardia intestinalis* and with *E. coli* are more prevalent among children than among adults.* The authors suggested an explanation of these observations on the supposition that infections had been lost among the adults. The evidence, however, was indirect, and the purpose of the present note is to record certain observations which seem to furnish fairly satisfactory reasons for believing that an infection with *E. coli*, at all events, may disappear from the intestine in the course of time.

The individual whose infection has been followed was first examined for intestinal protozoa at approximately fortnightly intervals during the first three months of 1916. These examinations were negative. In the first week of August, 1916, further observations were made, when cysts of *E. coli* were detected in the stools. It cannot be definitely concluded that the infection was acquired just previous to that date, but since the early examinations were negative and since the person has from the beginning of 1916 been engaged on the protozoological examination of stools, it seems not unlikely that infection occurred in the laboratory between March and August, 1916. In November, 1916, a series of daily examinations was commenced, and was continued for three months. The main object of these examinations was to obtain data which would give a rough idea of the course of the infection over a considerable period of time. The method adopted was to count the number of cysts contained in six cover-slip preparations made from different parts of the stool. The accompanying chart (fig. 1) shows the average number of cysts for each preparation for a period of two months.† The method employed cannot be expected to give more than a rough idea of the course of the infection. Cysts of *E. coli* are usually irregularly distributed in the stool whether they are numerous or not. On one occasion six preparations contained respectively 76, 43, 39, 197, 143 and 79 cysts. Again, on a day when the cysts were much less numerous, six preparations contained respectively the following numbers of cysts—2, 7, 1, 0, 1, 4. The

* The figures were: Among children—*G. intestinalis*, 14·1 per cent.; *E. coli*, 11·1 per cent. Among adults—6·0 per cent. and 6·7 per cent., respectively.

† It should be mentioned that there was only one stool daily, although on two occasions, December 10th and January 11th no stools were passed.

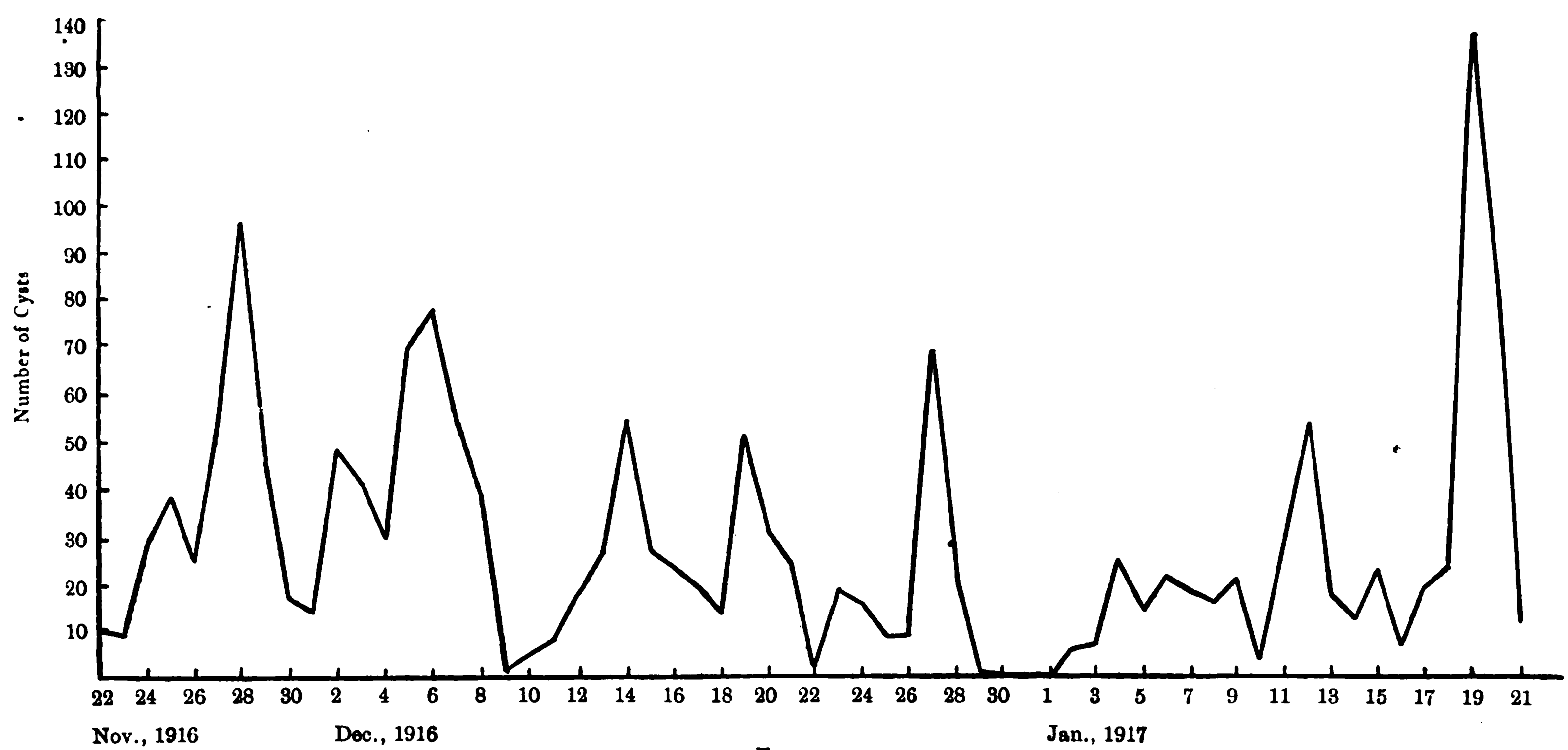

Number of Cysts
140
130
120
110
100
90
80
70
60
50
40
30
20
10
22 24 26 28 30 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 1 3 5 7 9 11 13 15 17 19 21
Nov., 1916
Dec., 1916
Jan., 1917

FIG. 1.

preparations each day were made as uniformly as possible, though slight variations in this respect doubtless occurred from day to day. It is believed, however, in spite of the defects of the method, that the main features of the course of the infection are brought out, and the rise and fall in the number of cysts produced daily is a feature which must be familiar to anyone who has followed infected cases for any length of time. It is this feature that gives in a very general sense an indication of periodicity in cyst production (cf. Porter's (1916) results for Giardia). An examination of the chart shows that the maximal values occur at intervals of 8, 8, 5, 8, 8, 8 and 7 days respectively, and the minimal values at intervals of 8, 8, 9, 4, 9, 10 and 6 days respectively. There is thus no conclusive evidence of *regular* periodicity in cyst production. It should be remembered, also, that we have here the data for a single case only, and it is unlikely that all infections with *E. coli* would behave in an exactly similar way.

Daily observation of the case was discontinued in February, 1917, only occasional examinations being made until July, 1917. Without exception these were positive. Thereafter, twelve months elapsed before the case was again examined. Several observations in July, 1918, gave negative results, and in view of the long succession of positive findings in the early history of the case, it seemed of interest to discover how long the negative period that had been encountered would extend. From 18th July, 1918, to 15th February, 1919, eighty negative examinations have been made, or an average of three examinations every eight days during a period of seven months. There now arises the difficult question of deciding whether the infection has entirely disappeared or whether a very long negative period has set in. Many cases might be cited from records obtained in this laboratory showing long intervals during which no cysts were found in the stools, although the cases were known to be infected. All these cases, however, are complicated by the fact that they were also infected with *E. histolytica* and had received treatment with emetine. It is not certain how far this treatment had an inhibitory effect on the concurrent infection with *E. coli*. Again, in instances where *E. coli* is recorded at a late examination (e.g. in one case at the fifty-fifth examination after three months' observation) there is no certain proof that the man had not recently become infected, i.e. not long before the positive finding was recorded. Finally, there are cases where *E. coli* has been found only once in a long series of examinations (e.g. one positive record out of sixty-five examinations extending over a period of five months). In such cases, there is no absolute certainty that the sample submitted, on the occasion when *E. coli* was found, was genuine. These criticisms, however, do not apply to the case which forms the subject of this note. Emetine was not administered in any form, and there was no doubt about the genuineness of the samples of stools examined. That the case has remained negative for seven months seems to indicate without much doubt, although not with absolute certainty, that the infection has disappeared entirely. It may be affirmed, anyhow, that should *E. coli* again be found in the same case, there will be no means of proving that re-infection has not occurred.

The facts of the present case may thus be summarised. Infection was known to exist for a year—from August, 1916, to July, 1917. A year elapsed without examination. Since July, 1918, frequent negative examinations have been recorded, and it is believed that the infection has been lost.

We have no means of deciding whether the course and the duration of the *E. coli* infection here described are normal (see Wenyon and O'Connor, 1917, p. 71). It is doubtless true that many cases remain infected for much longer than a year. James (1914) has mentioned the case of a negro known to have had an infection with *E. coli* lasting at least six years. We do not know, however, how often infections of short duration occur. If they are at all common, they may give rise to a factor which should be borne in mind when dealing with the comparative incidence of infection among different sections of any population examined for intestinal protozoa. As has already been mentioned, we have recorded significant differences in the incidence of infection with *Giardia intestinalis* and *Entamoeba coli* among Liverpool children and adults.

There does not appear to be any reason why *E. coli* should not disappear spontaneously. All the amoebae in the intestine may perish in some way, or they may all encyst simultaneously and all be evacuated in the encysted form. In either event the infection would be lost.

REFERENCES

DOBELL and STEVENSON (1918). A note on the Duration of Infections with *Entamoeba histolytica*. *Trans. Trop. Med. & Hyg.* Vol. XI, pp. 168-175.

JAMES (1914). A Study of the *Entamoebae* of Man in the Panama Canal Zone. *Ann. Trop. Med. & Parasitol.* Vol. VIII, pp.133-320.

MATTHEWS and SMITH (1919). The Spread and Incidence of Intestinal Protozoal Infections in the Population of Great Britain. III. Children. *Ann. Trop. Med. & Parasitol.* Vol. XII, pp. 363-371.

PORTER (1916). An enumerative study of the cysts of *Giardia* (*Lamblia*) *intestinalis* in human dysenteric faeces. *Lancet*, 10th June, pp. 1166-1169.

WENYON and O'CONNOR (1917). Human Intestinal Protozoa in the Near East. *London. Wellcome Bureau of Scientific Research.*

TWO PARASITES OF *NAJA NIGRICOLLIS*

BY

J. W. S. MACFIE

(*Received for publication* 16 *October*, 1918)

(PLATE I)

I

TRYPANOSOMA VOLTARIAE, sp. n.

(Plate I, figs. 1 to 4)

This trypanosome was found in the blood of a snake in the Gold Coast Colony, West Africa. The host was identified as *Naja nigricollis*.

The infection was a very scanty one, only about half a dozen trypanosomes being found in each of the blood-films obtained. The parasites were not seen alive. The description is therefore based entirely on the appearance of the parasite in blood-films stained with Leishman's stain. The trypanosome was monomorphic; all the specimens examined having a similar shape and being of about the same size.

All the trypanosomes found in the films were looped, and had the posterior extremity bent at a right angle to the rest of the body (figs. 1 to 4). The posterior end of the body extended for a considerable distance beyond the blepharoplast as a snout, which was always more or less folded. The body, which stained a blue colour, was compact posteriorly, but anteriorly frayed out into a number of striae which blended with the undulating membrane. The blepharoplast was small, rounded or oval, and compact, and was situated at some distance posterior to the nucleus. The nucleus was oval, measured about 3μ long and 2μ broad, and lay on the same side of the body as the undulating membrane at a point slightly posterior to the middle. The undulating membrane was well developed and arranged in ample folds along the external border of the loop formed by the body of the trypanosome. The flagellum terminated anteriorly in a free portion. The average measurements of five parasites were as follows:—Total length, 50μ; breadth (not including the undulating membrane) at the level of the nucleus, 4μ; posterior extremity to blepharoplast, 17μ; blepharoplast to nucleus, 4μ; free portion of the flagellum, 8μ.

This trypanosome, so far as I am able to ascertain, differs from any of the species hitherto found in snakes. In some respects, however, it resembles the small forms of *T. primeti*, a species found in the blood of water snakes in Tonkin, and described by Mathis and Leger (1909). These forms of *T. primeti* appear to be somewhat longer (57μ) and broader (7μ) than the trypanosome of *Naja nigricollis* described above, the distance from the blepharoplast to the nucleus (2μ) is shorter, and the free portion of the flagellum (13μ) is longer. Moreover, with the small forms are associated others about twice their size, both being comprised in the species *T. primeti* by Mathis and Leger. In the snake examined at Accra no trypanosomes were found resembling the large forms of *T. primeti*. Too much importance must not be attached to the small differences in measurements, because in the case of the trypanosome of *Naja nigricollis* only a very few individuals were examined, and in the case of *T. primeti* nothing is said about the range of variation. Mathis and Leger do not mention any forms intermediate in size between the large and small forms of *T. primeti*, but they appear to have been satisfied that only a single species of trypanosome was represented, and considered that the small forms were the young forms.

It should be mentioned that Wenyon (1908) has described a trypanosome, *T. najae*, found in *Naja nigricollis* in the Sudan, but as he saw it only in fresh blood, and as his drawings made from living specimens are mere sketches, it is not possible to compare it with the parasite found in the Gold Coast. For the latter parasite I propose the name *Trypanosoma voltariae*.

REFERENCE

Mathis, C., and Leger, M. (1909.) Sur un Nouveau Trypanosome des Serpents du Tonkin. *Comptes rendus de la Soc. de Biol.*, LXVII, pp. 572-574.

Wenyon, C. M. (1908). Report of Travelling Pathologist and Protozoologist. *Third Report of the Wellcome Research Laboratories at the Gordon College, Khartoum*, pp. 142-143.

II

PLASMODIUM MESNILI, Bouet

(Plate I, figs. 5 to 24)

Pigmented parasites of the red blood corpuscles have been described by several authors as occurring in snakes, but hitherto only gametocyte forms have been seen in the blood, and these appear to have belonged to but a single species. The name *Plasmodium mesnili*, given by Bouet (1909) to the species described by him, has therefore been applied generally to these parasites.

In a specimen of *Naja nigricollis* killed in 1916 at Accra in the Gold Coast, West Africa, the blood was found to be heavily infected with pigmented parasites, and as certain stages of the life-history were exemplified which have not previously been described, a brief account of them will be given. I have to thank Dr. A. Ingram for very kindly sending me the materials from this snake, namely two blood-films stained with Leishman's stain.

FORMS SEEN IN THE BLOOD

A considerable proportion of the erythrocytes of the snake were infected by parasites, many of them doubly, and some trebly. The most conspicuous forms were the gametocytes, but there were also a great many amoeboid forms, and a few which appeared to be undergoing schizogony. A few parasites, both young trophozoites or merozoites and rounded-up gametocytes, were seen free, but it is possible that this was the result of some post-mortem change or of mechanical injury to the corpuscles in the act of making the films. The infected erythrocytes stained similarly to the uninfected, and showed neither polychromatophilic changes nor stipplings (excepting the macules referred to below). Pigmented leucocytes were very abundant. As the trophozoites contained very little pigment and the gametocytes a large quantity, it may be surmised that much of the pigment in the leucocytes was derived from the latter after their death and disintegration.

In addition to the pigmented parasites there were a few haemogregarines similar to those figured by Wenyon (1908) as occurring in *Naja nigricollis*.

SCHIZOGONY. The youngest forms were usually situated at the ends of the corpuscles, and were oval or slightly irregular bodies with a small mass of chromatin at one pole. Somewhat larger forms were frequently ring shaped with a large central vacuole. Older parasites were often very irregular in shape, their cytoplasm thrust out as delicate pseudopodia, indicating a considerable degree of amoeboid activity (figs. 6 to 11). The trophozoites stained a pale blue colour. When fully grown they measured about 8μ in length. Pigment in the form of rather fine granules of a brown colour was sometimes present, but was generally scanty or inconspicuous and often entirely absent. The trophozoites did not cause any marked deformity of the erythrocytes or displacement of the nucleus.

Two or more trophozoites were frequently present in the same erythrocyte. In some such cases the cytoplasm of two parasites appeared to have fused, giving rise to a form suggesting schizogonic multiplication (figs. 9 and 10), but differing from that which I believe to be the true multiplication form (fig. 14). Such forms are indeed the very reverse of division forms, being produced by the conjunction of separate individuals.

Schizogony appeared to take place by the nucleus dividing into two with the formation of a simple dumb-bell figure, the formation of a binucleate parasite, and the subsequent separation of this into two daughter cells of about the same size (fig. 14). No indication of the formation of more than two merozoites was seen. The number of parasites undergoing schizogony in the blood was very small, whereas the number of trophozoites, both young and further developed forms, was large. It is possible therefore that schizogony occurs mainly in the organs.

GAMETOCYTES. The gametocytes were of two distinct types, the cytoplasm of the one staining a deep blue with Leishman's stain, that of the other remaining almost uncoloured. The former are considered to be the females, and the latter the males. The male and female gametocytes were present in about equal numbers: 105 male and 95 female forms occurring in a series of 200 consecutive individuals counted. Whilst still quite small, the male and female gametocytes were readily recognised and easily distinguished from one another by their staining reactions. The gametocytes when young lay usually at the ends of the corpuscles (fig. 20), when a little older they lay laterally, and eventually looped round the nucleus of the corpuscle, sometimes surrounding it completely. Many corpuscles contained two gametocytes of either the same or of different sexes (figs. 18, 19, 23). As a rule, a single gametocyte of either sex, even when fully grown, produced but little distortion of the erythrocyte, but considerable enlargement of the cell and displacement of its nucleus was caused by double or multiple infections, and a similar result was caused by a single gametocyte if it developed at the end of the corpuscle instead of growing round the nucleus in the usual halter-like manner. The gametocytes contained a large quantity of dark brown pigment in the form of coarse grains.

Both male and female gametocytes were occasionally seen which had two nuclei (fig. 22), but as they were of large size, caused distortion of the erythrocyte, and often showed two vacuoles, it is probable that they were in reality each compounded of two parasites lying side by side whose individual outlines were invisible. This interpretation is in agreement with that given by Woodcock (1911) of similar forms of *Halteridium frangillae* observed by him in the blood of a chaffinch. The gametocytes of *Haemocystidium simondi* described by Dobell (1911) as showing nuclear division are considered by Woodcock to be subject to the same explanation.

The male gametocyte. The male gametocyte stained very faintly with Leishman's stain. When fully developed it measured about 15μ by 5μ. It contained a large amount of pigment in rather coarse grains scattered irregularly about its substance (fig. 16), but sometimes collected into clumps at the extremities (fig. 19). The nucleus consisted usually of a group of coarse chromatin granules arranged in a rather compact mass near the middle of the parasite. In some specimens, however, the nuclear chromatin was more abundant, forming a band across the middle of the parasite (fig. 16) or a large central area lightly dotted with fine granules (fig. 17). The male gametocyte frequently showed a large rounded vacuole (fig. 15), which was, however, inconspicuous in the preparations studied owing to the very faint staining of the parasite with Leishman's stain.

The female gametocyte. The female gametocyte stained a deep blue colour with Leishman's stain. When fully developed it measured about 16μ by 6μ. The female gametocyte contained a large quantity of pigment in coarse granules of a dark brown colour. The nucleus was indistinct in most specimens, but consisted of chromatin granules arranged so as to form an oval or more elongated body about the middle of the parasite. A rounded vacuole was present in many of the female gametocytes, and was a conspicuous object by contrast with the deep blue colour of the cytoplasm surrounding it. Sometimes two vacuoles were seen in what appeared to be a single gametocyte, but this was probably to be explained by there being two gametocytes lying side by side in the same cell. A few of the erythrocytes containing female gametocytes showed red macules (fig. 24) of a characteristic scarlet colour similar to those described by Wenyon (1908).

NOMENCLATURE OF THE PARASITE

So far as the gametocytes are concerned, the parasite which has just been described resembles that observed by Wenyon (1909) in the Sudan and originally called by him *Haemocystidium najae*, a name which he subsequently (1915) admitted to be a synonym for *Plasmodium mesnili*. These forms very closely resemble *Haemoproteus*, and, in fact, França (1917) in his recent work 'Sur la classification des hémosporidies' has included them in this Genus under the name *Haemoproteus najae*. The occurrence in the blood of other stages of the parasite besides the gametocytes, however, renders the retention of this organism in the Genus *Haemoproteus* inadmissible.

Wenyon (1915) has proposed to group the pigmented parasites of cold-blooded animals, which have been variously named *Haemoproteus*, *Haemocystidium*, *Haemamoeba*, and *Plasmodium*, into two genera; 'firstly, the genus Plasmodium, which includes parasites which go through the schizogony stage in the red blood corpuscles and produce gametocytes in these cells too; secondly, the genus Haemoproteus, including parasites which do not reproduce by schizogony in the red blood corpuscles, which cells only contain the gametes in varying stages of growth.' In accordance with this classification, and because at that time only gametocytes had been found in the blood, Wenyon placed the pigmented parasite of the erythrocytes of snakes in the Genus *Haemoproteus*, but the description given above shows that it should more properly be included in the Genus *Plasmodium*.

REFERENCES

BOUET, G. (1909). Sur deux hémocytozoaires pigmentés des reptiles. *Comp. rend. Soc. Biol.*, Vol. LXVI, pp. 43-45. (Jan. 9, 1909).

DOBELL, C. (1911). Contributions to the Life-History of *Haemocystidium simondi*, Castellani and Willey. *Festschrift zum Sechzigsten Geburtstag Richard Hertwig*, Gustav Fischer, Jena, Bd. I, pp. 123-132.

FRANÇA, C. (1917). Sur la classification des hémosporidies. *Jornal de Sciências Matemáticas, Fisicas e Naturais*, 3rd series, No. 1.

WENYON, C. M. (1908). Report of Travelling Pathologist and Protozoologist. *Third Report of the Wellcome Research Laboratories at the Gordon College, Khartoum*, pp. 150-152.

——— (1915). The Pigmented Parasites of Cold-blooded Animals, with some Notes on a Plasmodium of the Trinidad Iguana. *Journ. Trop. Med. & Hyg.*, Vol. XVIII, pp. 133-140.

WOODCOCK, H. M. (1911). On an unusual condition observed in *Halteridium*. *Zoologische Anzeiger* (Leipzig), Bd. XXXVIII, pp. 465-471.

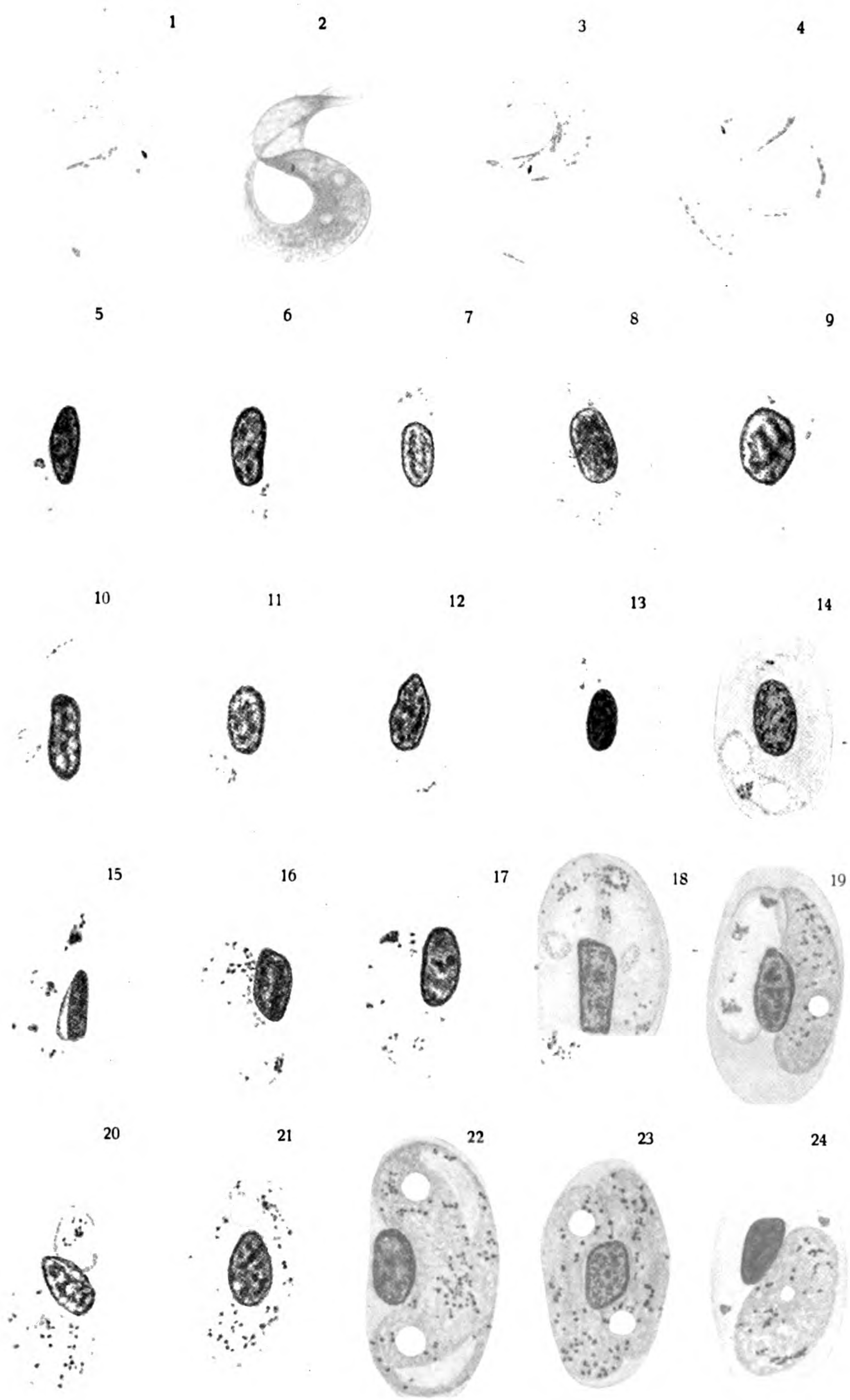
EXPLANATION OF PLATE

Two parasites of *Naja nigricollis*. Drawings by Miss M. Rhodes from specimens stained with Leishman's stain.

Figs. 1 to 4. *Trypanosoma voltariae*, sp. n. × 1500.

Figs 5 to 24. *Plasmodium mesnili*, Bouet. × 1500. Figs. 5 to 13, various forms of trophozoites; figs. 6 to 11, showing amoeboid forms of the parasite. Fig. 14, dividing form. Figs. 15 to 18, male gametocytes. Fig. 19, double infection with a male and a female gametocyte. Figs. 20 to 24, female gametocytes; fig. 20, two immature female gametocytes in a single erythrocyte; fig. 21, parasite encircling the nucleus of the erythrocyte; figs. 22 and 23, double infections; fig. 24, erythrocyte invaded by a female gametocyte, and showing two red macules.

PLATE I



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ON THE GENITAL ARMATURE OF THE FEMALE TSETSE-FLIES *(GLOSSINA)*

BY

ALWEN M. EVANS, M.Sc.

(*Received for publication* 13 *March*, 1919)

INTRODUCTION

It is well known that before 1911 the classification of the genus *Glossina* was based on highly unsatisfactory characters. Consequently the determination of specimens was a matter of great uncertainty, and this difficulty still exists in a large measure as far as the females of *Glossina* are concerned. The present paper is a record of investigations, the object of which was to discover characters of systematic value in the armature of these latter. In the two more specialised groups of which *G. palpalis* and *G. morsitans* were taken as types, the enquiry met with slight success in so far as the differentiation of the individual species included in these groups is concerned, but in the *Fusca* group the armature of each species was found to exhibit at least one distinctive character, and it has been discovered that six of the members of this latter group possess internal chitinous structures, which are specifically highly characteristic.

A fact of systematic interest which has come to light is that the armature of the females falls into three clearly defined structural types which correspond to the three groups into which *Glossina* is divided on the basis of the male armature by Newstead (1911).

I wish to thank Professor Newstead, F.R.S., to whom I am indebted for the indispensable assistance which he has most kindly rendered to the furtherance of this investigation.

GENERAL ACCOUNT OF THE MORPHOLOGY OF THE FEMALE ARMATURE

The external armature of the female *Glossina brevipalpis*, Newst., is briefly described by Stuhlmann under the name of *G. fusca*, Walker (1907, pp. 57, 58). He refers to a 'hufeisenformiger Chitinkorper' which, he suggests, may represent the

IX° segment, and he states that within it lies the genital opening, below which is the anus, separated from the former by a very small plate. Were this the case, then the relative position of the genital and anal apertures in the females of *Glossina* would be the reverse of that which holds in other members of the Diptera. It is, however, a simple matter to demonstrate by dissection (fig. 1, A)

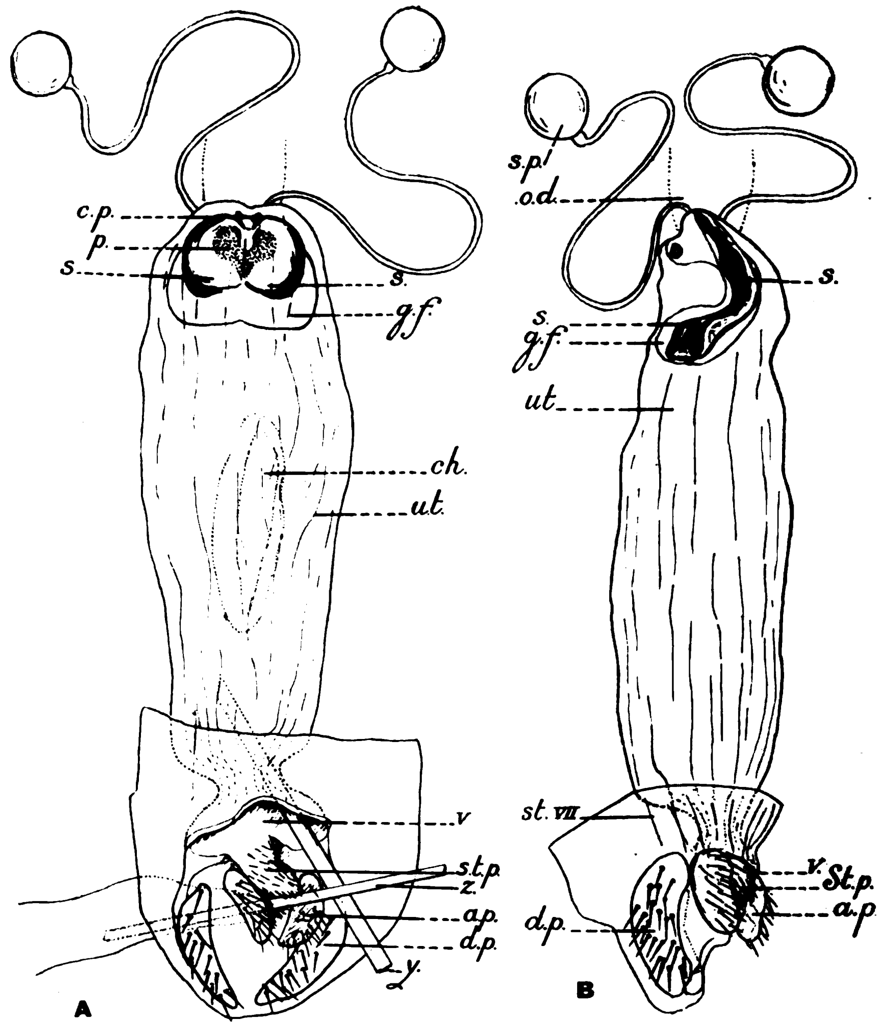

FIG. 1. A. *Glossina fusca* : ♀. Diagrammatic representation of external armature together with extended uterus after maceration in KOH. Ventral aspect. *a.p.*, anal plate; *c.p.*, process of signum; *ch.*, chorion; *d.p.*, dorsal plate; *g.f.*, genital fossae; *o.d.*, oviduct; *p.*, petaloid marking on signum; *s.*, signum; *sp.*, spermatheca; *st.* VII, seventh sternite; *ut.*, uterus; *v.*, vulva; *y and z.*, bristles inserted into vulva and anus respectively.

B. *Glossina fuscipleuris* : lateral aspect.

that the opening between the arms of the 'horseshoe' (Chitinhufeisens) leads directly into the rectum, and that the vulva (*v.*) is a broad slit-like opening between the hinder border of the VII° sternite and the base of the small piece of chitin (*st. p.*) which separates the apertures. Thus the genital orifice, as in other Diptera, lies ventral to, not dorsal to the anus. We may now proceed to a comparison between the external armature of *G. fusca* and that of the blow fly, *Calliphora erythrocephala*. In the latter case the ovipositor bears, terminally, four projecting plates, which surround the anal orifice, and are respectively referred to by Lowne (1893-95) as the 'sternal plate,' median and ventral; the 'dorsal scale,' median and dorsal; and a pair of 'lateral scales.' The vulva (*v.*) is a horizontal slit between the base of the sternal plate and the border of the VII° sternite. We may therefore conclude that the small triangular plate (*st. p.*) in *G. fusca* is the homologue of the sternal plate of *C. erythrocephala;* this name will be used in describing it. The anal scales of the blow-fly which lie lateral to the anus are undoubtedly represented in *G. fusca* by the 'Chitinhufeisens,' which, when examined microscopically, is seen to consist of a pair of projecting, slightly chitinised, scale-like plates bearing flexible spines, and here referred to as the 'anal plates' (*a. p.*). In *G. fusca* the median *dorsal scale* of the blow-fly is apparently unrepresented, but dorsal and external to the anal plates are paired, elongate, chitinisations of the integument, the *dorsal plates* (*d. p.*), which, as Stuhlmann (*l. c.*) suggests in the case of *G. brevipalpis*, are probably the tergites of the VIII° segment. It is the form of these plates which is the most variable feature of the external armature of the *Fusca* group of *Glossina*.

The position occupied by the several parts of the armature in relation to the rest of the abdomen (in *G. palpalis*) is indicated in fig. 2. The plates are borne by the terminal wall of the abdomen, a vertical membrane stretched between the arched posterior edge of the VIII° tergite and the posterior margin of the VII° sternite, from which latter it is separated by the slit-like opening, the vulva.

The internal armature. In fig. 1, A, a bristle (*y*) is inserted through the vulva into the uterus, which in *G. fusca* and other members of this group bears at the anterior extremity of its *dorsal* wall the structure *g. f.* This is a thick semi-transparent mass of tissue which persists after fifteen minutes' maceration in boiling KOH. As no reference could be found to such a structure in the literature on *Glossina*, the vagina of *Calliphora erythrocephala* was examined, and it was found that the organ situated in the dorsal wall, and described by Lowne (1893-95) as the 'genital fossae,' is of similar character to that here referred to as *g. f.* in *G. fusca*. Further, there occur on the internal surface of the genital fossae in *C. erythrocephala* two paired curved plates of

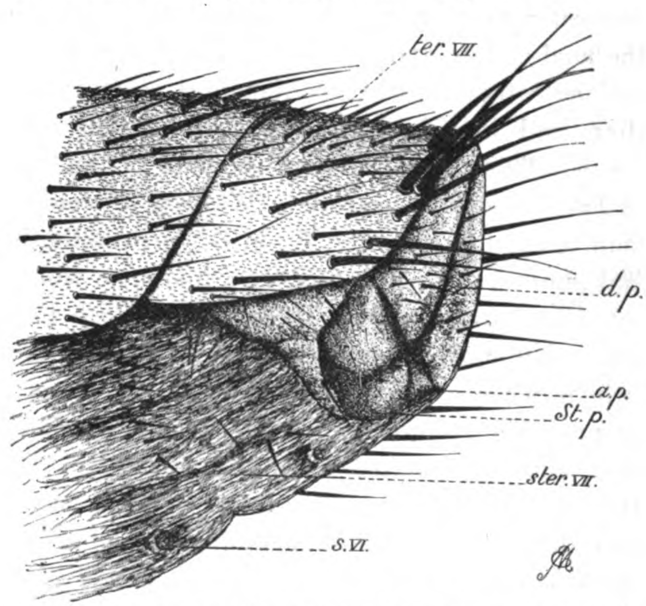

FIG. 2. *Glossina palpalis* : ♀. Terminal segments of abdomen, ventro-lateral aspect. *s.* VI, spiracle of sixth segment; *ster.* VII, seventh sternite.

yellow chitin, which, though figured (Plate XLVII), are not named by Lowne (1893-95). They are represented in typical *G. fusca* (fig. 4) by a peculiar symmetrical structure composed chiefly of a pair of darkly chitinous, hollow, sub-conical elements (fig. 4, *e*), which will be referred to provisionally as the *signum*, a term applied by Pierce (1914) to 'an internal armature of the bursa' in certain Lepidoptera. Although, as Lowne (1893-95, p. 577) states that the 'young uterus' is termed 'the bursa,' there seem to be some grounds for regarding these structures as homologous, I do not commit myself to this view since I have not had the opportunity of examining fresh material of female *Glossina*, but merely use Pierce's term in the sense that it refers to internal armature.

TECHNIQUE

The method employed is as follows: The terminal portion of the abdomen is cut off and placed in a test-tube containing 10 per cent. KOH. The latter is allowed to boil in a water bath for fifteen minutes, after which the specimen is well washed in water and the dissolved tissues expressed. The best result will be obtained if the abdominal wall is slit laterally, almost to the extremity, so that, in mounting, the specimen can be spread out with the plates of the armature lying neither above nor below any other portion of the integument. Unless a permanent preparation is required, the specimen may be mounted in glycerine for examination. In the former case it is dehydrated, cleared in oil of cloves, and mounted in Canada balsam.

To dissect out the signum, in the *Fusca* group remove the last four segments, and, if the presence of a larva is indicated, the whole abdomen. After the specimen has been macerated for fifteen minutes in KOH, and washed in water, the abdominal walls are slit and the contents teased out with a pair of needles. With the aid of a lens a pair of sub-spherical yellow bodies will be detected; these are the spermathecae, they lie in close proximity to the distal end of the uterus, to which they are connected by their much convoluted ducts. If fossae are present they will appear as a gelatinous mass occupying the extremity of the uterus, and on the surface will be seen a dark red-brown or ochraceous, symmetrical structure—the signum. When found, the genital fossae with the signum should be isolated and mounted in glycerine, or after dehydration in Canada balsam.

TABLE FOR THE DETERMINATION OF GROUPS

1.	Dorsal Plates absent	*Morsitans* Group (III)
	Dorsal Plates present	2
2.	Medio-dorsal Plate absent...	*Fusca* Group (I)
	Medio-dorsal Plate present	*Palpalis* Group (II)

Table for the Determination of Species of Fusca Group

1.	Signum of uterus absent	2
	Signum of uterus present	3
2.	Hamate sclerites at base of sternal plate ...	*G. brevipalpis*
	No hamate sclerites at base of sternal plate ...	*G. longipennis*
3.	Dorsal plates narrowly crescentic	4
	Dorsal plates not narrowly crescentic	7
4.	Signum markedly elongate and strongly flexed ...	*G. fuscipleuris*
	Signum not markedly elongate or strongly flexed	5
5.	Signum consisting of two narrow, pale, parallel strips	*G. nigrofusca*
	Signum not consisting of two narrow, pale, parallel strips	6
6.	Signum chiefly composed of a pair of conical or hemispherical lobes	*G. fusca*
	Signum composed of two long, convergent bands separated by a deep median cleft	*G. medicorum*
7.	Dorsal plates of great extent; signum consisting of two entirely separate, paired sclerites ...	*G. severini*
8.	Dorsal plates sub-triangular and not very large; signum consisting of a single plate	*G. tabaniformis*

Group I. Fusca Group (figs. 3-14). *External armature consisting of five plates—one pair dorsal, one pair lateral, and a single median sternal one; medio-dorsal plate absent. Signum generally well developed.*

External armature. Much greater in extent than in either of the other two groups, the dimensions varying between about 1·1 × 0·8 mm. and 0·8 × 0·7 mm. The dorsal plates (*d. p.*) are usually surrounded by a considerable area of unchitinised, membraneous, integument which separates them from the anal plates (*a. p.*). The outline of the dorsal plates is generally elongated; their long axes lying obliquely and approaching one another dorsally. They always bear stout, black, spines. The anal plates (*a. p.*) usually project almost vertically from the posterior surface of the abdomen, to which they are only attached basally. They can be readily turned outwards with dissecting needles.

Internal armature. In the species *G. fusca*, *G. tabaniformis*, *G. fuscipleuris*, *G. medicorum*, *G. nigrofusca* and *G. severini*, there occurs associated with the internal genitalia the peculiar chitinous structure here referred to as the signum. This is always of symmetrical form, and affords, apart from the external armature, important morphological characters for the distinction of species.

Glossina fusca, Walker

External armature of the female (fig. 3). The dorsal plates (*d. p.*) are sub-crescentic, the ends being more or less narrowly rounded or truncated. The greatest width is generally not more than one-fourth of the total length, and stiff spines are borne on

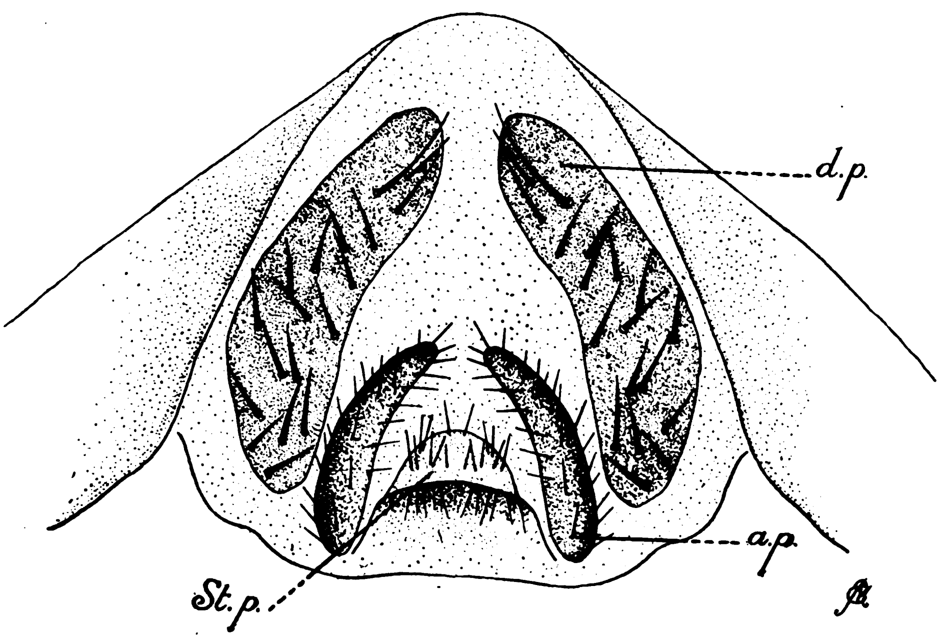

FIG. 3. *Glossina fusca*: ♀. External armature, × c.80. Lettering as in Fig. 1.

the entire surface. The anal plates (*a. p.*) project almost vertically one at each side of the sternal plate, and, when mounted in dorso-ventral aspect (fig. 3), each presents a narrow crescentic outline. When viewed in a horizontal position they are seen to have a somewhat rectangular form (fig. 1, *a. p.*) They bear setae which are much smaller, finer, and more flexible than those borne on the dorsal plate.

Signum of the uterus (fig. 4). The genital fossae are oblong-ovate in outline, the long axis being transverse. The signum of a typical *G. fusca* is composed mainly of a pair of sub-conical, hollow and highly chitinous lobes (*e.*), the colour varying from dark mahogany-brown to black. The inner surface of each lobe on either side of the median line, in front usually bears more or less strongly defined ridges. In dorso-ventral view a crescentic sclerite (*cr.*), which may

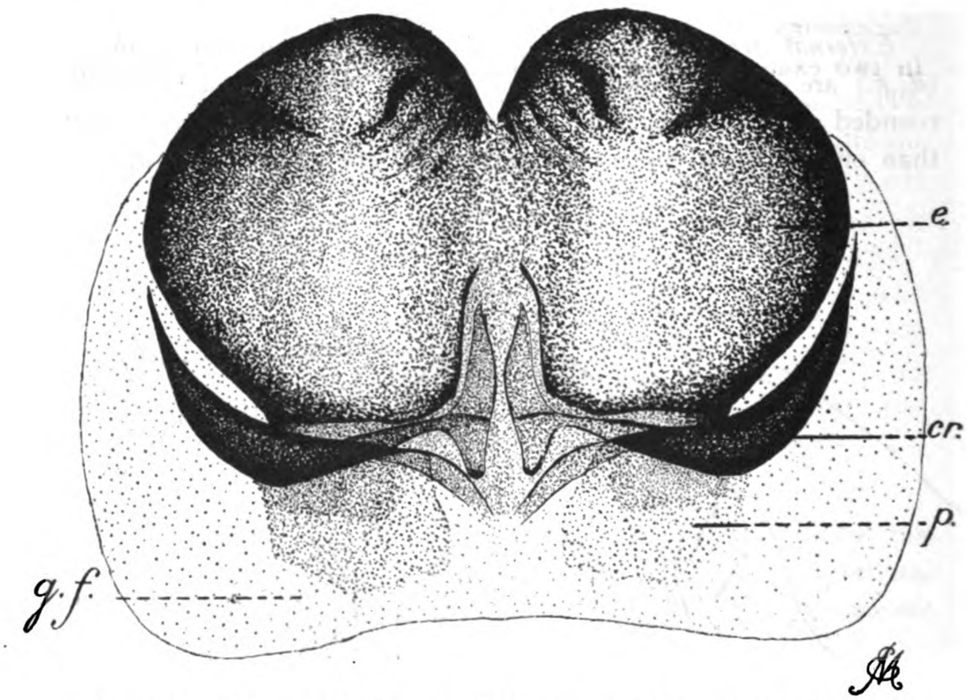

FIG. 4. *Glossina fusca* : ♀. Signum, × *c.*155. *cr.*, crescentic sclerite; *e.*, main elements; *p.*, posterior plate; other lettering as in Fig. 1.

appear densely black, bounds or overlies the posterior or postero-lateral border of each lobe. In lateral view this is seen to be in reality a curved plate of chitin projecting from the base of the lobe. At its upper extremity it is sharply bent, so that the posterior portion appears in surface view as a pale ochraceous plate (fig. 4, *p.*) completing the signum behind.

Type of female armature taken at Tainsu, Wenchi, N. Ashanti, 24.4.10 (Dr. A. Kinghorn). Five other typical examples were from the following localities:—Sunyani, Ashanti (2), 18.10.13 (Dr. F. H.

Storey); Volta River, Kpong, Gold Coast, 9.1918 (Dr. P. D. Oakley); and Tekiman Territory, Ashanti, 4.1912 (Dr. T. E. Fell).

The nine examples of *G. fusca* from the Katanga district of the Congo Free State taken by Dr. J. Schwetz were found to exhibit a marked deviation from the form of signum described above. The main elements or lobes (fig. 1, A, *g.*) are sub-hemispherical, semi-transparent, and of ochraceous colour; they bear in front a median collar-like projection (*a. p.*), and in addition a pair of distinct dorsal thickenings gives rise to the conspicuous, petaloid marking (*p.*). In two examples from the Belgian Congo (ex. coll. Musée Royal d'Histoire Naturelle de Belgique), the internal wall was partially unchitinised and the condition was somewhat intermediate between the typical form and that of the Katanga specimens, though it more closely resembled the former. The signum of specimens from Buamba Forest, Semliki Valley, 2,300-2,805 feet, Uganda Protectorate, 3-7 November, 1911, was largely unchitinised posteriorly, and anteriorly presented features in common with the specimens from Katanga; the median anterior projection and petaloid markings were conspicuously present.

Glossina nigrofusca, Newstead

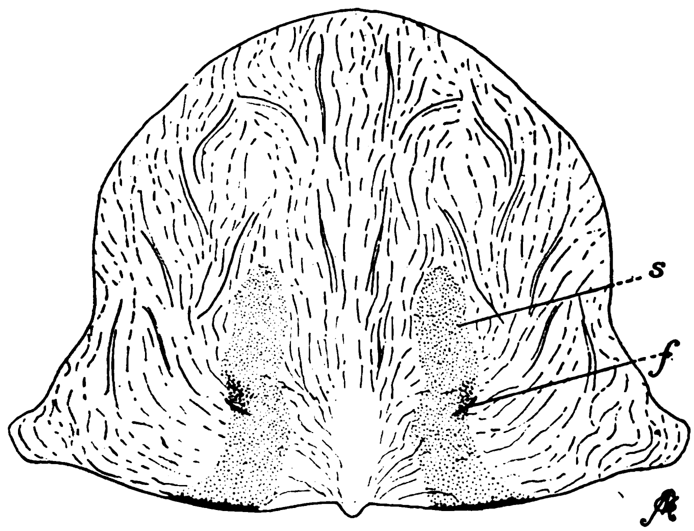

FIG. 5. *Glossina nigrofusca*: ♀. Signum, × *c.*100. *f.*, fold in signum.

External armature of the female. Resembles that of *G. fusca.*

Signum of the uterus (fig. 5). This is slighlty developed, consisting solely of a pair of small elongate strips of pale yellow chitin. The surrounding membrane is thrown into innumerable folds of intricate character, a pair of which traverses the bars of the signum, giving rise to a fold (fig. 5, *f.*) near the middle distance.

Type of female armature taken at Sunyani, Ashanti, 9.6.13 (Dr. F. H. Storey), other examples at Fiapri, Ashanti, Gold Coast (Dr. F. H. Storey) received 17.6.13; and Boonso, Birrim District, Gold Coast, 9.1912 (Dr. David Duff). The form of signum here described was met with also in two specimens from Tain River, Nsoko, W. Ashanti (Dr. A. Kinghorn), one taken 7.5.10, the other 21.5.10. The antennae were, however, not specifically identical with those of *G. nigrofusca*, but resembled those of *G. fusca*, while the thoracic markings were paler than in typical forms of either of these species. A parallel case has been found in the males; a specimen from Ashanti (Dr. A. Kinghorn) having internal armature typical of *G. nigrofusca*, antennae resembling those of *G. fusca*, and pale thoracic markings.

Glossina fuscipleuris, Austen

External armature of the female. Differs in no essential respects from that of *G. fusca*.

Signum of the uterus (fig. 6). A narrowly elongate structure the length about four times the greatest width. It is strongly flexed in the middle of its length (fig. 6, C.), the two limbs being almost at right angles. Fig 6, A, shews the signum in dorso-ventral aspect, the internal surface uppermost, having been straightened by pressure. In this position it appears as a ligulate, strongly ochraceous, lamina (*l.*) of somewhat irregular outline with a more or less deep bifurcation distally. Proximally a stem-like portion projects backwards carrying a pair of small lateral expansions (*l. e.*). This projecting process consists of a pair of very thick strongly chitinous ridges (*t. p.*), which form a prominent keel beneath the posterior surface of the lamina (fig. 6, C.). In the middle length of the latter arise at the borders a pair of band-like anterior thickenings (*t. a.*), which run forward supporting the lamina and converging distally. Medianly the chitin is thin, and may give place to one, two or more small apertures. When mounted without manipulation the signum tends to lie in a lateral position as shown in fig. 6, C. Viewed thus the most prominent feature is the black keel-like posterior ridge, while the anterior portion mainly consists of the thickening (*t. a.*).

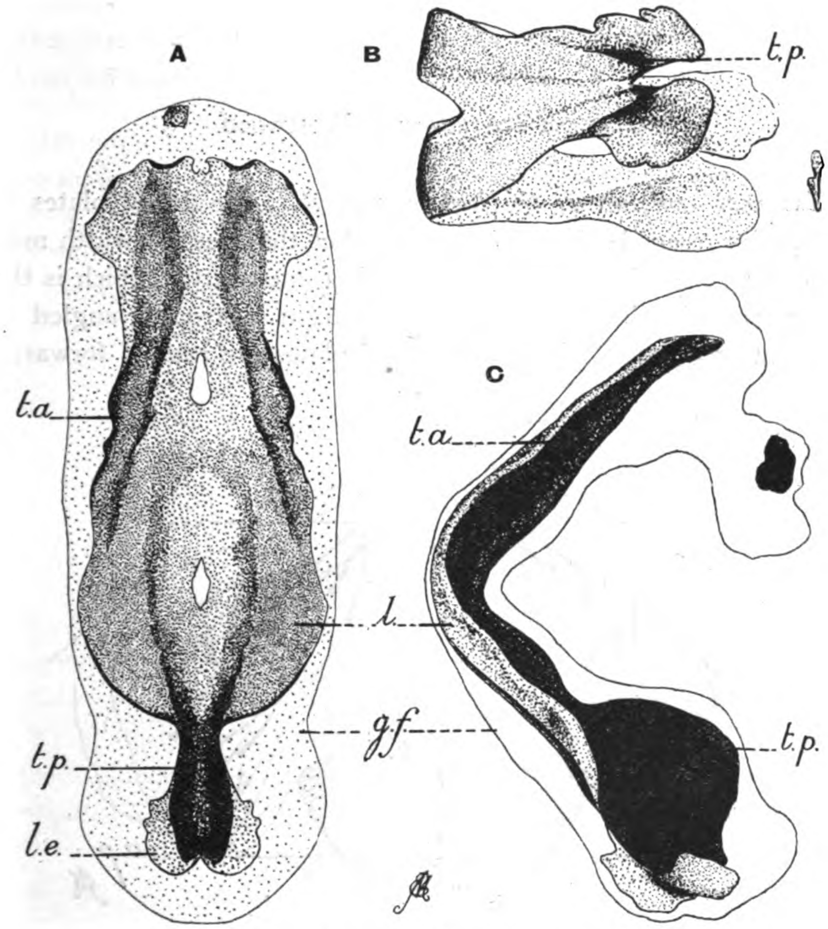

FIG. 6. *Glossina fuscipleuris*: ♀. Signum, × *c.*100. A. flattened specimen, dorso-ventral aspect; B. folded condition; C. flexed condition, lateral aspect; *l.*, lamina; *l.e.*, lateral expansions of posterior projection; *t.a.*, anterior thickening; *t.p.*, posterior thickening.

Type of female armature from the Belgian Congo (ex-coll. Musée Royal d'Histoire Naturelle de Belgique); no further data available. Two others were taken at Daru Forest, Uganda Protectorate, 25-29.10.1911 (Dr. S. A. Neave), and one at Buamba

Forest, Semliki Valley (2,300-2,800 feet), 3-7.11.1911 (Dr. S. A. Neave). In the latter specimen the signum was doubled on itself (fig. 6, B), and the posterior pair of thickenings (*t. p.*) were much shorter and less prominent than in the other specimens examined.

Glossina tabaniformis, Westwood

External armature of the female (fig. 7). The dorsal plates in this species resemble those of *G. fusca*, but the greatest width may be equal to one-third the length instead of one-quarter, which is the average ratio in *G. fusca*. The outer margin is strongly angled at the point of greatest width. The anal plates are rotated forwards

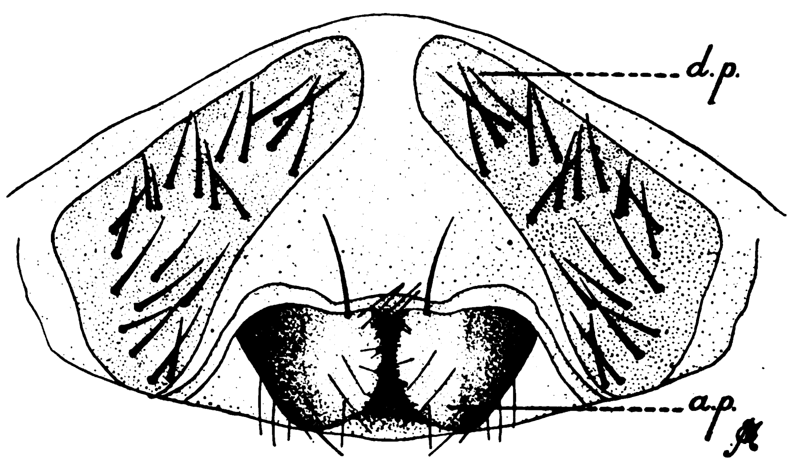

FIG. 7. *Glossina tabaniformis*: ♀. External armature, × c.80.

to a marked degree so that their free edges project ventrally, and when mounted with slight pressure they obscure from view the sternal plate. The condition has also been observed in isolated specimens of other species, but it is only in *G. tabaniformis* that it appears to be a constant character.

Signum of the uterus (fig. 8). The signum consists of a lyriform lamina somewhat depressed medianly. A transverse constriction divides it into two unequal portions, the proximal roughly twice as long as, and one and a half times as broad as, the distal. The former is sub-ovate in outline and bears two or three terminal processes diverging to a varying extent. The anterior division terminates in two projections. The whole is strengthened by a pair of curved bands of thickened chitin.

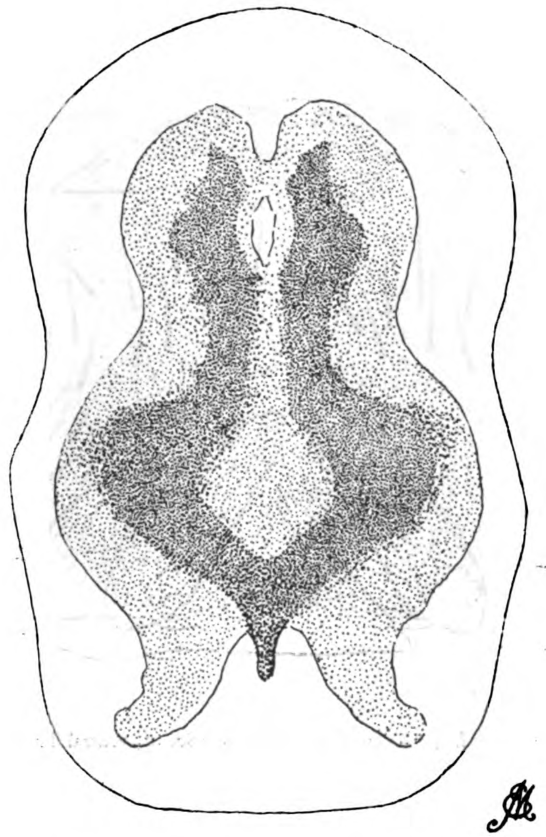

FIG. 8. *Glossina tabaniformis* : ♀. Signum, × *c*.155.

Type of female armature taken at Yombi Yombi, Leverville, Congo, 3.10.13 (Dr. Arbrassart). Two were taken at Yakusu (?), Haut Congo (Rev. Sutton-Smith) and another in Nigeria (Dr. T. R. Leonard).

Glossina severini, Newstead

External armature of the female (fig. 9). The characteristic feature of the armature of this species is the great extent of the dorsal plates. The external borders are strongly convex; the internal distally straight, and closely approximated in the median line, for a distance of half the total height of the armature. Proximally the latter diverge to form the concave inner edges of the ventral limb (*v.*) of the plate. The other plates are similar to those in *G. fusca*.

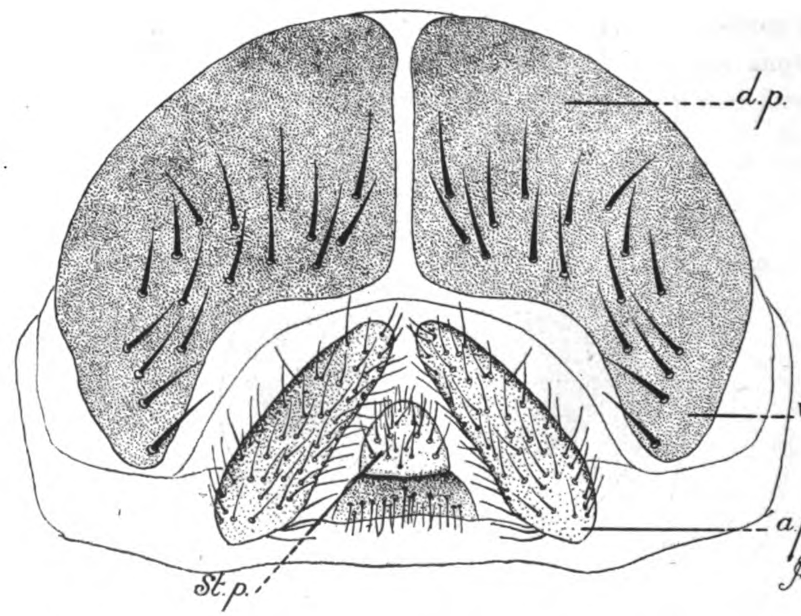

FIG. 9. *Glossini severini*: ♀. External armature, × *c*.80. *v.*, ventral limb of dorsal plate.

Signum of the uterus (fig. 10). The outline of the signum in dorso-ventral aspect (fig. 10) is oblong, the length being one and a half times the greatest width. *It is composed of a pair of plates separated throughout their length.* Each plate consists of an elongate proximal limb surmounted by a broader distal piece, the latter medianly closely approaching that of the opposite side.

Outwardly the plates are curved upwards so as to embrace the lateral walls of the genital fossae.

Type of female armature from the Belgian Congo (ex-coll. Musée Royal d'Histoire Naturelle de Belgique); no further data available.

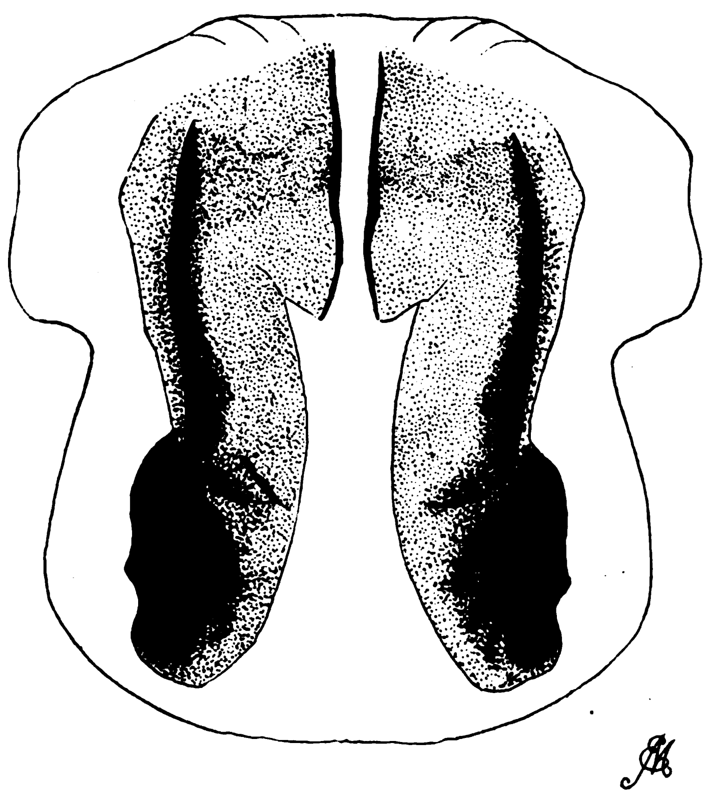

FIG. 10. *Glossina severini* : ♀. Signum, × c.155.

In a second specimen from this region the ventral limb of the dorsal plate was more prolonged than in that of the one figured here.

Glossina medicorum, Austen

External armature of the female (fig. 11). The dorsal plates are of a type frequently met with in *G. fusca*. They are almost uniformly narrow, the long axes slightly convex, and the extremities obliquely truncate. The lateral plates are inclined towards one another, and when mounted largely obscure from view the ventral plate.

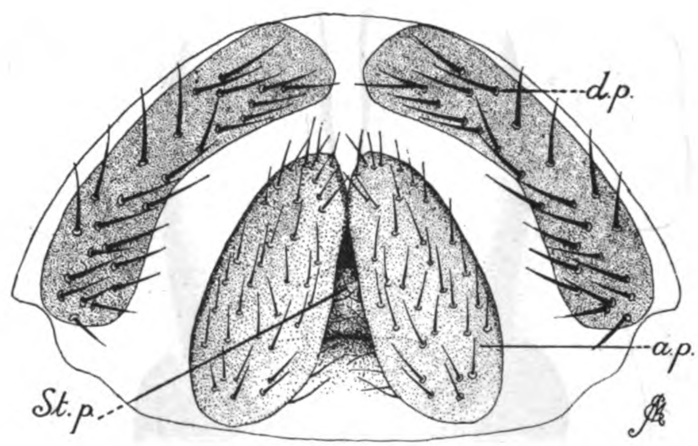

FIG. 11. *Glossina medicorum* : ♀. External armature, × *c*.80.

Signum of the uterus (fig. 12). The outline of the fossae in dorso-ventral aspect is elongated; the proximal portion cordiform, an appearance produced by the presence of two convergent ridges, separated by a deep median cleft. The length is twice the greatest width. The signum consists of two distally convergent ochraceous bands which clothe the crests of the two ridges and posteriorly are sharply curved to meet one another in the median line. A pair of crenulated, chitinous thickenings (*c. r.*) of the wall of the fossae extend from the narrow anterior extremities of the sclerites toward the border of the fossae, and in the region of their termination lie one or two irregular groups of dark granules. All four specimens exhibited to a well marked degree the aristal character described by Newstead (1913).

Type of female armature, from Black Volta River, N. Ashanti, 13.4.10 (Dr. A. Kinghorn). A second was without data; a third was from Volta River, P.A. Gl. Rapids, G.F.S., 1.12 (Dr. A. M. Dowdall), and a fourth from Volta River, 30. m. N. Kpong, 9.1911, Gold Coast (Dr. P. D. Oakley).

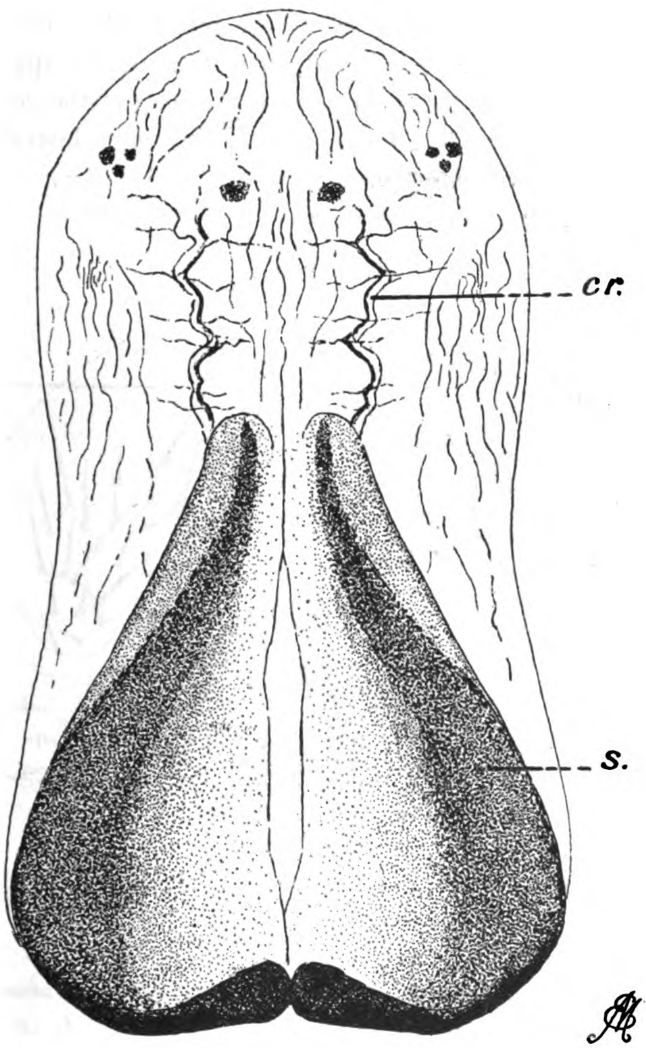

Fig. 12. ***Glossina mediocrum* : ♀. Signum, × c.155. *cr.*, crenulated thickening of wall of genital fossa.**

Glossina brevipalpis, Newstead

External armature of the female (fig. 13). The dorsal plates are relatively broader than in any other members of the group, the greatest width being about two-thirds the greatest length. The obliquely-placed inner edges are almost straight. The margin is curved externally, bluntly pointed apically and obliquely truncate proximally. Spines are confined to the inner halves of the plates. The lateral plates are broad and their free edges approach one another medially. In the membrane at the base of the ventral plate occurs a pair of *hamate sclerites* (*h. s.*), the outer lateral edges of which are irregularly dentate.

Signum of the uterus. Absent.

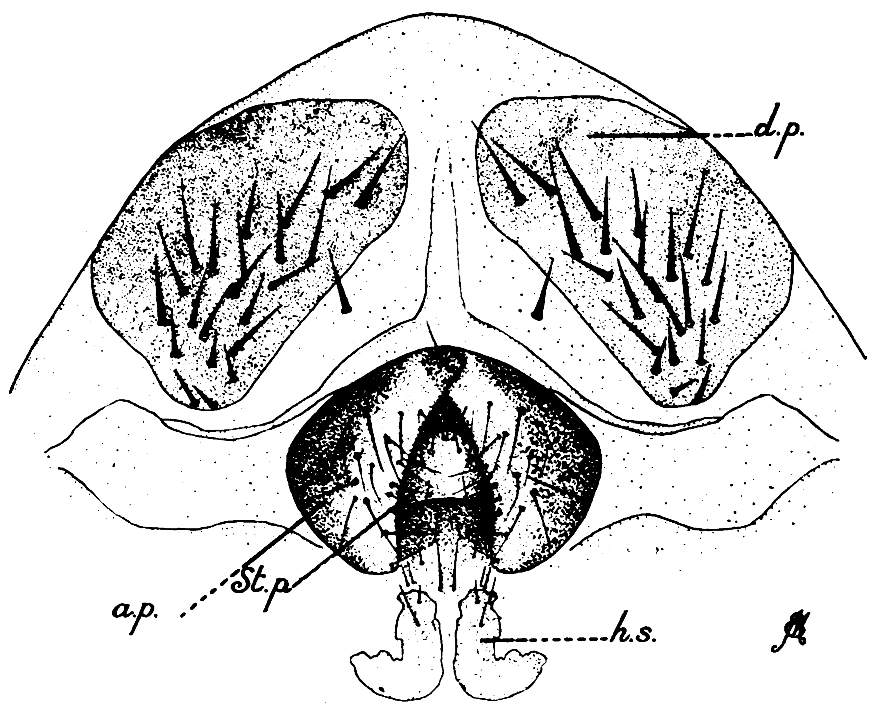

FIG. 13. *Glossina brevipalpis* : ♀. External armature, × c.80. *h.s.*, hamate sclerite.

The type of female armature was taken at Ng'ani Nyassa, 1912 (Dr. M. Sanderson); two examples at Katanga, Belgian Congo (Dr. J. Schwetz), another Juba River, East Africa (Dr. R. P. Filleul) and a fifth at Makulu, Congo, 11.7.04 (Drs. Dutton and Todd).

Glossina longipennis, Corti

External armature of the female (fig. 14). The external armature is unusually prominent, owing to the fact that the dorsal plates do not lie in the same plane, but are opposed to one another at a considerable angle, carrying out the lateral plates, which project from the extremity of the abdomen to a very marked extent. The dorsal plates are sub-triangular in outline, and their inner edges are adjacent from the apex to a distance approaching one-third of the length of the plate. Setae are confined to the proximal two-thirds of the inner half of each plate.

Signum of the uterus Absent.

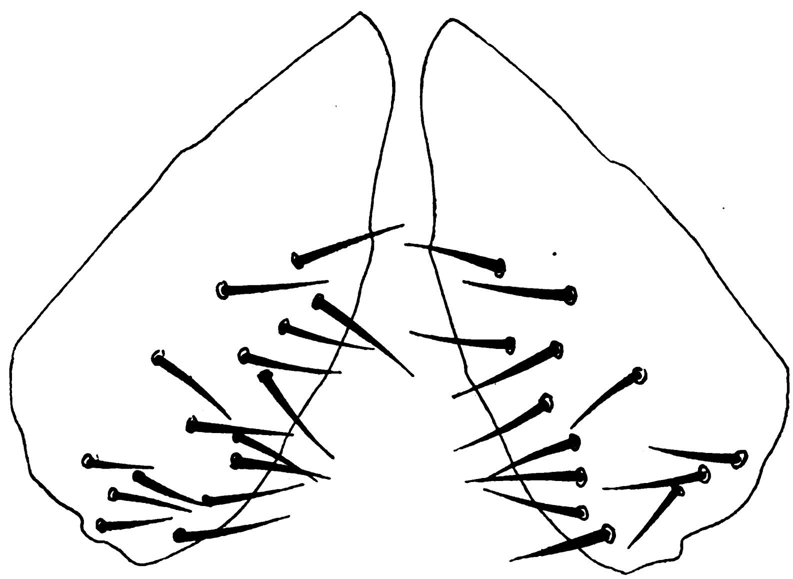

FIG. 14. *Glossina longipennis* : ♀. Dorsal plates, × c.80.

Type of female armature from Nairobi, British East Africa, 28.5.12 (Dr. A. D. Milne). In two other examples examined, one from this region, a second from Entebbe, Uganda (Dr. Christy), the dorsal plates were entirely membraneous; their position merely indicated by the presence of the dark spines.

GROUP II. PALPALIS GROUP (figs. 15, 16). *External armature, consisting of six plates: in addition to those present in Group I, there is a small medio-dorsal plate. Signum of uterus absent.*

External armature. Mean dimensions 0·6 × 0·5 mm. This structure presents an essentially compact aspect; the various plates, which are of comparatively great superficial extent, are of such form and disposition that, together they almost entirely fill the space in which the armature lies. The dorsal plates (*d. p.*) are sub-triangular in outline, their inner edges lying parallel and in close proximity. They bear spines smaller but similar in character to those borne by the dorsal plates of *G. fusca*. The anal plates (*a. p.*) are broadly triangular, and are closely applied to the underlying integument, to which they are attached by all but their free inner edges. Thus they cannot be reflexed as can those of the *Fusca* Group. Between the lateral and dorsal plates occurs a small oval sclerite (*m.d.p.*), which possibly corresponds to the 'dorsal scale' of the Blow-fly, but which is absent in the *Fusca* Group. It may, on the other hand, be a detached median portion of the VIII° tergite.

Glossina palpalis, Robineau-Desvoidy

External armature (fig. 15). The outline of the containing area is roughly circular. The dorsal plates (*d. p.*) are of chitin varying from brown to pale ochraceous colour, in one case they were as transparent as the surrounding membraneous integument. The general shape is that of a quadrant, the curved border lying externally, and the vertical inner edges parallel and closely approximated. The latter are sometimes almost entire, but frequently a variable number of indentations causes a jagged and irregular outline. In some cases the two plates may be connected medially by one or more bridges of chitin. The transverse proximal border of each dorsal plate is usually to a greater or less extent emarginate in the middle of its length, with the result that the inner angular portion, which is devoid of long spines, is more or less constricted off from the rest of the plate. The anal plates (*a. p.*), when mounted, consist of a broadly sub-triangular portion which lies almost parallel with the integument, and is folded externally so as to overlie the smaller basal portion from which it arises. The free edge is directed towards the sternal plate and the distal border appears to have become secondarily fused with the integument. The sternal plate (*st. p.*) is broadly rounded apically and, as in the lateral plates, the basal portion lies below that seen in surface view. The width at the base is greater than the length. The medio-dorsal plate is variable in size, and may consist of one, two, or three sclerites.

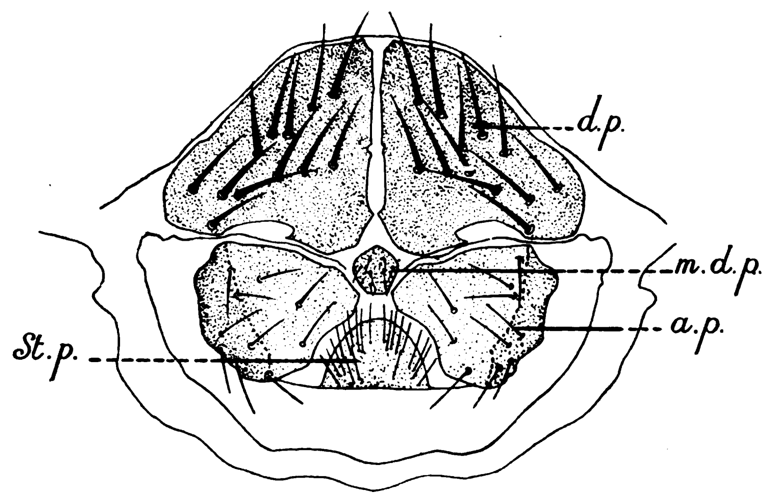

FIG. 15. *Glossina palpalis* : ♀. External armature, × *c.*90. *m.d.p.*, medio-dorsal plate.

Type of female armature taken at Kintampo, N. Ashanti, April to July, 1913 (Dr. A. Ingram); ten other specimens from this locality were examined. Eight were taken at Volta River, Gold Coast, 1913 (Dr. A. M. Dowdall), and two of a 'small dark grey form', Illorin, N. Nigeria (Dr. J. W. Scott Macfie).

Glossina palpalis, Rob.-Desv., race *fuscipes*, Newst.

External armature of the female. Resembled that of *G. palpalis*, but the constriction of the angular portion of the dorsal plate was very well marked. Further, the presence of well developed spines in this median region was a constant feature, and frequently these were arranged in a definite 'V' shaped group, the apex directed dorsally and lying in the median line between the dorsal plates at about the middle length. The presence or absence of these medianly-placed spines can usually be determined by examination of the terminal wall of the untreated dry specimen under the low power of the microscope. Before attributing any definite value to this character, however, it is hoped by examination of further material to discover whether or not this course is justifiable.

Six specimens examined were taken at Nimule, Uganda, July, 1911 (Dr. R. G. McConnel).

Glossina caliginea, Austen

External armature of the female. Examination of four specimens revealed no features of distinction which could be used to separate this species from *G. palpalis*. The examples were taken at Yewa River, South Nigeria, 10-12.9.1911 (Captain L. E. H. Holmfrey).

Glossina pallicera, Bigot

External armature of the female. The dorsal plates, unlike those of *G. palpalis*, are not right angled internally, but in one specimen the two shorter sides of the plate enclose a widely obtuse angle, and thus expose above the anal and sternal plates a comparatively large triangular median area of unchitinised membrane. In the other specimen examined the dorsal plates are bounded by a continuous curved margin, the outline of the plate being ovoid. In both cases the greatest width of the plate was one-half of the greatest length, while in *G. palpalis* this ratio is approximately 2 : 3. The sternal plate was, in both examples, proportionally longer and narrower than that of any other member of the *Palpalis* Group, the width at the base being considerably less than the greatest length; in the second specimen it was markedly attenuated and digitiform in outline. The medio-dorsal plate was of very small extent in both cases. The first example was taken at Côte d'Ivoire, 1910 (Prof. E. Roubaud, ex-coll. Museum, Paris), the second at Tetchari, Tanoso, W. Ashanti, 4.8.10 (Dr. A. Kinghorn).

Glossina tachinoides, Westwood

External armature of the female (fig. 16). Dorsal plates right angled internally but much narrower and more elongated than in *G. palpalis*. The height is rather less than twice the greatest width. The other plates resemble those of *G. palpalis*.

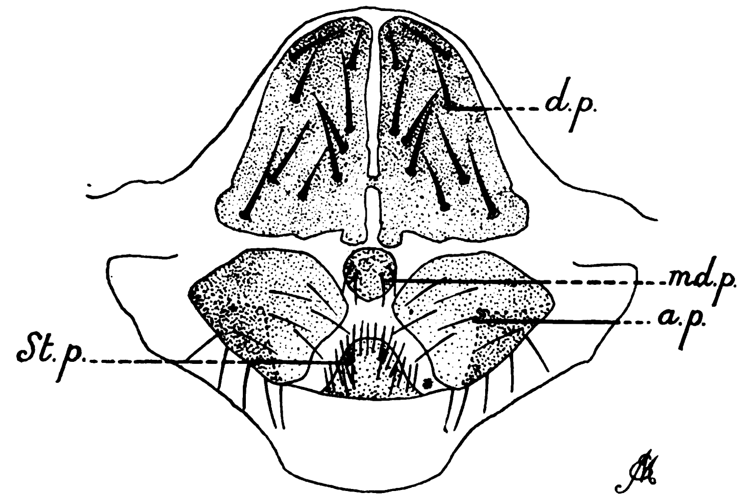

FIG. 16. *Glossina tachinoides* : ♀. External armature, × *c*.90.

Type of female armature taken at Lorha, Gold Coast, 1915 (Dr. J. F. Corson); a second specimen bore the same data. Other specimens were from Salago, Gold Coast, June 12th (Dr. Lefanu).

GROUP III. MORSITANS GROUP (figs. 17, 18). *External armature, consisting of a pair of fused anal plates and a median sternal plate. Dorsal plates generally absent. Signum of uterus absent.*

External armature. Mean dimensions 0·4 × 0·2 mm. The condition and extent of the armature exhibit very considerable reduction from that of the *Fusca* Group. In consequence of the loss or reduction of the dorsal plates, the area in which the armature lies is much shallower than in the case of Groups I or II, and is roughly in the form of an ellipse, the long axis of which runs transversely. The anal plates (*a.p.*) are of almost membraneous character, the walls collapsing when mounted under slight pressure after treatment in boiling KOH. They are united dorsally to form a continuous arc surrounding the free edge of the sternal plate. The median portion

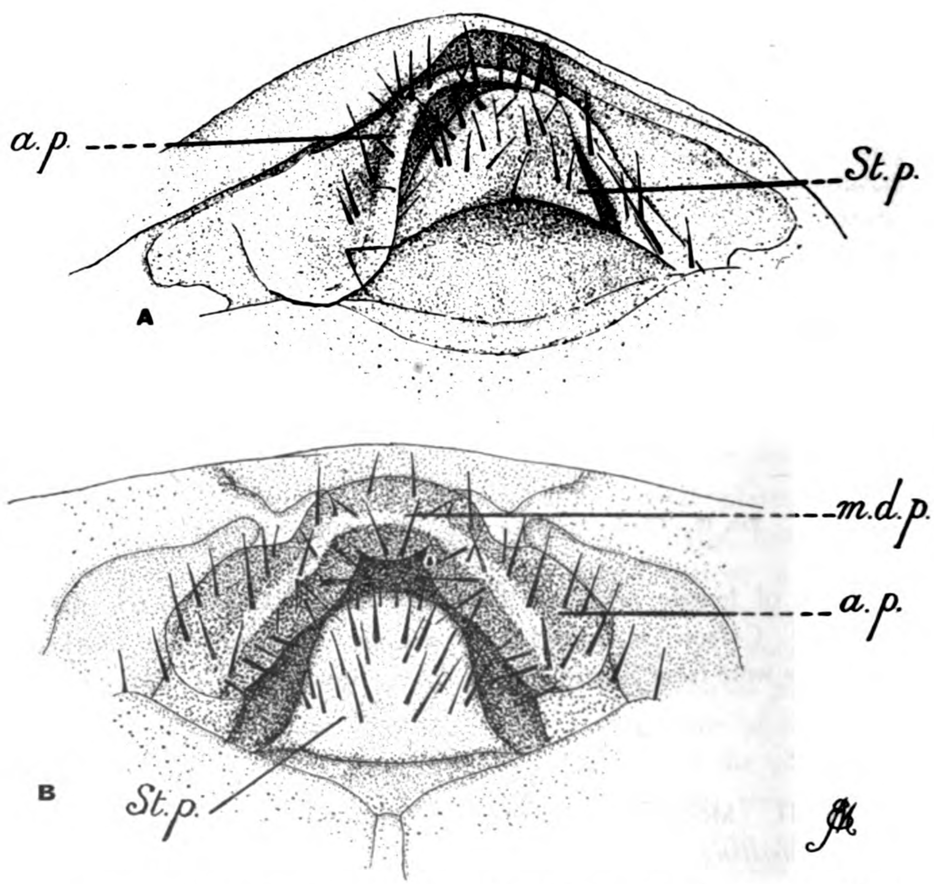

FIG. 17. *Glossina morsitans* : ♀. External armature, × *c.*155. A. mounted without maceration; B. mounted with slight pressure after maceration.

of the arc (*m. d. p.*) projects dorsally as shown in fig. 17, B, and possibly represents the 'medio-dorsal scale', which has become fused laterally with the anal plates. *G. austeni* was exceptional in the possession of a pair of small, though well developed dorsal plates.

Glossina morsitans, Westwood

External armature of the female (fig. 17). Dorsal plates generally absent, but when present extremely thin, not bearing dark spines. For other characters see pp. 53-4.

Nine of the examples were taken by Prof. R. Newstead and Dr. J. B. Davey during the Nyasaland Expedition of 1911. Seven others were from Zambesi, 1907 (Kinghorn and Montgomery).

Glossina austeni, Newstead

External armature of the female (fig. 18). This is distinguished from that of all other members of the *Morsitans* Group by the possession of a pair of small, but well defined, dorsal plates (*d.p.* and A) bearing relatively very long spines, the latter of a similar character

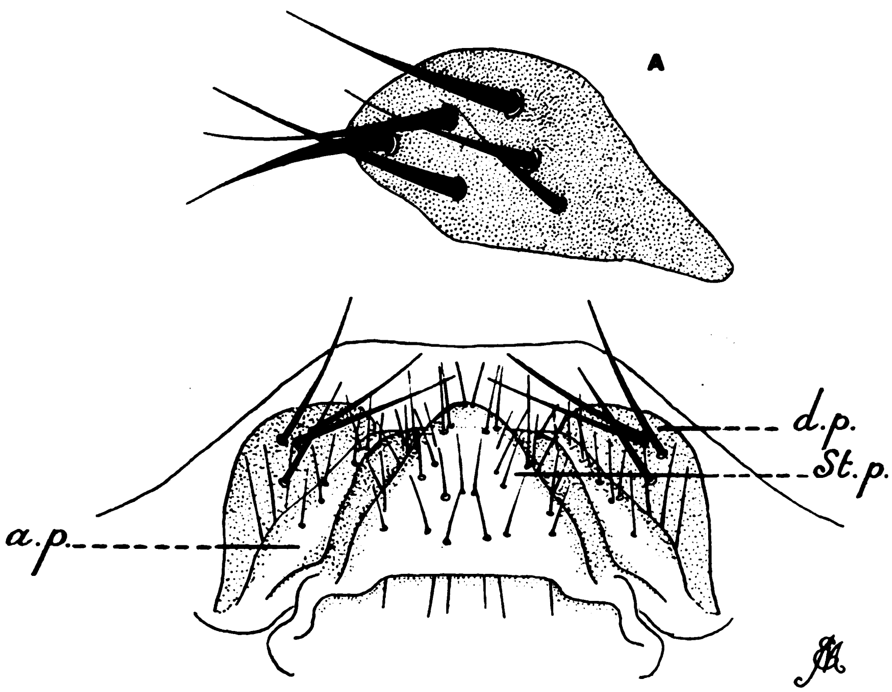

FIG. 18. *Glossina austeni*: ♀. External armature, × c.155. A. dorsal plate, × c.240.

to those borne on the dorsal plate in *G. fusca*. The dorsal plates are sub-triangular in form and widely separated.

The three examples examined were taken at Jubaland, 20.8.12 (Dr. R. P. Filleul).

Other species of this group were investigated, but none of these shewed any marked features distinguishing the armature from that of *G. morsitans*. The localities from which they were taken were as follows:—

G. submorsitans, Newstead. Makongo, Gold Coast, 14.10.14 (Dr. J. F. Corson); Prang, Gold Coast, 10.10.14 (Dr. J. F. Corson).

G. pallidipes, Austen. Two specimens at Juba River, East Africa (Dr. R. P. Filleul), and two others at Alexandra, Gosha, Jubaland, 6.1912 (Dr. R. P. Filleul).

G. longipalpis, Wiedemann. Two specimens taken at Edinam, Ashanti, 3.5.13 (Dr. F. H. Storey).

REFERENCES

LOWNE, B. T. (1893-95). The Blow Fly, Vol. II, pp. 745-6 and 675-77.

NEWSTEAD, R. (1911). A Revision of the Tsetse-flies (*Glossina*), based on a Study of the Male Genital Armature. *Bull. Ent. Res.*, Vol. II, May, 1911.

—— (1913). A New Tsetse-fly from the Congo Free State, and the Occurrence of *Glossina austeni* in German East Africa. *Ann. Trop. Med. & Parasitol.*, Vol. VII, No. 2, June, 1913.

PIERCE, F. N. (1914). The Genitalia of the Geometridae.

STUHLMANN, F. (1907). Beitrage zur Kenntniss der Tsetsefliege *Arbeiten aus. dem. Kaiserlichen Gesund*, Bd. XXVI, Heft. 3, pp. 57, 58.

STRONGYLIDAE IN HORSES

VII. *CYLICOSTOMUM PATERATUM* sp. n.

BY

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AND

J. W. S. MACFIE

(*Received for publication* 10 *March*, 1919)

SIZE AND SHAPE. A moderately small species of the GENUS *Cylicostomum*, the female being slightly larger than the male. Twenty females and ten males were measured. The males were from 8·0 to 9·5 mm. in length, average 8·6 mm.; the females from 8·4 to 11 mm. in length, average 9·5 mm.; the greatest breadth, in those worms which were properly orientated, averaged, males 380μ, females 393μ.

HEAD. A well-marked neck separated the head from the body.

Mouth collar. Marked off from the rest of the skin by a definite constriction.

Head papillae. Sub-median, pointed and projecting, their extremities separated off from the remainder by lateral notches; lateral, prominent.

Mouth capsule. Ellipsoidal in transverse section, the ratio of the lateral diameter to the dorso-ventral diameter of the anterior opening of the buccal capsule being about 1 to 1·3. When the worm is properly orientated the walls of the mouth capsule seen in optical section are wedge-shaped, being slender anteriorly and stout posteriorly; the inner surface is deeply notched at the level of the insertion of the internal leaf crown (fig. 1). When viewed laterally the walls of the buccal capsule converge considerably from before backwards (fig. 3). In properly orientated worms the antero-posterior diameter (i.e., the distance from the anterior to the posterior opening) of the buccal capsule varies in the males from 25μ to 28·5μ, average 26·7μ, and in the females from 26·5μ to 28μ, average 27·2μ. When the worm is lying on its side the mouth capsule appears less deep owing to the fact that the walls of the buccal capsule are set obliquely (fig. 3). In the males the lateral diameter of the buccal capsule at the anterior opening varies from

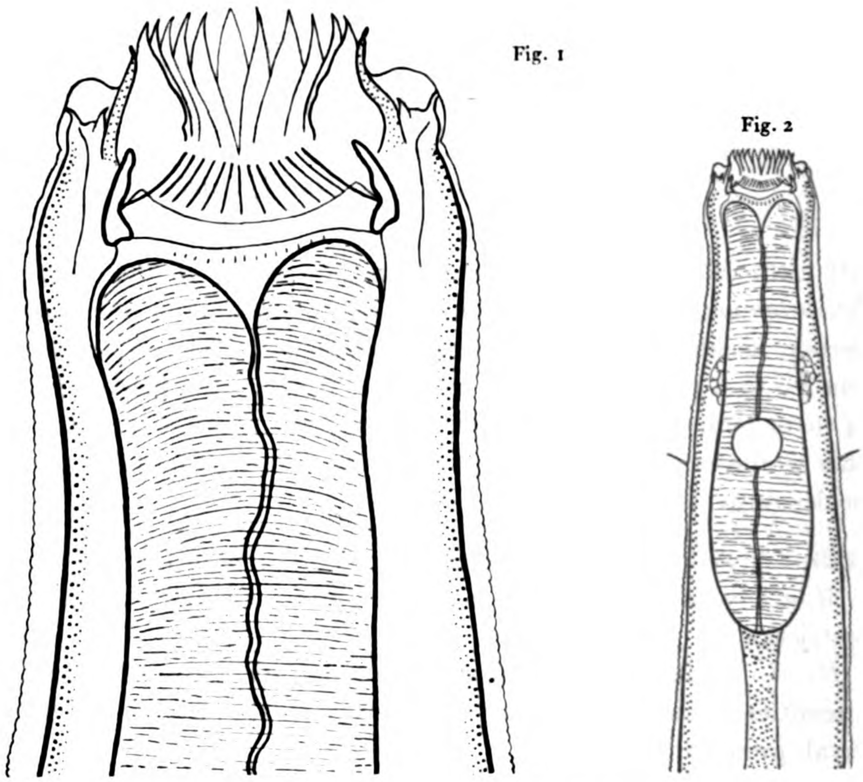


Fig. 1

Fig. 2

FIGS. 1-2. *Cylicostomum pateratum* sp. n.

Anterior extremity, ventral view. Fig. 1 × 360. Fig. 2 × 90.

80μ to 87μ, average 85μ, and at the posterior opening from 75μ to 84μ, average 79μ; in the females the lateral diameter of the buccal capsule at the anterior opening varies from 78μ to 97μ, average 89μ, and at the posterior opening from 80μ to 95μ, average 88μ. The ratio of the lateral diameter of the anterior opening of the buccal capsule to that of the posterior opening is therefore about 1 to 1. The ratio of the lateral diameter of the buccal capsule at the anterior opening to the antero-posterior diameter is in both sexes about 3·25 to 1.

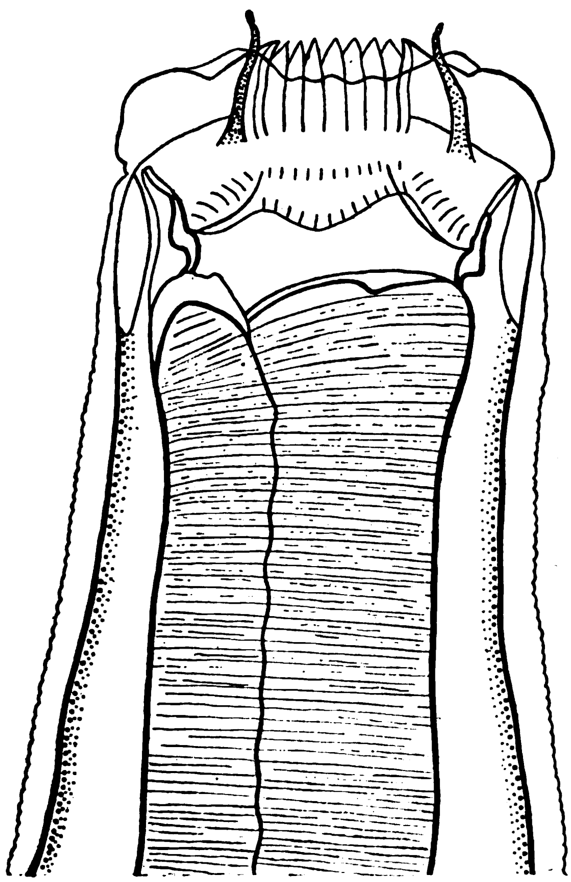
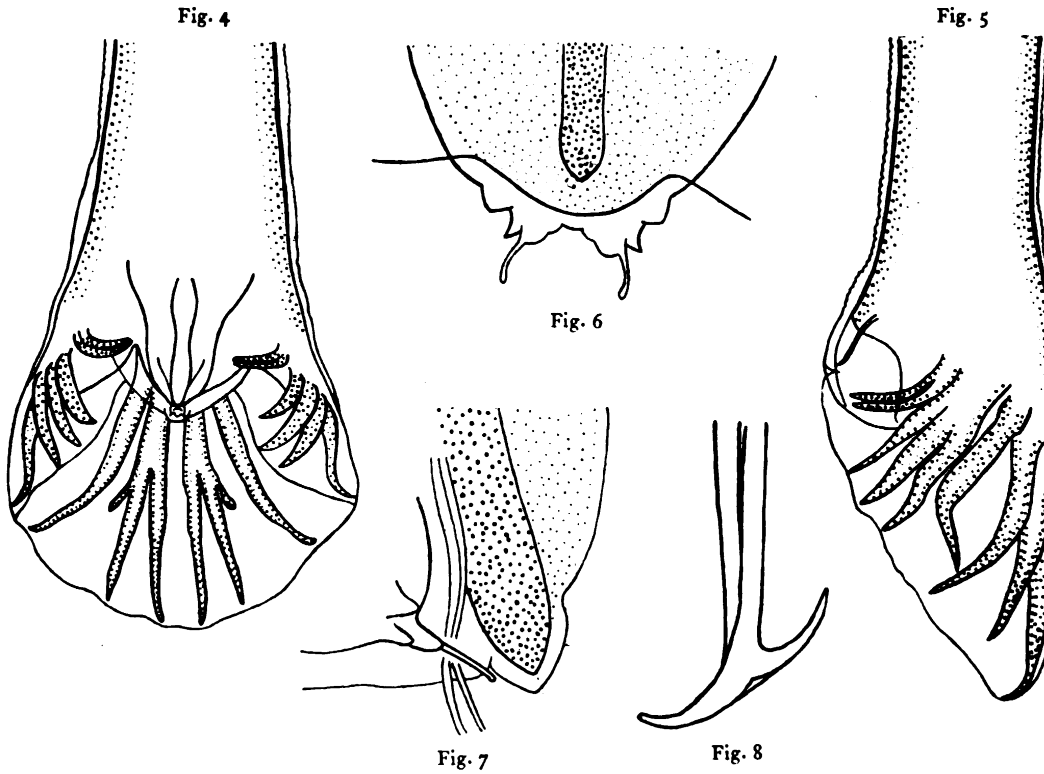


FIG. 3. *Cylicostomum pateratum* sp. n.
Anterior extremity, lateral view, × 360.

Dorsal oesophageal gutter. Does not project into the buccal capsule.

Leaf crowns. The external leaf crown consists of about twenty-four large pointed elements arising from the mouth collar. The internal leaf crown consists of numerous long narrow elements arising from a sinuous line situated deep in the mouth capsule (figs. 1 and 3).

OESOPHAGUS. The length in ten males varied from 530μ to 602μ, average 559μ, and the greatest breadth from 131μ to 155μ, average 146μ; the ratio of breadth to length is 1 to 3·8. In twelve females the length ranged from 561μ to 595μ, average 582μ, and the greatest breadth from 136μ to 171μ, average 149μ; the ratio of breadth to length is 1 to 3·9. The ratio of the length of the oesophagus to that of the worm is, in the male 1 to 15, and in the female 1 to 16.



FIGS. 4-8. *Cylicostomum pateratum* sp. n.

Fig. 4: Posterior extremity of male, ventral view, × 90.
Fig. 5: Posterior extremity of male, lateral view, × 90.
Fig. 6: Genital appendages, ventral view, × 360.
Fig. 7: Genital cone and appendages, lateral view, × 360.
Fig. 8: End of spicules × 1360.

EXCRETORY BLADDER. Lies just behind the nerve ring. The distance of its posterior margin from the posterior end of the oesophagus varies from 208μ to 280μ, average 244μ.

CERVICAL PAPILLAE. Lie at about the same level as the excretory bladder.

POSTERIOR EXTREMITY OF MALE. The dorsal lobe of the bursa is short, about semicircular (fig. 4). In eight worms the length of the main trunk of the posterior ray from the tip to the point of origin of the postero-external rays varied from 391μ to 495μ, average 442μ. The ratio of the average length of the main trunk of the posterior ray to the average length of the male worm is 1 to 19·5.

Genital Cone. The dermal collar is well developed on both the ventral and dorsal surfaces of the genital cone. The genital appendages on each side are represented by slight elevations furnished with three conical processes, the innermost bearing a long delicate finger-like process (fig. 6).

Spicules. The ends of the spicules are barbed as shown in fig. 8.

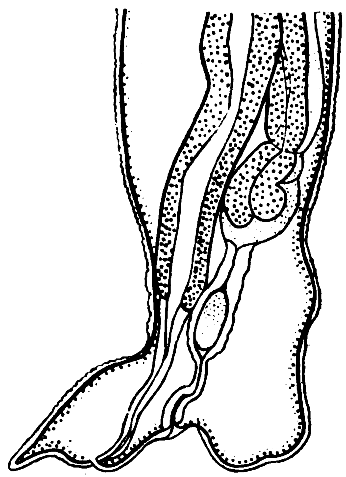


FIG. 9. *Cylicostomum pateratum* sp. n.
Posterior extremity of female, lateral view, × 90.

POSTERIOR EXTREMITY OF THE FEMALE. The end of the body is bent dorsally at right angles. The ventral prominence is large and projecting. The tail is very short and conical (fig. 9). In six worms the distance between the anus and vulva varied from 91μ to 116μ, average 104μ, and the distance measured straight along the middle line of the tail from the tip to a line drawn horizontally through the anus varied from 105μ to 143μ, average 114μ.

DIAGNOSIS. The following are the chief diagnostic characters of this worm:—

1. Size, moderately small; average length, males 8·6 mm. and females 9·5 mm.

2. Buccal capsule: anterior opening ellipsoidal, ratio of lateral to dorso-ventral diameter of anterior opening of capsule is 1 to 1·3; walls when seen in optical section, in properly orientated worms, are wedge-shaped, being slender anteriorly and stout posteriorly; the inner surface is deeply notched; ratio of breadth at anterior opening to antero-posterior diameter 3·25 to 1.

3. Dorsal oesophagal gutter does not project into buccal capsule.

4. Dorsal lobe of bursa short, almost semicircular; ratio of length of posterior ray to total length of male worm 1 to 19·5. The genital appendages are slight elevations furnished with three conical processes, of which the innermost is largest and bears a long delicate finger-like process.

5. Termination of female body bent dorsally at right angles; ventral prominence large and projecting, tail short and conical.

This species clearly belongs to the *catinatum-alveatum* group. In the character of the mouth capsule it most closely resembles *C. goldi*, but can be distinguished from this worm by its larger size, by the appearance of the walls of the mouth capsule when seen in optical section, by the fact that the line of origin of the internal leaf crown is sinuous and situated at a much deeper level than in *C. goldi*, by the fact that the tail of the female is bent dorsally at right angles whereas in *C. goldi* this is not quite the case, and by the appearance of the accessory bodies of the genital cone.

STUDIES IN THE TREATMENT OF MALARIA

XXII. INTRAMUSCULAR INJECTIONS OF QUININE BIHYDROCHLORIDE GRAINS 15 ON EACH OF TWO CONSECUTIVE DAYS ONLY, IN MALIGNANT TERTIAN MALARIA

BY

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Undertaken at the request of the War Office

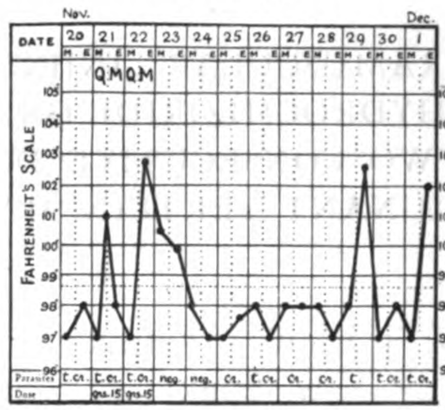
(Received for publication 2 February, 1919)

In previous studies (1917 and 1919) we have recorded the results of intramuscular injections of quinine bihydrochloride in simple tertian malaria. The present observations refer to malignant tertian malaria.

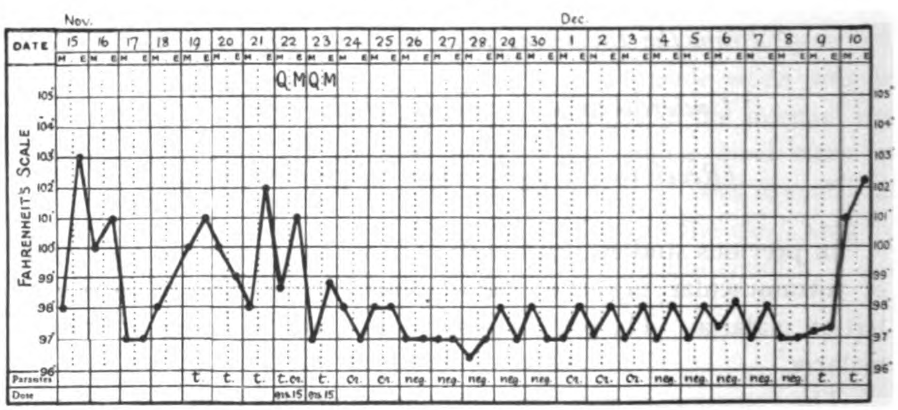
The treatment was an intramuscular injection of quinine bihydrochloride (grains 15 in 2 c.c. of water) on each of two consecutive days only. All the cases were adult males infected either in West Africa (Dakar) or in Macedonia.

In every instance a diagnosis of malignant tertian malaria was made microscopically, and in all cases trophozoites were present in

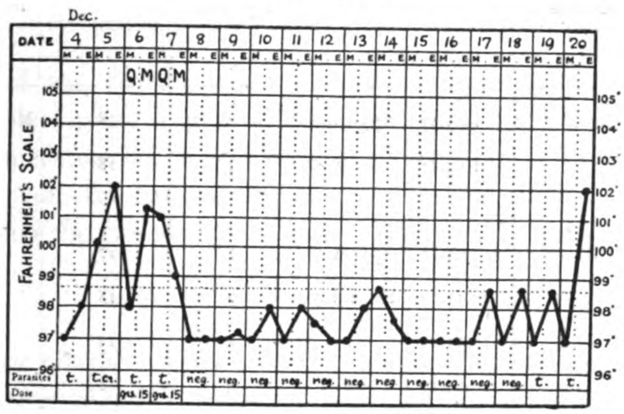
Case 1353



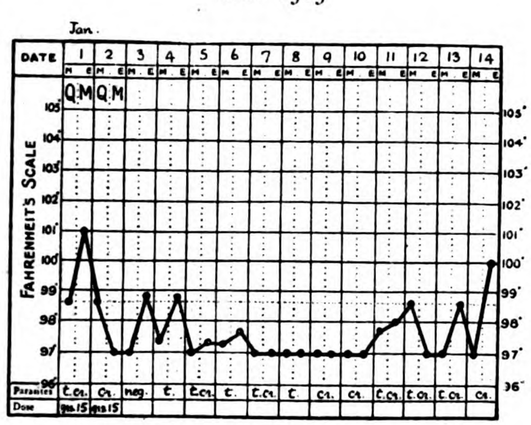
Case 1355



CASE 1359



CASE 1363



TABLE

Summary of results of intramuscular injections of quinine grains 15 on each of two consecutive days only in malignant tertian mal[illegible]

* S. = Salonika. W.A. = West Africa.

Number of case	*Place of infection	Interval (in months) between first admission to a hospital with malaria and present treatment	Interval (in months) between leaving infected area and present treatment	Interval (in months) between arrival in England and present treatment	Date of end of treatment	Temperature fell to normal in — days after first injection	Trophozoites disappeared from cutaneous blood in — days after first injection	Trophozoite relapse occurred in — days after first injection	Febrile relapse (above 100° F.) occurred in — days after first injection	Remarks
1347	S.	...	...	...	12.3.17	Apyrexia	2	8	No record	Crescents on 8th [illegible]
1348	S.	...	...	...	16.3.17	3	1	18	...	Crescents persist[illegible] tically through[illegible] Quinine orally, [illegible] on 20th day.
1349	S.	...	...	...	3.4.17	Apyrexia	1	17	18	
1350	W.A.	1	1	0	17.11.17	Same day	2	18	19	Crescents on 4th-[illegible] 13th-15th and [illegible]
1351	W.A.	1	1	0	17.11.17	1	4	13	18	Crescents on 5th-[illegible] and 12th days
1352	W.A.	1	1	0	17.11.17	1	3	8	11	Crescents on 6th [illegible] days.
1353	W.A.	1	1	0	22.11.17	3	2	5	8	Crescents on 1st [illegible] 4th-7th days.
1354	W.A.	1	1	0	22.11.17	2	2	8	11	Crescents on 2nd [illegible] 6th days.
1355	W.A.	1	1	0	23.11.17	1	2	17	18	Crescents on 2nd [illegible] 9th-11th days
1356	W.A.	1	1	0	28.11.17	2	3	17	16	Crescents on 2nd [illegible] 6th-9th, 11th [illegible] 16th days.
1357	W.A.	1	1	0	28.11.17	Apyrexia	2	6	9	Crescents on 2nd-[illegible]
1358	W.A.	1	1	0	6.12.17	Same day	2	21	22	Crescents on 6th [illegible] and 11th-21st [illegible]
1359	W.A.	1	1	0	7.12.17	2	2	13	14	
1360	...	...	...	...	17.12.17	1	2	7	9	Crescents on 2nd [illegible] and 7th days
1361	...	...	...	...	2.1.18	Apyrexia	2	15	15	Crescents on 1st [illegible] 6th-12th and 1[illegible]
1362	...	...	...	...	2.1.18	Apyrexia	1	6	15	
1363	...	...	...	...	2.1.18	1	1	3	13	Crescents on 1st [illegible]
1364	S.	37	1	1	28.8.18	2	2	15	16	
1365	S.	26	1	0	23.9.18	1	2	7	8	Crescents on 1st-[illegible]
1366	W.A.	1	1	0	23.11.18	2	2	14	16	
1367	W.A.	1	1	0	23.11.18	2	2	15	19	Crescents on 10th [illegible]
1368	W.A.	1	1	0	25.11.18	2	2	20	23	Crescents on 4th-[illegible] 13th-18th days
1369	S.	5	3	1	2.12.18	2	2	9	10	Crescents on 2nd [illegible]
1370	W.A.	2	2	1	2.12.18	1	2	17	18	
1371	S.	4	3	2	3.12.18	Apyrexia	1	7	...	Crescents on 3rd [illegible] Quinine orally, [illegible] on 8th day.
1372	S.	34	3	2	3.12.18	Apyrexia	1	16	...	Quinine orally, [illegible] on 17th day.
1373	W.A.	1	1	0	3.12.18	1	1	19	20	Crescents on 2nd [illegible] 18th and 19th [illegible]
1374	S.	16	1	1	4.12.18	Apyrexia	2	7	8	Crescents on 7th [illegible]
1375	S.	3	4	3	4.12.18	Apyrexia	1	8	...	Quinine orally, [illegible] on 10th day.

the blood on the day treatment commenced. Blood examinations were made daily.

In the temperature charts:—

t. = malignant tertian trophozoites.
cr. = crescents.
neg. = no parasites found.
Q.M. = intramuscular injection of quinine bihydrochloride grains 15.

In nine of the twenty-nine cases (Nos. 1347-1375) treatment commenced during an apyrexial period; in the remaining twenty cases the temperature fell to normal within three days. Trophozoites disappeared from the cutaneous blood within four days. Observations regarding the crescents will be found in the 'Remarks' column of the table.

Relapses

A parasitic relapse occurred in all cases, trophozoites reappearing in the cutaneous blood in three to twenty-one days; in Cases 1353 and 1363 trophozoites were absent for respectively three and two days only. Febrile relapses occurred in eight to twenty-three days.

CONCLUSION

An intramuscular injection of quinine bihydrochloride grains 15 in 2 c.c. of water on each of two consecutive days only, causes the cessation of febrile paroxysms and effects the disappearance of trophozoites from the cutaneous blood in malignant tertian malaria. The action, however, is only temporary, a relapse occurring within three weeks, occasionally within a few days.

REFERENCE

Stephens, J. W. W., Yorke, W., Blacklock, B., Macfie, J. W. S., Cooper, C. F., and Carter, H. F. (1917 and 1919). *Ann. Trop. Med. & Parasitol.*, Vol. XI, p. 113, and Vol. XII, p. 402.

STUDIES IN THE TREATMENT OF MALARIA

XXIII. ORAL ADMINISTRATION OF QUININE SULPHATE GRAINS 30 ON EACH OF TWO CONSECUTIVE DAYS WEEKLY, OVER A PERIOD OF FIVE WEEKS, IN MALIGNANT TERTIAN MALARIA

BY

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Undertaken at the request of the War Office

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All the cases were adult males, infected in West Africa. In every instance a diagnosis of malignant tertian malaria was made microscopically, and in all cases trophozoites were present in the blood on the day treatment commenced. Blood examinations of ordinary thin films were made daily.

The records of the observations are given in the tables, in which:—

0	= absence of fever and trophozoites.
1, 2, etc.	= number of trophozoite febrile relapses weekly.
1*, 2*, etc.	= number of non-trophozoite febrile attacks weekly.
t^1, t^2, etc.	= number of non-febrile trophozoite relapses weekly.
c^1, c^2, etc.	= number of days on which crescents were present weekly.

NOTE.—A rise of temperature above 100° F., of which the nature is unknown is termed a *febrile attack*. A similar rise of temperature accompanied by trophozoites in the blood at the time, or within three days, is termed a *trophozoite febrile relapse* or *true relapse*. The term *paroxysm* is used indifferently to denote any febrile disturbance of 100° F. or more.

Table I.

Results of oral administration of quinine sulphate grains 30 on each of two consecutive days weekly for 5 weeks, in malignant tertian malaria.

Number of case	Date of end of treatment	Temperature fell to normal in — days after first dose	Trophozoites disappeared from cutaneous blood in — days after first dose	Number of febrile paroxysms (trophozoite and non-trophozoite) and results of blood examinations during treatment — Week of treatment: 1st	2nd	3rd	4th	5th
1376	24.11.17	1	3	o ‡ c^2	o c^1	o c^1	2	t^3 c^3
1377	29.11.17	1	2	o	o c^1	o	1*	o
1378	29.11.17	3	2	o	o	o	o	o
1379	29.11.17	1	4	o	o	o	o	o
1380	29.11.17	2	2	o	o	o	o	o
1381	29.11.17	Same day	4	o c^3	o c^2	o	o c^1	o
1382	29.11.17	3	2	o c^1	o	o	o	o
1383	29.11.17	Same day	2	o c^1	o	o	o	o
1384	1.12.17	1	2	o	o	o	o	o
1385	1.12.17	1	2	o	1*	o	o	o
1386	6.12.17	1	2	o c^3	o	o	o	o
1387	13.12.17	1	2	o c^2	o c^3	o c^1	o c^1	o
1388	13.12.17	Apyrexia	2	o c^2	1*	t^1	o	o
1389	13.12.17	1	2	o c^2	o c^1	o	o	o
1390	13.12.17	Same day	3	o	o	o	o	o
1391	20.12.17	1	1	o	o	o	o	o
1392	17.11.17	2	2	o	o c^2	o c^2	...	...
1393	29.11.17	2	4	o c^1	o c^1	o c^1	...	...

‡ Four days are allowed for the initial fever to subside and for the disappearance of trophozoites from the blood. The absence of fever and trophozoites in this column refer to the last two days of the week only.

TABLE II.

Summary of Table I.

Week of treatment	1st	2nd	3rd	4th	5th
Number of cases treated	**18**	**18**	**18**	**16**	**16**
Number of cases having trophozoite febrile relapses	0	0	0	1	0
Number of cases having non-trophozoite febrile attacks	0	2	0	1	0
Grand total of all febrile cases	**0**	**2**	**0**	**2**	**0**
Total number of trophozoite febrile relapses ...	0	0	0	2	0
Total number of non-trophozoite febrile attacks	0	2	0	1	0
Grand total of all febrile paroxysms ...	**0**	**2**	**0**	**3**	**0**
Number of trophozoite cases(febrile and non-febrile)	0	0	1	1	1
Number of crescent cases	9	7	4	2	1

TABLE III.

Analysis of TABLE II.

Week of treatment	1st	2nd	3rd	4th	5th	Average per week
Percentage of trophozoite febrile relapse cases per cases treated ...	0	0	0	6·0	0	**1·2**
Percentage of all febrile (trophozoite and non-trophozoite) cases per cases treated	0	11·0	0	12·5	0	**4·7**
Percentage of all trophozoite (febrile and non-febrile) cases per cases treated	0	0	5·5	6·0	6·0	**3·5**
Percentage of crescent cases per cases treated	50·0	39·0	22·0	12·5	6·0	**26·0**

In one of the eighteen cases (Nos. 1376-1393) treatment was commenced during an apyrexial period; in the remaining seventeen cases the temperature fell to normal either on the same day or in one to three days.

Trophozoites disappeared from the blood in one to four days.

Relapses

During treatment. Owing to the discharge from hospital of two cases in the fourth week, the number of cases treated was eighteen in the first four weeks and sixteen in the fifth week.

The average weekly number, over a period of five weeks, of cases which had (1) trophozoite febrile relapses was 1·2 per cent. of cases treated, (2) non-trophozoite febrile attacks 3·5 per cent., (3) febrile paroxysms, trophozoite and non-trophozoite, 4·7 per cent., (4) trophozoite relapses, febrile and non-febrile, 3·5 per cent., and (5) crescent relapses, 26 per cent.

After treatment. The cases were not observed.

SUMMARY

As a palliative, quinine sulphate grains 30 on each of two consecutive days weekly, over a period of five weeks, suffices to keep the blood free from trophozoites and to prevent relapses in the great majority of cases. It is noteworthy that the percentage of cases having crescents in the peripheral blood diminishes each week, viz., from 50 per cent. in the first week to 6 per cent. in the fifth week of treatment.

STUDIES IN THE TREATMENT OF MALARIA

XXIV. THE DISAPPEARANCE OF CRESCENTS UNDER QUININE TREATMENT

BY

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Undertaken at the request of the War Office

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In a previous study (1917) we have referred to various contradictory statements in the literature on this subject. We record here observations made by us on eighty-nine crescent cases.

The cases were divided into three groups according to the daily dose of quinine administered:—

GROUP A. Quinine sulphate in solution orally, grains 20 daily. (Cases 1394-1413).
GROUP B. Quinine sulphate in solution orally, grains 30 daily. (Cases 1414-1451).
GROUP C. Quinine sulphate in solution orally, grains 45 daily. (Cases 1452-1482).

All the cases had crescents in the blood on the day treatment commenced. Blood examinations were made once weekly, thick films being used. As these observations were made in 1916 at the beginning of our work on malaria, information as to place and duration of infection is not available.

The results are given in the table, from which it will be seen that at the end of four weeks' treatment crescents had disappeared from 50 per cent. of cases treated when the daily dose of quinine was grains 20, and from over 90 per cent. when the daily dose was grains 30 or 45.

TABLE

Showing the disappearance of crescents under quinine treatment.

A. Quinine sulphate grains 20 daily. (Cases 1394-1413).

	At beginning of treatment	Week of treatment			
		1st	2nd	3rd	4th
Number of cases observed	20	20	20	20	20
Number of cases showing crescents	20	19	18	13	10
Percentage of cases showing crescents	100%	95%	90%	63%	50%

B. Quinine sulphate grains 30 daily. (Cases 1414-1451)

	At beginning of treatment	Week of treatment			
		1st	2nd	3rd	4th
Number of cases observed	38	38	38	38	38
Number of cases showing crescents	38	29	17	8	3
Percentage of cases showing crescents	100%	76%	45%	21%	8%

C. Quinine sulphate grains 45 daily. (Cases 1452-1482).

	At beginning of treatment	Week of treatment			
		1st	2nd	3rd	4th
Number of cases observed	31	31	31	31	31
Number of cases showing crescents	31	20	14	11	2
Percentage of cases showing crescents	100%	65%	45%	35%	6%

CONCLUSION

Under quinine treatment grains 30 or 45 daily, crescents do not persist in the cutaneous blood in the majority of cases for more than three weeks.

REFERENCE

STEPHENS, J. W. W., YORKE, W., BLACKLOCK, B., MACFIE, J. W. S., and COOPER, C. F. (1917.) *Ann. Trop. Med. & Parasitol.*, Vol. XI, p. 92.

STUDIES IN THE TREATMENT OF MALARIA

XXV. ARSENIC IN MALIGNANT TERTIAN MALARIA

BY

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Undertaken at the request of the War Office

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The observations recorded in this paper were made in order to determine the effects of arsenic in malignant tertian malaria.

All the cases were adult males, infected in either Macedonia or West Africa. In every instance a diagnosis of malignant tertian malaria was made microscopically, and in all cases trophozoites were present in the blood on the day treatment commenced. Blood examinations were made daily.

In the tables and charts:—

t. = malignant tertian trophozoites.
c. = malignant tertian gametes (crescents).
neg. = no parasites found.
Q.M. = intramuscular injection of quinine bihydrochloride.
A. = oral administration of Liquor arsenicalis.
N. = intravenous injection of novarsenobillon.

Four sets of observations were made as follows:—

GROUP A

A single intravenous injection of novarsenobillon in doses varying from gramme 0·45 to gramme 0·9 was given in fourteen cases

(Nos. 1483 to 1496). The results are recorded in Table I and in Charts 1485, 1493, and 1494.

It will be seen that the treatment had little if any effect on trophozoites or on the temperature; in the great majority of cases it was found necessary, for clinical reasons, to administer quinine in from three to seven days.

TABLE I.

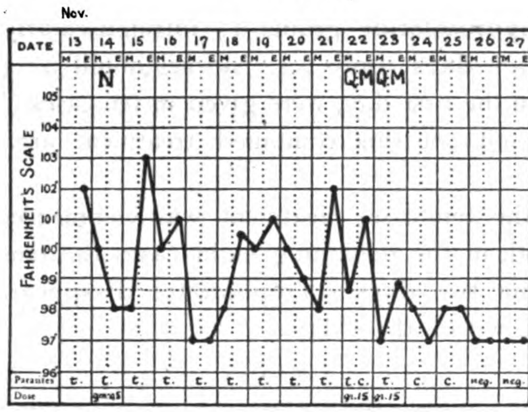
Results of a single intravenous injection of novarsenobillon in malignant tertian malaria.

* W.A. = West Africa.

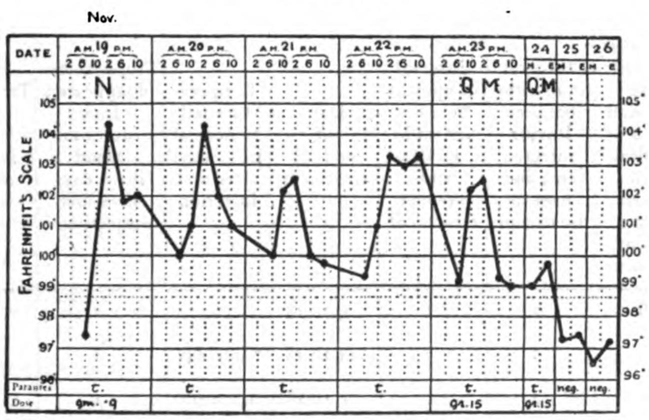
Number of case	*Place of infection	Interval (in months) between first admission to a hospital with malaria and present treatment	Interval (in months) between leaving infected area and present treatment	Interval (in months) between arrival in England and present treatment	Date of injection	Dose in grammes	Parasitic findings								
							Day of injection	1st day after	2nd day after	3rd day after	4th day after	5th day after	6th day after	7th day after	8th day after
1483	W.A.	1	1	0	13.11.17	0·45	t	t	t	t†	...	...	...	...	...
1484	W.A.	1	1	0	13.11.17	„	t	c	c	t c	c	c	t c	t c†	...
1485	W.A.	1	1	0	14.11.17	„	t	t	t	t	t	t	t	t	t c†
1486	W.A.	1	1	0	14.11.17	„	t	t	t†	...	...	...	...	...	...
1487	W.A.	1	1	0	16.11.17	„	t	t	t	t	t c†	...	...	...	...
1488	...	...	...	...	26.11.17	„	t c	t c	c	t c	c	t c	t	t c	t c
1489	...	...	...	...	17.1.18	„	t	t	neg.	neg.	t†	...	...	...	...
1490	W.A.	1	1	0	26.11.17	0·6	t	t	t†	...	...	...	...	...	...
1491	W.A.	1	1	0	26.11.17	„	t	t	t	neg.	c	t	t	t	t
1492	W.A.	1	1	0	19.11.18	0·9	t	t	t	t	t†	...	...	...	...
1493	W.A.	1	1	0	19.11.18	„	t	t	t	t	t†	...	...	...	...
1494	W.A.	1	1	0	20.11.18	„	t	t	t	t	t†	...	...	...	...
1495	W.A.	1	1	0	23.11.18	„	t c	t	t	neg.	c	t c	t	t c	t
1496	W.A.	1	1	0	27.11.18	„	t	neg.	neg.	c	t c	t†	...	...	...

† Quinine administered.

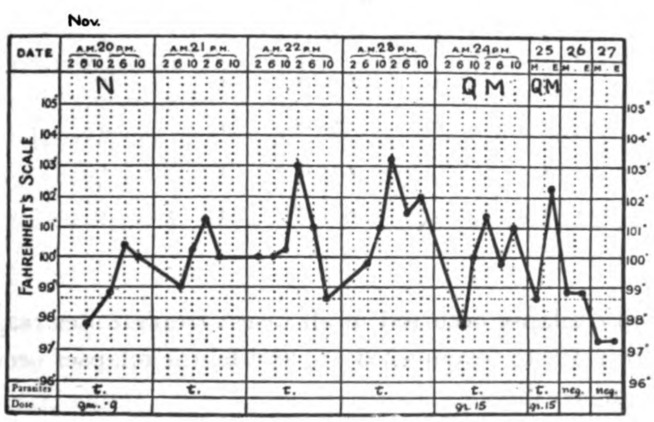
CASE 1485



CASE 1493



CASE 1494



GROUP B

Quinine bihydrochloride grains 15 intramuscularly on each of two consecutive days only + novarsenobillon gramme 0·9 intravenously on the first day, were given in ten cases (Nos. 1497 to 1506). The results of this combined treatment are recorded in Table II.

In two of the ten cases treatment was commenced during an apyrexial period, in the remaining eight cases the temperature fell to normal within two days. Trophozoites disappeared from the cutaneous blood within three days.

TABLE II.

Summary of results of administration of quinine bihydrochloride grains 15 intramuscularly on each of two consecutive days + novarsenobillon gramme ·9 intravenously on the first day, in malignant tertian malaria.

* S. = Salonika. W.A. = West Africa.

Number of case	*Place of infection	Interval (in months) between first admission to a hospital with malaria and present treatment	Interval (in months) between leaving infected area and present treatment	Interval (in months) between arrival in England and present treatment	Date of end of treatment	Temperature fell to normal in — days after first injection	Trophozoites disappeared from cutaneous blood in — days after first injection	Trophozoite relapse occurred in — days after first injection	Febrile relapse (above 100° F.) occurred in — days after first injection
1497	S.	26	1	1	9.10.18	2	1	8	11
1498	S.	39	3	3	14.10.18	Same day	1	15	...
1499	S.	27	2	1	14.10.18	Apyrexia	1	6	7
1500	S.	25	2	1	22.10.18	1	2	12	12
1501	S.	28	2	1	24.10.18	Apyrexia	1	8	7
1502	S.	18	2	1	26.10.18	1	3	8	7
1503	S.	2	2	2	28.10.18	1	1	9	15
1504	W.A.	1	1	0	2.11.18	1	2	17	18
1505	W.A.	1	1	0	2.11.18	1	1	16	17
1506	W.A.	1	1	0	2.11.18	1	1	15	16

Relapses

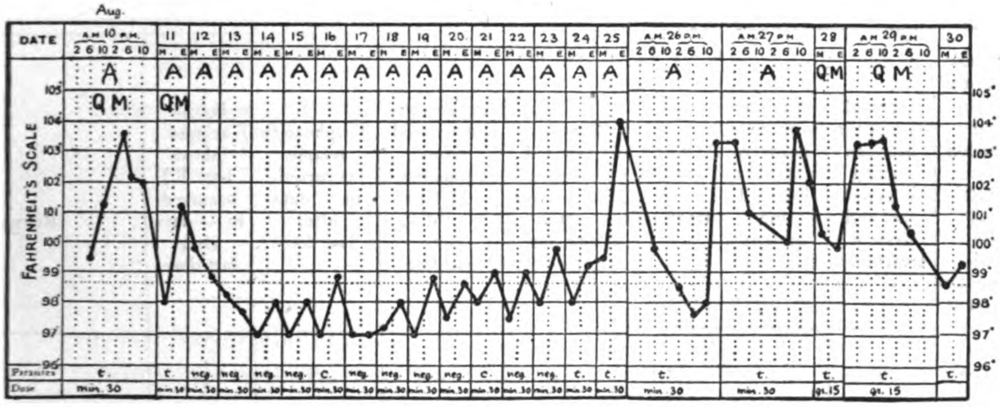
A parasitic relapse occurred in all cases; trophozoites reappeared in the blood in six to seventeen days, and febrile relapses occurred in seven to eighteen days.

A comparison of these results with those obtained by the injection of quinine bihydrochloride grains 15 intramuscularly on each of two consecutive days only, in malignant tertian malaria, recorded in a previous paper (1919), shows that the supplementary intravenous injection of novarsenobillon had no effect, all the cases relapsing within three weeks in both series.

GROUP C

Quinine bihydrochloride grains 15 intramuscularly on each of two consecutive days only + Liq. arsenicalis minims 30 by the mouth daily (Case 1507). The result of this combined treatment in the single case treated is shown in Chart 1507.

CASE 1507



The temperature fell to normal in two days, and trophozoites disappeared from the cutaneous blood in two days after commencement of treatment. Trophozoites reappeared on the fourteenth day and a febrile relapse occurred on the fifteenth day. As the condition from the fifteenth day onwards was uncontrolled, parasitic febrile attacks occurring each day, it was found necessary to abandon the treatment on the nineteenth day. From this we conclude that the daily administration of Liq. arsenicalis minims 30 had no effect, the temporary disappearance of symptoms and parasites being solely due to the two intramuscular doses of quinine bihydrochloride.

GROUP D

Liquor arsenicalis minims 30 by the mouth daily for 16 days + Quinine bihydrochloride grains 15 intramuscularly on the first and second, eighth and ninth, fifteenth and sixteenth days, were given in sixteen cases (Nos. 1508-1523).

The result of this combined treatment is recorded in Table III. The temperature fell to normal within three days. Trophozoites disappeared from the cutaneous blood within three days, except in one case (1519) in which they persisted throughout.

Relapses

A relapse occurred in every case, trophozoites reappearing in the peripheral blood in one to thirty-eight days, average eleven days, after cessation of treatment.

TABLE III.

Summary of results of oral administration of Liquor arsenicalis, minims 30 daily for 16 days + quinine bihydrochloride grains 15 intra-muscularly on the 1st and 2nd, 8th and 9th, 15th and 16th days, in malignant tertian malaria.

* S. = Salonika.

Number of case	*Place of infection	Interval (in months) between first admission to a hospital with malaria and present treatment	Interval (in months) between leaving infected area and present treatment	Interval (in months) between arrival in England and present treatment	Date of end of treatment	Temperature fell to normal in — days after first dose	Trophozoites disappeared from cutaneous blood in — days after first dose	Trophozoite relapse occurred in — days after last dose	Febrile relapse (above 100° F.) occurred in — days after last dose	Remarks
1508		No	records		8.1.19	1	2	38	39	
1509					21.1.19	3	3	16	...	Measles on 18th day.
1510					4.2.19	3	2	9	...	Quinine orally, grs. 45 [illegible] 10th day.
1511					5.2.19	2	2	8	...	Quinine orally, grs. 4[illegible] 9th day.
1512					6.2.19	Same day	1	5	5	
1513					11.2.19	Same day	1	24	...	101° F. on 12th and 1[illegible] days. Quinine ora[illegible] grs. 45 on 27th day.
1514					13.2.19	Apyrexia	1	20	...	Quinine orally, grs. 45 [illegible] 20th day.
1515					25.2.19	3	2	6	6	Crescents throughout treatment.
1516					27.2.19	1	1	5	...	Quinine orally, grs. 45 [illegible] 6th day.
1517	S.	6	4	0	27.2.19	1	1	26	28	
1518		No	records		28.2.19	1	1	5	...	Trophozoites on 8th day [illegible] treatment. Quinine orally, grs. 45 on [illegible] day.
1519	S.	5	1	1	5.3.19	1	Present throughout	...	...	Quinine orally, grs. [illegible] 8th day.
1520		No	records		6.3.19	Same day	1	9	...	Crescents throughout. Discharged on 9th d[illegible]
1521					7.3.19	Same day	1	1	...	Quinine orally, grs. 30 [illegible] 7th day.
1522					8.3.19	Apyrexia	3	2	...	Quinine orally, grs. 30 [illegible] 10th day.
1523	S.	6	1	1	11.3.19	2	3	10	...	Quinine orally, grs. 30 [illegible] 12th day.

TABLE IV.

Summary of the Treatments.

Treatment	Number of cases observed	Number of cases which relapsed	Percentage of cases which relapsed
A. Novarsenobillon intravenously gm. ·45 to gm. ·9 ...	14	14	100 %
B. Quinine intramuscularly grs. 15 × 2 + Novarsenobillon intravenously gm. ·9	10	10	100 %
C. Quinine intramuscularly grs. 15 × 2 + Liquor arsenicalis orally min. 30 daily for 18 days	1	1	...
D. Quinine intramuscularly grs. 15 × 6 + Liquor arsenicalis orally min. 30 daily for 16 days	16	16	100 %
Control. Quinine intramuscularly grs. 15 × 2 ... ... (*Study XXII*)	29	29	100 %

CONCLUSIONS*

1. Novarsenobillon in the doses used is of no value in the treatment of malignant tertian malaria.

2. A combination of arsenic (Novarsenobillon or Liquor arsenicalis) with quinine in the doses used is not more effective than quinine alone.

* The results recorded in this paper should be compared with those obtained in simple tertian malaria. (*Ann. Trop. Med. & Parasitol.*, Vol. XII, p. 371.)

THE INTESTINAL PROTOZOAL INFECTIONS AMONG CONVALESCENT DYSENTERICS EXAMINED AT THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

(THIRD REPORT)

BY

J. R. MATTHEWS, M.A.

AND

A. MALINS SMITH, M.A.

(*Received for publication February,* 1919)

INTRODUCTORY

The Second Report (1917) on the protozoological examination of dysenteric patients received at Liverpool dealt with cases admitted to hospital during the period May 1st to December 31st, 1916. That report contained a full account of the results obtained from the examination of 1,713 cases of dysentery and discussed various questions connected with the protozoological examination of stools. The present report deals with 2,355 cases that have been examined for intestinal protozoa during the years 1917 and 1918.

MATERIAL

So far as we know, the majority of the cases examined were invalided to this country from France. A small number that came under our observation had been to the East, but we have no idea what proportion of the total number of cases had served on the French front only or how many had been in the East before coming to France. We are therefore unable to make any comparison of the incidence of infection among 'Eastern' and 'French' cases. We believe, however, that the cases here dealt with constitute a fair sample of the 'convalescent dysenterics' that have returned to this country from various fronts during the past two years.

ANALYSIS OF RESULTS

Of the 2,355 cases examined, 1,158, or 49·2 per cent., were discovered to be infected with one or more of the intestinal protozoa found in man. An analysis of the 1,158 infected cases is given in Table I. The results previously obtained from 1,713 cases (Second Report, 1917) are shown for comparison.

TABLE I.

Showing incidence of protozoal infections among 2,355 convalescent dysenterics

Protozoon	Number of cases infected	Percentage of total cases	Previous results (Second Report) 1917
Entamoeba histolytica	306	13·0	10·9
E. coli	703	29·8	29·4
*E. nana**	279	16·6	...
Giardia intestinalis	352	14·9	18·5
Chilomastix mesnili	87	3·7	3·5
Trichomonas intestinalis	11	0·5	1·0

* The percentage of infection with *E. nana* is based on the last 1,674 cases examined. During the early part of the period covered by this report we were not familiar with this protozoon.

The results, on the whole, agree fairly closely with those published in 1917. The incidence of infection with *E. histolytica* in the present series of cases is somewhat higher than that obtained previously, but it will be shown later that the difference can be explained.

DETAILS OF EXAMINATIONS MADE

In order to appreciate fully the general results shown in Table I, it is necessary to know the number of examinations made. This matter has so frequently been ignored that we wish to draw attention to its importance again.

The total number of examinations performed was 14,130, which is an average of six per case. This average is obtained from two classes of cases, however, and does not give any idea of the actual distribution of the total number of examinations. The great majority of those cases in which *E. histolytica* was found received more than six examinations each. In fact, 204 *E. histolytica* cases, each examined at least six times, had in all 7,483 examinations, an average of 36·7 per case. Apart from these, therefore, there remain 2,151 cases having 6,647 examinations, which is an average of only three per case. The actual distribution is as follows:—

2,355	cases	had	at least one	examination	each.
1,935	"	"	"	two	"	"
1,480	"	"	"	three	"	"
595	"	"	"	four	"	"
400	"	"	"	five	"	"
319	"	"	"	six	"	"

It is unfortunate that nearly 40 per cent. of our cases should have left hospital with only one or two examinations.

Table II gives the findings of *E. histolytica*, *E. coli* and *G. intestinalis* for the number of cases examined at each stage of the above analysis. The increase in the number of specific infections detected at each succeeding examination is thus indicated.

TABLE II.

Examination	Number of cases examined	*E. hist.* cases	Per cent. of total cases	*E. coli* cases	Per cent. of total cases	*Giardia* cases	Per cent. of total cases
First ...	2,355	184	7·8	366	15·5	211	9·0
Second ...	1,935	244	10·4	551	23·4	278	11·8
Third ...	1,480	283	12·0	642	27·3	309	13·1
Fourth ...	595	295	12·5	659	28·0	322	13·7
Fifth ...	400	300	12·7	666	28·3	330	14·0
Sixth ...	319	301	12·8	670	28·5	332	14·1
Ultimately ...		306	13·0	703	29·8	352	14·9

Five infections with *E. histolytica* detected after the sixth examination were found in the following order: two at the seventh, one at the eighth, one at the eleventh, and one at the twelfth examination.* Thirty-three infections with *E. coli* not discovered until after the sixth examination appeared as follows:—

Infection detected at the	7th	examination in	3	cases.
,, ,,	8th	,,	4	,,
,, ,,	9th	,,	2	,,
,, ,,	10th	,,	1	case.
,, ,,	11th	,,	1	,,
,, ,,	12th	,,	2	cases.
,, ,,	14th	,,	2	,,
,, ,,	16th	,,	2	,,
,, ,,	17th	,,	2	,,
,, ,,	18th	,,	3	,,
,, ,,	19th	,,	2	,,
,, ,,	21st	,,	1	case.
,, ,,	24th	,,	1	,,
,, ,,	25th	,,	1	,,
,, ,,	26th	,,	2	cases.
,, ,,	28th	,,	1	case.
,, ,,	29th	,,	1	,,
,, ,,	45th	,,	1	,,
,, ,,	55th	,,	1	,,

Twenty infections with *G. intestinalis* discovered after the sixth examination were found in the following order:—

Infection detected at the	7th	examination in	3	cases.
,, ,,	8th	,,	1	case.
,, ,,	12th	,,	2	cases.
,, ,,	15th	,,	1	case.
,, ,,	16th	,,	1	,,
,, ,,	17th	,,	2	cases.
,, ,,	19th	,,	1	case.
,, ,,	22nd	,,	1	,,
,, ,,	23rd	,,	1	,,
,, ,,	26th	,,	1	,,
,, ,,	27th	,,	1	,,
,, ,,	30th	,,	1	,,
,, ,,	31st	,,	1	,,
,, ,,	34th	,,	1	,,
,, ,,	45th	,,	1	,,
,, ,,	64th	,,	1	,,

* Of course very few non-*E. histolytica* cases were examined more than six times.

INCIDENCE OF INFECTION WITH *E. HISTOLYTICA*

It is clear from Table II that if all the cases had received six examinations each the percentage of infection would have been much higher than that recorded. A full discussion of the real incidence of infection with various protozoa was given in our Second Report, and we shall refer to the question here very briefly. We gave reasons for believing that by a system of six examinations per case the incidence of infection with *E. histolytica* would be about 18 per cent. At the first examination we had found 6 per cent., i.e. the number of positive cases discovered by one examination per case was one-third of the number that would have been found had each case been examined six times. These calculations have been fully confirmed by Mackinnon (1918), who succeeded in examining a large series of cases (1,680) six times each. Of this number 4·3 per cent. were found infected with *E. histolytica* at the first examination. By the sixth examination the percentage had increased to 12·4. It will be observed from Table II that 7·8 per cent. of the present series of cases were found at the first examination to be infected with *E. histolytica*, and we may calculate, therefore that about 23 per cent. would have been discovered had all the cases been examined six times. The result for the first examination is higher than that obtained in our former series, namely 6 per cent. The difference is due to the inclusion in the present series of those infections with *E. histolytica* whose cysts measure less than 10μ in diameter. These small cysts were not recorded before the year 1917, for they were not identified as cysts of the pathogenic amoeba. Our early records, therefore, were based on the diagnosis of cysts exceeding 10μ in diameter. Among the 306 *E. histolytica* cases included in this report, 110 were infected with strains producing cysts under 10μ in diameter, while in 215 infections cysts larger than 10μ were found. These figures give 325 infections in all. In nineteen cases both large and small cysts were found. In the 306 *E. histolytica* cases then, 36 per cent. showed an infection with small cysts. This result agrees closely with that recorded by Dobell and Jepps (1917), but is considerably lower than that reported by Mackinnon (1918). If we had recorded the presence of the larger cysts only the incidence of infection with

E. histolytica would have been 9·1 per cent. instead of 13, and the former result would have been similar to that obtained in our previous report.

INCIDENCE OF INFECTION WITH *E. COLI*, *G. INTESTINALIS* AND *C. MESNILI*

While we have calculated the probable incidence of infection with *E. histolytica* on a system of six examinations per case, it is possible for us to obtain a more accurate idea of the real incidence of infection with some of the other intestinal protozoa. For this purpose we shall make use of 204 *E. histolytica* cases that received at least six examinations each. Table III shows the number of cases infected with *E. coli*, *G. intestinalis* and *C. mesnili* among these 204 cases.

TABLE III.

Showing number of cases infected with *E. coli*, *G. intestinalis* and *C. mesnili* among 204 *E. histolytica* cases examined a large number of times.

Number of cases examined	Number of examinations per case	*Entamoeba coli*		*Giardia intestinalis*		*C. mesnili*	
		Cases	Per cent.	Cases	Per cent.	Cases	Per cent.
204	1	42	20·6	17	8·3	7	3·4
204	2	62	30·4	25	12·2	10	4·9
204	3	78	38·2	29	14·2	11	5·4
204	4	86	42·1	30	14·7	12	5·9
204	5	91	44·6	35	17·1	13	6·4
204	6	93	45·5	36	17·6	14	6·8
204	Average of 36·7	122	59·8	54	26·4	29	14·2

Table III shows clearly that the increase in the number of infected cases found is the direct result of the increasing number of examinations made upon each case. The number of *G. intestinalis* cases detected at the first examination was 17, or 8·3 per cent., which agrees closely with the result for the whole series of cases (see Table II). Ultimately, however, 26·4 per cent. of the 204

E. histolytica cases were discovered to have *Giardia*, a result which agrees very closely with an observation made in our Second Report (see Table V of that report), where, among 110 *E. histolytica* cases 26·3 per cent. were ultimately found to be infected with *Giardia*. It is probable, therefore, that if the whole series of 2,355 cases had been examined many times each the incidence of infection with *Giardia* would have been found to approximate to 30 per cent.

Similar observations may be made regarding the incidence of infection with *E. coli*. Table III shows that the percentage increases from 20·6 at the first examination to 59·8 per cent. ultimately. There is reason to believe, however, that *E. coli* occurs more commonly among *E. histolytica* cases than in a random selection of cases. At the third examination of the 204 cases considered in Table III it will be seen that 38·2 per cent. were infected with *E. coli*, while in the total series of 2,355 cases with an average of three examinations per case only 29·8 per cent. were found infected. If we suppose that the percentage figures for *E. coli* given in Table III are approximately 10 per cent. higher than they would be for an unselected series of cases, we obtain 50 per cent. as the probable real incidence of infection with this amoeba. This agrees with the conclusion reached in our former report.

Particulars have also been given in Table III for *C. mesnili*. Among the 204 selected cases, 14·2 per cent. were ultimately found to be infected with this flagellate. In our former report we concluded that 'it was not improbable that the real incidence of infection was 12 to 15 per cent.,' and the present data give further evidence in support of that conclusion.

ORGANISMS OTHER THAN PROTOZOA

The commonest helminthic infection was *Trichuris trichiura*, the eggs of this worm being found in 110 cases (4·7 per cent.) *Ascaris lumbricoides* was recorded in ten cases. Eggs of *Taenia* sp. were observed in three cases, *Oxyuris vermicularis* in two cases and *Strongyloides* sp. on one occasion. Iodine cysts (Wenyon) were found in forty-one cases (1·7 per cent.).

RÉSUMÉ OF RESULTS FOR ALL THE DYSENTERY CASES EXAMINED AT LIVERPOOL SINCE MAY, 1916

It may be useful here to bring together the results of all the work done on the examination of dysenteric patients at the Liverpool School of Tropical Medicine since May, 1916. Altogether 4,068 cases have been dealt with, and 23,024 microscopic examinations of the stools performed. Protozoal infections were discovered in 1,976 cases (48·5 per cent.), an analysis of the infected cases being shown in Table IV.

TABLE IV.

Protozoon	Number of cases infected	Percentage of total cases examined
E. histolytica	494	12·1
E. coli	1,208	29·7
*E. nana**	279	16·6
G. intestinalis	669	16·4
C. mesnili	148	3·6
T. intestinalis	29	0·7

* Based on last 1,674 cases examined.

REFERENCES

CARTER, MACKINNON, MATTHEWS, and SMITH (1917). Protozoological Investigation of cases of dysentery conducted at the Liverpool School of Tropical Medicine (Second Report). *Annals. Trop. Med. & Parasitol.*, Vol. XI, pp. 27-68.

DOBELL and JEPPS (1917). On the Three Common Intestinal Entamoebae of Man. *Brit. Med. Journ.*, May 12, pp. 607-612.

MACKINNON (1918). Notes on the Intestinal Protozoal Infections of 1,683 men examined at the University War Hospital, Southampton. *Lancet*, September 21, pp. 386-389.

THE SPREAD AND INCIDENCE OF INTESTINAL PROTOZOAL INFECTIONS IN THE POPULATION OF GREAT BRITAIN

IV. ASYLUM PATIENTS

V. UNIVERSITY AND SCHOOL CADETS

BY

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AND

A. MALINS SMITH, M.A.

(*Received for publication* 18 *March*, 1919)

IV. ASYLUM PATIENTS

In continuation of our general investigation of the intestinal protozoa of the population of this country (Matthews and Smith, 1919) we examined (June, 1918 to February, 1919) specimens of faeces from two hundred and seven patients of the Lancashire County Asylum, Whittingham. This we were enabled to do by the kind permission of Dr. J. F. Gemmel, who also helped us by furnishing information as to the age, length of residence, and state of health of the patients whose stools were examined. The patients were all males, and ranged in age from 17 to 87, the average age being 48. The protozoal infections found among these patients, as the result of a single examination, were as follows:—

Entamoeba histolytica	9·7	per cent.
E. coli	45·9	"
E. nana	12·1	"
Giardia intestinalis	3·4	"
Chilomastix mesnili	23·2	"

Besides these protozoal infections we found the eggs of *Trichuris trichiura* in 3·4 per cent. of the cases, and those of *Oxyuris vermicularis* in 1·9 per cent.

In Table I the above figures are given together with the results for other sections of the population examined by us and also for dysenteric and non-dysenteric returned soldiers. All the figures, with the exceptions stated, are the results of a single examination.

	Never out of Britain.				Returned Soldiers	
	Asylum Patients	Adult civilians	Army Recruits	Children	Dysenteric Convalescents	Non-dysenteric Convalescents
No. examined	207	450	1098	548	4068	450
E. histolytica ...	9·7	1·5	5·6	1·8	7·0	6·4
E. coli	45·9	6·7	18·2	11·1	15·2	14·2
E. nana	12·1	2·4	5·5	2·7	—	—
G. intestinalis ...	3·4	6·0	7·0	14·1	9·9	6·0*
C. mesnili	23·2	1·5	2 cases	1·8	3·6 (3 exams. per case)	2·0 (2 exams. per case)

We have stated in previous papers that at least five hundred cases should be examined before reliable figures can be obtained as to the true incidence of the various protozoal infections in any population. Unfortunately in the case of Asylum patients we have not been able to examine more than two hundred and seven, a number too small to give reliable comparative figures. We can therefore only indicate one or two outstanding features which we think are significant, without considering any of the results as definitely established.

The first striking feature is the high percentage of almost all the infections among the Asylum patients. Infections with all the protozoa but *Giardia intestinalis* are more numerous than in any other group we have examined, not excepting those who have been to tropical and sub-tropical countries. In spite of the small numbers examined this seems to represent some real difference in the present group, and it may perhaps be suggested that the infections spread more rapidly and more widely within this isolated population because of the well-known lack of cleanly and careful personal habits among the insane.

* In Matthews and Smith (1919) Army Recruits, Table V, this value is given in mistake as 10·8. The present figure is correct.

A further noteworthy result is shown in the figures for *Giardia intestinalis* infections. These are a complete exception to all the remaining results, for not only are they not higher than in the other population groups, but they are considerably lower than in any other group. This fact seems to us to fit in with the suggestion we put forward in our paper on the infection of children, namely that *G. intestinalis* is mainly a parasite of children and becomes rarer in older people. The present group of Asylum patients with average age of 48 years is the oldest section of the population which we have examined, and it appears significant that, while all other infections are so common, this one should be rare. It is particularly striking to find *G. intestinalis* comparatively uncommon, since infections with *C. mesnili*, the other common flagellate of the human intestine, are very numerous, being many times more common than in any other group we have examined. Both flagellates were in every case found in the encysted state in the faeces, and we cannot suggest any cause of the remarkably different incidence of the two, other than that *G. intestinalis* tends to disappear in older people, while *C. mesnili* does not.

We hoped at the outset of the investigation to obtain some evidence as to the effect of continued residence in the institution upon the incidence of infection and also to find out whether those numerous patients, who suffer from colitis, were more or less heavily infected than the remainder, but the number of the patients examined has not been sufficient to give us any evidence upon these points.

We tender our hearty thanks to Dr. J. F. Gemmel, the Medical Superintendent of the Asylum, and also to Sergt. Fann, R.A.M.C., for his efficient help in the collection of the specimens.

SUMMARY

Two hundred and seven Asylum patients have been examined for intestinal protozoa. All the protozoa, but *G. intestinalis*, were found more commonly than in any other population group we have examined. *G. intestinalis*, on the other hand, was less common than in any other group.

V. UNIVERSITY AND SCHOOL CADETS

We began in April, 1918, an examination of the stools of youths in the Liverpool University Platoon of the Manchester University Officers' Training Corps, and also of the older boys in a high-class secondary school near Liverpool.

We examined in all only forty-one such persons, all males and ranging in age from 15 to 19 years. As it seems now very improbable that the investigation will be continued, we give without comment the results so far obtained. None of those reported on had ever been out of this country.

No. examined	41	
E. histolytica	1	case
E. coli	11	cases
E. nana	1	case
G. intestinalis	1	"
C. mesnili	—	

The results are from a single examination. Our object was to examine a number of youths from a higher social class and from better homes than the Army recruits whom we had previously reported on. We can only say that infections of the various protozoa exist among such persons.

REFERENCE

MATTHEWS, J. R., and SMITH, A. MALINS (1919). The Spread and Incidence of Intestinal Protozoal Infections in the Population of Great Britain. I. Civilians in Liverpool Royal Infirmary. II. Army Recruits. III. Children. *Ann. Trop. Med. & Parasitol.* Vol. XII, p. 349.

AWARD OF THE MARY KINGSLEY MEDAL

At a meeting of the Committee of the School, held on the 10th March, 1919, it was resolved to award this medal to J. W. S. Macfie, M.A., M.B., D.Sc., in recognition of his distinguished scientific work in Tropical Medicine.

Dr. Macfie was appointed to the West African Medical Staff, Northern Nigeria, in June, 1910. In 1913 he was seconded to the Medical Research Institute, Lagos, as bacteriologist, and during that year took part in the Yellow Fever investigation in West Africa. In 1914 he became pathologist at Accra, and in March, 1917, was seconded to the Liverpool School of Tropical Medicine to undertake research on the treatment of malaria.

During the last nine years Dr. Macfie has published many valuable contributions to science, notably on trypanosomiasis in man and domestic stock.

The medal was presented to him by the Chairman of the School at a luncheon given on the 12th April, 1919.

At the commencement of this number will be found a list of the medalists.

J. W. S. Macfie.

STUDIES IN THE TREATMENT OF MALARIA

XXVI. THE ACTION OF ARSENIC AND OF QUININE ON QUARTAN MALARIA

BY

LIEUT.-COL. J. W. W. STEPHENS, R.A.M.C.

W. YORKE

B. BLACKLOCK

AND

J. W. S. MACFIE

From the Liverpool School of Tropical Medicine

Undertaken at the request of the War Office

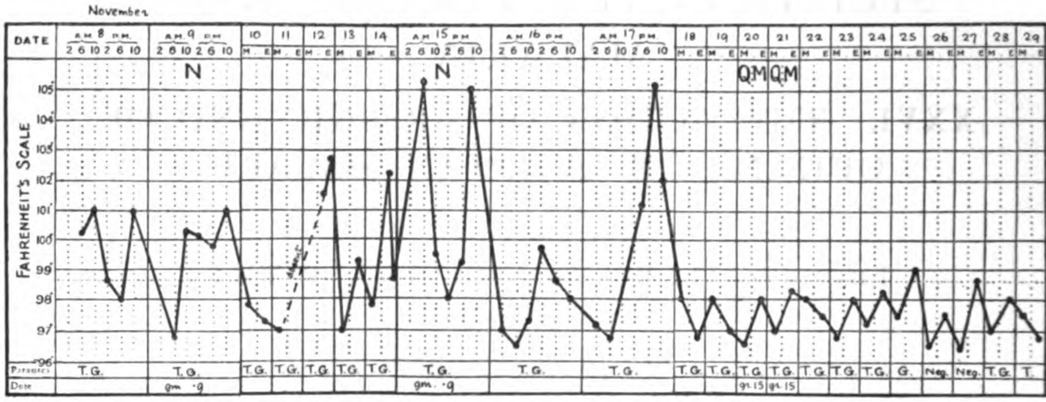
(Received for publication 1 *May,* 1919)

Two intravenous injections of novarsenobillon 0·9 gramme were given at an interval of approximately a week in two cases (Nos. 1524 and 1525). The results, which are given in the temperature charts, show that the treatment had no appreciable effect either on the temperature or on the parasites. After it had been ascertained that novarsenobillon was ineffective, an intramuscular injection of quinine bihydrochloride grains 15 on each of two consecutive days was given in each of the cases. From the charts it will be seen that in neither case did the injections cause the disappearance of the parasites, trophozoites and gametes persisting. In one case (1524) the temperature was controlled, in the other (1525) it was not.

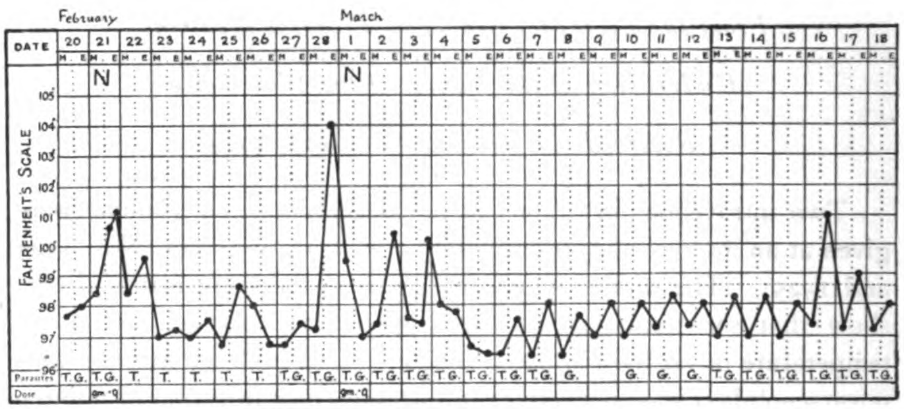
T. = quartan trophozoites or schizonts.
G. = quartan gametes.
Neg. = no parasites found.
N. = intravenous injection of novarsenobillon.
Q.M. = intramuscular injection of quinine bihydrochloride.
Q.O. = oral administration of quinine sulphate.

A comparison with the results given in Studies XVI, XXV, and XXVII shows a remarkable difference in the action of novarsenobillon and quinine on the three species of malaria parasites.

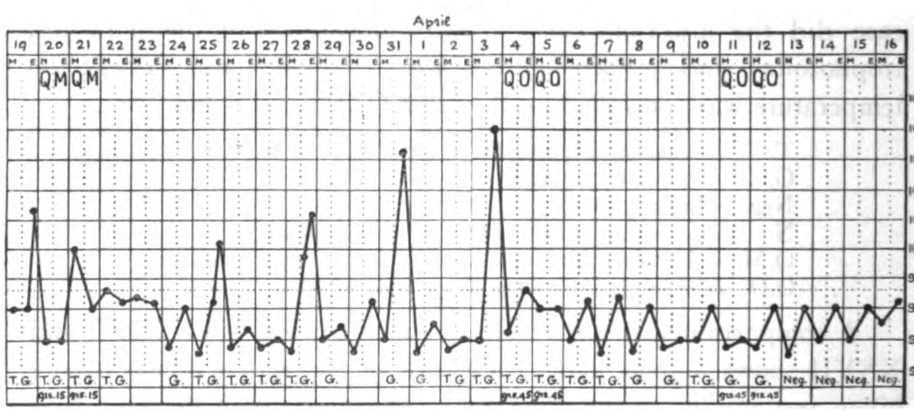
Case 1524



Case 1525 (Part I)



Case 1525 (Part II)



NOVARSENOBILLON

The action of novarsenobillon on *Plasmodium vivax* is marked. In this infection its action is even more rapid and efficient than that of quinine, a single intravenous injection of 0·9 gramme causing the disappearance from the cutaneous blood of all stages of the parasites within twenty-four hours. In the case of *P. falciparum* and *P. malariae* novarsenobillon in the same dosage has no appreciable effect on the temperature or on the parasites.

QUININE

An intramuscular injection of the bihydrochloride of quinine grains 15 on each of two consecutive days only, exerts in the case of *P. vivax* a constant and rapid effect both on the temperature and the parasites; in the case of *P. falciparum* the action on the temperature and trophozoites is also well defined, though relapses occur more quickly than in the case of *P. vivax*, whilst in the two cases of *P. malariae* treated in the same way there is little if any effect on the parasites, but in one of the two cases the temperature was controlled.

REFERENCES

Stephens, J. W. W., Yorke, W., Blacklock, B., Macfie, J. W. S., Cooper, C. F., and Carter, H. F. (1918 and 1919). *Ann. Trop. Med. & Parasitol.*, Vol. XII, p. 211, and Vol. XIII, pp. 75 and 101.

STUDIES IN THE TREATMENT OF MALARIA

XXVII. INTRAVENOUS INJECTIONS OF NOVARSENOBILLON AND INTRAMUSCULAR INJECTIONS OF QUININE BIHYDROCHLORIDE IN SIMPLE TERTIAN MALARIA

BY

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Undertaken at the request of the War Office

(*Received for publication* 5 *May*, 1919)

The observations made by us can be divided into three groups.

GROUP I. A single intravenous injection of novarsenobillon 0·9 gramme on the first day with an intramuscular injection of quinine bihydrochloride grains 15 on the first and second days of treatment.

GROUP II. An intravenous injection of novarsenobillon 0·9 gramme on the first, eighth, and fifteenth days, with intramuscular injections of quinine bihydrochloride grains 15 on the first and second, eighth and ninth, and fifteenth and sixteenth days of treatment.

GROUP III (control series). An injection of quinine bihydrochloride grains 15 on each of two consecutive days only.

GROUP I (Cases 1526 to 1566)

In nine of the forty-one cases (Table I) treatment was commenced during an apyrexial period. In thirty cases the temperature fell to normal within one day, in one case (1533) in two days. In one case pneumonia developed on the second day of treatment.

In all the cases parasites disappeared from the blood in one day.

Relapses

Thirteen of the forty-one cases (32 per cent.) relapsed within the sixty-days' observation period. Parasites reappeared in thirteen to forty-eight days (average twenty-nine days), and febrile relapses occurred in fourteen to forty-eight days.

Two of the cases (1543 and 1551) which did not relapse within the sixty-day observation period did so on the sixty-fourth and sixty-fifth days respectively.

GROUP II (Cases 1567 to 1578)

In five of the twelve cases (Table II) treatment was commenced during an apyrexial period; in the remaining seven the temperature fell to normal within two days.

In all cases parasites disappeared from the cutaneous blood in one day.

Relapses

One case relapsed on the last, i.e. the sixtieth day of the post-treatment observation period.

In one case (1578) the observation period was less than sixty days, viz., forty-five days; consequently the minimum number of relapses, those actually observed, is 8 per cent. and the possible maximum number 17 per cent.

One case (1574) which did not relapse within the sixty-day observation period did so on the sixty-eighth day.

TABLE I.

mary of results of a single intravenous injection of novarsenobillon gramme 0·9 on the 1st day, with an intramuscular injection of quinine bihydrochloride grains 15 on the 1st and 2nd days of treatment, in simple tertian malaria.

* E.A. = East Africa. F. = France. I. = India. M. = Mesopotamia. S. = Salonika.

*Place of infection	Interval (in months) between first admission to a hospital with malaria and present treatment	Interval (in months) between leaving infected area and present treatment	Interval (in months) between arrival in England and present treatment	Date of end of treatment	Temperature fell to normal in — days after first dose	Parasites disappeared from cutaneous blood in — days after first dose	Parasitic relapse occurred in — days after last dose	Febrile relapse (above 100° F.) occurred in — days after last dose	Observation period (in days) in cases which did not relapse	Remarks.
S.	28	10	9	3.10.18	Same day	1	...	...	127	
S.	13	9	9	3.10.18	1	1	...	...	62	
S.	15	...	...	3.10.18	1	1	...	...	124	
S.	21	3	2	8.10.18	1	1	...	...	106	
S.	14	4	2	9.10.18	Same day	1	...	...	72	
I.	16	7	3	9.10.18	Same day	1	...	...	7[illegible]	
S.	28	5	5	10.10.18	Apyrexia	1	...	...	6[illegible]	
E.A.	15	9	7	10.10.18	2	1	35	...	..	Quinine orally, grs. 45 on 44th day.
E.A.	8	8	7	11.10.18	Apyrexia	1	27	29	..	
S.	16	5	4	11.10.18	1	1	...	...	103	
E.A.	15	9	7	11.10.18	Same day	1	39	39	...	
F.	14	13	13	11.10.18	Same day	1	...	...	113	
...	...	...	...	11.10.18	Same day	1	...	...	70	
E.A.	10	9	7	12.10.18	Apyrexia	1	...	...	80	
S.	28	2	2	13.10.18	Same day	1	...	...	68	
E.A.	26	7	1	13.10.18	Apyrexia	1	...	...	69	
S.	42	...	1	15.10.18	Apyrexia	1	...	...	118	
S.	14	2	2	16.10.18	1	1	...	...	63	Relapsed on 64th day.
S.	14	10	4	17.10.18	1	1	28	...	...	Quinine orally, grs. 45 on 37th day.
M.	28	27	4	20.10.18	1	1	...	...	116	
S.	26	7	7	20.10.18	Apyrexia	1	...	...	100	

TABLE I—*Continued*

Number of case.	*Place of infection	Interval (in months) between first admission to a hospital with malaria and present treatment	Interval (in months) between leaving infected area and present treatment	Interval (in months) between arrival in England and present treatment	Date of end of treatment	Temperature fell to normal in — days after first dose	Parasites disappeared from cutaneous blood in — days after first dose	Parasitic relapse occurred in — days after last dose	Febrile relapse (above 100° F.) occurred in — days after last dose	Observation period (in days) in cases which did not relapse	Remarks.
1547	S.	7	5	4	22.10.18	1	1	...	...	101	
1548	S.	28	2	2	23.10.18	Same day	1	...	...	111	
1549	S.	11	4	3	24.10.18	Same day	1	...	...	97	
1550	S.	12	1	0	25.10.18	Same day	1	...	...	97	
1551	S.	27	7	6	25.10.18	1	1	...	...	64	Relapsed on 65th d
1552	...	...	...	...	25.10.18	Apyrexia	1	13	14	...	
1553	S.	28	4	3	25.10.18	Apyrexia	1	20	21	...	
1554	S.	7	4	2	27.10.18	1	1	...	...	96	
1555	S.	13	5	4	27.10.18	Same day	1	...	...	63	
1556	E.A.	10	7	6	27.10.18	Apyrexia	1	43	...	...	Quinine orally, gr. on 44th day.
1557	E.A.	9	7	5	27.10.18	1	1	18	18	...	
1558	S.	23	2	2	27.10.18	Same day	1	...	...	82	
1559	S.	27	2	2	27.10.18	1	1	48	48	...	
1560	S.	...	2	1	29.10.18	1	1	...	...	92	
1561	S.	29	8	8	31.10.18	1	1	31	...	...	Quinine orally, grs. on 32nd day.
1562	S.	24	6	5	1.11.18	1	1	30	30	...	
1563	I.	0	3	2	10.11.18	1	1	...	...	69	
1564	E.A.	14	8	7	12.11.18	Same day	1	25	...	...	Quinine orally, grs. on 27th day.
1565	S.	8	8	2	26.11.18	1	1	17	...	...	Quinine orally, grs. on 18th day.
1566	S.	1	2	2	29.11.18	...	1	...	...	64	Pneumonia on [illegible] day of treatment.

TABLE II.

Summary of results of an intravenous injection of novarsenobillon gramme 0·9 on the 1st, 8th and 15th days, with intramuscular injections of quinine bihydrochloride grains 15 on the 1st and 2nd, 8th and 9th, 15th and 16th days of treatment, in simple tertian malaria.

* E.A. = East Africa. I. = India. S. = Salonika.

[N]umber of [c]ase.	*Place of infection	Interval (in months) between first admission to a hospital with malaria and present treatment	Interval (in months) between leaving infected area and present treatment	Interval (in months) between arrival in England and present treatment	Date of end of treatment	Temperature fell to normal in — days after first dose	Parasites disappeared from cutaneous blood in — days after first dose	Parasitic relapse occurred in — days after last dose	Febrile relapse (above 100° F.) occurred in — days after last dose	Observation period (in days) in cases which did not relapse	Remarks.
567	E.A.	14	11	9	1.12.18	Apyrexia	1	60	...	...	Quinine orally, grs. 30 on 61st day.
568	E.A.	12	11	9	1.12.18	Apyrexia	1	...	...	65	
569	S.	25	3	2	1.12.18	2	1	...	...	71	
570	S.	2	5	4	4.12.18	2	1	...	...	65	
571	S.	13	4	4	4.12.18	1	1	...	...	65	
572	S.	28	2	2	6.12.18	Apyrexia	1	...	...	66	
573	S.	18	7	7	8.12.18	Apyrexia	1	...	...	82	100·2° F. on last day of treatment
574	I.	12	7	5	11.12.18	1	1	...	...	67	Relapsed on 68th day.
575	S.	6	3	2	11.12.18	2	1	...	...	76	
576	S.	31	3	3	12.12.18	1	1	...	...	60	
577	S.	24	15	6	14.12.18	Same day	1	...	...	76	
578	S.	17	8	7	15.12.18	Apyrexia	1	...	...	45	

TABLE III.

Summary of results of an intramuscular injection of quinine bihydrochloride grains 15, on each of two days only, in simple tertian malaria.

* E.A. = East Africa. I. = India. M. = Mesopotamia. S. = Salonika.

Number of case.	*Place of infection	Interval (in months) between first admission to a hospital with malaria and present treatment	Interval (in months) between leaving infected area and present treatment	Interval (in months) between arrival in England and present treatment	Date of end of treatment	Temperature fell to normal in — days after first dose	Parasites disappeared from cutaneous blood in — days after first dose	Parasitic relapse occurred in — days after last dose	Febrile relapse (above 100° F.) occurred in — days after last dose	Observation period (in days) in cases which did not relapse	Remarks.
1579	S.	29	3	3	1.11.18	Same day	1	29	...	...	Quinine on 32nd day
1580	S.	14	6	5	2.11.18	Apyrexia	2	20	...	...	Quinine orally, grs. [illegible] on 22nd day.
1581	S.	30	2	2	5.11.18	2	2	20	21	...	
1582	M.	29	29	5	5.11.18	1	3	20	23	...	
1583	S.	22	6	5	6.11.18	1	2	...	...	77	
1584	S.	...	6	5	6.11.18	Apyrexia	1	22	24	...	
1585	S.	15	4	3	9.11.18	Same day	1	22	20	...	
1586	S.	29	3	2	9.11.18	Same day	1	...	...	80	
1587	S.	8	8	2	9.11.18	Apyrexia	1	13	14	...	
1588	E.A.	10	9	3	10.11.18	1	1	18	18	...	
1589	...	...	...	...	26.11.18	Same day	1	...	...	37	
1590	E.A.	37	...	5	1.12.18	Same day	1	18	18	...	
1591	S.	16	4	3	11.12.18	Same day	1	10	...	...	Quinine orally, grs. [illegible] on 11th day.
1592	S.	27	2	1	14.12.18	Same day	1	...	...	60	
1593	E.A.	10	6	0	15.12.18	2	1	30	...	...	Quinine orally, grs. [illegible] on 32nd day.
1594	I.	34	7	2	21.12.18	1	2	51	52	...	
1595	S.	4	1	1	9.1.19	Same day	1	21	...	...	Discharged on [illegible] day.
1596	...	...	...	...	9.3.19	Same day	1	13	15	...	

TABLE IV.

Comparison of curative results obtained from the different treatments.

Treatment		Number of cases observed after treatment	Number of cases which relapsed within 60 days	Number of cases not relapsing but observed for less than 60 days	Percentage of cases which relapsed	
					Minimum	Maximum
Group I.	Novarsenobillon gm. 0·9 intravenously on the 1st day, with quinine bihydrochl., grs. 15 intramuscularly on the 1st and 2nd days	41	13	0	32%	32%
Group II.	Novarsenobillon gm. 0·9, intravenously on the 1st, 8th and 15th days, with quinine bihydrochl. grs. 15 intramuscularly on the 1st and 2nd, 8th and 9th, 15th and 16th days	12	1	1	8%	17%
Group III.	Quinine bihydrochl. grs. 15 on each of two days only (Control Series)	18	14	1	78%	83%
*Study XVI. (Table III.)	Novarsenobillon gm. 0·9 intravenously (single injection) ...	21	19	1	90%	95%

* *Vide* Reference.

GROUP III (Cases 1579 to 1596)

In three of the eighteen cases (Table III) treatment was commenced during an apyrexial period. In the remaining fifteen, the temperature fell to normal within two days.

In seventeen cases parasites disappeared from the cutaneous blood within two days, in one case within three days.

Relapses

Fourteen of the eighteen cases relapsed within the sixty-days' observation period. Parasites reappeared in ten to fifty-one days (average twenty-two days) and febrile relapses occurred in fourteen to fifty-two days.

In one case (1589) the observation period was less than sixty days, viz., thirty-seven days; consequently the minimum number of relapses is fourteen (78 per cent.) and the possible maximum fifteen (83 per cent.).

CONCLUSION

A combination of novarsenobillon and quinine is more effective than either novarsenobillon or quinine alone.

REFERENCE

STEPHENS, J. W. W., YORKE, W., BLACKLOCK, B., MACFIE, J. W. S., COOPER, C. F., and CARTER, H. F. *Ann. Trop. Med. & Parasitol.*, Vol. XII, p. 215.

FILARIA PERTENUE, N.SP., PROVOQUANT UNE DERMO-FILARIOSE CHELOÏDIFORME CHEZ *CEPHALOPHUS SYLVICULTOR*

PAR

J. RODHAIN

(PLATES II AND III)

(*Received for publication* 8 *April*, 1919)

Le nématode dont nous donnons ici une description, a été recueilli chez la grande Céphalope: *Cephalophus sylvicultor* à Bambili sur l'Uele au Congo Belge.

L'antilope, qu'on nous apporta tuée, portait au haut des cuisses deux taches glabres au niveau desquelles la peau noire, légèrement saillante et luisante, avait pris l'aspect qu'ont les grosses chéloïdes des tatouages nègres. Ces plaques cutanées, allongées, ayant 1 et ½ centimètre de large sur plus de 5 cen. de long, occupaient une position sensiblement symétrique. Toutes deux légèrement incurvées se rapprochaient en haut et en dedans de la base d'insertion de la queue, et s'en écartaient obliquement en bas et en dehors.

Un examen microscopique rapide des raclures prélevées au scalpel dans le tissu induré, montra à côté de débris d'helminthes des embryons filariens. Des fragments de la lésion vermineuse furent fixés à l'alcool et au formol pour étude ultérieure. Celle-ci commencée à Léopoldville au début de 1915 a pu être complètée récemment au laboratoire de M. E. Roubaud à l'Institut Pasteur à Paris.

DESCRIPTION DE LA LÉSION ANATOMIQUE

Macroscopiquement, la pseudochéloïde présente à la section l'aspect harolacé du tissu fibreux, mais parsemé de nombreux points et trainées jaunâtres. Ces dernières correspondent aux trajets tortueux des vers. Elle s'étend en profondeur, de l'épiderme

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The observations made by us can be divided into three groups.

GROUP I. A single intravenous injection of novarsenobillon 0·9 gramme on the first day with an intramuscular injection of quinine bihydrochloride grains 15 on the first and second days of treatment.

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GROUP III (control series). An injection of quinine bihydrochloride grains 15 on each of two consecutive days only.

jusqu'au contact des muscles fessiers, atteignant 1 centimètre de plus grande épaisseur.

Les *coupes microscopiques* montrent que l'épaisissement et l'induration de la peau sont dus à *l'hypertrophie scléreuse* du derme provoquée par l'irritation chronique causée par les nématodes qui habitent le chorion. Les helminthes, pliés et repliés parfois coudés sur eux-mêmes dans le tissu conjonctif fibreux, y déterminent une réaction inflammatoire du type essentiellement chronique. Celle-ci se reconnait au voisinage immédiat des parasites. Ils sont entourés d'une véritable gangue de pus épais constitué par les éléments conjonctifs dissociés et nécrosés entre lesquels se sont infiltrés des phagocytes mononucléaires, petits et grands lymphocytes entremêlés de macrophages et plasmocytes. Là où les fragments de nématodes se voisinent, les gangues de pus confluent, il se forme dans le tractus fibreux des noyaux de tissu infiltré, friable, véritable début de petites cavités (Planche II, figs. 1 et 2).

En dehors des parasites, la trame conjonctivale du chorion réagit par la formation de tissu fibreux dense dont on peut observer les divers stades de différenciation. L'hypertrophie scléreuse qui en résulte, entraine secondairement l'anémie de la couche papillaire du derme, l'amincissement de l'épiderme, l'atrophie et la disparition des follicules pileux et des glandes sébacées, la réduction des glandes sudoripares. L'épiderme au niveau de la lésion filarienne prend l'aspect luisant cicatriciel.

Les parasites siègent dans les couches profondes du derme et ne pénètrent que rarement dans les couches supérieures. Dans celles-ci se produit une stase lymphatique que traduit la dilatation du réseau sous-papillaire. Au niveau de la chéloïde, le tissu cellulaire souscutané a disparu et le derme hypertrophié, s'étend jusqu'à la couche musculaire des fessiers auxquels la tumeur est adhérente. Les filaires, du moins leurs extrémités, se déplacent dans les tissus qu'ils irritent. Leurs anciens trajets se reconnaissent sur les coupes, aux trainées de pus caractéristiques, dont les canalicules vides de parasites sont bientôt envahis par les éléments migrateurs de leurs parois même. Ultérieurement ces trajets sont comblés par du tissu de néoformation dérivé des fibroblastes suivant un véritable processus cicatriciel.

Ces lésions sont en plus d'un point comparables aux nodules fibrômateux produits par l'*Onchocerca volvulus* chez l'homme. Mais ici les parasites siègent dans les canaux ou les ganglions lymphatiques. Ils sont moins nombreux et plus immobiles dans leurs tumeurs. Leur localisation fibreuse est plus stricte. Plus volumineux, ils créent dans leurs fibromes de véritables cavités dans lesquelles les extrémités de mâles et de femelles voisinent. Dans la chéloïde filarienne de *Cephalophus sylvicultor*, ces cavités ont leur homologue dans les noyaux de pus où les fragments de vers sont rapprochés et dehors desquelles nous avons pu aussi isoler des extrémités d'helminthes.

DESCRIPTION DES FILAIRES

En dissociant sous le binoculaire des coupes épaisses de la chéloïde vermineuse, nous en avons extrait des fragments de vers, parmi lesquels les extrémités antérieures et postérieures de mâle et de femelle dont l'étude à permis la classification zoologique du parasite. Il s'agit d'une des *Filariinae* que nous rattachons au genre *Filaria* tel que celui-ci persiste actuellement.

Le corps est filiforme, atténué à ses deux extrémités dont la postérieure est plus amincie que l'antérieure. La cuticule est lisse, présentant au niveau des courbures ou des coudures du ver de nombreux plissements et montrant aux forts grossissements sous un éclairage favorable une très fine striation transversale. La tête est plus où moins règulièrement arrondie, parfois très légèrement aciculée vers son sommet, au niveau de la bouche. Celle-ci chez certains exemplaires apparait proéminente mais est toujours sans lèvres distinctes ni papilles visibles.* L'oesophage est droit sans dilatations. L'intestin dès son origine est plus large que la partie terminale de l'oesophage.

Mâle. Longueur inconnue.† Plus étroit que la femelle, il ne mesure que 72μ à 78μ de largeur en son plein développement. Son extrémité antérieure régulièrement rètrécie n'atteint plus que 61μ de large immédiatement en arrière de la bouche. L'extrémité

* Lorsqu' au cours des manipulations de la préparation des parasites, l'extrémité céphalique est comprimée, la partie buccale devient nettement saillante, et il apparait une indication de démarcation entre la tête et le corps. Chez les individus non déformés cette délimitation n'est pas visible.

† Le plus long fragment de mâle que nous ayons obtenu ne mesurait que 5 millimetres 670μ. C'etait une extrémité postérieure.

postérieure est plus étroite encore, généralement recourbée sur elle-même sans orientation uniforme de l'incurvation qui dépend de la situation momentanée occupée par le ver dans le tissu.

Le cloaque s'ouvre à 21 μ de l'extrémité caudale, qui à son niveau ne mesure plus que 27 μ de large. Les papilles sous forme de tubercules microscopiques, dont les plus volumineux ne dépassent la cuticule que de 1·5 μ, paraissent au nombre de six paires dont quatre préanales, une para-anale et une postanale.* Les deux spicules sont très inégaux. L'un court et large, à bords épais, concave sur sa face antérieure, se termine en pointe obtuse. Il engaine partiellement le spicule long à son point d'emergeance. Il est large de 6 μ à 8 μ à sa base et long de 36 μ à 43 μ. L'autre long et mince, arrondi, mesure au total 242 μ, atteignant son maximum de largeur de 5·6 μ près de sa base d'insertion. Il présente généralement une partie interne proximale longue de 208 μ et une partie externe distale recourbée vers l'arrière et mesurant 34 μ (Planche III, fig. 3A).

Femelle. Longueur inconnue.† Plus large que le mâle, elle atteint jusque 104 μ d'epaisseur.‡

L'anus s'ouvre à 37 μ ou 41 μ de l'extrémité caudale, au devant d'une dépression ovalaire qui termine le ver et qui montre une structure interne que l'état de conservation de nos parasites ne nous a pas permis de préciser.

L'utérus comprend deux tubes génitaux quelquefois d'inégale longueur, qui s'étendent en arrière jusqu'à 493 μ de l'extrémité postérieure. Ils se réunissent en avant, en un ovejecteur unique. Celui-ci, long de 286 μ et large de 16 μ, présentant au moins une inflexion, peut-être poursuivi jusqu'à 52 μ en arrière de la bouche. Il présente près de son extrémité antérieure un épaississement annulaire. Je ne suis pas parvenu à situer exactement l'orifice vulvaire qui n'est certainement pas saillant. Les tubes ovariques

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* Pour déterminer définitivement le nombre exact et la situation précise de ces papilles l'examen d'exemplaires extraits de tissu frais sera nécessaire. Les papilles sont microscopiques et sans l'avis autorisé de M. L. Gedoelst nous n'aurions pas osé considérer les proéminences cuticulaires que nous avions observées comme des papilles.

† Le plus long fragment de ♀ que nous ayons obtenu, mesurait 9½ millimètres à partir de l'extrémité postérieure. Il représentait certainement les ¾ de l'adulte car les deux tubes genitaux contenaient à leurs extrémités brisées des oeufs à embryons complètement formés. La longueur totale du ver femelle ne dépassera probablement pas 12 millimètres.

‡ Les mensurations out été faites sur des sections de vers fixés dans les tissus et montrant les deux tubes ovariques contenant des oeufs embryonnés. En réalité les dimensions oscillent entre 81 μ et 104 μ.

contiennent des oeufs embryonnés montrant des microfilaires vivantes. L'une de celles-ci échappée de sa coque ovulaire dans le tube utérin même, mesurait 65 μ de long sur 3 μ de large. La femelle est ovovivipare (Planche III, fig. 3B).

Nous appellerons *Filaria pertenue* cette filaire entièrement différente de *Dirofilaria kuelzii*, Rodenwaldt (1910), qui parasite le tissu conjonctif souscutané et intramusculaire de *Cephalophus maxwelli* au Cameroun, et que L. Gedoelst (1916) a retrouvé au Congo Belge. Il ne nous est pas possible de dire combien de filaires vivent dans une même tumeur chéloïdienne. Leur nombre parait être considérable, car hors d'un fragment de tumeur ayant près de 1 centimètre cube de volume, nous avons isolé cinq extrémités postérieures de mâles, et trois de femelles.

Ainsi que je l'ai dit précédemment, les vers, du moins leurs extrémités, se déplacent dans le tissu fibreux qu'ils habitent. Ces mouvements ont très vraisemblablement pour but d'assurer les contacts des sexes nécessaires pour la reproduction. Il est probable aussi, que la femelle effectue sa ponte dans la lésion fibromateuse même, quoique sur nos coupes nous n'ayons pas rencontré de microfilaires en dehors des tubes ovariques.

Un examen rapide du sang périphérique de la céphalophe parasitée n'a pas montré de microfilaires. Nos investigations n'ont pas porté sur les ganglions inguinaux drainant les régions où siègeaient les tumeurs chéloïdiennes.

Nous ne pouvons donc actuellement formuler aucune hypothèse fondée sur le mode de transmission de ce nouveau parasite.

BIBLIOGRAPHIE

GEDOELST, L. (1916). Notes sur la faune parasitaire du Congo Belge. *Revue Zoologique Africaine*, Vol. V, fasc. 1.

RODENWALDT, E. (1910). *Filaria kuelzii*, n.sp. *Arch. f. Schiffs. u. Trop. Hyg.*, XIV, p. 529.

EXPLANATION OF PLATES

Plate II

Chéloïde filarienne

Fig. 1. Coupe perpendiculaire à la peau et montrant : *a.* Les dilatations du réseau lymphatique sous-papillaire. *b.* Un noyau inflammatoire avec diverses sections de filaires.
(Microphotographie grossissant environ 70 fois.)

Fig. 2. Coupe montrant un fragment de filaire en section longitudinale, enveloppé dans sa gangue de pus.
(Microphotographie grossissant environ 140 fois.)

Fig. 1

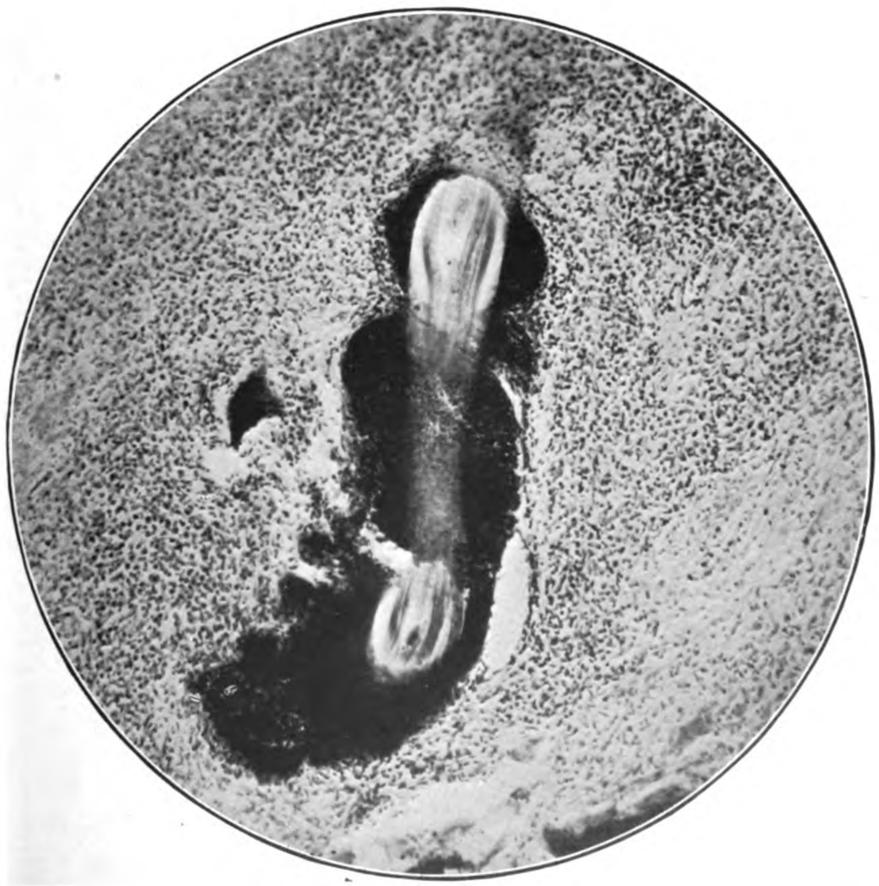

Fig. 2

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PLATE III

Filaria pertenue, n.sp.

Fig. 3. A. Extrémité postérieure du mâle. B. Extrémité antérieure de la femelle.
(Microphotographie grossissant environ 100 fois.)

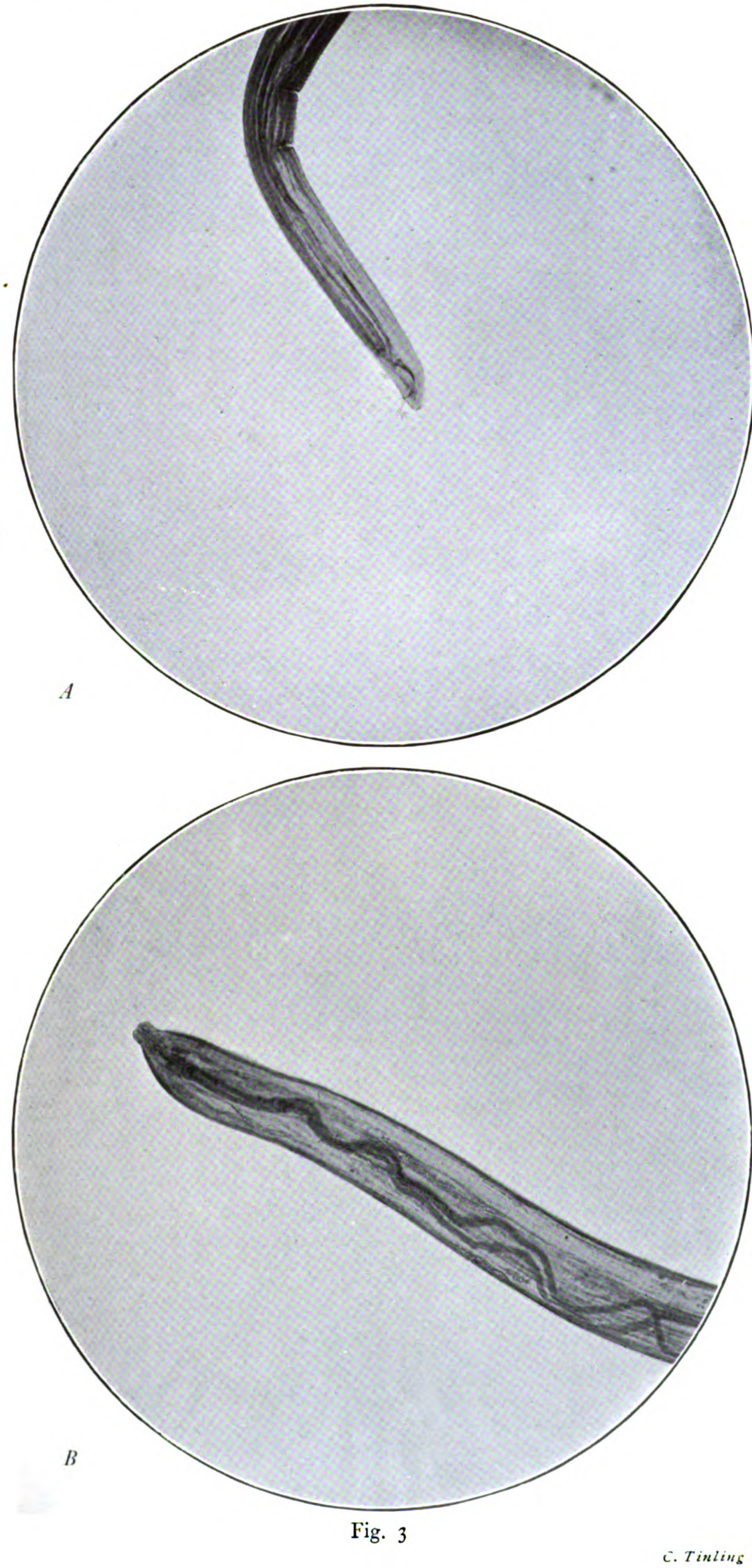

Fig. 3

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STUDIES IN THE TREATMENT OF MALARIA

XXVIII. QUITENINE HYDROCHLORIDE IN SIMPLE TERTIAN MALARIA

BY

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Undertaken at the request of the War Office

(*Received for publication* 10 *May*, 1919)

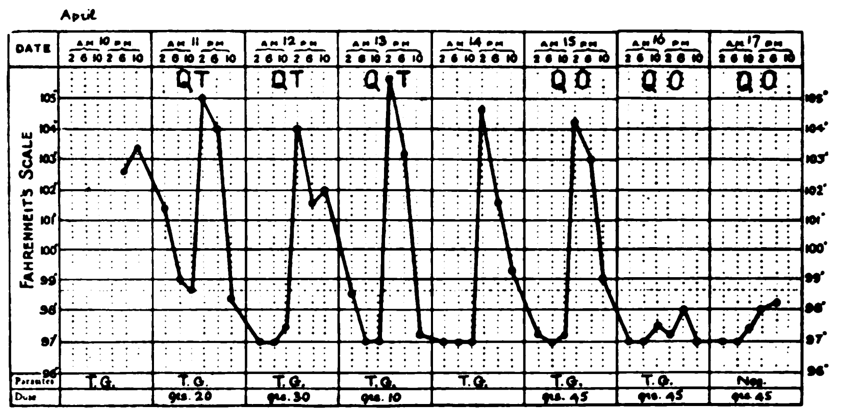
Sixty grains of this preparation* were supplied to us by the courtesy of Professor Ramsden.

The drug was administered by the mouth in one case (No. 1597), grains 20 being given on the first day, grains 30 on the second day and grains 10 on the third day of treatment, a total of grains 60. The result is shown in the chart, in which :—

T. = simple tertian trophozoites or schizonts.
G. = simple tertian gametes.
Neg. = no parasites found.
Q.T. = oral administration of quitenine.
Q.O. = oral administration of quinine sulphate.

* The quitenine given to the patient was prepared by Skraup's method by oxidising quinine with potassium permanganate whereby its vinyl group is converted into a carboxyl group.

CASE 1597



CONCLUSION

Quitenine hydrochloride in the doses used is of no value in the treatment of simple tertian malaria.

STUDIES IN THE TREATMENT OF MALARIA

XXIX. ORAL ADMINISTRATION OF LIQUOR ARSENICALIS MINIMS 30 DAILY FOR 16 DAYS WITH QUININE BIHYDROCHLORIDE GRAINS 15 INTRAMUSCULARLY ON THE 1ST AND 2ND, 8TH AND 9TH, 15TH AND 16TH DAYS, IN SIMPLE TERTIAN MALARIA

BY

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In a previous paper (1919) we recorded the results of administering Liquor arsenicalis in large doses, minims 30 daily, over a period of eight weeks in combination with two initial intramuscular injections of quinine bihydrochloride grains 15 on two days only. This treatment, which was administered in thirty-two cases, was followed by four relapses only (12·5 per cent.) within an observation period of sixty days after cessation of treatment.

The present observations were undertaken in order to ascertain whether an equally favourable result could be got with a similar treatment of shorter duration.

The records of the observations are given in the tables and charts, in which :—

T. = simple tertian trophozoites or schizonts.
G. = simple tertian gametes.
Neg. = no parasites found.
A. = oral administration of liquor arsenicalis minims 30.
Q.M. = intramuscular injection of quinine bihydrochloride grains 15.

Liquor arsenicalis minims 30 daily were given for sixteen days and an intramuscular injection of quinine bihydrochloride grains 15 on the first and second, eighth and ninth, fifteenth and sixteenth days.

Cases 1598—1642

Forty-five cases (Table I) were treated in this manner. All were adult males, the majority of whom were infected either in Macedonia or in East Africa. In every instance a diagnosis of simple tertian malaria was made microscopically, and in all cases parasites were present in the blood on the day treatment commenced. Blood examinations were made daily.

In four of the forty-five cases treatment was commenced during an apyrexial period. In thirty-nine of the remaining forty-one the temperature fell to normal within three days, and in one case in four days; in the remaining case (1630), owing to intercurrent disease, the temperature was uncontrolled until the fourteenth day.

In forty-one cases parasites disappeared from the cutaneous blood in one to two days, in three cases in three days, and in one case in four days.

Relapses. In nine of the forty-five cases parasitic relapses occurred in seventeen to fifty-six days, and febrile relapses in twenty-six to fifty-six days, after cessation of treatment. In twenty-one cases the observation period was less than sixty days;* consequently the minimum percentage of cases which relapsed (those actually observed) is 20 per cent., and the possible maximum (those actually observed + the number of cases discharged before completion of the sixty-day post-treatment observation period) is 66·6 per cent. Two of the cases (1600 and 1616) which did not relapse within the sixty-day observation period, did so on the sixty-eighth and sixty-fifth days respectively.

* This was due to the demobilization which took place subsequent to the date of the Armistice, 11.11.18.

Table I.

Summary of results of oral administration of Liquor arsenicalis, minims 30 daily, for 16 days + quinine bihydrochloride, grains 15, intramuscularly, on the 1st and 2nd, 8th and 9th, 15th and 16th days, in simple tertian malaria.

* E. = Egypt. E.A. = East Africa. I. = India. M. = Mesopotamia. P. = Palestine. S. = Salonika.

Number of case	*Place of infection	Interval (in months) between first admission to a hospital with malaria and present treatment	Interval (in months) between leaving infected area and present treatment	Interval (in months) between arrival in England and present treatment	Date of end of treatment	Temperature fell to normal in — days after first dose	Parasites disappeared from cutaneous blood in — days after first dose	Parasitic relapse occurred in — days after last dose	Febrile relapse (above 100° F.) occurred in — days after last dose	Observation period (in days) in cases which did not relapse	Remarks
1598	M.	29	29	6	15.12.18	1	1	...	...	50	
1599	E.A.	11	10	4	15.12.18	3	1	18	...	...	Quinine orally, grs. 30 on 24th day.
1600	S.	...	7	6	15.12.18	2	1	...	...	67	Relapsed on 68th day.
1601	E.A.	21	11	8	17.12.18	2	1	48	47	...	
1602	S.	30	4	4	17.12.18	Apyrexia	1	...	...	46	
1603	S.	18	4	4	18.12.18	3	2	...	...	65	
1604	E.	2	8	2	18.12.18	3	2	...	...	65	
1605	S.	16	5	4	18.12.18	1	2	...	...	98	
1606	S.	14	1	1	20.12.18	Same day	1	...	...	61	
1607	S.	31	2	2	21.12.18	Apyrexia	1	...	...	70	
1608	S.	35	9	7	21.12.18	1	3	...	...	70	
1609	S.	1	1	1	24.12.18	1	4	...	...	74	
1610	S.	41	18	4	24.12.18	Same day	1	31	32	...	
1611	S.	14	4	4	25.12.18	1	1	...	...	50	
1612	E.	28	5	5	28.12.18	1	1	...	...	89	
1613	S.	28	3	3	15.1.19	1	1	...	...	76	
1614	S.	15	4	4	18.1.19	1	1	...	...	63	
1615	...	...	...	...	21.1.19	2	2	56	56	...	
1616	...	...	...	...	23.1.19	1	2	...	...	64	Relapsed on 65th day.
1617	...	...	...	...	28.1.19	1	2	...	...	50	
1618	P.	4	1	1	31.1.19	2	2	...	...	62	
1619	E.	5	...	2	4.2.19	3	1	...	...	70	

TABLE I—*continued*

Summary of results of oral administration of Liquor arsenicalis, minims 30 daily, for 16 days + quinine bihydrochloride, grains 15, intramuscul[illegible] on the 1st and 2nd, 8th and 9th, 15th and 16th days, in simple tertian malaria.

* E. = Egypt. E.A. = East Africa. I. = India. M. = Mesopotamia. P. = Palestine. S. = Salonika.

Number of case	*Place of infection	Interval (in months) between first admission to a hospital with malaria and present treatment	Interval (in months) between leaving infected area and present treatment	Interval (in months) between arrival in England and present treatment	Date of end of treatment	Temperature fell to normal in — days after first dose	Parasites disappeared from cutaneous blood in — days after first dose	Parasitic relapse occurred in — days after last dose	Febrile relapse (above 100° F.) occurred in — days after last dose	Observation period (in days) in cases which did not relapse	Remarks
1620	E.A.	11	8	2	4.2.19	2	3	28	...	...	Quinine orally,[illegible] on 29th day.
1621	E.A.	11	2	2	5.2.19	Same day	2	...	...	70	
1622	S.	20	2	2	5.2.19	Apyrexia	1	...	...	58	102·8° F. on [illegible] and 102·4° F[illegible] 28th days.
1623	P.	3	1	1	13.2.19	Same day	1	34	35	...	
1624	S.	5	2	1	14.2.19	1	1	...	...	14	
1625	I.	11	3	3	19.2.19	Apyrexia	1	...	...	52	
1626	...	...	...	...	22.2.19	1	2	17	...	...	Quinine orally,[illegible] on 20th day.
1627	E.A.	20	17	9	23.2.19	2	1	...	...	5	
1628	...	...	...	...	25.2.19	1	1	...	...	32	
1629	...	...	...	...	25.2.19	2	1	...	...	32	
1630	...	...	...	...	25.2.19	14	2	...	...	21	
1631	P.	8	...	2	25.2.19	2	1	...	...	50	
1632	E.	3	3	0	26.2.19	1	1	25	26	...	101·4° F. and[illegible] on 3rd and[illegible]
1633	E.A.	11	6	0	27.2.19	3	2	23	24	...	
1634	...	...	...	...	27.2.19	1	2	...	...	30	
1635	...	...	...	...	27.2.19	2	1	...	...	13	102° F. on 7[illegible]
1636	...	...	...	...	28.2.19	2	1	...	...	18	
1637	...	...	...	...	2.3.19	1	1	...	...	16	
1638	...	...	...	...	2.3.19	4	3	...	...	15	
1639	...	...	...	...	2.3.19	Same day	2	...	...	17	
1640	...	...	...	...	5.3.19	Same day	2	...	...	13	
1641	S.	8	5	2	6.3.19	1	1	...	...	60	
1642	E.	21	...	1	13.3.19	2	2	...	...	21	

TABLE II.

Summary of results of intramuscular injections of quinine bihydrochloride grains 15 on each of two consecutive days only in simple tertian malaria (control series).

Number of case	Date of end of treatment	Temperature fell to normal in — days after first injection	Parasites disappeared from cutaneous blood in — days after first injection	Parasitic relapse occurred in — days after last dose	Febrile relapse (above 100°F.) occurred in — days after last dose	Observation period (in days) in cases which did not relapse	Remarks
1643	9.3.19	1	1	13	15	...	
1644	22.3.19	2	4-5	19	22	...	
1645	30.3.19	2	5-6	16	18	...	
1646	4.4.19	1	2-12	12	12	...	
1647	5.4.19	1	2-11	18	18	...	
1648	6.4.19	Apyrexia	...	9	22	...	
1649	6.4.19	1	3-10	21	23	...	
1650	7.4.19	4	2-4	4	25	...	
1651	9.4.19	1	2	...	...	20	
1652	9.4.19	Same day	1-7	16	16	...	101°F. on 9th day. 102·6°F. on 10th day.
1653	10.4.19	1	2-5	10	13	...	
1654	10.4.19	1	2-5	17	18	...	
1655	10.4.19	Apyrexia	1-5	15	...	...	Quinine orally, grs. 45, on 21st day.
1656	10.4.19	2	2-5	12	14	...	
1657	11.4.19	1	1-4	17	18	...	
1658	11.4.19	Apyrexia	1-5	13	15	...	
1659	11.4.19	Same day	1-4	...	...	24	
1660	13.4.19	Apyrexia	1-3	14	...	...	Quinine orally, grs. 45, on 19th day.

CONTROL SERIES (Cases 1643—1660)

Quinine bihydrochloride grains 15 on each of two consecutive days only.

In four of the eighteen cases (Table II) treatment commenced during an apyrexial period; in thirteen cases the temperature fell to normal in two days, and in the remaining case in four days. Blood examinations were too infrequent to give a figure for the time of disappearance of parasites from the cutaneous blood.

Relapses. In sixteen of the eighteen cases parasitic relapses occurred in four to twenty-one days, and febrile relapses in twelve to twenty-five days, after cessation of treatment. In the two cases (1651 and 1659) which did not relapse the observation period was less than sixty days, viz., twenty and twenty-four days respectively. Consequently the minimum percentage of cases which relapsed (those actually observed) is 88·8 per cent., and the possible maximum (those actually observed + the number discharged before the completion of the sixty-day post-treatment observation period) is 100 per cent.

CONCLUSION

It is not possible to form a definite estimate of the value of this treatment, as almost half the cases were insufficiently observed after cessation of treatment.

REFERENCE

Stephens, J. W. W., Yorke, W., Blacklock, B., Macfie, J. W. S., Cooper, C. F., and Carter, H. F. (1919.) *Ann. Trop. Med. & Parasitol.*, Vol. XII, p. 371.

STUDIES IN THE TREATMENT OF MALARIA

XXX. AT WHAT TIME AFTER CESSATION OF QUININE TREATMENT DO RELAPSES OCCUR IN SIMPLE TERTIAN MALARIA?

(SECOND COMMUNICATION)

BY

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Undertaken at the request of the War Office

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In a previous paper (1918) we have recorded the time incidence of the relapses (270) among 405 cases of simple tertian malaria. We have now at our disposal the data with regard to an additional 395 cases, and propose to record the time incidence of all the relapses (582) in the 800 cases. Before doing so we desire to indicate certain points which bear on the question at issue.

1. So far as possible all cases were observed for at least sixty days after the cessation of treatment. As only forty-six cases were discharged before this time we can determine with considerable accuracy the incidence of relapses up to the end of this period. Many cases were observed for varying periods exceeding sixty days,

but as the number of cases diminished through discharges from hospital the figures for periods after sixty days become less accurate.

2. All observations were made under the conditions of life prevailing in a military hospital in this country. We are, therefore, not in a position to say whether the incidence of relapses would be the same where other conditions may prevail.

3. By the word 'relapse' we mean a parasitic relapse, febrile or non-febrile, i.e., that parasites have reappeared in the blood after a negative period induced by treatment. In *long* treatments, lasting say eight weeks, only those cases can be considered, in which the blood was *negative* on the last day of treatment or on the following day, the condition of the blood during treatment being disregarded; this, of course, does not apply to *short* treatments lasting one or two days, where several days may elapse before the blood becomes negative.

4. All the treatments were quinine treatments.

In the tables:—

Q.O.	= Quinine sulphate or hydrochloride orally.
Q.M.	= Quinine bihydrochloride intramuscularly.
Q.M.alk.	= Quinine alkaloid intramuscularly.
Q.V.	= Quinine bihydrochloride intravenously.
10 or 15 × 1	= Grains 10 or 15 once.
5 × 2	= Grains 5 on each of two consecutive days.
5 × 7	= Grains 5 on each of seven consecutive days.
[–]	= In Table V, number of cases discharged without having relapsed.

An examination of the protocols shows that in any treatment the majority of relapses occur during the first thirty days of the observation period.

In Table I the percentage of relapses in each twenty-day period is given, from which it will be seen that in the different treatments the percentages vary from 46·2 to 100 in the first, 0 to 30·8 in the second, 0 to 7·7 in the third, and 0 to 1·5 in the fourth twenty-day period. A careful examination of these variations, and of the variations in the treatments, e.g., (1) dose, (2) mode of treatment, (3) duration of treatment, has failed to reveal any correlation between the two. We conclude, therefore, that the variations in the incidence of relapses are not dependent on differences of treatment, but are due simply to the fact that the numbers in certain of the treatments are

TABLE I.

Showing the percentage of relapses occurring during each 20-day period after various quinine treatments in simple tertian malaria.

Observation period after cessation of treatment. Days	**1** Q.O. 5×2	**2** Q.O. 10×2	**3** Q.O. 15×2	**4** Q.O. 30×2	**5** Q.O. 45×2	**6** Q.O. 60×2	**7** Q.O. 90×2	**8** Q.O. 120×2	**9** Q.O. 5×7 for 8 weeks
1-20	100·0	100·0	93·0	100·0	77·8	85·7	75·2	88·9	88·6
21-40	...	...	7·0	...	22·2	14·3	21·2	11·1	8·6
41-60	...	...	...	...	...	...	3·5	...	2·8
61-80	...	...	...	...	...	...	...	...	...
81-100	...	...	...	...	...	...	...	...	...

Observation period after cessation of treatment. Days	**10** Q.O. 15×7 for 8 weeks	**11** Q.O. 20×7 for 14 weeks	**12** Q.O. 30×7 for 8 weeks	**13** Q.O. 30×7 for 3 weeks, 45×7 for 1 week	**14** Q.O. 45×7 for 3-8 weeks	**15** Q.O. 10×2 for 8 weeks	**16** Q.O. 15×2 for 8 weeks	**17** Q.O. 45×2 for 8 weeks	**18** Q.O. 90×2 for 3 weeks
1-20	92·3	100·0	70·6	88·2	71·4	100·0	80·4	90·6	46·2
21-40	3·8	...	26·4	5·9	28·6	...	13·0	6·2	30·8
41-60	3·8	...	3·0	5·9	...	...	6·6	1·5	7·7
61-80	...	...	...	...	...	...	...	1·5	0·0
81-100	...	...	...	...	...	...	...	...	0·0

Observation period after cessation of treatment. Days	**19** Q.M. 15×2	**20** Q.M. alk. 15-60 ×2	**21** Q.M. and Q·O· 30 for 12 days	**22** Q.V. 10 or 15×1	**23** Q.V. 10 or 15×6	Range
1-20	89·3	71·0	69·2	100·0	100·0	46·2 — 100·0
21-40	10·7	29·0	23·1	...	...	0·0 — 30·8
41-60	...	...	7·7	...	...	0·0 — 7·7
61-80	...	...	...	...	...	0·0 — 1·5
81-100	...	...	...	...	...	0·0 — 0·0

small. It is, therefore, justifiable to consider the time incidence of the relapses (582) as a whole.

The time incidence of relapses can be considered in three ways.

1. *In reference to the relapses themselves, i.e. the percentage of the total relapses which occur during each period of time.*

This is shown in Table II and Graph 1, from which it follows that if in any treatment we know the number of relapses which have occurred in the first twenty days after cessation of treatment, then we can predict the total number of relapses in the second and third twenty days; e.g. if eighty-three relapses have occurred in the first twenty days then only thirteen or fourteen will occur in the second twenty days, and two or three in the third twenty days, and none or one in the fourth twenty days.

2. *In reference to the total cases treated, i.e. the percentage of the total cases treated which relapse during each period of time.*

This is shown in Table III and Graph 2, from which it follows that if in any treatment we know the percentage of cases treated which relapse in the first twenty days after the cessation of treatment, then we can predict the incidence of relapses in the second and third twenty days, e.g., if 60 per cent. of the total cases treated relapse in the first twenty days, then about 10 per cent. of the total cases treated will relapse in the second twenty days, about 2 per cent. of the total cases treated in the third twenty days, and about 0·2 per cent. in the fourth twenty days.

3. *In reference to remainders, i.e. the incidence among the cases treated less those who have previously relapsed.*

This is shown in Table IV and Graph 3, from which it follows that if in any treatment we know the percentage of cases treated which relapse in the first twenty days, then we can predict the percentage of the remainder that will relapse in the second, third and fourth twenty days, e.g., if 60 per cent. relapse in the first twenty days then about 26 per cent. of the remainder will relapse in the second, 7 to 8 per cent. of the remainder in the third, and about 1 per cent. of the remainder in the fourth twenty-day period.

TABLE II.

Showing the percentage of total relapses (582) which occurred during each 20-day period.

Observation period after cessation of treatment. Days	Relapses	Percentage
1-20	484	83·20
21-40	80	13·74
41-60	15	2·58
61-80	1	0·17
81-100	0	0·00
101-120	1	0·17
121-140	1	0·17
Total	582	100·03

TABLE III.

Showing the percentage of cases treated which relapsed during each 20-day period after cessation of treatment.

Observation period after cessation of treatment. Days	Original number of cases treated less those discharged without having relapsed : average for each 20-day period	Relapses	Percentage
1-20	798·7	484	60·6
21-40	786·6	80	10·2
41-60	763·5	15	2·0
61-80	666.2	1	0·2
81-100	605·7	0	0·0

TABLE IV.

Showing what percentage of cases, not having previously relapsed, did so during each 20-day period after cessation of treatment.

Observation period after cessation of treatment. Days	Average number of cases in hospital during each 20-day period, less the relapses during the previous 20-day periods	Relapses	Percentage
1-20	798·7	484	60·6
21-40	302·6	80	26·4
41-60	199·5	15	7·5
61-80	87·2	1	1·1
81-100	25·7	0	0·0

REFERENCE

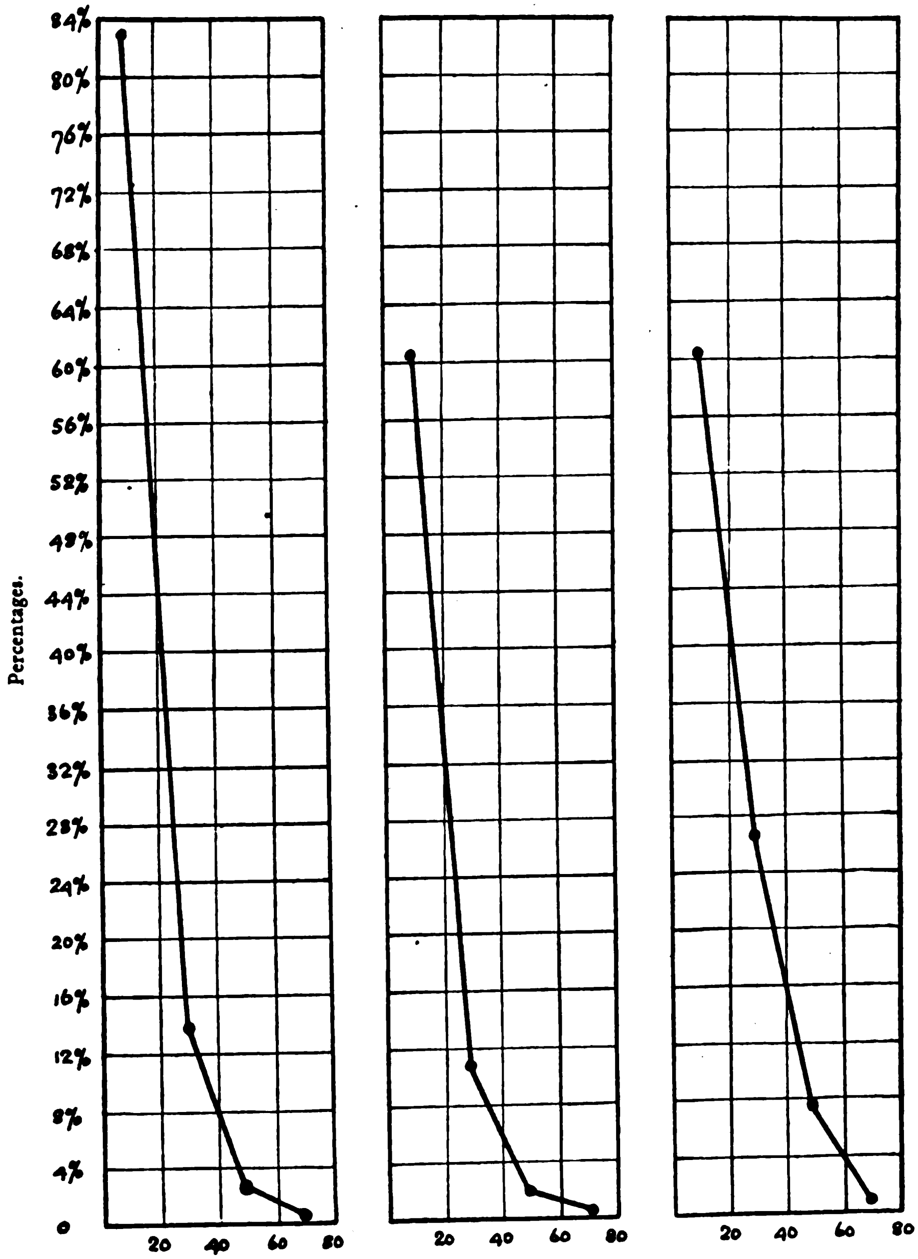
STEPHENS, J. W. W., YORKE, W., BLACKLOCK, B., MACFIE, J. W. S., COOPER, C. F., and CARTER, H. F. (1918). *Ann. Trop. Med. & Parasitol.*, Vol., XI, p. 425.

23 Q.V. 10 or 15 × 6 Cases : 12 Relapses : 11 Cases	 Relapses	Number of cases discharged without having relapsed	Original number of cases treated less those discharged without having relapsed	Average for each 20-day period	Relapses	Average number of cases in hospital during each 20-day period less the number relapsing during the previous 20-day periods	Day
12	0	...	800		10		1
12	0	...	800		5		2
12	0	...	800		12		3
12	0	...	800		8		4
12	0	...	800		9		5
12	0	...	800		8		6
12	1	...	800		11		7
11	1	1	800		20		8
10	4	...	799		35		9
6	3	1	799	798·7	43	798·7	10
3	1	...	798		29		11
2	0	...	798		46		12
2	0	...	798		45		13
2	0	...	798		36		14
2	0	...	798		55		15
2	1	1	798		44		16
1	0	...	797		21		17
1	0	...	797		18		18
1	0	...	797		14		19
1	0	1	797		15 (484)		20
1	0	2	796		16		21
1	0	...	794		8		22
1	0	1	794		7		23
1	0	1	793		5		24
1	0	2	792		9		25

GRAPH 1. Percentage of total relapses in each 20-day period.

GRAPH 2. Percentage of cases treated which relapse in each 20-day period.

GRAPH 3. Percentage of cases treated not having previously relapsed which do so in each 20-day period.



Days after cessation of treatment.

THE PHAGOCYTOSIS OF ERYTHROCYTES BY AN AMOEBA OF THE *LIMAX* TYPE

BY

WARRINGTON YORKE

AND

J. W. S. MACFIE

(*Received for publication* 21 *March*, 1919)

During the past ten or twelve years much has been written on the question of the differentiation of the vegetative stages of *Entamoeba histolytica* and *Entamoeba coli*. One of the main characters which has been held to be of use for this purpose concerns the ability of the parasites to ingest red corpuscles, but even on this point there is considerable difference of opinion.

Amongst the rules laid down by Wenyon and O'Connor (1917) as a guide to the diagnosis of amoebae in the stools is the following:—'If amoebae containing red blood corpuscles are present in a stool, whether evidently dysenteric or not, they are *E. histolytica*, and mean that some active dysenteric process is going on.' On the other hand James (1914), discussing this question, states that occasionally he has found one or two red cells in *E. coli*. In order to test the powers of *E. coli* to ingest red blood corpuscles, Wenyon and O'Connor mixed a portion of perfectly fresh stool, containing free *E. coli* in large numbers, with a quantity of finger blood; the mixture was placed at once into the incubator and was examined from time to time. Although the amoebae were seen to be moving freely amongst the blood cells, none of the latter were ingested. From two experiments of this nature Wenyon and O'Connor conclude that *E. coli* does not readily ingest red blood cells under the conditions of the experiment. The authors also state that they failed to induce *E. histolytica* to take up red cells under similar conditions. From these observations they infer 'that if amoebae are found with phagocyted red blood corpuscles they are certainly *E. histolytica*, and are taking part in some active dysenteric process.'

The very fact that in the above experiments *E. histolytica* failed to ingest red cells suggests to our minds that the conditions of experiment were such that no definite conclusion can be drawn regarding the capacity of *E. coli* to phagocytose erythrocytes. The following observations made by us are of interest in so far as they prove that the capacity to ingest red blood corpuscles is not peculiar to *E. histolytica*. Our experiments were conducted with an amoeba of the *Limax* type obtained from the faeces of a native at Freetown, Sierra Leone, in 1914, and subsequently maintained in the Liverpool laboratory by cultivation on Musgrave and Clegg's medium. It was found that if a small portion of a fresh culture containing abundant actively motile amoebae were mixed with a little blood and placed in the incubator, phagocytosis of the red corpuscles did not take place. When, however, sub-cultures of the amoebae were made on Musgrave and Clegg's medium containing about one-third of its volume of blood, it was found that, after a few hours' incubation, large numbers of the amoebae contained red cells, sometimes as many as twenty in a single amoeba.

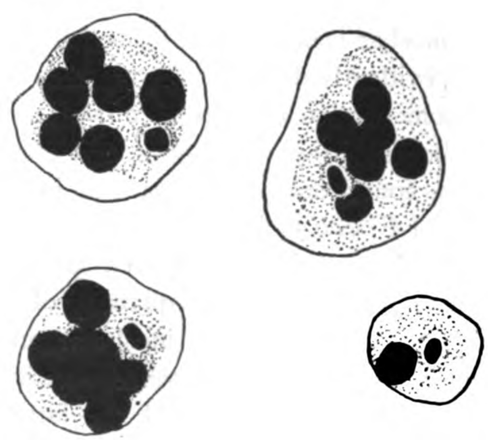


FIG. Showing phagocytosis of erythrocytes by an Amoeba of the *Limax* type. × 1000.

These two experiments show that although no phagocytosis of erythrocytes is obtained by merely incubating a mixture consisting of a scraping of an amoeba culture and blood, yet under conditions more favourable for the growth and metabolism of the amoeba active phagocytosis does occur. Wenyon and O'Connor have themselves suggested a similar explanation to account for their failure to induce *E. histolytica* to ingest red cells in experiments outside the body, and conclude that the amoebae found in the stool to contain red cells 'must be amoebae which have escaped from some definite active lesion of the gut, where they have been living as tissue parasites.' It appears to us that there is no reason to doubt that if *E. coli* happens to encounter blood when in a state of full activity, phagocytosis of erythrocytes may occur. In this connection it may be recalled that many observers have drawn attention to the fact that *E. coli* can ingest cysts of *E. histolytica* and intestinal flagellates. If this be so, it is difficult to believe that they are incapable of ingesting erythrocytes.

REFERENCES

James, W. (1914). *Ann. Trop. Med. and Parasitol.*, Vol. VIII., No. 2, p. 133.
Wenyon and O'Connor (1917). *Journal Roy. Army Med. Corps*, Vol. XXVIII., No. 2. p. 151.

STRONGYLIDAE IN HORSES

VIII. SPECIES FOUND IN AMERICAN HORSES

BY

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AND

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(*Received for publication 7 June*, 1919)

PLATES IV-VI

In the course of this investigation parasitic worms were examined from fourteen horses which had recently come from the United States of America and had died shortly after their arrival in this country, and from one foal (No. 14) which, although bred in this country, had been kept in a field infected by the American horses. We were ourselves present at the post-mortem examinations of five of the animals, and from the others material of one sort or another was sent to us by Col. Brittlebank, A.V.S., C.M.G., to whom we wish once more to express our thanks for the facilities he so kindly afforded us in our investigations and for the materials with which he was good enough to supply us.

We have also examined a small collection of materials from mules in the Argentine, and some parasitic worms from two U.S.A. horses which had died in France.

In seven previous papers (1918-19) we have described in detail a number of the worms, particularly those new to science; and in the present article we propose to give a list of all the *Strongylidae* found and to discuss briefly the pathological findings.

I. The Species of *Strongylidae* identified.

In Table I a list is given of the species of *Strongylidae* found in the American horses (including the foal); the Table shows also what species were found in each animal examined. It is quite likely that the horses harboured additional species because the materials obtained were sometimes small in amount or of a selected kind (e.g. aneurysms), and it has not yet been possible to examine completely the very large number of specimens collected on other occasions. In dealing with members of this family, it would be necessary to examine with a microscope every single worm collected in order to be certain that no species had been overlooked.

TABLE I.

Species of *Strongylidae* found in fifteen U.S.A. horses examined in England.

Species	U.S.A. horses examined in England														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Strongylus															
S. edentatus	+	...	+	...	+	...	...	+	...	...	...	...	+	+	...
S. equinus	...	+	+	+	+	+	+	+	+	+	...	+	+	...	+
S. vulgaris	...	...	+	...	...	+	...	+	+	+	+	...	+	+	+
Triodontophorus															
T. intermedius	...	...	...	+	+	...	...	...	...	...	...	...	...	+	...
T. brevicauda	...	...	...	...	...	...	...	...	...	...	...	...	...	+	...
T. tenuicollis	...	...	...	...	...	...	...	...	...	...	...	...	...	+	...
Gyalocephalus															
G. capitatus	...	...	+	...	+	+	...	...	...	...	...	...	...	+	...
G. equi	...	...	...	...	...	+	...	...	...	...	...	...	...	+	...
Cylicostomum															
C. bicoronatum	...	...	...	...	...	...	...	...	...	...	...	...	...	+	...
C. calicatum	+	...	...	...	...	...	...	...	+	+	...	+	+	...	+
C. coronatum	+	...	+	+	...	...	...	...	+	+	...	...	+	...	+
C. insigne	...	...	...	...	+	...	...	...	...	...	...	...	...	+	...
C. labiatum	...	...	+	...	+	...	...	...	...	...	...	...	...	...	...
C. labratum...	...	...	+	...	+	...	...	...	...	...	...	...	...	...	...
C. longibursatum	+	+	+	...	+	...	...	...	...	...	...	...	...	...	+
C. minutum	...	...	...	...	+	+	...	...	...	...	...	+	...	...	...
C. nassatum var. parvum ...	...	...	+	+	+	+	...	...	...	...	...	+	...	...	...
C. pateratum	...	...	...	+	+	...	...	...	...	...	...	...	...	...	...
C. poculatum	...	...	...	...	...	...	...	...	...	...	...	...	...	+	...
C. pseudo-catinatum ...	...	...	+	+	+	+	...	...	...	...	...	+	...	+	...

All the *Strongylidae* found by us belonged to the sub-family *Strongylinae*, that is *Strongylidae* having a chitinous buccal capsule.

For purposes of comparison, we give in Table II a complete list of all the *Strongylinae* in horses, donkeys and mules recorded from different parts of the world.

TABLE II.

Species of *Strongylinae* found in horses and donkeys in various parts of the world.

Species	Egypt (Looss)	Canada (Ransom)	England (Leiper Boulenger)	U.S.A. horses in England (Yorke and Macfie)
Strongylus				
S. edentatus, Müller, 1784	+	+	+	+
S. equinus, (Looss), 1900	+	+	+	+
S. vulgaris, (Looss), 1900	+	+	+	+
Triodontophorus				
T. brevicauda, Boulenger, 1916	...	+	+	+
T. intermedius, Sweet, 1908	...	+	+	+
T. minor, Looss, 1900	+	...	...	...
T. serratus, Looss, 1900	+	...	+	...
T. tenuicollis, Boulenger, 1916	...	+	+	+
Gyalocephalus				
G. capitatus, Looss, 1900	+	+	+	+
G. equi, Yorke and Macfie, 1918	...	...	...	+
Cylicostomum				
C. alveatum, Looss, 1900	+	...	...	...
C. auriculatum, Looss, 1900	+	...	...	...
C. bicoronatum, Looss, 1900	+	+	+	+
C. calicatum, Looss, 1900	+	+	+	+
C. catinatum, Looss, 1900	+	+	...	...
C. coronatum, Looss, 1900	+	+	+	+
C. elongatum, Looss, 1900...	+	+	...	...
C. euproctus, Boulenger, 1917	...	...	+	...
C. goldi, Boulenger, 1917	...	+	+	...
C. insigne, Boulenger, 1917	...	+	+	+
C. labiatum, Looss, 1901	+	+	...	+
C. labratum, Looss, 1900	+	+	...	+
C. longibursatum, Yorke and Macfie, 1918 ...	...	...	...	+
C. mettami, Leiper, 1913	...	...	+	...
C. minutum, Yorke and Macfie, 1918	...	...	...	+
C. nassatum, Looss, 1900	+	+	+	...
C. nassatum var. parvum, Yorke and Macfie, 1918	+	...	...	+
C. pateratum, Yorke and Macfie, 1919	...	...	...	+
C. poculatum, Looss, 1900	+	+	+	+
C. pseudo-catinatum, Yorke and Macfie, 1919 ...	...	...	...	+
C. radiatum, Looss, 1900	+	+	...	...
C. tetracanthum, (Mehlis), 1831	+	...	...	...
Oesophagodontus				
O. robustus, (Giles), 1892	...	+	+	...

In the materials from Argentine mules *Strongylus edentatus*, *S. equinus*, *S. vulgaris* and *Cylicostomum insigne* were found. The parasites from two horses which had died in France were in the one case *Cylicostomum insigne*, and in the other *Strongylus edentatus*, *S. equinus*, *S. vulgaris*, *Gyalocephalus capitatus* and *Cylicostomum longibursatum*.

PATHOLOGY

Intestinal Wall. The caecum in a number of the infected animals exhibited marked pathological changes. Areas of the caecal wall were greatly thickened and intensely engorged with blood. The mucous membrane was studded with small circular lesions, the points of attachment of worms belonging to the GENUS *Strongylus* (fig. 1). A greater or smaller number of nodules, varying in size from that of a pea to less than that of a pin's head, were scattered throughout the mucous membrane of the caecum and upper portion of the colon (fig. 2). Some of the nodules were calcified; on opening others with a needle they were found to be cysts lying in the mucous membrane, or sub-mucous tissue, and containing worms which varied in size with the dimensions of the cysts (fig. 3). Sections through the affected areas showed congestion of the blood vessels, great thickening of the gut wall, the presence of worms lying coiled up in the cysts in the mucous membrane (fig. 4) and sub-mucous tissue (fig. 5), and finally a general and marked endarteritis (fig. 6).

A considerable number of worms removed from these cysts were examined. Without exception they proved to be larvae of the Genus *Cylicostomum*. This was ascertained to be the case because we were fortunate enough to find a few of the larvae undergoing their final moult, and were thus enabled to distinguish the characters of the mouth parts of both the last larval stage and the young adult. The larvae found in the different cysts varied greatly in size, some were small (4 mm.) whilst others were much larger (11·5 mm.). As practically all the specimens examined by us were in the same stage of development—the final stage with provisional buccal capsule—it is clear that the variation in size was not due to age, but to the fact that the larvae represented different species.

It is interesting to note that larvae similar to those removed from the cysts were also found free in the lumen of the caecum and colon.

A few exceptionally large cysts were found in the duodenum and colon of one animal (Foal 14). On opening these they were found to contain blood-stained pus-like matter, and larvae, or young adults, of *Strongylus edentatus* and *S. vulgaris*.

Aneurysms of the Mesenteric Artery. Besides the general endarteritis observed in sections of the gut wall, aneurysms varying considerably in size, were found in the mesenteric artery and its branches in many of the horses. In every aneurysm examined by us, worms were found embedded in the fibrinous clot (figs. 7 and 8). These on microscopic examination proved to be larvae of the GENUS *Strongylus*. A considerable number of these larvae were measured, they varied in length from 10 mm. to 16 mm. Many were found undergoing their final moult, and we were thus enabled to determine the character of the mouth parts of the last larval stage and those of the young adult in the same individual. The mouth capsule of these moulting specimens was invariably that of *Strongylus vulgaris*. Judging from the appearance of the provisional buccal capsule, and from the slight variations in size, we are inclined to believe that the larvae of this species of *Strongylus* only were present in the aneurysms.

Peri-renal Cysts. In the peri-renal tissue of one animal (Foal 14), a large number of cysts were found. The capsules of the kidneys were adherent, and in the cortex of the organs themselves there were some caseous cysts. Embedded in the fatty tissue surrounding the kidneys were a large number of worms, each coiled up in a cyst which contained also pus and sometimes a little discoloured blood. The worms were of various sizes, the largest being unmistakably immature adults of *Strongylus edentatus*. The mouth parts of the smaller individuals were all alike, and as one or two were moulting and showed both the larval and the adult forms of the buccal capsule, it was possible to identify the other larvae as those of *S. edentatus*.

Lungs. We were informed that at the autopsies performed on many of the animals which had succumbed to sclerostomiasis, lesions were found in the lungs. A portion of the 'anterior' lobe of the lung of one of these animals was examined by us. The pleura was greatly thickened and gelatinous, and the lung tissue itself was solid in places. Sections showed a greatly thickened pleura and a pneumonic condition of the lung. Bacteriological examination revealed nothing definite beyond the presence of a staphylococcus and a bacillus of the *B. coli* group.

No larvae were found in the specimens examined, and consequently we are unable to associate this pneumonic condition directly with the *Strongylidae* infections.

PATHOGENICITY

Summing up our observations, we may say, as regards the GENUS *Cylicostomum*, that the adults (sexually mature) inhabit the colon and caecum, and that the larval stages are found both encysted in the mucous membrane and sub-mucous tissue of the same parts of the intestine and also actually free in the lumen of the gut.

As regards the GENUS *Strongylus*, the adults (sexually mature) inhabit the colon and caecum, attaching themselves firmly to the wall of the intestine. The larvae of *S. vulgaris* were found in aneurysms of the mesenteric artery in large numbers, and occasionally in cysts of the colon and duodenum. The larvae of *S. edentatus* were found on one occasion in the peri-renal tissue and in cysts of the colon and duodenum. The larvae of *S. equinus* were not identified. Many of the worms in the aneurysms and peri-renal cysts had reached the adult form, and it seems incredible that they could migrate to the gut from these situations in this stage of their development; they are, therefore, to be regarded as side-tracked.

There can be no doubt that the worms, taken as a whole, produce serious changes in the intestinal wall and mesenteric arteries which finally result in death. When one attempts to assess the importance of the rôle played by the individual genera, one is faced with considerable difficulty owing to the rareness with which a pure infection with a single genus is encountered. So far as our observations go, we can state that the adult *Strongylus* produces lesions in the intestinal wall at the points where it attaches itself, and, furthermore, that the larvae of one species, *S. vulgaris*, causes grave lesions in the mesenteric artery and its branches. Probably members of the GENUS *Strongylus* have by far the greatest pathological significance and are mainly responsible for the changes in the gut wall mentioned above, and for the symptoms (emaciation, weakness, anemia, oedema, etc.) exhibited.

Regarding the *Cylicostomes*, there can be no doubt that when they are sufficiently numerous the encystment of their larvae in the gut wall is of serious moment.

REFERENCE

Yorke, W., and Macfie, J. W. S. (1918-19). *Ann. Trop. Med. & Parasitol.*, Vols. XI and XII.

EXPLANATION OF PLATES

PLATE IV

Fig. 1. Photograph of caecum of horse, showing worms of the GENUS *Strongylus* attached to the gut wall.

Fig. 2. Photograph of caecum of horse, showing verminous cysts.

Fig. 1

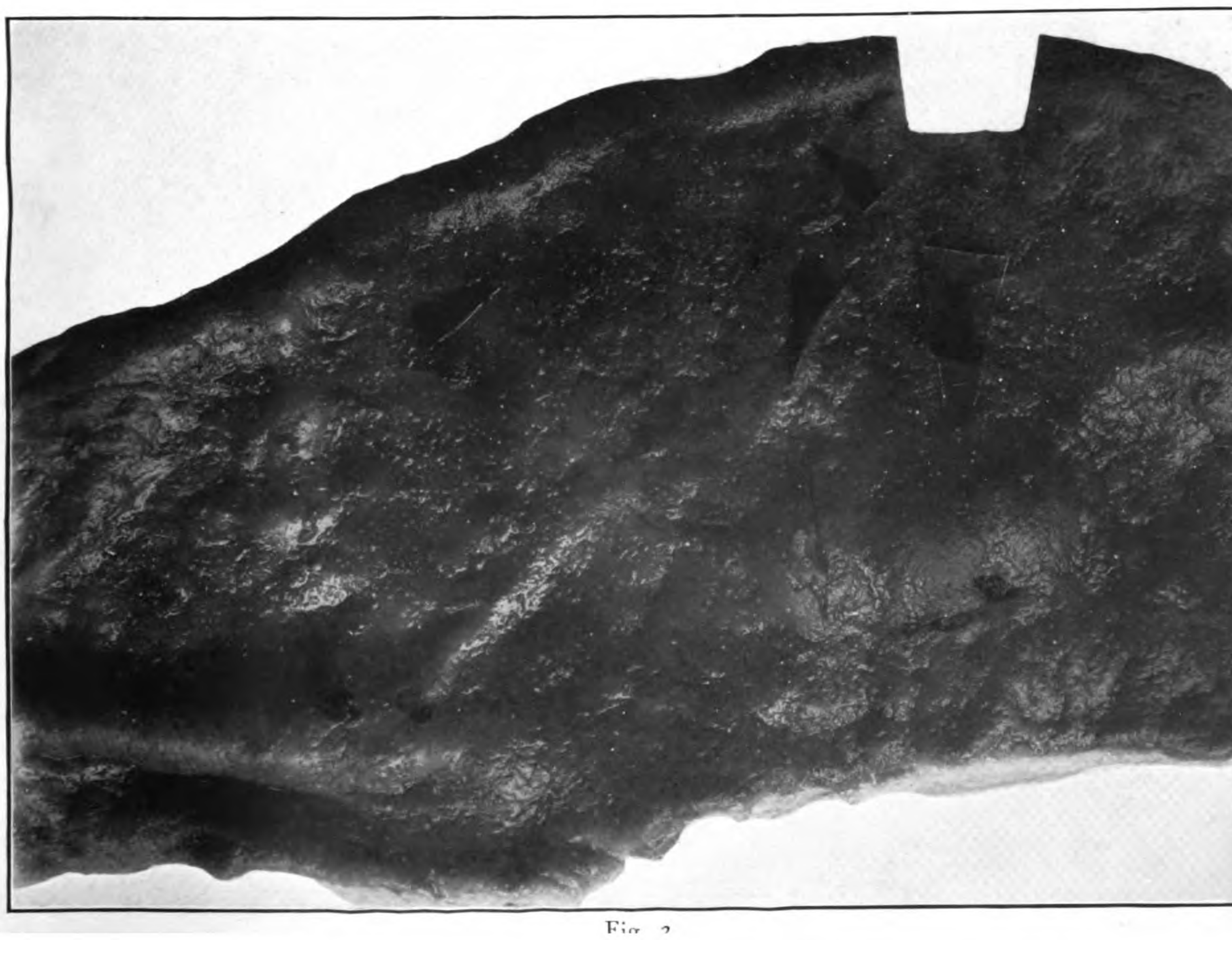

Fig. 2

PLATE V

Fig. 3. Micro-photograph of final larval stage of a cylicostome lying encysted in mucous membrane of colon of horse. × 16.

Fig. 4. Microphotograph of section through caecum showing cylicostome larva encysted in mucous membrane. × 16.

Fig. 5. Microphotograph of section through caecum showing cylicostome larva encysted in sub-mucous tissue. × 16.

Fig. 6. Microphotograph of section through caecum showing cylicostome larva encysted in the mucous membranc and thickening of arterial wall. × 16.

Fig. 3

Fig. 4

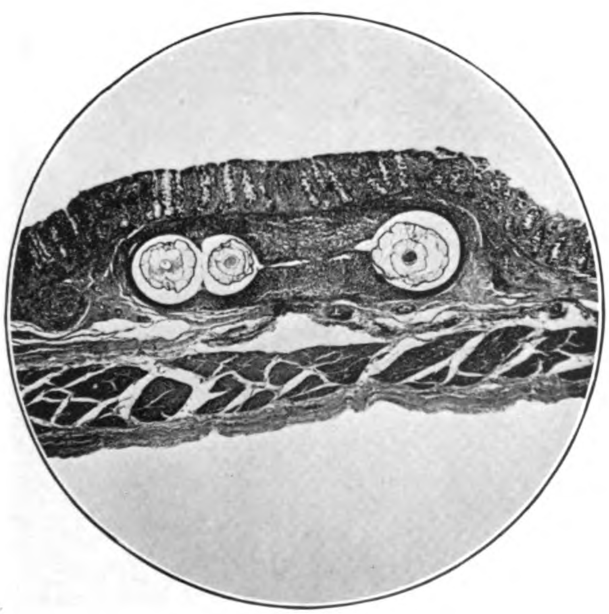

Fig. 5

Fig. 6

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PLATE VI

Fig. 7. Photograph of mesenteric aneurism of horse showing larvae of *Strongylus vulgaris*.

Fig. 8. Photograph of mesenteric aneurism of horse showing larvae of *Strongylus vulgaris*.

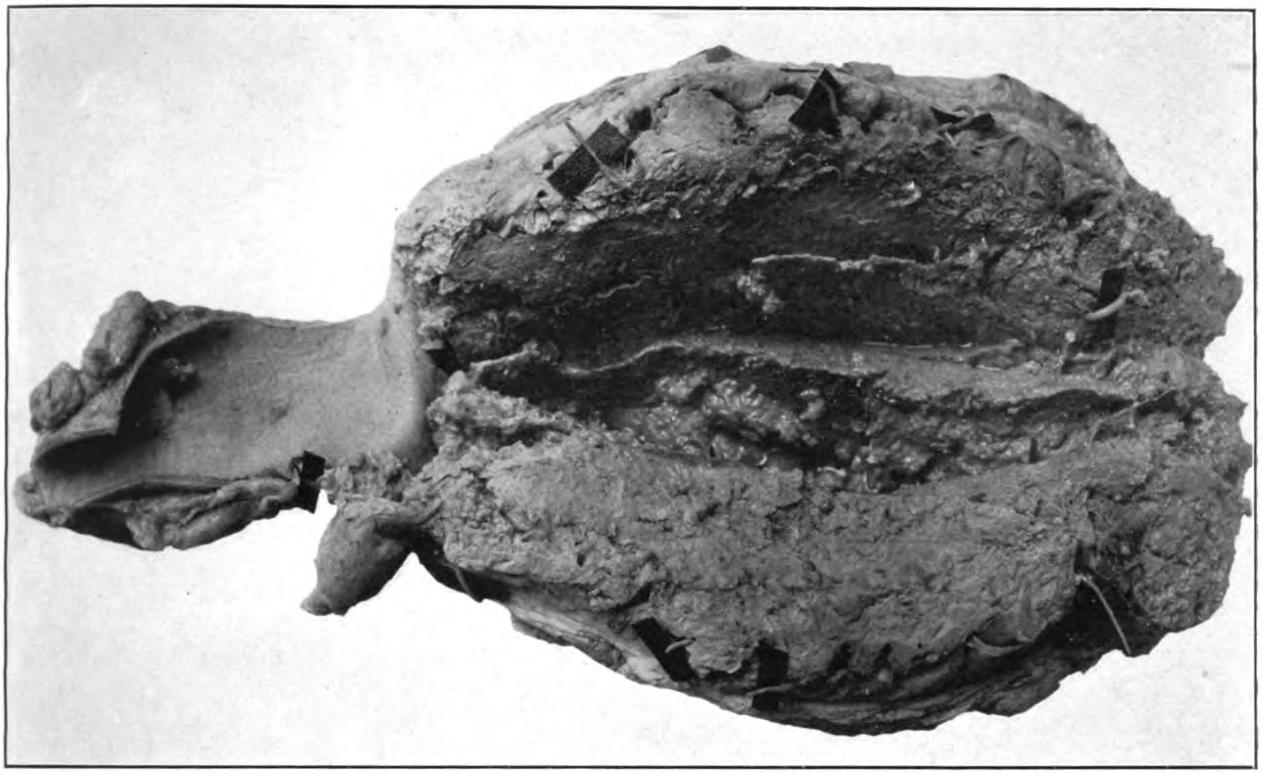

Fig. 7

Fig. 8

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ON THE DISTRIBUTION AND DESTRUCTION OF QUININE IN ANIMAL TISSUES

BY

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(A Report to the Medical Research Committee)

(Received for publication June 20, 1919)

INTRODUCTION

In a recent paper by Ramsden, Lipkin, and Whitley (1918), it was shown that 'Quinine introduced into an animal in large doses accumulates in most of the tissues at very much higher concentrations than in the blood,' and also that 'the liver of rabbits, guinea-pigs, oxen and sheep rapidly attacks quinine post mortem.'

It was shown also (p. 256) that even a concentration of 16·6 mgm. of quinine per litre, of blood, a concentration so high as to be almost intolerable to the patient, and which had been maintained at this height for at least thirty-three hours, nevertheless failed to effect a radical cure in a case of malaria.

The experiments recorded in the present paper continue the investigation, and are concerned mainly with

1. The distribution of ingested quinine in animal tissues.
2. The power of various tissues to destroy quinine.
3. The nature of the quinine-destroying agent and the conditions favouring its activity.
4. The substances resulting from such destruction of quinine.

DISTRIBUTION OF INGESTED QUININE

The methods used for the extraction of the quinine from tissues are those described by Ramsden and Lipkin in previous papers (1918*a*, 1918*b*).

1. *The Ammonium Sulphate Method*, serviceable for most tissues, e.g., spleen, kidney, suprarenal, muscle, bone marrow, testis.

I have now tested this method with salivary and thyroid glands and intestinal wall, and find it equally reliable.

Tissue	Animal	Weight of tissue grms.	Quinine given mgms.	Quinine found mgm.
Thyroid	Sheep	10	0·5	0·5
(Submaxillary) Salivary gland	"	7·5	0·5	0·5

NOTE.—(1) In the estimation of quinine in the walls of the large and small intestine it was found desirable to treat the ammonium sulphate filtrate with 5% of its volume of 25% lead acetate solution and filter before extraction with ether, thereby removing certain impurities which themselves give Tanret turbidity; (2) Losses of less than 5% are to be regarded as within the range of experimental error.

About 5 grms. of the tissue is transferred direct from the animal into a weighed flask containing about 10 c.c. of a saturated aqueous solution of $(NH_4)_2SO_4$ + 0·6 per cent. H_2SO_4. The flask is re-weighed, then boiled for two minutes, and its contents filtered under pressure through a Gooch crucible or a Buchner filter.

The residue is pulped in a mortar with glass-powder and again boiled up in the original flask with successive lots of acidulated $(NH_4)_2SO_4$ solution. The combined filtrates are shaken with ether to extract 'oily' matter, then alkalised strongly with ammonia and again shaken up with ether to extract the quinine. Evaporate each lot of ether as it separates in one of the tubes guaged for nephelometry. Dissolve the residual quinine by boiling it with a sufficiency of saturated ammonium sulphate and estimate nephelometrically against quinine standards.

2. *The alcohol extraction method for brain and fat.*

Weigh out about 10 grammes of tissue, grind up to a fine pulp with powdered glass, and transfer it to a flask with the aid of boiling absolute alcohol.

Boil and filter into a graduated cylinder. Repeat the boiling and filtering with three further lots of alcohol. Note volume of filtrate, pour it into five times its volume of 1 per cent. H_2SO_4, and shake thoroughly for five minutes. Extract the fats and lipoids by shaking with three successive lots of pure ether. For every 100 c.c. of fat-free liquid add 5 c.c. of 25 per cent. Pb. acetate solution, filter off aliquot portion of the whole into a stoppered cylinder. Saturate with

$(NH_4)_2SO_4$, pipette off the layer of alcohol-ether which separates, and again extract with ether until the extracts are colourless. Alkalise with NH_4OH, extract quinine with four successive lots of ether, evaporating each lot as separated in one of the tubes gauged for nephelometry. Dissolve the residual quinine by boiling it with a sufficiency of known volume of saturated aqueous ammonium sulphate and estimate nephelometrically.

EXPERIMENT I. Guinea-pig, 755 grammes in weight. 0·4 grammes of quinine dissolved in 10 c.c. of water with enough HCl to effect its solution was injected into the peritoneal cavity. The solution was not acid to Congo red, or methyl orange, although acid to litmus, and its osmotic pressure was calculated as about half that of 0·9 per cent. NaCl. The animal died fifteen minutes after the injection. The peritoneal cavity, which was in parts denuded of its epithelial lining, contained 5 c.c. of turbid faintly yellow fluid full of leucocytes and masses of rolled-up epithelial cells. Each organ investigated was rinsed free from peritoneal fluid by salt solution and wiped dry before weighing. Blood was taken from the left ventricle, urine from the bladder. The intestines were congested, but nothing else abnormal was noticeable. There were no notable symptoms during the period between the injection and the death of the animal.

Tissue	Grammes of tissue taken	Mgm. quinine found	Mgm. quinine per 100 grammes of tissue
Suprarenal	0·35	4·23	1210·0
Peritoneal Fluid	2·15	14·6	679·0
Kidney	2·27	1·93	85·0
Spleen	0·75	0·285	38·0
Liver	3·25	1·21	37·5
Pancreas	1·53	0·23	15·0
Muscle	2·30	0·20	9·0
Blood	1·15	0·055	4·73
Brain	2·68	0·065	2·86
Urine	2·3 c.c.	0·06	2·6 per 100 c.c.

Hence in fifteen minutes 91 per cent. of the quinine had been absorbed. The very high quinine content of the suprarenal confirms the previous results of Ramsden, Lipkin and Whitley (1918), and this notwithstanding the probability that, owing to the short interval elapsing before death, quinine accumulation had not reached its maximum.

EXPERIMENT II. Guinea-pig, 463 grammes in weight. 0·4 grammes of quinine dissolved in 2·5 c.c. of water, with enough HCl to effect its solution, was injected into the gluteal muscle. The injected fluid was not acid to Congo red. The animal showed great restlessness after fifteen minutes, and was killed by a blow on the occiput seventy-five minutes after the injection. The site of the injection showed nothing remarkable, and no fluid or exudate could be found there.

Tissue	Grammes of tissue taken	Mgm. quinine found	Mgm. quinine per 100 grammes of tissue
Suprarenal	0·22	0·05	25·25
Thyroid	0·10	0·025	25·0
Spleen	0·3	0·068	22·9
Bone marrow	0·07	0·009	13·0
Small intestine (wall)	0·30	0·037	12·35
Kidney	3·10	0·26	8·3
Brain	2·44	0·14	5·9
Large intestine (wall)	2·02	0·09	4·8
Blood	3·84	0·14	3·7
Muscle	4·9	0·14	2·73
Heart muscle	1·17	0·029	2·5
Liver	1·82	0·04	2·5
Bile	2·4 c.c.	0·006	0·25 per 100 c.c.

Although the suprarenals have again the highest percentage of quinine, their lead is small, and the concentration is very much less than with intraperitoneal injections.

EXPERIMENT III. Buck rabbit, weight 1380 grammes. 1250 mgm. quinine dissolved in 5 c.c. of water with just enough HCl to effect its solution was injected into the gluteal muscle of the leg. The fluid injected was not acid to Congo red. The animal died in convulsions in fifteen minutes.

Tissue	Grammes of tissue taken	Mgm. quinine found	Mgm. quinine per 100 grammes of tissue
Suprarenal	0·21	0·062	29·76
Kidney	4·24	1·25	29·5
Large intestine (wall)	0·32	0·05	17·4
Small intestine (wall)	0·48	0·08	16·66
Spleen	0·32	0·036	11·16
Bone marrow	0·37	0·03	8·3
Muscle	1·33	0·102	7·7
Brain	2·65	0·12	4·54
Liver	2·35	0·09	3·85
Testes	0·78	0·02	3·03
Blood	6·87	0·08	1·17

Urine turbid with phosphates. Quinine, blood and albumen absent.

The short time elapsing before death was probably not long enough to permit of the differences of distribution in the various tissues attaining their maximum.

EXPERIMENT IV. Buck rabbit, weight 1410 grammes. 0·500 mgm. quinine in 1 c.c. of water with enough HCl to effect its solution injected into the gluteal region. The animal died seventy minutes after the injection.

Tissue	Grammes of tissue taken	Mgm. quinine found	Mgm. quinine per 100 grammes of tissue
Suprarenal	0·25	0·07	28·6
Spleen	0·24	0·057	24·0
Kidney	4·07	0·80	20·0
Lung	0·57	0·06	11·16
Fat	0·52	0·058	11·1
Marrow	0·645	0·05	8·6
Muscle	1·20	0·08	7·0
Heart muscle	1·23	0·066	5·4
Large intestine (wall)	1·88	0·086	4·6
Liver	3·35	0·9	2·8
Brain	2·51	0·067	2·7
Small intestine (wall)	1·40	0·037	2·7
Testes	1·36	0·028	2·1
Stomach	3·89	0·047	1·23
Lymph gland	0·68	0·006	0·9
Blood arterial	8·43	0·101	1·2
Blood venous	4·71	0·068	1·46

Venous blood was taken from the iliac vein on the side of the injection; arterial blood from the left ventricle. It is noteworthy that the venous blood coming from the side of injection is richer in quinine than the arterial blood from the heart. The lymph glands examined were from the cervical chain. The site of the injection showed nothing abnormal.

EXPERIMENT V. Guinea-pig, weight 685 grammes. 400 mgm. quinine in 2 c.c. of solution injected into the gluteal muscle. Animal killed sixty minutes later.

Tissue	Grammes of tissue taken	Mgm. quinine found	Mgm. quinine per 100 grammes of tissue
Suprarenal	0·25	0·09	38·0
Spleen	0·63	0·15	24·0
Small intestine (wall)	0·45	0·06	14·6
Bone marrow	0·68	0·08	12·6
Kidney	2·46	0·21	8·8
Large intestine (wall)	0·64	0·03	5·3
Brain	2·54	0·12	4·8
Blood	5·85	0·15	2·6
Fat	1·32	0·03	2·3
Liver	6·44	0·14	2·3
Muscle	2·45	0·056	2·3
Lymph gland	0·64	0·005	0·8

It should be noted that (*a*) no haemolysis was found even in the animals poisoned with quinine, (*b*) quinine rapidly disappeared from the site of injection.

The following table furnishes a conspectus of the accumulation observed in the different tissues:—

	Intramuscular Injections								Intraperitoneal Injection	
Animal	Guinea-pig		Guinea-pig		Buck rabbit		Buck rabbit		Guinea-pig	
Mgms. Q. per 100 grammes body weight	86·4		58·4		35·4		90·6		53	
Time after injection	75 min.		60 min.		70 min.		15 min.		15 min.	
Tissue	Mgm. Q. per 100 grammes of tissue	Tissue Q. / Blood Q.	Mgm. Q. per 100 grammes of tissue	Tissue Q. / Blood Q.	Mgm. Q. per 100 grammes of tissue	Tissue Q. / Blood Q.	Mgm. Q. per 100 grammes of tissue	Tissue Q. / Blood Q.	Mgm. Q. per 100 grammes of tissue	Tissue (/ Blood (
Blood	3·7	—	2·6	—	1·2	—	1·177	—	4·73	—
Suprarenal	25·25	7·0	38·0	14·6	28·6	23·83	29·76	25·8	1210·0	256
Spleen	22·9	6·0	24·0	9·23	24·0	20·0	11·16	9·7	38·0	8
Kidney	8·3	2·3	8·8	3·4	20·0	16·66	29·5	25·5	85·0	18
Liver	2·5	0·7	2·3	0·88	2·8	2·33	3·85	3·3	37·5	8
Muscle	2·73	0·75	2·3	0·88	7·0	5·83	7·7	6·6	9·0	2
Brain	5·9	1·6	4·8	1·85	2·7	2·25	4·54	4·0	3·0	0·6
Large intestine (wall)	4·8	1·3	5·3	2·04	4·6	3·83	17·4	14·9	...	...
Small intestine (wall)	12·35	3·3	14·6	5·6	4·7	3·91	16·6	14·2	...	...
Lymph gland ...	...	...	0·8	0·3	0·9	0·75	...	...	...	...
Bone marrow ...	13·0	3·5	12·6	4·8	8·6	7·16	8·3	7·0	...	...
Heart muscle	2·5	0·7	...	...	5·4	4·5	...	...	...	...
Fat	...	...	2·3	0·8	11·1	9·25	...	...	...	...
Thyroid G.	25·0	6·8	...	...	...	...	...	...	...	...
Testicle	...	...	...	...	2·1	1·75	3·03	2·6	...	...
Lung	...	...	...	...	11·16	9·3	...	...	...	...
Stomach	...	...	...	...	2·3	1·9	...	...	...	...
Peritoneal fluid ...	...	...	...	...	...	...	...	...	14·7	3·0

Partition of quinine between corpuscles and plasma of the blood

In the experiments recorded above, samples of blood were taken up (4 c.c. usually) in a syringe containing 1 c.c. of 1 per cent. potassium oxalate. This was centrifuged at once and the quinine content of the plasma and corpuscles estimated separately.

Rabbit (1) 100 grammes of Plasma contain 2·4 mgm. quinine.
100 grammes of corpuscles contain 1·26 mgm. quinine.

$$\frac{\text{Plasma quinine}}{\text{Corpuscle quinine}} = \frac{2}{1}$$

Rabbit (2) 100 grammes of Plasma contain 0·81 mgm. quinine.
100 grammes of corpuscles contain 0·39 mgm. quinine.

$$\frac{\text{Plasma quinine}}{\text{Corpuscle quinine}} = \frac{2 \cdot 1}{1}$$

Guinea-pig 100 grammes of Plasma contain 1·98 mgm. quinine.
100 grammes of corpuscle contain 0·62 mgm. quinine.

$$\frac{\text{Plasma quinine}}{\text{Corpuscle quinine}} = \frac{3 \cdot 2}{1}$$

The blood from a case of blackwater fever gave the ratio

$$\frac{\text{Plasma quinine}}{\text{Corpuscle quinine}} = \frac{2 \cdot 2}{1}$$

In a previous paper (6), one observation on the blood of a guinea-pig gave a ratio

$$\frac{\text{Serum quinine}}{\text{Corpuscle quinine}} = \frac{3}{1}$$

ACCUMULATION OF QUININE IN EXCISED TISSUES

A. *Guinea-pig's suprarenal gland* cut in two and immersed in 10 grammes of 0·9 per cent. NaCl containing 1 mgm. quinine.

B. *Guinea-pig's suprarenal* cut in two, immersed in 15 grammes of 0·9 per cent. NaCl containing 15 mgm. quinine.

C. 0·96 *gramme of guinea-pig's spleen* immersed in 10 grammes of 0·9 per cent. NaCl containing 1 mgm. of quinine. All were kept for forty-eight hours at 35°C., and in each case the quinine content of the tissue and fluid were estimated separately.

	Grammes of tissue taken	Grammes of medium	Mgms. quinine in 100 grammes of bathing fluid		Mgms. quinine per 100 grammes tissue	Quinine concentration in tissue / Quinine concentration in Medium
			Initially	Finally		
A.	0·21	10	10	2·5	307·1	123·0
B.	0·28	15	100	18·0	2677·4	154·3
C.	0·96	10	10	2·8	77·0	22·5

It is seen that the excised tissues, and especially the suprarenal gland, under conditions where their vital processes must be reduced to a low ebb, accumulate quinine from the environment at enormous relative concentration just as they do during life, a fact which indicates that the accumulation is dependent on some specific chemical or physical affinity between quinine and one or other of the cell constituents—it is tempting to associate it with the lipoids of the cortex. To get further light on the question, attempts were made to find out whether the accumulation was mainly in the cortex or in the medulla. Glands containing large amounts of quinine, ranging from 250 to 385 mgm. per 100 grammes of tissue in the corresponding glands of the other side, were incised and immersed in Christensen's Herapathite reagent for eighteen hours at laboratory temperature. After freezing and section, neither crystalline nor (black) amorphous Herapathite could be seen in either cortex or medulla, notwithstanding the large amount of quinine present.

DESTRUCTION OF QUININE IN TISSUES

The material employed for most of these experiments was obtained in the animal experiments already described. Each tissue investigated was divided symmetrically into two approximately equal portions; the quinine of one portion was estimated at once, and that of the other estimated after incubation in 0·8 per cent. sodium fluoride solution at 35°C. As the initial concentrations of quinine differ considerably in the various tissues, the results are not strictly comparable *inter se*, but indicate merely whether quinine is destroyed or not.

	Tissue examined	Grammes tissue taken	Quinine in mgm. per 100 grammes of tissue estimated at once	Quinine in mgm. per 100 grammes of tissue after incubation	Hours of incubation	% Destruction
Expt. I.— Guinea-pig	Muscle	4·75	2·73	2·00	18	26·7
	S. Intestine	0·32	12·3	4·1	18	66·76
	Blood	3·84	3·7	3·6	18	2·7
	Spleen	0·3	22·9	22·2	18	3·0
Expt. III.— Rabbit	L. Intestine	2·71	17·4	3·0	21	83·0
	S. Intestine	0·9	16·6	2·7	21	83·6
Expt. IV.— Rabbit	Kidney	3·98	20·0	9·0	21	55·0
	Spleen	0·14	24·0	23·81	24	0·8
	Testis	1·30	2·1	2·0	26	5·0
	L. Intestine	2·53	4·6	0·0	24	100·0
	S. Intestine	2·23	2·7	2·2	24	18·5
	Muscle	1·20	7·0	4·0	24	43·0
	Marrow	0·65	8·6	8·32	24	3·3
Guinea-pig	Kidney	2·45	85·0	80·0	18¾	6·0
	Suprarenal	0·32	1210·0	1200·0	18½	0·8
	Muscle	3·49	9·0	6·2	17¾	31·0
	Peritoneal F.	2·41	14·7	14·7	16¾	0·0
	Pancreas	1·53	15·0	11·0	18½	37·0
	Thyroid	10·0	1 mgm.	1 mgm.	24	0·0
	Salivary G.	7·5	1	1	24	0·0

In the following estimations, the tissues examined were pulped in a mortar and added to a solution of quinine in 0·8 per cent. NaF and incubated for varying periods at 35° C. All were fresh, but taken from various guinea-pigs.

Tissue	Grammes tissue taken	Quinine added mgm.	Found	Incubation period hours	Loss %
Lymph gland	6·3	1	0·95	63	5·0
,, ...	3·6	1	0·91	17	9·0
,, ...	5·0	1	1·0	23	0·0
Kidney	4·7	1	0·93	21	7·0
,, ...	3·6	1	0·83	21	16·6
Suprarenal	2·5	1	0·95	63	5·0
,, ...	0·76	1	0·95	23	5·0
Bone marrow	0·84	1	0·97	21	3·0
,, ...	0·69	1	0·96	21	4·0
Spleen	2·7	1	0·95	21	5·0

MAIN CONCLUSIONS CONCERNING THE ACCUMULATION AND DESTRUCTION OF QUININE IN TISSUES

Tissue	Relative accumulating power	Relative destructive power
Liver	(++)	+++++
Spleen	+++	nil
Bone marrow	++	nil
Kidney	(+++)	++
Suprarenal	+++++	nil
Muscle	++	++
Intestinal wall	(++)	++++
Pancreas	(++)	++
Lymph gland	nil	nil or slight

In column 2, tentative conclusions regarding tissues with execretory or destroying powers are indicated by brackets.

PERFUSION EXPERIMENT

A liver from a young sheep, freshly killed, was perfused for two hours at 37° C. with 2 litres of oxygenated Locke's solution containing 200 mgm. of quinine hydrochloride not acid to Congo red. After perfusion, the liver was gently squeezed. The total quantity of fluid collected was 2024 c.c.

Weight of liver before perfusion ... 978·6 grm.
„ „ after „ ... 952·4 „

A portion of the liver was estimated for its quinine content—the total calculated for the whole liver was 18·68 mgm. quinine.

As the total perfusion fluid finally contained 36·8 mgm. of quinine, 72·26 per cent. of the quinine had been destroyed in two hours. The final concentrations of quinine in the liver and perfusion fluid, weight for weight, were approximately equal.

A weighed portion of the liver was pulped and incubated in 0·8 per cent. NaF at 35° C. for forty-five hours. The whole of its quinine was then found to have disappeared. In the perfusion fluid similarly incubated only 8·8 per cent. of its quinine had disappeared. During the perfusion 978·6 grammes of liver destroyed 144·52 mgm. of quinine, i.e. 1 gramme of liver destroyed 0·148 mgm. of quinine (cf. Experiment A of last paper, p. 231, where 0·4 mgm. quinine was destroyed by 1 gramme of guinea-pig's liver post-mortem).

At this rate 1·7 grammes (27 grains) of quinine would have been destroyed in twenty-four hours.

OBSERVATIONS ON THE QUININE DESTROYING AGENT FOUND IN LIVER

Thermolability

10 per cent. emulsions of guinea-pig liver and ox liver in 0·9 per cent. NaCl. Boiled for three minutes on a water bath. Quinine added and the mixture allowed to stand for twenty hours at 37° C.

Tissue	Animal	Mgm. quinine added	Found	Loss %
Liver	Sheep	0·2	1·193	3·35
„	Guinea-pig ...	1·0	0·93	7·0
„	Ox	1·0	0·95	5·0
„	Guinea-pig ...	1·0	0·901	9·1

The experiments were repeated with unboiled liver extracts.

Animal	Mgm. quinine added	Found	Loss %
Sheep	0·2	0·14	30
Guinea-pig	1·0	0·47	53
Ox	1·0	0·74	26
Guinea-pig	1·0	0·58	41

It is evident that the active agent is thermolabile.

Influence of the Reaction of the Substrate

The experiments recorded below show that the enzyme acts best in neutral solution, and that alkalinity is more inhibitory than acidity.

To three flasks A, B and C, each containing 10 c.c. of a 10 per cent. emulsion of fresh guinea-pig's liver in a 0·9 per cent. NaCl and 1 mgm. of quinine, was added:—

(*a*) 10 c.c. of 0·9 % NaCl.
(*b*) 10 c.c. of 0·4 HCl.
(*c*) 10 c.c. of 0·9 % Na_2CO_3.

All were kept at 35° C. for twenty hours and the quinine then estimated.

	Mgm. Quinine added	Found	Loss %
A. Neutral	1	0·71	28·6
B. Acid	1	0·83	16·7
C. Alkaline	1	0·91	9·1

The experiment was repeated with ox liver:—

	Mgm. Quinine added	Found	Loss %
A. Neutral	1	0·62	37·5
B. Acid	1	0·78	21·4
C. Alkaline	1	0·89	10·7

Repeated with dried ox liver:—

	Mgm. Quinine added	Found	Loss %
A. Neutral	1	0·77	23·0
B. Acid	1	0·87	13·0
C. Alkaline	1	0·91	9·1

Repeated with guinea-pig liver:—

A. 10 c.c. of 10 % extract + 1 mgm. Q. + 10 c.c. 9 % NaCl.
B. 10 c.c. of 10 % extract + 1 mgm. Q. + 10 c.c. $\frac{N}{10}$ H_2SO_4.
C. 10 c.c. of 10 % extract + 1 mgm. Q. + 10 c.c. $\frac{N}{10}$ NaOH.

Reaction	Mgm. Quinine added	Found	Loss %
A. Neutral	1	0·62	37·5
B. Acid	1	0·74	26·7
C. Alkaline	1	0·83	16·7

The Influence of Oxygen

There was some indication in the paper by Ramsden, Lipkin and Whitley (1918) (p. 231), that a free supply of air promoted the destruction of quinine by liver pulp.

The following experiments show that oxygen is absolutely essential. 10 c.c. of 50 per cent. fresh liver extract in 0·8 per cent. NaF solution was incubated for twenty hours at 35° C. In Case A, a current of air was gently drawn over the mixture without mechanical disturbance of the surface; in Case B, no air was drawn over the mixture:—

	Mgm. Quinine added	Found	Loss %
A.	1	0·43	57·0
B.	1	0·62	38·0
This was repeated.			
A.	1	0·40	60·0
B.	1	0·61	39·0

A. 20 c.c. of fresh guinea-pig's liver extract in 0·9 per cent. NaCl + 1 mgm. quinine in solution mixed, and a current of air drawn over the mixture at laboratory temperature for twenty-one hours.

B. 20 c.c. of the same liver extract placed in a flask and 1 mgm. of quinine in solution in a tube inside the flask. The flask was exhausted and filled with hydrogen. The quinine was then tilted into the liver extract and the sealed flask allowed to stand for twenty-one hours.

	Mgm. Quinine added	Found	Loss %
A.	1	0·4	60·0
B.	1	0·83	16·7

As complete removal of oxygen was uncertain, the experiment was repeated with extra precautions.

A. 10 c.c. of a 0·50 per cent. extract of guinea-pig's liver in 0·8 per cent. + 5 drops of ammonium bisulphide solution + 1 mgm. quinine in solution. The flask was exhausted and then filled with oxygen and sealed.

B. 10 c.c. of 0·8 per cent. NaF solution + 5 drops of strong ammonium bisulphide solution + 1 mgm. of quinine in solution.

C. 10 c.c. of same liver extract + 5 drops of strong ammonium sulphide solution, 1 mgm. quinine in solution placed in a small test-tube inside the flask. The flask was exhausted and then filled with hydrogen freed from all traces of oxygen by passage through a red hot copper tube. This was repeated three times before the final sealing.

The quinine solution was then tipped into the liver extract. All the flasks were allowed to stand for twenty hours at laboratory temperature, and were heated to 100° C. for two minutes before they were opened.

	Mgm. Quinine added	Found	Loss %
A.	1	0·23	77·0
B.	1	0·97	3·0
C.	1	0·93	6·2

The losses in B and C have no significance, as they are attributable to experimental error.

Influence of Hydrogen Peroxide on the Ferment

A. 10 c.c. of fresh extract of guinea-pig's liver in 9 per cent. NaCl + 10 c.c. of distilled water and 1 mgm. quinine.

B. 10 c.c. of same liver extract + 10 c.c. of neutral H_2O_2 + 1 mgm. quinine.

C. 10 c.c. of same liver extract boiled and cooled + 10 c.c. of carefully neutralised H_2O_2 + 1 mgm. quinine.

	Mgm. Quinine added	Found	Loss %
A.	1	0·58	41·2
B.	1	0·76	23·1
C.	1	0·95	5·0

Evidently hydrogen peroxide is detrimental. In the control experiment C, the hydrogen peroxide had not affected the quinine.

Repeating the experiment with bile salts instead of hydrogen peroxide showed that they had no effect on the destruction.

RAPIDITY OF QUININE DESTRUCTION

A series of flasks containing 10 c.c. of 25 per cent. guinea-pig liver extract in 0·8 per cent. NaF + 1 mgm. quinine. The quinine content of the flasks was estimated after known intervals of time.

Mgm. quinine added	Found	Time of exposure quinine in the liver extract	Loss %	% of total destruction
1	0·83	1 minute	16·67	32·56
1	0·68	15 „	31·04	60·62
1	0·62	30 „	37·5	73·24
1	0·58	60 „	41·18	80·43
1	0·55	3 hours	44·4	86·72
1	0·52	24 „	47·37	92·54
1	0·48	48 „	51·2	100·00

It will be seen that the rate of destruction is at first considerable but soon becomes slow—in the first fifteen minutes 37 per cent. quinine was destroyed, in the second 6·8 per cent., and in the next thirty minutes only 3·7 per cent.

PURIFICATION OF THE ENZYME

It had already been shown that 50 per cent. alcohol extracts the active principle from liver. It is possible by a process of fractional precipitation with alcohol to obtain the ferment in purer form. 500 grammes of minced liver were extracted with 25 per cent. alcohol and filtered till clear. The filtrate was red with haemoglobin. Alcohol was then added until by volume 36 per cent. was present, and the mixture was filtered. By further successive additions of alcohol and filtering after each addition, solutions were obtained with 48·8 per cent., 59 per cent., 68·8 per cent., 83 per cent. and 92 per cent., respectively.

A 20 c.c. sample of each filtrate was incubated for twenty-one hours with 1 mgm. quinine, and the destruction of quinine estimated with the following results:—

Percentage volume of alcohol in filtrate	Mgm. quinine added	Found	Loss
25·0	1	0·62	37·8
36·0	1	0·64	35·5
48·8	1	0·66	33·3
59·0	1	0·77	23·0
68·8	1	0·97	0·0
83·0	1	0·96	4·0
92·0	1	0·97	5·0
92·0	0	0·0	

It is clear that either the active agent was completely precipitated when the alcohol reached 68·8 per cent. and upwards by volume, and that up to 59 per cent. very little was precipitated, or that more than 59 per cent. inhibits its activity.

PREPARATION OF ENZYME BY ALCOHOL PRECIPITATION

415 grm. of minced guinea-pig liver were extracted with 50 per cent. alcohol and allowed to stand for two hours and then filtered clear.

To the straw-coloured filtrate absolute alcohol was added until 70 per cent. was present. The precipitate was allowed to stand for four hours, then filtered off, and dried in a dessicator.

The product was a whitish-yellow powder which gave no purpurogallin with pyrogallic acid, but contained catalase and haemoglobin (guiacum reaction). 0·4 grm. of the powder was shaken with 20 c.c. of water and filtered—the filtrate was colourless, faintly opalescent, and contained protein in small amount. 10 c.c. of it was incubated with 1 mgm. quinine for eighteen hours—50 per cent. of the quinine was destroyed.

Two weeks later the experiment was repeated: only 37·5 per cent of the quinine was destroyed—apparently the powder deteriorates on standing. An attempt was made to purify the enzyme after the principle found effective for fibrin ferment by Schmidt. 250 grms. of minced fresh guinea-pig liver were extracted with absolute alcohol for twenty-four hours, and then shaken with ether, dried in a dessicator and ground to a fine powder. The powder, when shaken up with distilled water and filtered gave a clear solution poor in protein. 1 grm. of the powder was incubated at 35° C. with 1 mgm. quinine + 20 c.c. of distilled water for twenty-one hours. Its action was feeble—only 26 per cent. of the quinine was destroyed.

1 grm. of dried crude powdered ox liver, tested similarly, destroyed nearly as much, namely 17 per cent. It was evident that by exposure to absolute alcohol most of the enzyme had been inactivated.

THE PRODUCTS OF QUININE DESTRUCTION BY LIVER

Having found that the liver destroyed quinine only in the presence of oxygen, an attempt was made to identify the products formed. Quitenine ($C_{18}H_{22}N_2O_2COOH$), a well-known oxidation product of quinine ($C_{18}H_{22}N_2O_2CH : CH_2$), was an obvious possibility, since Kerner (1870) had described its occurrence in the urine of patients taking quinine, and although this had been denied by Merkel (1902) and by Giemsa and Schaumann (1907), Dr. Nierenstein (1919) had informed me that he had found it in the urine in the earliest stages of quinine excretion.

Professor Ramsden kindly prepared some pure quitenine by Skraup's (1880) method, and together we studied some of its properties. In addition to the properties assigned to it by Skraup and Nierenstein, we find that although it gives with bromine water and ammonia an apparent Thalleioquin reaction, the initial colour is much bluer though it goes green on standing, and that, unlike the quinine pigment, this blue compound cannot be extracted by shaking with chloroform. This insolubility in chloroform furnishes a useful means of distinguishing it from quinine.

Crystals of quitenine from alcoholic solution are transparent, and show varying 'relief' when examined through a rotating Nicol's prism. When heated in water the crystals fragment, and become opaque at temperature well below 80° C. Quitenine picrate is insoluble in ether. Tanret's reagent is not nearly so delicate a precipitant for quitenine as for quinine, although, as in the case of quinine, the presence of much ammonium sulphate greatly enhances its delicacy. With Christensen's reagent some darkening occurs, but no polarising crystals are obtainable.

EXPERIMENT 1. $1\frac{1}{4}$ lbs. of freshly minced sheep liver in 200 c.c. 0·9 per cent. NaCl with 5 grms. of quinine dissolved with the aid of a minimum of HCl, was incubated at 35° C. for eighteen hours. The suspension was raised to 100° C. for a few moments, 'defaecated' and filtered. The filtrate was saturated with picric acid and filtered clear. The precipitate was suspended in 100 c.c. of 1·0 per cent. HCl, and the picric acid was completely extracted by shaking with ether. The acid aqueous layer was rendered alkaline with NaOH, and the quinine then removed by ether. A current of CO_2 was passed through the alkaline aqueous liquid, and the flocculent precipitate formed was dissolved in boiling alcohol water mixture (two parts of water to one of alcohol). The clear filtrate obtained on cooling deposited clear colourless crystals which gave all the reactions and possessed all the properties of quitenine.

The anhydrous crystals melted at 233° C. The total amount of quitenine obtained weighed about 500 mgm.

EXPERIMENT 2. 800 grms. of guinea-pig liver, finely minced, suspended in 200 c.c. 0·8 per cent. NaF containing 1 grm. of quinine in solution, incubated for eighteen hours at 35° C.

Traces of quitenine were found.

EXPERIMENT 3. 350 grms. of minced guinea-pig's liver in 0·8 per cent. NaF + 4 grms. of dissolved quinine in it, were incubated for twenty hours at 35° C.

Following the same procedure as in Experiment 1, no precipitate was obtained with the CO_2. The solution was evaporated to dryness and the residue extracted with absolute alcohol. No quitenine, but an interesting and apparently new quinine derivative was obtained as an amorphous residue soluble in alcohol, water, acids and alkalies, and giving with bromine water and ammonia a green pigment which was not extracted by chloroform. Its picrate was soluble in ether. With Christensen's Herapathite reagent on a slide it gave colourless long needle crystals. It gave a strong yellow turbidity with Tanret's reagent.

EXPERIMENT 4. 375 grms. of guinea-pig liver incubated for forty-eight hours to 35° C. with 2·5 grms. of quinine HCl—quitenine was found in small quantity, only 24 mgm. (anhydrous) being obtained.

QUITENINE IN URINE

To test the influence of quitenine on the malarial parasite, a patient, aged 23, was given, on the 11th of April, four 5-grain doses of quitenine hydrochloride in solution orally, six such doses on the 12th, and two 5-grain doses on the 13th. The patient had typical malarial rigors from the 10th to the 15th, inclusive. The quitenine had no effect on the clinical symptoms or on the presence of parasites in the blood. On the 15th, the patient was given 45 grains of quinine—no further rigor occurred, and no malarial parasites could be found in the blood on the 17th.

The urine collected in the twenty-four hours between the 12th and 13th measured 1500 c.c.; 750 c.c. of this was examined for quitenine by the method already described, by which I found I could easily detect as little as 3 mgms. in 100 c.c. of urine, but no quitenine whatever was found. No quitenine could be found in a later sample of urine. The faeces were not investigated, but it may be safely assumed, in view of Kerner's (2) observations on animals, that most of the quitenine had been absorbed. It would seem that in the doses given, quitenine is not only itself ineffective but is broken up into therapeutically ineffective compounds, since little, if any, appeared in the urine.

NOTE ON A CASE OF BLACKWATER FEVER

Three 15-grain doses of quinine sulphate in solution were given orally to a man, aged 23, who had been suffering from malaria for two years, and whose blood contained malignant tertian parasites. Nine hours after the last dose of quinine, the patient vomited, had a rigor, passed dark urine and became jaundiced.

A sample of urine passed three hours after the onset of the rigor was dark red in colour, and contained oxyhaemoglobin, methaemoglobin, urobilin in great excess, albumen in fair amount, and débris consisting of cellular and amorphous matter and haematin.

A sample of blood taken sixteen hours after the onset of blackwater was found to contain 0·67 mgm. of quinine per 100 grms. of blood (7·1 mgm. quinine per litre of blood).

$$\frac{\text{Plasma quinine concentration}}{\text{Corpuscle quinine concentration}} = \frac{2.2}{1}$$

The blood was centrifuged immediately, the serum was deep reddish brown in colour and gave a strong spectrum of mixed oxyhaemoglobin and methaemoglobin. Urobilin was present.

A sample of urine passed ten minutes after taking the blood sample was estimated to contain 35 mgm. of quinine per litre of urine.

$$\frac{\text{Urine quinine concentration}}{\text{Blood quinine concentration}} = \frac{8.73}{1}$$

This remarkably low ratio has been noticed before in blackwater fever (cf. Ramsden, Lipkin and Whitley (1918), page 246).

Haemoglobin was found in the urine until sixty hours, and quinine until sixty-four hours after the last dose of quinine.

EXAMINATION OF THE FAECES

Twenty-six grms. of moist faeces passed eighteen hours after the onset of blackwater was suspended in 1 per cent. H_2SO_4 and saturated with $(NH_4)_2SO_4$, 50 c.c. of absolute alcohol added, and the whole mixture boiled and filtered. The residue was washed with more hot alcohol. The combined filtrates were shaken up with ether. The ether-alcohol layer which separated was very dark brown, almost black in colour. This was pipetted off into another vessel and allowed to stand. A large quantity of blackish brown material separated out.

A few drops of this ether extract poured on to a filter paper turned gradually from deep brown to green, yellow and red (a play of colours due apparently to oxidation by the air).

The black precipitate from the ether was washed free from stercobilin with ether and dried. It was insoluble in water, acids, alkalies, chloroform, benzene, amyl alcohol, toluene, ether, but very freely soluble in alcohol. The alcoholic solution showed no spectral absorption bands, and was dark brown in colour. It turned purple on standing, especially if the alcoholic solution was acidified with hydrochloric acid. This purple pigment proved to be cholecyanin—the acid solution showing three well marked absorption bands. One thin band between the C and D lines, another broader band on the green side of the D line, and a third very broad band between b and F lines.

On rendering alkaline with ammonia the purple colour was replaced by yellow, showing only one feeble absorption band on the D line; the purple colour reappeared on acidification.

The purple pigment could be extracted in ether, chloroform, toluene or benzene, giving solutions nearly pure blue in colour.

It seems highly probable that the initial black substance, certainly not itself cholecyanin although so easily converted into it, is a cholecyaninogen. None could be found in the urine.

NOTE ON THE THALLEIOQUIN REACTION

A great drawback to this very useful test for the quinine group of alkaloids is its lack of sensitiveness.

The success of the test depends, among other factors, on—

1. The concentration of the quinine in the solution to be tested.
2. The avoiding of excess of bromine.

Ramsden and Lipkin (1918*a*) found that, using 10 c.c. of solution, the least concentration of quinine certain to give a positive reaction with suitable precautions was 1 in 400,000, and that 0·25 mgm. of quinine could thus be detected.

By the following procedure, in which quinine is first isolated as described for the herapath test and then dealt with in strong solution, 0·004 mgm. of quinine can easily be detected in 40 c.c. of water (1 in 10,000,000):—

1. Dissolve 5 grms. of $(NH_4)_2SO_4$ in every 10 c.c. of the quinine solution, alkalise with ammonia and extract the quinine by shaking with two successive lots of purified ether, transferring each lot as it separates to a small silica crucible in a water bath. Aspiration of air from its interior greatly accelerates drying.

2. Dissolve the residue when quite dry in a minimum of ether squirted repeatedly down the side of the crucible by means of a small teat pipette.

3. Bring the ether solution in minimum drops on to a warm microscope slide, in such a way that the residue left by its evaporation is spread over a minimal area.

4. Cover the area of the residue with a very small drop of 0·4 per cent. HCl, now add a small drop (1/30 c.c.) of weak bromine

water and immediately after a small drop of strong ammonia. A green colour is obtained at once. On addition of a drop of strong H_2SO_4, the green colour turns to a distinct red.

I have found that the following modification of the thalleioquin test, although its rationale is by no means clear, serves to distinguish quinotoxin from quinine. To 5 c.c. of the solution containing quinine or quinotoxin add a small drop of 0·5 per cent. Congo red in water. Add bromine water till the blue colour is discharged and replaced by a yellow colour. Add ammonia at once and extract the pigment with chloroform. Quinine gives a green, quinotoxin a red colour, both extracted by chloroform, but the red much more rapidly. One part of quinotoxin in twenty parts of quinine can be detected by this means.

DISCUSSION

(Written jointly with Professor Ramsden)

One of the main objects of the work has been to get fresh light on the question, how it is that quinine, although almost always successful in causing the disappearance of malarial parasites from the circulating blood and effecting a temporary cure, nevertheless almost always fails to obviate eventual relapse. That this failure is not due to any lack of quinine in the general blood-stream, at least up to almost intolerable amounts throughout a period of many hours, has been shown by Ramsden, Lipkin and Whitley (1918*b*) in collaboration with Professors Stephens and Yorke.

Assuming for the moment that quinine is directly parasiticidal, and also that its metabolites may be neglected, various explanations may be suggested for this failure:—

1. The existence of quinine-resistant parasites or of a specially resistant phase in their life history.
2. Parasites may find safety outside the blood, either in other body-fluids or inside cells free from quinine.
3. Parasites may find 'backwaters' maintained free from quinine inside the blood vascular system.

No conclusive evidence can be adduced for any of these possibilities, but the data showing the quinine-destroying powers and quinine content of the various tissues have obvious bearings on the last two of them, although subject to the disadvantages that post-mortem destruction is not a very safe guide to destruction in vivo and that the average quinine-content of a tissue is not necessarily representative of all the cells of that tissue.

It should also be pointed out that so far as extracellular parasites are concerned the data showing the quinine-content of various tissues, although of much interest in relation to absorption and storage of quinine and the physico-chemical conditions inside the cells, do not justify any conclusion as to the *effective* concentration of the drug inside the cell—since much of it might be harmless to the parasite if precipitated or absorbed by the colloids or dissolved in the lipoids of the host cell, or in some non-toxic chemical combination.

As regards the hypothetical places of safety in 'backwaters' inside the blood-vascular system, it may be pointed out that if quinine alone is parasiticidal, they must satisfy the following requirements:—

(*a*) Parasites must be able to remain in them throughout such periods as the concentration of quinine in the general blood stream is actually maintained at a toxic level.

(*b*) They must have a blood supply such that even in many hours only a small proportion of the total blood passes into them—otherwise quinine would not be present in quantity in the general blood-stream so many hours after a dose, as in fact it is (cf. (1918*b*) page 241).

(*c*) They must be surrounded by tissues which remove quinine from them at least as rapidly as fresh supplies are brought up.

Of the powers of the various tissues to remove quinine from the blood, the data obtained give only imperfect evidence—this power must depend not only on the ability of the tissue to destroy or to excrete the drug but also on its storage capacity, and in the case of those tissues which destroy or excrete quinine this can only be dimly guessed at.

It is, however, clear that the tissues mainly responsible for the destruction of quinine are the liver, intestinal wall, muscles and kidneys, and that the blood and spleen have little or no such power. It is clear, also, that each of the tissues examined, except the lymph glands and red blood corpuscles, store much more quinine weight for weight than does the blood or the blood plasma, and that this storage capacity is especially high in the suprarenals, spleen and kidney.

The considerable variations in relative storage capacity of the different tissues in different experiments (cf. suprarenals with intravenous and intraperitoneal injections) may possibly depend on variations in the physiological condition of the tissues and in their blood supply.

Failing exhaustive anatomical knowledge of the 'backwaters' which may exist in various regions of the blood vascular system, and therefore considering only the bone-marrow and the spleen in which their existence is beyond question, it is clear that since neither of these tissues is capable of destroying quinine post-mortem, such quinine-removing power as they possess probably depends solely on their storage power, and of this nothing is known except that it is greater than in many other tissues and might conceivably be adequate for maintaining quinine-free 'backwaters' (especially, perhaps, in the bone-marrow) provided the access of fresh blood is sufficiently slow.

The lymph-glands are remarkable as being the only tissue in which the quinine concentration is consistently less than that in the blood. Whether this is due to poor blood supply or to relative impermeability to quinine is uncertain. That it is probably not due to quinine destruction is indicated by the absence of such in the one post-mortem experiment made.

If the parasiticidal effect of quinine in malaria be not due to quinine itself but to one or other of its metabolites, other possibilities arise for the survival of the parasite, e.g. they might be safe in *any* backwater if the toxic concentration of the effective metabolite is maintained only in the blood of the quinine-destroying tissues. Until the existence of such metabolite has been actually demonstrated, further discussion would however scarcely be profitable.

That the active metabolite, if it exists, is neither quitenine nor any of its metabolite products, is strongly indicated by the observation that this substance had no therapeutic value in malaria and was not excreted as such in the urine, and by Kerner's (2) observation that it has little or no toxicity for animals although readily absorbed by them. It would appear that with the oxidation in the liver of the 'vinyl' group of quinine (R.CH : CH_2) into the carboxyl group of quitenine (R.COOH) all therapeutic activity is lost.

Whether the whole of the quinine which is metabolised in the body is converted into quitenine can only be proved by quantitative work. If it is, the only metabolite of quinine which could conceivably be therapeutically active would be the intermediate aldehyde R.CHO. The unknown substance found in 'hepatised quinine' (Experiment 3, p. 168) is of special interest in this connection, and deserves further investigation.

SUMMARY

1. Evidence is given bearing on the possibility that there exist in the blood-vascular system, regions kept almost free from quinine throughout a period of quinine medication.

2. The quinine content of tissues has been more extensively studied. Accumulation at much higher concentration in most tissues than in the blood is confirmed.

3. The suprarenal body is pre-eminent in this respect, although not so markedly with intramuscular as with intraperitoneal injections. Fairly large accumulations may occur also in the spleen and kidney: the lymph glands contain much less quinine than the surrounding blood.

4. The liver, kidney, muscle, intestinal wall, and probably pancreas, have considerable power to destroy quinine post-mortem, and, therefore, presumably during life. The blood, spleen, suprarenal bodies, bone-marrow, lymphatic, salivary, and thyroid glands have little or no such power.

5. The quinine-destroying agent extracted from the liver is thermolabile, inactivated at 100° C. and acts best in neutral media. Its action is rapid at first, but soon falls off. It does not act at all in the absence of oxygen, and is hindered by hydrogen peroxide. It can be crudely 'purified' by fractional precipitation with alcohol.

6. Quitenine is formed by the action of liver pulp on quinine.

7. Quitenine given by the mouth, in ample doses to a malarial

patient, was therapeutically inert. As none appeared in the urine, this is probably true also of its metabolites.

8. In the faeces of a case of blackwater fever, a brown pigment is described which, although not itself cholecyanin, readily yields this body.

9. New tests for quitenine and quinotoxin are described.

10. By an improved procedure, the thalleioquin test is rendered capable of detecting easily 0·004 mgm. of quinine.

In conclusion, I wish to record my thanks to Professor W. Ramsden for the valuable help and advice he unstintingly gave me during this work, and for his collaboration in discussing the results of the investigation.

I am indebted also to Professors J. W. W. Stephens and W. Yorke for help with clinical experiments and animal injections.

REFERENCES

GIEMSA, G., and SCHAUMANN, H. (1907). *Archiv. f. Schiffs. u. Tropen. Hygiene*, Beiheft. 3.

KERNER, G. (1870). Beiträge zur Kenntniss der Chininresorptin. *Archiv. f. d. gesammte Physiologie.*

MERKEL, A. (1902). Stoffwechselproducte des Chinins. *Archiv. f. Exp. Path. u. Pharm.*, Vol. XLVII.

NIERENSTEIN, A. M. (1919). 'Quitenine,' a disintegration product of quinine found in urine. *Journ. Roy. Army Med. Corps*, Vol. XXXII, pp. 218-219.

RAMSDEN, W., and LIPKIN I. J. (1918). Detection and estimation of quinine in blood and urine. *Ann. Trop. Med. & Parasitol.*, Vol. XI, pp. 434-464. *Brit. Med. Journ.*, May, 1918.

RAMSDEN, W., LIPKIN, I. J., and WHITLEY E. (1918). On quinine in animal tissues and liquids with methods for its estimation. *Ann. Trop. Med. & Parasitol.*, Vol. XII, pp. 223-258.

SKRAUP (1880). *Monatshefte für Chemie*, Vol. X, p. 39. *Berichte d. deut. Chem. Gesell*, Vol. XII, 1104.

CASES OF ACUTE AMOEBIC DYSENTERY IN ASYLUM PATIENTS NEVER OUT OF ENGLAND

BY

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In a previous paper issued from this Laboratory, Matthews and Smith (1919) recorded the results of the protozoological examinations of the stools of two hundred and seven patients at Whittingham Asylum. These stools were examined as fresh as possible, yet, as they were not examined at the asylum itself, several hours (in actual practice from about eight to about thirty) necessarily elapsed before the examinations could be made. Through the kind introduction of Professor Warrington Yorke, I was recently enabled to carry on examinations at an asylum itself, namely the Lancashire County Asylum at Rainhill. In these circumstances the stools could be examined very soon after being passed. Care was taken that the dysenteric stools in particular should be examined as fresh as possible. Some were examined within half an hour of being passed, and five hours was the longest time which elapsed before a dysenteric stool was examined. My visit coincided with a considerable outbreak of the so-called 'asylum' dysentery, and I had the opportunity of examining, during my stay of some two months, the stools of sixty patients suffering from acute dysentery. In three of these patients I found free amoebae containing ingested blood corpuscles, i.e. *Entamoeba histolytica*.

It has previously been considered that asylum dysentery was of bacillary origin. Dr. Gettings (1915), Pathologist at Wakefield Asylum, isolated, at the height of the 1913 epidemic, Flexner's bacillus from 50 per cent. of the cases he examined. This leaves half the cases unaccounted for, and by no means precludes the possibility of some of the cases being amoebic. In the discussion on Dr. Gettings' paper, it was stated that ipecacuanha was used very largely by the visiting physicians at Wakefield Asylum, nearly a century ago. From this fact, and from the fact that relapses occur in asylum cases, it was suggested that some of the Wakefield cases might possibly have been amoebic and the possibility still remains, as Dr. Gettings confessed that he had not examined for the amoeba.

RESULTS OF EXAMINATIONS OF THE RAINHILL PATIENTS

1. *The acute cases*

I have included among these every case in which blood and pus, or pus alone, or blood and mucus were present in the stool. I have not included those cases with blood alone, which might have had other origin, e.g., haemorrhoids, nor with mucus alone, as mucus is commonly present in colitis and post-dysenteric conditions. I examined the stools of sixty patients showing acute dysentery, as thus defined, and of these 3 (5 per cent.) showed the free *Entamoeba histolytica* containing red blood corpuscles. Two of these patients (males) had never been out of England, and one (a female) had lived in Rouen during girlhood, but had been nowhere else outside this country. The occurrence of these cases is of considerable interest, and I, therefore, append some details of each case.

J. Ho., aged 39, male, admitted February 18th, 1918, from Wakefield Asylum, admitted there July 21st, 1917, from Pontefract, his native place; was never abroad, was in poor health on admission with cardiac and kidney disease. His stool was first examined on April 9th, 1919, when it was normal and negative. On April 13th he developed symptoms of dysentery, and an examination of his stool on April 14th revealed the presence of blood, mucus and pus and *Trichomonas* flagellates. On April 17th his stool was again examined, and large numbers of motile amoebae containing red blood corpuscles were present in the bloody mucus. There were present also heavy infections of *Trichomonas* and *Chilomastix*. He was given half a grain of emetine hydrochloride subcutaneously twice daily on the 20th and 21st April. On the 22nd his stool was negative. The treatment was accidentally discontinued from April 22nd to 30th, and his stool was positive on April 23rd. A twelve days' course of emetine, as before, lasting from May 1st to 12th, resulted in gradual improvement. The highest temperature during the illness was 100·4° F.

J. Ha., aged 32, male, admitted December 20th, 1912, born in Scotland, never abroad, lived lately in Warrington, no illness at Rainhill until present attack, but had some symptoms of kidney disease. On April 17th, 1919, the first examination of his stool was made. He was found to be passing cysts of *Entamoeba histolytica*. The stool was normal. On April 25th he developed symptoms of dysentery and had mucus, pus and blood in his stools, which were, however, negative for protozoa. On April 28th amoebae were present in the stools. Owing to the absence of erythrocytes these could not be diagnosed. On April 29th, however, amoebae with ingested red blood corpuscles were seen. A course of 12 grains of emetine hydrochloride, subcutaneously, 1 grain per day, was given from May 1st to 12th. This caused immediate marked improvement in the number and consistency of the stools, and the patient soon completely recovered. Highest temperature 99·4° F.

L. P., aged 37, female, admitted 21st June, 1900, born near Liverpool, was always delicate, but no definite illness, lived in Rouen for some time when a little girl, no known illness there. No other journey abroad. Her stool was examined once only on April 29th, 1919, when blood, mucus and pus were present and also amoebae of *E. histolytica* with ingested red blood corpuscles. A course of emetine hydrochloride was given from May 2nd to 13th. The number of stools passed in the first week of her illness was thirty-five (April 29th to May 5th). From May 5th to 12th, while the emetine was being given, the number was reduced to sixteen. and in the following six days it was fifteen, so that marked improvement resulted. Highest temperature 101·6° F.

Thus the fact of acute amoebic dysentery occurring in an asylum is definitely established. No exactitude can be claimed for the figure—5 per cent.—showing the proportion of amoebic to all acute cases. Possibly it may be higher than this in other asylums, and it is, even at Rainhill, almost certainly a minimum. As the examinations of the case J. Ho. showed (cf. Wenyon and O'Connor (1917), pp. 65 and 66), even acute cases do not pass amoebae in every stool, and in the great majority of the cases only one stool was examined. There seems to be no doubt, however, that the majority of the cases were bacillary in origin. It is commonly stated, see Wenyon and O'Connor (1917) and Willmore and Shearman (1918), that a great preponderance of pus in the stools, as seen under the microscope, is a characteristic of bacillary dysentery. I noted those cases in which pus was the most abundant constituent on microscopic examination, and these numbered thirty-six out of fifty-nine, i.e., 60 per cent. In these thirty-six no amoebae were found, the three amoebic cases being found among the twenty-three cases in whose stools pus was a minor constituent or altogether absent. The preponderance of stools in which pus was the most important constituent and the finding of amoebae in only a small proportion of the cases renders it probable that the outbreak was mainly bacillary. It will be noted later (see Table IV) that the percentage of carriers of *E. histolytica* in the asylum was smaller than that found in Whittingham Asylum. It is probable, if an outbreak of dysentery should occur in an asylum with a higher percentage of *E. histolytica* carriers, that the percentage of acute amoebic cases would be higher than at Rainhill. One aspect of the case J. Ha. may, perhaps, be emphasised in this connection. He was a carrier with normal stool on his first examination, and according to present views (see Dobell and Stevenson (1918), but see also Matthews (1919)) had probably been a carrier for a considerable period. Yet his dysenteric symptoms did not appear until the height of an outbreak of dysentery which was undoubtedly mainly bacillary in origin. No bacteriological examinations of his stools were made, and it is possible that the primary cause of his attack was a bacillary one. A case of dysentery whose exciting cause was Flexner's bacillus, for example, would probably pass *E. histolytica* amoebae, if the patient was already a carrier of *E. histolytica*. In such a case *E. histolytica* might only cause a secondary aggravation of the condition. In the absence of bacteriological examinations no definite statement on this point can be made.

As bearing on the question of the place of origin of the acute dysentery cases, it may be noted that some of the cases were patients who had recently been admitted to the asylum. Of the fifty-nine* acute cases, fourteen were patients admitted within the three

* One of the 60 acute cases previously mentioned was found at post-mortem to be a case of carcinoma of the rectum.

months previous to the onset of their attack. Seven had dysenteric stools within one month of their arrival in the asylum, and of these four had never been abroad, while three had been in the tropics. Five showed symptoms of dysentery within a fortnight after admission (the exact intervals being fourteen, twelve, six, five and one day respectively), and of these three had never been abroad, while two had visited the tropics. It cannot, of course, be in any case proved that the disease originated outside the asylum, but it seems probable from the figures that the asylum receives from the outside population, both from those who have been in the tropics and those who have never been abroad, occasional cases of dysentery.

2. *The general population of the asylum*

The total number of asylum patients examined, inclusive of the acute cases, was five hundred and four. The majority of these patients were without any record of physical ailments. The commonest ailment was dysentery, and sixty-nine of the patients were suffering from, or had a record of, this disease. Twenty-five had renal disease. Nine of these twenty-five had also acute dysentery. In two of these nine cases the dysentery was amoebic. Other illnesses, apart from mental trouble, were comparatively rare. The average age of the patients examined was 42 years.

Table I gives the result of the examination of these five hundred and four patients for intestinal protozoa. The figures are based on one examination per case.

The stools of a few of the patients were examined more than once, and, as usual, new infections were found at later examinations than the first. Sixy-two patients received two examinations each, eleven three each, three four each, and two five each. The examinations subsequent to the first resulted in finding new infections as follows:—*Entamoeba histolytica*, four; *E. coli*, seven; *E. nana*, three; *Giardia intestinalis*, four; *Chilomastix mesnili*, six; *Trichomonas intestinalis*, two; and '*I*' *bodies*, one. The results of these later examinations have not been included in any of the tables given in this paper.

In addition to the infections recorded in Table I, five cases with 'I' bodies (1·0 per cent.) were discovered, and also *Oxyuris* eggs in one case, *Taenia sp.* eggs in two cases, and *Trichuris* eggs in one case. As was to be expected, there is no significant difference between the infections of the men and those of the women. *Giardia intestinalis* is comparatively rarer in the latter, but a larger number of cases would be necessary before any importance could be attached to this difference.

An attempt was made to ascertain if there was any significant increase in the number of infections as the length of stay of the patients increased. The difficulty at once presented itself that subdivisions of such a—for statistical purposes—comparatively small number as five hundred are too small to give significant results. It may be worth while to note, however, that there were sixty-three patients examined who entered the asylum after January 1st, 1919

TABLE I.

	Number examined	*Entamoeba histolytica* infections per cent.	*E. coli.* per cent.	*E. nana.* per cent.	*Giardia intestinalis* per cent.	*Chilomastix mesnili* per cent.	*Trichomonas intestinalis* per cent.
Men... ...	285	3·5	20·1	3·1	6·7	6·2	0·7
Women ...	219	5·0	22·8	2·7	2·7	7·3	0·9
Total ...	504	4·2	21·4	3·0	5·0	6·7	0·8

(the examinations were carried on from April 7th to May 15th, 1919), and among these sixty-three, there occurred one infection with *Entamoeba histolytica*, ten with *E. coli*, three with *E. nana*, and one with *Giardia intestinalis*. Thus, as was to be expected, infections with the various protozoa are being received from outside. The only other figures on this point which I have thought worth recording are found in Table II, where the five hundred and four patients examined have been divided into two almost equal groups according as they have been more than or less than four complete years resident in the asylum.

The significance of these figures is at present very difficult to estimate, and I shall not attempt to draw conclusions from them.

Every stool examined was seen in bulk, and the presence or absence of mucus and blood noted. The stool was further classified as loose, semi-formed or formed. The formed stools numbered

TABLE II.

	Number examined	*Entamoeba histolytica* per cent.	*E. coli.* per cent.	*E. nana.* per cent.	*Giardia intestinalis* per cent.	*Chilomastix mesnili* per cent.	*Trichomonas intestinalis* per cent.
Resident less than four years ...	249	2·0	21·6	3·6	6·0	5·2	0·4
Resident more than four years	255	6·4	21·2	2·4	4·0	8·2	1·2

exactly half of the total number examined. With the exception of one case of flagellate trichomonas in a formed stool, the free forms of the flagellates and of the entamoebae were found in the loose or semi-formed stools only.

Table III shows the percentage of the intestinal protozoa found in the formed stools on the one hand and the loose and the semi-formed stools on the other. The only figure worthy of note is that for *G. intestinalis*. The larger number of infections of this flagellate found in the loose stools agrees with the results of Wenyon and O'Connor (1917) on this point.

TABLE III

	Number examined	*Entamoeba histolytica* per cent.	*E. coli.* per cent.	*E. nana.* per cent.	*Giardia intestinalis* per cent.	*Chilomastix mesnili* per cent.	*Trichomonas intestinalis* per cent.
Formed stools	252	4·8	24·0	3·6	3·2	7·2	0·4
All others ...	252	3·6	19·2	2·4	6·8	6·4	1·2

A note may be made of the fact that in one of the men's wards, from which the stools of sixty-nine patients were examined, the percentage of *G. intestinalis* was found to be 14½ and that of *C. mesnili* 11½. These figures, particularly the former, are significantly larger than those for the whole number examined. The variation may be due to chance, as the number examined was not large, but on the other hand it may be correlated with the fact that this ward contained, in the words of Dr. Cowen, the Medical

Superintendent, 'patients who are a very degenerate lot and are in many cases of faulty habits.'

Finally Table IV gives the results for Rainhill, compared with other sections of the population already examined and reported on. As only thirty-four of the five hundred and four patients at Rainhill had, so far as could be traced, ever been abroad, the figures are to be looked upon as representing the infections of a section of the home population, and are to be compared with the groups of that population rather than with the results for returned soldiers.

TABLE IV.

	Home Population				Returned Soldiers		
	Rainhill Asylum	Whittingham Asylum	Adult Civilians, Hospital population	Children, Hospital population	Army Recruits	Dysenteric Convalescents	Non-dysenteric Convalescents
Number examined	504	207	450	548	1,098	4,068	450
Entamoeba histolytica	4·2	9·7	1·5	1·8	5·6	7·0	6·4
E. coli. ...	21·4	45·9	6·7	11·1	18·2	15·2	14·2
E. nana. ...	3·0	12·1	2·4	2·7	5·5	...	...
Giardia intestinalis	5·0	3·4	6·0	14·1	7·0	9·9	6·0
Chilomastix mesnili ...	6·7	23·2	1·5	1·8	0·2	3·6 (3 exams. per case)	2·0 (2 exams. per case)

It may be noted that the various protozoal infections, with the exception of *G. intestinalis*, are much less common in the patients at Rainhill Asylum than we found them to be among those at Whittingham. It may be that wide differences would be found in the various asylums in this respect, differences depending on factors at present unknown. The comparative isolation of an asylum population would tend to prevent these differences, once established, from being obliterated. Though the number of *G. intestinalis* infections is higher than at Whittingham, yet the difference is not so marked as to call for any revision of the theory put forward in previous papers by Matthews and myself, namely that this flagellate tends to disappear from older people. In fact, the smaller percentage of *G. intestinalis* among those who have been longer in the asylum (see Table II) would form some slight further evidence in favour of our view.

I wish to thank very heartily Dr. Cowen, Medical Superintendent at Rainhill and Dr. Watson, Pathologist, for their great help and kindness during the course of my investigation. But for their cordial co-operation the work would have been impossible.

SUMMARY

Five hundred and four asylum patients have been examined for intestinal protozoa. Fifty-nine of these had acute dysentery and in three of the cases vegetative *E. histolytica* were found in the stools.

All the usual protozoa were found in the stools, but the infections were distinctly less numerous than in the patients of the asylum previously examined.

REFERENCES

Gettings (1915). Bacillary Dysentery. *Trans. Trop. Med. and Hyg.*, Vol. VIII., p. 111.

Wenyon and O'Connor (1917). Human Intestinal Protozoa in the Near East. *Wellcome Bureau of Scientific Research, London.*

Willmore and Shearman (1918). On the differential diagnosis of the dysenteries. *Lancet* CXCV., 2, p. 200.

Dobell and Stevenson (1918). A note on the duration of infections with *Entamoeba histolytica. Trans. Trop. Med. and Hyg.*, Vol. XI., pp. 168-175.

Matthews (1919). The course and duration of an infection of *Entamoeba coli. Ann. Trop. Med. and Parasitol.*, Vol. XIII., p. 17.

Matthews and Smith (1919). The spread and incidence of intestinal protozoal infections in the population of Great Britain. IV. Asylum patients. V. University and school cadets. *Ann. Trop. Med. & Parasitol.*, Vol. XIII, p. 91.

THE EXPERIMENTAL INFECTION, IN ENGLAND, OF *ANOPHELES PLUMBEUS*, HAL., WITH *PLASMODIUM VIVAX*. (SPOROZOITES IN SALIVARY GLANDS.)

(PRELIMINARY NOTE)

BY

B. BLACKLOCK

AND

H. F. CARTER

(*Received for publication* 24 *July*, 1919)

Specimens bred at the Liverpool School of Tropical Medicine from the pupae, collected in England, of *A. plumbeus*, Hal., were fed on a volunteer patient, suffering from simple tertian malaria, acquired in Salonica. On 9.7.19 the patient had a rigor at 9 p.m. and the blood was positive; on 10.7.19 at 6 p.m. the blood of the patient showed a moderate infection of trophozoites and gametes. The *Anopheles* were now fed on him, and six of them which had fully engorged were separated and placed in glass globes in an incubator, kept at a temperature of about 28° C. The mosquitoes received no meal of blood either before or after the infective feed, but were given raisins. The first mosquito died on 13.7.19, the second on 14.7.19, the third on 15.7.19; no evidence of infection was found in any of them, but the presence of blood in the gut rendered the examinations unsatisfactory. The fourth mosquito, however, which died on 18.7.19 was found to be infected, twelve oöcysts being found in the gut, some of them in an advanced state of development. The fifth mosquito was killed on 21.7.19 at 10 a.m., and infections of both the gut and salivary glands were found. Both glands were infected with sporozoites in large numbers, the distal extremities of the lobes being chiefly involved.

We have also obtained oöcysts (thirty-six, varying in size from 18μ to 30μ) in the gut of a female killed on the thirteenth day after the infective feed, and kept at laboratory temperature.

NOTE ON A CASE OF MULTIPLE INFECTION BY *DRACUNCULUS MEDINENSIS*

BY

B. BLACKLOCK

AND

CAPT. W. R. O'FARRELL, R.A.M.C.

PLATE VII

(*Received for publication* 22 *July*, 1919)

The following case of guineaworm infection presents some features of interest, namely the number of worms with which the patient was infected, and the somewhat severe symptoms caused by their presence and condition.

The patient, an Indian, aged 15, from Nizampur village in the district of Alibag, was admitted to the Tropical Ward of the Royal Infirmary, Liverpool, on June 3rd, 1919. He stated that he had only been away from India about two months, that neither he nor the members of his family had suffered from this disease, but that it was common in his village.

Local condition. Both feet and the right hand were affected, the lesions being distributed as follows:—

Right foot: (1) Immediately above the internal malleolus; (2) below and behind the internal malleolus; (3) the outer side of the first phalanx of the second toe.

Left foot: (1) On the dorsum of the fourth metatarsal; (2) below and behind the external malleolus; (3) below and two inches in front of external malleolus; (4) on the inner aspect of the dorsum of the first metatarsal.

Right hand: (1) On the radial side of the base of first metacarpal; (2) on the dorsum of the first phalanx of the middle finger.

In all these sites the lesions were of much the same character. The superficial layers of the skin had been removed over wide areas, especially of the feet, probably by previous applications. The lesions caused by the worms consisted of a more or less circular area—measuring from 2 to 3 centimetres in diameter—of raised tissue with a central orifice. In the majority of the lesions the tissue round the opening was scaly and white, but in two there was a prominent area of red granulation tissue extending 1 centimetre around the orifice. From several of the sites portions of worm of varying length were protruding, and from all the sites, by gentle pressure, a small amount of pus could be obtained. Both feet and the right hand was swollen, oedematous, and tender.

The appearance presented by the patient's feet and hand are shown in Plate VII, figs. 1-4.

Among the portions of worm protruding from the openings, no head was discovered. They had been removed previous to admission. Examination of the discharge from the lesions showed that although larvae were present, they were motionless. Larvae removed from the body of the adult females also proved to be dead.

General condition. The temperature was 98·4° F., pulse 94°.

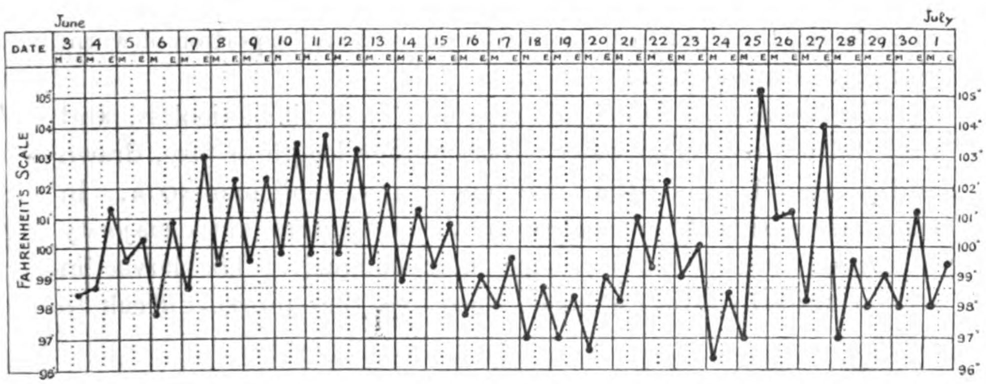
Blood. No parasites were observed either in fresh or stained preparations. Eosinophilia was noted to the extent of 14 per cent. (500 leucocytes counted).

Urine. Clear, sp. g. 1009, acid, no alb., blood, or sugar. No deposit macroscopic or microscopic.

Faeces. There was a small quantity of apparently pure blood passed with the first motion, but this was possibly present owing to the scybalous nature of the motion. It was not observed on subsequent examinations. Ova of *Ascaris lumbricoides* and *Trichurus trichiura* were found, and later ancylostome ova also.

Treatment. In view of the condition of the lesions, neither gradual extraction of the worms nor the injection of them or the tissues with a view to preventing the spread of sepsis appeared to hold out much chance of success. It was considered possible that the intravenous injection of antimony might be of some value in this direction. On the 6th and 8th June the patient received an injection of 1 grain of tartar emetic. On the 7th June the patient had some epistaxis and coughed up some blood-stained sputum, but there is no evidence that this was due to the injection, and no such symptoms followed the second injection. On the 7th of June the portion of worm presenting on the right wrist disappeared from view; on the 8th, three inches of worm were discharged from the left foot; on the 9th, fresh lesions were observed, one on the right wrist, from which a portion of worm emerged, and another on the left foot; also five inches of worm were discharged from the right foot; all the openings were discharging freely pus tinged with blood.

At this stage the rapid progress of the septic condition accompanied by enlarged and painful glands in the groin necessitated surgical interference. It was, therefore, not possible to come to any conclusion as to the effect of treatment by intravenous injection of antimony in this case of guineaworm disease, as the case did not afford the drug a fair trial. During this period the patient's temperature rose nightly to over 102° F. (see chart), his pulse and



respiration also being rapid. X-ray examination of the limbs was negative. On June 12th, Mr. Thelwall Thomas opened up the worm tracks in the feet, and removed portions of several worms, ligatures being applied to the portion remaining in the tissues. No tail portions were obtained, but the next day at dressing the terminal portions of two worms were removed easily, one from the left foot, eight inches in length, and one from the right hand, each bearing the typical curved tail. Further terminal portions of tails were obtained at subsequent dates. The following table shows the period during which the portions of worms were obtained:—

TABLE

Giving total lengths of portions of worms obtained from each limb, with the number of tails found.

Date	Limb	Total length of portions in inches	Tails
(1) From 8.6.19 to 19.6.19	Left foot	49	3
(2) From 13.6.19 to 15.6.19 ...	Right hand	16	2
(3) From 5.6.19 to 7.19	Right foot	57	3
Total		122	8

On 25.6.19 the patient had a rigor, temperature 105° F., but no malarial parasites were found on several examinations. Quinine was given and appeared to exercise some effect on the temperature. The right foot was tender and swollen. On 3.7.19, under a general anaesthetic, the right foot was further incised and a portion of worm extracted, bearing a tail.

The larvae. As stated above, these were all motionless, and they did not recover in water. The average length of fifty, drawn and measured, was 616μ, maximum 737μ, minimum 490μ. In transverse section of the female worm the larvae were, in the majority of cases, cut transversely, some more obliquely. The larvae in section stained readily, whereas, when free, they proved difficult to stain.

EXPLANATION OF PLATE

Figs. 1-3. Showing the appearances presented by the feet.

Fig. 4. Showing the appearance presented by the right hand.

PLATE VII

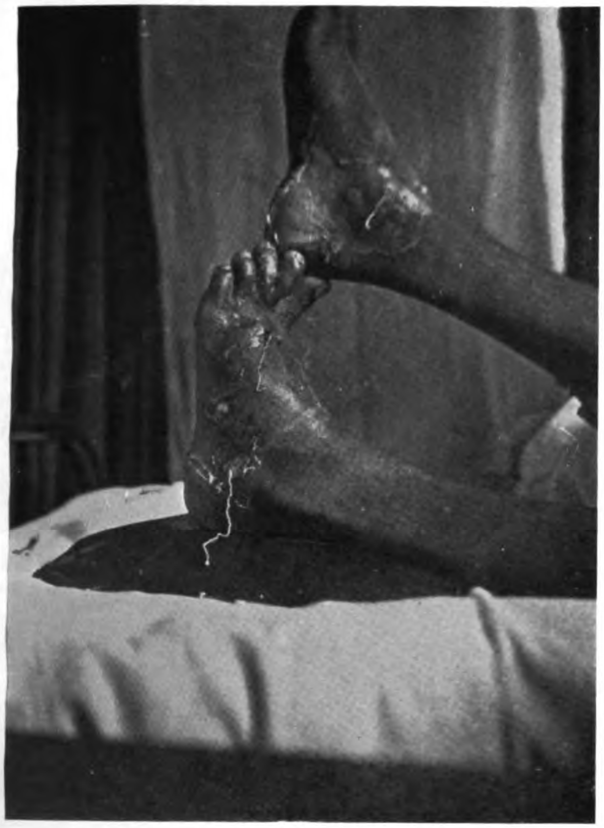


Fig. 1

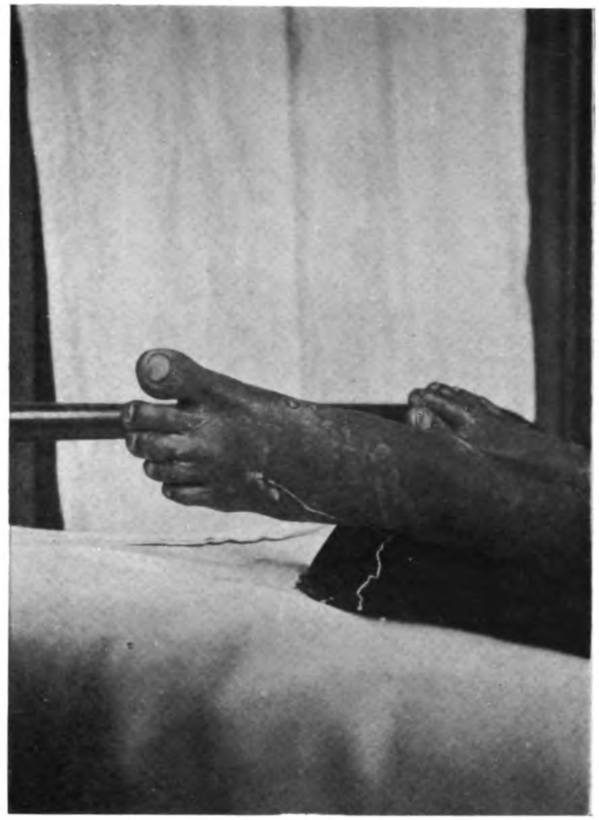


Fig. 2

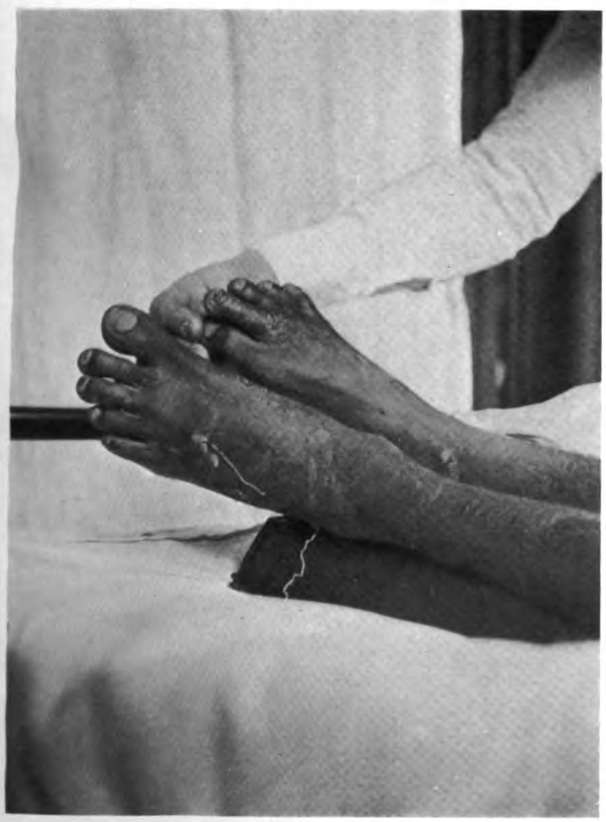


Fig. 3

Fig. 4

C. Tinling & Co., Ltd.,

COINCIDENT MALARIA AND ENTERIC FEVER

BY

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(*Received for publication* 17 *June*, 1919)

'Twenty years ago, when it was alleged before the Indian Plague Commission that anti-plague inoculation had cured eczema, gonorrhoea, and other miscellaneous infections, I thought the matter undeserving of examination. I took the same view when it was reported in connection with anti-typhoid inoculation that it rendered the patients much less susceptible to malaria.' These words were spoken by Sir Almroth Wright (1919) in the course of a paper read at a meeting of the Royal Society of Medicine and reproduced also in the *Lancet*.

During the last few months, in a report to the Tropical Diseases Research Committee, I mentioned certain peculiarities which were noticeable in cases of coincident enteric fever and malaria, and as having some bearing on the second of the above two passages the following account may be of interest.

Every worker in the tropics has met with cases of enteric fever in which malaria supervenes, but in the majority, if not in all, of these about to be described both conditions were present together. Lyon has published some cases, but his paper is not available, and I cannot tell, therefore, whether his experiences coincide with my own. Far be it from me to attempt to revive the old term of 'Typhomalaria,' for unless this is distinctly understood to imply merely malaria occurring in a typhoid patient, the term is liable to mislead in giving the idea of a single and distinct disease. When, however, we have a dual infection from the beginning, certain peculiarities manifest themselves which are worthy of special remark.

It has been stated by Sir Patrick Manson that in malarious subjects the oncoming of typhoid is often preceded by three or four paroxysms exactly like those of ordinary ague. In such cases quinine is usually given early, and its failure to check the disease should warn us to be guarded in giving a prognosis. *Vice-versâ*, well marked malaria-like fluctuations of the temperature and the appearance of parasites in the blood in the course of a continued fever do not exclude typhoid. These cases are 'typhomalarial,' that is typhoid fever with a malarial complication.

One must remember in discussing this question that any disease in a malarial subject is prone to take on an intermittent or periodic character; thus one meets with cases of true lobar pneumonia, for example, with a distinctly periodic remission until the crisis takes place; this must not be confused with the 'pneumonic form of malaria,' such as occurred in some of the Salonika troops.

No attempt will be made to discuss the question of the simulation of malaria by typhoid nor that of typhoid by malaria, but that of the definite co-existence of the two conditions. There are, of course, three periods at which this combination may take place. Firstly, at the onset; in such cases the usually described 'Typhoid chart' is departed from. The rise is not of the staircase type at all, but with rigors, high fever, sweating, partial remissions, strongly suggestive of and usually diagnosed as malaria, especially as similar attacks may be repeated for two or three days with, it is true, smaller remissions as a rule. Quinine is given, the rigors and marked oscillations are checked, but the temperature remains persistently high and the typical typhoid condition develops.

Secondly, the attack begins in the customary insidious manner of most cases of enteric fever, then, about the second or third week, rigors and oscillations occur giving rise to considerable anxiety as to urgent complications. Administration of quinine brings about a cessation of these oscillations, and the typhoid resumes its course.

Thirdly, the malaria may occur, as already stated, at the termination of the enteric fever attack or during convalescence. In some of these cases one sees in a patient suffering from *Plasmodium vivax* infection fluctuations of temperature strongly suggestive of tertian ague (though, as stated, rarely falling actually to normal), but there are the furred tongue with red tip and edges, the loose pea-soup stools, the listlessness and other clinical appearances of enteric fever. Again, with concomitant *P. falciparum* and enteric infection the chart may be one of subtertian malaria with intermissions, but the appearance clinically is that of an enteric patient.

The following are brief descriptions of some cases recently met with:—

1. P.Y., male, aged 28 years.

Was admitted with a temperature of 101° F.; previous duration uncertain; was said to have 'had fits a few days ago,' before coming to hospital, but nothing definite as to the nature or character of these could be ascertained. His chart showed that during his stay in hospital the highest temperature was 101·4° F. One week after admission it fell to normal for 24 hours, and four days later reached normal to stay there. The debility was but slight, and recovery rapid and uneventful. On the day of admission the blood was examined and gave an agglutination of *B. typhosus* in high dilution and also of *B. paratyphosus* A. in low dilution (1 : 30 only), probably a group reaction; *P. falciparum* was seen in smears. The malarial element is clear; the question is whether there had been an old attack of typhoid fever leaving residual agglutinins in the serum. The titre was high, so that if there had been a previous attack the patient could hardly have forgotten it, yet no history of any prolonged fever could be obtained.

2. J.W., male, aged 12 years.

This patient gave a history of 'fever and headache' for three weeks prior to coming to hospital; on admission the temperature was 101° F., and the tongue was coated. Subtertian parasites were found in the blood and quinine was given. In view of the history and of the facts that the temperature in four days, though lower, had not become normal, and that the patient appeared ill, a Widal reaction was asked for and a strong agglutination of *B. typhosus* was obtained. So much of the illness as was observable, namely, at the end of the third week and after, showed a temperature like that of a mild case of enteric fever at that period. The malarial infection did not appear to have any appreciable effect on the temperature. He had had no quinine or any other form of medicinal treatment before coming to hospital. Ten days afterwards the temperature reached normal and stayed there; convalescence was rapid and uninterrupted.

3. B.M., female, aged 21 years.

This patient was admitted to hospital with a history of 'fever and headache' for the preceding four days; there had been no shivering attacks. Her temperature was 102° F. on coming to hospital. Blood examination revealed the presence of *P. falciparum*; the serum gave a marked agglutination of *B. typhosus* in low dilution (up to 1 in 50), but blood culture yielded a growth of *B. typhosus*. The tongue was coated, with red tip and edges, and the general aspect was that of an enteric patient. In view of the presence of malarial parasites quinine was given, but otherwise the treatment was that of enteric fever. During the succeeding five days the temperature once fell to 99° F., but except for this remained between 102° and 104°. On the ninth day of illness it fell rapidly to 98°, and remained normal till the day of discharge from hospital three weeks after admission (Chart 1).

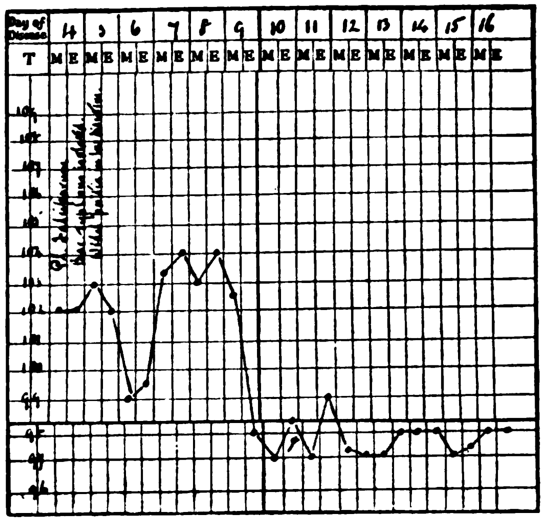

CHART I

This is interesting because, if, as would appear to be the case, there was definite infection with *B. typhosus*, the attack was exceptionally mild. No previous history of any prolonged fever could be obtained whereby the Widal result might be regarded as a residual agglutination reaction. Moreover, the patient presented a typhoid appearance, and the positive result occurring in comparatively low dilution (1 : 50 only) would be accounted for by the early stage of the disease, the fifth day. Other possible explanations are either that the attack was abortive (by no means synonymous with a mild attack), or what is less likely, the patient was not actually suffering from an attack of enteric fever, but that the agglutination was given owing to a previous infection, or lastly, that she was a 'healthy carrier' without having undergone a previous attack and that the presence of the bacilli was merely a temporary, transient condition during which one was fortunate enough to obtain a culture. This last is a far-fetched explanation, but in whichever way the question is regarded there are difficulties of interpretation.

4. A.W., male, aged 26 years.

This man was admitted to hospital with a history of 'fever, headache, and chills' during the preceding eight days. The temperature on admission was 103° F., the tongue furred in the centre, but with red tip and edges. In view of the history smears of blood were taken in the admission room and examination revealed *P. falciparum* and pigmented mononuclears. Quinine was given by mouth and later, as the temperature remained elevated, by injection in large doses. This, however, was not effectual and the Medical Officer, suspicious of 'something besides malaria being at the root of the trouble,' sent up the blood for agglutination tests. *B. typhosus* was agglutinated in high dilution. The patient had by this time been ill for three weeks, but during the following seven days the fever abated, the temperature becoming normal on the twenty-ninth day of disease and remaining there (Chart 2).

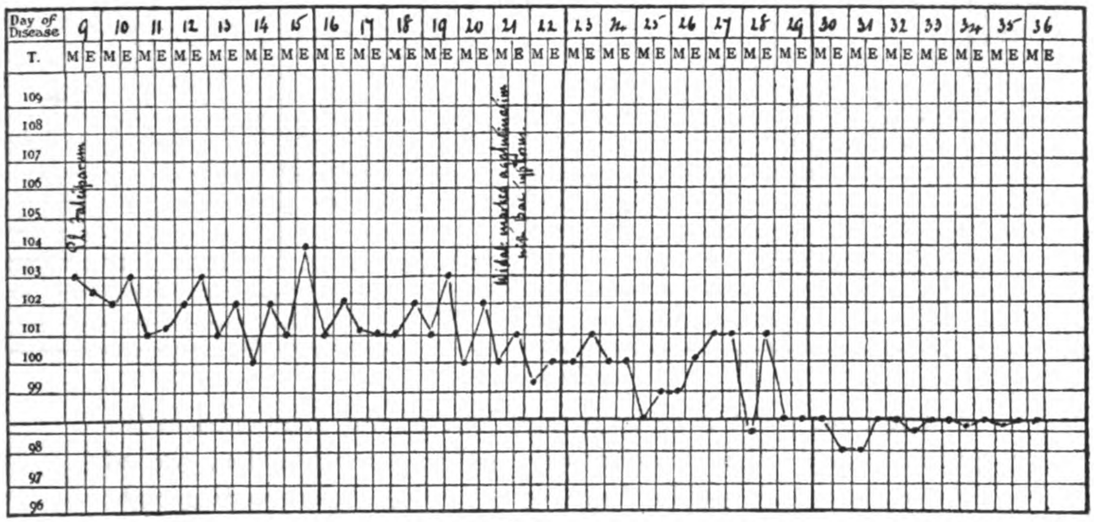

CHART 2

Two points to be noticed in this case are, firstly, the rapid fall of the temperature to normal to remain there instead of showing the usual terminal fluctuations of a case of enteric fever; secondly, the combination of malaria with enteric did not prove any more severe than the latter alone. This fact has been noticed in several instances.

5. R.D., female, aged 17 years.

Prior to admission to hospital this patient had been ill for eight days with 'fever, pains in the head and abdomen.' The temperature in the taking-in room was found to be 105° F.; the pulse was 115; the spleen was enlarged, and there was abdominal tenderness, especially in the right iliac region. The blood was examined and *P. falciparum* found in considerable numbers, while the Widal reaction gave a marked agglutination of *B. typhosus*; the degree of this was greater in the higher dilutions than the lower—a reversed reaction (pro-agglutinoid). Four days later there is a note to the effect that the patient was delirious at night, but this subsided, and a week afterwards it is stated that 'she is sleeping well.' Improvement set in with a sudden fall of temperature from 102° to 98° and this was maintained for eight days, when, without any return of the fever, the patient became dull and apathetic, took nourishment badly, and grew progressively weaker. Malarial parasites were not again found in the blood, and there was no further rise of temperature till 24 hours before death, which took place on the fifty-sixth day of disease. Her condition during the last three weeks of life was that of a post-typhoid psychosis, a sort of lethargy (Chart 3).

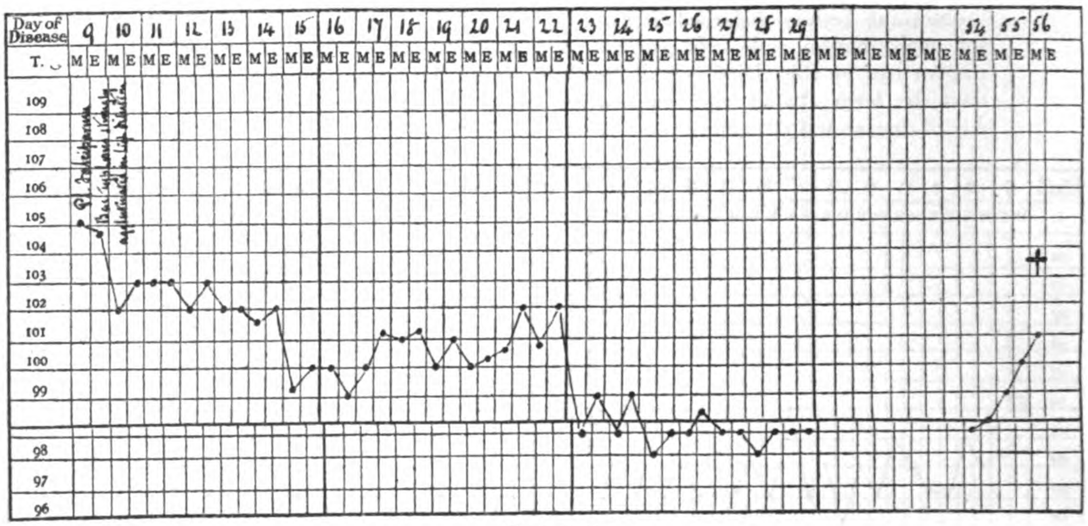

CHART 3

The same point is to be noticed here as was mentioned in the previous case. The temperature, 105° F. on admission, varied between 102° and 103° for four days. During the succeeding eight days it never exceeded 102°, and at the end of the third week of illness dropped rapidly from 102° to 98° and thereafter, except when 99° was registered on three occasions, it remained subnormal till the last twenty-four hours of life, during which it rose to 101°. The febrile course is thus seen to have been a mild one, and the presence of *P. falciparum* did not appear to render it any more severe; the fever was of an unusual type for the third week of typhoid fever, but examination of the blood again during that period did not result in the finding of any malaria parasites to account for the irregularity or the sudden drop.

6. Z.P., male, aged 53 years.

Prior to coming to hospital this patient had for eight days, so he stated, been suffering from 'fever, pains all over, and cough.' The temperature on admission was 101·6° F.; the stools were loose and offensive. Blood examination on day of admission: *P. falciparum* present; the Widal reaction was indefinite: there was considerable loss of motility and attempts at agglutination, that is, there were small clumps of bacilli, but the majority were isolated though motionless, and this was noticed only in the lower dilutions. At the end of the third week of illness the temperature became normal. On the fourteenth day of disease the patient's condition was poor, he was very weak, and the pulse soft and feeble, but after the fall of the temperature he rapidly improved and convalescence was uninterrupted. The blood was again tested for agglutination, and gave a well marked positive result with *B. paratyphosus* A; negative with *B. typhosus* and *B. paratyphosus* B. No malaria parasites found in smears. With the exception of a single occasion on which 99° was recorded, it will be noticed again in this case that after once falling to normal the temperature did not again rise and never showed any of the usual terminal oscillations of most enteric patients (Chart 4).

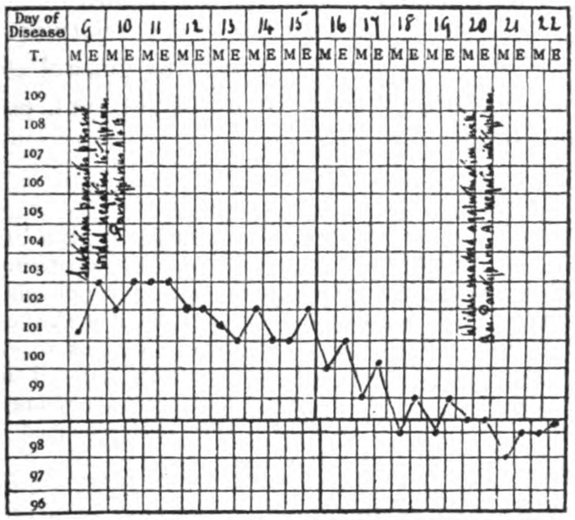

CHART 4

7. R.P., male, aged 7 years.

This boy was admitted to hospital on the eighth day of illness, complaining of 'fever and pains in the stomach.' His temperature was 103° F., the pulse was rapid, 124 per minute, the tongue coated but with red tip and edges. The abdomen was tumid, but liver and spleen were both palpable. *P. falciparum* was found in smears of blood sent up on the following day, and on the tenth day of illness a Widal test gave a strong agglutination of *B. typhosus* in high dilution. Progress was very satisfactory; the temperature never rose above 103° F., and reached normal on the eighteenth day of disease (Chart 5).

CHART 5

Here again, in spite of the double infection with malaria and typhoid, the course was unusually mild; no history could be obtained of any prolonged fever or of anything of a typhoid-like nature which might have left residual agglutinins; the relatives denied any previous illness of a serious nature, and the general condition was that of a typhoid patient—feverish with bright eyes, painful, tumid abdomen, and so forth.

8. G.L., female, aged 4 years.

This child had been ill with 'fever and abdominal pains' for a fortnight before being brought to hospital. On admission the temperature was 102° F., the tongue coated, edges and tip clean. Blood examination on the same day revealed the presence of *P. falciparum*. Five days later as the temperature was still irregular a Widal test was asked for and the result was an agglutination of *B. typhosus;* on this occasion no malarial parasites were detected though carefully sought for. On the evening of the second day after this the temperature touched normal, rose again next day to 100° F. and thereafter remained normal. Convalescence was uninterrupted (Chart 6).

The general aspect of the patient was that of a child suffering from enteric fever, and the rapid defervescence is again here worthy of note.

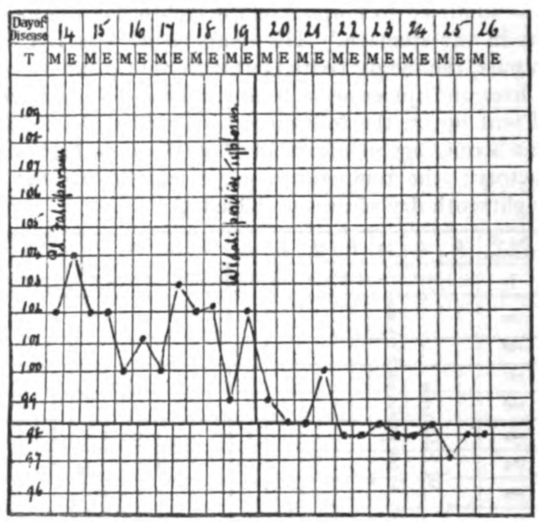

Chart 6

The suggestion may be made that we were dealing here with a patient suffering from a mild or from an abortive attack of enteric fever, and that the true, if not the only, cause of the fever on and after admission was the presence of the malarial parasites.

Everyone knows how anomalous are the forms of enteric fever as met with in children, but against the above explanation is the fact that the temperature continuing irregular was the reason for making a Widal test, and that on that occasion prolonged search failed to reveal the presence of any malarial parasites.

9. A.A., male, aged 23 years.

This patient was admitted to hospital with a temperature of 103° F., a coated tongue, and loose stools. The duration of the present illness could not be determined with accuracy, because he had suffered a few weeks earlier from influenza and broncho-pneumonia (during the epidemic of the so-called Spanish Influenza), and had not thoroughly recovered from this, though convalescing and gaining strength, when he again 'felt feverish, had pains in the limbs, and severe headache.' There was no history of any previous attack of enteric fever nor of any illness suggestive of such. Examination of the blood was undertaken both for agglutination reactions and for the presence of malarial parasites. *P. vivax* was found, and the Widal test gave a well-marked agglutination with *B. typhosus*, negative with the Paratyphoids. As the chart shows (Chart 7) the temperature each morning for four days after admission was 100° F., and from 1 to 3 degrees higher each evening. On the fifth day it became normal, or rather subnormal, with a rapid fall and remained so till the patient left hospital after a stay of four weeks. Convalescence was gradual, but steady and uninterrupted.

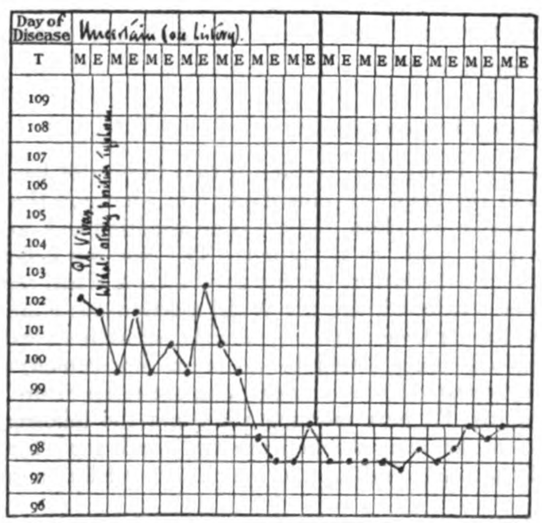

CHART 7

It will be seen here that the temperature for the short time during which it remained elevated after the patient came to hospital was not like that of benign tertian malaria, but resembled more that of enteric fever in the third week; the rapid, almost critical, fall, however, during the night of the fourth day after admission is more suggestive of an uncomplicated malaria brought under control. The headache was continuous and the general appearance very suggestive of enteric fever, while the course of convalescence resembled this also rather than an ordinary attack of malaria.

There is, of course, the possibility that the attack of 'influenza and broncho-pneumonia' had been wrongly diagnosed, and had been enteric fever from the start; this case would then come under the category of malaria developing towards the end of an attack of enteric fever. Against this it may be stated that the patient had been in hospital for his attack of 'influenza' and under the charge of a medical officer who is always careful to send to the laboratory a specimen, and if necessary repeated specimens from any case in which enteric fever is suspected, or, in fact, of almost any case of fever at all before starting to treat the patient for malaria, as so many do. It is a common fault, I believe, in all tropical countries to treat every case of 'fever' as malarial, and only when quinine is found ineffectual are means taken to verify or refute the diagnosis. Here, with a laboratory on the spot, this is rarely done, the blood being taken in the great majority of cases for examination before treatment is begun.

10. A.S., male, aged 8 years.

This patient was brought to hospital with a history of having suffered from 'fever and pains in the head' for nine days. On admission the temperature was only 99° F., the tongue was clean, there was no vomiting and no distension of the abdomen. The spleen could be easily felt. The case was believed to be one of malaria, but in view of the history of nine days' fever a Widal test was also carried out. *P. falciparum* was found, and the Widal reaction was positive, more marked in high dilutions than in lower. (I have already reported several instances of this which, in children out here, I find by no means uncommon). Quinine, which was given at once, appeared to have no effect on the temperature. Some 36 hours afterwards it was 104° F., next morning 100°, and the following morning 103°. It then came down and remained between 100° and 102° for ten days, then oscillated again for a couple of days, after which time it fell to normal and remained there (Chart 8).

The course of the illness and the convalescence were both like those usually met with in mild cases of enteric fever, the temperature reaching normal on the twenty-eighth day of illness and the patient being sufficiently convalescent to leave hospital after a stay of thirty-seven days, or forty-six days after the onset of the fever.

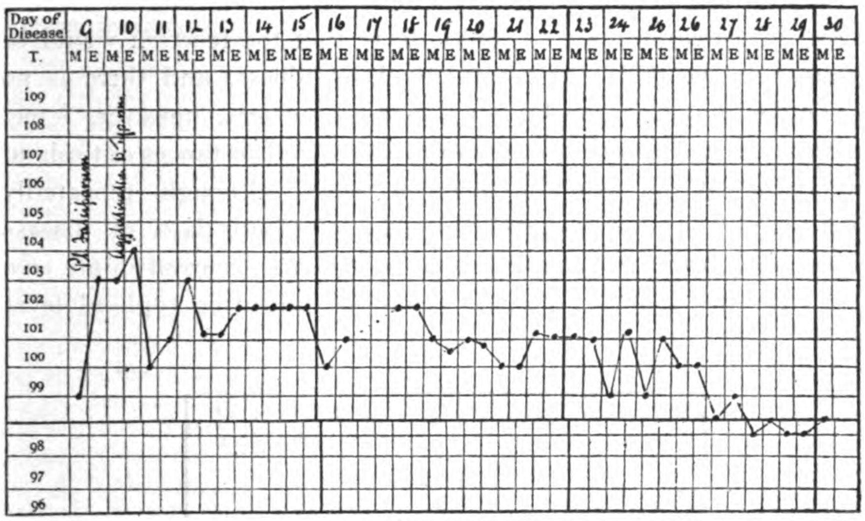

CHART 8

This is very typical of the old 'typho-malaria,' and is inserted here for that reason. There is nothing otherwise worthy of special note except that the Widal reaction was a 'reversed' one.

11. A.G., female, aged 11 years.

This patient was admitted to hospital on the twenty-first day of illness having suffered with fever, headache, and constipation. She had a coated tongue, and had been vomiting, it was stated, shortly before admission. The abdomen was neither distended, tender, nor painful; the temperature was 103° F.; the heart's action was rapid, 124 per minute, and examination of the lungs showed a few rhonchi at the upper parts on both sides. The blood was examined both for agglutination and for malarial parasites. The serum agglutinated *B. typhosus* in lower dilutions (up to 1 in 50), but not in 1 in 100 or over; *P. falciparum* was present, both ring-forms and crescents in considerable numbers. Blood culture was negative, as one would expect at this stage of disease—the end of the third week—but *B. typhosus* was isolated from the stools. The chart shows a rapid fall of temperature following the administration of quinine (Chart 9).

The question here is: Can the whole condition be ascribed to malaria? The clinical state certainly supports this, and the rapid effect of quinine further backs it up. One point against this is not very strong, namely, the positive Widal reaction in comparatively low dilutions only. Now, there was no history of any previous attack of prolonged fever which might allow us to interpret the Widal result as a residual reaction, or to the fact of the patient being a carrier (the presence of bacilli in the stools would support equally the last idea or the fact of a recent attack). One would naturally expect in a definite case of enteric fever that the reaction would be stronger at the end of the third week of illness, and there is no reason for doubting the history. Some time ago, however, I met two cases in which, though they were undoubted instances of typhoid fever, the Widal reaction was negative, although repeatedly tried, until the twenty-eighth and thirty-second days of disease respectively; while a still more striking instance occurred in my own case. In spite of every clinical symptom indicating typhoid fever

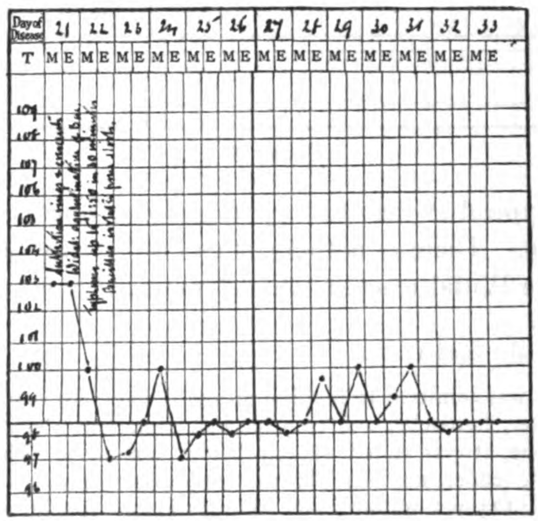

CHART 9

and repeated blood examinations, it was not until the onset of a second relapse and the forty-eighth day of illness that a positive agglutination of *B. typhosus* was obtained, and then only in 1 in 50, though seven to ten days later a marked agglutination in high dilution was obtained, and is still, though it is now over six months since the fever disappeared.

All one can say in the case of A. G., is that the patient was excreting typhoid bacilli in the faeces while suffering also from subtertian malaria, but whether the presence of the bacilli was due to her being a carrier in the ordinary sense of the term or a patient suffering with a recent attack it is not possible to say with certainty.

12. L.R., male, aged 20 years.

The statement given by this patient on coming to hospital was that he had been suffering with fever and headaches for two days previously; though he had not been feeling really well for three or four days prior to that. The temperature on admission was 102° F., the tongue was slightly coated, but moist, the spleen was distinctly enlarged, and the liver was also palpable. Examination of the blood showed the presence of *P. falciparum*, while a Widal test gave an agglutination of *B. paratyphosus* A, up to 1 in 50 and of *B. typhosus* to 1 in 30 only. The temperature became normal five days after admission and the case was regarded as probably one of Paratyphoid infection only, the agglutination of *B. typhosus* being of the nature of a group reaction. Some ten to twelve days later the patient felt quite well, and was grumbling at being still kept in hospital, but on the blood being again examined, more as a matter of curiosity to see whether the reaction was the same as before, it was found that agglutination of *B. typhosus* was very marked up to 1 in 500 at least, higher was not tried, while *B. paratyphosus*, A, was agglutinated up to 1 in 50 only as before. The stools were also examined and *B. typhosus* was isolated from them; *B. paratyphosus*, if present, was not isolated. Smears of the blood examined the same day revealed the presence of *P. falciparum* and also *P. vivax*, both in large numbers, one or two in every field of the smear, and several corpuscles contained two and occasionally three parasites. The patient was told that he could not leave just then, at which he was very angry, stating that he felt perfectly well. The temperature being taken frequently during the day showed a rise at 2 p.m. to 102° that day, but with very little discomfort, and after this never exceeded 100°, and on treatment with quinine soon became normal again, and the patient left a month after his first admission (Chart 10).

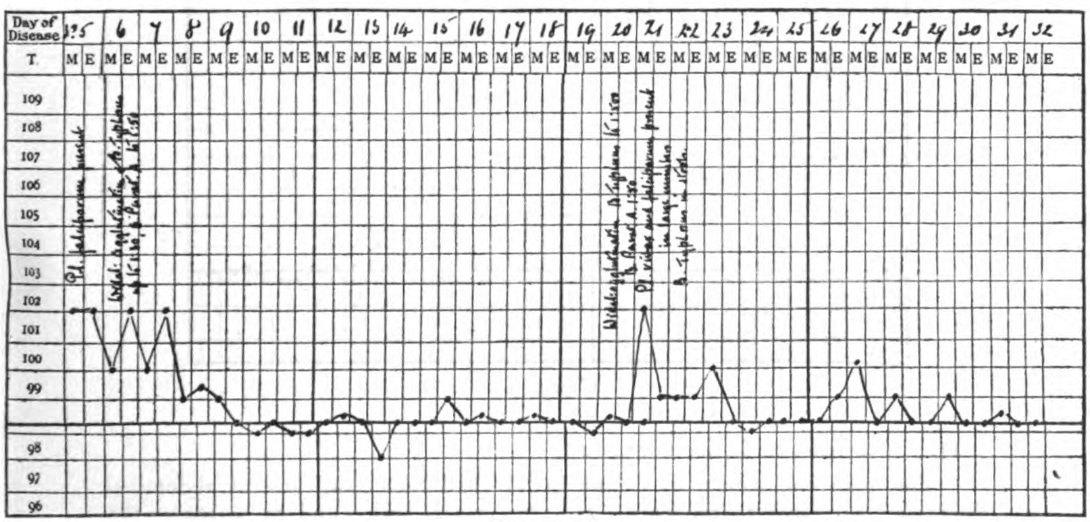

CHART 10

There are several points worthy of note in this case: firstly, the possible double infection with *B. typhosus* and *B. paratyphosus* A, the reaction being more marked with the latter in the early stage and with the former later; secondly, the coincident infection with *P. falciparum*; thirdly, the exceptional mildness of the mixed infections, the temperature becoming normal early in the second week of illness; fourthly, the rapid recovery so that the patient chafed strongly at being detained more than a fortnight in hospital; and lastly, the large infection with subtertian and benign tertian parasites without any feeling of malaise.

13. F.S., female, aged 6 years.

This child was brought to the hospital with a history of 'fever, pains in the head and stomach' for six days. The temperature in the taking-in room was found to be 102° F., the tongue was coated and the abdomen distended, and the child was admitted. Smears taken on the day of admission showed *P. falciparum*, and, as the fever persisted, a Widal test was carried out three days later and agglutination of *B. typhosus* was obtained in high dilution. Except for considerable weakness and listlessness during the third week of illness nothing remarkable occurred, and after touching normal on the seventeenth day of disease, the temperature came down finally two days later and remained normal (Chart 11).

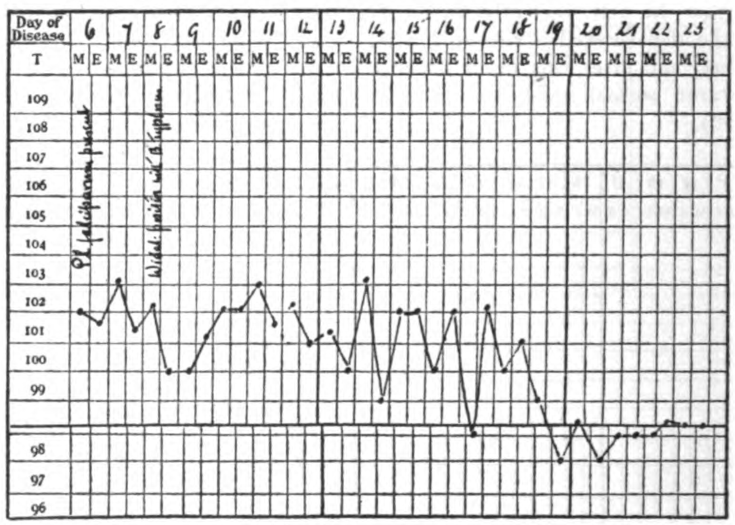

CHART 11

14. S.S., male, adult, age not known.

This man complained of feeling 'chilly and then hot,' and of abdominal discomfort for eight to ten days. His temperature was found to be 102°; the blood was examined and malarial parasites were found. Two days later spots suggestive of typhoid rash were noticed, and the stools were also 'typhoid-like,' so the blood was sent for a Widal examination and the serum gave an agglutination of *B. typhosus* up to 1 in 50 distinct, partial in 1 in 100. By this time the temperature was normal (see Chart 12). Except for a recurrence of the malaria a week later there were no further untoward symptoms.

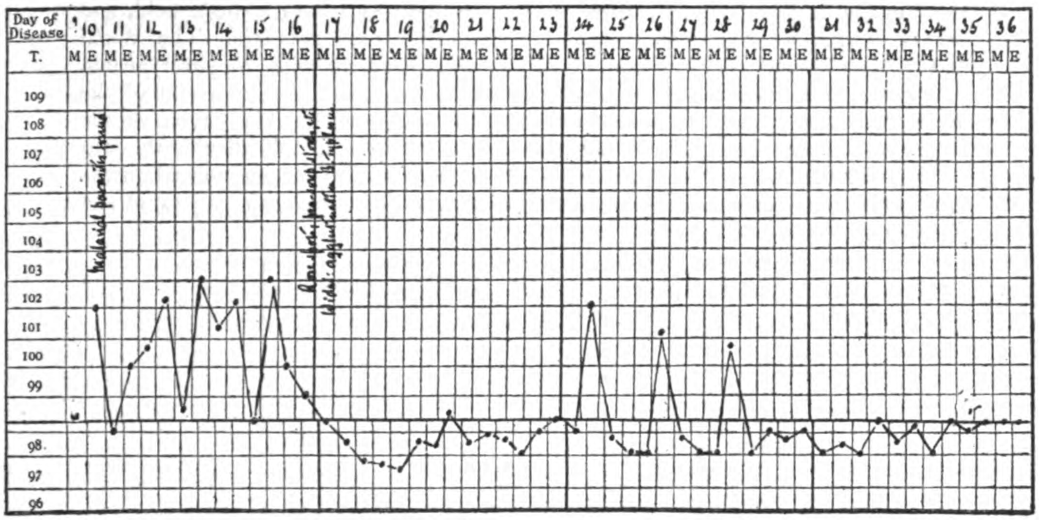

CHART 12

The record of temperature in this patient is that of uncomplicated malaria. The symptoms of enteric fever, I am informed by the medical attendant, were quite distinct; rose spots, tender abdomen with some distension, offensive 'pea-soup' stools. This man had never had any previous attack of fever so far as he was aware, he had only come out from England a few months before and he had never had any prophylactic typhoid inoculation.

As the patient had been feeling ill for some eight to ten days before admission and the spots were first seen six days afterwards, it would appear that the malarial element was the main cause of the early indisposition. If we take it that the rash came out on the seventh or even tenth day of disease (enteric), the fall of the temperature and the generally good condition of the patient point to the case being an abortive one from the enteric aspect. Of a certainty the malaria had no adverse effect upon the enteric fever.

15. C.P., male, aged 28 years.

This patient was admitted to hospital stating that he had been suffering with 'fever' for the previous nine days; apart from 'feeling feverish,' he stated that he did 'not feel at all ill,' nor did he appear so. Beyond a temperature of 101° F., a somewhat coated tongue and a just palpable spleen, there was nothing objective made out. There was no abdominal distension or pain. He had never suffered from any previous attack of fever of any prolonged duration, and had always been very healthy. The blood was examined by smears for malarial parasites, by cultivation and for Widal's agglutination reaction. The smears showed a heavy infection with *P. falciparum*, often as many as eight or ten in a single field, and several corpuscles contained two and occasionally three parasites. The Widal test gave a strong agglutination of *B. typhosus*, negative with *B. paratyphosus*, A and B. The culture was negative as regards the blood, but from a stool the *B. typhosus* was present in large numbers and was isolated. The course of the illness was exceptionally mild; the patient was most cheerful and ridiculed the idea of his being ill enough to be kept in bed. The temperature became normal on the fourteenth day of disease; convalescence and recovery were exceptionally rapid and uninterrupted (Chart 13).

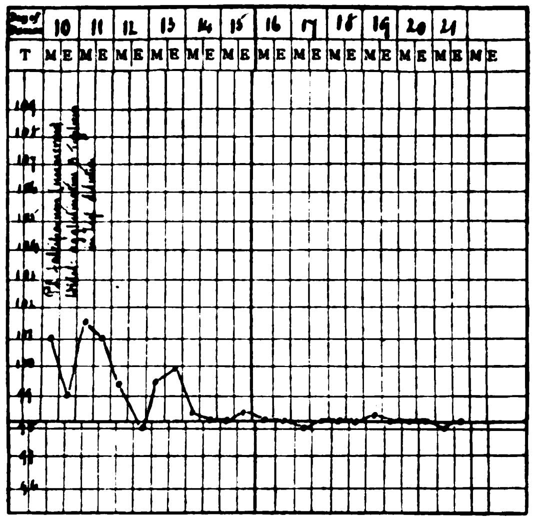

CHART 13

Very little comment is needed. There was no history obtainable of any previous illness pointing to enteric, and the patient was very intelligent and his relatives bore out his assertion. The Widal reaction cannot thus be interpreted as residual from a previous attack. Isolation from the blood would hardly be expected as late as the tenth day of disease; hence the attempt, which was successful, to isolate the organism from the excreta. He had not been inoculated, in fact, none of the cases here recorded had had inoculation.

The number of cases described is too small to allow of any generalisations, but so far as one may venture on statements based on so few cases, and feeling that those statements may and probably will have to be modified as further instances are met with, one may note provisionally:—

1. That the effect of dual infection with malaria and enteric fever does not merit any graver prognosis than the latter alone, and that in many, if not most, of the above the temperature fell early and the attack proved to be mild.

   Judging from the accounts given, it may be argued that the cases were mild from the enteric point of view, and that the quinine dealt successfully with the malarial element of the illness. It may be so, but, on the other hand, enteric fever in Jamaica, at all events in Kingston, is by no means a mild disease. The mortality is high, and in those who recover, convalescence is as a rule prolonged, and the debility marked; therefore it is, *prima facie*, hardly credible, at least it is extremely improbable, that all these cases in which malaria and enteric fever were coincident should be exceptionally mild.

   Further, malaria here is also a serious condition; this is the opinion of medical men who have practised in Jamaica for many years, and the laboratory records show that of the many specimens sent up for examination 80 per cent. of those revealing parasites show *P. falciparum*, and the recorded mortality from malaria alone is high.

2. We are, therefore, driven to the conclusion that the presence simultaneously of two diseases, one protozoal, the other bacterial in nature, each of which is usually severe in type and effects, leads to the production of an illness of comparative mildness in both these respects.

3. Sir Almroth Wright's quotation at the beginning of this paper is to the effect that anti-typhoid inoculation appeared to reduce susceptibility to malaria. If we regard the attack as equivalent to inoculation, then it would appear (as in Cases 12 and 15) that the malarial infection, though a heavy one, caused very little constitutional upset, and that the patients experienced no malaise and wondered why they should stay in bed when they felt quite well. In the above cases, however, there are instances which are in favour of the corollary to this, viz., that the presence of malaria coincidently with enteric fever appears to modify greatly the severity of the latter. The point I wish to

emphasise is not quite the converse of the typhoid and malaria statement: that as anti-typhoid inoculation appears to reduce susceptibility to malaria, so malaria reduces susceptibility to typhoid, for I do not think this is the case. The majority of persons out here suffer at some time with malarial infection, and, as stated, four out of five are infected with *P. falciparum*; nevertheless, enteric fever is a severe disease here with a comparatively high mortality. But when the two conditions, typhoid and malaria, are present together in the initial stages of the former, the course of each is modified, and favourably so; in other words, the presence of malarial parasites in the blood appears to have a mitigating effect on the severity or the course of an attack of enteric fever.

4. There arises the question as to whether, *in the tropics*, quinine has a beneficial or any effect on the severity and course of enteric fever. I say expressly 'in the tropics' because in temperate climates one has had repeated proof of the old dicta 'any fever which does not yield to quinine is not malarial', and 'in any case of fever of more than a week's duration suspect enteric.' The effect, if any, is but slight, judging from a very large number of cases of ordinary enteric fever seen here. Of course, in a patient who has been subject to malarial attacks the exhibition of quinine may modify slightly the course of fever at the onset of enteric, but as a rule it is the failure of quinine to mitigate or reduce the temperature that brings up the suspicion of the presence of enteric fever, especially when the blood is not submitted to examination for malarial parasites. In other words, from experience of a large number of cases one may say that quinine has very little effect on ordinary cases of enteric fever.

5. Can malaria of itself lead to a positive Widal reaction in the absence of enteric fever? (It may be stated again here that none of the cases quoted had ever been given anti-typhoid inoculation, so that the reaction obtained was not due to inoculation agglutinins.)

With a view to testing this aspect of the question, I have for some months past, when taking smears from patients suffering from malarial fever, taken blood also for the agglutination reaction. I have tested some two hundred cases in this way and in no instance have I obtained definite positive results in uncomplicated malaria. Occasionally a loss of motility and a weak attempt at agglutination might be noticed in dilutions up to 1 in 20, rarely possibly 1 in 30, but never higher than this. So far, therefore, it may be said that the Widal reaction is not given by patients suffering from uncomplicated malaria. We may safely say that in high dilutions the reaction is specific, in lower, group agglutinins come into play, while in very low dilutions, even normal blood at times appears capable of giving some degree of agglutination. In the above cases the majority came to hospital too late for blood culture to yield successful results, and one had to rely largely on the Widal reaction and the isolation of the bacilli from the excreta. In one case, B. M., the patient was seen on the fourth day, and the organism was obtained by blood culture.

SUMMARY

1. Anti-typhoid inoculation, it has been reported, reduces susceptibility to malaria.

2. Enteric fever in Jamaica is a severe affection with comparatively high mortality.

3. Malarial infection in Jamaica is also a severe condition, in a large majority of instances of the subtertian variety.

4. Quinine has not any marked effect on uncomplicated cases of enteric fever.

5. The serum of patients suffering from uncomplicated malarial fever will not give a positive reaction to Widal's agglutination test.

6. Coincident enteric fever and malaria (that is, when a patient is seen early in the attack of enteric fever and examination of the blood reveals the presence of malarial parasites at this early stage), in many instances at least is remarkably mild, in type and in

course, and recovery is usually rapid and complete, more so than in the case of either affection separately.

I desire to express my acknowledgments and thanks to Dr. T. R. Matthews for allowing me access to these cases, for eliciting histories, interviewing friends and relatives, and facilitating me in obtaining specimens for examination from the patients.

REFERENCES

LYON (1900). *Johns Hopkins Hosp. Rep.* Vol. VIII, cited by Kolle and Wassermann, 'Handbuch der pathogenen Mikro-organismen,' Vol. III, p. 777.

MANSON, Sir PATRICK (1917). Tropical Diseases, 6th Edition, pp. 117, 380.

SCOTT, H. H. (1913). *Practitioner*, Nov.

—— (1915). Enteric Fever in Kingston and its suburbs. *Ann. Trop. Med. & Parasitol.* Vol. IX, p. 239.

WRIGHT, Sir ALMROTH (1919). *Lancet*, March 29th.

THE METABOLISM OF WHITE RACES LIVING IN THE TROPICS

II. THE COMPOSITION OF THE URINE

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(*Received for publication* 17 *June*, 1919)

In the course of a study of the effects of a tropical climate upon a white working race, a number of urines have been analysed to ascertain whether they showed any marked variation in the quantities of constituents from those given in the physiological text-books as the average excretions for dwellers in a temperate climate.

Investigations on the urine of white men living in the tropics have previously been carried out by a few workers only, and these have been done mostly with the object of inquiring whether the changed conditions bring about an altered mechanism for regulating the thermal equilibrium of the body.

The only systematic work of any extent is that of Eijkman (1893), in Java, who estimated the calorific value of the food of nineteen Europeans, and drew up a balance sheet between the nitrogen of the food and that excreted in the urine and faeces. In the course of these experiments he analysed the daily urine of his subjects over a period of fourteen days.

A few workers have recorded the results of observations upon themselves made during a short visit to the tropics, and have compared these with figures obtained in Europe before or after their journey. Among these latter may be mentioned Schilling and Jaffé (Schilling (1909)), who compared the composition of the diet taken by themselves in Europe with that consumed during a short stay in West Africa; in addition they determined the nitrogen excreted in the urine and faeces in both places. Rancke (1900), Wick (1910), Glogner (1909), and a few others have also published the results of a few observations made on themselves.

The results have been recorded of the examination of the urine of a number of American soldiers serving in the Philippines, as well as of natives of these islands, but these observations have been mainly confined to volume and specific gravity (Chamberlain (1911)).

A certain amount of work has been done on the composition of the urine of native races in the tropics. Eijkman (1893) analysed the urine of a few Malays for comparison with his Europeans, whilst McCay (1912) has recorded observations on a large number of urines of Bengalis, and Aron (1909) has examined urines of natives in Manilla. More recently Campbell (1917) published the results of analyses of the daily excretion of a few native medical students in Singapore. In most instances it was found that the urines of natives showed marked differences from the European standards, thus, for example, the nitrogen was generally much lower, which may be accounted for by the native diets being generally poorer in protein, and richer in carbohydrate than the diet usually consumed by the white man.

The results of these previous investigations will be discussed in reference to those recorded in Townsville in the following pages.

In a previous publication (Young (1915)) the results were recorded of an investigation carried out in Townsville into the partition of nitrogen amongst the various nitrogenous constituents of the urine. It was found that the total nitrogen in the urine was somewhat low, but the proportions of this nitrogen in the individual compounds agreed approximately with those found elsewhere.

It is a common belief that in the tropics the excessive loss of water through the skin causes a corresponding decrease in volume of the urine, and that the continued passing of concentrated urine is responsible for a large amount of kidney disease. In the present investigations, therefore, the main object has been to compare the concentration of urine with that found in Europe, and attention has been confined to the volume, specific gravity, total nitrogen, freezing point (osmotic pressure) and certain inorganic constituents.

METHODS

The following methods were employed in these experiments. The specific gravity was determined at 15° C. with a Mohr's balance, the total nitrogen by Kjeldahl's method, and the sodium chloride by Volhard's method, i.e. by adding a known amount of silver nitrate, filtering off the silver chloride, and estimating the silver nitrate left unchanged by titration with potassium sulphocyanide in presence of a ferric salt. The phosphate was determined by titration with a uranium solution. The freezing point of the urines was obtained in the usual manner.

The results are put together in the accompanying Table 1, all the figures being obtained during the hot summer months. The subjects were drawn from various walks in life, and comprise persons doing laboratory work, a medical man in practice, a newspaper reporter, hospital wardsmen, several men doing office work in the city, a shop assistant and several labourers. The figures in the table refer to the whole urine passed in twenty-four hours, the subjects living on their ordinary diet.

As is well known, with a freely chosen diet considerable variations are observed from day to day in the quantity of the various substances excreted in the urine, so that no great accuracy can be claimed for a single twenty-four hours' sample. The following figures, however, were found for a number of persons of very different occupations, and it will be noticed that on the whole they vary from the usual standard in the same way, so that the average figures may be taken as an indication of the type of urine found in tropical Australia.

TABLE I.

Subject	Volume c.c.	Specific Gravity	Total Nitrogen grams.	Grams Sodium Total	Grams Chloride per 100 c.c.	Freezing point degrees C.	P_2O_5 grams.
1 (a)	1116	1·016	8·7	6·14	0·55	1·028	...
(b)	1000	1·017	7·8	3·60	0·32	0·935	...
(c)	525	1·027	8·0	3·60	0·68	1·726	...
(d)	515	1·026	6·8	4·75	0·92	1·907	...
2 (a)	830	1·023	10·2	5·41	0·65	1·437	...
(b)	644	1·023	8·0	3·69	0·57	1·466	...
3 (a)	885	1·025	11·0	8·15	0·92	1·643	...
(b)	814	1·032	14·7	6·95	0·85	2·095	...
4 (a)	718	1·030	10·9	8·55	1·19	2·049	...
(b)	753	1·031	11·7	10·32	1·37	2·102	...
5 (a)	624	1·032	9·8	6·73	1·08	2·119	...
(b)	556	1·031	...	4·50	0·81	2·025	...
6	965	1·026	11·7	12·35	1·28	1·869	...
7	582	1·028	6·5	6·29	1·08	1·694	...
8	874	1·027	15·0	4·82	0·55	1·929	...
9	738	1·031	14·3	5·64	0·76	2·105	...
10	852	1·024	11·7	6·69	0·79	1·714	...
11	702	1·023	9·2	5·68	0·81	1·675	...
12	875	1·021	9·7	8·85	1·01	1·613	1·24
13	593	1·022	6·9	7·24	1·22	1·752	1·57
14	1390	1·023	15·6	15·37	1·06	1·748	0·81
15	634	1·031	11·6	5·41	0·85	2·259	2·61
16	905	1·027	12·7	8·94	0·99	1·970	2·24
17	920	1·020	8·9	7·65	0·83	1·581	2·06
18	688	1·026	10·8	6·03	0·88	1·987	1·34
19	655	1·025	8·7	5·59	0·85	1·611	2·04
20	375	1·018	3·1	2·95	0·79	1·360	0·57
21	718	1·026	9·6	6·78	0·94	1·925	1·86
22	1070	1·024	14·3	10·38	0·98	...	2·61
23	922	1·027	12·4	12·76	1·32	...	1·76
24	782	1·025	10·8	7·38	0·94	...	1·96
25	621	1·023	8·8	5·00	0·49	...	1·56
Mean ...	782	1·0254	10·4	7·0	1·13	1·761	1·73
Maximum ...	1487	1·032	17·3	15·4	1·39	2·259	2·61
Minimum ...	375	1·013	3·1	2·95	0·32	0·935	0·57

22, 23, 24 and 25 are the averages over one week in each case.

The total volume, specific gravity, and inorganic salts

The figures given in the text-books (Starling (1912)) as the average for temperate climates are:—

	European	Townsville averages
Volume c.c.	1500	782
Specific Gravity	1·014 to 1·020	1·0254
Freezing point	-0·87° to -2·71°	-0·935° to -2·259°
Sodium Chloride	15·0 grams	7·0 grams
Phosphate	2·5 grams	1·73 grams

When compared with these it is seen that the volume is very considerably lower, the average being only 782 c.c. per day. The gravity is correspondingly higher. The freezing points of the urines come in every case within the range given for temperate climates, the maximum being – 2·259° C. with a mean depression of 1·76° C.

The sodium chloride shows a very interesting difference. The quantity was in every case but one (No. 8) far below the European figures. The average amount passed was found to be only seven grams per day. When the actual concentration of this salt is considered it is seen that it is not very different from that found in temperate climates, namely about 1 or 1·2 per 100 c.c. The figures actually found vary from 0·32 to 1·39 grams per 100 c.c. with an average of 1·13. This small amount of salt in the urine is thus instrumental in maintaining the freezing point about the same as that found in Europe. The small total quantity of chlorides present in the urine may be accounted for by the excessive perspiration, and a quantity of sodium chloride being excreted in this way.

The amount of water and sodium chloride lost in the sweat

The quantity of water lost in perspiration in the tropics is very considerable. Eijkman (1893) estimated that in his subjects, laboratory workers, an average of 1730 c.c. was lost per day in the perspiration and from the lungs. This, however, seems a small amount for a man doing manual labour in a hot climate such as that of North Queensland, and where all the work is carried out by white people, who work mostly to the same hours as in a temperate climate, that is during the hottest part of the day. Hunt (1912) in a paper upon the effects of a dry hot climate upon the body, describes his experiences during a march in the Deccan in India, with the dry bulb temperature at 104° F., and states that none of his party consumed less than three gallons (13·6 litres) of water per day, a quantity which he considers as the minimum advisable. He states that even with this quantity of liquid the flow of urine was by no means free. Most of this water, therefore, must have passed out by the skin and lungs.

In a warm moist climate, where there is little evaporation, the slightest exertion causes profuse sweating, and this saturates and clogs the clothing, and thus further interferes with free evaporation from the skin. This induces further excretion of sweat, a sort of vicious circle is set up, and the body is not able to utilise the sweat economically. The result of an increased consumption of water is an increased perspiration, which adds to the discomfort, and it is only when a very large amount of water is taken that any marked increase in the quantity of urine is observed. In spite of this discomfort the slightest exertion causes an increased desire for liquid, and a large quantity of water is consumed, and thus a corresponding amount of water is excreted by the skin.

In some experiments carried out in Townsville the loss in body weight was observed during a brisk walk of seventy minutes at the rate of about four miles an hour, the thermometer at the time being 80° to 85° F. dry bulb, and 70° to 80° F. wet bulb. The loss of weight in some subjects reached as much as 1100 grams, most of which would be due to loss of sweat. Moreover, in these experiments only the water actually evaporated was determined, the subjects being weighed in their clothing, which was saturated with sweat, so that the total loss of water must actually have been much greater than this quantity.

Losses of water such as this must be quite a common occurrence with people doing manual labour in the tropics. In discussing this question with a local carpenter, the author was informed that in the workshop it is customary for the men—four in number—to fill in the morning a large bucket, capable of holding nine to ten litres, with water, and from time to time during the day these men dip their pannikins into it and drink. The whole contents of the bucket are consumed during the day, and it generally has to be replenished before the day's work is over. In addition these men will drink a large amount of tea and other liquids outside and at their homes. The great bulk of this water must be excreted in the sweat. It seems probable, therefore, that with manual work at least four or five litres of water per day must be passed through the skin.

With four workers in the laboratory the amount of actual liquid taken in was found to average between three and four litres per day per man, whilst the average volume of urine passed was only 817 c.c.

In order to estimate the quantity of sodium chloride which may be removed from the body in the sweat, determinations of this salt were made in the sweat of two subjects. The sweat was collected in a room in which the air was practically saturated with moisture at 95° to 96° F. The body was well washed down beforehand, so as to remove all old sweat residues. Generally two samples collected at an interval of half an hour agreed well in the sodium chloride content, showing that under these conditions there was no concentration of sweat on the skin, and that the specimens represented a fair sample of the sweat excreted. The sodium chloride was estimated by a modification of Volhard's method.

With one subject, upon two occasions, samples of sweat were found to contain 0·36 and 0·31 grams of sodium chloride per 100 c.c. respectively. During the first experiment the subject lost 650 grams in body weight during an hour in the hot room. The loss in weight due to respiratory exchange* during this time, as

* The loss in weight due to the respiratory exchange was calculated in one experiment. The expired air was measured by breathing into a Zuntz meter and a sample of this air was analysed for oxygen and carbon dioxide. The subject breathed into the meter for five minutes just before going into the hot chamber, again after thirty-five minutes, and a third time after seventy minutes in the chamber. The volume of CO_2 evolved and oxygen absorbed in c.cs. per minute reduced to N.T.P. were :—

	CO_2 evolved	O_2 absorbed
Before entering	197	216
After 35 minutes	294	327
After 70 minutes	311	375

An average of these rates over the whole time gives as a rough estimate 19 litres of CO_2 evolved and 22 litres of O_2 absorbed, i.e. a total loss of about 7 grams in weight.

well as the loss in water from the lungs with the inspired air saturated at 96° to 97°, may be neglected, so that the loss of weight represents loss by sweat. The sodium chloride excreted in the sweat during this time was thus 2·34 grams.

Samples of sweat from a second subject were collected under the same conditions, and were found to contain 0·22 grams of sodium chloride per 100 c.c. During the hour and a half which the subject spent in the hot chamber, he lost 2,000 grams in weight, corresponding thus to 4·4 grams of sodium chloride from the skin. In another experiment on this subject the sweat was found to contain 0·11 grams of sodium chloride per 100 c.c.

Hunt (1912) found that his sweat contained 0·18 to 0·20 grams per 100 c.c., and he obtained higher figures (0·4) with other subjects. The quantity of sodium chloride excreted in the sweat may therefore be taken as from 0·1 to 0·4 grams per 100 c.c. A daily loss of sweat of several litres would, therefore, correspond to a good many grams of sodium chloride, and the deficiency of this salt in the urines under examination (7 grams instead of 15) may easily be accounted for by increased excretion by way of the skin.

The fact that so much sodium chloride is excreted in the sweat does not mean that the kidneys are relieved of any work, but rather the other way, since this salt after passing from the blood through the glomeruli of the kidney must have been subsequently reabsorbed in the tubules of that organ.

The phosphates

The quantity of phosphates expressed as phosphoric acid averaged 1·73 grams per day with a maximum of 2·61 and a minimum of 0·57. The normal figure given by the text-books is 2·5 grams, so that the phosphate was slightly lower than the standard. The concentration of phosphate was, however, much higher than the standard, 0·25 grams per 100 c.c. as against 0·17 grams. In samples of urine collected here, it is a common thing for phosphates to separate out within a very short time after the urine has been passed, and in many cases the urine is actually voided in a cloudy condition, which is due to precipitation of the phosphates, since it clears at once upon the addition of acid.

The total nitrogen

The urines examined in Townsville gave an average daily figure of 10·4 grams of nitrogen. Considerable variations were found in the same individuals at different times. In the following table are seen the daily averages of the nitrogen secreted by four persons over different periods:—

Subject	Date	Days	Volume	Specific Gravity	Nitrogen
1	March, 1913	9	986	1·026	11·7
	December, 1913	8	772	1·030	11·6
	February, 1914	7	1070	1·027	14·3
	January, 1915	7	849	1·026	12·4
	Average ...				12·1
2	September, 1914	4	922	1·029	12·4
	January, 1915	4	735	1·030	11·2
	Average ...				11·8
3	March, 1913	7	621	1·026	8·8
	January, 1915	7	946	1·025	7·8
	March, 1917	7	667	...	9·2
	Average ...				8·6
4	April, 1914	7	782	1·025	10·8
	January, 1917	4	737	1·023	9·1
	Average ...				10·1

Although in these four cases the average nitrogen differed fairly considerably from time to time, yet in general it was markedly lower than the European average, the mean of all four persons being 10·6 grams per day.

The figures all point to the fact that the nitrogen is below the European standard.

Urines collected during the cool season

Northern Australia has a hot and a cool season, and during the latter (May to October) very different climatic conditions are observed, the wet bulb thermometer is much lower and occasional spells of what is felt by the inhabitants to be cool weather are experienced. A few urines have been collected during this season for comparison, and these are given in the table below, the numbers being the averages of urine collected for several days.

TABLE II.

	No.	Volume c.c.	Specific Gravity	Total Nitrogen grms.	NaCl. grms.	NaCl. per cent.
	1	1437	1·017	12·1	...	...
	2	1037	1·021	11·7	...	...
	3	1372	1·012	10·5	...	...
	4	1367	1·014	11·0	...	...
	5	1214	1·016	9·5	9·87	0·80
	6	1912	1·016	14·5	9·95	0·59
	7	1766	1·018	7·9	8·24	0·72
	8	1441	1·020	9·4	13·58	0·97
	9	878	1·027	10·2	9·15	1·07
	10	1151	1·015	9·7	5·86	0·48
Average	5-10	1357·5	1·0187	10·2	9·44	0·77
Same subject hot Season		770	1·0257	9·95	6·55	0·86

These figures show a decidedly larger volume and smaller gravity than those obtained in the hot season, whilst the sodium chloride is larger in amount, but less in concentration. The urines of the last six subjects are averaged separately in the table for comparison with those of the same subjects during the hot season.

DISCUSSION

The records in the literature of the composition of the urine passed by the white man in the tropics are not very plentiful, and often contradictory in the results obtained.

Eijkman (1893) found that his nineteen European subjects in Java over fourteen days gave the following daily averages:—

Volume 1,442 c.c., specific gravity 1·017, nitrogen 13·04 grams. These urines, therefore, did not differ to any marked extent from those found in Europe as regards concentration, volume and nitrogen. Other workers have recorded the results of observations on themselves. Thus Plehn (1908) in West Africa found that his urine had an average daily volume of 1,075 c.c., and a specific gravity of 1·025, whilst Glogner (1909) found the volume practically identical in Sumatra and Berlin.

Neuhaus (1893), during a journey round the world in 1893, tested the average volume and gravity of the urine which he passed daily at various places during his journey. In the tropics the volume varied from 1,100 to 1,200 c.cs., and the specific gravity from 1 029 to 1·033, whilst outside the tropics the volumes ranged from 1,353 to 1,609 c.c., with a gravity of 1·021 to 1·023. His urine was thus more concentrated in the hotter parts of the world.

Chamberlain (1911) took the gravities of single specimens of urine of five hundred and ninety-six soldiers in the Philippines upon two occasions at a year's interval, and he obtained the figures 1·099 and 1·097. He concluded that the urine was therefore little different in gravity from that found in a temperate climate.

In a recent research Campbell (1917) found that his own urine in Singapore conformed in volume and gravity to the European standard, being 1,560 c.c. and 1·012 respectively. It is noteworthy in the last instance that the sodium chloride content averaged only 8·10 grams, or 0·52 per cent.

When the averages found in Northern Australia are compared with the figures quoted above, it is seen that they differ in that the volumes obtained elsewhere were much higher than those recorded in Australia, whilst the specific gravities were generally lower.

A comparison of the nitrogen with that found in Europe and by other workers in the tropics shows also certain differences. Our ideas of the metabolic changes which the protein taken in the food undergoes in the animal body have altered considerably in the last decade. The modern view assumes that the proteins are broken down by the digestive enzymes of the intestines into their constituent amino acids, which are then absorbed. The greater part of these are denitrified, the bulk of the nitrogen being quickly eliminated in the urine as urea. A small portion goes to make good the wear and tear of the tissues. The nitrogen in the urine represents, therefore, mainly the substances produced directly from the proteins of the food (exogenous metabolism, Folin), which vary in quantity with the amount of food taken. In addition it contains the nitrogenous substances such as creatinine produced by metabolism of the tissues (endogenous metabolism, Folin), which, according to Folin, are not materially different in quantity whether the diet is rich or poor in protein. The nitrogen in the urine is not an accurate measure of the quantity of protein metabolised, since a certain quantity is excreted into the intestine and passes out with the faeces, a certain quantity may be retained in the body, and a small amount is also lost in other secretions of the body such as the sweat. Still the total nitrogen in the urine may be taken as a rough indication of this protein.

In ordinary life when an indiscriminate diet is consumed, naturally great variations occur from day to day in the quantity of nitrogen passed in the urine, but the average figure as given in the text-books on physiology for temperate climates is about 15 or 16 grams of nitrogen per twenty-four hours.

Pflüger and Bohland, and Bleibtreu and Bohland (quoted by Eijkman) give rather less than this, namely 12·67 and 14·93 grams per day.

With regard to the nitrogen in the urine of white people in the tropics, Eijkman found that the urine of his nineteen European subjects on an ordinary mixed diet contained a daily average of 13·04 grams. He concluded, therefore, that an acclimatised European in the tropics passed as much nitrogen in his urine as he did in Europe.

Schiller and Jaffé (Schilling (1909)) during a short visit to West Africa, carried out experiments on themselves, and compared these with observations made in Europe before and after their journey. They found that in both parts of the world nitrogen equilibrium was approximately maintained on 17 grams of nitrogen a day, so that no appreciable difference was observed in the urine nitrogen.

Similarly Campbell (1917) in Singapore found that his own daily urine contained on the average 15·3 grams of nitrogen, similar again to the European standard.

These results would indicate that the quantity of protein required in the tropics is not different from that consumed elsewhere, and in fact Eijkman's analyses of the food eaten by his subjects, conformed in every respect to the usual standards. A similar conclusion was also arrived at by other researchers.

On the other hand Ranke (1900) published a comparison of his own diet during a visit of a few months to Brazil. This diet showed that although the protein was not much different, yet the total calorific value was much less in the tropics than in Europe. Glogner explained Ranke's observations as probably due to a loss of appetite, which he states is a common experience during the first period of residence in the tropics, and he concluded that Ranke had not resided in the tropics long enough to have passed this stage.

With regard to the nitrogen in the urines analysed in Townsville the average obtained was very much lower than that of the European text-books, and did not agree, therefore, with the results recorded by others in the tropics.

A certain difference in the nitrogen might be expected in a hot climate, on account of the larger quantity of water excreted by the skin, which takes with it a small quantity of nitrogen. Eijkman found the nitrogen lost in this way by Malays to be about 0·76 to 1·36 grams, and he estimated that Europeans in Java lost about 1·6 grams per day.

Benedict (1906), outside the tropics, found that during rest the nitrogen secreted in the sweat was approximately 0·071 grams per day, but during muscular exercise he found as much as 0·13 to 0·22 grams per hour.

In experiments carried out in this laboratory and already referred to, samples of sweat were obtained and determinations were made of the nitrogen in them. This was found to be 0·040 and 0·057 grams per 100 c.c. upon two occasions in one subject, and 0·033 and 0·030 grams per 100 c.c. in the other; the two subjects thus lost only 0·26 and 0·66 grams of nitrogen respectively during an hour in the hot chamber.

Allowing a man to lose from three to four litres of water a day by way of the skin, this would only account for a daily loss of 1 to 2 grams of nitrogen by this means. If this be allowed for it would bring the average nitrogen to from 11 to 12 grams per day, and a further allowance of 10 per cent. lost in the faeces would bring the total to about 13 to 13·5 grams, which, taking the higher figure, corresponds to about 87 grams of protein, a figure still below that usually accepted as a standard (viz., about 100 grams (Voit)).

Eijkman found that his subjects in Java consumed on an average 99·6 grams of protein, of which 88·2 grams were actually absorbed, the rest being lost in the faeces. Taking Eijkman's average figures for nitrogen in the urine, 13·04 grams, and making the above allowances for sweat and loss in the faeces, it works out at about 101 grams protein per day, a number in close agreement with that found. It would appear, therefore, that with the subjects experimented on in Townsville the protein actually katabolised was less than that found by Eijkman in Java.

When an explanation is sought for these different results, a possible one suggests itself in the different conditions under which life is carried on in the Australian tropics. Eijkman's subjects, for example, were living in Java, where cheap native labour is to be obtained in abundance, and it is the rule to rest during the early and hot portion of the afternoon. In Queensland, on the other hand, as has been pointed out already, work is carried on by white people, and it is the exception to rest during the hotter hours of the day.

The results of examinations of the urine of native races in the tropics show differences from that of white people. As a rule, the native consumes less protein and more carbohydrate than the white man, and as might be expected, therefore, the urines are lower in nitrogen. Eijkman found that the daily urine of thirteen Malays gave an average of only 8·08 grams of nitrogen. Campbell, for native medical students in Singapore, obtained figures varying from 6·64 to 9·25 grams of nitrogen per day. On the other hand, Aron, in the Philippines, found a higher number, the nitrogen in the daily urine of the Philippino being stated to vary from 10 to 12 grams.

McCay (1912) has made a large number of observations upon the urine of Bengalis in India, and has compared them with the standards for Europeans. Very decided differences were found to exist, as is seen in McCay's table which is given below. However, if the Townsville urines be placed side by side with this table, the differences from the European standards are almost as striking for some constituents as those given for the Bengalis. The urea and sulphate given below were obtained from five people, and are the daily averages for a week in each case.

TABLE III

	European	Bengali	Townsville
Volume c c.	1440	1200	784
Specific gravity	1020	1013	1025
Urea grams	35	13	20·7
Total nitrogen grams ...	18	6	10·4
Freezing point	−2·5° C.	−1·24° C.	−1·76° C.
Chlorides grams ...	15·00	10·00	7·53
Phosphates grams... ...	3·50	0·98	1·73
Uric acid grams	0·75	0·45	0·48
Sulphates grams	2·50	1·88	2·01

The average daily excretion of nitrogen found in Townsville (10·4 grams) may be compared with that obtained by Chittenden (1911) from one hundred and eight university students in the United States, the average daily nitrogen in the urine being 12·87 grams.

It is interesting also to compare the samples for one week obtained from four men doing laboratory work in Townsville with those averages given by Hamill and Schryver (1906) for seven men doing similar work in the laboratory of University College, London.

	Weight kilos	Total N. grams
1	67	14·3
2	61	12·4
3	63	10·8
4	48	8·8
Average	59·8	11·6
Schryver and Hamill	72·4	13·5

Although the average nitrogen is actually less in Townsville, yet when considered per kilogram of body weight the figures are practically the same (0·194 and 0·186 grams), and both correspond to an amount of protein very much below what used to be considered as the daily standard (approximately 100 grams of protein), even when all due allowances are made for the nitrogen lost in the faeces.

ALBUMINURIA

It has frequently been stated that the higher concentration of the urine in the tropics causes a greater amount of kidney disease than is found in temperate climates; moreover, in the opinion of many medical men in Northern Australia, a greater incidence of kidney troubles is found there. As reliable figures to test this could not be found, an attempt was made to obtain some indication by ascertaining the prevalence of albuminuria in Townsville.

A number of samples of urines from out-patients of the General Hospital were collected and tested for the presence of albumin. In no case did the subject show any other clinical symptoms of kidney trouble. The tests employed were the boiling test, the salicyl sulphonic test and the ferrocyanide test, and no urine that did not give all these tests was accepted as definitely containing albumin. The urine from six hundred and sixty-three patients was examined, including three hundred and sixty men and three hundred and three women, and a positive reaction was obtained in fifty-seven cases, forty-two of them being men and fifteen women. This would correspond to a total percentage of 8·6; amongst the men the percentage was 11·7, and amongst the women 4·9.

No attempt is made to draw any conclusions from these figures, but it is thought of interest to put them on record. The much higher percentage amongst men than women is worthy of note, nineteen out of forty-two of the cases in males being in men above the age of forty years.

SUMMARY

The twenty-four hours' urine collected from a number of persons living in North Queensland and of different occupations was analysed. The daily volume was very much less than the European standards given in the text-books, the average volume being only 784 c.c. This volume was increased considerably in the cooler weather.

The specific gravity was very much higher, whilst the freezing point did not differ very much from that found in Europe, thus the osmotic pressure was not very much higher.

A striking difference was noticed in the quantity of sodium chloride excreted in the urine, which was very low, and this may be accounted for by the large loss of water in the sweat which carries with it this salt. It is calculated that a man doing manual labour in the tropics must lose several grams of sodium chloride per day through the skin, which would readily account for the deficiency in the urine.

The total nitrogen showed a lower figure than that found in Europe, which cannot be accounted for by loss of nitrogen from the skin, since it is shown that this can only amount to 1 or 2 grams per day under normal circumstances.

These results differ from those obtained by Eijkman and other observers in other parts of the tropics.

An examination of the urine for albumin of persons not showing any other symptoms of kidney disease showed a high percentage of albuminuria, which was more marked in men than in women.

REFERENCES

ARON (1909). *Philip. Journ. Sci.* B. Vol. IV, p. 225.
BENEDICT (1906). *Journ. Biol. Chem.* Vol. I. p. 263.
CAMPBELL (1907). *Journ. Straits Branch R. A. Soc.*, p. 57.
CHAMBERLAIN (1911). *Philip. Journ. Sci.* B. Vol. VI, p. 427.
CHITTENDEN (1911). Quoted by McCay (1912), p. 118.
EIJKMAN (1893). *Virchow's Archiv.* Vol. CXXXI, p. 170, and Vol. CXXXIII, p. 105.
GLOGNER (1909). *Arch. Schiffs. Trop. Hyg.* Vol. XIII, p. 169.
HAMILL and SCHRYVER (1906). *Proc. Physiol. Soc.* in *Journ. Physiol.* Vol. XXXIV, p. 10.
HUNT (1912). *Journ. Hyg.* Vol. XII, p. 482.
McCAY (1912). The Protein Element in Nutrition. London, p. 156.
NEUHAUS (1893). *Virchow's Arch.* Vol. CXXXIV, p. 373.
PLEHN (1908). Quoted by Wick (1910).
RANKE (1900). Quoted by Schilling (1909).
SCHILLING (1909). Tropenhygiene. Leipzig, p. 514.
STARLING (1912), Principles of Human Physiology, London.
WICK (1910). *Arch. Schiffs. Trop. Hyg.* Vol. XIV, p. 106.
YOUNG (1915). *Ann. Trop. Med. & Parisitol.* Vol. IX, p. 91.

ON THE ENDEMIC TSUTSUGAMUSHI DISEASE OF FORMOSA

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PLATES VIII AND IX

I. INTRODUCTION

In the Karenko District of eastern Formosa there is endemic in certain localities an exanthematous fever. Attention was first called to it in 1908 by its prevalence among the police engaged in building guard-lines against the savage Batran tribe of the Mokkui valley. Hence the disease goes by the name of 'Batran or Mokkui fever,' and is also called 'Horin fever,' as it frequently affects people who enter the virgin forests adjoining the village of Horin. Further, of recent years the fever has appeared among inlander* immigrants in the Yoshino, Toyoda, Hayashida and other plantations, where a number of people fell victims to the disease.

During the summer campaign of 1914 against the Taroko head-hunters of East Formosa, while acting as chief Medical Officer, I was able to examine minutely cases of the fever occurring chiefly in the Mokkui valley. My observations made it clear that the fever, which was always accompanied by swelling of the lymphatic gland system was analogous to the Tsutsugamushi or Kedani disease well known in the northern districts of Japan proper. I afterwards made more detailed investigations and laboratory experiments by permission of the Chief of the 'Commission devoted to the Study of the Endemic and Epidemic Diseases in Formosa,' and the essential features of my five reports on the disease from 1914 to the present time are summarized in the present paper, in which I use the familiar term 'Tsutsugamushi disease,' though originally,

* By the term 'inlander' the Japanese settlers in Formosa are meant.

from the clinical point of view, I called the disease 'exanthematous bubonic fever.'

II. ENDEMIOLOGICAL

A. Geographical Distribution

The localities where the fever prevails or mite-infested areas exist, or are supposed to exist, are as follows:—

(*a*) *The Karenko district.* This district is more intensely infected than others, the following foci being known:—(1) Mokkui and Rokei valley, (2) a part of Yoshino plantation, (3) Kotobuki-mura and the western part of Toyoda plantation, (4) Hayashida plantation, Horin-sho and neighbouring forests, (5) Maribashi plantation, (6) Seisui valley, (7) Shinjo and Takkiri valley, etc. Some details of the prevalence in these localities are given later.

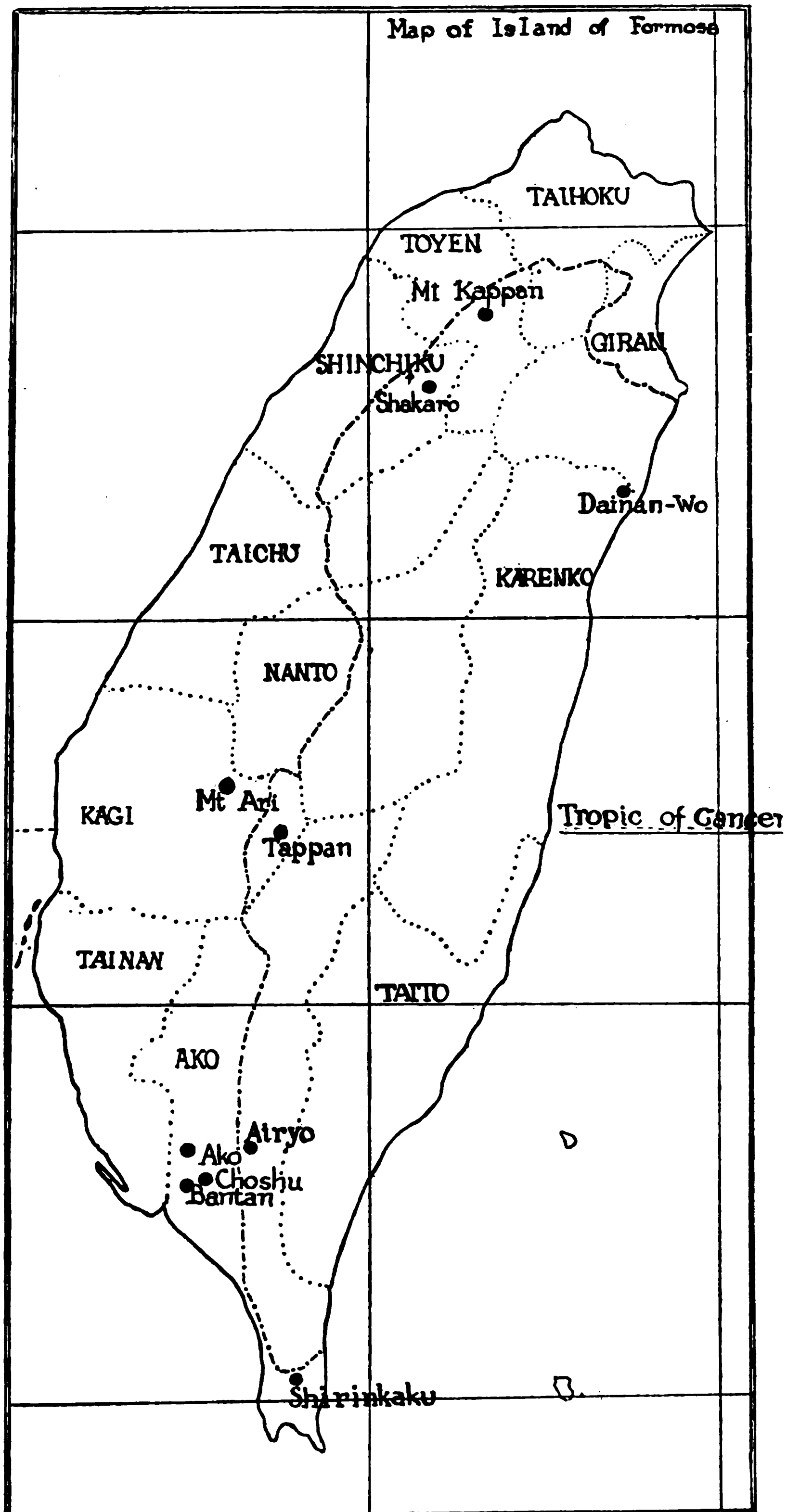
(*b*) *The Giran district.* In 1914, during the operations against the Nanwo tribe of savages, a certain number of policemen were attacked by the disease while serving in the southern branch of the Dainanwo river valley. Five typical cases came under my observation. In 1918, the total number of patients (including out-patients) infected in the Dainanwo valley amounted to about fifty, although cases had not been reported in the preceding three years.

(*c*) *The Toyen district.* In 1918 (November 15th), a police-inspector, stationed at the aboriginal boundary line of Mount Kappan, was admitted into the Government Taihoku Hospital suffering from a typical attack. It is evident, therefore, that an endemic focus exists in the mountainous areas.

(*d*) *The Shinchiku district.* In 1917, at least one typical case was recorded among the police stationed in the village of Shakaro.

(*e*) *The Kagi district.* Several cases of the fever were recorded by Drs. Kato, Sano and Senouye in 1911-13. They were said to have been infected in the forest region of Mount Ari. Recently a case occurred in the savage village of Tappan. So that it is clear that the disease occurs in mountainous areas.

(*f*) *The Ako district.* In 1914, three policemen, two in an aboriginal village of the Airyo valley and one at Maruyama, near Shirinkaku, Koshun, were affected by the fever. In 1915, one police sub-inspector. In 1916, two Japanese and three Formosans. In 1918, two Formosans were affected in the plains from Ako to Choshu.



Endemic locality ------- Aborigine boundary
.... District boundary

From other districts of Formosa, viz., Taihoku, Taichu, Nanto, Tainan and Taito, no cases have been reported, and no mite areas are at present known.

B. DETAILS OF THE PREVALENCE IN THE KARENKO DISTRICT

(*a*) *The expeditionary police forces.* Twenty-five cases, of which four proved fatal, were reported among the Batran sub-division of three thousand eight hundred men during an eighty days' campaign. Another sub-division of one thousand two hundred in the Takkiri valley had only four cases, one of which ended fatally. Not a single case was reported throughout the whole campaign among the military division which manœuvred across the central mountain range running from the west to the east coast.

(*b*) *Boundary line Guard.* Among the Japanese police serving on the guard line between Hoppo and Keiko, a thirty-mile front, twenty cases were reported during 1913-14, two of which proved fatal. Of the guard posts on the line, those along the Rokei river and Rigyo lake, and also those on the Tamoran hill, were so seriously affected with the disease that nearly all the guards and their families fell sick one after another.

(*c*) *Inlanders' plantations.* Three plantations of immigrants from Japan, i.e., the Yoshino, Toyoda and Hayashida plantations, were established under the control of the Government Industry Bureau in the District. The Yoshino plantation, founded in 1911, lies close to the foot of the mountain range, two miles westward of Karenko town. Fever cases appeared among the immigrants in 1912. It is said that it is at the foot of the mountains on the site of deserted aboriginal villages that people get bitten by some unseen insects.

On the Toyoda plantation in 1914, thirty-eight immigrants suddenly fell sick after having been engaged in felling a forest west of the railway.

In 1915, a colony of fifteen houses was established in this area, so the fever still persists.

The Hayashida plantation, situated a few miles eastward of the Horin village, was established in 1914. In the first year the fever raged so violently among the new settlers that thirteen out of ninety cases of the disease proved fatal; subsequently the epidemic declined year by year.

The morbidity and mortality in these three plantations are shown below.

TABLE I.

Tsutsugamushi disease in the three plantations.

	Yoshino		Toyoda		Hayashida		Total	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
1913	25	2	0	0	...	...	25	2
1914	30	1	38	0	90	13	158	15
1915	27	4	32	1	49	6	108	11
1916	7	0	30	0	10	2	47	2
1917	12	3	8	0	3	1	23	4

(*d*) *The Horin-sho and Maribashi plantations.* Horin and the neighbouring forest region have been noted for the disease, as the latter bears the name 'Horin fever' in the district. In 1910, members of an engineering party of the Government railway department fell sick with the Horin fever. Cases also occurred among people working in the forest, such as camphor collectors, wood choppers, charcoal burners; and even farmers are also very often affected with the disease. When the Maribashi plantation of the Yensuiko Sugar Manufacturing Co., consisting of inlanders and Formosans, was founded in 1914, there were twenty-five cases, with two deaths, but in the following years the fever declined.

(*e*) *Other localities.* There are cases notified from other localities of the district, chiefly among camphor collectors and guardmen on the Seisui valley, tunnel workers at Sappa, sugar-cane planters in Kada and Kotobuki-mura, Getsubi and Kompo-sho, etc.

The following table gives the figures of cases notified in the Karenko district during 1913-17 for Formosans and Japanese:—

TABLE II.

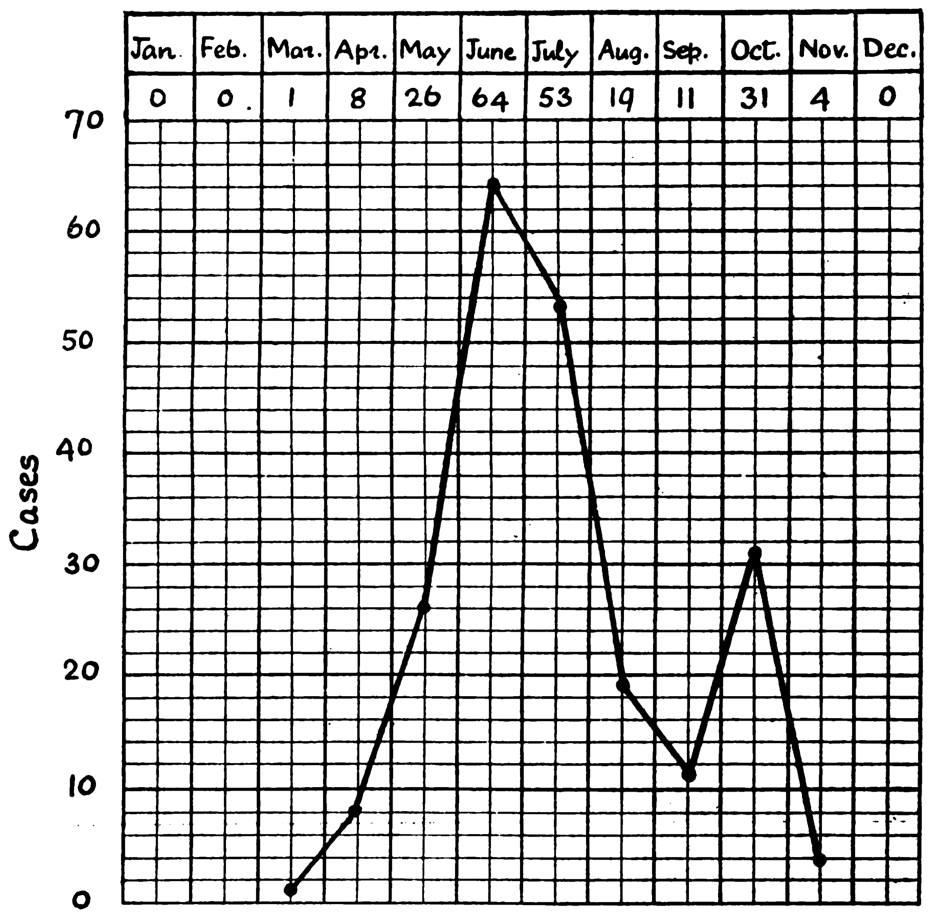
Tsutsugamushi disease in the Karenko district during 1913—17.

	Cases	Deaths	Percentage
1913	60	4	6·6
1914	305	33	10·8
1915	118	11	9·3
1916	60	5	8·3
1917	72	6	8·3

The endemiological conditions in Karenko district are as follows :—

(1) *Local features.* Noxious, i.e., mite-infected areas, are always limited (1) to the valleys of rivers; (2) to uncultivated but fertile fields covered with tall grass, mostly lying at the foot of mountains; and (3) to the flat or sloping ground covered with thick forest.

(2) *Influence of seasons.* The fever generally makes its appearance in April and persists till July, then it declines for a while, only to rise once more in October, subsiding in November, as the following curve shows :—



Monthly occurrence of the Tsutsugamushi disease in Yoshino and Hayashida plantation, 1914-15.

(3) *Race.* Inlanders, especially fresh immigrants, show the greatest susceptibility toward the virus, while the Formosans are far less susceptible and the aborigines seem to be almost immune against the infection, although one old aboriginal informed me that 'patau,' the minute red mite, which is so abundant in the noxious areas, caused fever by its bite.

(4) *Age and sex.* Adult men, being much more exposed to the danger of being bitten by the red mites than old men, children or females, show a correspondingly higher case incidence.

TABLE III.
Shewing influence of race and sex.

	1914			1915		
	Cases	Deaths	Percentage	Cases	Deaths	Percentage
Japanese male	242	27	11·15	77	7	9·09
female ...	57	4	7·00	23	2	8·84
Formosans male ...	14	2	14·2	17	2	11·77
female ...	1	0	...	1	0	...

(5) *The annual case incidence and mortality.* These rates vary greatly according to locality and year, as shown in the following table:—

TABLE IV.
Case-incidence and mortality in the three plantations.

Plantation		Population	Cases	Case incidence per 1,000	Deaths	Mortality, per 1,000
1914	Yoshino	1,236	27	21·8	1	0·81
	Hayashida ...	522	89	170·3	13	24·9
	Toyoda	681	38	55·7	0	...
1915	Yoshino	1,604	27	16·83	4	2·49
	Hayashida ...	634	49	77·30	6	9·46
	Toyoda	738	32	35·23	1	1·35
1916	Yoshino	1,694	7	4·13	0	...
	Hayashida ...	729	10	13·71	2	2·74
	Toyoda	863	30	34·75	0	...
1917	Yoshino	1,823	12	6·6	3	1·64
	Hayashida ...	740	3	4·05	1	1·35
	Toyoda	1,100	8	0·73	0	...

III. ETIOLOGICAL RESEARCHES

A. Preliminary Examinations on the Causative Virus and Mode of its Invasion

In 1914, I made a series of bacteriological investigations in order to determine the causative organism of the so-called 'undetermined fever' of the Karenko lazaret, and came to the following results:—

(*a*) Serum reactions with typhoid and paratyphoid bacilli, and with Horiuchi's bacillus of the Manchurian exanthematous fever, proved negative.

(*b*) All attempts to cultivate a specific organism from the patients' blood, urine and dejecta as well as from the spleen, post-mortem, failed.

(*c*) Smear preparations were made from patients' blood, lymphatic glands, and cutaneous ulcers, as well as from the spleen and liver, post-mortem, and stained with Giemsa. Neither bacterial nor protozoal organisms, nor cell inclusions could be found.

(*d*) Finally, blood drawn from the *vena mediana* of a patient at the acme of the fever was injected hypodermically into a native monkey (*Macacus cyclops*) with a definite positive reaction.

There is, on the other hand, no doubt about the fact that the virus invades the body through the skin, because, when carefully examined, an ulcer, the seat of invasion, is to be found in all cases, and it is recognizable where the mite has attacked the patient. It is usually accompanied by swelling of the adjacent lymphatic glands, or in other cases by a lymphangeitis apparently connecting the spot with the lymphatic gland. From these facts I easily drew an analogy between this endemic fever and the Tsutsugamushi disease endemic in the northern part of Japan, caused by the bite of the tsutsugamushi or akamushi mite. To ascertain the accuracy of the inference, I collected and examined rats in an endemic area, and found, as I expected, red mites parasitic on the rodents, and these are in every respect identical with the Japanese examples.

My preliminary conclusions concerning the etiology of the endemic fever are as follows:—

(1) The causative virus of the endemic fever is obscure, very likely of an ultra-microscopic nature.

(2) Invasion, without exception, takes place through the epidermis.

(3) This is effected through the bites of the minute red mites found in the endemic areas.

B. THE TSUTSUGAMUSHI OR AKAMUSHI, THE VIRUS CARRIER.

Zoological studies of the Japanese red mite, the fever transmitter, well known as tsutsugamushi, akamushi or kedani, have been reported, each independently, by Profs. Nagayo, Kawamura, Miyajima, Tanaka, and their collaborators, and the life cycle of the mite has now been made clear by the discovery of its parental form by these authors in 1916. According to my researches, the Formosan species of the pathogenic red mite is identical with the Japanese tsutsugamushi or akamushi, the larva of *Trombicula akamushi*,* Brumpt. And the parental forms of our species agree with those of the Japanese ones.

In the endemic area in Formosa, the red mites are found in nature parasitic on such rodents as *Mus rattus rufescens* (the common house rat of the island), *M. decumanus*, *M. musculus*, *M. agrarius*, etc., or on such insectivora as *Crocidura muschata*, commonly swarming in the interior of the ear of the animals. Sometimes pheasants, chickens, even dogs and cats, are infested by the mites. People coming out of a mite-infested field or forest occasionally carry a number of mites attached to various parts of the body such as the groin, scrotum, axilla, etc.

The parasitic red mite, 'patau,' of Formosan Ami aborigines, is the hexapodal larva of *Tr. akamushi*, Brumpt, of ovoid shape and of orange red colour; its capitulum consisting chiefly of the buccal organs. The mandibles are chelate chelicerae, each terminating in a single hook. The palpus is stout, consisting of five segments, of which the last one bears long hairs, and the penultimate one a claw. The scutum on the anterior part of the dorsal surface of the body is somewhat rectangular, and bears seven plumose hairs. One pair of the later is long and tactile (*pilus tactilis*) and attached to the pseudostigmata. One pair of eyes, most conspicuously red in colour, each consisting of two groups of four to five granules, is

* Given as *Leptus akamushi* (Brumpt) MIYAJIMA and OKUMURA. *Kitasato Archives*. Vol. I, No. 1, p. 8 (1917). Eds.

placed on the dorsum near the posterior corner of the scutum. The integument is soft and finely folded, bearing a certain number of plumose hairs (about thirty pairs), which are curved, with branched lateral ones mostly on the convex side. The type of our mite is the so-called 'thin haired,' owing to its sparsely hirsute character in contradistinction to the so-called 'coarse haired' species, *Tr. pseudo-akamushi*, Tanaka, another Japanese species. The legs are strong and have five segments excluding the coxal one. Each terminal segment carries three prominent claws. On the coxal segment of the anterior legs the stigmatic spiracle is distinctly seen. The following are the measurements of the akamushi from the Karenko district:—

	Free in Field	From a Rat	From a Man
	m.m.	m.m.	m.m.
Body length	0·196	0·265	0·4
Body width	0·16	0·2	0·3
Capitulum, length	0.085	0·63	0·09
Capitulum, width	0·057	0·05	0·05
Scutum, length	0·066	0·066	...
Sensory hair, length	0·044	0·04	...
Distance of spiracles	0·096	0·104	0·127
Distance of eyes	0·072	0·074	0·085
Palp, length	...	0·052	0·054
Palp, width	...	0·022	0·022
Chelicera hook, length	0·022	0·022	0·027
Body hair, length	0·057	0·048	0·05
Leg I, length including claw	0·204	0·19	0·21
Leg II, length	0·16	0·156	0·165
Leg III, length	0·22	0·2	0·23

The akamushi is active in its movement, and attaches itself to the host with alacrity. While it sucks, it continually moves its hind legs; and when filled to repletion it becomes paler in colour, oblong in shape and markedly larger. When an ablated rat's ear, with numerous mites, is kept *in vitro* with soil, the mites leave the host, and the fully fed ones creep into the soil where they moult and become nymphs. As observed in deutovum, the puparium has a peculiar spur on the anterior dorsum. After several days of the resting stage an eight-legged nymph emerges. The nymph is of the peculiar form of a figure of 8, characteristic of the genus *Trombicula* of Berlese, the abdominal constriction dividing the body into two distinct parts. The cephalothorax bears the mouth parts and the anterior pairs of legs, while the anterior part of the abdominal region carries two pairs of posterior legs. The colour of the body is pale, faintly reddish, and densely covered with colourless hairs. The mandibles are chelate, and the palpi consist of five segments; the terminal segment is calvate, while the penultimate has a single claw-like process. On the area sensilligera of the crista metopica, a pair of long tactile hairs (*pili sensoriales*) occur. The eye is of rudimentary character, has no lens and is represented by a mass of reddish corpuscles. On the ventral surface of the distal portion of the abdomen are the genital orifice with two pairs of suckers, and the anus. The leg consists of six joints, excluding the coxal joint, end tarsi bearing two claws, with no pulvilli. The first pair of legs is the largest of all, its apical joint being the strongest. A specimen of nymph bred *in vitro* gave the following measurements:—

	m.m.
Body length (with hairs)	0·65
Cephalothorax, length	0·26
Abdomen, length	0·45
Abdomen, width	0·36
Mandible, length	0·2
Palp, length	0·16
Genital orifice, length	0·05
Leg I, length	0·4
Leg II, length	0·24
Leg III, length	0·23
Leg IV, length	0·3
Posterior body hair, length	0·047
Sensory hair, length	0·13
Terminal Joint of Leg I	0·11 × 0·044

An attempt to develop the nymph on vegetable matter *in vitro* was unsuccessful. At the end of the nymphal stage it passed again into a quiescent condition and in due time moulted into the adul mite.

The adult form of tsutsugamushi or akamushi is found in the soil of infested fields, it is a tiny creature of the peculiar 8-shaped form; pale greyish or reddish in colour, with one pair of rudimentary eyes. The measurements of an adult male animal are as follows:—

	m.m.
Body length	0·94
Body width	0·5
Cephalothorax, length	0·2
Abdomen, length	0·74
Abdomen, width	0·5
Palp, length	0·2
Genital orifice, length	0·07
Leg I, length	0·62
Leg II, length	0·32
Leg III, length	0·31
Leg IV, length	0·4
Hair of posterior body, length	0·054
Sensory hair, length	0·13
Distance of pseudostigmata	0·054
Terminal joint of Leg I	0·18 × 0·07

In a certain infested field at Karenko another species of *Trombicula* larva was found abundantly and living freely on grasses, and according to my observations not parasitic on man. It is dark red in colour, and much larger and more rapid in its movements than the parasitic akamushi. The adult form of this species is found in the soil; its body is 8-shaped, of red colour, and the eye is provided with a well developed lens. I provisionally named it *Trombicula pseudo-akamushi* (non Tanaka), as I am inclined to consider it a new species, although Prof. Miyajima believes that the said larva is identical with the European *Leptus autumnalis*, and that the adult animal is identical with *Trombicula mediocris*, Berl, of Java.

Recently I found another species of red mite which infests the domestic fowl at Karenko. As its specific peculiarity, the fowl mite has fan-shaped sensory hairs on the scutum, and it is easily distinguished from akamushi which also often occurs on fowls.

It seems highly probable that the mite acquires the virus from the adult form and transfers it to its offspring, as in the case of the Texas fever tick. As to the fact that rodents carry the virus, we can accept the authority of Profs. Miyajima and Kawamura, who independently made successful experiments. Accordingly, the persistence of the disease in a definite area is readily explained. The further spreading of mites seems to be chiefly due to the migration of such hosts as rodents, birds, etc.

In the well-known Chinese work, entitled 'Honzo komoku' (System of Natural History), edited by Li Shiting in the 16th century, a certain 'sand mite' has been described as a fever carrier. The passage reads:—

> 'In the Moorish region in South China the so-called "Shashutsu" (literally sand mite) are found in enormous numbers. They are so small that they cannot be seen by the naked eye. If people wade through shallow water or pass through a wood by night, the sand mite will fasten upon and subsequently burrow under the skin, causing a feeling similar to that of a slight prick, each spot being inflamed afterwards to the size of a lentil. The patient dies if the mite reaches his heart. The disease appeared first in Reinan (South Fookiang). In order to avoid infection, the mite on the skin must be scratched off with a blade of grass and some lettuce juice be immediately applied on the spot. If the mite lies already deep under the skin, it is necessary to dig it out by means of a needle. The mite looks like a scrabble mite.' (Cited from Chûgoho.)

The sand mite of South China and the tsutsugamushi of Japan were first identified about ninety years ago by Genkei Ohtomo and his son, both experienced physicians of the Akita district.

Now, from a zoo-geographical standpoint, Formosa belongs to the Indian or Oriental region, as is amply exemplified by the similarity of fauna, especially the venomous snakes and mosquitos. Therefore, it might safely be conjectured that the sand mite of South

China is probably akin to our red mite in Formosa, just as the old physicians deduced its identity with the Japanese mite.

According to Dr. Schüffner's paper, the pseudotyphus of Deli, Sumatra, is caused by the bite of certain acarine larvae. Further South, the Mossman fever of North Australia seems to be a disease with a similar cause. So it would be a matter of profound interest to carry out further investigations on these fever-carrying mites distributed so widely from Northern Japan to the Far South. I believe that further study will very likely show a wider distribution of the tsutsugamushi or allied acarine mites and diseases caused by them in various parts of the tropics.

C. Animal Experiments

(*a*) *Inoculation of the virus.* Several monkeys, inoculated with 0·5 to 1 c.c. of blood from cases in the exanthematous stage, fell sick with fever and bubo, apparently indicating actual infection; in one case (♀) a typical spleen was found at the autopsy. Other monkeys inoculated with patients' blood or emulsion of spleen and lymphatic glands of infected monkeys as well as the spleen from human autopsy material, failed to show any signs of positive infection.

(*b*) *Mite bites.* Several monkeys were kept in the mite-infested area of Yoshino plantation. The mites were found in large numbers on the chest, eyelid, etc., of the animals. The bites were inflamed for some days, healing without ulcers. In the meantime the lymphatic glands became swollen and tender, and fever appeared after several days of incubation. The animals thus infected eventually recovered.

Some species of indigenous monkeys are therefore definitely susceptible to the virus through inoculation of patients' blood or through bites of the red mite in an infected field, whereas others seem resistent; this is probably due to an immunity arising from mite bite in their previous habitat.

III. CLINICAL OBSERVATIONS

A. Symptomatology

According to my observations, the Formosan tsutsugamushi disease has, after the bite of infected larvae of *Tr. akamushi*, an incubation period ranging from four to ten days or more. As prodromal symptoms, some patients complain of headache, general malaise, pains in the joints and loss of appetite for a few days.

With or without these initial symptoms the fever begins with or without, usually with, a chill, seldom with a definite rigor. The body temperature gradually or rapidly rises to 39 to 40° C., and remains at this point for a variable time, falling gradually or somewhat critically. The duration of the fever varies, according to the severity of the case, from ten days to three weeks or more. In grave cases, the high temperature continues for two to three weeks, and then lysis follows.

In moderate cases, again, the duration is shorter, and the fever ends by lysis or crisis in the course of about two weeks. In still milder cases, the fever, generally remittent, lasts for only one to two weeks. Sometimes it is so slight that these cases are regarded as apyrexial forms.

At the site of the bite there is a red, non-itching area, with a red mite in the centre, which is inclined to necrose but soon heals, or there may develop an ulcer, with a scab, about 5 mm. in diameter. The ulcer heals in one to two weeks, generally leaving some pigmentation. They usually occur on soft folded areas of skin, such as the pudenda, inner thigh, belly, scrotum or axillary region. The ulcer is usually single, exceptionally there are two or three.

An important sign is adenitis in the region of the ulcer, seldom accompanied by lymphangeitis. The primary bubo affects the inguinal, femoral, axillary or other lymphatic glands, while later secondary enlargement of the glands occurs. The swollen glands vary in size, from that of a lentil to that of a pigeon's egg; they are single or multiple, painful on palpation. The bubo does not adhere to the surrounding tissues and gradually returns to its normal size; in one case suppuration of a cubital bubo has been recorded.

Some days after the beginning of the attack, eruptions appear on different parts of the body, e.g., the chest, back, face and extremities. They are non-itching, reddish papules, and fade on pressure. They develop for a few days and then gradually disappear, leaving pigmentation.

In grave cases, however, the papules sometimes become confluent and persist, mostly on the face; in mild cases they very often fail to appear. Icterus has never been observed.

In the majority of cases the face is congested and somewhat oedematous during the exanthematous period, and the conjunctiva is injected. In two cases I observed transitory dimness of vision probably due to keratitis.

The tongue is coated and moist, but in severe cases dry. The pharynx and tonsils are injected and generally swollen. Nausea or vomiting occurs, and in grave cases hiccough. Constipation is the rule, but diarrhoea may occur in grave cases.

The urine is albuminous, and the diazo-reaction is usually more or less positive. The spleen and liver are often enlarged and tender.

Bronchitis occurred in 34 per cent. of 118 cases. The pulse is generally rapid, but in some cases it is slow and dicrotic, as in typhoid fever. In grave cases it is soft and frequent, cardiac failure occurring in the second or third week. Leucopenia is characteristic of the fever, the leucocytes falling to as low as 3,600 per c.mm. The leucopenia is most marked at the acme of the fever, and then diminishes gradually. There is also a decrease in coagulability of the blood.

In general the patients complain of headache, dizziness, tinnitus, partial deafness, etc. In severe cases there is delirium, stupor or coma. In some cases stiffness of the neck occurs, and the muscles, especially those of the extremities, become very sensitive to pressure. The knee-jerk, skin and cremasteric reflexes are usually present. Nervous symptoms generally disappear after subsidence of the fever, while in grave cases they remain after the apyrexia, a peculiarity of the disease.

Relapses have not been observed, although in some cases high fever returns after defervescence; such cases are usually fatal.

As to reinfection, I have observed several cases, two with an intermission of a few months, and one after about two years. Recovery from a first attack generally ensures a relative immunity, as a second attack is milder, and natives and early immigrants are less liable to infection.

Among seventeen deaths, six occurred in the second, eight in the third, two in the fourth and one in the seventh week.

The death rate varies greatly according to locality and year. It amounted to from 0 per cent. (1914) to 3 per cent. (1915) in the Toyoda plantation, and from 15 per cent. (1914) to 12 per cent.

(1915) in the Hayashida plantation. In the Karenko district the death rates were 10·5 per cent. (1914), 9·3 per cent. (1915), and 11·66 per cent. (1916).

B. Treatment

The site of the bite should be treated quickly and energetically, best by extirpation or cauterisation, otherwise the treatment is symptomatic only.

C. Diagnosis and Differential Diagnosis

A typical case of the disease may easily be recognised by the presence of an ulcer, buboes, and fever with exanthemata.

From typhus fever, with which it has a certain similarity especially in the rash, it can be distinguished by the buboes. Incidentally, it is worth noting that in Formosa, typhus fever is unknown up to date.

In dengue fever, which is sometimes epidemic in the island, glandular enlargement is exceptionally observed, but the ulcer is unknown. In measles, the nature of the rash, the absence of ulcer and bubo, as well as its epidemic character, enable a diagnosis to be made.

From venereal ulcers and bubo, the present disease may be differentiated by the character of the ulcer, by the bubo and exanthem, and more especially by the history.

From other ulcers, lymphangeitis and adenitis, caused by suppurative cocci, a diagnosis can be established by microscopical examination as well as by the appearance of the rash.

Weil's disease, caused by *Leptospira icterohaemorrhagiae*, Inada, is always accompanied by icterus, and has never been observed in this island.

Seven-day fever, or the autumnal fever of Fukuoka and Shizuoka-ken, is a spirochaetal disease caused by *Leptospira hebdomadis*, Ido, one suspected case of which was observed by Dr. Ohnishi at Taihoku. These are differentiated from the tsutsugamushi disease by the presence of icterus and the causal spirochaetae or by serum reactions.

IV. SOME REMARKS ON THE JAPANESE AND FORMOSAN TSUTSUGAMUSHI DISEASE AND ALLIED ENDEMIC GLANDULAR FEVERS

Though the identity of the endemic fever of northern Japan and Formosa, as can be seen in my earlier papers, is recognised, there still exists some differences between them, which may be summarized as follows:—

(1) The mortality of the tsutsugamushi disease in Niigata and Yamagata districts in 1917 is estimated at 30 per cent. and 50 per cent. respectively, while that of the Formosan fever amounted in recent years to about 10 per cent. per annum.

(2) The Japanese fever appears regularly in the hot season from July towards the end of September, the mean temperature in these months being above 71·2° F. at Nagaoka in the Niigata district. The Formosan fever generally begins in April and disappears in November.* The free season in the Yoshino plantation is in the months with a mean temperature below 75·0° F.

(3) The endemic areas of Japanese fever are strictly restricted to certain parts of river banks and alluvial deltas or islets formed in the river, which are subjected to annual inundation, as the name 'Japanese flood or river fever' given by Prof. E. Baelz indicates. In Karenko district, however, the endemic areas are situated in river valleys or fertile plains and woodlands, either at the foot of mountains or on elevations free from inundation.

(4) The host of the tsutsugamushi or akamushi mite of Japan is the field mouse, *Microtus montebelli*; those of the Formosan mite are common rodents and insectivora and other wild or domestic animals, e.g., chickens, pheasants, and dogs.

(5) While the exanthem occurred in nearly all of the cases in the Yamagata district (Ohnuma and Naganobori), it is absent in 5 per cent. of Niigata cases (Commissioner's report), and thirty-five non-exanthematous among one hundred and four cases were recorded in 1915 in the Karenko district.

(6) The respiratory system was affected in 48 per cent. of the cases in Niigata; in 34 per cent. of Formosan cases in 1915.

(7) It is said that the Japanese monkey is susceptible to the virus

* It is reported from Ako district office that four typical cases of the disease were observed by a local physician in January, 1919.

of the tsutsugamushi disease, but this is not the case with the Formosan virus.

(8) The virus-transmitting red mite of Japan and Formosa is the larva of *Trombicula akamushi*, Br.

Dr. Schüffner has observed, since 1902, a special type of fever in Deli, Sumatra. He gave the name 'pseudo-typhus' to the disease, and believes it to be a variant of the tsutsugamushi disease. The following is a summary of his paper (1913):—

> 'In Deli, pseudo-typhus, with a mortality of 3 per cent., is found throughout the year. So far as is known, people contract the disease through the bite of *Trombidium* larvae. In a quarter of an hour after the bite, violent itching is felt. An initial ulcer occurs in the groin, thigh or neck in most cases. The adjacent lymphatic glands swell to the size of a pigeon's egg. A characteristic eruption appears on the second or third day, develops for several days, and then slowly disappears. It occurs in 70 per cent. of the native cases. The course of the fever resembles that of typhoid fever, but it begins and terminates more acutely. The blood shows on an average a leucocytosis of 12,000, in which the increase of the lymphocytes and decrease of the polymorphonuclears is remarkable. Inoculation experiments with monkeys gave no result.'

Ashburn and Craig noted a similar disease in the Philippine Islands in 1908, but gave no information about the mode of transmission.

Noc and Gantron reported two cases of undetermined fever observed at the Saigon Military Hospital in the autumn of 1914. These cases were very similar to those seen by Schüffner at Deli.

Dowden reported a suspected case of Kedani river fever observed in the Federated Malay States, viz., that of an European, aged 20 years, admitted into hospital on the fourteenth day of the disease in the typhoid state. A profuse and haemorrhagic rash appeared on the eighth day. Glandular enlargement was marked, and there were neuritic symptoms.

Weir reports the disease and the red mite from Corea.

The Mossman fever which occurs in the Mossman district, North Queensland, and affects cane-cutters, etc., was first reported by

Smithson in 1910, and recently studied by Drs. Breinl, Priestly and Fielding; it has a great similarity to tsutsugamushi disease and Schüffner's pseudo-typhus. It is characterised by an irregular remittent fever from three days to three weeks, accompanied by painless swelling of lymphatic glands and the appearance of a macular or vesicular rash.

The above-mentioned endemic fevers, the Sumatran pseudo-typhus, Australian Mossman fever, and Philippine, Saigon and Malay fever, are exanthematous glandular fever, having many features in common. They are most probably caused by the bite of mites.

Two cases resembling tsutsugamushi fever, said to have been caused by the bite of a tick, *Amblyomma* sp., have been reported from Karenko. Whether or no the cases were truly tick fever or tsutsugamushi disease due to mite bite could not be determined.

VI. PROPHYLAXIS

As the mode of infection of the endemic disease of Formosa has been clearly defined, preventive measures based on its aetiology should be adopted.

(1) All parts of the body should be, in a mite-infested area, properly protected. Prof. Hayashi's or Prof. Nagayo's mite-proof suit is recommended for this purpose.

(2) All articles of clothing, including loin-cloths, used in an infected field, should be taken off outside the dwelling-house, and be fumigated with sulphur or boiled or dipped into a disinfectant solution.

(3) At the same time, hot baths with the use of soap should be taken regularly. Vleminkx's solution, Kummerfield's mixture, 'disinfectol' solution, etc., may be applied to those parts of the body liable to mite bite.

(4) The skin of the body should be carefully examined after field work has been done. Examine with a lens any red spot for a red mite in its centre. If the mite is found, the spot should be excised or the mite should be removed with a needle.

(5) The best prophylactic measure is to bring the infested areas

under cultivation. Before doing so, it is indispensable to burn all bushes and grasses. The clearing of the areas must be done during the free season.

(6) It is very important to educate the inhabitants of the infested localities as to the nature of the disease and its transmitter.

REFERENCES

AKAGI, K., and RIN, T. (1918). On a case of Tsutsugamushi Disease occurring in Kagi district, Formosa. *Taiwan Igakkai Zassbi*, No. 182-3.

ASHBURN, P. M., and CRAIG, C. F. (1908). A comparative study of Tsutsugamushi Disease and Spotted or Tick Fever of Montana. *Philippine Journ. of Sci. B.* Vol. III, p. 1.

BREINL, PRIESTLEY, and FIELDING (1914). On the occurrence and pathology of endemic glandular fever, a specific fever occurring in the Mossman district of North Queensland. *Med. Journ. of Australia.* Vol. i, p. 391.

DOWDEN, R. (1915). A suspected case of Kedani River Fever in the Federated Malay States. *Indian Medical Gazette.* Vol. L, p. 208.

HATORI, J. (1915-18). Reports I-IV on the Exanthematous Bubonic Fever (Tsutsugamushi Disease), endemic in Formosa. *Taiwan Igakkai Zassbi.* Nos. 147, 150, 170, 182.

——— (1916). On the Exanthematous Bubonic Fever of Formosa, and its identity with the Tsutsugamushi Disease of Japan. *Nippon Eisei Gakkai Zassbi.* Vol. XI.

HAYASHI, N. (1917). Piroplasma-like bodies as the cause of the Tsutsugamushi Disease. *Kyoto Igaku Zassbi.* Vol. XIV.

HAYASHI, N., and MUKOYAMA, T. (1918). Studies on the Tsutsugamushi Disease in 1917. *Cbuo Igakkai Zassbi.* No. 138.

HOKUETSU IGAKKAI (1904). The First Report on the Investigation of the Tsutsugamushi Disease.

KATO, S. (1910). On an Exanthematous Fever observed in Kagi district. *Taiwan Igakkai Zassbi.* No. 100.

KAWAMURA, R., ARIMA, E., and HATTORI, T. (1913). On the Pathological Anatomy of the Tsutsugamushi Disease. *Nippon Byori Gakkai*, Trans. Vol. IV.

——— (1914). On the Blood Changes of the Tsutsugamushi Disease. *Hokuetsu Igakkai Zassbi.* 29th annual set.

KAWAMURA, R., and HATTORI, T. (1915). Histological Studies of the Exanthem of Tsutsugamushi Disease. *Hokuetsu Igakkai Zassbi.* 30th Annual Set.

——— (1916). On the Causative Virus of the Tsutsugamushi Disease. *Hokuetsu Igakkai Zassbi.* 30th annual Set.

KAWAMURA, R., and KOMAGATA, K. (1916). On the Morphology of the Akamushi. *Hokuetsu Igakkai Zassbi.* 31st Annual Set.

KAWAMURA, R., and YAMAGUCHI, M. (1916). On the Adult and Nymph of the Akamushi. *Hokuetsu Igakkai Zassbi.* 31st Annual Set.

——— (1916). On the Anatomy of the Adult and the Life Cycle of the Akamushi. *Hokuetsu Igakkai Zassbi.* 31st Annual Set.

——— (1917). Researches on the Etiology of the Tsutsugamushi Disease. *Hokuetsu Igakkai Zassbi.* 32nd Annual Set.

KITAJIMA, T., and MIYAJIMA, M. (1909-10). Studies on the Tsutsugamushi Disease. *Saikingaku Zassbi.* Report III-IV.

——— (1918). Studien ueber die Tsutsugamushi-Krankheit, I. *Kitasato Arch. Exper. Med.* Vol. II.

MIYAJIMA, M. (1917). On the Life Cycle of the Akamushi, Carrier of the Nippon River Fever. *Kitasato Arch. Exper. Med.* Vol. I.

MIYAJIMA, M., and OKUMURA, T. (1917). Comparative Studies on the Japanese, Korean and Formosan Akamushi and Allied Species. *Saikingaku Zassbi.* No. 266.

——— (1916). Comparative Studies on the Larvae of *Trombidiidae. Saikingaku Zassbi.* No. 254.

——— (1918) On the Akamushi Body, or *Clamydomycetes Tsutsugamusbi. Saikingaku Zassbi* No. 269.

NAGAYO, M., MIYAKAWA, Y., MITAMURA, T., and IMAMURA, A. (1915). On the Tsutsugamushi Disease. *Tokyo Igakkai Zassbi.* Vol. XXIX.

——— (1917). On the Nymph and Prosopon of the Tsutsugamushi (*Leptotrombidium akamusbi*), Carrier of the Tsutsugamushi Disease. *Journ. Exper. Med.* Vol. XXV, p. 255.

——— (1917). On the Culture of the Causative Virus of Tsutsugamushi Disease. *Ijisbinbun*, No. 979.

NAGAYO, M., MIYAKAWA, Y., MITAMURA, T., and TAMIYA, T. (1918). On the Causative Virus of Tsutsugamushi Disease in the body of the adult Tsutsugamushi Mite. *Densenbyo Kenkyujo Gakuyukai Zassbi.* Vol. II, No. 1.

NAKAGAWA, K. (1913). On an Exanthematous Fever prevailing at Karenko District. *Taiwan Igakkai Zassbi.* No. 125.

NOC, and GANTRON (1915). Deux casde fièvre indéterminée rappelant le pseudo-typhus de Deli observés à Saigon. *Bull. Soc. Med. Cbir. Indo-Cbina.* Vol. VI, p. 108.

OGATA, M. (1911-17). II-VIII Mittheilungen ueber die aetiologie der Tsutsugamushi Krankheit. *Mittbeilung d. Med. Facult. d. Kais.-Jap. Univ. z. Tokio.* Vol. X-XVII.

——— (1918). On the Tsutsugamushi-eumycetes. *Eiseigaku Densenbyogaku Zassbi.* Vol. XIII, No. 6.

OHNISHI, S. (1918). On the Seven-days Fever observed in Formosa. *Taiwan Igakkai Zassbi.* No. 186.

SANO, Y. (1914). On the undetermined Exanthematous Fever. *Taiwan Igakkai Zassbi.* No. 138.

SCHUEFFNER (1913). Pseudotyphus in Deli, Sumatra. *Comptes rendus des traveax du Troisième Congrès Biennal*, Saigon, p. 309.

SENOUYE, Y. (1917). On a case of Tsutsugamushi Disease infected in Mt. Ari region. *Taiwan Igakkai Zassbi.*

TANAKA, K. (1899). Ueber die Aetiologie und Pathogenese der Kedani Krankheit. *Centralbl. f. Bakt. u. infect. Kr. I.* Abt. Bd. XVI.

——— (1906). Ueber meine Japanische Kedani Krankheit. *Ibid.* Bd. XLII.

——— (1918). Differentiations of the Kedani, pseudo-akamushi, Japanese *Leptus autumnalis* and its adults. *Ikaijibo.* No. 1228.

TSURUKI, T. (1915). The Tsutsugamushi Disease in Yamagata-ken.

YERSIN, A., and VASSAL, J. J. (1908). Typhus fever in Indo-China. *Pbilippine Journ. Sci.*. B. Vol. III, p. 131.

EXPLANATION OF PLATES

Plate VIII

Fig. 1. View of Mokkui Valley at the time of the expeditionary campaign, 1914.

Fig. 2. Larva of *Tr. akamushi*. × 100.

Fig. 3. Nymph of *Tr. akamushi*.

Fig. 4. Adult of *Tr. akamushi*.

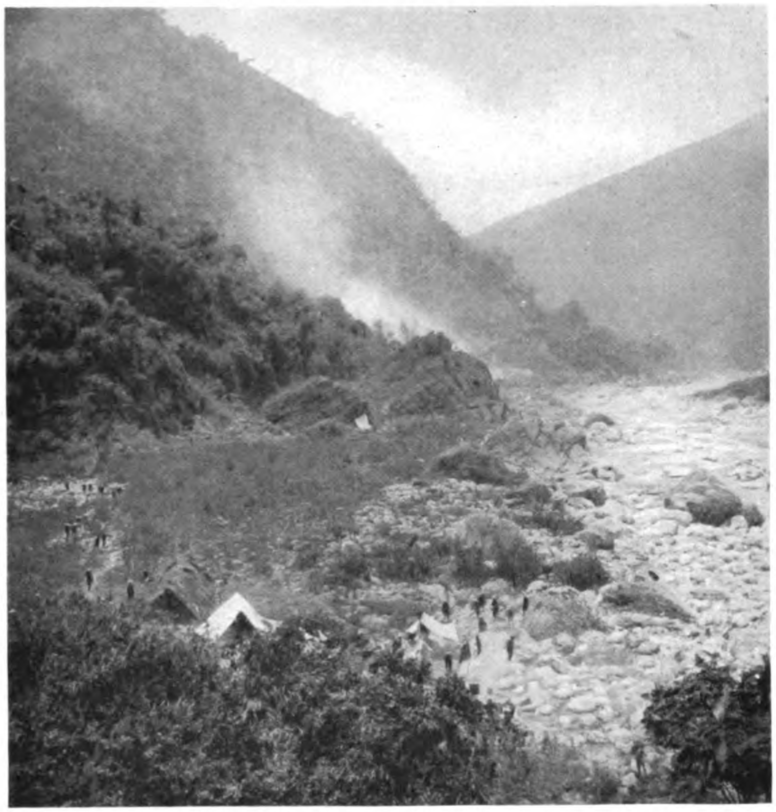


Fig. 1

Fig. 2

Fig. 3

Fig. 4

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PLATE IX

Fig. 1. An ulcer on scrotum caused by bite of virulent akamushi.

Fig. 2. Patient in acute stage [after Dr. Ohta]. □, denotes ulcer; o, inguinal bubos.

Fig. 1

Fig. 2

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NOTES ON THE BIONOMICS OF *STEGOMYIA FASCIATA*, FABR.

(PART I)

BY

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INTRODUCTION

Since the discovery of the rôle of *Stegomyia fasciata* in the transmission of yellow fever, much attention has been devoted to the study of the life history and habits of this mosquito in Europe, Africa and America.

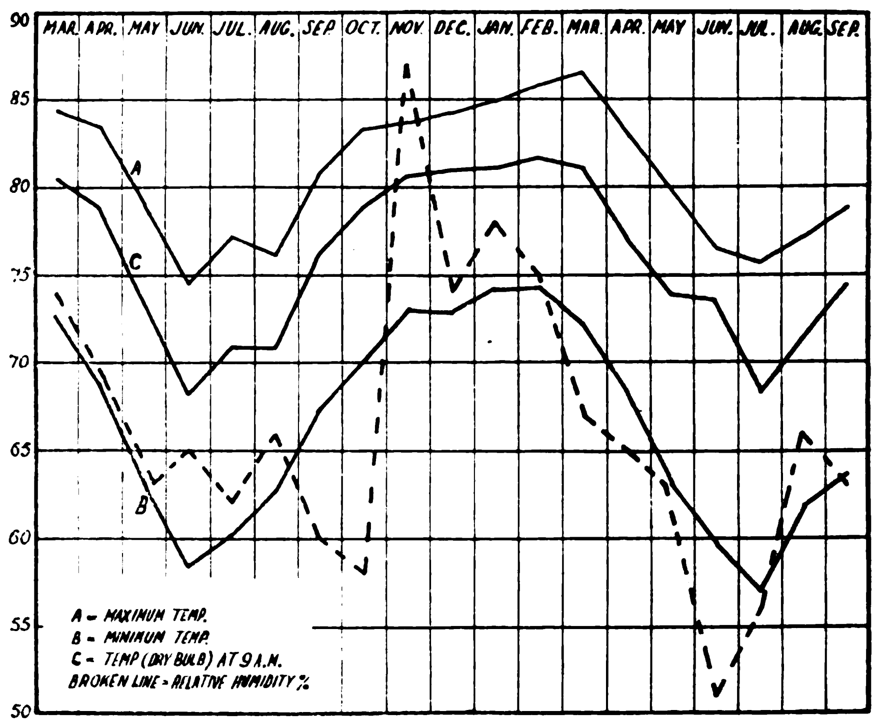
Notwithstanding the vital importance of such knowledge to Australia, no research work of any importance, other than Taylor's inquiry into the distribution of the species in the coastal towns of Queensland, has been undertaken in Australia, either to confirm or to supplement the result of investigations carried out elsewhere by Finlay, Goeldi, MacGregor, Carrol, Reed, Newstead, Boyce, Bacot and many other workers, to whom we are indebted almost entirely for our knowledge of the bionomics of this widely distributed species.

The necessity for breeding large numbers of *Stegomyia fasciata* to meet the requirements of this Institute appeared a favourable opportunity to undertake some of this hitherto neglected work. Although very incomplete, it has been considered advisable to record the results of these observations and experiments at this stage, rather than to defer their publication until further contemplated experiments have been undertaken.

It will be observed that where similar information has been sought, I have followed very closely the methods and technique of Bacot (1916), whose paper has been freely consulted as being by far the most complete and comprehensive of its kind yet published.

The various stages of *Stegomyia fasciata* have been so fully and accurately described by earlier writers that it is quite unnecessary to attempt to add anything to their descriptions, nor can new facts be added regarding the seasonal prevalence and distribution of this mosquito in Townsville to the published records of Taylor (1915).

The accompanying chart shows the temperatures and humidity readings taken in the Institute grounds during the period in which the experiments were carried out.



METHODS OF KEEPING AND FEEDING ADULT MOSQUITOES

In these experiments the adult mosquitoes were kept in small cages, as commonly used, measuring 12 by 8 by 7 inches, and consisting of a light wooden frame covered with cheese cloth on three sides and one end, and zinc on the bottom. The remaining end was fitted with a calico sleeve sufficiently large to permit of the passage of the arm and necessary water containers. A glass panel occupied one-third of one side of each cage, but it was considered to be of no advantage when light fabric was used for screening the remainder of the cage. The mosquitoes were offered food daily, the following substances being tried at different times, viz., blood, banana, peptone and sugar solution, milk and sugar, etc. After some initial trials blood was found to be the most satisfactory for the females and banana for the males, these foods being used subsequently to the exclusion of all others.

Feeding experiments with small animals, such as guinea-pigs, rats and puppies, were, as a rule unsuccessful, even when the animal was left in the mosquito cage overnight. The most satisfactory method of feeding with blood, and the one adopted throughout the following experiments, was found to be the presentation of the arm and hand each morning for a period of from ten to twenty minutes according to circumstances, it being noticed, for example, that during hot and humid weather the mosquitoes fed more readily than in cool weather.

In some current experiments, in which it is desired to feed *Stegomyia* on dog's blood, successful results are obtained by the following method :—One of the cages previously referred to is placed upon the floor and the dog's head thrust into it, the animal being kept in a recumbent position and the sleeve of the cage secured behind its head with one hand whilst the other presses the body to the floor to prevent the animal rising. To ensure successful feeding it is found necessary to shave or clip the hair on the face and ears. Other methods of feeding upon dogs were tried, but found to be unsatisfactory.

I. EGGS

The maximum number of eggs laid by a fertilised captive female

From a long series of observations it has been found that the average egg production of a fertilised captive female fed on blood is about seven hundred and fifty, covering a laying period of from forty to seventy-two days. None of my records cover a longer period of productiveness, although females have been kept in captivity up to a maximum of ninety-three days. It was found impossible, however, owing to absence from Townsville, to continue blood feeding after the sixty-sixth day, banana being substituted for blood, with the invariable result that egg production ceased on the change of food.

The following record may be cited as typical of many illustrating the rate of egg production. A female, bred for five generations in the laboratory, was segregated with two males and fed exclusively on human blood. The first feed was taken four days after her emergence, and the first batch of eggs laid three days later. During the first thirty-one days of her life she fed eight times and laid four hundred and thirty-seven eggs. During the succeeding thirty-one days she fed six times and laid two hundred and sixty eggs. Two more feeds were taken and fifty-five eggs laid during the following ten days. In all she fed sixteen times and laid seven hundred and fifty-two eggs during the seventy-two days of her life. On the day following the laying of the last batch of eggs she was accidentally destroyed. A complete record of these eggs was not kept to determine the percentage of fertility, but a certain proportion of the batches were kept under observation and found to produce between 80 per cent. and 90 per cent. of larvae.

The selection of situation for oviposition

(*a*) Females in captivity

The receptacles usually provided in the cages for oviposition were dishes 3 inches to 4 inches in diameter, and contained about ½-inch to 1 inch of water. The favourite position of the ovipositing female appears to be resting on the sides of the receptacle with the apex of the abdomen just touching the water. Very often eggs are laid on the surface film of the water as the female moves about on it, but occasionally as many as fifteen eggs are laid in a batch. A considerable proportion of the eggs laid near the margin of the receptacles are drawn against the sides by capillary attraction, and become stranded as the water dries. Under more natural conditions these doubtless form the bulk of the resistant eggs which lie dormant until the supply of water is replenished. When wet filter paper is provided the eggs are usually scattered over its surface, but occasionally they are laid so close together as to overlap.

(*b*) Wild females

It has been frequently noticed that almost any vessel containing water and left in or near the Institute building would attract the females of *Stegomyia* and offer a suitable place for oviposition.

In view of the opinion held by some other observers that these mosquitoes show a decided preference for certain positions and certain rooms, the investigations detailed in Experiment No. 1 were undertaken with the object of confirming, or otherwise, the results of my casual observations.

EXPERIMENT I

No.	Position	Nature of Receptacle	Contents	*Stegomyia* eggs laid	Remarks
1	Workroom on floor ...	Enamel bucket ...	Tap water	+	
2	Workroom on bench ...	Earthenware sink ...	Tap water	+	
3	Workroom on bench ...	Pickle bottle	Tap water and sea water equal parts	+	
4	Workroom on bench ...	Pickle bottle	Rain water	+	
5	Workroom on bench ...	Tin used as water bath	Rain water	+	Also *Culex* sp.
6	Hallway	Glvd. iron cistern 12 ft. from floor	Rain water	+	
7	Bathroom on bench ...	Glvd. tin	Tap water	+	
8	Bathroom on floor ...	Earthenware jar ...	Tap water	+	Also *Culex fatigans*.
9	Water Closet	Cast iron cistern not used for some time	Tap water	+	
10	Sterilising room on bench	Enamel bowl	Tap water and dirty tubes	+	
11	Ster. room under bench	Earthenware jar ...	Tap water and dirty slides	+	Also *Culex fatigans* and *C. ? sitiens*
12	Animal house on shelf 8 ft. from floor	Glvd. iron dish ...	Tap water	+	
13	Animal house on shelf 8 ft. from floor	Enamel bowl	Tap water	+	
14	Under roof of Institute ...	Glvd. iron dish ...	Tap water	−	
15	Under roof of Institute ...	Glvd. iron dish ...	Tap water	−	
16	Under roof of Institute ...	Enamel bowl	Tap water	−	

The investigations on the whole tend to show that in Townsville, at any rate, *Stegomyia* do not appear to show a preference for certain rooms or situations, but will oviposit in almost any receptacle containing water. This finding is noteworthy in view of the fact that Bacot's experiments gave entirely different results. In a set of twelve receptacles under observation he found *Stegomyia* ovipositing in only one—a card cream jar in the kitchen. It must be noticed, however, that he found *Stegomyia* ovipositing in such positions as the following: in safe stands in gallery, in a tin bowl in yard and in tins and wooden tub in the mosquito house.

Baits to attract pregnant females

Experiments to ascertain if ovipositing females prefer contaminated water to clear

The following experiments (see Experiment II) were carried out in a cage containing a large number of *Stegomyia*, the females of which were given opportunities of feeding regularly on human blood. The glass dishes each contained 200 c.c. tap water, in addition to the organic matter, and were placed as far apart as the size of the cage would permit. Except in the case of leaves, which stood for forty-eight hours, none of the cultures were permitted to stand for more than twenty-four hours before being introduced into the cage. One trial was carried on a second day owing to the small number of eggs laid on the first.

Bacot, who carried out similar experiments, remarks: 'While the evidence of selection . . . appears definite, it is very far from being the unanimous unvarying character that might be expected from a deep-seated instinct coupled with larval needs. On the contrary, it carries with it a certain suggestion of bias on the part of the females in reference to their own tastes largely parallel, but not necessarily identical, with provision for their offspring. . . .'

It appears to me that these remarks might be fittingly applied to the results of my experiments. It might be observed, however, that sugar and water appeared to be the most attractive bait, yet it has been shown that sugar gave unfavourable results as a larvae food.

EXPERIMENT II

No.	Contents of dish	No. of eggs laid	Percentage of total	Remarks
	(1st SERIES)			
1	Tap water	30	8·6	No smell
2	Tap water plus 0·5 grm. dried mango leaf, standing for 2 days	30	8·6	Did not smell strongly
3	Tap water plus 0·5 grm. fresh fowl faeces	120	34·3	Did not smell strongly
4	Tap water plus 0·5 grm. freshly killed cockroach	170	48·5	Smelled strongly
	Total eggs laid	350		
	(2nd SERIES)			
1	Tap water	40	6·66	
2	Tap water plus 0·5 grm. dry pawpaw leaf allowed to decompose for 2 days	20	3·34	Did not smell strongly
3	Tap water plus 0·5 grm. dried cockroach	186	31·00	Slight smell
4	Tap water plus 1cc. ox bile	160	26·66	Fairly strong smell
5	Tap water plus 0·5 grm. Sodium Taurocholate	194	32·34	Fairly strong smell
	Total eggs laid	600		
	(3rd SERIES) 1st Count			
1	Tap water	14	21·5	On account of the few eggs laid the dishes were placed in the cage again on the following day and a second count was made 48 hours later.
2	Tap water plus 0·5 horse faeces ...	20	30·8	
3	Tap water plus 1 cc. urine 3 days old ...	31	47·7	
	Total eggs laid	65		
	(3rd SERIES) 2nd Count			
1	Tap water	120	24·0	Did not smell
2	Tap water plus 0·5 horse faeces ...	90	18·0	Smelling
3	Tap water plus 1 cc. urine 3 days old ...	290	58·0	Smelling strongly
	Total eggs laid	500		
	(4th SERIES)			
1	Rain water	43	20·5	At the conclusion all dishes had a very slight smell
2	Tap water plus 0·5 grm. powdered rice	40	19·0	
3	Tap water plus 10 grm. sugar	127	60·5	
	Total eggs laid	210		

Table I contains a summary of this experiment (Experiment II).

TABLE I.

Contents of Receptacles	Percentage of the total eggs laid in each series
Tap water and pawpaw leaf	3·34
Tap water (2nd series)	6·66
Tap water (1st series)	8·6
Tap water and mango leaf	8·6
Tap water and horse faeces (2nd count)	18·0
Tap water and powdered rice	19·0
Rain water	20·5
Tap water (1st count)	21·5
Tap water (2nd count)	24·0
Tap water and ox bile	26·66
Tap water and horse faeces (1st count)	30·8
Tap water and dry cockroach	31·0
Tap water and sodium taurocholate	32·34
Tap water and fowl faeces	34·3
Tap water and urine (1st count)	47·7
Tap water and fresh cockroach	48·5
Tap water and urine (2nd count)	58·0
Tap water and sugar	60·5

Experiments to determine whether Stegomyia *would oviposit on sea water*

For this purpose two buckets, each containing 4 litres of water, were exposed in a room where *Stegomyia* were fairly numerous. Bucket 'A' contained sea water drawn from the harbour, and bucket 'B' tap water from the town service pipes. The sea water was exposed continuously for a period of two months (19th April to 19th June), during which time no eggs were laid on it, whilst the other containing tap water was exposed for one month (19th May to 19th June) and was frequently visited by both *Stegomyia* and *Culex* *fatigans* for oviposition. In all, four hundred and ninety-one eggs of *Stegomyia* were laid.

A further series of experiments were then carried out to determine the amount of dilution necessary before *Stegomyia* could be induced to oviposit upon sea water mixed with rain water. For this purpose jars containing various percentages of sea water, from 10 per cent. to undiluted sea water, were exposed in one of the stock-breeding cages containing numerous females, which fed regularly on human blood. As each jar was found to contain eggs it was removed from the cage, and that portion of the experiment was considered finished. In the case of the jars containing 80 per cent. to 100 per cent. sea water, the vessels were kept under observation for a considerable period and until there was no further possibility of oviposition taking place. The results are shown in Table 2.

TABLE 2.

Number of eggs of *Stegomyia fasciata* laid on different percentages of sea water.

Percentage of sea water in rain water	Number of eggs laid	Remarks
10	?	Numerous eggs were laid but were not counted
20	?	
30	12	
40	43	
50	24	
60	28	
70	20	
80	nil	
90	nil	
100	nil	

Hatching of Eggs of Stegomyia

The period elapsing between the deposition of the eggs and the emergence of the young larvae is extremely variable. In the routine of breeding *Stegomyia* in this Institute, it has been found that the great majority of eggs laid overnight in the small dishes of water provided for the purpose hatched during the second and third day following in summer, and during the third and fourth day in winter. A certain proportion of eggs of some batches, however, do not hatch within this period, but do so after a further period of immersion, this period being, as I have ascertained, so long as four and a half months. Had further observations been made in this direction, it is very probable that the period of delayed hatching of continuously immersed eggs would have been found to be sometimes longer, since Bacot has shown that under these conditions hatching may be delayed from two to five months.

The influence of periods of drying upon the hatching of eggs

Theobald (1903) first drew attention to the fact that eggs of *Stegomyia fasciata* are capable of withstanding the effects of long periods of dessication. Since then, several others have published the results of experiments to ascertain the maximum period of viability of these eggs under various conditions of dry storage, and to elucidate the problems concerning the factors which bring about their subsequent hatching. Bacot believes that the incubation period of the eggs in Freetown is from thirty to forty hours, but that hatching of any given batch may be distributed over a lengthy period if subjected to periods of drying. The whole of the eggs of a batch may hatch as soon as they are replaced in water, or a proportion of them only may do so. Others may resist the first or second immersion and yield to a subsequent one. The principal factors which act as stimuli to hatching appear to be temperature and humidity. As he points out, a response to cooling would well serve the needs of the species in allowing of the fullest advantage being taken of the facilities for breeding in small temporary accumulations of rain water.

With the object of confirming the above observations under local conditions a number of experiments (III, IV, V) were carried out, which will be found to accord generally with the conclusions arrived at by earlier investigators.

Experiments to show the effects on eggs of drying and replacement of water

Experiment III

Twenty eggs laid overnight were placed in a tube $1\frac{1}{2}$ inches in diameter, containing 15 c.c. of tap water, which completely evaporated and was replaced on the days shown.

Days	3	5	7	8	11	14	15	20	21	Total
Number of eggs hatched	2	4	...	9	2	...	1	...	...	18
Days when found dry (indicated –) ...	...	...	–	...	...	–	...	–	...	...
Days when water replaced (indicated +)	...	...	...	+	...	...	+	...	+	...

Thus eighteen eggs (90 per cent.) hatched in fifteen days and after two dryings; two eggs (10 per cent.) were found to be collapsed when examined on 24th day.

Experiment IV

Twenty-five eggs laid overnight were placed in a petri dish 3 inches in diameter, containing 10 c.c. of tap water, which completely evaporated and was replaced on the days shown.

Days	2	3	4	5	6	8	10	11	12	15	18
Number of eggs hatched	3	8	...	...	5	1	...	...	...	...	...
Days when found dry (indicated –) ...	...	...	–	...	...	–	–	...	–	–	–
Days when water replaced (indicated +)	...	...	...	+	...	+	+	...	+	+	+

Experiment IV (*continued*).

Days	20	21	23	26	28	29	32	33	34	Total
Number of eggs hatched	...	7	...	...	...	...	...	...	1	25
Days when found dry (indicated –) ...	–	...	–	...	–	...	–	...	...	...
Days when water replaced (indicated +)	+	...	+	...	...	+	...	+	...	...

Thus 100 per cent. of the eggs hatched in thirty-four days and after ten dryings and ten replacements of water.

EXPERIMENT V

Twenty-eight eggs laid overnight were placed in a petri dish 4 inches in diameter, containing 15 c.c. of tap water, which completely evaporated and was replaced on the days shown.

Days	2	3	4	5	7	9	11	13	16	17	20	22
Number of eggs hatched	6	...	13	...	...	...	...	...	...	...	...	...
Days when found dry (indicated –)	...	–	...	–	–	–	–	–	–	...	–	–
Days when water replaced (indicated +)	...	+	...	+	+	+	+	+	...	+	+	...

EXPERIMENT V (*continued*)

Days	23	25	26	27	29	31	32	34	38	Total
Number of eggs hatched	...	...	...	...	...	...	2	...	4	25
Days when found dry (indicated –) ...	...	–	...	...	–	...	...	–	–	...
Days when water replaced (indicated +)	+	...	+	...	...	+	...	+	+	...

Thus twenty-five eggs (89·2 per cent.) hatched in thirty-eight days and after thirteen dryings and replacements of water. The remaining three eggs were found to be uncapped on the thirty-ninth day, but no larvae were seen. Assuming that these three eggs were uncapped by larvae, 100 per cent. of the eggs in this experiment may be said to have hatched.

The viability of eggs after long periods of dry storage

The longest period during which the eggs of *Stegomyia fasciata* are known to have remained viable in dry storage appears to be two hundred and sixty-two days (Bacot (1916)). For his experiment, Bacot collected eggs laid in Sierra Leone in January, from whence they were sent to England and immersed on 20th October. Within two hours of immersion the eggs hatched freely.

The possibility of the change of climate and environment influencing the period of viability, as shown by Bacot's experiment, suggested the desirability of carrying out some experiments wholly in Townsville. In one test (Experiment VI) eggs were kept in a viable condition in dry storage for a period of two hundred and fifty-seven days. In two other tests eggs retained their viability for over two hundred days (216 and 218), and in five other tests for over one hundred and fifty days (198, 172, 169, 163, 154). The eggs used in Experiment VI were taken from the stock-breeding cages and were known, from other experiments, to be of normal fertility. Those in 'A' were stored on a shelf in the laboratory, excepting in the case of 'A' 7, which was placed between the iron roof and the ceiling of an upstairs room of the Institute building. The eggs used in 'B' were stored on a layer of sand on the roof of an out-building, where they were protected from the rain by a flat sheet of iron resting on four legs about 18 inches high. Since Bacot and others have shown that in order to withstand long periods of drying eggs must pass through a preliminary incubation period in contact with water, all the eggs used in 'A' and 'B' were incubated as shown in Experiment VI.

Experiment VI

A. Indoors.

No.	No. of eggs laid	Date of laying	Maximum possible time in contact with water before storage	Nature of storage receptacles	Date of immersion	No. of eggs hatched after immersion	Viable for days	Percentage of eggs hatched
			Hours.					
1	24	30.4.17	25	Muslin covered petri dish	28.8.17	14	120	58·3
2	25	1.5.17	27	Muslin covered petri dish	28.8.17	2	119	8·0
3	154	5.5.17	33	Muslin covered tube ...	28.8.17	32	114	20·8
4	68	8.5.17	35	Glass covered petri dish	28.10.17	33	172	48·5
5	92	13.5.17	40	Glass covered petri dish	28.10.17	40	167	43·5
6	61	23.5.17	91	Glass covered petri dish	28.12.17	32	216	52·4
7	200 (approx.)	29.4.17	113	Glass covered petri dish	11.1.18	2	257	1·0 (approx.)

B. Outdoors.

No.	No. of eggs laid	Date of laying	Maximum possible time in contact with water before storage	Nature of storage receptacles	Date of immersion	No. of eggs hatched after immersion	Viable for days	Percentage of eggs hatched
1	41	7.4.17	43	Muslin covered tube ...	28.8.17	25	142	60·9
2	108	4.5.17	41	Muslin covered tube ...	28.8.17	25	115	23·15
3	70	17.5.17	40	Gelatine capsule	28.10.17	29	163	41·4
4	44	23.5.17	113	Muslin covered tube ...	28.10.17	2	154	4·5
5	62	2.6.17	138	Glass covered dish ...	11.1.18	1	218	1·6
6	110	23.6.17	109	Muslin covered dish ...	11.1.18	1	198	0·9

In a further series of experiments to determine the maximum period of viability after long periods of drying, thirty-two batches of eggs, numbering from thirty to one thousand, were stored for periods varying from three hundred and ninety-one days to five hundred and seventy-nine days and then subjected to stimuli to induce hatching. In all of these experiments negative results were obtained.

Resistance of eggs to drying over Calcium chloride

A number of experiments were carried out to determine the effect of Calcium chloride on eggs. Apparently this method of drying was too severe. The results are shown below:—

Eggs dried for 7 days ... 80 to 90 per cent. of the eggs hatched.
,, ,, ,, 19 ,, ... 8 to 15 per cent. ,, ,, ,,
,, ,, ,, 26 ,, ... None hatched.

Stimuli to the hatching of resistant eggs

Cooling of resistant eggs as stimulus to hatching

Bacot has shown that a rapid fall of a few degrees in temperature acts as a decided stimulus to the hatching of eggs which have been dried and have resisted subsequent immersion, or which have resisted the influence of the addition of fresh water.

In Experiment VII will be found the results of investigations which appear to fully confirm the first of these findings, although in these the eggs were not first subjected to periods of wetting and drying. In this experiment eggs were collected from the stock-breeding cages and kept in water until placed in the ice chest. Those eggs which failed to hatch within three days were considered to be 'overdue,' this 'overdue' period being shown below. Just before cooling, the eggs were placed in a dish containing 200 c.c. of tap water and removed to the ice chest, where they remained from two to forty-eight hours as shown, the temperature falling from about 80° F. to between 51·8° and 57·2° F.

EXPERIMENT VII

Expt.	Eggs laid	Cooled	Days Overdue	No. of eggs	No. hatched within 2 hours	No. hatched within 24 hours	No. hatched within 48 hours	Percentage hatched
1	25.3.17	3.4.17	6	6	6	...	...	100·0
2	13.4.17	24.4.17	8	16	10	...	...	62·5
3	17.4.17	2.5.17	12	6	2	...	...	33·3
4	4.5.17	26.5.17	19	35	8	5	13	76·0
5	8.6.17	27.6.17	16	24	14	...	...	58·3
6	13.6.17	27.6.17	11	8	6	...	...	75·0
7	19.6.17	27.6.17	5	108	45	...	...	41·7
8	2.1.18	18.1.18	13	25	14	4	1	76·0

Thus in this experiment of two hundred and twenty-eight eggs which failed to hatch during continuous immersion in water for periods varying from five to nineteen days longer than is generally required by non-resistant eggs, one hundred and twenty-eight were induced to hatch after periods of cooling ranging from two to forty-eight hours. Of these one hundred and twenty-eight eggs, one hundred and five hatched within two hours of being placed in the ice chest, nine within twenty-four hours, and fourteen within forty-eight hours.

The effect of lysol on resistant eggs

In certain experiments carried out at this Institute, it was found necessary to sterilize the eggs of *Stegomyia*, lysol being selected as a suitable agent. It was noted that after a short period of immersion in the disinfectant, resistant eggs hatched out soon after being submerged in water. This finding suggested the following experiment (VIII).

The eggs used were from the stock-breeding cages, and proved to be of average fertility. They remained in or on water from the time of laying until immersed for thirty seconds in lysol (of the strength retailed by the manufacturers), the overdue period being reckoned from the third day after laying to the date of immersion in the disinfectant. After treatment in lysol, the eggs were drained on filter paper and then immersed in water, the controls remaining in water throughout. The results of this treatment are shown in Experiment VIII.

EXPERIMENT VIII

Expt.	No. of eggs treated	No. of control eggs	Date of treatment	No. of days over-due in hatching	No. of eggs hatched inside 2 hours.		No. of eggs hatched in 24 hours		Percentage hatched after lysol
					Treated	Control	Treated	Control	
1	13	3	20.5.17	11	7	0	5	0	93·3
2	2	3	28.5.17	7	2	0	...	0	100·0
3	2	3	28.5.17	7	2	0	...	0	100·0
4	5	3	28.5.17	7	2	0	2	0	80·0
5	3	...	28.5.17	6	2	...	1	...	100·0
6	3	...	29.5.17	8	2	...	1	...	100·0
7	7	1	4.6.17	7	7	0	...	1	100·0
8	5	7	5.6.17	8	3	0	2	0	100·0
9	2	5	7.6.17	10	2	0	...	1	100·0
10	4	...	8.6.17	11	2	...	2	...	100·0
11	6	6	11.6.17	14	3	0	...	0	50·0
12	3	3	14.6.17	17	3	0	...	0	100·0
13	3	...	15.6.17	18	1	...	...	...	33·3

In this experiment (VIII), fifty-eight resistant eggs were treated with lysol, whilst thirty-four were used as controls. Of the former, thirty-eight hatched within one to two hours following their re-immersion in water after the lysol treatment, and thirteen others followed during the succeeding twenty-four hours. In all 87·9 per cent. hatched. Of the thirty-four controls, none hatched during the first one to two hours following the commencement of the experiment, and two only during the first twenty-four hours.

The effect of 2·5 per cent. soap solution on resistant eggs

Bacot, experimenting with larvicides, found that petroleum soft soap emulsion 1-8000, acted as a stimulus to the hatching of resistant eggs in a similar manner to lysol. The present observations show that soap solutions without petroleum also have a similar action.

In my experiment (Experiment IX) eggs were taken from the stock-breeding cages and kept continuously in or floating upon water up to the time of their immersion in the soap solution. The overdue period was reckoned, as in the former experiments, from three days after laying to the time of immersion in soap solution. The eggs were placed in the solution without preliminary drying and, after about five minutes' immersion, were returned to tap water.

EXPERIMENT IX

Expt.	No. of eggs treated	No. of control eggs	Date of treatment	No. of days overdue in hatching	No. of eggs hatched inside 2 hours		No. of eggs hatched in 24 hours		Percentage
					Treated	Control	Treated	Control	
1	4	3	20.6.17	13	3	0	1	0	100·0
2	4	3	20.6.17	13	1	0	0	0	25·0
3	5	2	20.6.17	15	1	0	0	0	20·0
4	6	...	22.6.17	17	5	...	0	...	83·3
5	4	3	22.6.17	20	3	0	0	0	75·0
6	6	3	24.6.17	20	4	0	0	0	66·7
7	5	5	25.6.17	23	4	0	0	1	80·0
8	4	5	25.6.17	23	4	0	...	0	100·0
9	5	5	25.6.17	23	3	0	0	0	60·0
10	10	...	27.6.17	25-27	7	...	0	...	70·0

Thus fifty-three resistant eggs were treated with soap solution and twenty-nine used as controls; thirty-five of the former hatched within one to two hours of their re-immersion in tap water following five minutes' immersion in the solution, and one hatched during the twenty-four hours following the treatment. In all 69·2 per cent. hatched within twenty-four hours. Of the seventeen which failed to hatch, two (one in No. 2 and one in No. 3) were found to be infertile and fifteen to contain either living or dead larvae. Only one of the control eggs hatched.

The influence of submergence of eggs upon hatching

EXPERIMENT X

In order to ascertain the difference, if any, in the hatching period of submerged eggs and those floating on the surface film, forty-five eggs laid during the night of 28th April were divided into two batches on the following morning, 'A' containing twenty-two eggs and 'B' twenty-three eggs. The former ('A') were submerged on filter paper in a dish containing 300 c.c. of tap water, whilst the latter ('B') were allowed to float on the surface film of a similarly prepared dish.

Batch 'A' (submerged) hatched as follows: eighteen on the third day and one on the twenty-third day. The three remaining eggs were taken from the water, dried and dissected on the twenty-eighth day, when they were found to be collapsed. In batch 'B' (floating), twenty-one eggs hatched on the third day and one on each of the fourth and fifth day.

From this one experiment it would appear that the position of the eggs in the containers, i.e., whether floating or submerged, has little influence upon the hatching, since in 'A' 81·8 per cent. of the total eggs, i.e., 94·7 per cent. of the total *fertile* eggs in the batch, hatched on the third day, and in 'B' 91·3 per cent. of the total. This view is supported by observations during the routine work of breeding *Stegomyia*.

The effect of sea water upon the hatching of eggs

On dry storage eggs

EXPERIMENT XI

Three hundred eggs, after having been dry stored for a period of three weeks, were divided into two equal batches, 'A' being placed in 300 c.c. of sea water and 'B' in 300 c.c. of tap water. The effects of this immersion are shown in Table 3.

TABLE 3.

"A" In sea water		"B" In tap water	
Total number of eggs hatched at the end of :—		Total number of eggs hatched at the end of :—	
15 minutes ...	20	15 minutes ...	nil
30 minutes ...	40	30 minutes ...	30
60 minutes ...	96	90 minutes ...	34
27 hours ...	110	2 hours ...	70
		5 hours ...	85
		27 hours ...	125
Percentage hatched ...	77·3	Percentage hatched ...	83·3

On freshly laid eggs

EXPERIMENT XII

Two hundred eggs which were laid during the previous night were transferred in the morning from the dish of fresh water to a jar containing sea water. After fifteen days' immersion in sea water they were divided into ten batches of twenty eggs each, and placed in bottles, (1) containing tap water, (2) containing 10 per cent. sea water, (3) containing 20 per cent. sea water, and so on up to 90 per cent. sea water. The subsequent history of these eggs is shown in Table 4.

TABLE 4.

No.	Contents of bottle	No. of eggs hatched	Length of larval life or subsequent stage of development reached
1	Tap water ...	17	16 adults reared
2	Tap water plus 10% sea water ...	5	5 adults reared
3	" " 20% " ...	4	4 adults reared
4	" " 30% " ...	5	4 adults and 1 pupa reared
5	" " 40% " ...	2	1 adult reared, 1 larva lived three days
6	" " 50% " ...	5	5 larvae lived three days
7	" " 60% " ...	1	lived one day
8	" " 70% " ...	1	lived under 16 hours
9	" " 80% " ...	1	lived under 16 hours
10	" " 90% " ...	0	—

The effects on hatching of submergence in small or large quantities of water

Bacot's experiments to elucidate the above question failed to bring forth any very conclusive evidence, but it appeared from them that slightly increased mortality and delayed hatching followed as a result of submergence in a small quantity of water. Nor do the present experiments afford convincing evidence, but such as has been adduced is in support of Bacot's finding regarding the increased mortality amongst eggs submerged in small quantities of water.

In the first of these experiments, thirty eggs laid during the night of 25th May were divided into two batches and submerged on filter paper, 'A' in 300 c.c. and 'B' in 1,300 c.c. of tap water.

In the second, sixty eggs laid on 30th May were similarly treated. The results are readily seen in Tables 5 and 6.

TABLE 5.

Batch	Day																	Mortality
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	28	
'A'	6	1	...	...	...	...	...	2	4	...	...	...	...	...	...	...	...	13·3%
'B'	8	2	1	...	...	2	...	...	1	1	...	...	...	...	...	...	...	nil

The two remaining eggs in 'A' failed to hatch by the twenty-eighth day.

TABLE 6.

Batch	Day																				Mortality
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
'A'	...	25	...	...	2	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	**6·6%**
'B'	...	20	...	3	4	...	...	2	...	1	...	...	...	...	...	...	...	...	...	...	**nil**

The two remaining eggs in 'A' which failed to hatch by the sixtieth day were dried and found to be collapsed (dead).

The influence of temperature and environment on the hatching of eggs and the development of the resulting larvae

In Experiment XIII, ninety eggs recently laid were divided into three batches each containing thirty eggs. Each batch was placed in 200 c.c. of tap water with 0·5 gram of polished rice.

'A' was kept in the laboratory at room temperature, i.e., about 78° F.

'B' was kept in an incubator at a temperature of about 95° F.

'C' was kept in an ice chest at a temperature varying from 56° to 59° F.

For details of the results see Experiment XIII.

Experiment XIII

Batch	Eggs	Day												
		3	4	5	6	7	8	9	10	11	12	13	14	15
'A'	30	1L.	5L.	3L.	1L.	2L.	12L.	...	...	1P.	2P.	1P.	...	5P. 3M.
'B'	30	1L.	...	...	...	2L.	9L.	...	2L.	...	...	3L.	...	1P. (a)
'C'	30	...	...	...	...	...	2L.	5L.	1L.	...	1L.	...	4L.	2L.

Experiment XIII (*continued*)

Batch	Eggs	Day												
		16	17	18	19	20	22	23	24	25	26	27	38	103
'A'	30	1P. 2M.	3P. 1M.	3M.	2M.	2M.	2P.	2P.	6P.	1P. 3M.	2M.	6M.	...	...
'B'	30	...	...	...	...	...	...	...	...	L. (b)	...	...	...	...
'C'	30	...	...	...	...	...	...	...	...	...	...	...	1P. (c)	L. (d)

Note :—'L.' = larva
'P.' = pupa
'M.' = adult

(*a*) pupated and died on 15th day
(*b*) the last larva died on 25th day
(*c*) this pupa died two days later
(*d*) the last larva died on 103rd day

Thus of thirty eggs kept under the above conditions in the laboratory, twenty-four, or 80 per cent., produced larvae, all of which pupated and subsequently produced adults. Of the thirty kept in the incubator, seventeen, or 56·6 per cent., produced larvae, only one of which pupated; this one died on the same day. The last surviving larva died on the twenty-fifth day. Of the thirty eggs placed in the ice chest, fifteen, or 50 per cent., hatched, of which number only one pupated. This pupa died two days later. The last surviving larva died on the hundred and third day.

In Experiment XIV, one hundred and ten eggs were divided into three batches, 'A' and 'B' containing thirty-eight eggs and 'C' thirty-four eggs. The methods of storing and the temperature were the same as the corresponding batches in Experiment XIII. The results are shown in the following statement:—

EXPERIMENT XIV

Batch	Eggs	Day								
		5	6	7	8	9	10	11	12	13
'A'	38	...	...	4L.	8L.	2L.	1L.	...	9L. 1P.	...
'B'	38	4L.	...	...	6L.	...	...	8L.	...	1P. (*a*)
'C'	34	...	...	...	...	1L.	...	...	...	1L.

EXPERIMENT XIV (*continued*).

Batch	Eggs	Day								
		14	15	16	17	18	19	20	21	47
'A'	38	... 3P.	2P. 1M.	1P.	2P. 4M.	... 2M.	2P.	... 1M.	... 2M.	...
'B'	38	...	...	...	...	...	...	...	L. (*b*)	...
'C'	34	...	...	12L.	1L.	...	...	...	...	L. (*c*)

(*a*) This pupa lived only two days.

(*b*) The last surviving larva died on 21st day.

(*c*) The last surviving larva died on 47th day.

Thus of thirty-eight eggs kept in the laboratory twenty-four (63·1 per cent.) hatched. Of these twenty-four larvae eleven (45·8 per cent.) pupated, and ten of the eleven (90·9 per cent.) produced adults. The percentage of adults reared from the thirty-eight eggs was therefore 26·3. Of thirty-eight eggs placed in the incubator eighteen (47·3 per cent.) hatched, one of which reached the pupal stage but survived only two days. Fifteen of the thirty-four eggs placed in the ice chest produced larvae, none of which pupated, although one lived to the forty-seventh day. The low percentage of hatching in 'A,' Experiment XIV, as compared with 'A,' Experiment XIII, is not accounted for.

The influence of sunlight, diffused light, and darkness on the hatching of eggs and development of subsequent stages

EXPERIMENT XV

In this experiment, one hundred and five eggs were divided into three batches, which were treated in the following manner:—

Batch 'A,' containing forty-five eggs placed in 1,300 c.c. tap water with a small quantity of fowl faeces, was stored on the verandah, where it was exposed to direct sunlight during the day and to the lower temperature of night.

Batch 'B,' containing thirty eggs placed in 1,300 c.c. tap water with a small quantity of fowl faeces, was stored in a photographic dark room.

Batch 'C,' containing thirty eggs, was treated similarly to 'A' and 'B,' excepting that it was stored in the laboratory.

The results are shown in the tabulated statement XV.

EXPERIMENT XV

Batch	Eggs	Day									
		3	4	6	7	8	9	10	11	12	13
'A' exposed to direct sunlight	45	...	35L.	...	...	...	...	...	...	12P.	5P. 1M.
'B' darkroom	30	...	24L.	...	...	5P.	5P.	3P.	1P. 8M.	... 1M.	... 4M.
'C' exposed to diffused sunlight	30	16L.	...	2L.	2L.	8P.	...	8P.	... 13M.	2P. 3M.	2P.

EXPERIMENT XV (*continued*).

Batch	Eggs	Day								
		14	15	16	17	18	19	21	29	32
'A' exposed to direct sunlight	45	2P. 5M.	6P. 2M.	... 15M.	1P. 1M.	... 1M.	...	... 1M.	1P.	... 1M.
'B' darkroom	30	... 1M.	...	...	...	1P.	... 1M.	...	...	...
'C' exposed to diffused sunlight	30	... 3M.	... 1M.	...	...	...	...	...	...	...

Thus in 'A,' thirty-five (77·7 per cent.) larvae hatched from forty-five eggs, and twenty-seven (77·1 per cent.) of these larvae ultimately produced adults.

In 'B,' twenty-four (80 per cent.) larvae hatched from thirty eggs, of which number fifteen (62·5 per cent.) produced adults.

In 'C,' twenty (66·6 per cent.) larvae hatched from thirty eggs, all of which larvae produced adults.

It will be seen that the lowest percentage of hatching occurred in the laboratory (Batch 'C'), where 100 per cent. of those which did hatch ultimately produced adults. It should be noted, however, that no observations were made on the unhatched eggs after the thirty-second day; it is quite possible, therefore, that all, or a large proportion of them, may have been naturally resistant.

2. LARVAE

The effect of light or absence of light on the development of larvae

EXPERIMENT XVI

Twenty larvae which hatched between 9.45 a.m. and 11.45 a.m. on 24th March were divided into two equal batches, and each batch placed in a bottle containing 5 or 6 grains of rice and 10 ozs. tap water. One bottle ('A') was stored in the photographic darkroom, the other ('B') on the laboratory windowsill. All the larvae, excepting two of those in 'A,' pupated between 5 p.m. on 27th March and 8.45 on 28th March. On the next day (29th) one of the pupae in 'A' died. On the 30th (about 10 a.m.) the remaining seven pupae in 'A' and the full number (ten) in 'B' produced adults.

In this experiment it would appear that the development of the larvae under artificial conditions and at room temperature is not influenced by the presence or absence of light.

Experiment XVII.

'A.'	On verandah exposed to the sun	Food given, 0·3 per cent. in 1300 c.c. of water.
'B.'	In dark-room	
'C.'	In workroom	
'D.'	In ice chest and room temperature alternately	

h.	Larvae.	Day.															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	25	...	...	...	...	...	...	...	...	2P.	5P. 1F.	2P. 2M. 3F.	6P. 2M. 2F.	1P. 5F.	2P. 1M.	1P. 1M.	1P. 2M
	24	...	...	...	5P.	...	5P.	3P.	1P. 6F. 3M.	4P. 2F.	1P. 3F. 1M.	... 4M.	...	...	...	1P.	...
	20	...	...	...	...	...	8P.	...	8P.	... 10F. 3M.	2P. 2F. 1M.	2P.	... 3F.	... 1F.	...	...	...
	32	..	...	...	...	...	...	...	...	...	1P.	1P.	2P.	4P.	2P. 1M.	2P. 3F.	... 2F. 4M.

Experiment XVII (*continued*).

	Larvae.	Day.															
		17	18	19	21	22	23	24	25	26	27	28	29	30	31	33	36
	25	...	... 1M.	...	...	...	...	...	...	1P.	...	...	... 1M.	...	...	...	...
	24	... 1M.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	20	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
	32	10P. 2F.	5P. 2F. 1M.	... 1F. 1M.	... 1F. 1M.	... 1M.	...	... 3F. 2M.	...	... 1F. 1M.	...	1P.	...	...	1P.	?	?

Note.—'M' = Male. 'F' = Female. 'P' = Pupa.

Thus in 'A' 21 larvae completed their life cycle—84 per cent. reared in 28 days. In 'B' 20 larvae completed their life cycle—83·3 per cent. reared in 16 days. In 'C' all larvae completed their life cycle—100 per cent. reared in 12 days. In 'D' 31 larvae completed their life cycle—96 per cent. reared in 36 days.

The effect of temperature and darkness on the development of recently hatched larvae

The larvae in these two experiments (Experiments XVIII and XIX) hatched overnight. In Experiment XVIII, thirty larvae were divided into two equal batches, 'A' and 'B,' each of which was placed in 100 c.c. tap water containing 0·25 grams of fowl faeces. 'A' was kept in an incubator at 98·6° F. and 'B' in a second incubator which was not artificially heated, the temperature being about 78·8° F. The results are shown hereunder:—

EXPERIMENT XVIII

Batch	Larvae	Day.										
		1	5	7	8	9	11	12	13	14	16	18
'A'	15	...	1P. (a)	...	L. (b)	...	...	...	...	...	...	...
'B'	15	...	...	...	6P.	6P. 1M.	... 4M.	2P. 4M.	... 1M.	... 3M.	1P.	... 1M.

NOTE.—'L' = Larva. 'P' = Pupa. 'M' = Adult.

(*a*) The first larva pupated on the fifth day and died on the seventh.

(*b*) The remaining larvae were all dead on the eighth day.

Thus in 'A' (temperature 98·6° F.) only one larva pupated, and this lived only two days. The remaining larvae died by the eighth day.

In 'B' (temperature 78·8° F.) only 6·7 per cent. of the larvae failed to produce adults.

EXPERIMENT XIX

Batch	Larvae	Days					
		10	11	12	13	14	15
'A'	10	L.(a)	...	...	...	...	...
'B'	10	3P.	5P.	1P.	1P. 4M.	... 2M.	... 1M.

(a) All the larvae were dead on the tenth day.

Thus in 'A' (temperature 98·6° F.) none of the larvae survived, but in 'B' (temperature 78·8° F.) only 10 per cent. of the larvae failed to produce adults.

From these experiments it would appear that excessive heat and not darkness was responsible for the mortality. In both experiments (XVIII 'B' and XIX 'B') the larval period was longer than usual, i.e., from ten to thirteen days in the latter.

Subsequent records of experiments will show that the lengthened larval period was probably due to unsuitable feeding, since it has been shown in Experiment XVI that the absence of light does not retard larval development.

Quantity and nature of food, and its influence on larval development

In this experiment (XX) forty newly hatched larvae were placed in each of five vessels containing 300 c.c. tap water and 0·1 per cent. of food as follows:—

P.R. 0·3 grm. polished rice.
M.L. 0·3 grm. dry mango leaf.
F.F. 0·3 grm. dry fowl faeces.
B. 0·3 c.c. broth, previously exposed to the air for two days, with luxuriant growth of bacteria.
F.M. 0·3 grm. fresh fly maggot (dead).

The average laboratory temperature during the experiment was about 71° F. The results are shown in the corresponding statement (XX).

Experiment XX

Batch	Larvae	Day 8	9	10	11	12	13	14	15	16
P.R.	40	1P.	11P.	4P.	8P. 3M.	3P.	1P.	...	3P. 6M.	... 1M.
M.L.	40	...	...	...	...	...	...	...	...	1P.
F.F.	40	1P.	3P.	4P.	1P.	1P. 4M.	3P. 5M.	... 2M.	1M.	1P.
B.	40*	...	...	...	...	...	...	...	...	...
F.M.	40	1P.	...	... 1M.	1P.	...	2P.	... 1M.	...	... 2M.

Experiment XX (*Continued*).

Batch	Larvae	Day 17	18	19	20	21	22	23	24
P.R.	40	... 1M.	... 2M.	...	2P.	...	...	... 1M.	...
M.L.	40	...	...	... 1M.	...	...	...	...	...
F.F.	40	1P.	... 1M.	1P.	...	1P. 1M.	... 2M.	1P.	... 1M.
B.	40*	...	...	...	...	...	...	...	...
F.M.	40	...	...	...	...	...	...	...	...

* All died in larval stage.

The mortality totals in the above 0·1 per cent. foods are therefore seen to be:—P.R. 65·0 per cent., M.L. 97·5 per cent., F.F. 57·5 per cent., B. 100 per cent., and F.M. 90 per cent.

Experiment XXI

The number of larvae used in this experiment varied between ten and thirteen to each batch. All were recently hatched. Each batch was liberated in a vessel containing 400 c.c. tap water and 0·25 per cent. of food, as follows:—

M.L. 1·0 gram dry mango leaf.
D.C. 1·0 ,, dried cockroach.
H.D. 1·0 ,, dried horse faeces.
P.R. 1·0 ,, polished rice.
G.S. 2·0 c.c. goat serum.

The results are shown in the corresponding statement (XXI).

Experiment XXI

Batch	Larvae	Day								
		5	6	7	8	9	10	11	12	13
M.L.	13	1P.	1P.	1P.	... 3M.	2P.	1P. 2M.	1P. 1M.	1P. 1M.	1M.
D.C.	10	...	4P.	2P.	... 4M.	2P. 2M.	... 2M.	...	...	...
H.D.	10	...	...	...	...	1P.	...	2P.	... 3M.	1P.
P.R.	13	11P.	... 11M.	...	...	...	...	...	...	...
G.S.	13	...	1P.	2P.	1P. 2M.	3P. 2M.	...	... 3M.	...	...

Experiment XXI (*continued*).

Batch	Larvae	Day							
		14	15	16	17	18	19	20	21
M.L.	13	...	...	1P.	...	1P. 1M.	1P. 1M.	...	... 1M.
D.C.	10	...	1P.	...	... 1M.	...	...	...	...
H.D.	10	1P.	... 1M.	...	... 1M.	...	...	...	...
P.R.	13	...	...	...	...	1P.	...	... 1M.	...
G.S.	13	2P.	1P.	1P.	... 3M.	... 1M.	... 1M.	...	...

Thus the mortality totals in 0·25 per cent. of the foods shown above are as follows:—M.L. 15·4 per cent., D.C. 10 per cent., H.D. 50 per cent., P.R. 7·7 per cent., and G.S. 7·7 per cent.

EXPERIMENT XXII

From nine to seventeen recently hatched larvae were liberated in each of nine vessels containing 400 c.c. tap water and 0·3 per cent. of food, as follows:—

M.L. 1·2 gram. dry mango leaf.
D.C. ,, dry cockroach.
H.D. ,, dry horse faeces.
P.R. ,, polished rice.
P. ,, peptone.
P.S. 0·15 per cent. peptone solution, tweny-four hours old, filtered.
B.C. 1 ,, agar slant, B. coli.
B.C.2 ½ ,, ,, ,, ,,
H.U. 4 c.c. human urine.

The results are shown in the corresponding statement (XXII).

EXPERIMENT XXII.

Batch	Larvae	Day									
		5	6	7	8	9	10	11	12	14	18
M.L.	17	3P.	6P.	... 4M.	5P. 4M.	...	1P. 6M.	...	1P.	... 2M.	...
D.C.	17	2P.	5P.	6P. 1M.	... 4M.	3P. 5M.	...	... 4M.	...	...	...
H.D.	9	2P.	2P.	... 2M.	...	...	... 2M.	...	...	...	...
P.R.	9	5P.	2P.	...	... 7M.	...	...	...	...	...	...
P.	15	...	1P.	...	...	...	...	...	...	...	...
P.S.	9	5P.	3P. 1M.	... 3M.	1P. 4M.	...	... 1M.	...	...	...	...
B.C.	11	7P.	2P. 1M.	... 5M.	... 2M.	...	...	... 1M.	...	...	...
B.C.2	10	5P.	1P.	... 5M.	...	...	... 1M.	1P.	...	1P. 1M.	... 1M.
H.U.	9	4P.	... 1M.	... 2M.	1P. 1M.	1P. 1M.	... 1M.	...	...	1P.	... 1M.

Thus the mortality totals in 0·3 per cent. of the foods shown are as follows:—M.L. 5·9 per cent., D.C. 5·9 per cent., H.D. 55·5 per cent., P.R. 22·2 per cent., P. 93 per cent., P.S. nil, B.C. 18·2 per cent., B.C.2 20 per cent., and H.U. 22·2 per cent.

The results of these three experiments are tabulated in Table 7.

TABLE 7.

Batch	Nature of food	Percentage of food to water	Percentage of mortality
P.R.	Polished rice	0·1 0·25 0·3	65·0 7·7 22·2
M.L.	Mango leaf	0·1 0·25 0·3	97·5 15·4 5·9
F.F.	Fowl faeces	0·1 0·25 0·3	57·5 ... ...
B.	Broth	0·1 0·25 0·3	100·0 ... ...
F.M.	Fly maggot	0·1 0·25 0·3	90·0 ... ...
D.C.	Cockroach	0·1 0·25 0·3	... 10·0 5·9
H.D.	Horse faeces	0·1 0·25 0·3	... 50·0 55·5
G.S.	Goat serum	0·1 0·25 0·3	... 7·7 ...
P.	Peptone	0·1 0·25 0·3	... ... 93·3
P.S.	Peptone Solution	0·15	nil
B.C.	1 agar slant *B. coli*	...	18·2
B.C.2	½ agar slant *B. coli*	...	20·0
H.U.	Human urine	1·0	22·2

EXPERIMENT XXIII

'A.' 200 c.c. of tap water containing 0·5 per cent. rice starch.
'B.' ,, ,, ,, with the addition of 5 per cent. sugar.
'C.' ,, ,, ,, with the addition of half the white of an egg.
'D.' ,, ,, ,, containing piece of banana (removed after the third day).

The results are shown in the corresponding tabulated statement (XXIII).

Batch	Larvae	Day								
		7	8	9	10	11	12	13	14	15
'A'	22	...	...	...	...	...	...	3P.	...	...
'B'	20	L. (a)	...	...	...	...	...	...	...	...
'C'	20	...	...	9P.	1P.	... 1M.	... 6M.	3P. 2M.	...	1P. 3M.
'D'	30	1P.	8P.	5P.	6P.	... 1M.	4P. 4M.	... 2M.	1P. 9M.	2P. 5M.

EXPERIMENT XXIII (*continued*).

Batch	Larvae	Day								
		16	17	18	19	21	22	23	24	25
'A'	22	... 3M.	...	11P.	...	1P.	1P.	... 11M.	2P.	... 4M.
'B'	20	...	...	...	...	...	...	L. (b)	...	...
'C'	20	2P. 1M.	...	(c)	(d)	...	...	...	...	1P.
'D'	30	...	... 6M.	...	(e)	...	...	...	...	...

(*a*) Some of the larvae now in second skin.
(*b*) Larvae in second and third skin.
(*c*) Two pupae and one larva died.
(*d*) One pupa died.
(*e*) The remaining larvae died.

Mortality	'A'	... ...	18·2 per cent.
,,	'B'	... ...	100 ,,
,,	'C'	... ...	35 ,,
,,	'D'	... ...	10 ,,

The effects of an increase of temperature on larvae and pupae of Stegomyia

A number of recently hatched larvae were placed in water in beakers heated over a water bath. When the temperature reached 114·8° F., 75 per cent. of the larvae were found to be dead, and the remainder failed to develop when the water was gradually reduced to room temperature.

Further experiments were then carried out with older larvae, i.e., larvae in their third skin, and with pupae. The procedure was the same as in the first experiment. The results of these experiments are seen in the tabulated statement (XXIV).

EXPERIMENT XXIV

	Temperature	Number used		Number subsequently reared	Number killed by heating	Mortality
		Larvae	Pupae			
1	90° F.	10	3	all	nil	nil
2	95° F.	10	3	all	nil	nil
3	100° F.	10	3	all	nil	nil
4	105° F.	10	3	10	3 larvae	23 %
5	110° F.	10	3	4	2 pupae 7 larvae	69·2 %
6	115° F.	10	3	nil	all	100 %

From the foregoing statement it will be seen that the approximate maximum temperature that the larvae and pupae are capable of withstanding without great mortality is about 105° F.; beyond this temperature there is a very great increase in the mortaliy. Bacot showed that a temperature of 112° F. caused a mortality of 50 per cent., and that higher temperatures caused the death of all larvae and pupae experimented with.

The survival of larvae and pupae out of water

EXPERIMENT XXV

(1) On filter paper kept slightly moist

Thirty-four larvae and five pupae were placed on wet filter paper and kept moist by adding a few drops of water each day. Two adult mosquitoes emerged on the following day, two others on the second day and the remaining one on the fourth day. The larvae were left on the paper until the fifteenth day, when only two of them remained alive. These two were then transferred to a bottle of water, but failed to reach the pupal stage.

(2) On wet filter paper and allowed to dry in muslin covered dish

Larvae and pupae were placed on wet filter paper in a muslin covered dish and allowed to dry gradually. Two larvae and one pupa were removed to water at the end of thirty-two hours, when the paper was nearly dry, and all of them eventually developed into adults.

Of two larvae and one pupa removed at the end of forty-seven and a half hours, only the latter was alive, but this died soon after in a dish of water. Another pupa, however, was alive after fifty and a half hours on the paper, but was dead at seventy-two hours. None of the larvae appeared to have survived beyond thirty-two hours.

(3) On wet filter paper and allowed to dry in a glass covered dish

Thirty-one larvae were placed on wet filter paper and put on a glass covered petri dish. After sixty-five hours in this dish seven larvae were removed to water, where one of the seven eventually matured. The remaining twenty-four larvae were transferred to water after having been on the paper for eighty-nine hours. Of these five were alive, but one died during the following twenty-four hours. The remaining four lived for nine days, after which no further record of them was kept.

3. ADULTS

Length of life of adult mosquitoes in captivity when unfed and fed on various foods

The mosquitoes used in this experiment (XXVI) were recently emerged laboratory-bred insects, all of which (except A.) were given an opportunity of feeding daily during the course of the experiment. The various batches were kept in similar cages and treated similarly to each other, excepting with regard to the food given. The average daily temperature was between 71° F. and 80° F.

A. Not fed.
B. Fed on syrup.
C. Milk and sugar.
D. Banana.
E. Blood once (females); banana afterwards.
F. Blood for sixty-one days, then bananas (females); banana only for males.

The results are shown in the following tabulated statement:—

EXPERIMENT XXVI

Expt.	Number of adults used	Day 2	4	7	12	15	19	26	30	35	47	51	58	61	68	93
A'	25F. 12M.	9F. 8M.	15F. 4M.	1F.	...	...	...	...	...	...	...	...	...	...	...	...
B'	7F. 4M.	...	2M.	2F.	...	2F.	2F. 1M.	1F.	1M.	...	...	...	...	...	...	...
C'	7F. 2M.	...	...	1F.	1F. 2M.	2F.	2F.	...	...	...	...	...	...	...	...	...
D'	5F. 5M.	...	...	...	...	...	1F. 1M.	1M.	1F. 1M.	1F. 1M.	1F. 1M.	...	...	...	1F.	...
E'	3F. 1M.	...	...	...	...	...	...	1F. (e)	1M.	1F.	...	...	...	1F.	...	...
F'	15F. 5M.	...	...	...	5F.	...	5F. 1M.	...	1F. 2M.	...	1F. 1M.	1F.	1F.	1M.	...	1F.

(c) = Escaped from cage. F. = Females. M. = Males.

Retention of eggs by gravid females

Oviposition on dry surfaces

It is a generally accepted belief that either water or a damp surface is essential to oviposition, and that if such is not available the gravid female will retain her eggs for a long period—until her death or until favourable conditions are made available. My experience in the routine of breeding *Stegomyia*, as well as the results of a number of experiments undertaken especially to confirm former observations, convince me that this belief is well founded.

Oviposition on oily surface

That similar results follow the provision in the breeding cages of only water having a surface film of oil is shown by the following experiments:—

EXPERIMENT XXVII

On the morning of 11th September, two gravid females and four males were placed in a breeding cage containing a dish of tap water covered with a thin film of kerosene. For several days previously the females had fed on human blood and the males on banana, which foods were offered daily during the progress of the experiment. The following observations were subsequently recorded:—

11th September (afternoon), one female fed. 13th, one female fed. 14th, one female fed; three males drowned. 17th, a female drowned. 19th, the remaining male drowned, three others liberated in cage. The remaining female fed on 29th September and on 4th October. No eggs laid up to this date. A dish of tap water without oil on surface was put into the cage, and the first dish (with oil) removed. 5th October, forty-six eggs laid over-night, the first oviposition during her twenty-four days of captivity. Female fed in afternoon. 15th October, seventy-nine eggs laid over-night. Dish of fresh water removed and replaced by one containing water with oily film. 16th October, female fed. 22nd October, female fed, and at intervals of about three days until her death. One male drowned. 24th October, one male drowned. 25th October, one male drowned. 8th November, female drowned.

Egg laying by unfertilised females

We have noticed on several occasions that certain batches of eggs produced females only, and that when segregated and fed on human blood these unfertilised insects were capable of laying eggs.

In this connection the following experiment is of interest as showing the increase which takes place in egg production after fertilisation.

The mosquitoes used in this experiment, seven in number, were reared from a batch of eggs which produced females only, and as soon as possible after hatching they were segregated in such manner as to preclude the possibility of fertilization. Opportunities for feeding on human blood were given frequently from the date of emergence, i.e., 7th September. After thirty days of captivity in this manner, two males were liberated in the cage with the five surviving females for seventeen hours, with the results shown in Experiment XXVIII.

EXPERIMENT XXVIII

Dates on which eggs were laid	Number of eggs laid	Approx. number proved fertile	Fertility	Deaths to date	Feeds given to date	Remarks
22.9.17	5	...	...	2	14	
24.9.17	6	...	...	...	16	
1.10.17	85	...	...	...	19	Total number of eggs laid by unfertilised females : 105
8.10.17	9	...	...	...	23	Total number of feeds given : 23
4 p.m. 8.10.17 to 9 a.m. 9.10.17	(17 hours) 2 males liberated in cage with 5 surviving females.					
10.10.17	60	30	+	...	28	
12-13.10.17	78	78	+	...	...	
15.10.17	12	3	+	...	31	
17.10.17	31	31	+	...	33	
18.10.17	60	60	+	...	35	
22.10.17	140	140	+	...	39	
25.10.17	52	38	+	1	41	
29.10.17	120	100	+	...	...	
2.11.17	72	60	+	1	...	
7.11.17	140	100	+	1	44	Total number of eggs laid by fertilised females : 765
8.11.17	...	...	...	2	...	Total number of feeds given to fertilised females : 21

From the foregoing it will be seen that seven unfertilised females did not lay eggs during the first fifteen days, and that during this period two died. During the following fifteen days the remaining five females laid one hundred and five infertile eggs and were then mated with two males. During the thirty days following the introduction of the males seven hundred and sixty-five eggs were laid, of which number approximately six hundred and forty proved fertile.

Experiments to ascertain whether Stegomyia fasciata *would lay eggs when fed on food other than blood*

From time to time various batches of mosquitoes were given food other than blood for periods ranging from twenty-five days to one hundred and forty days. The following foods were experimented with:—

Concentrated sugar solutions	4	experiments.
Concentrated peptone and sugar solution	3	,,
Concentrated sugar and haemoglobin solution	3	,,
Milk and sugar	2	,,
Banana	1	,,
Peptone solution	2	,,
Syrup	1	,,
Honey	1	,,
Dates	1	,,
Apple	1	,,

In each of the three experiments in which peptone and sugar were given as food a number of eggs were laid, about 60 per cent. of which were fertile. In all other experiments there was no egg production.

ACKNOWLEDGMENT

In conclusion, I desire to thank Mr. G. F. Hill, Entomologist to the Institute, for his help and advice in the preparation of the manuscript of this paper for publication.

ANCYLOSTOMA CEYLANICUM IN THE CAT IN DURBAN

BY

B. BLACKLOCK

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Material from Durban was sent by Dr. F. G. Cawston on 10th June, 1919, with the following notes:—

'*Ankylostoma duodenale* from a cat in Durban. The gut also contained a tapeworm, and death seems to have been caused by the numerous Ankylostoma.'

The material consisted of ten female worms, of which nine were complete and in a good state of preservation.

It is not possible in the absence of specimens to describe the characters of the male bursa, but the general appearance of the females, the measurements and the arrangement of the chitinous buccal capsule and teeth have been studied.

Of the nine complete specimens the minimum length is 7·0 mm., the maximum 9·5 mm., the average 8·3 mm. The arrangement of the teeth is characteristic. There is a pair of large ventral teeth and also a very small pair situated at the base of these on a deeper plane. The measurements and mouth structure are, in fact, such that this parasite cannot be distinguished from *A. ceylanicum* (Looss). This parasite was recorded by Yorke and Blacklock (1915) in seven dogs in Sierra Leone, and was further referred to by them (1917) subsequent to Macfie's discovery of the same parasite in four of ten dogs at Accra (1916).

That this parasite should be found in Durban is of interest, more especially so if, as Dr. Cawston surmises, it should be the cause there of fatal disease in cats.

REFERENCES

MACFIE, J. W. S. (1916). Report of the Accra Laboratory for the year 1916.

YORKE AND BLACKLOCK, (1915). Ankylostomiasis in Dogs in Sierra Leone. *Ann. Trop. Med. & Parasitol.*, Vol. IX, p. 425.

——— (1917). The Occurrence of *Ankylostoma ceylanicum* in West African Dogs. *Ann. Trop. Med. & Parasitol.*, Vol. XI, p. 69.

THE MECHANISM OF THE SPONTANEOUS ELIMINATION OF YELLOW FEVER FROM ENDEMIC CENTRES

BY

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First: This inquiry is limited to places in regions in which yellow fever is endemic: biologically, to places in which *Stegomyia* is abundant and active at all seasons: geographically, to tropical America.

The mechanism of its spontaneous elimination from places north or south of this endemic zone is too well known to be discussed, viz., the death or inactivity of *Stegomyia* caused by cold weather.

Secondly: It is limited to places in the above regions in which elimination was not due to the sanitary measures taken for this purpose.

The mechanism by which this has been accomplished is in effect the same as I have just mentioned as occurring north and south of the endemic zone, i.e., the control of the insect host. It differs from the elimination due to cold weather only by the degree of destruction of *Stegomyia* and the method of accomplishing this. Indeed, in places far south—as in Key West—the degree of the elimination of *Stegomyia* by the advent of winter may not exceed that of a well-conducted campaign against their breeding-places in Panama.

Note that in such towns it is not necessary to exterminate *Stegomyia* to eliminate yellow fever. If the number of mosquitoes be brought below the 'critical number' for yellow fever—an idea we owe to Ross as applied to malaria—at that place the disease will die out. Note, too, that this 'critical number' for any place will vary directly as the proportion of men immune to yellow fever to the total population: thus, if with one hundred cases of yellow fever introduced into a community in which all were susceptible to yellow fever, the number of *Stegomyia* were such that exactly one hundred men would be infected from them, the disease would neither die out nor increase. This would be the critical number of *Stegomyia* for that place and time. With less mosquitoes than this, less than one hundred men would be infected and the fever would die out. If more, it would increase. Now if one-fourth of the inhabitants are immune to yellow fever, obviously the same number of mosquitoes which infected one hundred men before would now infect only seventy-five—one-fourth of their bites going to immunes, and hence wasted—and the disease would die out. The number of mosquitoes required to infect one hundred more men, and hence perpetuate the fever, would have to be increased by one-third above the first number.

Obviously then, this critical number, below which the *Stegomyia* must be brought to eliminate fever, is less in a town as the proportion of susceptible people increases, and more intensive work is required to eliminate it from such a community, other things being equal, than from one in which a large proportion of the population is immune. This will be referred to later.

Yellow fever has been driven from the great permanent endemic foci of Havana, Vera Cruz, Panama, Rio, Santos and Para by sanitary measures, i.e., by control (lessening) of *Stegomyia*. It has also disappeared from a number of former endemic foci in which no measures were taken and in which *Stegomyia* are still abundant and active. This is so well known that it only requires mention. Among them I name Georgetown and Demarara in British Guiana—the former the seat of the several epidemics on which Blair's invaluable monographs are based—free now as are Kingston and Port Royal in Jamaica. Add Port-au-Prince, Jacmel, Cape Haytien and San Domingo, in none of which can sanitation have been a factor, for there had been none. Yellow fever was virulent in all four for years, and to the last named is accredited the evil distinction of being the place where this disease was first introduced to the white race. St. Thomas, accounted a danger to naval vessels for many years as always infected, has been free for over twenty-five years; as has St. Lucia, the site of severe epidemics, except for one epidemic in 1901 from infection introduced from Brazil (Low).

Except St. Lucia none of these have, I think, reported yellow fever for from twenty to thirty years.

Havana was freed from yellow fever by sanitary measures—control of *Stegomyia* breeding. Nothing of the sort was done at Puerto Principe, where there was a sharp epidemic in 1899. I think the same is true of Santiago de Cuba, with an epidemic the same year, but I have less knowledge of the sanitary measures taken there. Cienfuegos and Matanzas, to my positive knowledge, were free from yellow fever long before this sanitary measure was instituted for them. All four of these Cuban towns are free now, and have long been free. La Guira—once of evil fame—Maracaibo, Cartagena and Corinto, all formerly infected, have shown no fever for a number of years. I could add many others.

What is the status of these towns as regards yellow fever? Has the disease simply ceased to appear, being still existent, or has it, that is the parasite, ceased to exist in this community? In other words, does the 'Spontaneous Elimination,' of which I have purposed to show the 'Mechanism,' occur? This really is the first proposition to be proven.

The reappearance of fever in certain towns after periods during which it has not been reported, has made us, especially those of us charged with Maritime Quarantine, slow to accept a place, especially a seaport, as free from yellow fever simply because none has been reported there for some time; and this without impugning the good faith of the local health authorities. Yellow fever can exist among the children of a town, especially if there be much negro blood among the people, and be scarcely capable of diagnosis. It would practically not be reported among such. *Non-report of yellow fever does not of itself imply its non-existence.*

We need not consider the old theory that although it is not appearing in men, yet the organism is still alive and growing in fomites ready to attack any susceptible man who is exposed to it. And yet I am persuaded that part of this idea, that of the permanence of the condition, is the parent of the concept of 'latent yellow fever.' That it is carried on indefinitely 'in endemic centres' by 'recurrent attacks among the indigenes' is the doctrine of a Commission of the Pasteur Institute. The rôle of human carriers without symptoms in thus continuing the disease would be added by some writers. This has been further modified by the doctrine of 'larval yellow fever': that the strain has been so attenuated by passing through the resistant indigenes that the disease is taken lightly even by newcomers (Europeans) and hence not recognized, although immunizing them. In any case the general belief has been that a town or district infected with yellow fever in the tropics rarely frees itself, that is without sanitation, from the infection.

It may not be easy to pronounce such a town free from yellow fever. Have we then no test to determine the presence of yellow fever, the existence of the parasite, in a community? The *positive* test is simple and convincing. If a case of yellow fever is contracted in that community, yellow fever exists there. The *negative* must be that people suceptible to yellow fever, and in whom its diagnosis would be made if they developed it, live in this community so as to be exposed to yellow fever if it exists therein and are *not* found to develop it.

Negative evidence is convincing in proportion to its mass, and to accept this negative test as convincing we must have large numbers of exposures of such people, i.e., many people exposed over a considerable period of time, and the more intimate the local relations with the native population among which the fever is supposed to be latent the better.

So far as 'larval yellow fever' is concerned, the advocates of this phase of the disease admit, or rather assert, that the passage of the organism through newcomers restores its virulence and the disease becomes recognizable. Prolonged residence then, of large numbers of newcomers in the infected environment should do away with this camouflage of the disease, for the existence of which, indeed, I have seen no satisfactory evidence. Is not this test fair? Let us apply it. I think it will be admitted without argument that Georgetown and Demarara, Kingston and Port Royal are now free from yellow fever. We received men at Ancon who had contracted yellow fever in Corinto in 1905 and 1906, when it was also at Managua and Valencia. We kept an Inspector at Corinto from 1908 to 1912, a man who had not had yellow fever. He reported no yellow fever, no sanitary measures taken and *Stegomyia* in abundance. In the spring of 1912, five thousand U.S. Marines landed at Corinto, making this place their base of operations. They occupied this place and Managua and Valencia all summer. No yellow fever occurred among them. Could there have been any in Corinto? We have had Marines at Jacmel, at Cape Haytien, at Port-au-Prince and at San Domingo. Prior to this, and when we had no control of port sanitation, we had naval vessels lying at these ports and landing parties and no yellow fever among either garrisons, crews or landing parties. Yet in 1905, of two naval vessels lying in Panama Bay, Panama being infected, one became infected—seven cases and two deaths—although they took all the precautions their regulations called for, certainly more than were possible in the close harbour of Port-au-Prince or when sending armed men ashore. So in Guayaquil, in 1907, I think, one naval vessel lay in the harbour and she developed yellow fever aboard, losing her Commander. All of these San Domingan towns had had yellow fever badly and for many years, infecting the vessels, naval and others, in their harbours.

In Maracaibo, in 1916, we found Americans working the oil wells. There was a large number of them, scattered all over the town; they were practically all susceptible to yellow fever and they had been there some eighteen months or over. Maracaibo was alive with *Stegomyia*. There had been no yellow fever among the Americans. Obviously there was none in Maracaibo.

Baranquilla is the Caribbean port of entry to the Colombian highland. Through it pass each year some thousands of people susceptible to yellow fever: Colombians from the plateau, Americans and Europeans. These people, going either way, wait in town from one to six days for vessels. No yellow fever has been reported among them since 1907 or 1908. It could scarcely have been missed. Guiteras and myself in 1916 made a most careful examination of the mortuary records of the town since 1911, and we are sure that yellow fever has not been prevalent among the children since that date. Baranquilla is innocent of any yellow fever sanitation. If Baranquilla stays free, Cartagena is free also.

Why multiply examples? I could give a number of others, but I think I have shown that by our test *strictly applied* towns in the tropics *do* free themselves from yellow fever, *and that without sanitary work*. This is the first part of my proposition. The examples I have given show that this is not rare.

By what mechanism is yellow fever thus eliminated?

If we assume that one attack of yellow fever gives, usually, a permanent immunity to that disease the argument will be easier to follow. I, myself, believe that this immunity is generally permanent, as much so as that from small-pox or measles. Other men do not. It will be seen that this assumption of permanence is not essential to the argument for the mechanism presented.

From the known facts of the conveyance of yellow fever, it is obvious that the conditions for the continued existence of yellow fever in a community are three: the carriers, active *Stegomyia calopus* and susceptible men: all present at the same time, the insects having access to both classes of men.

Parasites exist only in an infected mosquito or in an infected man. They live in the mosquito only during its life, and only a short time, infective to mosquitoes, in man.

Here, then, are two postulates:—

(1) Since the parasites in the mosquito live only during the life of the host, say, ten days, no interval greater than ten days may elapse between the date when some sick man infective to mosquitoes is bitten by them and the date when one of the mosquitoes infected by him feeds on a man susceptible to yellow fever without the death of the parasites, and hence the extinction of yellow fever in that community.

(2) Susceptible people, then, are necessary for the continuance of yellow fever in a community. Such people must not only be present, but must be present under certain conditions of time and place with relation to the *Stegomyia* infected from other people with yellow fever. If in a community there be no susceptible people fulfilling these conditions, yellow fever will disappear.

Now let us consider a community in the tropics in which yellow fever is present, *Stegomyia* abundant and active at all seasons, and with susceptible people. Parasites, of course, are present in those sick of yellow fever, and, since *Stegomyia* are active all the year round, this place will be an *endemic focus* of yellow fever.

Obviously, if one attack of yellow fever produces in general a permanent immunity, such a community will have in time no people susceptible to yellow fever left, unless there is an introduction of such people. Yellow fever would then disappear, and, as soon as the infected mosquitoes died off (within our ten days), the parasites would disappear and the community be free from infection. Indeed, yellow fever would doubtless disappear before there were 'no people susceptible to yellow fever left,' because, under the doctrine of chances, there would be no susceptible people left fulfilling the conditions of time and place mentioned above before there were absolutely none at all, possibly long before. Once free, it would remain free for ever, unless the same three factors for conveyance are again brought together. In the natural course of events a new generation would grow up susceptible to yellow fever, susceptible immigrants move in, and *Stegomyia* breed to the limit; but unless the *parasite* be again introduced the community would remain free from yellow fever. In such a community, growing naturally, an epidemic would result if the parasites were introduced some years after it had been free of infection, the maximum age of the natives then developing the yellow fever depending on the length of the interval of freedom.

An immigration of susceptible people, then, is necessary for the continuation of yellow fever in a community, and if this immigration fails, or fails to fulfil certain conditions, yellow fever disappears. This mechanism I have called the 'elimination of yellow fever by failure of the human host.'

This immigration might be of susceptible people from some other place, or of infants born in the place itself. This is just as true an introduction of susceptible people as the other. As old Blair says, 'truly are they new-comers.' If these additions to the susceptible population *conjointly* fulfil the conditions necessary for the continuance of yellow fever, as I have stated them, it will continue; if they do not, it will disappear.

The effect in continuing yellow fever of each class of these additions to the susceptible population—men from outside the community, and babies born in it—will depend on many factors, but among others on its amount, increasing for each class as that class increases.

Both classes of immigration then affect the continuance of yellow fever, and theoretically either one may be sufficient to continue it. Yet the proportional effect of the introduction of an adult and the birth of a baby in keeping up the infection is very different, that of an adult immigrant being very much the greater; so that to supply the susceptible people necessary to keep up yellow fever, it requires a very much larger number of babies to be born than of susceptible immigrants. Adult immigrants are of far more importance in thus keeping up yellow fever in proportion to their numbers than babies, and a town receiving no susceptible immigration needs to be much larger to be a permanent focus of yellow fever than if it did receive such immigration. Gorgas, indeed, in 1916 expressed himself as believing that immigration of susceptible adults was necessary to continue yellow fever, that it could not be kept up by the births alone. I cannot think so.

How does our explanation agree with the known epidemiology of yellow fever and with the facts we have adduced in our first proposition? On this agreement will depend whether we hold it tenable or not. Obviously, if our explanation holds, it will be the small and moderate sized towns receiving little immigration which will free themselves of yellow fever; the large cities and those with much susceptible immigration not doing so. And so we find:

(1) The towns I have mentioned as freeing themselves of yellow fever are all small, or of moderate size, and out of the way of commerce, receiving little immigration of suceptible people, and this phenomenon is evidently common among such towns.

(2) It is the large towns and those with considerable susceptible immigration: Guayaquil, Havana, Vera Cruz, Rio, etc., which did not free themselves, or are not yet free, from yellow fever.

(3) Besides these there are certain communities of small towns between which the travel relations are so close that they must be held, for this mechanism, as a single large town. Indeed, in such a group of inter-related towns the propagation of yellow fever would be decidedly slower than in a single town of their joint population, and the fever would therefore last longer and be less apt to disappear than in the single larger town. Such a section is Yucatan and Campeche—the large sisal haciendas being indeed towns—and this section has not freed itself from yellow fever, but seems to be a permanent endemic focus of yellow fever needing sanitation for its elimination.

(4) Also, towns which were badly infected while prosperous—hence large and with much immigration—as Georgetown and

Demarara, Port-au-Prince and Cartagena, become free from yellow fever as their trade declines. And see how Guayaquil reverses this!

(5) Also we find these small towns liable to epidemics of yellow fever from time to time, when a sufficient number of susceptible people have accumulated and the parasite is again introduced, and that when this occurs, as at Buenaventura in 1915 and 1916, 'children and people who have moved in during the past ten years were attacked.' The last epidemic here was twelve years previously. The occurrence of epidemics among the native-born is, to me, *proof* that yellow fever had not been general, whether 'latent' or not, since they were, say, three or four years old.

It is this recurrence of fever which has given rise to the belief in 'latent' or 'larval' yellow fever held by many very eminent men. Unquestionably yellow fever may, and at times does, exist unrecognized among the native children of a community; showing itself only, or rather being recognized only, when it attacks some stranger. Here we have true recrudescence whenever an influx of strangers occurs.

This view is too well known to require elaboration. It is true, and I will not pretend to predicate how long such a condition may last; nor deny that, under some conditions, it may last indefinitely and by this means alone keep the place or area of communicating places a permanent focus of yellow fever, and without cases occurring sufficiently marked to compel recognition. What I do deny is that this is the rule. Indeed, I believe that it is rather the *rule* for yellow fever to disappear, to disappear completely, from isolated communities of moderate size *and this without sanitary work or diminution of Stegomyia.*

The instances given prove that the spontaneous disappearance of yellow fever is not rare. An analysis of them would show that, in the absence of adult immigration and of inter-travel, this is to be expected to occur in a large proportion of towns, and that after this, even when there are influxes of strangers, outbreaks in such towns do not occur, unless they are in communication with some infected focus. These outbreaks, then, are re-infections and not recrudescences of 'latent' or 'larval' yellow fever.

I think our explanation then agrees, and agrees completely, with the facts as we have found them, and with the known epidemiology of yellow fever. Not only is it consistent with the facts observed, but the deductions from it are in accordance with other facts not hitherto noted, or at least not stressed. On this kind of evidence we are accustomed to accept other laws of nature.

It is obvious that the explanation I have given of the spontaneous elimination of yellow fever depends absolutely on the doctrine that an attack of yellow fever confers immunity against another attack. In proportion as that immunity is permanent and general, the chance of the exhaustion of the susceptible human material by a definite number of cases of fever, that is at a definite time, to the point of causing the disappearance of disease is greater than if the immunity be of short duration and uncertain. If this immunity be not permanent, but yet endures for some time, the disappearance of yellow fever by the mechanism I have outlined can still occur. This is evident. It is also evident that the men in our community who lose their immunity through lapse of time add to the supply of susceptible material just as immigrants would. In proportion as attacks recur frequently and at short intervals, so will the chance of failure of the human host, to the point of causing disappearance of the disease, diminish. If 'frequently recurring attacks of the indigenes' are the rule, and these recurrences are indefinite, they might very well continue the fever in a very mild or 'larval' form indefinitely, independently of immigration or new births. The *possibility* of disappearance by the mechanism we have given is then not dependent upon the permanence of the immunity given by one attack, but the *chance* of its occurring in any place at any definite time is directly dependent on it and reaches its maximum when, as we believe, one attack gives permanent immunity. The frequency with which yellow fever has disappeared when immigration was lacking is evidence against recurrent attacks.

Similarly, it fails in the presence of a permanent reservoir host for the micro-organism of yellow fever infective to *Stegomyia* or otherwise capable of communicating it to man, analogous to those for the trypanosomes of sleeping sickness. The existence of animal hosts, other than man, with the same reactions to the parasite as man—i.e., in which an immunity was caused by an infection, temporary or permanent—would not seriously affect it. Such animals, if present, would simply count as a certain extra number of men.

The evidence for the existence of a permanent reservoir host seems to me to have no basis in observation. The evidence against it, or rather against it being an animal associated with man in the places in which yellow fever has been studied, of necessity negative, is considerable.

Nevertheless, it would be much easier to accept the American origin of yellow fever, for which the historical evidence is very strong, if we had evidence of the existence of such a host in Columbian and pre-Columbian times in the Antilles or on the Carribbean litoral.

One thing we must note, however: The *fact* that yellow fever does disappear from towns in the tropics without sanitary work and with *Stegomyia* abundant is true. The explanation I have given is logical: it is in accord with what we know of the epidemiology of yellow fever, and I believe it to be true. Nevertheless, it is only a deduction from observed facts, not the fact itself.

One other thing. For a town which has freed itself from yellow fever *by the failure of the human host* to remain *permanently* free from yellow fever, *isolation* from infected places is necessary. When yellow fever has been eliminated *by the control of the insect host*, this isolation is not necessary *as long as this control continues to be efficient*, because with the control of the insect host, yellow fever is not communicable, and such parasites as are brought in by infected men or infected mosquitoes would, at the most, establish a very temporary focus of infection. If the control were complete, infected men would transmit no parasites. It is to be noted, however, that the reduction of *Stegomyia* sufficient to eliminate yellow fever from a town in the tropics would nearly always be less than that required some years later to prevent its spread, because there will then be a larger proportion of susceptible people than at first. Hence the mosquito control must be more intensive.

It is worth contrasting these two methods for the elimination of yellow fever. That by the control of the insect host is unquestionably the method of election for the sanitarian, while the method by which it is eliminated *in nature* is by failure (control) of the human host.

For malaria the method by which it has been eliminated *in nature* is by control of the insect host (agricultural drainage).

Malaria confers minimal immunity, and human 'carriers' without symptoms are in effect reservoir hosts.

Every one has noted the great diminution of yellow fever which has been going on since the fall of the tropical sugar industry in the smaller West Indian Islands, Hayti and the Guianas, and which is still in progress. It is due to a variety of causes:—

(1) To the diminished trade and commercial importance of the Islands, and the Carribbean litoral, and hence diminished immigration to this region.

(2) The substitution of steam for sailing vessels has enormously lessened the number of infections (parasites) carried between ports, thus lessening the re-infection of such ports as had cleared themselves of yellow fever. Sailing vessels frequently carried *Stegomyia* breeding in their water supply, as well as infected men, while iron steam vessels very rarely carried the former, and hence, in spite of the shortened voyage, were much less efficient in the transport of parasites. Sailing vessels, too, lay longer in port, had larger crews per unit of carrying capacity and were under laxer discipline, thus furnishing a much larger number of susceptible men ashore—temporary immigrants—in the ports they visited.

(3) With the loss of its commercial importance, indeed before it, came the diminished strategic importance of the Carribbean Sea and the practical withdrawal of European fleets and garrisons. In the 18th and the early part of the 19th centuries, the Carribbean was the rendezvous of the fleets of Great Britain, France and Spain, and to a certain extent, of the United States. The war vessels, sailing ships then, lay long in port; some in permanence as receiving ships for crews fresh brought from Europe. They carried large crews, one thousand men or more, and their shore parties added markedly to the susceptible population of the Carribbean seaports. They were a prime factor in carrying yellow fever from port to port both in infected men and in infected mosquitoes. It is worth noting how very generally the introduction of yellow fever in the different ports is ascribed to a naval vessel.

The rôle of the European garrison, with its frequent drafts from Europe and its massing of susceptible men, was the same in continuing the infection at the garrisoned port.

(4) The extinction of the great permanent foci of Havana, Vera

Cruz, Panama and Rio. With this a number of less important places were freed from yellow fever by sanitary work in control of the insect host; Santos, Para, Manaos, Iquitos, Pernambuco and Caracas. This enabled such ports, on the Carribbean especially, as could spontaneously free themselves of yellow fever to remain free, being no longer exposed to infection, or rather much less so exposed. The smaller ports, freeing themselves from yellow fever from time to time by failure of susceptible people, had been doubtless continually re-infected from the permanent foci, whenever they had accumulated sufficient susceptible material, by birth or immigration, for the spread of the fever. Even the Great War has helped in this, stopping European immigration, and by the commercial depression it caused lessening travel in the American countries in which yellow fever prevailed. To it is probably due the freeing of Maracaibo, Cucuta and Bucaramanga.

It is facts like these which justify the plan of the International Health Board for the elimination of yellow fever from the earth, a plan already operative at Coro and at Guayaquil. In my judgment it is entirely feasible. Many places in which yellow fever exists will need but minimal sanitary work to turn the scale against it, and the freeing of one place from yellow fever frequently prevents the infection of some other, making an endless chain of good. Many of the higher forms of life have permanently disappeared from the earth, some in our own times, but this is the first attempt made for this purpose against a micro-organism pathogenic to man. Its accomplishment will mark an era in sanitation.

THE METABOLISM OF WHITE RACES LIVING IN THE TROPICS

III. THE INFLUENCE OF EXTERNAL TEMPERATURE AND RATE OF COOLING UPON THE RESPIRATORY METABOLISM

BY

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A considerable amount of attention has been devoted to the question whether the human organism responds to changes in external temperature by changes in the quantity of heat produced; that is to say, whether changes in the heat production play any part in maintaining a constant body temperature. The action of low temperature has been studied by Loewy (1890), Johansson (1896), Rubner and Lewaschew (1897), and others, by observing the respiratory metabolism under different conditions, and the results point to the conclusion that an increased metabolism is only produced when the cold is sufficient to stimulate the muscles to voluntary or involuntary action, and to cause shivering. The increased metabolism produced on a cold day is due, therefore, to the cold air acting on the skin and producing an increased muscular activity or tone.

With regard to high external temperatures, such as exist in the tropics, the question is of considerable interest as to whether the body adapts itself to the altered conditions by a decrease in its heat production, or whether the temperature is regulated entirely by heat loss, i.e., by physical means.

A summary of the work done on the subject of metabolism in the tropics from this point of view has been given by Breinl and Young (1919) in an article on the settlement of Tropical Australia. The general results of these investigations are that a decrease in heat production does not play any important part in the maintenance of body temperature.

The action of high temperatures and humidity upon the respiratory gas exchange has been investigated mostly under artificial conditions in a temperate climate, and the results are not very definite, and in some cases contradictory. Briefly all that can be said is that there appears to be an ill-defined zone of temperature, about 15° to 25° C. (59° to 77° F.) in which the metabolism is at a minimum.

The effects of extreme humid heat upon the respiratory metabolism have been studied by Harvey Sutton (1908). When the wet bulb temperature of the surroundings rose above a critical point, as previously shown by Haldane, so that no evaporation of sweat could take place, the body temperature rose, and this caused an increased metabolism and corresponding increased production of heat, which again further increased the body temperature; a vicious circle was thus established, resulting in a gradually increasing rate in the rise of body temperature, until dangerous conditions were reached.

Eijkman (1896), working under natural conditions actually in the tropics, determined the respiratory exchange of eleven Europeans and twelve Malays, and compared the average amount of oxygen consumed and carbon dioxide expired with the averages obtained by workers in Europe. Using the Zuntz-Geppert method, he obtained, as the result of thirty-seven experiments on Europeans and forty-eight on Malays, an average of 245·7 c.cs. of oxygen absorbed per minute by the Europeans, and 215·5 by the Malays; these he compared with figures obtained by the same method in Europe by Geppert, Loewy and others, namely, 250·3 c.cs. per minute. In both cases the numbers are calculated to a body weight of 64 kilograms. He concluded from these that there was no evidence of any decreased heat production. It is obvious that the number of experiments is far too small to draw definite conclusions.

Observations were carried out, also under natural conditions, by Osborne (1912) during the summer in Victoria, Australia, where the temperature frequently rises above 100° F., and he found a decided increase with the higher external temperatures, both in the pulmonary ventilation and in the carbon dioxide produced.

The reaction of the metabolism to surrounding conditions has been investigated recently by Hill and his colleagues (1914) in England, by determining the respiratory exchange from day to day under natural conditions, and co-ordinating the changes he observed not with the actual air temperature, but with the cooling power of the atmosphere as determined by the katathermometers, two thermometers with large bulbs filled with alcohol. This instrument was invented by Hill to measure the rate of cooling due to atmospheric conditions on a body at a temperature in the neighbourhood of that of the human body. The two instruments employed (dry and wet bulb katathermometers) give the rate of cooling, the dry one by convection and radiation, and the wet by convection, radiation and evaporation. He found that in experiments done in the open air, the greatest metabolism took place on days on which the greatest rate of cooling was observed. He also found that in experiments done indoors where the rate of cooling was low, the metabolism was very markedly less than outdoors upon the same day, provided that the cooling power outdoors was very much higher than indoors. In short, the metabolism ran parallel with the cooling power of the atmosphere, and not with the actual temperatures as observed with the ordinary dry and wet bulb thermometers. He attributes the efficacy of the open air treatment of pulmonary complaints to the increased metabolism produced by the greater degree of cooling outside.

The present paper contains the results of experiments extending over three years, the subjects living in the tropics (19° South), with the object of investigating the changes in the respiratory metabolism under different atmospheric conditions. Observations have been carried out in the hot season of the year (November to May) and in the cooler season (June to October). In these experiments a similar procedure was followed to that of Hill, the cooling power of the atmosphere being observed by means of the katathermometers as well as the temperatures with the ordinary dry and wet bulb thermometers.

The writer wishes to acknowledge his indebtedness to Mr. J. W. Fielding, chief assistant of this Institute, who not only acted as one of the subjects, but helped with the various observations recorded.

METHODS EMPLOYED

The subject lay at ease in a deck chair, with a foot rest, and remained without movement during the experiment. The usual canvas of the chair was removed and replaced by a covering made from an open-mesh string hammock, so that the body should be freely exposed on all sides. After lying for about thirty minutes, the subject breathed for exactly twenty minutes through a mouthpiece fitted with inspiratory and expiratory valves into a Zuntz portable respiration meter, the nose being closed by means of a spring clip. The meter was previously tested by driving through it a known volume of air from a large aspirator at the rate of between 5 and 6 litres per minute, and a correction determined from the mean of a large number (100) of such tests, and applied to the volume of expired air in each experiment. This correction was very small, amounting to an addition of 2 c.c. per 100 c.c. measured. Certain modifications were employed in collecting the sample of expired gases for analysis. A mixing vessel was interposed between the mouthpiece and the meter, as recommended by Bohr (see Krogh (1916)), consisting of a cubical metal box of about 2 litres capacity. The sample was drawn out through the usual opening attached to the small box at the top of the meter, but the proportionate sampling arrangement employed by Zuntz was replaced by a gas sampling tube as designed by Huntly (see Haldane (1912)), which allowed of the sample being collected at an even rate over the whole period, merely by allowing the mercury with which the sampling tube was filled to run out through an exit tube of a suitable bore so as to collect about 180 to 200 c.c. in the time. The water acidulated with sulphuric acid, used by Zuntz, was thus dispensed with, as it was found that this absorbed a small quantity of carbon dioxide, especially if the analysis were postponed for any time. The sample was analysed by means of the Haldane apparatus, at least two analyses of each sample being performed.

Table I shows a series of pairs of observations, done at different times to test the method, each pair consisting of consecutive twenty minutes' breathing into the meter. The two observations were substantially the same in each case.

TABLE I.

Subject	Vol. of expired air Litres per 20 min.	Analysis of expired Air		CO_2 eliminated c.c. per min.	O_2 consumed c.c. per min.	Resp. Quot.
		CO_2%	O_2%			
W. J. Y. (1)	110	3·66	16·50	199	254	0·78
	112	3·69	16·51	205	260	0·79
(2)	108	3·49	16·74	186	236	0·79
	111	3·37	16·55	197	250	0·79
(3)	87·5	3·72	16·28	161	214	0·75
	86·0	3·80	16·09	162	220	0·74
J. W. F.	120	3·67	16·82	217	253	0·86
	116	3·58	16·89	205	241	0·85

Whilst the breathing was going on, the following observations were made by the assistant at the beginning, half-way through, and at the end of the time, and the averages of the readings so obtained taken as the figure for the whole time:—

1. The readings of the ordinary dry and wet bulb thermometers.
2. The temperature of the expired gases in the meter.
3. The temperatures, taken with dry and wet bulb thermometers, of the air between the skin and the clothing (called by Hill skin-shirt temperatures).
4. The times taken for the dry and wet bulb katathermometers to cool from 110° to 100° F. and 100° to 90°. As a rule, only one determination was made with the dry bulb instrument, as the time taken was generally too long to allow of a series of observations.
5. The pulse rate was noted, and the rectal temperature of the subject was taken before and after each experiment. The pulse rate did not show any great variations, the two extremes with W. J. Y. being 64 and 76 per minute, and with J. W. F. 60 and 70 per minute.

The volume of expired gases was calculated dry to 760 mm. pressure and 0° C., and for the calculation of the oxygen absorbed, a correction was made for the difference in volume of inspired and expired air as given by Haldane (1912). The percentages of oxygen and carbon dioxide in the inspired air were taken as 20·90 and 0·03 respectively, the figures given with outside air by the Haldane gas analysis apparatus employed in the experiments.

The experiments were carried out on two subjects, W. J. Y., 38 years at commencement and 45·4 kilograms in body weight, and J.W.F., 27 years and 56·2 kilograms body weight, and were always done in the mornings about the same time after breakfast. Neither subject altered in weight during the whole period by more than a few tenths of a kilogram. A number of preliminary experiments were performed in order to get the subjects accustomed to the mouthpiece.

In all but a few experiments the same amount of clothing was worn, consisting of a light shirt, cotton trousers, socks and shoes. Most of the experiments out of doors were carried out in the shade of a large mango tree, and a few on the verandah of the first storey of the Institute. The accompanying tables give the details and results of the experiments.

AIR TEMPERATURE

The climate in the coastal districts of North Queensland is monsoonal, and the rains occur during the hot months of the year (November to April), during which time the readings of the wet bulb thermometer are very high, and typical moist tropical weather is experienced. During the hot season the outside dry bulb temperatures lay between 80° and 91° F. (26·7° and 32·8° C.), and the wet bulb was with a few exceptions always between 75 and 80° F. (23·9° and 26·7° C.), whilst higher wet bulb temperatures (over 80° F.) were observed indoors. In the cooler season experiments were carried out with much lower air temperatures, the lowest dry bulb reading being 62·8° F and the lowest wet bulb 51·4° F.

THE SKIN-SHIRT TEMPERATURE

It is a well-known fact that there is always a layer of stagnant air between the clothing and the skin which is usually at a higher temperature and at a greater degree of saturation with moisture than the external air. The more the body perspires and blocks the pores of the clothing the greater becomes the degree of saturation of this air. In a cool climate the difference in temperature between this air immediately surrounding the body and the outer air is much more marked.

For comparison with the experiments of Hill, dry and wet bulb temperatures were taken by two thermometers which were prevented from touching the skin by being enclosed in a small wire cage. The bulb of one was covered with a woven cotton glove and was dipped in water before inserting between the skin and the shirt. The figures obtained in these experiments under various conditions are given in the tables.

It was observed that when the subjects were out of doors the skin-shirt temperatures ran roughly parallel with the air temperatures, and were several degrees above these. In the hot season the difference between the dry bulb temperatures of the skin-shirt air and the outer air was less marked than between the wet bulb temperature.

Hill has published a number of skin-shirt temperatures which he collected in England, and it is noticeable that many of his figures taken out of doors in winter with the subject at rest, are as high as those registered in North Queensland in a tropical summer (e.g., he observed wet bulb temperatures of 88° on several occasions). It must be borne in mind that the subjects of Hill's experiments were wearing heavy clothing, whereas the clothing worn here was of the lightest description. These figures thus show the influence of the clothing on the temperature of this air, and bear out the truth of Rubner's remark that a clothed man always lives in a tropical climate as far as his body is concerned.

The effect of moving air upon the skin-shirt temperature were also studied by Hill who showed that these greatly effected this temperature; on a windy day he recorded skin-shirt temperatures as low as 64° F. wet and 74° F. dry, whilst temperatures lower than these were also observed by him when the rush of air was made by cycling rapidly downhill into the wind. The effect of air movement in the experiments done in Townsville was not nearly so marked as in those quoted above.

The figures recorded in the cooler season of the year show much lower skin-shirt temperatures. A wet bulb skin-shirt temperature as low as 64·8° F. was observed on a very windy, showery day in

June, the subject at the time feeling so cold that he was shivering. Much higher temperatures were observed when the subject was in the sun; thus, on one occasion skin-shirt temperatures of 103·6° dry bulb and 99·6° wet bulb were observed, and on another 98·7° dry and 90·7° wet bulb.

THE KATATHERMOMETERS AND RATE OF COOLING

It has been recognised that the wet and dry bulb thermometers do not give a true indication of the effect of the atmosphere upon the human body. Anyone who has lived in a hot moist climate knows the immense difference in comfort between moving and still air, yet little change may be observed in the actual air temperatures under the two conditions. Since the comfort or discomfort of the body is determined by the rate at which cooling can take place, and not by the actual temperature of the surroundings, Hill has devised the katathermometers, which measure the rate of the heat loss, i.e., the cooling power of the atmosphere under the existing conditions. In the tropics when the outer temperatures are high, the evaporation of the sweat plays a much greater part in the cooling mechanism of the body than it does in a temperate climate, so that the rate of cooling of the wet bulb katathermometer is a closer representation of the actual conditions than the dry bulb instrument.

In these experiments the time taken for the dry katathermometer to fall from 110° to 100° only was generally recorded, on account of the long time taken to cool to 90°: the wet bulb was generally carried on to 90°. These times are given in the tables in seconds. It has been found convenient for comparison of the figures to express the rate of cooling over the whole ten degrees in degrees lost per minute; these figures are also given in some of the tables.

It was observed by Osborne (1916) in Melbourne, that the wet bulb katathermometer is extremely sensitive to air currents, and that successive readings outdoors, especially in dry weather, may show very considerable variations. Katathermometer observations carried out here have shown this objection to be justified, especially as regards the wet bulb instrument. If a series of readings be taken in rapid succession in still air indoors, or outdoors on a still day, fairly consistent times may be obtained, and the same is true if a steady wind be blowing. On a day, however, with the wind coming in gusts great variations may be observed. Experience in Townsville has shown how very seldom days occur in which a series of observations can be done out of doors without fairly large variations, and what would be felt as a still tropical day is often not so calm when tested by the wet katathermometer. Several observations with the wet katathermometer are given in Table II to illustrate these points.

TABLE II.

No.	Air Temperature		Wet Bulb Katathermometer		
	Dry B.	Wet B.	110°—100°	100°—90°	
			seconds	seconds	
(1)	88·1	81·7	76	152	Indoors with doors closed. 10 minutes readings.
	89·0	81·9	73	151	
	89·0	81·9	77	155	
(2)	63·3	52·0	17	20	Outdoors, cool season breeze blowing. 5 minutes readings.
	...	...	15	20	
	64·7	51·8	15	22	
	65·8	52·8	16	21	
(3)	87·1	80·2	67	128	Hot season. Outdoors, wind in gusts. 5 minutes readings.
	...	...	48	89	
	...	...	52	83	
	...	...	76	124	
(4)	74·0	66·3	39	60	Outdoors, cool season. Breezy day. 10 minutes reading.
	75·2	66·9	46	56	
	76·2	67·3	32	51	
(5)	82·4	77·2	56	104	Day felt extremely close and uncomfortable. 5 minutes readings.
	82·0	76·5	49	74	
	81·8	76·3	57	94	
	82·0	77·0	62	99	
	82·0	77·0	61	114	

Nos. 1 and 2 show that constant readings could be obtained indoors, as well as outdoors when a strong steady wind was blowing; Nos. 3 and 4 show the effects of gusts of wind, and No. 5 shows the variations over half an hour on a day that was classed to the senses as very close and uncomfortable.

On account of these variations a series of observations was made with the wet katathermometer at intervals during the whole time of each experiment, and the average of the series taken as a representation of the cooling power during the time. With the dry katathermometer the time occupied in cooling, especially in the hot season, was much longer; thus, a sudden gust of wind would not produce so noticeable an effect.

The times recorded for the katathermometers to cool, in most cases, are of course much longer than those recorded by Hill in England. Indoors, in the hot season, the average time taken for the wet bulb katathermometer to cool from 110° to 100° was 74 seconds, and for the dry bulb instrument 330 seconds, approaching in fact some of the observations recorded by Hill in an artificially heated chamber, and similar to conditions found by him in weaving sheds in England, where the wet bulb temperature was 80° F. and more.

In the outdoor experiments considerable variations were noted, the rate of cooling from 110° to 100° varying from 250 seconds dry bulb in the hot season to 42 in the cool season, and 60 seconds wet bulb down to 16 seconds in the cool season.

It is interesting to note that on one or two days in June the time of cooling of the katathermometers was quite as low as some of the times recorded by Hill in England in winter (Table V, No. 16, and Table VI, No. 12).

THE RESPIRATORY METABOLISM

The experiments carried out indoors, Tables III and IV, were all performed in the same room with doors closed, so that in the comparison between the two seasons the only differences in outer conditions were those due to atmospheric temperature. Indoors, during the hot season experiments, the air temperatures averaged 87° F. dry bulb and 80° F. wet bulb, whilst in the cool season the averages were much lower, 71° to 74° F. dry bulb and 63° to 67° wet bulb. The rate of cooling indoors, as measured by the katathermometers, did not vary to any great extent on the different days of each season, but a marked difference was observed between the average rates of cooling during the hot and cool weather. With both subjects the average quantities of oxygen consumed and carbon dioxide eliminated were greater in the hot season. Thus, in subject W. J. Y., the average oxygen consumed was 248 c.c. in the hot

Subject W. J. Y. Indoor Experiments.

	Date	Air Temp. Dry B.	Air Temp. Wet B.	Skin-Shirt Temp. Dry B.	Skin-Shirt Temp. Wet B.	Katathermometers 110°—100° Time in Secs. Dry B.	Katathermometers 110°—100° Time in Secs. Wet B.	Katathermometers 110°—100° Rate of cooling Degrees per min. Dry B.	Katathermometers 110°—100° Rate of cooling Degrees per min. Wet B.	Vol. of air expired Litres per 20 min.	Analysis of expired air. CO_2%	Analysis of expired air. O_2%	CO_2 eliminated c.c. per min.	O_2 consumed c.c. per min.	Resp. Quot.	Rect. Temp.
Hot Season	1.3.16	88.5	81.8	92.9	88.3	345	76	1.7	7.9	109	3.66	16.50	198	254	0.78	99.2
	2.3.16	87.4	80.2	91.1	84.3	288	66	2.1	9.1	103	3.47	16.80	177	223	0.79	99.4
	3.3.16	88.1	80.1	92.0	87.5	301	72	2.0	8.3	121	3.56	16.83	214	258	0.83	99.0
	4.3.16	89.5	81.9	92.0	87.5	306	69	2.0	8.7	113	3.69	16.51	205	261	0.79	99.2
	5.3.16	87.6	80.3	91.1	85.1	294	72	2.0	8.3	115	3.54	16.98	202	234	0.86	99.2
	6.3.16	87.2	80.0	92.0	83.2	274	71	2.2	8.4	108	3.49	16.74	186	236	0.79	99.1
	7.3.16	87.3	80.2	91.8	85.3	290	71	2.1	8.4	111	3.57	16.65	197	250	0.79	99.1
	20.3.16	86.5	79.2	91.5	84.2	334	75	1.8	8.0	106	3.79	16.33	199	258	0.77	99.0
	21.3.16	85.3	78.3	92.1	86.7	314	73	1.9	8.2	115	3.91	16.37	224	275	0.77	99.2
	29.3.16	84.0	75.7	89.8	80.1	313	80	1.9	7.5	103	3.80	16.59	196	231	0.85	98.8
	4.4.16	82.8	78.4	89.6	83.4	302	82	2.0	7.3	105	3.92	16.41	204	246	0.83	98.9
Cool Season 1	22.5.18	72.7	65.1	84.6	73.5	192	62	3.1	9.7	88	3.61	16.54	157	203	0.78	98.4
	23.5.18	74.5	69.8	83.9	74.2	205	62	2.9	9.7	79	3.76	16.24	147	197	0.75	98.2
	24.5.18	72.5	64.7	81.2	74.0	194	60	3.1	10.0	87	3.68	16.29	159	212	0.75	98.5
Cool Season 2	20.6.19	72.1	61.5	85.1	71.8	192	56	3.1	10.7	85	3.70	16.19	156	213	0.74	98.4
	23.6.19	69.2	61.5	82.7	74.8	173	56	3.5	10.7	87	3.92	16.10	168	220	0.76	98.2
	24.6.19	72.2	65.0	81.0	73.5	195	57	3.1	10.5	93	4.12	16.09	191	235	0.81	98.2
	25.6.19	72.6	62.2	82.7	74.0	193	54	3.1	11.1	88	3.67	16.41	161	210	0.81	98.4
	26.6.19	71.5	62.6	82.3	70.5	173	56	3.5	10.7	87	3.95	16.10	169	219	0.77	98.2
	27.6.19	73.1	68.1	83.2	75.3	188	58	3.2	10.3	82	3.83	16.26	156	202	0.78	98.0
	3.7.19	69.6	60.5	80.6	74.4	173	54	3.5	11.1	88	3.74	16.15	164	210	0.78	98.5
	4.7.19	68.2	58.1	78.5	69.3	165	53	3.6	11.3	90	3.93	16.00	175	233	0.75	98.6
	9.7.19	72.2	64.0	81.8	71.6	189	55	3.2	10.9	86	3.80	16.09	162	220	0.74	98.1
	11.7.19	69.2	64.5	79.0	71.4	226	55	2.7	10.9	96	3.83	16.31	182	231	0.79	98.7
	18.7.19	74.1	65.9	82.8	73.8	199	58	3.0	10.3	92	3.56	16.37	168	223	0.73	98.0
AVERAGES																
Hot Season ...	—	87.0	80.0	91.0	85.0	305	73	2.0	8.2	110	...	...	200	248	0.80	...
Cool Season 1 ...	—	73.0	67.0	83.0	74.0	197	61	3.0	9.8	85	...	...	155	204	0.76	...
Cool Season 2 ...	—	71.0	63.0	82.0	73.0	191	55	3.1	10.9	89	...	...	168	219	0.77	...

season, and the average carbon dioxide eliminated was 200 c.c., whereas in the cooler weather series averages of 204 and 219 for the oxygen and 155 and 168 for the carbon dioxide were obtained. Taking, for convenience, the minimum absorption of oxygen in each series as a basis, the extreme variation observed in the hot season series was 23 per cent., and in the two cool seasons series 8 per cent. and 16 per cent. of the minimum respectively. It is noticeable further, that the total quantity of air breathed in this subject was distinctly greater with the higher external temperatures.

With the other subject, J. W. F. (Table IV), similar observations were recorded indoors, the average for the oxygen being 256 and 222 c.c., and for the carbon dioxide eliminated 217 and 183 c.c. respectively in the hotter and cooler weather.

In the experiments done out of doors the external conditions were more varied. Large variations were observed in the wet and dry bulb air temperatures in the hot season, and the cooling power of the atmosphere, as measured by the katathermometers, also showed considerable differences from day to day. Thus, with the air temperatures above 80° dry and 70° wet bulb, the extreme variations in the time that the dry bulb katathermometers took to cool from 110° to 100° were 250 and 94 seconds, and the wet bulb instrument 60 to 35 seconds, whilst in the cooler weather, with lower air temperatures, times taken were on more than one occasion as low as 45 seconds for the dry bulb and 19 seconds for the wet bulb katathermometers. In these outdoor experiments, however, it was also noticeable that the metabolism was generally higher with the higher outer temperatures.

The comparison was rendered more difficult in the outdoor experiments by the fact that on certain days in the cooler weather the subject felt decidedly cold, often sufficiently so to be shivering, and on these occasions an increased metabolism was observed (Table V, Nos. 14, 15, 16 and Table VI, Nos. 10, 11, 12). An example is No. 16 in Table V, done on a windy day in June with air temperatures of 64·3° dry bulb and 51·4° wet bulb, in which the subject was so cold as to be shivering, in spite of the fact that he was wearing a coat; the rectal temperature fell 0·8° during the period. On these days it was observed that the temperatures between the skin and shirt were much lower.

TABLE IV.

Subject J. W. F. Indoor Experiments.

	Date	Air Temp.		Skin-Shirt Temp.		Katathermometers 110°—100° Time in Secs.		Katathermometers 110°—100° Rate of cooling Degrees per min.		Vol. of air expired Litres per 20 min.	Analysis of expired air.		CO_2 eliminated c.c. per min.	O_2 consumed c.c. per min.	Resp. Quot.	Rect. Temp.
		Dry B.	Wet B.	Dry B.	Wet B.	Dry B.	Wet B.	Dry B.	Wet B.		CO_2%	O_2%				
Hot Season	22.3.16	85·9	78·3	91·5	85·5	322	70	1·9	8·6	116	3·62	16·73	208	253	0·82	98·8
	23.3.16	84·8	76·1	90·0	85·3	328	69	1·8	8·6	119	3·67	16·82	217	253	0·86	99·2
	24.3.16	86·1	75·6	90·4	84·3	316	66	1·9	8·8	127	3·63	16·85	228	267	0·85	99·2
	25.3.16	85·8	75·8	90·5	84·4	322	70	1·9	8·6	116	3·58	16·89	205	241	0·85	99·0
	5.4.16	83·0	77·6	89·4	84·8	282	87	2·1	6·9	121	3·74	16·66	225	268	0·84	99·0
Cool Season	24.5.18	73·9	67·9	83·6	74·7	183	58	3·3	10·3	122	3·04	17·52	183	214	0·86	98·6
	29.5.18	75·2	69·8	86·0	76·9	195	59	3·1	10·2	111	3·34	16·99	184	228	0·81	99·0
	5.6.18	73·5	64·3	85·1	72·9	183	58*	3·3	10·3	118	3·41	16·95	182	223	0·82	98·8
AVERAGES.																
Hot Season	—	85·0	77·0	90·0	85·0	314	72	1·9	8·3	120	...	...	217	256	0·84	....
Cool Season	—	74·0	67·0	85·0	75·0	187	58	3·2	10·3	117	...	...	183	222	0·83	....

TABLE V.

Subject W. J. Y. Outdoor Experiments.

No.	Date	Air Temp.		Skin-Shirt Temp.		Katathermometers 110°—100° Time in Secs.		Katathermometers 110°—100° Rate of cooling Degrees per min.		Vol. of air expired Litres per 20 min.	Analysis of expired air.		CO_2 eliminated c.c. per min.	O_2 consumed c.c. per min.	Resp. Quot.	Rect. Temp.	Remarks
		Dry B.	Wet B.	Dry B.	Wet B.	Dry B.	Wet B.	Dry B.	Wet B.		CO_2%	O_2%					
1	7.3.16	90·3	79·5	93·3	83·7	250	45	2·4	13·3	107	3·49	16·75	186	234	0·79	99·4	Light breeze; sweating
2	8.3.16	91·1	79·8	92·7	84·2	231	45	2·6	13·3	114	3·62	16·56	205	261	0·78	99·1	Uncomfortably hot; sweating.
3	9.3.16	89·9	78·4	92·0	83·4	228	49	2·6	12·2	115	3·44	16·88	196	243	0·81	99·2	Light breeze; not sweating
4	14.3.16	87·1	78·6	90·7	83·6	189	42	3·2	14·3	112	3·56	16·73	198	245	0·81	98·8	Breeze fairly strong; sun obscured by clouds.
5	15.3.16	84·2	76·8	90·3	89·1	119	44	5·0	13·6	105	3·56	16·63	186	237	0·78	99·2	Gusty winds, rain at end; D.B. Temp. falling 5°.
6	16.3.16	85·0	76·1	90·3	83·7	244	60	2·5	10·0	103	3·70	16·56	190	236	0·80	99·0	Very close and overcast.
7	20.3.16	86·4	76·5	90·6	81·8	141	40	4·2	15·0	108	3·71	16·57	199	245	0·80	99·3	Breezy day.
8	21.3.16	86·7	76·8	90·8	83·2	162	40	3·7	15·0	125	3·77	16·27	201	264	0·76	99·0	Light Breeze
9	27.3.16	85·0	74·0	89·1	78·7	157	43	3·8	14·0	105	3·71	16·53	196	241	0·80	99·3	Light breeze.
10	29.3.16	82·2	72·5	86·6	74·9	123	38	4·9	15·8	111	3·78	16·77	209	237	0·88	99·0	Breezy day.
11	4.4.16	83·2	75·0	88·5	78·9	166	48	3·6	12·5	92	3·83	16·34	174	216	0·81	98·9	Rather close.
12	6.4.16	86·4	69·0	89·8	74·5	172	39	3·5	15·4	96	3·89	16·30	186	232	0·80	99·4	Fair breeze. Note lower wet bulb.
13	17.4.16	82.4	69·8	87·8	74·4	162	30	3·7	20·0	96	3·81	16·28	182	235	0·77	98·7	Strong breeze.
14	24.6.16	71·9	56·7	80·6	66·6	119	27	5·0	22·2	98	4·01	16·17	194	243	0·80	99·4	Faint breeze; feeling distinctly cold.
15	29.6.16	62·8	58·4	73·3	65·4	73	36	8·2	16·7	108	3·86	16·46	206	250	0·82	98·8	Breezy; Rectal Temp. fell to 97·8; shivering.
16	30.6.16	64·3	51·4	77·0	63·1	44	19	13·6	31·4	119	3·89	16·44	230	278	0·83	98·6	Breezy; Rectal Temp. fell to 97·8; shivering.
17	30.3.17	83·8	75·0	87·8	78·9	155	44	3·9	13·6	98	3·46	16·64	167	220	0·76	98·8	Light breeze.
18	13.4.17	84·0	73·0	88·7	76·6	230	51	2·6	11·7	99	3·57	16·55	174	211	0·82	98·4	Close
19	24.4.17	84·2	71·7	86·4	74·8	174	42	3·4	14·3	98	3·51	16·87	168	204	0·82	98·9	Moderate breeze.
20	22.5.18	75·1	62·9	81·0	69·1	120	34	5·0	17·6	120	3·49	16·66	208	269	0·77	98·0	Strong breeze; feeling cold towards end.
21	28.5.18	75·6	65·3	81·0	70·2	88	28	6·8	21·4	92	3·50	16·72	150	204	0·79	98·2	Strong breeze.
22	...	73·0	59·0	78·0	67·0	85	27	7·1	22·2	96	...	...	170	215	0·79	...	Average of 11 observations in cool season. Table VII.
23	25.2.16	87·0	79·9	90·9	84·0	123	40	...	...	107	3·66	16·16	191	275	0·72	98·9	In shade.
"	"	...	...	103·6	99·6	...	53	...	...	119	3·38	16·85	202	252	0·80	99·3	Sitting in sun for 30 min. before breathing into meter.
24	8.3.16	...	...	98·7	90·7	...	54	...	...	116	3·47	16·87	201	248	0·81	99·1	The same. Compare No. 2 done on same day in shade.

Another question investigated was whether the cooling power of the atmosphere observed during the hot season, as measured by the katathermometer, influenced the metabolism in any way. The figures did not show any definite changes in the metabolism with rate of cooling. In the cooler weather a high rate of metabolism was observed in a few cases on days which had a high rate of cooling, but it was noticeable that this occurred only when the subject felt cold.

No such marked changes were observed as those obtained by Hill in England, who found in outdoor experiments that the greatest rate of metabolism coincided with the greatest rate of cooling.

Observations were made on the effects of increasing the rate of cooling by performing experiments, first indoors and then outdoors, upon the same day, in the same manner as done by Hill (1912).

The subject in each case reclined motionless for at least 40 minutes under each set of conditions before breathing into the meter. Tables VII and VIII give the results of a number of such experiments, the indoor experiments being carried out in a closed room. In the hot season experiments no increase in the metabolism was observed out of doors, although the cooling power was considerably higher outside. The difference in comfort indoors in a closed room and outdoors in the breeze was very marked. Moving air was thus without any definite effect when the outer temperatures were high (over 80° dry bulb and 70° wet bulb), showing that the increased rate of cooling was insufficient to reduce the body temperature during the experiment. With the lower external temperatures observed in the cool season differences were observed. The pulmonary ventilation was generally greater out of doors, and in certain instances there was a decided increase in carbon dioxide eliminated and oxygen consumed. This occurred on windy days when the cooling power of the atmosphere was high and when the subject felt cold: on these days the rate of cooling was often sufficient to reduce the rectal temperature. On the whole, however, no marked increase in the oxygen consumed was observed, and it is noteworthy that the averages for the eleven observations on W. J. Y. showed no increase out of doors excepting that the total volume expired was greater. In several experiments the increased ventila-

TABLE VI.

Subject J. W. F. Outdoor Experiments.

No.	Date	Air Temp.		Skin-Shirt Temp.		Katathermometers 110°—100°				Vol. of air expired Litres per 20 min.	Analysis of expired air.		CO_2 eliminated c.c. per min.	O_2 consumed c.c. per min.	Resp. Quot.	Rect. Temp.	Remarks.
						Time in Secs.		Rate of cooling Degrees per min.									
		Dry B.	Wet B.	Dry B.	Wet B.	Dry B.	Wet B.	Dry B.	Wet B.		CO_2%	O_2%					
1	13.3.16	89·0	77·3	92·5	85·7	194	38	3·1	15·8	132	3·47	17·10	228	261	0·87	99·5	Good breeze.
2	14.3.16	87·6	78·2	89·5	84·9	207	49	2·9	12·2	143	3·74	16·90	266	295	0·90	99·2	Slight breeze.
3	15.3.16	86·5	78·2	91·3	85·3	164	50	3·7	12·0	112	3·52	16·92	195	232	0·84	99·2	Wind in gusts; sweating.
4	16.3.16	82·6	75·6	89·8	85·7	236	51	2·5	11·8	115	3·80	16·40	217	273	0·80	99·1	Still day.
5	22.3.16	86·8	76·7	93·1	83·9	161	50	3·7	12·0	122	3·61	16·91	219	253	0·86	98·8	Still day.
6	24.3.16	86·4	72·3	89·7	78·3	170	39	3·5	15·4	127	3·67	16·85	231	267	0·87	99·6	Good breeze.
7	5.4.16	83·7	75·9	88·9	79·0	94	35	6·4	17·1	113	3·76	16·73	211	245	0·86	98·4	
8	6.4.16	84·7	66·5	88·0	74·4	129	30	4·6	20·0	111	3·99	16·53	220	251	0·88	99·5	Note lower wet bulb.
9	7.4.16	84·3	69·7	86·8	76·8	134	31	4·5	19·4	110	4·09	16·30	223	263	0·85	99·4	Strong wind.
10	25.6.16	74·5	65·8	84·4	77·8	127	39	4·7	15·4	119	4·07	16·41	240	277	0·87	...	Still day.
11	29.6.16	64·0	59·7	78·9	72·7	93	40	6·4	15·0	109	4·03	16·33	218	260	0·84	99·3	Raining; breezy; feeling cold.
12	30.6.16	64·0	51·2	77·2	64·8	42	16	14·3	17·5	116	4·03	16·51	231	262	0·88	99·5	Strong wind; feeling cold; Rectal temp. fell from 99·5 to 99·0.
13	11.4.17	83·2	73·1	88·1	81·0	191	49	3·1	12·2	136	3·37	17·26	228	256	0·89	99·1	
14	17.4.17	85·4	73·9	88·4	79·4	205	52	2·9	11·5	131	3·35	17·18	218	254	0·86	99·7	
15	24.5.18	73·3	65·6	79·8	70·7	76	31	7·9	19·3	138	2·94	17·76	201	224	0·90	98·6	
16	29.5.18	75·0	68·0	83·7	72·4	79	26	7·6	23·1	112	3·45	16·88	191	236	0·81	99·0	
17	5.6.18	80·5	61·8	86·2	71·6	112	24	3·4	25·0	127	3·28	16·93	206	266	0·77	98·8	

Subject W. J. Y. Effects of Wind. Experiments done Indoors and Outdoors.

No.	Date	Air Temp. Dry B.	Air Temp. Wet B.	Skin-Shirt Temp. Dry B.	Skin-Shirt Temp. Wet B.	Katathermometers, Time in Secs. 110°—100° Dry B.	110°—100° Wet B.	110°—90° Dry B.	110°—90° Wet B.	Vol. of air expired Litres per 20 min.	Analysis of expired air CO_2%	O_2%	CO_2 eliminated c.c. per min.	O_2 consumed c.c. per min.	Resp. Quot.	Remarks.	
1	20.3.16	86·5	73·2	91·5	84·2	334	75	1259	230	106	3·79	16·33	199	258	0·77	In	
		86·9	76·6	90·6	81·8	141	40	439	106	108	3·71	16·57	199	245	0·80	Out	
2	21.3.16	85·3	78·3	92·1	86·7	314	73	1050	217	115	3·91	16·37	224	275	0·77	In	
		86·7	76·8	90·8	83·2	162	40	642	110	108	3·77	16·27	201	264	0·76	Out	
3	29.3.16	84·0	75·7	89·8	80·1	313	80	1028	212	103	3·80	16·59	196	231	0·85	In	
		82·2	72·5	86·6	74·9	123	38	300	82	112	3·78	16·77	209	237	0·88	Out	
4	4.4.16	82·8	78·4	89·6	83·4	302	82	927	234	105	3·92	16·41	204	246	0·83	In	
		83·2	75·0	85·6	78·9	166	48	496	121	92	3·83	16·34	174	216	0·81	Out	
5	22.5.18	72·7	65·1	84·6	73·5	192	62	484	156	88	3·61	16·54	157	203	0·78	In	Cooler season.
		75·1	62·9	81·0	69·1	120	34	283	73	120	3·49	16·66	208	269	0·77	Out	Feeling cold; strong
6	28.5.18	74·5	69·8	83·9	74·2	205	62	514	160	79	3·76	16·24	147	197	0·75	In	breeze; Rectal temp.
		75·6	65·3	81·0	70·2	88	28	216	67	92	3·50	16·72	157	204	0·79	Out	fell to 98°.
7	20.6.19	72·1	61·5	85·1	71·8	192	56	480	136	85	3·70	16·19	156	213	0·74	In	
		72·2	54·2	77·2	61·2	87	30	207	63	92	3·39	16·75	154	202	0·76	Out	
8	23.6.19	69·2	61·5	82·7	74·8	173	56	426	135	87	3·92	16·10	168	220	0·76	In	
		71·4	60·0	81·4	73·6	127	37	306	87	88	3·57	16·69	158	196	0·80	Out	Very little breeze.
9	24.6.19	72·2	65·0	81·0	73·5	195	57	485	141	93	4·12	16·09	191	235	0·81	In	
		78·4	64·3	83·2	72·6	109	26	298	59	90	3·61	16·47	161	211	0·76	Out	Gusty breeze.
10	25.6.19	72·6	62·2	82·7	74·0	193	54	494	133	88	3·67	16·41	161	210	0·81	In	
		72·4	55·5	76·3	65·4	63	19	165	46	106	3·44	16·89	181	223	0·77	Out	Good breeze; feeling cold.
11	26.6.19	71·5	62·6	82·3	70·5	173	56	450	137	87	3·95	16·10	169	219	0·77	In	
		72·5	59·3	79·2	65·8	73	28	224	60	94	3·56	16·66	166	210	0·79	Out	Very slight wind.
12	27.6.19	73·1	68·1	83·2	75·3	188	58	479	150	82	3·83	16·26	156	202	0·78	In	
		73·4	65·3	77·0	67·3	68	26	182	59	97	3·49	16·90	167	202	0·83	Out	Moderate wind.
13	3.7.19	69·6	60·5	80·6	74·4	173	54	427	129	88	3·74	16·15	164	210	0·78	In	
		70·5	59·0	79·3	67·8	111	33	313	78	93	3·87	16·45	179	216	0·83	Out	Wind in gusts.
14	4.7.19	68·2	58·1	78·5	69·3	165	53	402	127	90	3·93	16·00	175	233	0·75	In	
		68·0	51·3	76·1	60·5	55	24	154	54	103	3·62	16·67	184	228	0·81	Out	Good breeze.
15	9.7.19	72·2	64·0	81·8	71·6	189	55	482	135	86	3·80	16·09	162	220	0·74	In	
		72·9	59·5	75·8	64·7	97	26	263	57	102	3·54	16·61	179	232	0·77	Out	Wind in gusts; uneven
16	11.7.19	69·2	64·5	79·0	71·4	226	55	478	135	96	3·83	16·31	182	231	0·79	In	cooling.
		71·2	61·3	78·7	70·4	67	24	185	58	92	3·73	16·38	171	219	0·78	Out	
17	18.7.19	74·1	65·9	82·8	73·8	199	58	518	149	92	3·56	16·37	168	223	0·73	In	
		76·4	62·0	80·3	73·5	74	24	203	57	99	3·54	16·53	174	229	0·76	Out	Very strong wind.
								Average Results.									
		85·0	78·0	91·0	84·0	316	78	1066	223	107	...	...	206	252	0·80	In	4 days in hot season.
		85·0	75·0	88·0	81·0	148	41	469	105	105	...	...	196	240	0·81	Out	Nos. 1—4.
		87·0	80·0	91·0	85·0	305	73	...	...	110	...	...	200	248	0·80	All indoor experiments hot season 11 observations.	
		87·0	77·0	90·0	82·0	183	45	...	...	109	...	...	195	242	0·80	All outdoor experiments same period 11 observations.	
		74·0	67·0	84·0	74·0	198	62	499	158	84	...	...	153	200	0·76	In	2 days in cool season, 1918
		75·0	64·0	81·0	70·0	104	31	249	70	106	...	...	184	236	0·78	Out	Nos. 5 and 6.
		71·0	63·0	82·0	73·0	191	55	468	137	88	...	...	168	219	0·77	In	11 days in cool season, 1919.
		73·0	59·0	78·0	67·0	85	27	228	62	96	...	...	170	215	0·79	Out	Nos. 7—17.

TABLE VIII.

Subject J. W. F. Effects of Wind. Experiments done Indoors and Outdoors.

No.	Date	Air Temp.		Skin-Shirt Temp.		Katathermometers, Time in Secs.				Vol. of expired air Litres per 20 min.	Analysis of expired air		CO_2 eliminated c.c. per min.	O_2 consumed c.c. per min.	Resp. Quot.		Remarks.
						110°—100°		110°—90°									
		Dry B.	Wet B.	Dry B.	Wet B.	Dry B.	Wet B.	Dry B.	Wet B.		CO_2%	O_2%					
1	22.3.16	85·9	78·3	91·5	85·5	322	70	1212	206	116	3·62	16·73	208	253	0·82	In	
		86·8	76·7	93·1	83·9	161	50	...	121	122	3·61	16·91	219	253	0·86	Out	
2	24.3.16	86·1	75·6	90·4	84·3	316	66	...	202	127	3·63	16·85	228	267	0·85	In	
		86·4	72·3	89·7	78·3	170	39	542	97	127	3·67	16·85	231	267	0·87	Out	Good breeze blowing.
3	5.4.16	83·0	77·6	89·4	84·8	282	87	...	234	121	3·74	16·66	225	268	0·84	In	
		83·7	75·9	88·9	79·0	94	35	912	91	113	3·76	16·73	211	245	0·86	Out	
4	24.5.18	73·9	67·9	83·6	74·7	183	58	469	146	122	3·02	17·52	183	214	0·85	In	
		73·3	65·6	79·8	70·7	76	31	217	71	138	2·94	17·76	201	224	0·90	Out	
5	29.5.18	75·2	69·8	86·0	76·9	195	59	498	151	111	3·34	16·99	184	228	0·80	In	
		76·9	68·0	83·7	72·4	79	26	209	64	112	3·45	16·88	191	236	0·81	Out	Not much wind.
6	5.6.18	73·5	64·3	85·1	72·9	183	58	458	135	118	3·41	16·95	182	223	0·82	In	Resp. rate 13·5.
		80·5	61·8	86·2	71·6	112	24	285	58	127	3·28	16·93	205	266	0·77	Out	Resp. rate 14·5, feeling cold.
		AVERAGE RESULTS.															
		85·0	77·0	90·0	85·0	311	76	...	214	121	...	...	220	263	0·84	In	Hot season experiment.
		86·0	76·0	91·0	80·0	175	45	...	136	121	...	...	220	255	0·86	Out	Nos. 1—3.
		74·0	67·0	85·0	75·0	187	58	475	144	116	...	...	187	226	0·82	In	Cool season.
		77·0	65·0	83·0	72·0	89	27	237	64	127	...	...	203	247	0·83	Out	Nos. 4—6.

tion may have caused a washing out of carbonic acid from the tissues, since on some occasions the respiratory quotient was slightly higher out of doors. The difference in the rate of cooling indoors and outdoors was on no occasion so great as on those days recorded by Hill as 'bracing,' when he observed a very large increase in the metabolism out of doors. The rate of cooling due to air movement must therefore be increased beyond a certain limit in order to stimulate the body to an increase in respiratory metabolism. These results agree in the main with Wolpert's observations that moving air has very little effect upon the carbon dioxide output between 78° and 95° F., but below this it produces an increase.

One experiment was carried out under similar conditions to those investigated by Harvey Sutton (1908) with high temperatures and so high a saturation of moisture as to prevent all cooling through evaporation of sweat. This was done in a small chamber heated artificially. Before entering the chamber the subject (W. J. Y.) breathed in the usual manner for five minutes into the meter, and a sample was taken for analysis. After thirty minutes in the hot room, a second breathing into the meter was performed, and again after seventy minutes. The figures obtained are seen in Table IX.

TABLE IX.

Air Temp.		Wet-bulb Kata. 110°-100°	Rectal Temp.	Pulse	Vol. expired Litres per min.	CO_2 c.c. per min.	O_2 c.c. per min.	Resp. Quot.	
D.B.	W.B.								
81·6	69·5	63	99·5	76	6·4	198	216	0·91	Before entering.
97·8	93·0	...	100·5	112	8·6	294	327	0·90	After 30 mins. in hot room.
100·3	96·0	241	103·0	168	9·7	311	375	0·83	After 70 mins.

The subject was naked and was perspiring profusely during the experiment, losing 650 grms. in weight, practically all of which would be due to sweat. The result was similar to those recorded by Sutton, the rectal temperature rose, slowly at first, more rapidly later, and the metabolism was increased. It was not observed, however, that the respiratory quotient rose towards unity as described by Sutton. The last breathing was extremely trying, the mouthpiece proving somewhat uncomfortable under the existing conditions, and was accompanied by a sensation of suffocation, which may have affected the breathing in this case.

It is interesting to note here some observations which were made during exercise. The effort consisted in walking on the flat for fifteen minutes, at the rate of three miles per hour, and the meter was carried on a wooden stand strapped to the shoulders like a knapsack, the total weight carried being approximately 13 kilograms. These experiments are detailed in Table X. The temperatures given are the shade temperatures at the time; the walking, however, was performed in the sun. The calories, corresponding to the oxygen consumed, are calculated from the data given by Cathcart (1915).

TABLE X.

	Shade Temp.		Rectal Temp.	Vol. expired Litres per min.	CO_2 c.c. per min.	O_2 c.c. per min.	Resp. Quot.	Resp. Rate	Calories per min.	
	Dry B.	Wet B.								
W. J. Y. (1)	83·8	75·0	98·8	4·9	167	220	0·76	12¼	1·06	Rest.
			99·6	19·4	781	959	0·81	19¼	4·62	Exercise.
(2)	84·0	73·0	98·4	4·9	174	211	0·82	12½	1·02	Rest.
			100·2	20·7	901	1119	0·81	26½	5·40	Exercise.
J. W. F. (3)	83·2	73·1	99·1	6·8	228	256	0·89	14¾	1·26	Rest.
			99·8	17·4	698	836	0·83	20¼	4·04	Exercise.
(4)	83·4	73·9	99·7	6·6	218	254	0·86	16¼	1·24	Rest.
			100·0	20·2	1007	1260	0·80	20½	6·05	Exercise.

As already pointed out, in the tropics the loss of heat from the body takes place to a greater extent by evaporation of the perspiration than it does in a temperate climate. It is of interest, therefore, to calculate the amount of water which would have to be evaporated to neutralise the heat produced in these experiments, assuming for the time that all heat was lost in this way.

The largest production of heat with subject W. J. Y. amounted to 5·4 calories per minute; 1 litre of water evaporated at 98·6°

requires 582 calories, so that to neutralise this amount of heat would require the evaporation of about 9 c.c. per minute or 540 c.c. per hour.

With the second subject (J. W. F.) in Experiment No. 4, 6·5 calories were produced, and the evaporation of 10·4 c.c. of water per minute would be required to neutralise this heat, or 626 c.c. per hour.

In another research (1919) the loss in body weight of the same two subjects during walking exercise was measured, the subjects being weighed in their clothing, so that the loss in weight practically represents the perspiration evaporated. The first subject (W. J. Y.), in walking on a hot day (D. B. 87·8°, W. B. 79·7°) for sixty minutes at about three to three and a half miles per hour, lost 740 grms. in weight, and the second subject (J. W. F.), walking the same distance in seventy-six minutes, lost 900 grms. or about 710 grms. per hour.

It is thus seen that even with a high moist temperature sufficient evaporation can take place to neutralise the heat produced by such exercise as that performed, even if this evaporation were the only source of heat loss. These experiments, however, caused profuse perspiration, and it shows how necessary it is in the tropics to supply the body with sufficient water (cf. Hunt (1912)).

DISCUSSION

The ventilation of the lungs, carbon dioxide expired and oxygen consumed, were greater in the summer than in the cooler weather, excepting on a few days when the subjects were cooling sufficiently rapidly to feel cold and to produce shivering, on which occasions larger metabolism was observed.

The results agree, therefore, with those found by Osborne (1912), who found that the pulmonary ventilation and the carbon dioxide produced varied directly with the external temperatures, and who pointed out also that the rate of respiration is noticeably higher with higher external temperatures (over 100° F.).

An increased rate of respiration in the tropics has been noticed by other observers; thus Chamberlain (1911), as the result of extended observations on six hundred and eight American soldiers stationed in the Philippine Islands, found an average respiration rate of 19·3 per minute, as against 17 to 18 per minute in temperate climates. An increased rate of respiration would in itself cause a certain small increase in the carbon dioxide produced.

With one of the subjects (W. J. Y.) the rectal temperatures in the cool weather were in most cases lower than in the summer, which would account for a certain difference in the metabolism; with the other subject, however, this was not the case, the rectal temperature did not show any marked variation in the two seasons, being generally about 99° in all the experiments. Osborne also states that he was unable to observe any rise in rectal temperature whilst at rest which would have caused the increase observed in the metabolism.

A discussion of the various conditions which may affect the basal metabolism, i.e., the metabolism with complete muscular rest and in the post-absorptive condition, has been published by Benedict (1915), in which he sums up the results of the large number of experiments which have been carried out in the Nutrition Laboratory of the Carnegie Institute, Washington. These experiments have been made on a large variety of individuals, and have extended over a number of years. He pointed out how numerous the factors are which may affect the basal metabolism. Besides those more obvious factors directly connected with the mass of the organism, such as body weight, body surface, muscular development, etc., there are others which, though less obvious, have a marked effect. He has noted considerable changes (as much as 30 per cent.) from day to day in the oxygen absorption of normal individuals, and small changes in the course of twenty-four hours even in fasting subjects, which changes could not be attributed to changes in the active mass of the body.

Benedict and Cathcart (1913), experimenting with a professional athlete, found that after a prolonged period of severe work the metabolism remained high, although gradually decreasing, long after the external evidence of muscular activity had ceased. They further found that the metabolism of this individual, determined from day to day, showed variations which in some cases might be traced to conditions existing prior to the experiment; for instance, when he chose a rough road on his way to the laboratory the extra exertion affected the subsequent determination, and the same was true in the winter, when his walk was impeded by slippery roads.

Benedict concludes, therefore, that besides the active mass of the organism, 'the stimulus to cellular activity' existing at the time the measurement of the metabolism is made, is an important factor in determining the metabolism observed.

The metabolism at any time is thus determined not only by the conditions which obtain at the time of the actual experiment, but also by the conditions which have gone before, the results of which on the cellular activity of the organism have not had time to disappear.

The foregoing observations suggest an explanation at any rate for part of the increased metabolism found in the summer in these experiments. In a tropical summer with a hot moist atmosphere, when the least exertion is accompanied by profuse sweating, a very slight amount of muscular work will cause an increase in the body temperature and a corresponding increase in the metabolic activity. The increased activity produced by the exertion of dressing and walking to the laboratory would persist, and would not be materially reduced by the subsequent rest, before and during the actual measurement. In the summer here, it has frequently been noted how rapidly the body temperature rises with exercise and falls again only very slowly when the exercise has ceased. It was frequently observed that the subject was perspiring freely during the experiments. In the cooler season the effects of the earlier exertion would not be so great, and the greater cooling power of the atmosphere would further cause the resting period to modify the activity to a greater extent. The body temperature during the hot weather seldom changed very much during the actual experiment, whilst in the cooler season in the outdoor experiments it often showed a decided fall. The amount of actual exertion before the experiments began was roughly the same every day, yet the body temperature of one subject was usually greater in the hot weather.

One might thus expect to find the metabolism working at a slightly higher level in the very hot weather. It may be noted here that very cold weather might possibly produce a similar effect, since the increased muscular activity produced by the cold might also persist for some time.

That the metabolism was not wholly influenced by the immediate conditions is also seen by the fact that during the winter the rate of cooling of the katathermometers inside with doors closed was of the same order as that frequently found out of doors in the summer, although the oxygen consumed was lower in the former experiment.

The fact that the level of metabolism is affected by the general conditions under which the subject is living, and not only by the immediate experimental surroundings, has been entirely overlooked in experiments of comparative short duration, in which tropical conditions have been produced artificially, and it shows how these can never replace observations carried out under natural conditions upon subjects living continuously under these conditions.

SUMMARY

During the hot season of the year a greater metabolism was observed than in the cooler season, excepting on certain days in the latter, when the rate of cooling was sufficiently great to cause the subject to shiver. This greater metabolism is attributed to the effects of the ordinary activities of everyday life which had preceded the actual experiment, which in the hot moist weather produce a greater increase in body temperature, and consequently in the metabolism, than in the cool season. These effects also are reduced much more slowly during the hot season, when the cooling power of the atmosphere is low, than during the cool weather when the cooling power is much greater. The effects of such conditions prior to the actual measurement have thus a larger influence on the level of metabolism in the hot season.

With tropical heat the metabolism is at a high level on account of the increase in body temperature produced by even slight exertion, and which decreases only very gradually after the exertion has ceased. Cold may also increase the metabolism but by producing shivering and so increasing the muscular activity.

REFERENCES

BENEDICT (1915). *Journ. Biol. Chem.* Vol. XX, p. 263.

BREINL and YOUNG (1919). *Med. Journ. of Australia.* Vol. VI, i, pp. 353, 375 and 395 [reprinted in this issue].

CATHCART (1918). *Journ. R.A.M.C.* Vol. XXXI, p. 339.

CHAMBERLAIN (1911). *Philipp. Journ. Sci.* B. Vol. VI, p. 427.

EIJKMANN (1896). *Pflügers Archiv.* Vol. LXIV, p. 57.

HALDANE (1912). Methods of Air Analysis. London.

HILL, L. (1914). Local Government Board Reports. New Series, No. 100.

HUNT (1912). *Journ. Hyg.* Vol. XII, p. 479.

JOHANSSON (1896). *Skand. Arch. Physiol.* Vol. VII, p. 123.

KROGH (1916). The Respiratory Metabolism of Animals and Men. London.

LOEWY (1890). *Pflügers Arch.* Vol. XLVI, p. 189.

OSBORNE, W. A. (1910). *Journ. Physiol.* Vol. XLI, p. 353.

OSBORNE, W. A. (1916). *Proc. Roy. Soc. Vic.* New Series. Vol. XXIX.

SUTTON, HARVEY (1908). *Journ. Path. and Bact.* Vol. XIII, p. 68.

RUBNER, and LEWASCHEW (1897). *Arch. f. Hygiene.* Vol. XXIX, p. 1.

WOLPERT (1902). *Arch. f. Hygiene.* Vol. XLIII, p. 236.

THE HYPOPUS OF *CARPOGLYPHUS ANONYMUS*, HALLER

BY

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A quantity of dried figs received from the Port Sanitary Authority, Liverpool, were found to be heavily infested with mites and small beetles in all stages of development. The mites were found to be *Carpoglyphus anonymus* (Haller), and the beetles were *Carpophilus hemipterus* (Linn).

A few days later a single hypopial nymph was seen.

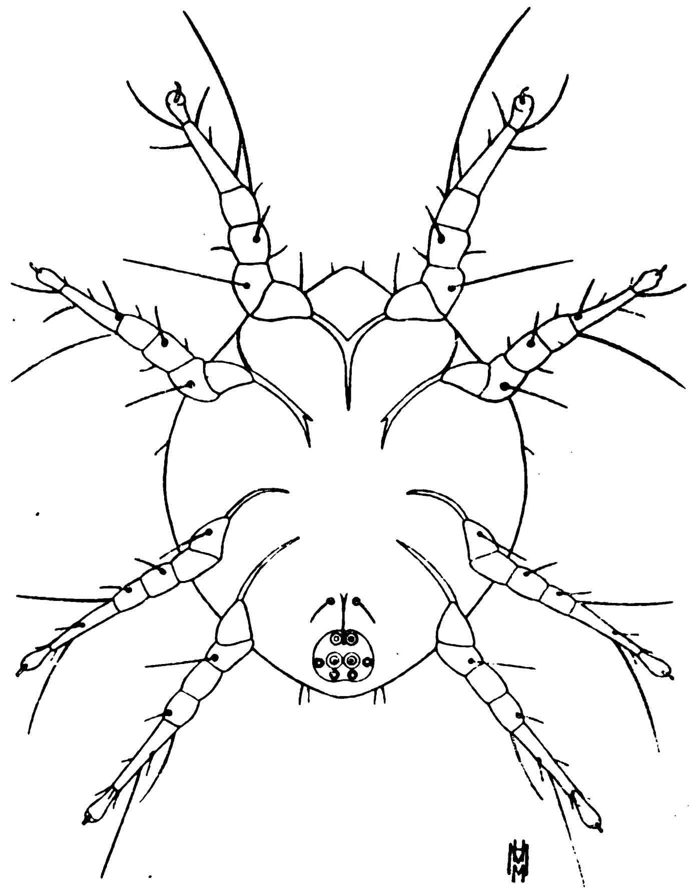
No other species of mites were present, and as the hypopus shows some points of similarity to the other stages of *Carpoglyphus anonymus*, there seems no doubt that it belongs to that species. Only one example of the hypopus was met with amongst a great number of specimens of the other stages.

This hypopus is broader in relation to its length, less strongly convex dorsally, and much more active than the normal nymphs of the species.

It has been considered worth while to figure and describe this hypopus, as Michael (1903) states that it is unknown.

The hypopial nymph forms a very interesting stage in the life of those species of mites in which it occurs. Its occurrence appears to be entirely confined to mites belonging to the family *Tyroglyphidae*, and it has not been recognised in all members of that family.

The hypopial nymph occurs apparently between the two normal nymphal stages, or after the first nymphal stage where only one occurs; the hypopial period being preceded and followed by an ecdysis in the usual way. By no means all individuals become hypopi, but usually only a relatively small number, and in some species, as probably is the case with that which is now described, it is of rare occurrence. The hypopi may develop into adults of either sex.



Hypopus of *Carpoglyphus anonymus*, Haller. Ventral aspect. × 215.

Their occurrence does not seem to be caused by unfavourable conditions, but is a provision for facilitating the distribution of the species, and to that end they are adapted for being transported by insects, larger mites, and other animals, by the development of relatively powerful suckers and in some cases large claws, as well as by being usually considerably more active in this than in other stages of their development.

The habit of the hypopi clinging to insects, etc., has frequently led to their being considered as parasites, but as they do not prey upon their host, but merely adhere to it in order to be transported from place to place, they are not true parasites, although on occasions when they occur in enormous numbers, as is sometimes the case, one may safely infer that they are deleterious to their host.

The integument of the hypopus differs from that of the adult, being of such consistency as to afford them greater protection, enabling them to endure greater degrees of heat and drought, such as they are liable to meet with while undergoing transportation.

DESCRIPTION OF THE HYPOPUS OF *CARPOGLYPHUS ANONYMUS*, Haller.

Colour: Translucent white, the legs very slightly tinged with pink.

Form: Broadly ovate, approaching to circular owing to its relative width. Dorsally it is rather convex, but ventrally it is flattened.

The cephalothorax projects between the first pair of legs as a blunt triangle, which bears two pairs of hairs, and below this is a very slightly developed rostrum.

A pair of short hairs are situated laterally at the posterior end of the cephalothorax; two short pairs at the posterior end of the body and a pair anterior to the sucker-plate.

The first pair of epimera are joined to the sternum, the other pairs are free.

The sucker-plate is strongly chitinised, and nearly circular, but is truncated posteriorly. The plate appears to be divided in the median line in front. At the anterior end of the plate there are a pair of suckers of medium size, close together; behind these are another rather larger pair also close together, lateral to these another pair of smaller suckers, and posterior to them an additional pair.

The legs are approximately equal in length, each bearing a well developed caroncle and a slightly developed claw. The legs also bear a number of hairs, of which the majority are short and slender.

The tibia of the first leg bears a hair about equal in length to that of the entire leg; the tibia of the second leg bears a similar hair, but it is only about half the length of the leg; the third and fourth legs each bears a similar and still shorter hair; there is also a well developed hair on the tarsus, which is longer than that on the tibia, particularly in the case of the fourth leg.

The integument bears a number of striations which on the cephalothorax lie roughly transversely, and on the abdomen in a more or less longitudinal direction.

The length of the specimen was 0·24 mm. and its breadth 0·20 mm.

REFERENCE

MICHAEL (1903). British *Tyroglyphidae*, Vol. II, p. 44.

XEROPHTHALMIA IN A NATIVE OF THE GOLD COAST

BY

J. W. S. MACFIE

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In a recent number of the *Journal of Tropical Medicine and Hygiene*, Archibald (1919) described three cases of epithelial xerosis of the conjunctiva in natives of the Sudan, a disease which, he remarked, apparently had not been previously described as occurring in the Tropics.

Epithelial xerosis of the conjunctiva, or Xerophthalmia, is a not-uncommon affection of natives in West Africa, but, as a rule, it causes no trouble, and so does not come to the notice of Medical Officers. It is for this reason presumably that its occurrence has not been previously recorded. Occasionally it happens, however, that a patient becomes alarmed about the white patches on his eyes, fearing that they may spread and obscure his vision. A case of this sort was sent to me a short time ago, and the opportunity was taken of attempting to transmit the disease to experimental animals.

CLINICAL HISTORY OF THE CASE

The patient was a native of Accra, Gold Coast, 23 years of age, a fitter by trade. He stated that six years ago he had an illness which he was told was 'Bright's disease,' the chief symptoms being cough, blood in his urine, and swelling of his legs. Soon after this illness white patches appeared on his eyes, and had persisted ever since. The white patches caused neither pain nor discomfort, were not spreading, and did not interfere with vision; but it was evident that the fear of blindness had become an obsession. The conjunctiva was not particularly dry; indeed, the patient complained that the secretion from his eyes was sometimes excessive, a condition which he attributed to dust and metal filings getting into his eyes when at work. There was no night blindness.

The general health of the patient was good. His diet was that customary for an adult native of his class, and was ample in every respect. His urine contained a considerable quantity of albumen, a circumstance which may have had something to do with the condition of his eyes.

On the conjunctiva of each eye, external to the corneal margin and a little below the middle line, there was a triangular dull-white patch, the superficial layers of which were easily scraped off, revealing brown pigmentation underneath. Direct smears made from the scrapings from the patches showed the usual cellular elements, and large numbers of Gram-positive, diphtheroid bacilli resembling *Bacillus xerosis*.

CULTURAL CHARACTERS OF THE BACILLUS

After washing one eye thoroughly with sterilised normal saline solution, a little of the white patch on it was scraped off with a sterile needle and used for inoculating media. In this way a pure culture of a bacillus was obtained which on agar grew slowly, at the end of forty-eight hours showing minute colonies, raised, rounded, and semi-transparent; on 'Nasgar' showed a growth similar to that on agar; and in broth produced after forty-eight hours a slight turbidity and a little fine granular deposit. The bacillus was a non-motile, Gram-positive, diphtheroid organism which was not acid-fast, and did not form spores.

BIOCHEMICAL REACTIONS OF THE BACILLUS

No change was produced in maltose, dextrin, inulin, starch, salicin, inosite, glycerol, erythritol, dulcitol, iso-dulcitol, mannitol or sorbitol. Acid was produced in glucose, laevulose, lactose, saccharose, galactose; and slight acidity in amygdalin. Gas was not liberated in any of the media employed. These tests were made in Hiss' serum-water medium, containing 1 per cent. of the carbohydrate or other substance, inoculated from a culture of the bacillus growing on blood serum.

The biochemical reactions of the diphtheroid bacilli found in the eye vary greatly; those of the organism isolated from this case differed from those of the bacillus recovered by Archibald from his Case I, the patient from whom a successful inoculation was made on to the eye of a rabbit, since the latter produced acid in galactose, rhamnose, maltose, lactose, erythritol, and dulcitol.

PATHOGENICITY OF THE BACILLUS

The bacillus was not pathogenic to guinea-pigs when inoculated sub-cutaneously.

Although diphtheroid bacilli are found in great numbers in the lesions of xerophthalmia, they are not, as a rule, regarded as the cause of the disease. This question, however, has been re-opened by Archibald, who succeeded in producing a xerotic area on the conjunctiva of a rabbit by applying to an abraded surface 'an emulsion of the viscid xerotic material' obtained from the eye of one of his cases. As it was possible that such material might contain, besides *B. xerosis*, other things which did not grow in the cultures, this procedure was not followed, but instead an emulsion was used of a culture growing well on agar after several sub-inoculations.

A little of the emulsion was applied to the eyes of the following animals after gently scratching the conjunctiva with a needle—one goat, one sheep, three rabbits, two guinea-pigs, two tame rats (*E. rattus*) and two monkeys (*Cercopithecus patas*). No xerotic patches were produced. The observation period was three weeks in the goat, sheep, one rabbit, and one guinea-pig, and ten weeks or longer in the other animals.

SUMMARY

(1) Epithelial xerosis of the conjunctiva is a not-uncommon affection of natives in West Africa.

(2) Attempts to reproduce the disease in animals, by means of the bacillus obtained from a case of this disease, were unsuccessful.

REFERENCE

ARCHIBALD, R. G. (1919.) Epithelial Xerosis of the Conjunctiva in Natives of the Sudan. *Journ. of Trop. Med. & Hyg.*, XXII, p. 81.

AN OBSERVATION ON THE EFFECT OF MALARIA IN LEUKAEMIA

BY

J. W. S. MACFIE

(*Received for publication 7 January, 1920*)

On a previous occasion (1917) it was shown that in a case of lymphatic leukaemia a great reduction in the number of leucocytes took place when the patient developed malaria. The leucocytes, which had numbered as many as 286,000 per c.mm. before the attack, were reduced to 59,000; but the ultimate effect was not observed, as the patient left Accra for a more healthy district and was consequently not seen again. The effect of an attack of malaria in leukaemia has again been observed recently, on this occasion in a case of the myelogenous type. For this reason, and because the examinations were in some respects more complete, a brief record may be of interest.

The patient was a native boy, aged 18 years, suffering from myelogenous leukaemia. He complained of nothing but a little pain and 'hardness' on the left side of the abdomen. His spleen was greatly enlarged, reaching downwards to a point two inches below the umbilicus; and he was anaemic. The superficial lymphatic glands were not palpably enlarged. He had not recently suffered from fever, and dated back the onset of his illness only six weeks. He was first examined by me on the 5th of August, 1919, and was subsequently seen weekly until the 9th September; that is, six examinations were made at intervals of seven days.

The treatment given was tartar emetic, to which was added quinine when it was found that the patient was infected with malaria. The details were as follows:--

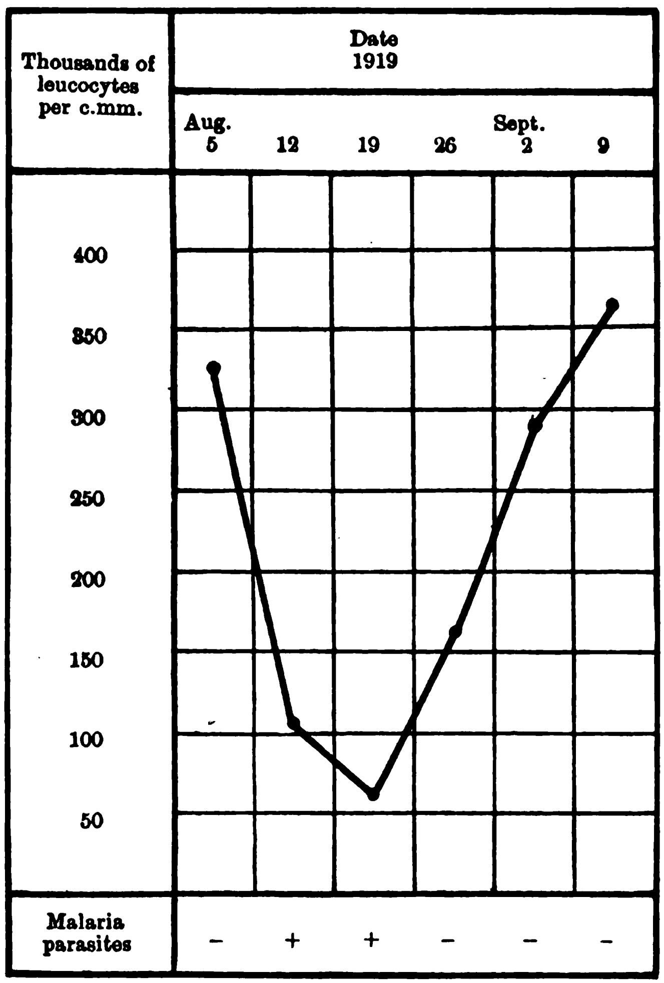
Intravenous injections of tartar emetic, one grain each, on August 6th, 9th, 11th, 13th, 17th, 19th, 22nd, 25th, 28th, 31st, September 2nd, 4th, 6th, and 8th—total 14 grains.

Quinine sulphate, 30 grains daily, by the mouth in solution, from August 20th to September 9th.

The results of the blood examinations are shown in the Table and in the Graph. It will be noted that the number of leucocytes per

SUCCESSIVE EXAMINATIONS OF THE BLOOD IN A CASE OF MYELOGENOUS LEUKAEMIA.

Date of observation	Blood Corpuscles per c.mm.		Examination for Malaria parasites	Remarks
	White	Red		
August 5th	326,250	3,047,000	Neg.	Intravenous injections of tartar emetic started on Aug. 6th.
12th	104,870	2,451,340	*P. falciparum*: not numerous	Morning temperature on Aug. 11th, 101° F.; on Aug. 13th, 100° F.
" 19th	62,900	3,334,400	*P. falciparum*: numerous	Quinine sulphate, 30 grains daily, orally, started on Aug. 20th.
" 26th	162,130	3,432,150	Neg.	
September 2nd	290,940	3,200,000	Neg.	
" 9th	364,375	2,906,250	Neg.	



c.mm. was reduced from 326,250 to 62,900 between the 5th of August and the 19th, a phenomenon which might have been attributed to either the treatment with antimony, which was started on the 6th, or to the intercurrent malaria (*P. falciparum*), which developed about the 12th of August. On the 20th of August quinine was given for the first time. Subsequently malaria parasites were not again found in the blood, and although the antimony treatment was continued the number of leucocytes increased steadily, so that on the 9th of September they were more numerous than they had been at the time of the first examination.

In myelogenous leukaemia, just as was previously observed in lymphatic leukaemia, a notable reduction in the number of leucocytes coincides with the appearance in the blood of malaria parasites. This reduction is not permanent, and is rapidly effaced by treatment with quinine.

Incidentally this case shows also, (1) that intravenous injections of tartar emetic in the doses given have no beneficial action in myelogenous leukaemia, and (2) that such injections do not prevent the development of an attack of malaria (*P. falciparum*).

REFERENCE

Macfie, J. W. S. (1917.) A Note on the Occurrence of Leukaemia in the Natives of West Africa. *Report of the Accra Laboratory for the year* 1916, pp. 39-42. J. and A. Churchill, London.

TROPICAL AUSTRALIA AND ITS SETTLEMENT

BY

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AND

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Reprinted from *Medical Journal of Australia*, Vol. VI, i, pp. 353, 375 and 395.

CONTENTS

I. INTRODUCTION

Tropical Australia and its settlement by a British race has formed the theme of numerous public utterances and of a good deal of writing in both the scientific and lay press. Sometimes these utterances have been dictated by utilitarian reasons alone, sometimes they have only formed part of a general political propaganda; many have been influenced by personal prejudice, and were put forth by writers whose experience of life in Northern Australia did not qualify them to express an opinion on this subject.

On many occasions, when the ultimate fate of Northern Australia has formed the subject of discussion at various scientific meetings, the speakers have applied indiscriminately to Northern Australia the outcome of experiences gained by themselves or by others in various parts of the tropics, without realising that Northern Australia occupies a different position, on account of the absence of a settled native population and its peculiar climatic conditions.

The following pages are the outcome of several years' investigation in and consideration of conditions obtaining in Northern Australia and their influence upon a settled white race. A résumé is included of previous investigations by other workers into the practicability of a white settlement of other parts of the tropics and the influence of tropical life upon a population of European descent. An attempt is made to sift facts and to review the results of previous investigations, published in numerous scattered journals, most of which, with the exception of the experiments carried out in the Philippine Islands, not only have been done in a haphazard way, but sweeping conclusions have been based upon a few unsystematic observations.

The problem of the settlement of tropical Australia by a white race is a very difficult and complicated one, since two factors enter into the question. In the first place, climatic conditions and their effect upon the white man are of paramount importance, since racial degeneration, brought about by climatic conditions alone, would decide the problem. At the same time the economic side of the question plays an important part and cannot be neglected, and a discussion on this aspect has been included.

Opportunity is taken here to thank Mr. H. A. Hunt, the Commonwealth Meteorologist, for putting at our disposal the climatic data, and also Mr. G. H. Knibbs, C.M.G., the Commonwealth Statistician, for help and suggestion in connexion with the statistical data.

II. THE CLIMATE OF NORTHERN AUSTRALIA

The Australian living in the southern parts of his continent generally possesses only a very uncertain knowledge of the climate of the northern parts. He assumes that the whole of the north above the tropic has a uniformly hot climate, that heat and mugginess persist and that a feeling of personal comfort is only a rare sensation. This popular conception arises from the general idea of 'tropics'; most people in their early youth associate with 'tropics' a land of impenetrable jungle, heat, swamp and fever, an impression gained by reading books of adventure and travel.

The scientific study of climates within the tropics shows, however, that there exists a great diversity of climatic conditions, regulated by a number of factors, such as the nearness to the ocean, elevation above sea level, proximity to high mountain ranges which control rainfall, prevailing wind and, above all, the amount and monthly distribution of the rainfall.

To describe climate from the point of view of its effects upon a human race is impossible with our present means. It is only to a certain extent that climate, as we feel it, finds a graphic expression in those meteorological observations usually recorded, which measure heat and humidity only, namely, the readings of the dry and wet bulb thermometers. From these readings is further calculated the relative humidity, that is, the extent to which the atmosphere is laden with moisture. The degree of personal comfort or discomfort experienced is indicated to some extent by these readings, but it is certain that many other factors play a large part in determining individual sensations.

Numerous attempts, summarized as far as 1908 by Hann, have been made to construct a 'discomfort scale,' but no satisfactory solution has yet been attained. Conspicuous amongst these is that of Cleveland Abbé, who suggested a 'curve of comfort,' based upon three factors, namely, air temperature, relative humidity and wind velocity. By plotting temperatures against humidities for a certain velocity of wind and estimating personal comfort or discomfort under these conditions, he obtained charts which correlated his personal sensation with the above factors.

Wet bulb temperatures alone were first used as a guide to comfort by Harrington, who termed them 'sensible temperatures' and mapped out wet bulb isotherms for the United States of America for the month of July. The importance of wet bulb readings has been further emphasised by Haldane (1905), who demonstrated experimentally that the regulation of body temperature above a certain wet bulb reading became disorganised and human energy was paralysed. He therefore suggested wet bulb temperatures as a standard by which to regulate conditions in factories, mines and workshops.

Griffith Taylor (1916) has suggested a tentative scale of discomfort, applying only to warmer regions, where humidity and temperature are the chief factors, a scale depending on wet bulb readings. He divided 'climate' into the following grades:—

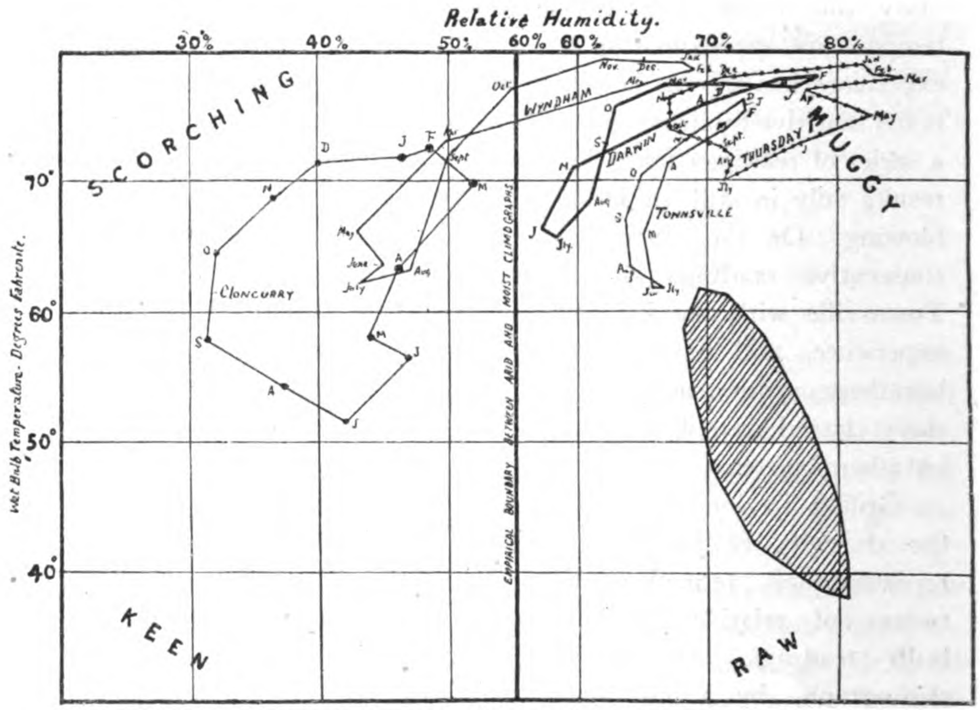
8·3°-12·7° C. (45°-55° F.) wet bulb—Most comfortable.
12·7°-15·5° C. (55°-60° F.) wet bulb—Very rarely uncomfortable.
15·5°-18·3° C. (60°-65° F.) wet bulb—Sometimes uncomfortable.
18·3°-21·1° C. (65°-70° F.) wet bulb—Often uncomfortable.
21·1°-23·7° C. (70°-75° F.) wet bulb—Usually uncomfortable.
Over 23·7° C. (over 75° F.) wet bulb—Continuously uncomfortable.

A rough-and-ready indication of the degree of discomfort, used by Osborne (1916), is the amount of clothing required to enable him to lie in an open-meshwork hammock. His experiences have led him to set a wet bulb of about 22·75° C. (73° F.) as an empirical standard above which truly tropical conditions arise.

Many authors, however, contend that wet bulb readings alone are not an unerring indication of the subjective sensation caused by climate. L. Hill (1914, 1916), for example, pointed out: 'It is no use to trust to the ordinary thermometer, either wet or dry, because it does not show heat loss. It only shows its own temperature, the average temperature of the furniture, surroundings and walls; it does not show the heat loss of the body. Whilst the thermometer is a static instrument indicating average temperature, the human body is a dynamic structure, continually producing and losing heat, while its temperature remains sensibly constant.' He deprecated the use of relative humidity as a criterion of comfort, and suggested the katathermometer, a new apparatus, which would register the rate of heat loss and thus indicate more closely the effect of temperature and atmospheric conditions upon the human body. This instrument consists of two thermometers, each having a large bulb filled with alcohol; one of these is kept dry—the dry bulb katathermometer; the other is kept moist by means of a wet cotton glove—the wet bulb katathermometer. Both are heated to 43·3° C. (110° F.) and the time which each takes to cool from 43·3° to 32·2° C. (110° to 90° F.) is noted. The dry bulb katathermometer thus measures the rate of cooling by convection and radiation, the wet bulb katathermometer that by convection, radiation and evaporation and thus measures not the actual air temperature but the rate of cooling of a moist body, due to the effects of the atmospheric condition at an actual temperature approximating to that of the human body. Practical experience has shown, however, that the wet bulb katathermometer is too sensitive to air currents. Osborne (1916) has pointed out that a series of readings taken in rapid succession yields fairly uniform results only in still air indoors, or outdoors if a steady breeze be blowing. On the other hand, when the wind comes in gusts, consecutive readings show great variations. Observations in Townsville with the katathermometers have confirmed Osborne's experience, and have proved that calm weather, as far as the katathermometer readings are concerned, is a rare exception, and days classed as still to personal sensation may not be so to the katathermometer.

Griffith Taylor (1916) attempted to overcome in a novel manner the difficulty of judging a climate, by introducing a graphic representation, termed a climograph, and plotting the monthly means of relative humidity against monthly means of wet bulb readings. He compiled as a standard a composite climograph, by using average figures for towns situated in regions where human energy is at its best, selecting for this purpose five towns in the southern and seven towns in the northern hemisphere, where the average monthly wet bulb readings ranged between 2·7° and 16·6° C. (37° and 62° F.). This climograph, according to Taylor, represents ideal conditions for the white race, and he terms it the 'white race climograph.' On the same principle he constructed a number of climographs for different parts of the world and differentiated in this way four extreme climatic types—hot and damp, hot and dry, cold and damp and cold and dry—and compared the climographs of Australian towns with these types (see fig. 1). The shape of the climograph also indicates the seasonal distribution of rainfall.

It is an ingenuous and striking method of comparing in a general way the climate of any given locality with a type, but does not convey any further information than the mean readings of dry and wet bulb thermometers, and it is significant that Taylor himself, when discussing the question of discomfort in relation to climate, uses the wet bulb readings as guide.



NOTE.—The shaded figure is the composite white race climograph based on twelve typical cities.

FIG. 1.

Bruce (1916) has proposed the use of dew point as an indicator of the effects of atmospheric conditions upon the human body, and comes to the conclusion that the dew point most desirable for human activity is 16·6° (62° F.), that is to say, that air saturated with moisture at 16·6° (62° F.) is neither muggy nor chilly. If the dew point, however, rises over 21·1° C. (70° F.) the conditions become exceedingly trying.

This principle has been applied to the average temperatures recorded in Townsville, and the results have indicated that the dew point at 9 p.m. is, as a rule, higher than at 9 a.m. or 3 p.m., although the conditions in the evenings are, on the whole, undoubtedly less trying than those prevalent during the day. Furthermore, this principle does not take into account the effects of other conditions, such as wind and sunshine.

It would appear, therefore, that neither humidity nor dew point give any more information in regard to personal comfort than the readings of dry and wet bulb thermometers.

Hunt, at Osborne's suggestion, constructed wet bulb isotherms for Australia—the only part of the world for which such complete isotherms have been published—attempting thus to represent graphically climatic conditions as far as personal comfort is concerned. As pointed out previously, wet bulb temperatures alone in this respect are only of limited value, since experience has shown that, although high wet bulb temperature, approaching the limit of safety, may be a guide to discomfort, yet lower ones, without the accompanying dry bulb readings, are indefinite. From our personal experience, wet bulb readings above a certain limit invariably denote discomfort, yet the dry bulb temperature even then modifies the degree of discomfort felt. For example, a wet bulb reading of 26·6° C. (80° F.) is never pleasant, but the degree of discomfort becomes exaggerated in direct proportion to the dry bulb reading. For these reasons it has been thought advantageous to make use simultaneously of the mean dry and wet bulb readings for the comparison of the various parts of Northern Australia. In the accompanying graphs (fig. 2) are plated the average monthly dry and wet bulb temperatures, taken at 9 a.m. over a period of five years, and the points are connected by lines for distinctness only, the 10° C. (50° F.) line being indicated in order to make a comparison easy. Since the temperatures are so dependent on the rainfall, average rainfalls obtained from Hunt's publications have been plotted in these charts. The relative positions of the graphs of the various towns are arranged diagrammatically as near as possible in accordance with their geographical position (compare map, fig. 3). Thus the coastal towns are represented on the outside of the diagrams and the inland towns in the interior. The graphs

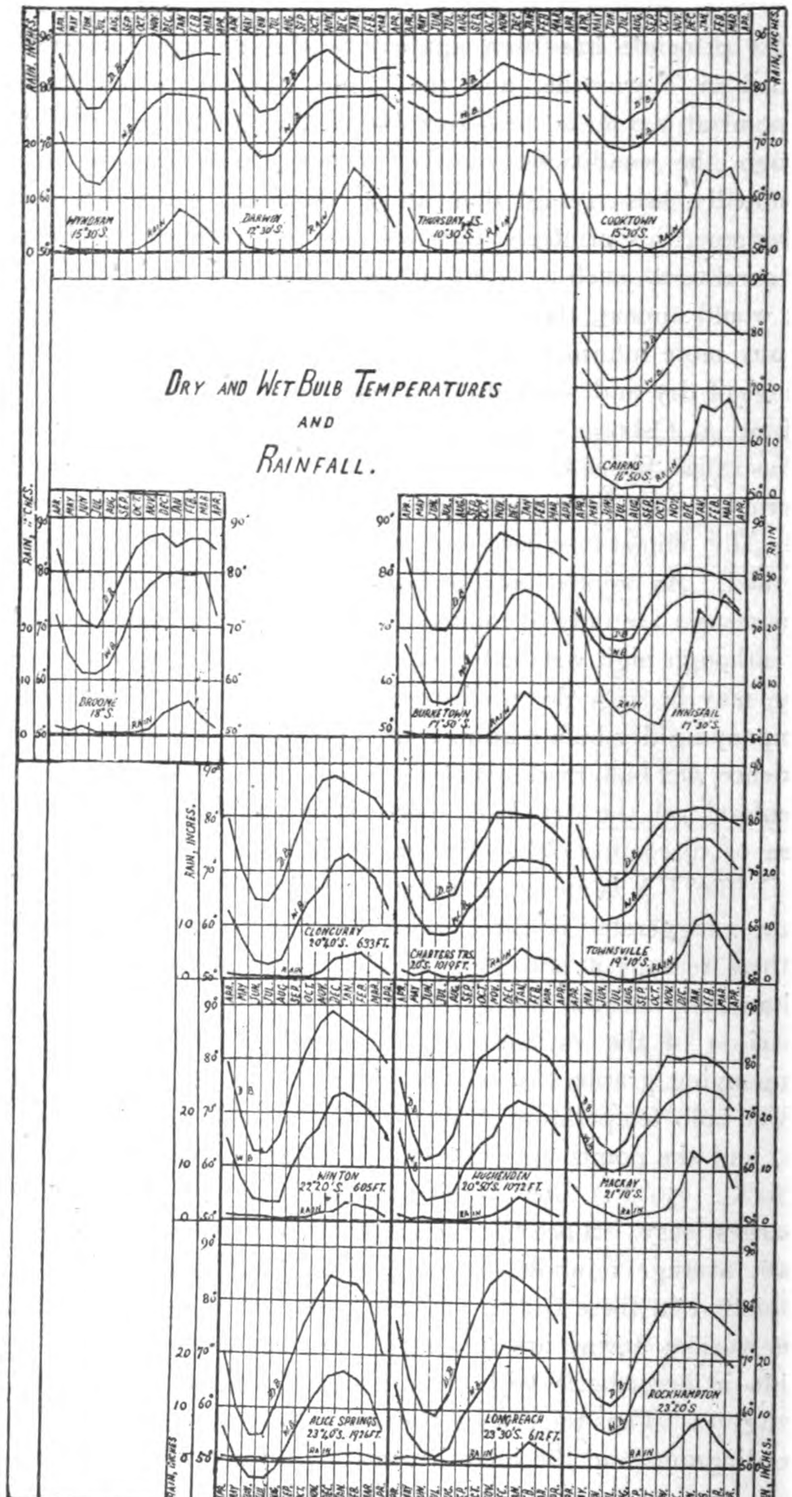

DRY AND WET BULB TEMPERATURES
AND
RAINFALL.
WYNDHAM 15°30'S.
DARWIN 12°30'S.
THURSDAY IS. 10°30'S.
COOKTOWN 15°30'S.
CAIRNS 16°50'S.
BROOME 18°S.
BURKETOWN 17°50'S.
INNISFAIL 17°30'S.
CLONCURRY 20°40'S. 633FT.
CHARTERS TRS. 20°S. 1019FT.
TOWNSVILLE 19°10'S.
WINTON 22°20'S. 605FT.
HUGHENDEN 20°50'S. 1072FT.
MACKAY 21°10'S.
ALICE SPRINGS 23°40'S. 1926FT.
LONGREACH 23°30'S. 612FT.
ROCKHAMPTON 23°20'S.
RAIN, INCHES.

FIG. 2.

commence with the month of April, as in this month a distinct change of season sets in; beginning from then, the average temperature falls (rapidly at first and more gradually afterwards) and begins to rise again on or about July. In this way both fall and rise are more conveniently displayed for comparison than if the graphs were commenced with January in the usual way. In order to make the comparison more complete, the monthly averages for the maximum and minimum temperatures are displayed in a similar manner on a second series of charts (fig. 4).

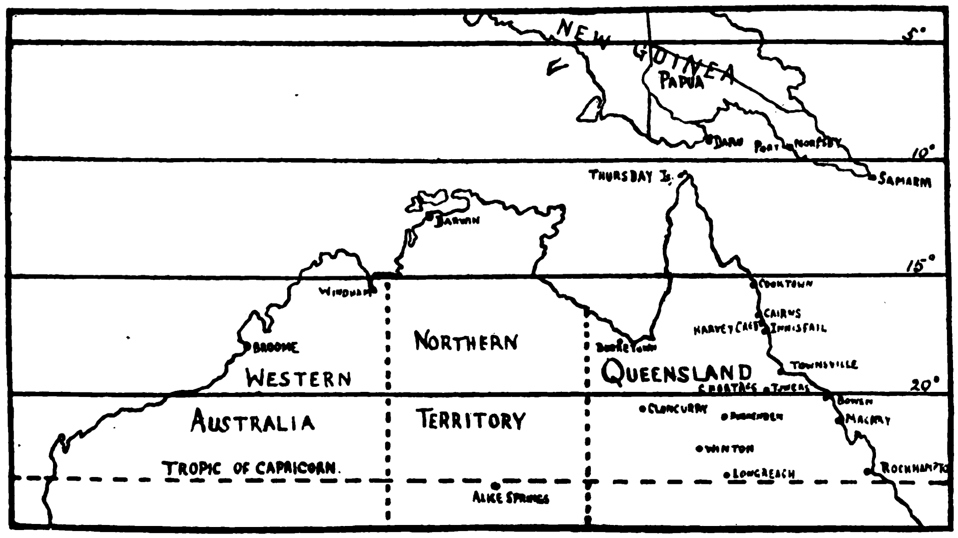


FIG. 3.

The graphs make it clear that the climate of the coastal towns differs essentially from that of the inland towns. The former towns have, on the whole, higher wet and lower dry bulb readings than the latter. With the coastal towns the average readings increase gradually with decreasing latitude and the contrast between the cool and hot season becomes less and less pronounced. The charts of Rockhampton and Thursday Island, the two extreme towns of Queensland, situated within the tropics, illustrate this contention. In Rockhampton (latitude 23° 24') the coolest month (July) has average dry and wet bulb readings of 16·3° C. (61·4° F.) and 13·3° C. (56° F.) respectively, and the hottest month (January) has readings of 27° C. (80·6° F.) and 22·7° C. (73° F.) respectively. The average dry bulb temperature is therefore 10·7° C. (19·2° F.) higher,

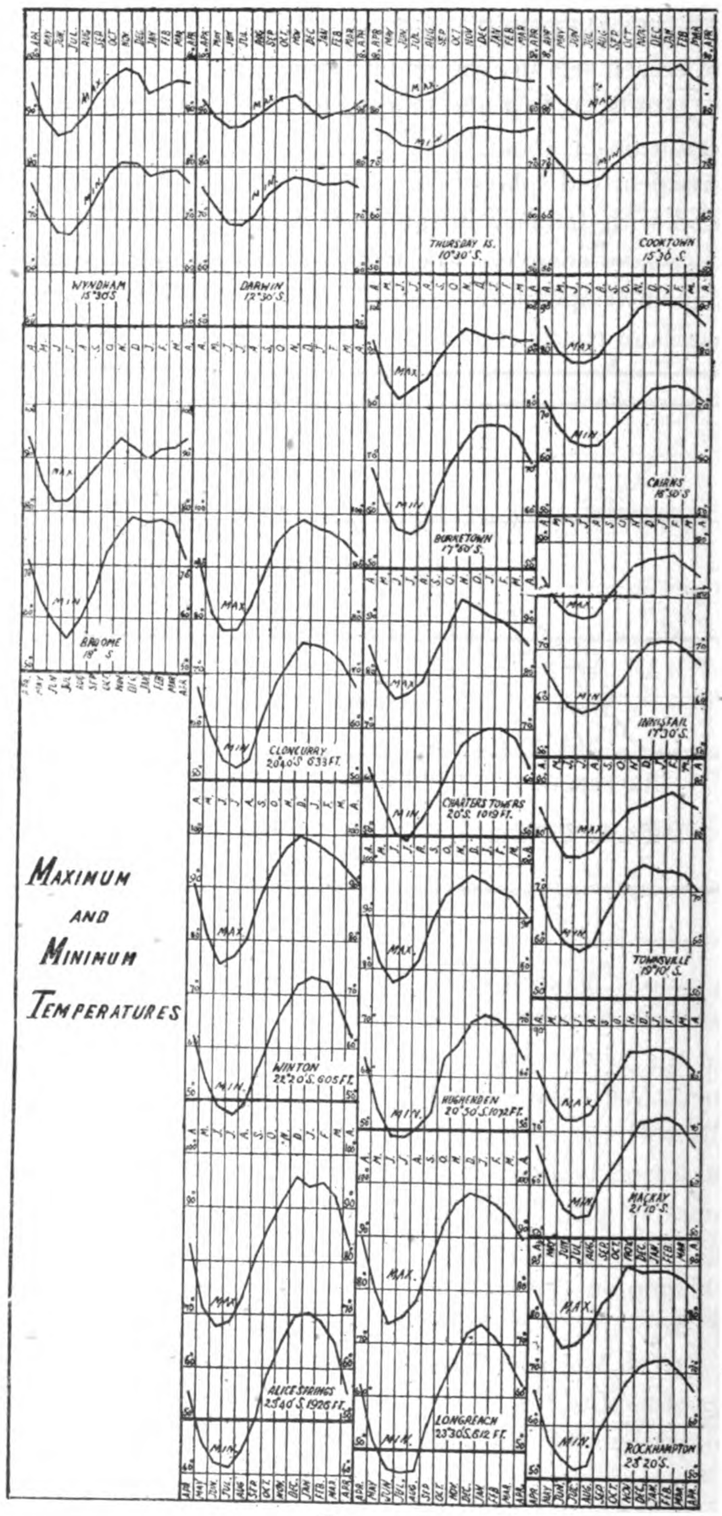

MAXIMUM AND MINIMUM TEMPERATURES
WYNDHAM 15°30'S.
DARWIN 12°30'S.
THURSDAY IS. 10°30'S.
COOKTOWN 15°30'S.
BROOME 18° S.
BURKETOWN 17°50'S.
CAIRNS 16°50'S.
CLONCURRY 20°40'S. 633 FT.
CHARTERS TOWERS 20°S. 1019 FT.
INNISFAIL 17°30'S.
TOWNSVILLE 19°10'S.
WINTON 22°20'S. 605 FT.
HUGHENDEN 20°50'S. 1072 FT.
MACKAY 21°10'S.
ALICE SPRINGS 23°40'S. 1926 FT.
LONGREACH 23°30'S. 612 FT.
ROCKHAMPTON 23°20'S.
MAX.
MIN.

FIG. 4.

and the average wet bulb reading is 9·4° C. (17° F.) higher in January than in July. In Thursday Island, however (latitude 10° 34′), the corresponding averages are 25·8° C. (78·3° F.) and 23·2° C. (73·8° F.) for July respectively and 28·1° C. (82·7° F.) and 25·9° C. (78·7° F.) for January, showing much smaller differences, namely, 2·3° and 2·7° C. (4·4° and 4·9° F.) respectively. In other coastal towns, such as Darwin, Wyndham and Broome, situated on the northern coast of Australia, the seasonal variations are more pronounced when compared with Thursday Island. During the hot season the average readings there are higher than in towns on the eastern coast in the same latitude. Broome and Innisfail, for example, both situated between 17° and 18° South, show this difference in a marked degree (see charts), but the geographical position alone explains this difference, since Innisfail lies within the region of the trade winds, and, in addition, possesses a larger average rainfall.

For comparison only, graphs representing the climates of Daru, Port Moresby and Samarai, all in Papua, have been added (fig. 5). These graphs do not differ essentially from those of Thursday Island.

In most of the inland towns of Northern Australia weather conditions are greatly influenced by altitude, and changes with latitude are consequently less pronounced.

The graphs representing the average maximum and minimum temperatures exhibit the same seasonal variations, which in the coastal towns become less marked with decreasing latitude and show smaller ranges of temperature. In the inland towns the average maximum readings in general are much higher and the average minimum readings much lower than in towns on the sea coast.

A comprehensive account of the distribution of rainfall throughout Northern Australia has been published by Hunt in a series of monographs issued by the Meteorological Bureau. The rainfall in North Queensland shows a seasonal distribution corresponding to the monsoonal type of climate, the heaviest falls taking place during the hot months (December to March), and only occasional showers occur during the cooler months.

There is a 'wet belt' on the north-eastern coast, the centre of which lies about Harvey Creek and which extends northward to a point beyond Port Douglas and southward as far as Halifax, where a very high general rainfall is registered (see Table I).

TABLE I.—RAINFALL

Cooktown	181·9 cm.	(71·6 inches)
Port Douglas	210·6 cm.	(82·9 inches)
Cairns	229·9 cm.	(90·5 inches)
Harvey Creek	426·0 cm.	(167·7 inches)
Innisfail	384·0 cm.	(151·2 inches)
Cardwell	218·6 cm.	(86·1 inches)
Halifax	226·6 cm.	(89·2 inches)
Ingham	204·5 cm.	(80·5 inches)
Townsville	125·2 cm.	(49·3 inches

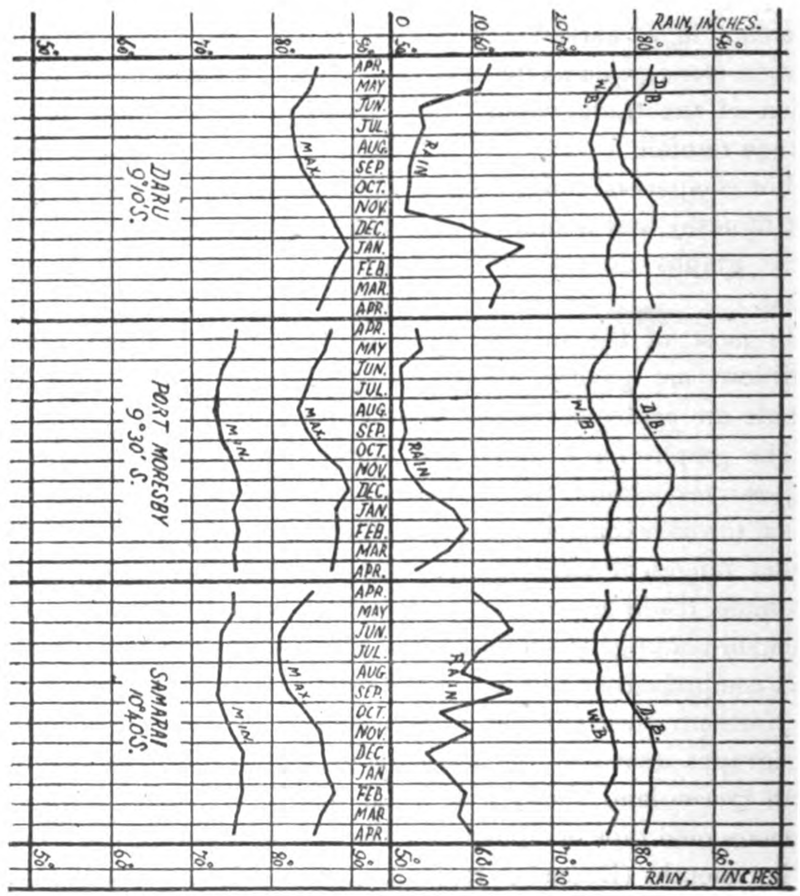


FIG. 5.

The inland towns, on the whole, are much drier than the coastal towns and show a similar seasonal distribution of rainfall.

In the coastal towns of other parts of Northern Australia conditions similar to those of North Queensland prevail, with the one difference that Broome and Wyndham show a comparatively small average rainfall, namely, 58·2 cm. and 71·4 cm. (22·9 inches and 28·1 inches) respectively. In New Guinea, Port Moresby and Daru, both situated within the monsoonal belt, possess in the same way a dry and wet season, whereas Samarai has its rainfall of 296·4 cm. (116·7 inches) more evenly distributed over the whole of the year.

III. SUNLIGHT IN THE TROPICS

The main difference between a temperate and a tropical climate lies in the greater intensity of the sun's rays in the tropics. This greater intensity is solely due to the less oblique path of the rays striking the earth, whereby they have thus passed through a smaller layer of atmosphere. As a result, a less degree of absorption and scattering has taken place, and the chemical and physical activities of these rays are therefore greater the nearer to the equator. This greater activity of the sun's rays manifests itself in everyday life. The newcomer to the tropics soon becomes aware that coloured materials, such as curtains, carpets and clothing fade quickly, and written matter in ink, when exposed to the sun, gradually gets fainter, and, after a time, almost disappears. Similarly, a number of chemical preparations decompose, rubber rapidly perishes and certain qualities of glass are altered. The frosting of glass flasks, microscopic slides and high-power lenses are examples only too well known to laboratory workers in the tropics.

The bactericidal action of sunlight has been known for a long time and has been investigated again recently by Clemesha (1912) in India, who exposed cultures of faecal bacteria to the sun. In other experiments he added large quantities of faeces to water contained in a tank with a large surface area (0·6 hectares, or 1 acre) exposed to the sunlight and examined the liquid from day to day for bacterial content. Furthermore, he studied the bacteriological flora of waters of natural lakes during the monsoonal and dry seasons. All these inquiries led him to conclude that the sun has a very powerful action in destroying faecal organisms in water.

Such changes as the above, together with numerous others of a similar nature, led to the question of the nature of the rays producing these results. Are they brought about by those rays of shorter wave length in the violet and ultra-violet portions of the solar spectrum (chemical rays), or are they to be attributed to rays of longer wave length, situated at the other end of the spectrum, the red and infra-red rays (heat rays)? The ultra-violet rays are known to increase chemical activity, and many chemical reactions and decompositions may be brought about by exposure to these rays. An example is the decomposition of oxalic acid into carbon monoxide, carbon dioxide and water when exposed to the sun in the presence of a uranium salt. This decomposition is almost entirely due to those rays of the solar spectrum in the ultra-violet extending from 550μ to 291μ (Freer (1912)). Observers in the Philippines attempted to make use of this decomposition of oxalic acid under standardised conditions, in order to compare the intensity of the ultra-violet rays of the sun in different parts of the world. The results, however, showed that the amount of decomposition in the Philippines and other parts of the tropics was inconstant and did not bear any definite relationship to latitude. The figures obtained by the same method in a temperate zone were sometimes as high, and even higher, than those found in the tropics.

The extensive observations of the Philippine workers have led them to the conclusion expressed by Aron (1911): 'That the spectrum of the sun's rays does not extend much, if any, further into the ultra-violet in Manila than in northern climates.' These observers do not agree with Woodruff (1905) and others, who attribute the effects of tropical sunlight on the human organism to the influence of ultra-violet rays only. Aron (1911) believes that 'the rays of the tropical sun, having greater wave length than those in the red and ultra-red end of the spectrum, play the most important rôle in producing the untoward effects generally attributed to tropical sunlight'; in other words, he attributes such effects to the heat rays alone.

Gibbs (1912), working in conjunction with Freer and Aron, expresses a somewhat similar opinion, and, if altitude and local meteoroligical conditions are taken into consideration, he does not believe that 'when the normal intensities are compared, the light of the tropics is different from the sunlight of any other regions.' Effects upon life in the tropics, usually attributed to sunlight, are, in his opinion, due to 'other meteorological modifications, which go to make up climate, namely, duration of sunshine, clouds, rainfall, winds and humidity, all of which affect the air temperature; the last is probably the most important fact and depends to a large extent upon the duration of sunshine.'

In Manila and Baguio, both in the Philippines, Aron (1911) and Gibbs (1912) carried out experiments on animals in order to study the effects of exposure to the sun's rays under varying conditions. Different experimental animals, such as rabbits and monkeys, were exposed to the midday sun for varying periods. These animals, when shielded from draughts, died after an exposure of from thirty-four minutes to about one and a half hours, and showed the post-mortem appearances characteristic of heat stroke. Black rabbits, as a rule, succumbed more quickly than light-coloured animals, and it was noticed that the sub-cutaneous temperatures, taken by means of a thermocouple, in the case of black rabbits, rose quicker and higher than in that of the lighter-coloured ones.

Aron, furthermore, carried out exposure experiments on dogs which had been tracheotomised and thus had part of their effective heat-regulating mechanism put out of action.* It is well known that dogs do not possess sweat glands on the body, but keep their body temperature from rising by means of an increased rate of respiration and an increased evaporation from their respiratory tract. These animals died after about an hour with typical symptoms of heat stroke and corresponding post-mortem appearances. In a similar manner the body temperature of tracheotomised rabbits rose on exposure to the sun more rapidly than that of normal rabbits.

When monkeys were exposed to an artificial draught during the experiment, or were protected by shade from the direct sun rays, no injurious effects were noticed. These experiments, according to Aron (1911), show that 'when placed under the fan animals lost the excessive heat which reached them by radiation from the sun. Rays including the ultra-violet were nevertheless present, and were

* Considering the relatively small surface of mouth, nose and throat above the tracheotomy wound, compared with the breathing surface of the lungs, it is evident that the operation had put out of action only an extremely small portion of the respiratory surface, and cannot altogether account for the quicker death of these animals.

absorbed by the body in the same manner and degree as by that of the control monkeys.'

These experiments have recently been repeated by Shaklee (1917) and further amplified, to ascertain whether it is possible for monkeys to become accustomed to the sun (acclimatised) by a gradually increasing daily exposure. His results differed from those of Aron, as several of his monkeys, even in the beginning of the experiment, lived for hours in the direct sunlight without being protected in any way. Summarising his results, he states that experimental monkeys, exposed to the sun in Manila, may die from heat stroke after varying periods, depending to a less extent upon the sun's rays than on other local conditions, such as proximity of a large, hot surface (ground or roof), high relative humidity of the atmosphere and low wind velocity. Monkeys, however, may become temporarily acclimatised by gradually increasing the time of exposure to the sun. This, in his opinion, increases the sensitiveness of the nervous mechanism which regulates body temperature by an increased rate of perspiration. The fact that administration of atropine, which impairs the function of the sweat glands, causes the death even of an acclimatised monkey on exposure, is evidence in support of this conception.

Animal experiments were also carried out by Schmidt, who, working in a temperate climate, exposed rabbits to the sun and found a rise in the anal temperature which was more pronounced in a black than in a white rabbit. In the same way Schilling (1909) observed a rise in the skin temperature of rabbits exposed to the sun, and several other authors have made similar observations. It must be kept in mind, however, that the temperature of rabbits varies considerably even under normal conditions, and the struggling alone, when the animal is handled, may cause a considerable rise.

It is not a new observation that animals, even in a temperate zone, may succumb to exposure to the sun, as the following example illustrates. In a laboratory in the north of England, in which monkeys were kept in a glass house, on more than one occasion during the summer months several animals died under the symptoms of heat stroke, showing an ante-mortem rectal temperature of 43·3° C. (110° F.), which still rose after death. The painting of the glass roof with a white wash reduced the inside temperature of the animal house and prevented death.

A consideration of the foregoing observations makes it evident that exposure to the direct sun's rays caused such an increase in body temperature that the animals finally succumbed to hyperpyrexia. In every case animals of dark colour died more quickly than those with light fur, on account of the greater absorption of heat.

The dark skin of most of the aboriginal races in the tropics, from the above point of view, would appear to be a disadvantage, and the explanation that dark skin affords protection against the effects of the sun merely by insulating the body against the deep penetration of harmful rays must be modified.

Attempts to elucidate the rôle played by the pigment in a protective sense are not lacking in the literature. Eijkman (1895), in Batavia, covered the bulbs of two thermometers with pieces of white and coloured human skin and placed the thermometers in the sun. He noticed that the brown skin caused a higher rise of the mercury (50·1° C.) than the white skin (47·5° C.).

Aron (1911) and Gibbs (1912), in the Philippines, experimenting with live skin, exposed white and coloured persons to the sun and recorded the skin temperatures of various parts of the body by means of a thermocouple. Their results were somewhat inconstant; the skin temperature invariably rose after a time to from three to four degrees above normal. Whilst Gibbs found temperatures distinctly higher for the dark skin, Aron noted that the white skin was always hotter than the brown, and that after prolonged exposure the temperature of the brown skin showed a more distinct fall. 'It may be said,' as Freer (1912) remarks in summarising the results of both investigators, 'that as regards rise in temperature on exposure to the sun, the white and brown skin (Filippino) are about equal, with a slight factor in favour of the white, but that in regard to the very dark skinned negro the temperature on exposure reaches a decidedly higher point than it does with either of the others.' In its physiological action, on the other hand, the dark skin is superior to the white skin. It absorbs a greater quantity of heat rays, warms up more quickly and reaches the point where perspiration commences earlier and the evaporation of the sweat causes heat loss and consequently affects the cooling of the body.

A consideration of the foregoing experiments and observations upon the effects of exposure to the sun suggests that any ill-effects are due not to light but to heat. These experiments, however, only take into consideration those effects known as sun stroke or heat stroke, and it is, moreover, an almost impossible task in such experiments to study the physiological action of the other rays with the entire exclusion of heat rays.

The effect of sunlight on living organisms has formed the subject of many publications, and many opinions have been put forward which are not founded upon facts but are only wild speculations. Woodruff (1905), for example, wrote an extensive monograph on the effects of tropical light on the white man, and his conclusions have been widely quoted in the literature. He contended that the ultra-violet rays of the tropical sun are inimical to white settlement, but, unfortunately, he based many of his arguments on false premises, and many of his statements were merely expressions of personal opinion. As a glaring example, the following sentence may be quoted: 'The southern hemisphere, except the tip of Patagonia, is north of 45° and therefore unfit for blondes (he assumes that a blonde race cannot live nearer to the equator than 50°), and even in New Zealand and Australia the native white families are already dying out or kept alive by constant new importation from home.' He assumes, further, that in New Zealand there is ample evidence of the physical decay of the white population. In view of such statements, entirely without foundation, it is difficult to consider any of his conclusions seriously.

IV. EFFECTS OF HEAT AND HUMIDITY ON THE ANIMAL ORGANISM

In all warm-blooded animals the temperature of the body under normal physiological conditions only varies slightly. This temperature is maintained by two processes—heat production and heat loss. The former is brought about by chemical processes, the combustion of food material inside the body, the latter by physical means, namely, radiation and convection and evaporation of sweat. In the clothed human being the heat production plays a much less important part in regulation of temperature than the heat loss by physical means, and it is evident that the extent to which these physical means operate must depend on the surroundings. Whereas in a cold climate heat loss takes place mainly by radiation and convection from the body, in a hot climate, where the air temperature often approaches or even exceeds that of the body, the heat loss through radiation can only be very small or nil, and heat is mainly lost by evaporation of sweat from the skin.

It was of great interest to inquire into the physiological reaction of the animal body to increased outer temperature, and a great deal of experimental work has been done in this direction. Attention has been paid to the body temperature and the metabolism in conjunction with the calorific value of the food, but a great deal of this work has been carried out in temperate climates under artificial conditions, and comparatively few observations have been made in the tropics.

(1) *Regulation of Body Temperature under Artificial Conditions*

Blagden and Fordyce (1775) noticed that their mouth temperatures did not rise above normal when remaining for fifteen minutes in a dry room heated to a temperature of 115° C. to 126·7° C., although the heat was sufficient to cook a beef steak. Exposure for the same length of time, however, in a moist room heated to 54·4° C. caused the body temperature to rise to 37·8° C. In recent times Haldane (1905) carried out his classical experiments on the effect of humid heat. His observations were made on human beings, both at rest and at work, in an artificially heated chamber, as well as underground in deep mines, in still air or exposed to artificial draughts. He concluded that the temperature of the human organism exposed to humid heat begins to rise after a period varying according to the individual and the conditions and then continues to do so. The rise in body temperature corresponds with the reading of the wet bulb temperature and is practically independent of the dry bulb temperature. During rest in still air, wet bulb temperature of about 31·1° C. (88° F.) could be borne without any abnormal rise in rectal temperature, but when the thermometer rose above that temperature the rectal temperature immediately commenced to rise, which rise was accompanied by an increase in the pulse rate, profuse sweating and dyspnoea, until finally exhaustion set in. During muscular exercise in still air this increase in body temperature commenced at a much lower wet bulb (27° C., or 80·6°F.,) and in moving air much higher wet bulb temperatures were required to produce the same effect.

These experiments were continued and amplified in Haldane's laboratory by Harvey Sutton (1908), who studied the changes in the gaseous metabolism of the body when exposed to humid heat. He observed a large increase in the metabolism, running parallel to the rise in body temperature; in fact, analogous conditions to those existent in fever. He concluded that 'all experiments point to the fact that once the balance of the mechanism of heat regulation in the human body has been definitely upset by high external temperatures, combined with almost total abolition of heat loss in evaporation, a vicious circle is established. The internal temperature rises and as a result the oxidation processes——and therefore the production of heat also increases—so that the body temperature rises still further, and so on. Once the ball has been set rolling, nothing seems to check its progress, and it slowly but surely gathers speed. The rectal temperature not only continues to rise, while the external temperature still remains constant, but the rise gradually increases in rapidity as if momentum were being gathered.'

Conditions similar to those under which the experiments of Haldane and Harvey Sutton were conducted, may, according to Pembrey (1913), give rise to heat stroke. He analysed the history of a large number of cases of heat stroke which had occurred amongst British troops in India, and came to the conclusion that heat stroke is brought about by a failure of the mechanism which regulates the body temperature, after prolonged exposure to excessive moist heat. In an excessively hot and moist atmosphere the cooling effect of the evaporating sweat is insufficient to prevent the body temperatures from rising, and a prolonged exposure to these unfavourable circumstances leads to a gradual rise in body temperature and ultimately to heat stroke.

Observations on the influence of wind and draughts on the body bring additional evidence to show that, with high external temperature, the loss of heat due to evaporation of sweat is the most important factor in regulating the body temperature. As far back as 1883 Herman suggested that the ill-effects due to bad ventilation and crowded rooms were caused by heat stagnation. This view was confirmed experimentally by Heyman, Paul and Ercklentz (1905), and later still by Hill, Flack, Rowlands and Walker (1913). Hill and his colleagues confined individuals in a small experimental chamber until the carbonic acid content rose from 1 per cent. to 1·5 per cent. in one instance and from 3 per cent. to 4 per cent. in another. The subjects experienced discomfort at a wet bulb temperature of 27·7° to 28·4° C. (82° to 85° F.) and this discomfort was considerably allayed when the air in the chamber was moved by a fan. No relief, however, was experienced when fresh air was breathed through mouthpiece and tube passing through the wall of the chamber. Subjects outside the chamber who breathed the chamber air suffered no discomfort, thus proving that the discomfort was caused not by the increased carbon dioxide of the air in the chamber, but by the heat stagnation, following the failure of evaporation of sweat.

The part played by the evaporating perspiration is illustrated in Zuntz and Tendlau's observations upon the body temperature of a man whose skin was devoid of sweat glands. The exposure to the summer sun of a temperate climate caused his temperature to rise to 39° C. (102·2° F.) and a slight amount of manual work produced the same effect. He was, however, able to find a substitute for the missing function by frequently soaking his shirt in water.

Such a condition as described above can be artificially produced by immersion in a hot-water bath, when the evaporation from the skin is prevented and the effects of perspiring are eliminated. Hill and Flack (1909) studied the pulse rate, blood pressure, body temperature, breathing volume and alveolar tensions of man in a hot bath. They found that immersion up to the neck in hot water (40·5° to 43·3° C., or 105° to 110° F.) for fifteen to thirty minutes caused an increase in the body temperature of 39·1° to 40·3° C. (102·5° to 104·6° F.). The pulse rate and respiration rate and volume rose and the blood pressure was lowered. In the alveolar air a notable fall in carbonic acid tension and a corresponding rise in oxygen tension were observed, caused by the washing-out effect of the increased breathing.

The foregoing observations make it clear that the main difference between temperature regulation in the tropics and in a temperate climate lies in the greater activity of the sweat glands, and the evaporation of the increased amount of sweat plays a more important part in the cooling of the body. It is a common experience in the tropics that the quantity of water secreted in the urine is greatly diminished during hot weather, in spite of the larger intake of fluid; the balance of the water leaving the body by way of the skin and the lungs is considerably larger.

The establishment of an equilibrium between the water secreted by the kidneys and that secreted by the sweat glands is probably of importance in the process of acclimatisation. Inhabitants of the tropics know only too well the discomfort caused by the frequent necessity of micturition during a sudden cold spell, even at times when the temperature is well above that regarded as comfortable and even warm in a temperate climate. Moreover, the feeling of subjective discomfort on such occasions is very marked, and this sensitiveness to cold has given rise to the popular belief that the blood of dwellers in the tropics is thinner than that of a person living in a temperate climate.

(2) *Changes in the Physiology of the White Man Living under Tropical Conditions*

The tradition that the white man cannot flourish in tropical climes has given rise to a great deal of controversy, and the possibility of an acclimatisation has constantly occupied the attention of authorities interested in the development of the tropics. It has been propounded by several observers with experience of life in tropical countries that, apart from diseases peculiar to the tropics, climate *per se* comprising heat, humidity and light make it impossible for a white race to settle and thrive. Attempts have therefore been made to study the physiology of the white man living in the tropics, in order to ascertain whether changes have actually taken place, and for comparison the investigations have been extended to native races. The results so far are by no means complete, and a decided answer is still to seek.

Little reflection is needed to appreciate the difficulties which stand in the way of a practical solution of the question. Tropical diseases have up to recent times been very prevalent in most parts and have been a large factor in preventing settlement by a white race. The pioneer work of Manson and Ross on the rôle of insect carriers in such diseases as filariasis and malaria has laid down new lines for the investigation of other tropical complaints. The practical application of the results has led to increased public health activities, and has thus created a new era in the permanent settlement of the tropics by inhabitants of European descent. Moreover, the tropics, with only few exceptions, such as the South American Republics, have only recently been settled; a second and third generation is rare, and it is impossible to obtain and select subjects in sufficient numbers for carrying out observations which would yield results which could not be attacked on the plea of insufficient time of observation. The conditions of life in general there, for example, housing, ventilation and food, are so different that the difficulty of obtaining figures suitable for a comparison of physiological functions is greatly increased and comparative standards in many respects are lacking.

A critical review of the literature on acclimatisation brings to light the fact that nearly all observations have been made on Europeans who have resided in the tropics for a comparatively short time. The majority were men chosen for service in the tropics on account of their physical fitness, and few of them could boast of an uninterrupted residence in the tropics for more than a few years at most.

As an example, in the work of the American observers in the Philippines, the figures relating to Europeans were obtained with male subjects only, and, furthermore, cannot be accepted, excepting on the supposition that changes due to climatic influences become established within a comparatively short time. The same criticism may be applied to experiments on metabolism carried out by Eijkman in Java, whose subjects had resided in the tropics for from four and a half to fifteen years, and out of nineteen Europeans only one could show a continuous residence for fifteen years. Results such as those of Rattray, Wick, Schilling and many others are the outcome of observations on one or a few subjects only, collected during a flying visit to the tropics.

Investigations on aboriginal races, although interesting from a comparative point of view, do not help to solve the problem, on account of the different mode of living, customs and personal habits.

(*a*) *Body Temperature in the Tropics.* The fact that the external temperature in the tropics is considerably higher than in a temperate climate suggested a raised body temperature. On account of the ease with which the body temperature can be taken, it is not surprising that the literature contains records of numerous observations, many collected during a sea voyage between Europe and the tropics, others on Europeans and native races living in the tropics.

John Davy, in 1839, published a number of observations made upon the mouth temperature of seven healthy men during a voyage from England to Ceylon, and he concluded that the temperature of a European increased whilst passing from a temperate to a warm climate, and that, in addition, the body temperature of residents in the tropics is slightly raised above normal. He amplified his work during a residence of three years in Barbados, and stated that his own temperature was higher by 0·5° C. (0·9° F.) when compared with his body temperature in England. Many others, including Reynaud and Blossville, Rattray, Brown-Séquard, Jousset, Maurel and others (referred to by Pembrey, 1898) confirmed this slight rise (less than 0·55° C., or 1° F.), which was more pronounced during the first few weeks of residence in the torrid zone, that is, during the time of acclimatisation. Crombie (see Pembrey, 1898) recorded the results of 1,288 observations upon his own mouth temperature in Bengal, and found it slightly higher (namely, 0·23° C., or 0·7° F.) than in England. Neuhauss (1893), who, during a voyage round the world, observed his own rectal temperatures, found the following differences (see Table II).

TABLE II.

Temperature of Air	6 a.m.	12 noon	10 p.m.	6 p.m.	Remarks
Temperate Zone Minimum Maximum 11·5° C. 19·1° C. (51·7° F.) (56·5° F.)	36·6° C. (97·9° F.) 55	36·9° C. (98·5° F.) 55	36·7° C. (98·2° F.) 56	37·1° C. (98·8° F.) 62	Temperate in rectum Pulse } Mean of 20 days
Tropical Zone Minimum Maximum 28·8° C. 26·6° C. (75·0° F.) (79·9° F.)	36·9° C. (98·5° F.) 60	37·3° C. (99·2° F.) 68	37·1° C. (98·8° F.) 64	37·3° C. (99·2° F.) 72	Temperate in rectum Pulse } Mean of 20 days

On the other hand, many observers have denied that there is any marked difference in the body temperature; amongst others were Thornly (1878) and Furnell (1878), and more recently Wick (1910), who could not observe any alteration in his body temperature during the passage from a temperate zone to the tropics.

Chamberlain (1911), as the result of 3,000 mouth temperatures, taken at quarterly intervals upon 600 healthy American soldiers living in the Philippines, arrived at a similar conclusion. According to him, the temperature of American soldiers doing duty in the Philippines showed no appreciable variation for season or for complexion type; it averaged 37° C. (98·7° F.), and this average differed little, if at all, from the mean normal temperature for white men living in the United States. In Plehn's (1898) opinion there is a slight rise in temperature during acclimatisation, but after continued residence this rise disappears.

The body temperature of dark-skinned native races in the tropics does not differ appreciably from that of Europeans. A. Jousset (1884) found that the average temperature of Hindoos, Chinese and negroes was 37·8° C. (100° F.), whereas that of Europeans under similar conditions was 37·87° C. (100·1° F.). Eijkman (1895) in Batavia compared the axillary temperature of Malays and Europeans, and found that the Malays had a slightly lower temperature (36·85° C., or 98·3° F.) when compared with Europeans (37° C., or 99° F.).

Young (1915) in a recent paper on this subject recorded a number of observations taken during the hot season in North Queensland. He pointed out that it is fallacious to speak of a normal mean body temperature, as that of a healthy person, even at rest in a cool climate, varies considerably during the twenty-four hours,* and considered it more rational to employ as standard the range of body temperature which has been observed in healthy individuals. He concluded that:—

* Benedict and Carpenter (Carnegie Institution monographs, No. 126, 1910), writing on this subject, state that 'the idea of constancy in body temperature has become so generally accepted that it is commonly believed that the body remains at the temperature of 98·6° F. or 37° C. without material change, other than that produced by disease. This impression is so firmly fixed that one finds to-day on all clinical thermometers a special mark opposite this temperature, to indicate what is supposed to be the normal temperature. It is somewhat difficult to place the exact history of this mark being selected, and it may be looked upon as more or less of a thermometric fetish, which has been worshipped by long-continued usage.'

1. The temperature of the mouth of Europeans living in the tropics is often considerably lower than that of the rectum and that this difference is generally much more marked after exercise, just as in temperate climates. The temperature of the mouth is thus not reliable as a measure of the body temperature.

2. During complete rest the rectal temperature did not show any marked variations from the limits of temperature observed in Europe.

3. A considerable rise in the rectal temperature was produced by slight muscular work and this was usually maintained for some time after the work had ceased.

These observations make it clear that the discrepancies in the records quoted above are in all probability partly due to the mode in which the temperatures were taken and partly to individual and daily variations.

At rest the body temperature in the tropics does not vary from that in a temperate climate, but with exercise the temperature rises more quickly and subsequently decreases more slowly than in a temperate zone. A good example of this is quoted by Young in respect to one of his subjects, who was in the habit of taking exercise in the early morning; during a period of eighteen days his average rectal temperature at 8 a.m. was 37·9° C. (100·3° F.). On three occasions on which the exercise was omitted the rectal temperature at the same time was only 37·5° (99·6° F.).

(*b*) *Rate of Respiration.* A comparison of the respiration rate of tropical inhabitants with that found in Europe has yielded conflicting evidence. According to Rattray (1870) the total volume of air expired was about 7 per cent. less than in a temperate climate, and he attributed this to a decreased rate of respiration, since spirometer measurements showed that the lung capacity was increased. These observations, made on a small number of subjects, were not confirmed by Jousset and by Plehn, who, on the contrary, found an increase in the respiration rate. Chamberlain (1911) made observations on 608 American soldiers in the Philippines. He found the average to be 19·3, which is much higher than the figure usually accepted as normal for Europeans in Europe, namely, 17 to 18. It may be mentioned here that Osborne (1910) carried out observations during the summer months in Victoria (Australia), and found that with a high outer temperature (37·7° C., or 100° F.) the pulmonary ventilation was increased.

In consideration of the fact that the human body loses a certain amount of water in the expired air and consequently heat due to the evaporation of the water, it might be expected that the body would make use of this means for cooling purposes and an increased respiration would be the outcome.

(*c*) *Blood Pressure.* Estimations of the blood pressure in the tropics have been made by a number of observers, with contradictory results. Musgrave and Sisson (1910) in Manila examined 97 Americans, 10 sisters of charity and 40 Filipinos. They graded the former two groups according to their length of tropical residence and found a decided decrease in the blood pressure, corresponding to the length of residence in a hot climate, and suggested that the lower blood pressure might be due to a lowered peripheral resistance, brought about either by a decrease in the vasometer tone or by splanchnic influences. Chamberlain (1911) took the blood pressure of 992 American soldiers in the Philippines and made 5,368 observations, and concluded that 'the average blood pressure of 115 to 118 millimetres found in these large bodies of men differed little, if any, from the accepted standard among males of the same age in a temperate zone when the 5-inch armlet is used.'

Breinl and Priestley (1914) estimated the blood pressure of North Queensland school children, and concluded from 573 observations that the climate as such has no marked influence on the blood pressure in children.

Theoretically a permanent change in the blood pressure in the tropics should not be anticipated, as a sound heart and sound arteries are adaptable to any change. Only temporary variations are observed, such as a rise in blood pressure on exertion or a lowering due to venous hyperaemia of the skin, similar to that produced by a hot bath, but both would only be evanescent and not peculiar to tropical life.

(*d*) *Blood Conditions of Europeans in the Tropics.* The existence of tropical anaemia has for a long time been regarded as an established fact, and most of the early text-books on tropical medicine speak of an anaemia due to climatic influences only. There is no doubt that the greater proportion of the cases in the time before microscopical examination became general were in all probability caused by malaria and hookworm (anchylostomiasis). The skin of the healthy European inhabiting a tropical climate appears to the newcomer pale and sallow, the degree of the sallowness depending on many factors, especially complexion and skin texture. The unprotected skin of individuals with red or fair hair is invariably more sallow and appears more anaemic for the same reason that it has, on account of the lack of pigment, a lesser defensive power against the action of the sun's rays. The paleness of the skin has naturally given rise to the conception that there exists a tropical anaemia and that 'thinness and poorness' of blood is a natural sequence of prolonged residence in the tropics. It is not unexpected that the advent of accurate microscopical methods has led to an investigation of blood conditions of Europeans in the tropics, and the results of these investigations have been rather surprising.

Marestang (1889), van der Scheer (1890) and Eijkman (1891) examined the blood of Europeans living in the Dutch East Indies without finding any marked differences when compared with figures obtained for Europe. Glogner (1892) in Sumatra, and Plehn (1892) in the Cameroons, carried out similar investigations with identical results. These observations, however, are not entirely conclusive, since they were based on a comparatively small number of estimations.

Work of a similar nature was carried out by the Americans in the Philippines. Wickline (1908) examined the blood of about 70 American soldiers, after a varying period of residence in the Philippines. The first examination was made three months after their arrival; it was repeated after the lapse of three months and twice again after an interval of approximately eight months. He discovered a gradual decrease in the amount of haemoglobin from 9·7 to 8·3 and a regular rise in the number of erythrocytes from 4·9 to 5·3 millions. Chamberlain (1911), following up Wickline's work, performed 1,718 red cell counts and 1,433 haemoglobin estimations of 702 soldiers, and concluded that 'the red cell counts, averaging 5·2 millions, rarely falling below 4·5, do not differ from the normal at present recognised for healthy young men in a temperate zone.' The haemoglobin figures are perhaps a little low, but not sufficient to indicate a definite anaemia.

Cuthbert (1911) collected similar observations from 21 healthy soldiers of European descent on the West Coast of Africa with a similar result. Wickline's and Chamberlain's figures were obtained from healthy subjects especially chosen for tropical service, and after a comparatively short residence in the tropics (two years), and are open to the objections already mentioned.

Breinl and Priestley (1914) made observations on apparently healthy school children in North Queensland of ages ranging between seven and fifteen years, most of whom were born and had lived all their life in North Queensland. Five hundred and eight blood counts were performed on 305 boys and 269 girls, and the haemoglobin content of the blood was estimated by the Fleischl-Miescher method.

They concluded from their investigations that there is no striking difference in the number of erythrocytes and the colour index in North Queensland children when compared with averages obtained in children of a temperature climate. The number of white blood corpuscles (leucocytes), according to Chamberlain (1911), also to Breinl and Priestley (1914), is slightly higher when compared with that generally accepted as the standard for a temperate climate.

Differential counts, which estimate the percentage of the various types of leucocytes, showed, according to these observers, a distinct alteration. The proportion of the polymorphonuclear neutrophile leucocytes appeared to be decreased to 56·1 per cent. (Chamberlain and Vedder (1911)) and 56·8 per cent. (Breinl and Priestley); 65 per cent. to 70 per cent. is generally accepted as average. The percentage of the eosinophile leucocytes was distinctly increased.

Chamberlain and Vedder (1911) studied the blood conditions of American soldiers and natives in the Philippines, and came to the conclusion that the Arneth blood picture of the soldiers showed a slight 'shift to the left,' that of the Filipinos a much more marked shift, which means that the nuclear condition of the polymorphonuclear neutrophile leucocytes in respect to the numbers of nuclear fragments was altered when compared with those of healthy Europeans living in a temperate climate; the number of cells with a smaller number of fragments (one to two) was considerably increased. Breinl and Priestley (1914) carried out analogous observations on North Queensland school children, and found that similar conditions prevailed and that the blood picture resembled closely that of the Filipinos. The authors concluded that the 'shift to the left' was due to the effect of a tropical climate upon the white race living in the tropics.

For comparison a number of Arneth counts on apparently healthy native children in the Northern Territory were performed, and their 'Arneth index' corresponded closely to that of North Queensland school children.

The significance of the altered Arneth index is uncertain and, according to Breinl and Priestley (1914), the presence of a large number of neutrophile leucocytes belonging to class one (possessing a single nucleus) 'indicates a greater activity of the bone marrow, but does not necessarily imply that the resistance of the organism is lowered' (to disease) 'in any way.'

A few observations on the specific gravity of blood were made by Eijkman (1896) in Java, and by Breinl and Priestley (unpublished) in Townsville. The results did not show any marked deviation from the average specific gravity of the blood considered normal for a temperate climate, and, when considered in conjunction with the previously mentioned blood work, proves that there is in general no evidence of an anaemia in the tropics due to climatic influences only. The popular belief of the 'thinness of the blood' after prolonged residence in the tropics is, apart from anaemia due to disease, from a scientific point of view only a myth.

(*e*) *Metabolism in the Tropics.* In a cold climate, as has been stated previously, the temperature equilibrium of man is maintained mainly by regulation of the heat loss through radiation, convection and evaporation (termed by Rubner physical heat regulation), whilst changes in the heat production (Rubner's chemical heat regulation) take place only to a small extent. In fact, Rubner has said that a man feels comfortably warm only when the chemical regulation of his heat balance is completely eliminated. In a cool climate chemical heat regulation can only be spoken of in the sense that the stimulating effect of cold on the skin causes reflex innervation of the muscles, resulting in their movement or increased tone, which brings about an increase in the production of heat, i.e., the increased metabolism is really due to the muscular action and not to cold *per se*.

The question naturally arose whether the chemical heat regulation plays any part in maintaining this equilibrium in the tropics where the external temperature is high; in other words, whether the body responds to these different conditions by a reduced combustion of food material. This problem has been attacked in two ways; first, by a comparison of the calorific value of the food actually katabolised by Europeans living in the tropics, with the average value found in temperate climates, and, secondly, by a comparison of the total metabolism in both parts of the world, as determined by the respiratory gas exchange, that is, the oxygen absorbed and the carbon dioxide eliminated, the experiments being carried out otherwise under exactly the same conditions.

The most important work in the first direction is that of Eijkman (1893), who investigated the food of seven Europeans living in the Dutch Indies. His experiments, lasting for several days, were carried out as follows:—At each meal each person under observation took a second portion of each dish, similar in quantity, as judged by the eye, to that which he consumed, and these duplicate meals were collected for each day and the protein, fat, carbohydrate, etc., in them estimated. At the same time the faeces were collected and analysed to determine the quantity of food actually absorbed.

Allowing for the loss in the faeces, he obtained the following figures for the average constituents of the food actually utilised:—

Protein	88·2 grms.
Fat	79·1 grms.
Carbohydrate	256·4 grms.
Ash	17·5 grms.
Alcohol	28·5 grms.

From the results he calculated that for a man weighing from 65 to 70 kilograms the heat production from the food amounted to 2,400 to 2,500 calories, a figure which does not differ from that given by Rubner for men doing a similar class of work in Euorpe, namely, 2,445 calories. The excretion of nitrogen in the urine and faeces showed that, on the whole, his subjects maintained nitrogen equilibrium during the experiment.

Eijkman concluded from these experiments that there was no evidence that chemical heat regulation played any significant part in the thermo-equilibrium of inhabitants of the tropics.

A different conclusion was arrived at by Ranke (1900), who published the results of observations upon himself extending over a few days, comparing the calorific value of his food in Europe and during a short residence in Brazil. In Europe the heat value of his daily food in two experiments was 3,527 and 3,514 calories, respectively. In Brazil, however, the calorific value of his food was much less, being only 2,812, 1,920 and 1,948 calories, respectively, in three series of observations.

Ranke's experiments have been criticized adversely by Glogner (1909), who conducted similar determinations upon himself at different times during his residence in Sumatra and Padang, and again after his return to Europe. He stated that loss of appetite is a common experience during the first period of residence in the tropics, and attributes the lower values obtained by Ranke to the fact that the latter had only been a few months in the tropics when the experiments were carried out.

Glogner's experiments, conducted after a six years' residence, and lasting for fourteen days, showed the average energy value of his daily food to be 2,118 calories, or 28·8 calories per kilogram of body weight per day. A similar experiment was performed in Europe fifteen years later—five years after his departure from the tropics—and then the daily average was found to be 2,038 calories, or 25·5 calories per kilogram of body weight. It is doubtful, however, whether any value can be placed on these observations, since the first series was made when the subject was 32 years old and weighed 71·1 kilograms, and the series for comparison when the subject was 47 years old and weighed 80·3 kilograms.

Experiments were carried out also upon themselves by Schilling and Jaffé (1909) during a visit to West Africa, and the results were compared with observations made in Berlin, both before and after their journey. There were no appreciable differences between the heat value of their food in both parts of the world, the average being 2,863 and 2,953 calories per day for Jaffé, and 2,953 and 3,021 calories per day for Schilling, in Europe and Africa respectively. In both places nitrogen equilibrium was approximately maintained on about 17 grammes of nitrogen per day.

In a similar way the nutritive value of the food of native races in the tropics has been estimated for comparison with that of white people. Eijkman (1893) examined the food of five Malays in a similar manner to that employed in his experiments quoted above, and found the following average for the food actually katabolised, after allowing for the material lost in the faeces:

Protein	59·9 grms.
Fat	25·0 grms.
Carbohydrate	462·0 grms.
Ash	13·2 grms.

The heat value of the food was about 2,349 calories for an average body weight of 50 kilograms, i.e., a figure slightly higher per kilogram of body weight than that given by Rubner for men in Europe. The amount of unabsorbed matter in the faeces was much greater in the Malays than in the Europeans, probably on account of the large preponderance of vegetable food in their diet.

An extensive inquiry has been made by McCay (1912) in India into the food of various native races. For Bengalis he found that the calorific value of the food was higher (3,196 calories) and the nitrogen lower than that of Europeans. A study of the diet and nutrition of the Filipinos was made by Aron (1909), and the average composition of the daily allowance in the Manila native prisons was given as:

Protein	55·9 grms.
Fat	27·0 grms.
Carbohydrate	510·0 grms.
Calories	2,646

An estimate of the calorific value of the food eaten by a Filipino living under normal conditions corresponded to from 2,500 to 2,600 calories, and when allowances were made for the smaller body weight of Filipinos (50 to 55 kilos), this value corresponded to the requirements of a working-man in Europe performing moderately hard labour.

Recently, Campbell (1917) examined the food of several native students (Chinese, Tamil, Malay and Brahmin), and obtained values ranging from 1,502 to 2,492 calories per day.

The results of these investigations into the food value in the tropics make it clear that in general the requirements in calories, either of white men or coloured natives, does not differ appreciably from European standards. The experiments quoted above indicate that the amount of heat produced by the combustion of food is not any less in the tropics than in a temperate climate, and that nature does not have recourse to a reduced metabolism to regulate body temperature.

The energy involved in the total process of oxidation (heat production) taking place in the animal body may be measured either by the actual heat value of the food digested (calorific value of the food), or again by the quantity of oxygen used up and carbon dioxide and water produced (the respiratory exchange), which measures the end products of this oxidation.

If metabolism would play any appreciable part in controlling the body temperature in the tropics, it would find its expression in a reduced oxygen absorption and carbon dioxide production.

Experiments upon the influence of high external temperature and humidity upon the respiratory exchange have been carried out from this point of view under artificial conditions upon man and animals, and on man in the tropics.

The general results of these experiments show that with cold-blooded animals the oxygen absorbed and carbon dioxide eliminated are directly proportional to the external temperature; in other words, there is no heat regulation, and the body temperature is that of the surroundings. With warm-blooded animals, on the other hand, the organism regulates its heat to a more or less extent. With the monotreme, *Ornithorhynchus*, the mechanism is almost entirely chemical; thus, with increasing external temperature the metabolism decreases until a temperature of 32° C. (89·6° F.) is reached, the body temperature remaining throughout almost constant. Above this the animal's temperature rises, thus indicating that the regulatory mechanism is failing, until finally, with increasing outer temperatures, the animal behaves like a cold-blooded animal.

In the rabbit the same is observed, but to a less degree, because the mechanism is complicated by loss of heat by physical means.

In man the elimination of carbon dioxide is not affected by rising external temperature in a constant manner. In some experiments, carried out under artificial conditions, it has been noticed that with rising temperature the carbon dioxide sinks in quantity at a temperature of about 15° C. (59° F.), reaches its minimum between 20° C. (68° F.) and 25° C. (77° F.), and then rises slightly. In other experiments, however, no such change has been observed. Generally speaking, all that can be said is that there appears to be an ill-defined zone of temperature in which the metabolism is at a minimum. When the external temperature and humidity are so high that the physical regulation fails, and a rise in body temperature results, this rise is accompanied by an increased metabolism, still further increasing with continued rise of body temperature, until dangerous conditions are reached, as already stated.

A study of the gaseous metabolism under actual tropical conditions was made by Eijkman in Java. By means of the Zuntz-Geppert method he determined the quantity of oxygen consumed and of carbon dioxide eliminated during rest by eleven Europeans and twelve Malays. He obtained the following averages as the result of forty-eight experiments on Malays and thirty-seven experiments on Europeans (see Table III). The numbers are calculated to a body weight of 64 kilos.

TABLE III.

	Oxygen absorbed per minute	Respiratory quotient
Europeans in Java	245·7	0·791
Malays in Java	251·5	0·880
Europeans in Europe	250·3	0·775

Eijkman interpreted these results as confirmatory of his other experiments already quoted, namely, that the heat production by the body in the tropics is not less than in a temperate climate, and that therefore change in heat production plays an insignificant part in the regulation of temperature. He attributes the higher respiratory quotient of the Malays to the larger proportion of carbohydrates in their diet.

In reviewing these researches on the metabolism in the tropics it is necessary, before drawing any conclusions, to consider the limitations of metabolic experiments in general. Contradictory results may be accounted for in some measure by different food, habits and customs in various countries in which the experiments have been carried out, and by individual variations. Moreover, up to the present no definite standard has been acknowledged.

A large number of observations upon metabolism during complete rest, which is termed basal metabolism, have been carried out during several years in the Nutrition Laboratory of the Carnegie

Institute in the United States by Benedict and his co-workers. Subjects included men and women of various ages, athletes, vegetarians, etc. The general conclusions drawn from these experiments have been embodied in a paper by Benedict (1915) upon the factors affecting basal metabolism, in which he pointed out that a much larger number of factors enter into this question than has been hitherto recognised. Although body weight plays an important part, there is no direct relationship between the body weight and the total heat production. Similarly with body surface, the general belief that heat production is determined by body surface is not strictly accurate; a careful analysis of metabolism measurements shows that the heat output is not proportional to the body surface. Other factors are the proportion of inert fat and active protoplasmic tissue in the body composition, and also the height and age. Finally, there are large variations from day to day in the same individual. Summing up his conclusion, Benedict says:

> It is clear that the basal metabolism of an individual is a function first of the total mass of active protoplasmic tissue and second of the stimulus to cellular activity at the time the measurement of the metabolism is made. Apparently at present no law can be laid down that will cover both of these important variables in the basal metabolism of an individual.

In the light of this work it is at once seen that units for comparing the metabolism are still only in the making, and a comparison is only possible of the averages of experiments on large numbers of individuals, extended over a long period of time, so as to eliminate changes which might be due to the temporary ascendency of any one factor. It is obvious, therefore, that researches which have been made hitherto on total metabolism have not been on a sufficiently large scale to justify any definite conclusion, and probably the discrepancies in the results of different experimenters may be accounted for in this way.

The researches into the total metabolism and heat production in man have so far not revealed any changes which might be attributed to the influence of climatic conditions. It has, however, repeatedly been pointed out that the dietary of the majority of the aboriginal races in the tropics contains a relatively small proportion of protein. This fact has naturally led to the presumption that dwellers in the tropics require a less amount of protein in their food.

Protein differs from the other food constituents in the respect that it is utilised by the organism in both directions, namely, for producing energy and building up tissue. It is, therefore, not unexpected that the protein requirements of tropical inhabitants has been studied in detail.

Eijkman (1893) in Java found that Malays consumed 73·3 grammes of protein per day, of which about 56 grammes were actually metabolised, an amount far below the standard usually accepted in Europe (100 grammes). An extensive series of observations on the protein content of the dietary of native races in India has been carried out by McCay (1912), who investigated its bearing upon the physique and general efficiency of his subjects. In his opinion the small quantity of protein generally consumed by the natives was not the result of want of desire, but of inability to procure a larger quantity, and he convinced himself that most natives would eat more if they could obtain it. He concluded that those races with a higher level of protein interchange were generally more robust, energetic, and more manly. In the same way the daily food of Filipino prisoners, according to Aron (1909), contained only 75 grammes of protein.

A much higher quantity of protein was utilised, according to Eijkman, by Europeans living in Java. He found that the food of his experimental subjects contained 99·6 grammes of protein, of which 88·2 grammes were actually absorbed, which amount corresponds to the normal protein requirements in Europe. Other observers who examined their food during a visit to the tropical zone, confirmed this. Thus Ranke, during his voyage to Brazil, found very little change in the protein of his food, and Jaffé and Schilling found that nitrogen equilibrium was maintained on the same amount of protein in Europe and in West Africa.

Unfortunately the whole question of the protein requirement of man is still unsolved. Atwater's standard of 125 grammes of protein a day, and Chittenden's standard of about 60 grammes, are both accepted by various workers, and recent experiments of Hindhede have shown that nitrogen equilibrium can easily be maintained upon a much lower amount of protein, provided that the diet contains sufficient calorific value. He himself maintained nitrogen equilibrium on 32 grammes of protein for 150 days. The question is further complicated by the necessity of accessory food products and their relation to the different constituents of the food, about which we are as yet in almost complete ignorance.

The protein metabolism has been further studied by the examination of the various constituents of the urine, since the final products of its degradation are excreted in the urine. Eijkman found that the urine of Europeans living in the East Indies did not differ appreciably from standards in Europe. He found the average volume for twenty-four hours as 1,442 c.cm., the specific gravity 1·017, whilst the average nitrogen content was 13·04 grammes. In the physiological text-books the average for Europe is generally given as 1,200 to 1,500 c.cm., with a specific gravity of 1·015 to 1·025, and a total nitrogen content of 14 to 18 grammes. He allowed for 1·6 grammes of nitrogen lost in the sweat, and concluded therefore that an acclimatized European passed as much nitrogen in his urine as he did in Europe.

Other observers, such as Wick, Ranke and Neuhauss, however, found that the urine is much more concentrated. Observations in this respect, extending over some time, have been made in Townsville by Young (1919). A number of urine samples collected during the hot months have been examined in this Institute, and the average figures were obtained for the daily urine of twenty-five persons, some of whom were engaged in manual labour, and others followed a sedentary occupation. The averages calculated are given in the Table IV, together with European standards:

TABLE IV.

	In Townsville	European standard
Quantity	782 c.cm.	1,500 c.cm.
Specific Gravity	1.025	1.015 to 1.020
Total Nitrogen	10.4 grm.	16 grm.
Sodium Chloride	7.00 grm.	15 grm.
Phosphates	1.73 grm.	2 to 3.5 grm.
Freezing Point	—0.935° to —2.259° C.	—0.87° to —2.71° C.

These observations reveal decided differences. The volume was much lower and the specific gravity higher. The total nitrogen, too, was smaller. The most striking difference lies in the quantity of sodium chloride, which may be accounted for by the increased quantity of sweat lost, which carries with it sodium chloride. Estimations of the amount of water lost from the skin during exercise in Townsville have yielded interesting figures. After a brisk walk of about seventy minutes with an external temperature of 24·4° C. (76° F.) wet bulb, as much as 1,100 grammes in weight have been lost. As the subjects of these experiments were weighed in their clothing, this figure only represents the water which had actually evaporated. Samples of sweat carefully collected contained from 0·1 to 0·3 gramme of sodium chloride per 100 c.cm., and the cutaneous excretion would account for several grammes of sodium chloride in the above experiment. The nitrogen lost in the sweat can only be small, and is almost negligible, since samples of sweat only contained 0·03 to 0·04 grammes per 100 c.cm.

The urine measured during the cold weather averaged a higher volume, 1,357 c.cm., a lower specific gravity, 1·019, and an increased salt content, 9·44 grammes, per day.

The total nitrogen in the urine in Northern Australia was decidedly below the European standard, even considering that a small amount (1 to 2 grammes per twenty-four hours) may leave the organism by way of the skin. There is thus an indication of a decrease in the amount of protein metabolised. Whether this is due to a smaller intake of food brought about by a want of appetite during the hot weather, so commonly observed, or whether other factors come into play, must be left an open question.

(*f*) *Effect of a Tropical Climate on the Nervous System.* In many of the writings on health and disease in the tropics attention has been drawn to the frequent occurrence of a mental condition resembling neurasthenia, of the same type as that found in temperate climates. It occurs mostly amongst Europeans, women and men, after a prolonged residence in the tropics, and is referred to in the literature as tropical neurasthenia. In its mildest form it manifests itself in a lability of the mental equilibrium, fits of depression alternate with states of exuberance; unwarranted irritability over trifling matters is hardly ever absent, leading to uncontrollable outbreaks of temper. It is associated with a loss of mental activity and power of concentration, lack of confidence and failing memory, all of which cause a decreased working capacity. The realisation of this state, and the futile attempts to overcome it by increased spurts of energy, tend to exaggerate the trouble. This condition may be stationary for a long time, influencing the career of the sufferer, or may get steadily worse and lead to a condition termed by Plehn 'tropical fury' (*Tropen Koller*), which often explains the committal of deeds of violence in an outburst of uncontrollable passion.

Amongst others, Fales (1907) has drawn attention to the occurrence of neurasthenia amongst men and women after a residence of a year or more in the Philippines, which in women is often associated with disturbance of menstruation and of the vasomotor system. In his opinion it is in most instances not directly attributable to parasitic invasions, but to climate only, and forms one of the chief drawbacks to acclimatization. Woodruff made the same observation in the same locality, and attributed the condition entirely to the effects of tropical light.

In 1913 the Society of Tropical Medicine devoted a meeting to the discussion of this subject, at which the President, Sir Havelock Charles, delivered an introductory address on 'Neurasthenia and its Bearing on the Decay of Northern Peoples in India.' He discussed the occurrence of 'Punjab head' in Bengal, which is characterised by shortness of temper, forgetfulness, sleeplessness and disinclination to work, etc., which corresponds to tropical neurasthenia. Similar 'heads' occur throughout India and other parts of the tropics, and affect Europeans after having lived in the tropics for a considerable period without furlough. In a number of cases, according to Havelock Charles, tropical disease may be held primarily responsible for the condition, but in many instances he considers climate alone as the root of the evil. Since 'the constitution of the northern races is developed in temperate latitudes,' he believes 'that its powers are injuriously affected by the climatic conditions of the hot zone, and this must be attributed to damage done mainly to the nervous system by a hot and humid climate.' Unfortunately his address, and a great deal of the discussion which followed, were marred by statements which were merely expressions of personal opinions, collected during a shorter or longer residence in the tropics, and often coloured by prejudice; actual data in support of these opinions were sadly lacking.

Basil Price (1913), who for many years examined the causes of invaliding home of members of the Church Missionary Society, found that neurasthenic conditions were the cause of 25 per cent. of invalidity in most countries.

A perusal of the general literature on the tropics confirms the fact that a complaint similar to neurasthenia is very prevalent amongst Europeans who have emigrated to the tropics, but it is difficult to gauge how far climatic influences alone may be held responsible, or what part is played by the altered conditions and habits of tropical life. The novelty of the strange environment often leads to an increased output of energy, and it is only after a time that the newcomer realises his energy capacity under the new conditions and begins to husband his strength, but often too late to avoid paying the penalty.

It is, however, beyond doubt that the monotony and discomfort of life and climate, the lack of pleasure and excitement, the big distances from the centres of civilisation and, in women especially, the confinement to the house during the hot hours of the day, all help towards producing a mental condition resembling neurasthenia.

(*g*) *Clothing in the Tropics.* The degree of bodily comfort in the tropics, in the same way as in a cold climate, is governed by a number of factors, many of which may be modified artificially. In a cold climate bodily comfort is attained by conservation of heat, in a hot climate by the allowance of a maximum amount of cooling, and the clothing is a very important factor in this respect.

The literature contains numerous accounts of experiments and suggestions on the most suitable kind of clothing for a tropical climate. The aboriginal inhabitants possess a dark pigmented skin, and, theoretically, therefore, a dark-coloured garment, allowing of ample ventilation, would appear to be the ideal, since it would imitate nature. Practice, however, does not bear out this assumption. It has been shown that a dark skin absorbs heat rays to a greater extent than does the white skin; its temperature, therefore, rises more quickly and the sweating mechanism is brought into action earlier and the cooling due to evaporation is more effective. A dark dress material absorbs heat in the same way as a dark skin, promotes perspiration, but at the same time acts as an obstacle to the free evaporation of sweat from the skin. There is between the skin and the clothing a space filled with stagnant air, which in fact contains more moisture than the surrounding air, on account of insufficient ventilation. In consequence, the dress material becomes more and more impregnated with moisture, and the meshes of the fabric clogged with water, and the degree of saturation of the air space between skin and clothing exaggerated. *Young has recorded observations in North Queensland upon the temperature between skin and shirt—'skin-shirt temperature'—which confirm and amplify Hill's (1914) previous experiments in the same direction, which were carried out in England. Wet and dry bulb thermometers were used, the bulbs of which were encased in wire cages to prevent contact. The temperatures thus observed were invariably several degrees higher than the outer temperatures. Hill's observations, made during an English winter, showed figures as high as those recorded in North Queensland, but his subjects wore heavy clothing suitable to winter conditions in Europe, whereas Young's subjects only wore a thin shirt. This is a clear proof that the quantity of clothing and the nature of the material is of paramount importance in determining the condition of the air surrounding the skin. The former does not require any further explanation; the latter, however, the nature of the material, may be further discussed here. It should possess two properties, firstly, that of absorbing moisture and allowing of a maximum surface for speedy evaporation—the difference between woollen, cotton and silk material is well known—and, secondly, it should prevent, as far as possible, penetration of heat waves.

Attempts to obtain this objective have been made by several workers, who experimented on the suitability of material of various colours for tropical use. This question has been discussed by Sambon (1907), who based suggestions upon experiments carried out by Baly upon the penetration of rays from an electric arc through a piece of dark pigmented skin derived from an Indian. Baly photographed the spectrum after passage of the rays through

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* See these *Annals*, p. 313.

the skin, and noticed that rays of shorter wave length than 3,600μ (ultra-violet) were entirely absorbed, proving thus that the skin pigment excludes the ultra-violet rays. From these observations Sambon suggested that, for tropical use, material coloured black, red or orange should be used, colours which eliminate the rays of short wave length. As all these colours absorb heat rays, he suggested at first a combination of white outer garments and coloured underwear, the former to reflect heat rays, the latter to exclude actinic rays. Later he suggested the manufacture of a special material, combining both advantages, and this has been put on the market as 'Solaro.' This fabric is composed of white and suitably coloured threads, woven in such a way as to present a warp (upper surface) of white, and a weft (under surface) of orange, red or black, and it has been proved by spectroscopical examination to be as impervious to actinic rays as the black skin.

Schmidt (1909) suggested the wearing of loose garments made of either thick light-coloured or thin dark-coloured material, and recommended the use of material similar to that suggested by Sambon.

The practical test of coloured underwear, however, has not proved it to be advantageous. Phalen (1910) conducted experiments on a large scale on soldiers in the Philippines. He supplied five hundred soldiers with orange-red underwear, and compared their well-being in the course of a year with another group wearing white underwear. He compared body weight, the number of red cells and haemoglobin, blood pressure, pulse and respiration rate, body temperature, and incidence of sickness in both groups. He found that the men wearing orange-red underwear showed changes due to heat, such as loss in body weight and haemoglobin and decreased blood pressure, more marked than the control group. Phalen concluded from his experiments that the coloured garments were more receptive to heat rays than were the white, since wearers complained persistently of greater heat, greater weight, and increased perspiration, and expressed the opinion that khaki clothing alone affords the same protection from chemical rays as the special underwear.

The question of clothing in the tropics has been dealt with from a more commonsense point of view by Gibbs (1917). 'Clothing which in the sun will cast a shade upon the body without hindering the air circulation and heat radiation will be the most desirable, and if a colour is used which will give a minimum of heat absorption the efficiency is increased. This ideal condition is fulfilled by the umbrella, and it is evident that a large white umbrella lined with a material of a colour agreeable to the eyes, for example, a shade of green, will be most efficient.' He states that, since custom prescribes that man shall cover his body, this mode of 'clothing' is in fact impracticable, and substitutes for the umbrella a large-brimmed helmet, casting a shadow over the back, shoulders and chest, and a loosely fitting white suit of material as thin as possible.

Judging by our own experience, the wearing of light-coloured suits of porous material assures the maximum amount of comfort possible, but it is obviously impossible for a labourer to wear white clothing at his work. In North Queensland he wears, as a rule, khaki-coloured trousers and a thick, dark-coloured flannel shirt, which prevents the sun's rays from penetrating to the skin, and absorbs perspiration, and thus allows a maximum degree of evaporation of the sweat.

On the whole, an extended inquiry amongst the residents in tropical Queensland has revealed such a diversity of opinions as to what type of clothing is the most comfortable that it appears presumptuous to lay down definite rules. Many prefer cotton for underwear, others pin their faith on wool, but all agree on the one point that outer as well as under garments should be as porous and as light in weight as possible.

(3) *Summary of Physiological Changes of the White Man in the Tropics*

A review of the known facts concerning the physiological changes of the white man transplanted to the tropics is, from a scientific point of view, disappointing. Throughout the literature published since the beginning of the last century may scrappy attempts have been made to obtain evidence in this respect; figures have been collected and experiments on metabolism and other physiological functions have been performed. In spite of the energy expended, the results are not conclusive; firstly, on account of the smallness of the number of observations recorded by the different individual observers, too small to eliminate individual variation and error, secondly, on account of the uncertainty of the generally accepted standards which may be considered normal for a temperate climate. The question of body temperature is a case in point. Several observers formed their conclusions by accepting 37° C. (98·6° F.) as a normal body temperature, and considering even a small rise above this as an indication of an increase in body temperature in the tropics. If a series of observations had been taken on a few subjects at intervals during the day and night, and the average calculated, discordant results would have been the outcome, even in Europe.

The same criticism applies to experiments regarding other physiological functions, as, for example, the total metabolism as measured by the gaseous exchange. As already pointed out, a great deal of work has been attempted in Europe, and recently on a much larger scale than hitherto in the large nutrition laboratories in the United States. In spite of these concentrated and intensive efforts, as Benedict has pointed out, no definite unit for the comparison of the metabolism of normal individuals has been discovered, and there does not exist at present a definite knowledge of the factors influencing individual variations.

Such changes as have been noted have been of a qualitative, but not of a quantitative, nature. Thus, with regard to maintenance of body temperature under tropical conditions, no evidence could be found in support of the view that heat, generated by internal combustion, is reduced in order to regulate the body temperature; even under the altered conditions the body still relies on physical means for this purpose. As convection and radiation of the body are greatly decreased with the high temperature, cooling must be brought about to a much larger extent by sweating and evaporation than in a temperate climate.

Again, the increased loss of fluid from the body by the skin is accompanied by a decrease in the quantity of the urine voided. As the sweat contains sodium chloride, an increased amount of this salt must be lost through the skin, which in its turn again leads to a decreased amount of salt excreted in the urine.

Blood examinations performed on a large scale have not proved the occurrence of a tropical anaemia as such, since the figures for haemoglobin and for the formed elements do not show a definite decrease. There is a certain alteration in the percentage of the various types of white blood corpuscles, and in the number of cell fragments in the polymorphonuclear neutrophile leucocytes, showing that the percentage of 'young leucocytes' in the circulation, that is, leuçocytes containing a smaller number of nuclei or nuclear fragments, is larger than in a temperate climate. This is expressed in haematological language as a 'shift of the Arneth index to the left.' The significance of this change is, however, not clear, but at any rate does not seem to indicate a decreased power for resisting disease, as supposed by the Philippine observers.

The concensus of opinion gleaned from experiences in various parts of the tropics seems to indicate that living in the tropics affects the nervous system, and that neurasthenia seems to be more prevalent than in a temperate climate. It is, however, impossible to obtain definite figures and data, and most statements are only based on personal experience. In the light of this it would be interesting to ascertain whether a detailed examination of the functions of the nervous system would reveal any definite alterations, which, on account of their frequent incidence, would have to be considered an outcome of life in a tropical climate.

It appears, therefore, possible that a closer study of the functions of central and peripheral nervous system might reveal definite alterations. It would be advisable to obtain figures for the promptitude and quality of the response given by the nervous system to various stimuli. Reaction times and responses to different stimuli are quantities readily capable of exact measurements by means of generally used apparatus.

An extensive investigation into the mental activities in general would perhaps yield figures of definite value. The testing of a great number of individuals in the tropics on similar lines to those applied in choosing aviators would be very interesting. The applying of the Binet-Simon test for mentality to a great number of school children, again, would furnish figures of definite value for deciding the presence or absence of racial degeneration. In the mentality tests the methods are rough, and only a very large number of tests, sufficiently large to eliminate personal error on the part of the observer and to minimise the influence of a few feeble-minded children, would yield figures of value. In short, all the efforts to detect physiological changes due to living in a tropical climate have not yielded definite results. It would, however, be premature to conclude that such changes do not exist, and it is possible that the advent of refined methods and more sensitive apparatus may in the future demonstrate definite alterations.

WHITE SETTLEMENT IN TROPICAL AUSTRALIA

(1) *General Conditions and Statistics*

When comparing tropical Australia with other countries situated in the torrid zone, it becomes apparent that Northern Australia occupies a somewhat unique position from more than one point of view. On the whole, the general conditions of a tropical country are modified to a much greater extent by climate *per se*, meaning seasonal incidence and degree of rainfall, than a country within the temperate zone. With the exception of a wet belt on the Queensland coast, mentioned previously, the rainfall is limited to a few months in the year, and during the remainder of the year at the most an occasional light shower disturbs the monotony of sunshine, and during about seven months of the year the weather conditions correspond to the 'dry tropics.' Vegetation, which is luxuriant during the wet season, dies down, and the green of the countryside gives place to a uniform brown. The shrivelled up undergrowth is often consumed by bush fires, which are said to be sometimes started by an accidental focussing of the sun's rays, and which sweep for miles over the country, leaving only the trees standing, bare of their bark.

Many phases of insect life, which are dependent on vegetation and moisture, die down during the dry months, only to become all-pervading again as soon as the rains start. These changes are more marked inland than on the coast, since many of the inland districts have a very small rainfall and the hot winds arising from the barren desert of Central Australia cause the inland plains of Northern Australia to approximate to a typical 'desert type' of climate, with cold nights and scorching hot days.

Another respect in which Northern Australia differs from other tropical regions is the sparcity of the aboriginal population. The natural conditions of Northern Australia seem to militate against a large aboriginal population, and the natives have never evolved beyond the nomad state and have for an unknown reason never made any attempt towards settlement. The uncertain food conditions, aggravated by droughts, have helped to keep down their numbers. After the arrival of Europeans, the inability of the aboriginals to change their nomadic habits has led to a decrease in their number and has prevented them from living alongside the white man. In consequence, in any part where a large white population exists, the black man has become extinct.

The white population is still very scanty, and is mostly concentrated in a few towns which are long distances apart.

The climatic conditions, especially the small and erratic seasonal rainfall, in conjunction with the scarcity of population, have created the unique position which Northern Australia holds to-day. The long dry season influences the insect life, and those tropical diseases which are transmitted by certain insects, such as the mosquito, are apt to show a corresponding seasonal incidence, and are to a certain extent kept in check. Other diseases, such as infectious and contagious ailments, are in a similar way kept down by the sterilizing action of the sunlight, which may kill the virus before it can spread over large and scantily populated areas.

The contention that the prevalence of certain diseases is favoured by larger and more evenly distributed rainfall is borne out by the fact that in districts situated within the wet belt a greater incidence of certain diseases is observed. Various fevers, such as endemic glandular fever, other scrub fevers of unknown aetiology, which are most probably insect-borne, occur throughout the wet belt, but are absent from other drier districts. In the same way hook-worm disease is far more prevalent in those places where the rainfall is higher, and is, as far as our present experience goes, practically absent from other districts showing similar local conditions but a smaller and strictly seasonal rainfall.

The effects of climate and surroundings upon any race finds a clear expression in the vital statistics, such as birth and death rates, infantile mortality rates, and expectation of life.

The statistical data for North Queensland and Northern Australia have not been published separately for comparison with similar statistics for the rest of Australia; moreover, it is impossible to obtain definite data as to the number of inhabitants in Australia, since the last census took place in 1911, and the population of Northern Australia is of a migratory character; any figures obtained are therefore only of approximate value. The Commonwealth Statistician, Mr. G. H. Knibbs, C.M.G., was able to supply information which throws light upon this question, and has kindly given permission to include his statements in the present publication.

The area and population of the tropical portion of the Commonwealth are shown in the following table:

TABLE V.

Area and population of the tropical portion of Australia.

Tropical Portion of—	Area in square miles	Population (exclusive of full-blooded aboriginals) at Census of—			
		1881	1891	1901	1911
Northern Territory	426,320	3,451	4,898	4,096	3,310
Queensland	359,000	56,041	108,986	145,982	157,112
Western Australia	364,000	661	3,711	4,664	4,998
Total	1,149,320	60,153	117,595	154,742	165,420

These figures relate to all that portion of the Commonwealth which lies to the north of the Tropic of Capricorn. For the tropical portions of Queensland and Western Australia population figures are available at the date of a census only. For the Northern Territory they are compiled quarterly, and the returns for December 31, 1917, give a total of 4,908—an increase of 1,598 since the census of 1911.

At the census of 1911 the total population of Queensland (exclusive of full-blooded aboriginals) was 605,813, so that at that date the tropical population of Queensland represented rather more than 25 per cent. of the total. It also represented about 95 per cent. of the total tropical population of Australia. It might consequently be presumed that any unhealthiness of tropical Australia would have left its impress on the vital statistics of Queensland. That this is not the case is clearly shown from the following comparison of the

Queensland death rates per thousand of population with those for the Commonwealth as a whole:

TABLE VI.

Comparison of Queensland and Commonwealth Death Rates, 1906 to 1917

	1906	1907	1908	1909	1910	1911	1912	1913	1914	1915	1916	1917
Queensland	9.50	10.31	10.26	9.70	9.71	10.65	10.96	10.39	9.97	11.00	11.09	9·64
Commonwealth	10.92	10.99	11.07	10.33	10.43	10.66	11.23	10.78	10.50	10.66	11.04	9.80

It will be seen that in ten out of the twelve years under review Queensland recorded a lighter death rate than the Commonwealth as a whole, and that in one of the other two years (viz., 1916) the difference was inappreciable. The remaining year (1915) was that in which Queensland suffered from the most severe drought ever known there, while the greater part of the remaining States experienced normal conditions, having suffered from drought in the preceding year.

Another test of salubrity often applied is that derived from a comparison of the deaths of infants under one year of age with the total births. The rate so deduced (i.e., the number of deaths of infants under one year of age per one thousand births) is what is generally known as the 'infantile mortality.' A comparison of the Queensland results with those of the Commonwealth as a whole for the past twelve years is furnished in the following table:

TABLE VII.

Comparison of Queensland and Commonwealth Infantile Mortality, 1906 to 1917

	1906	1907	1908	1909	1910	1911	1912	1913	1914	1915	1916	1917
Queensland	74.68	77.65	70.67	71.50	62.90	65.36	71.73	63.35	63.93	64.33	70.27	53.87
Commonwealth	83.26	81.06	77.78	71.56	74.81	68.49	71.74	72.21	71.47	67.52	70.33	55.91

In every one of the twelve years under review the infantile mortality of Queensland was more favourable than that of the Commonwealth as a whole. Further, in both the foregoing tables the comparison has been between Queensland on the one hand and the Commonwealth, inclusive of Queensland, on the other. A comparison between Queensland and the Commonwealth exclusive of Queensland would have given results even more favourable to Queensland than those deduced above.

In the absence of complete data in respect to the population and mortality of the tropical parts of Australia, it is impracticable to carry the test further, but the figures given above indicate that the vital statistics of the Commonwealth furnish no evidence of lack of salubrity in those parts. On the contrary, the State having 25 per cent. of its population within the tropics, and containing some 95 per cent. of the tropical population of the Commonwealth, has a record for general and infant mortality much more favourable than that for the Commonwealth as a whole. It may be noted that practically the whole of the State of Queensland lies in the north of the twenty-ninth parallel of south latitude.

Striking as the foregoing Queensland figures are in comparison with those for the whole of Australia, an even more telling result is obtained by comparing them with those of some of the leading European and other countries.

TABLE VIII.

Country	Year	Death Rate	Rate of Infantile Mortality
Queensland	1913	10.4	63
Commonwealth	1913	10.8]	72
Netherlands	1913	12.3	91
Denmark	1913	12.5	94
Ontario (Canada)	1913	12.7	117
Norway	1913	13.2	65
Sweden	1913	13.6	70
England and Wales	1913	13.8	108
U.S.A. (registration area)	1913	14.1	*
Switzerland	1913	14.3	96
Belgium	1913	14.8	120
Germany	1913	15.0	151
Scotland	1913	15.5	110
Ireland	1913	17.1	97
France	1913	17.7	98

* Not available.

TABLE VIII—*continued*

Country	Year	Rate	Rate of Infantile Mortality
Italy	1913	18·7	137
Japan	1913	19.5	150
Austria	1913	20.5	180
Jamaica	1913	21.7	171
Spain	1913	22.1	*
Hungary	1913	23.3	186
Rumania	1913	25.9	202
Ceylon	1913	28.4	189
Chile	1913	30.1	255

* Not available.

In addition to the foregoing, it may be pointed out that the mortality experience of Queensland has been continuously improving, both absolutely and also in relation to the experience of the Commonwealth as a whole. This is clearly brought out in the next table, showing the expectation of life at age 0 in each of the last three decades:

TABLE IX.

EXPECTATION OF LIFE AT AGE 0.

Decade	Males		Females	
	Queensland	C'wealth	Queensland	C'wealth
	Years	Years	Years	Years
1881-1890	41·330	47·199	49·754	50·844
1891-1900	49·512	51·076	55·800	54·756
1901-1910	54·203	55·200	59·294	58·837

In Mr. Knibbs's opinion, local statistics relating to individual towns would not give results sufficiently reliable to warrant definite conclusions, owing to the migratory character of much of the population, and, in connection with birth, owing to the tendency to move into the towns for purposes of confinement.

(2) *Housing*

The question of the construction of suitable dwellings in the tropics has been the object of a great deal of study and controversy. A suitable tropical residence should be so constructed that the interior is protected as much as possible from the direct rays of the sun, in order to prevent excessive heating of the walls of the room, and at the same time sufficient ventilation should be provided in order to secure the quickest possible cooling of the structure. Unfortunately, in the construction of the average dwelling house in North Queensland these principles have not been sufficiently considered. The greater proportion of the dwellings of the settlers on the land are entirely unsuitable, and a great number in the larger towns are far from ideal. A statement of a leading firm of architects with twenty years' experience in the north is of interest, namely, that they have never designed one cottage as they know a cottage should be designed, on account of the prejudice against the introduction of novel ideas.

The majority of the scattered habitations of the smaller settlers in north-western Queensland are built of galvanized iron on a wooden framework, without verandahs or any insulation, and the temperature of the interior during the hot hours of the day surpasses by far the outer air temperature.

The small dwellings in the towns are raised on piles, and contain as a rule four rooms; they possess a narrow verandah in front, and sometimes also on the side. The rooms are as a rule low, and the roof is composed of galvanized iron sheets and often does not possess an inner lining. The kitchen is nearly always detached and generally consists of a small cubicle built of galvanized iron only. Most of the larger wooden houses have a verandah all round, and higher rooms with ample ventilation.

A good type of tropical house may be seen in Darwin, where many of the houses are constructed after a design similar to houses in the Far East. The verandahs are wide and are closed in by bamboo shutters, which are kept shut during the heat of the day and thus prevent the heating up of the rooms and at the same time allow of sufficient ventilation; the shutters are opened as soon as the sun gets low, It is quite evident that a galvanized iron 'humpy,' without verandah, is the most unsuitable structure to reside in in a hot climate. During the day the heat is nearly unbearable, and the only advantage is the speedy cooling of the walls after sundown. The larger houses, built of timber, with open verandahs, are, on the whole, suitable for the climatic conditions if attention has been paid to several points.

The house should be raised off the ground and built on piles in order that the air should have free access to every part of the house. It should be so placed, if practicable, that it lies in the direction of the prevailing breeze, and windows and doors should as far as possible be opposite, in order to allow of the maximum of ventilation. The verandah should be sufficiently broad to protect the walls from the direct rays of the sun at any time, but the minimum width depends upon the latitude in the geographical sense. According to Schilling (1909), the houses in regions near to the equator require a verandah on the four sides, as the path of the sun is more vertical. In regions below the Tropic of Capricorn a verandah on the south side is not absolutely necessary, as the path of the sun is inclined from the north and the south side of the house is not struck by the direct rays at any time. The rooms should be spacious and with as many windows and doors as practical for the sake of ventilation and lighting. It is only too well known that mosquitoes and other insects choose dark corners and avoid light. A roof of galvanized iron is suitable provided that the necessary ventilation is allowed for. The roof should be slightly raised from the wall. The top gable should be provided with a ventilator and, if possible, there should be louvres on either side below the gable to provide additional ventilation. If the roof be closed in, there is a layer of hot, stagnant air between the roof and the ceilings of the rooms, which is heated by the sun to a higher temperature than the outside air, and this keeps the temperature of the rooms high after sunset.

We are indebted to Messrs. Lynch and Hunt, architects, of Townsville, whose activities extend throughout North Queensland, for the framing of the following recommendations for building small dwellings suitable for North Queensland, and costing approximately from £200 to £600:

Apart from any further items that may be found advisable, as many of the following recommendations as the site will permit should be embodied in every cottage :—

1. Buildings should face due east and have verandahs to front and back of not less than nine feet in width.

2. In addition to other necessary openings, there should also be doors and windows so arranged in every room to allow the wind to enter on the weather side and escape on the lee side. Doors should also be arranged so as to avoid the necessity of retracing one's steps to get to any section of the house.

3. Buildings should not be more than one room in depth.

4. The size of a room built under the most favourable circumstances should be regulated by the number of prospective occupants. Eight hundred cubic feet per head would be a fair minimum.

5. Side verandahs and excess widths to other verandahs should not be constructed at the expense of the size of the rooms. Large rooms and limited verandah space are infinitely better than small rooms and wide verandahs.

6. Blocks should be approximately 2 feet 6 inches above the ground. They should be either brick or concrete.

7. All buildings should be constructed to resist cyclones.

8. Fully exposed walls should be double-sheeted and ventilated.

9. Roofs should be fitted with ventilators.

10. Ceilings should stand two feet below the top of top plates. In this connexion studding should not be less than twelve feet in length for the smallest cottage and proportionately longer for larger cottages.

11. Fan-lights should be hung immediately under ceilings.

12. Ledges and corners should be avoided as far as possible.

13. Roofs, should be hipped and continuous, having eaves approximately two feet in width, to walls and verandahs.

Mr. C. D. Lynch has kindly put at our disposal a novel suggestion for building suitable houses, which would entail an expenditure of at least a thousand pounds, and which scheme is the outcome of twenty years' practical experience of house designing in North Queensland. The building, the idea of which is shown in the accompanying sketch (fig. VI), is constructed of reinforced concrete or brick, and possesses double outer walls, with an intervening and suitably ventilated air space of about three inches, thus ensuring coolness and dispensing with the necessity of verandahs. The floor space is sub-divided by wide corridors (3·5 to 4·5 metres, or about 12 to 15 feet) arranged in the form of a cross, on to which the rooms, placed at the corners of the house, open. The corridor may serve as a general living room, since it enjoys the maximum amount of ventilation, whatever the direction of the wind may be. A flat roof, surrounded by a balustrade, would, according to Mr. Lynch, add further to the roominess and comfort of the building.

Suggestions are found in the literature for cooling houses by artificial means. Attempts have been made to circulate artificially cooled air through buildings, but in practice this method has not proved satisfactory on account of the high initial expense of the necessary machinery and the technical skill required for its maintenance. Less costly, but at the same time less effective means, are fans run by electrical or water power; but as the majority of the northern towns of Queensland are without electrical power, and as the water rate is excessive and the supply often uncertain, fans in private houses have not come into general use.

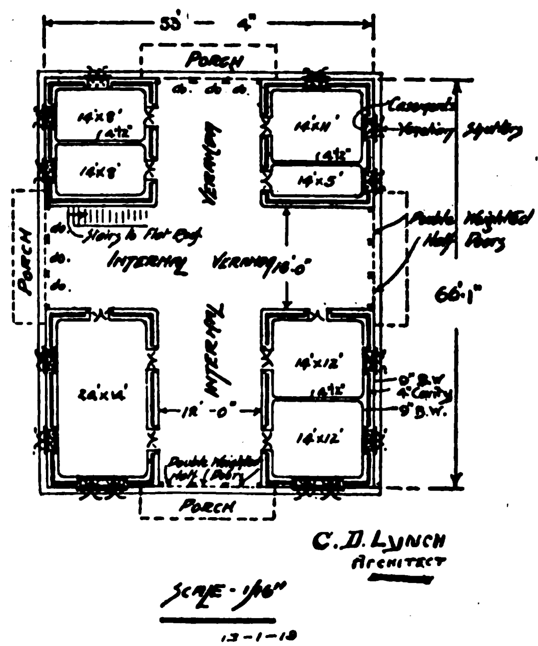

FIG. 6.
(Reduced one half from original drawing.)

The systematic laying out of towns in tropical Australia has unfortunately been sorely neglected, and most settlements have been allowed to grow up in a haphazard way. In the main streets of the larger towns ground has become too valuable to permit of an adequate space between buildings to allow free circulation of air. The main streets have often been laid out regardless of the prevailing winds. Flinders Street, the main street in Townsville, runs in the direction from north to south, the prevailing breezes blowing from south-east, and, as a result, the street has the welldeserved reputation of being the hottest corner in North Queensland. If, in the original laying out of the town, this point had been considered and a number of cross streets in the direction of the prevailing winds allowed for, a great deal of discomfort might have been spared.

GENERAL RÉSUMÉ

A consideration of the position in Northern Australia at present shows evidence that the progress in North Queensland, and to a still greater extent in the Northern Territory, has not been commensurate with the advance of time and with the undeveloped resources of the country. Certain townships, without doubt, have gone ahead, have increased in population and prosperity, but at the same time other towns have ceased to prosper, have decreased in population, and their inhabitants have migrated to the prospering townships. It is, however, difficult to get definite figures which would illustrate this point, as the census is only taken every ten years, but the relatively small increase of the population of the north when compared with the south of Queensland during the decade between two census (1901-1911) is significant. The total increase in population for Queensland during this time amounted to 108,657 souls; of these, only 7,577 represent the increase in the north. Considering that in 1911 25 per cent. of the total population of Queensland was living in the northern division, the increase in district was relatively much smaller than in the centre or in the south. Even if figures for the last seven years could be obtained, their value for gauging the progress of the country would be doubtful, on account of conditions brought about by the war which led to a nearly complete cessation of immigration.

The population of the Northern Territory has slightly increased lately. Even so, at the end of 1917 the total population is 4,908, and represents approximately one inhabitant to about 80 square miles.

It is therefore obvious that Northern Australia is not as favoured for settlement as the southern parts of the Commonwealth, and in this connection it was pointed out by Sir Thomas Anderson Stuart on the occasion of a meeting of the Royal Colonial Institute, in 1912, that the southern and more salubrious parts of Australia were not by any means over-populated yet, and that immigrants naturally preferred to settle there than to go further afield; thus, in the course of time they would find it an easier matter to obtain a settlement in the northern parts than in the south. It was simply a case of filling up, and the rapidity would depend on the rate of immigration. A similar conclusion was arrived at by Atlee Hunt (1915) regarding the Northern Territory. 'Should any new and rich mining field be discovered, the question of populating the Territory will settle itself; but in the absence of any such happening, it is submitted that the only course open is to wait until, in the natural course of things, the trend of population moves gradually northward.'

It is well known that economic conditions play an important part in the settlement of any country, and especially of the tropics. There is no doubt that life in the tropics is burdened with a great deal of discomfort, due to heat and other general conditions brought about by climate. For this reason the settlement of the tropics carries with it more difficulties than settlement in a temperate climate. This is borne out in practice by the experience that, throughout the world, the white population in tropical parts is largely of a migratory character. Tropical Australia does not form any exception to this rule. Even during a comparatively short residence one notices that the population is continuously changing, and that there exists a general desire amongst the inhabitants that their stay in the north shall be as short as possible, and the whole aspect of the majority of northern towns bear testimony to this. A lack of public interest is noticed everywhere, and has led to putting up with makeshifts. Houses are only rarely built for comfort, as their owners hope to occupy them only for a few years. Town improvements are often only of an ephemeral character, and short-sighted policies are the rule in most instances. This again is due to the unsettled mental condition of a migratory population which is unable to concentrate on anything but the most pressing immediate needs.

Many conditions co-operate in bringing about this state of affairs. North Queensland is the most recently settled part of Australia; it is an enormous stretch of country, with comparatively few lines of communication with the centres, and consequently it has received a great lack of consideration from the centres of government. A feeling of isolation is a natural outcome, and exaggerates migratory tendencies and the desire to get back to 'civilisation.'

The hot summer naturally militates against permanent settlement, especially since the housing, lack of water, etc., do not mitigate the severe discomforts. If a person residing in northern Europe were obliged to live during a severe winter in a draughty wooden house, without artificial means of heating, a strong desire to move to a more congenial climate would be created. In the same way one could not expect a white population to thrive in the tropics unless all possible means be adopted to alleviate climatic conditions.

Many newcomers arrive in the north with a prejudice against northern Australian heat, which has been instilled into them by their southern friends, and adds greatly to this discomfort. Dressed in their southern clothing they walk about perspiring, but never think that a cold day in the south, spent in a light cotton suit, would be equally, if not more, uncomfortable for the opposite reason.

In addition to climatic influences, the mode of employment in general tends to attract a migratory population. North Queensland is dependent almost entirely on raw products for supporting its inhabitants, and permanent institutions, such as factories, giving employment throughout the year, are lacking.

The settler in the north has thus to face conditions which, when compared with those of a temperate climate, render life in one way more uncomfortable, although in another way less strenuous on account of the lack of competition. During the hot season work is carried out under trying conditions; the least exertion causes profuse perspiration, the degree of discomfort depending mostly on the nature of the work and on the surroundings, but is well pronounced, even in people who follow a sedentary occupation. In addition, during the very hot months of the year the continuous pouring out of perspiration during the day is not relieved by a respite during the hot night, when, although at rest with doors and windows wide open, one perspires freely and wakens up in the morning unrefreshed and more tired than one felt on going to bed.

It requires but little thought to realise that this discomfort plays a still more important part in the life of the women. Their work—domestic duties—is carried out during the hottest part of the day, indoors in the hottest part of the house, and in most instances in that part which has been most neglected in construction. It is very difficult to obtain domestic help, on account of the small number of domestic servants available, whose wages are in consequence very high, demanding an expenditure which is in most instances prohibitive for a medium income. The wife of the settler on the land is in a still worse plight, as she has not only to do her own housework, but in many instances has to cook for the employees. There is, in fact, no eight-hour working day for these women.

As man is largely affected by his surroundings, and the quality and quantity of his work influenced by outer conditions, it can be readily understood that a tropical climate with a hot atmosphere laden with moisture, where the discomfort following any bodily exertion is great, is not conducive to a maximum output of energy, especially if the lack of competition makes the condition of life easy. There is no doubt that climate *per se* tends to affect greatly the quality and quantity of physical and mental work. A cold climate conduces to physical activity, a warm climate to lassitude, and the economic conditions are then the determining factor for the output of energy.

In North Queensland, with its remarkable natural resources, its remarkable recuperating power after a series of bad seasons, and its small population, the conditions of life are such that poverty is unknown and an easy living earned at a minimum cost of exertion, and the rate of wages is very high.

Another factor which plays a very important part in the determination of the quality of work is the abuse of alcohol, which is rampant amongst all classes of the community. A number of factors contribute to this abuse, such as the thirst caused by the heat, the temptation to quench this thirst owing to the great number of licensed public houses, the temporary stimulating effect of the slow poison, and last, but not least, the well-known open-hearted hospitality of the north, which finds its expression in 'shouting.'

The foregoing observations prove that in North Queensland both climate and economic conditions, namely, the lack of competition and high wages, have created a tendency in the same direction, towards a deterioration of labour, and it is impossible at present to apportion correctly the influence of either factor. It is quite probable that, in the future, when the population of Northern Australia has increased and competition has become keener, the quality of labour may improve, unless in the meanwhile a definite decay of the race, due to climate, has set in. Up to the present, however, no such decay is apparent, but it must be kept in mind that in a newly-settled country, with a floating population, which to a great extent is maintained by immigration, racial degeneration would not make itself felt until after a somewhat prolonged period.

The 'Great Experiment of White Australia' in most previous discussions has been considered entirely from one point of view, namely, the health point. It has been assumed that the possibility of a population of European descent to live, propagate and thrive in a tropical climate, alone would decide the question. In Northern Australia, however, the economic conditions are of equal importance and present a problem as far-reaching in its influence as health for the permanent settlement of tropical Australia.

LITERATURE

ABBE CLEVELAND, quoted by Hann (1908). Vol. I, p. 57.

ARON (1909). *Philippine Journal of Science*, B., Vol. IV, p. 225.

—— (1911). *Philippine Journal of Science*, B., Vol. VI, p. 102.

BENEDICT (1915). *Journal of Biol. Chem.*, Vol. XX, p. 263.

BLAGDEN, and FORDYCE (1775). *Phil. Trans. London*, Vol. XLV, p. 1, quoted from Schäfer's Text-book of Physiology, Vol. I, 1898.

BREINL, and PRIESTLEY (1914). *Annals of Trop. Medicine and Parasit.* Vol. VIII, p. 606.

BRUCE (1916). Moisture and Temperature in Air in Relation to Comfort and Health. Reprint of paper read before the Public Health Section of the Roy. Soc., N.S.W., November 14, 1916.

CAMPBELL (1917). *Journal Straits Branch, Roy. Asia. Soc.* No. 76

CHAMBERLAIN (1911). *Philippine Journal Med. Science.* Vol. VI, No. 6.

—— and VEDDER (1911). *Philippine Journal Science.* B., Vol. VI, p. 403.

CLEMESHA (1912). The Bacteriology of Surface Waters in the Tropics, London.

CUTHBERT (1911). *Journal Roy. Army Med. Corps*, Vol. XVII, p. 56.

DAVY, JOHN (1839). *Researches*, London. Vol. I, p. 161.

—— (1850). *Phil. Trans.*, p. 437.

EIJKMAN (1891). *Virchow's Archiv.* Vol. CXXVI, p. 113.

—— (1893). *Virchow's Archiv.* Vol. CXXXIII, p. 105.

—— (1895). *Virchow's Archiv.* Vol. CXL, p. 155.

—— (1896). *Virchow's Archiv.* Vol. CXLIII, p. 448.

FALES, L. (1907). Tropical Neurasthenia. *American Journ. Med. Sci.*, April.

FREER (1912). *Philippine Journal Science*, B. Vol VII, p. 2.

FURNELL (1878). *Lancet.* Vol. II, p. 110.

GIBBS (1912). *Philippine Journal of Science*, B. Vol. VII, p. 92.

GLOGNER (1892). *Virchow's Archiv.* Vol. CXXVIII, pp. 160, 180.

——— (1909). *Archiv für Schiffs and Tropenhyg.* Vol. XIII, p. 169.

HALDANE (1905.) *Journal of Hygiene.* Vol. V, p. 494.

HANN (1908). *Handbuch der Klimatologie*, Stuttgart. Vol. I, p. 57.

HARRINGTON, quoted by Hann (1908). Vol. I, p. 57.

HAVELOCK, CHARLES (1913). *Trans. Soc. Trop. Med. Hyg.* Vol. VII, No. 1, p. 1.

HERMANN (1883). *Archiv. f. Hyg.* Vol. I, p. 1.

HEYMAN, PAUL, and ERCKLENTZ (1905). *Zeitschrift für Hyg.* Vol. XLIX.

HILL, L. (1914). Reports Local Government Board, No. 100. New Series, London, p. 18.

——— (1916). *Phil. Trans.*, p. 214.

——— and FLACK (1909). Proceedings, Phys. Soc., *Journ. of Physiol.* Vol. XXXVIII.

HILL, FLACK, ROWLANDS, and WALKER (1913). Smithonian Miscellaneous Collections. Vol. XL (quoted by Hill, 1914).

HUNT, ATLEE (1915). The Northern Territory of Australia. Memorandum. Melbourne.

JOUSSET (1884). *Traité de l'acclimatement et de l'acclimatation*, Paris.

McCAY (1912). The Protein Element in Nutrition, London. p. 156.

MARESTANG (1889). *Arch. de Méd. Navale*, No. 2.

MUSGRAVE, and SISON (1910). *Philippine Journ. Science*, B. Vol. V, p. 325.

NEUHAUS (1893). *Virchow's Archiv.* Vol. CXXXIV, p. 365.

OSBORNE (1910). *Journal of Physiol.* Vol. XLI, p. 353.

——— (1916). *Proc. Roy. Soc. Vict.*, N.S. Vol. XXIX.

PEMBREY (1898). Animal Heat. Schäfer's *Text-book of Physiology.* Vol. I, p. 812.

——— (1913). *Journal Royal Army Med. Corps.* Vol. XXI, p. 156.

PHALEN (1910). *Philippine Journ. of Science*, B. Vol. V, p. 525.

PLEHN (1892). *Virchow's Archiv.* Vol. CXXIX, p. 285.

——— (1898). Die Kamerun Küste.

——— (1906). *Arch. f. Schiffs und Tropenhyg.* Vol. X, p. 220.

PRICE, BASIL (1913). *British Med. Journal*, November 15, p. 1290.

RANKE (1900). Quoted by Schilling (1909).

RATTRAY (1870.) *Proc. Roy. Soc.* Vol. II, p. 513.

SAMBON (1907). *Journ. Tropical Medicine and Hyg.* Vol. X, p. 67.

VAN DER SCHEER (1890). *Geneesk. Tijdschr. v. Nederl. Ind.* Vol. XXX.

SCHILLING (1909). *Archiv. f. Schiffs und Tropenhyg.* Vol. XIII, p. 1.

——— (1909). Tropenhygiene, Leipzig, p. 514.

SCHMIDT (1909). *Archiv. für Hyg.* Vol. LXIX.

——— (1909). Quoted by Aron (1911). p. 106.

SHAKLEE (1917). *Philippine Journal Science*, B. Vol. XII, p. 1.

SUTTON, HARVEY (1908). *Journal Path. and Bact.* Vol. XIII, p. 68.

TAYLOR, GRIFFITH (1916). The Control of Settlement by Humidity and Temperatures. Commonwealth Bureau of Meteorology. Bulletin 14.

THORNLEY (1878). *Lancet.* Vol. I, p. 554.

WICK (1910). *Archiv. f. Schiffs und Tropenhyg.* Vol. XIV, p. 106.

WICKLINE (1908). *Milit. Surgeon.* Vol. XXIII, p. 283.

WOODRUFF (1905). The Effects of Tropical Sunlight upon the White Man, New York.

YOUNG (1915). *Journal of Physiol.*, Vol. XLIX, p. 222.

——— (1919). Composition of Urine in Tropical Australia. *Ann. Trop. Med. & Parasitol.*, Vol. XIII, p. 215.

ZUNTZ, and TENDLAU, quoted by Pembrey (1913).

THE EXPERIMENTAL INFECTION IN ENGLAND OF *ANOPHELES PLUMBEUS*, STEPHENS, AND *ANOPHELES BIFURCATUS*, L., WITH *PLASMODIUM VIVAX*

BY

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HISTORICAL

The potentiality of *A. plumbeus* as a malaria carrier has been variously assessed. Theobald (1901),* in his Monograph of Culicidae, remarks that both *A. plumbeus* (= *A. nigripes*) and *A. bifurcatus* are malarial carriers, but are not nearly so abundant as *A. maculipennis* in England. Nuttall and Shipley (1901) give '*A. nigripes*?' as a carrier of malaria. Bacot (1918) says that there is no evidence that this species is able to carry malaria. Grove (1919), in a report to the Local Government Board, states '*A. plumbeus* from the rarity of its occurrence is certainly of very little danger. It has not yet been proved whether it is capable of transmitting the malarial parasites.'

These apparently irreconcilable statements confronted us in the commencement of our investigation, and it became necessary to ascertain which view should be considered the correct one. On making a search through the literature we found no mention of experimental work associated with *A. plumbeus* (vel *nigripes*) beyond that in the early Italian records, more especially the works of Bastianelli, Bignami and Grassi. These workers (1898) dissected amongst other mosquitoes one specimen which they identified as *A. nigripes*; the mosquitoes were caught in the room of four patients

* For literature consulted, see pp. 441-444.

suffering from malaria, probably malignant tertian; the specimen of *A. nigripes* was not infected. Grassi, Bignami and Bastianelli (1899[2]), in giving an account on February 5th of their work during the month of January, 1899, say that the need for experimenting with *A. bifurcatus* and *nigripes* has become evident, and that they propose to carry out such experiments within a short time. This work was evidently accomplished by June 18th of 1899, because on that date Grassi (1899) felt himself justified in stating that it was then proved that all the Italian species of the genus *Anopheles* propagate malaria.

It is between February 5th and June 18th, 1899, therefore, that we may expect to find the evidence upon which Grassi made this somewhat comprehensive statement, and the following is the evidence which we have obtained. Grassi, Bignami and Bastianelli (1899[1]) wrote, in May:—'We have together made experiments with *Anopheles bifurcatus* var. *nigripes* with blood containing gametes of tertian fever and with blood containing crescents; the results were undoubtedly positive.'

They amplify this bald statement in another place (1899[3]) on May 7th as follows:—'We have completed a series of experiments on *A. bifurcatus* (1). The individuals which were used inclined to the variety *nigripes*. We have experimented with these insects on a case of crescent infection and on cases of tertian infection. Both with crescent and tertian infections we have obtained positive results. The various stages of development observed in the walls of the intestine of *A. bifurcatus* correspond perfectly with those already noted by us in *A. claviger*, kept under the same conditions of temperature and examined at the same interval after the time of biting.'

These statements by Grassi and his collaborators are the sole evidence which we have been able to obtain of any reference to *A. plumbeus* (vel *nigripes*) in experimental work on malaria, and it appears to us, apart altogether from the unfortunate lack of detail in the description of the experiments themselves, that the views of the authors upon the identity of the mosquito with which they were working are of great interest. It will be noted that a change in the nomenclature of the mosquito has occurred during this short period. First of all there are references to *A. nigripes*, next comes a reference to *A. bifurcatus* var. *nigripes*, and in the conclusion of the account of the above experiment the mosquito is referred to simply as *A. bifurcatus*. The attitude of the authors towards the question of species is expressed by them (1899[1]) thus:—'Comparative observations made by Grassi in Calabria had led him to suspect also the other species of *Anopheles* found in Italy of being agents in transmitting malaria. These species, according to the researches of Ficalbi completed by Grassi, are the following:—

(1) *Anopheles bifurcatus* (there is a variety called *A. bifurcatus* var. *nigripes*).

(2) *Anopheles pseudopictus* (Grassi).

(3) *Anopheles superpictus* (Grassi).'

From this it would appear that they did not recognise *A. nigripes* as a separate and well defined species, but only as one particular form of *A. bifurcatus*. That this is in reality their considered opinion is made more evident by an important footnote by Grassi, indicated above (1), which runs:—'After the examination of a certain number of individuals collected in different localities, I hold with Ficalbi that *A. nigripes* is simply a variety of *A. bifurcatus*, an opinion which I intend to confirm by the examination of numerous individuals as well as of the larvae and ova.' No modification of this opinion on the part of Grassi has been traced by us.

From a study of the foregoing facts, therefore, we gain the information that:—

(1) This series of experiments of Grassi and his collaborators was carried out with wild mosquitoes, and not laboratory-bred insects.

(2) Whatever the true identity of the mosquitoes which they were using in these experiments, infection of the gut only was obtained.

(3) Grassi and his co-workers almost certainly experimented, not with *A. plumbeus*—a tree-hole breeder, but with a dark variety of *A. bifurcatus*.

Grassi, moreover, was aware that individuals of *A. bifurcatus* varied considerably in appearance, because he states explicitly (1903) that in June, 1899, he had, by himself, experimented successfully with '*l'Anopheles bifurcatus tipico*.'

It is of interest to note that at the time when the experiments recorded above were done by Grassi, Bignami and Bastianelli, Grassi had not observed mosquitoes breeding in tree-holes. Not until 1901 do we find any reference by him to such breeding-places, and when he did come upon them he referred to them very shortly under the heading *A. bifurcatus*, 'Ich fand in einer mit Wasser angefüllten Ausholung eines Baumes, larven des *A. bifurcatus* var. *nigripes*.' He shews in this statement no appreciation of the fact that this tree-hole breeder was a separate and definite species, nor that the adults and larvae of it were quite distinct from those of any known *A. bifurcatus*, whether small and dark, or large and 'tipico.' Not only is it evident that Grassi failed to recognise the importance of his capture, but in our opinion this was the only occasion upon which he encountered the true *A. nigripes* (*i.e.*, *plumbeus*).

Galli-Valerio and de Jongh (1903) did not associate this observation of Grassi's with the existence of a different species, because they say:—'We have often obtained from larvae very small forms of *A. bifurcatus* presenting the characters of *A. nigripes*, Staeger, but having observed all the range of variation in size between typical *A. bifurcatus* and typical *A. nigripes*, we agree with the opinion of Ficalbi, who says that it is not a case of a distinct species, but simply a form of *Anopheles bifurcatus*, which is liable to present very great variations in size.' Later, however, Galli-Valerio and de Jongh (1913), discussing their previous identification of Anopheline larvae in tree-holes in Plamont as *A. bifurcatus*, state that the tree-hole breeding larvae are *A. nigripes*:—'This is the first time,' they say, 'that the species has been found in the Canton of Vaud in Switzerland, and what we have previously called *A. bifurcatus* var. *nigripes* is simply a small form of *bifurcatus*.' They refer to the fact that Blanchard also was under the impression that *A. bifurcatus* and *A. nigripes* were identical. From this time onwards Galli-Valerio has studied *A. plumbeus* with great care, and yet recently (1917[2]) he writes, 'Mes recherches précédentes ont démontré l'existence en Suisse de trois espèces d'Anophélines: *A. maculipennis*, *A. bifurcatus* et *A. nigripes*. Tandis que pour les deux premières espèces, leur rôle dans la transmission de la malaria est démontré, la troisième n'a pas encore fait l'objet de recherches expérimentales a ce point de vue; mais il parait probable qu'elle intervient aussi dans la contamination et dans la transmission malarique, surtout dans les zones boisées.'

As a result of our examination of the literature on this subject, we agree with the opinion of those who up to 1919 held that *A. plumbeus* had not been convicted by any evidence, experimental or other, of being an agent in the carriage of malaria.

EXPERIMENTAL

I. Experiments with laboratory-bred *Anopheles plumbeus*

In a preliminary note (1919) we recorded the experimental infection of the gut and salivary glands of *Anopheles plumbeus* bred in the laboratory from pupae collected in England, and fed upon a patient whose blood contained *Plasmodium vivax*. More detailed information with regard to those experiments and additional ones conducted along the same lines are given in the present paper.

The work was carried out at the Liverpool School of Tropical Medicine during the period July to October, 1919. No experiments on the transmission of the disease to healthy human beings were made, because transmission experiments appear almost superfluous when such work as that of Mitzmain (1916) is taken into consideration. In his work with *Anopheles punctipennis*, Mitzmain found that a single mosquito which had sporozoites in its salivary glands was capable of infecting a series of healthy persons by single bites, and this even when the insect did not have an opportunity of taking a full meal of blood in biting.

Technique. Breeding. In the case of the first experiments the larvae of the pupae were obtained from Grasmere in the Lake District, they were taken from the tree-hole in which they were living and placed in a jar together with a quantity of the water and débris present in the cavity in the tree trunk, and brought to the laboratory by motor car; they withstood the journey well, and a number of adults subsequently emerged. An attempt made, at a later period, to send live pupae on moist moss from Wales by post proved unsuccessful; all the pupae died on the way. Larvae were found to be capable of surviving days of travelling if placed in bottles or tubes half-filled with the water from the tree-hole in which they were found. Our experience so far points to the greater hardiness of young larvae in this respect. When the material was brought to the laboratory it was placed in suitable glass breeding vessels and covered over with bell jars into which the emerged adults could ascend; in order to approach as closely as possible the natural conditions, the bell jar was encased in brown paper. Transference from the bell jar to the feeding apparatus was effected as required by means of a piece of wide glass tubing, over one end of which netting was fixed; the bell jar having been removed under mosquito netting, the mosquitoes, one at a time, were captured in the tube and then liberated into the feeding apparatus, which consisted of a wide glass cylinder covered at each end by netting kept in position by rubber bands. The cylinder was set upright in the bottom of a Petri dish, on which a piece of moist blotting paper was placed. The food provided, apart from the single meal of infected blood, consisted of raisins. Each day the blotting paper was moistened and a split and moistened raisin placed on the netting on the top of the feeding jar. The netting on the feeding apparatus and the blotting paper in the Petri dish were renewed daily at first, but later about every two days was found sufficient. In the incubator experiments a vessel containing water was placed in the incubator, and as evaporation proceeded fresh water was added.

Feeding. Anopheles plumbeus rarely attempted to feed on blood within the first twenty-four hours of emergence from the pupa, but after that period most of them fed readily and engorged themselves with blood, some even feeding well when it was obvious that they had recently partaken freely of the raisin diet provided.

Dissection. All mosquitoes which died after the infected feed were dissected, and at intervals others were killed and examined; fresh preparations of the mid-gut and salivary glands were made and examined in 0·85 per cent. sodium chloride; for permanent preparations the gut and glands were fixed in Bless' fluid, stained with haematein and differentiated in acid alcohol.

The results obtained by feeding and dissection are given below in Table I. Gametes were present in the peripheral blood of all patients used for the feeding experiments. The case upon which Experiments A and C were done had at the time of feeding numerous parasites in the peripheral blood and had a temperature of 103° F.

It is worthy of note that salivary gland infection was present in four of eight mosquitoes which were dissected after ten days or longer at 28° C. from the time of the infected feed.

TABLE I. Showing the results of feeding laboratory-bred *A. plumbeus* on patients infected with *P. vivax*.

Exp.	Date fed	Number					Temperature used	Remarks
		Fed	Dissected	Infected	Gut	Sal. Glands		
A.	11.7.19	2	2	0	0	0	Room	One, allowed to live, survived 71 days after infected feed
B.	10.7.19	6	6	3	3*	2	28°c.	
C.	11.7.19	2	2	0	0	0	28°c.	
D.	11.7.19	2	2	1	1	0	Room	
E.	14.7.19	6	5	3	3	2	28°c.	One died 1.8.19 but could not be dissected.

* One of these, which had not yet infection of the salivary glands, was killed on the 8th day. In four of the twelve oöcysts found in the gut, sporozoites were observed.

2. Experiments with laboratory-bred *Anopheles bifurcatus*

In order to obtain some idea of the relative infectibility of *A. plumbeus* and one of the better known anophelines which occur in England, we made some experiments with *A. bifurcatus* under the conditions which obtained in procuring salivary gland infection in *A. plumbeus*, with the exception that the experiments were carried out at a later period of the year and that the same patients were not used. The results are given in Table II, and are of interest. Experiment Q, in this control series, is especially interesting, because in this case the maturity of the gametes was tested by observing exflagellation, which was found to be proceeding freely before feeding was carried out.

TABLE II. Showing the results of feeding laboratory-bred *A. bifurcatus* on patients infected with *P. vivax*.

Exp.	Date fed	Number					Temperature used	Remarks
		Fed	Dissected	Infected	Gut	Sal. Glands		
M.	23.9.19	2	2	0	0	0	28°c.	
N.	29.9.19	8	8	0	0	0	28°c.	
Q.	17.10.19	12	12	1	1	0	28°c.	

In this experiment, thirteen mosquitoes were dissected, after ten days or longer at 28° C. from the time of the infected feed; no instance of salivary gland infection was obtained.

We hope to have an opportunity in the summer of making a rigidly comparative series of experiments with *A. plumbeus* and *A. bifurcatus* in order to determine with more accuracy their ability to acquire infection in laboratory experiments.

Previous experimental work with these species

The amount of such work recorded in the literature is very small. Neither of the two species used by us has previously been experimentally infected in this country, nor has either of them ever been found infected in nature. With regard to experiments conducted on mosquito infection in England, the only record is that of James (1917) who worked with *Anopheles maculipennis*. The mosquitoes which he used were apparently adults captured in huts and bedrooms of malaria patients at Sheerness; they were fed on a benign-tertian gamete carrier, and afterwards dissected. Infection was obtained in two mosquitoes, and was in each case confined to the gut.

SUMMARY

We have been able to infect laboratory-bred *A. plumbeus* with *P. vivax*. At 28° C. infections of the gut and salivary glands were obtained; at room temperature (max. 26° C., min. 17° C.) gut infection only was obtained. We have also produced infection of the gut with *P. vivax* in the case of *A. bifurcatus* at 28° C.

This is, we consider, the first experimental evidence produced that *A. plumbeus* is capable of becoming infected with a malaria parasite. As regards *A. bifurcatus* we have proved that in England the native form of this mosquito is capable of being infected with malaria at 28° C.

OBSERVATIONS ON *ANOPHELES (COELODIAZESIS) PLUMBEUS*, STEPHENS, WITH SPECIAL REFERENCE TO ITS BREEDING-PLACES, OCCURRENCE IN THE LIVERPOOL DISTRICT, AND POSSIBLE CONNECTION WITH THE SPREAD OF MALARIA

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PLATES X—XII

As will be gathered from the preceding paper, we have, during the last few months, given considerable attention to *Anopheles plumbeus*, Stephens, the bionomics of which species are probably less known than those of any European Anopheline mosquito. This lack of knowledge is aggravated by the scattered nature of the literature pertaining to this insect and by the not infrequent contradictory statements made regarding it. In recording our observations, therefore, it will not be inexpedient if, besides alluding to the more important work performed by other observers, we give some general account of the species.

SYNONYMY

Anopheles plumbeus was discovered by Haliday in the north of Ireland in 1828. But although this author gave a brief description of his specimens, he evidently doubted their being distinct from *A. bifurcatus*, L., and did not name them. Stephens, however, in the same journal and in a paper immediately following Haliday's assigned the name *plumbeus* to the description supplied by the latter writer.

In Denmark, in 1839, Staeger captured a female Anopheline which he described as *A. nigripes*. But the apparent rarity of *A. nigripes* and the resemblance of the adult to small dark forms of *A. bifurcatus* led to much confusion, and, until Eysell (1912) described its early stages and Galli-Valerio and de Jongh (1912[2]) its breeding habits, most continental authorities considered it a variety of this species. Such a conclusion was based chiefly upon incorrect identifications. Theobald (1901), however, not only recognised its validity but associated it with Stephens' species, a suggestion which Edwards (1912) accepted and extended by resuscitating the name *A. plumbeus*.

James (1911) described *A. barianensis* from the Western Himalayas. But Christophers (1916), after studying all its stages, decided that it was identical with the European form of *A. plumbeus*. This author also suggested that the North American *A. barberi*, Coq., would probably prove synonymous with *A. plumbeus*, but in the opinion of one of us (H. F. C.) the species are quite distinct, as the descriptions and figures of the American species given by Smith (1906) and Howard, Dyar and Knab (1912-1917) reveal important morphological differences.

The synonymy of *A. plumbeus* is therefore:—

Anopheles plumbeus, Stephens (1828)
Anopheles nigripes, Staeger (1839)
Anopheles barianensis, James (1911)

and to that of *A. bifurcatus* must be added:—

Anopheles nigripes, Ficalbi et auct. (nec Staeger)
Anopheles bifurcatus var. *nigripes*, Ficalbi et auct. (nec Staeger).

RELATIONSHIP WITH OTHER ANOPHELINES

A. plumbeus, Steph., *A. barberi*, Coq., and the south Indian *A. culiciformis*, Cogill, compose a group of species all of which show great selectivness in their habits.* They are primitive forms, and thus are related to the 'Anopheles' (sens. str.), 'Stethomyia' and 'Myzorhynchus' groups. Their eggs and larvae are peculiar, and present characters which have been considered of generic or sub-generic value. Dyar and Knab (1906) founded the genus *Coelodiazesis* on the larval characters of *A. barberi*, but subsequently

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* See note on p. 444.

Dyar (1918) reduced it to sub-generic rank. Independently, Eysell (1912) created the genus *Cyclophorus* for *A. plumbeus* on account of the striking characters of its eggs.

General Distribution

From what has been said above, it will be seen that *A. plumbeus* is very widely distributed. Owing to its selective habits and our comparatively recent discovery of them, records are meagre and, for the most part, disconnected. But it has been found in nearly all the European countries, including Scandinavia and Italy, and also in the Western Himalayas. The records show that it occurs at varying altitudes up to 8,000 ft., and apparently that it is restricted, or almost restricted, to forested regions. Yet it has been captured in towns, *e.g.*, Edinburgh, Grenoble, and in the suburbs of London, Paris and Bonn. Its supposed rarity has been due to the paucity of adults collected or observed, but since the discovery of its breeding-places it is becoming evident that it is much more common than was believed.

Characters of the Adult and Immature Stages of *A. plumbeus*

The adult has been described by several systematists since Haliday's time, and the early stages in some detail by Eysell (1912), Galli-Valerio and de Jongh (1912[2]), Martini (1915), Christophers (1916), and others (cf. p. 432 *re* Meinert's description). It will, therefore, be necessary to mention only the more important diagnostic characters.

A. plumbeus measures from 4 mm. to 5 mm. in length and is of a general dark colour with unspotted and densely scaled wings. The palpi are uniformly dark in both sexes, and the hair-tuft on the last two segments in the male is much reduced. The thorax, as in *A. barberi* and *A. culiciformis*, is relatively shorter in comparison with the width (not more than twice as long as wide) and more rounded than in other Anophelines; the median region of the mesonotum is broadly ashy-grey, with a central line of white scales anteriorly, and is sharply separated from the sides which are a rich dark brown colour.The legs are very dark, with inconspicuous pale spots at the apices of the femora and tibiae. The abdomen is blackish brown and hairy.

The egg measures 0·5-0·6 mm. long by 0·2 mm. wide. It is fusiform, broad in the middle and tapering sharply towards the ends, and is completely surrounded by a float of nearly uniform width.

The larva is dark, almost black, above, and whitish below, and when mature measures 5 mm. in length. As with the other members of this group, it is characterised by possessing long branched hairs on abdominal segments 1-6 inclusive, and by the presence of minute hairs only on the head. The clypeal hairs are simple, the antennae are almost devoid of minute spines on the shaft and the basal hair is very small. The abdomen bears dorsally five pairs of palmate hairs and laterally on segments 1-7 conspicuous radiate bristles, each composed of from three to seven setae. The ninth segment is strongly chitinised.

The pupa, like the larva, is pale ventrally; but unlike it, shows poor development of the abdominal hairs and spines.

ADULT HABITS

Comparatively little is known of the habits of the adult *A. plumbeus*. It is generally considered to be an essentially sylvan and 'wild' species, which occurs but rarely in houses. In woods, however, when observed at all, it has been recognised as a vicious and persistent biter, and Edwards (1916) states that it is a 'common blood-sucker in wooded districts in this country, often biting during the day.' For the most part, the statements made regarding the slight tendency of this mosquito to invade houses are based upon the occasional capture of one or two individuals in such places, but from the few serious observations made it would seem that this species is in no way averse to entering houses. Thus Burton (see Lang (1918)) states that near Shrewsbury *A. plumbeus* is not rare, 'It bites all day and night; I have never seen more than two trying to bite at the same time. It is found mostly in the woods, but will come into houses to bite more often than will *A. bifurcatus*.' Hesse (1918) says that the adults enter houses voluntarily.

That *A. plumbeus* has not been recorded more frequently in houses is, in our opinion, due chiefly to the fact, noted by Christophers (1916), that the females retire to the shelter of their breeding-places shortly after feeding. Unlike *A. maculipennis*, therefore, they will remain but a short time in the house, and the chances of seeing them except in the mornings and evenings will be small. In this connection Christophers, working with *A. plumbeus* in the Western Himalayas, states: 'The adults may be found during the day resting inside hollow trees. Most of the females taken in such situations contained blood in the gut, and as it was ascertained that in the mornings and evenings, but more especially the former, the female entered houses and bit freely, it is probable that such blood was to a large extent of human origin.' It is evident, therefore, that in this country much closer observation of the feeding habits of *A. plumbeus* is necessary.

A. plumbeus bites readily in the laboratory twenty-four hours after emergence, and will do so at any time of the day and in full daylight. Females, kept in glass cylinders, became engorged in from one and a half to four and a half minutes, but three minutes was the usual period of time required. Their avidity for blood was shown in several instances by females, which had distended themselves with blood half an hour to one hour earlier, re-engorging for a period of one minute or so. The bite was singularly devoid of pain and produced very slight local reaction; the usual character of the local reaction was the appearance of small white circular areas, each surrounded by a reddened zone, and this appearance was noticed within an hour, or up to ten hours, after the bite. The bite of a mosquito and its effect is largely a question of individuality, but it is worthy of note that, with some people at least, the bite of *A. plumbeus* may pass entirely unnoticed, and for a supposedly 'wild' species this is of interest. In our experience the bites of *A. bifurcatus* and the purely sylvan *O. nemorosus* or *O. maculatus* are distinctly more painful.

The great majority of the females under observation were not kept for the purpose of studying their biology and were only given one meal of blood each and often killed when perfectly vigorous. They fed readily on moistened raisins, however, and in several instances lived considerable periods on such diet.

Of twenty-four females which received one meal of blood and had free access to raisins: five lived less than ten days (one being killed); eleven lived 10-20 days (six killed); two lived 20-30 days (one killed on the 28th day); four lived 30-40 days (one killed on the 40th day); and two lived over 40 days. Of the last two, one, although active and vigorous, was killed on the 48th day, and the other died on the 75th day.* Most of the females had been kept in an incubator at 28° C., but the last mentioned had remained in the room, the temperature of which varied during its life from 12° C. to 26° C.

Of three females which received raisin diet only and were kept at room temperature (17° C.—21° C.) one lived 14 days and two 15 days each.

Of nine raisin-fed males the lengths of whose lives were ascertained, five died in less than 10 days, and the remaining four lived 29, 31, 33, and 47 days respectively. These four were all confined in cylinders with one or two females, and during their lives the last two were subjected to temperatures ranging from 12° C. to 28° C.

Mr. R. F. L. Burton of Shrewsbury has made many interesting observations on the swarming of the males of *A. plumbeus*. He states (in Lang (1918)) that on windless evenings from July to October they may always be found swarming in certain definite spots, and, in a personal communication, informs us that one such spot is about 100 yards from an elm-stump, the only breeding-place known to him. He states that this swarm usually consists of seven or less males and that it occurs on the outskirts of a wood and six feet from a slow, muddy stream. Mr. Burton has observed in other spots swarms consisting of as many as thirty individuals.

BREEDING-PLACES

A. plumbeus and the other species of *Coelodiazesis* are essentially tree-hole breeders, their larvae developing in the water contained in rot-holes. Many authorities—Martini (1915), Galli-Valerio (1917[2]), Eckstein (1918[1])—agree that they live exclusively in such places, and, unless situations be found where the biological conditions approximate to those peculiar to the rot-holes, they are probably correct. Apart from records which undoubtedly pertain to dark forms of *A. bifurcatus*, we have found three relating to the discovery of *A. plumbeus* larvae in places other than rot-holes. Evans (in Theobald (1910)) claims to have bred males from larvae

* This mosquito has been referred to in Table I of the preceding paper.

taken from a pool near Lasswade, Edinburgh, but as this gentleman made no reference to the striking characters of the then unknown larva, this record is not generally accepted. C. Blanchard (1918) described a young larva, which he suggested was that of *A. plumbeus* (*nigripes*), from a marsh, but later R. Blanchard (1918) stated that an error of identification had been made and that the specimens captured were the first stage larvae of *A. maculipennis*. The third record is deserving of much closer attention and was made by Theobald (1908). This author writes: 'We have found such as *A. nigripes*, Staeg. and *A. bifurcatus*, L. breeding in the water of peat cuttings in Wales and Somerset.' He has recently informed us privately that his identifications were made from adults reared from the larvae found. In an attempt to confirm this observation we recently examined the water of peat cuttings in this neighbourhood but without success, although in a hole in a birch tree, situated not more than 15 yards from the peat water, hibernating larvae of *A. plumbeus* were discovered. In this case, however, the water in the cutting was almost colourless and very different in appearance from that in which *A. plumbeus* larvae are usually found; but if, as is conceivable, the water in very old cuttings acquires a greater resemblance to that of the rot-holes, the possibility of its acting as a breeding-place must be considered.

A very good idea of the type of breeding-place selected by this Anopheline may be gained from Plates X and XI and from the table on pages 430 and 431. We have on one occasion only found the larvae at ground level (cf. Plate XI, figs. 3 and 4), and then the breeding-hole was well sheltered in the projecting portion of a root and by a mass of fallen leaves which almost completely covered it. The characteristic hollows formed by the root-buttresses of beech trees containing collections of clear water of a temporary nature have never yet yielded us *A. plumbeus* larvae; but in one instance an exceptionally deep hollow contained, so far as we could ascertain, four larvae of *O. geniculatus*. Galli-Valerio (1916) states that the larvae may also occur in holes in stumps of trees and in the forks between the boughs of beeches. In most cases examined by us the breeding-place has provided ample shelter for both larvae and adults, even although the tree itself was in an exposed position. In several instances the orifice of the hole was almost blocked by a piece of broken and rotten branch which came away at the slightest pull; or in private grounds and parks where the trees were more carefully tended it was occasionally found that potential breeding-places were present in sawn-off branches which, a few yards away, appeared perfectly sound. On investigation, a small orifice was visible at the lower edge of the cut surface which had rotted away behind and below, leaving internally a fair-sized hole. The depth of some of the rot-holes is considerable, sometimes three feet or more, so that, bearing in mind the frequently admirable shelter provided and the resultant protection from evaporation, the permanent nature of most of these breeding-places is easily understood. Eysell (1912), who at the time did not know of the type of breeding-place selected by this insect, remarked that in the abnormally hot dry summer of 1911 he was able to obtain more *A. plumbeus* material than ever; and he states that in this season only those mosquitoes breeding in permanent water reservoirs were numerous. Howard, Dyar and Knab (1917), speaking of *Anopheles barberi*, say 'This species occurs, with other mosquitoes breeding in tree-holes, in forested regions, often when the country is so dry that no other mosquitoes are found.'

Christophers and Khazan Chand (1916) found the larvae of *A. culiciformis* in about 10 per cent. of the tree-holes holding water, and as a rule this water was of a 'characteristic deep brown tint like strong tea or coffee.' We have made no estimate regarding the percentage of tree-holes harbouring *A. plumbeus* larvae, but the remark of these authors regarding the colour of the water is equally applicable to this species; we have never yet found larvae in holes in which the water was colourless (on filtration). The exact colour of the water in which larvae occur appears to vary from yellowish-brown to deep reddish-brown, and is evidently a solution of substances derived from the rotting tree and vegetable débris at the bottom.* Galli-Valerio (1916) states that in the laboratory the larvae develope better if kept in dark yellow containers and adds (1917[1]) that the water contains tannin. The depth of the surface water in

* An analysis of a sample of this water (specimen 1) made by Professor W. Ramsden, of Liverpool University, is given on p. 440. Specimen 2 was obtained from what appeared to be a suitable breeding-place, but no larvae were found. The absence of larvae from such water was so exceptional that we requested Professor Ramsden to examine it also.

the rot-holes varies greatly and may not be more than a few inches. Even in deep holes the accumulation of vegetable matter is sometimes so large that little water is evident.

From the table on p. 430 it will be seen that breeding-places have been found in various kinds of trees; some of these have already been recorded by other observers as harbouring larvae. To the list must be added silver-fir (*Abies pectinata*) (Galli-Valerio and de Jongh (1915)) and hornbeam (*Carpinus betulus*) (Mr. F. W. Edwards, personal communication). Apparently, therefore, the species of tree is only of importance from its tendency to form suitable breeding-places. Certain kinds appear to show a higher proportion of rot-holes in the trunks while others, particularly when old, may by their conformation offer more opportunities for the retention of water. Beech trees have been especially referred to in connection with *A. plumbeus* and old beeches possibly are more liable to form breeding-places than other species of tree. In our investigations, and more particularly those conducted in the vicinity of Liverpool, the search for breeding-places was largely confined to comparatively small trees (the diameter of the main trunk seldom being more than eighteen inches), and of these sycamores easily ranked first in providing us with larvae. Near Simla Christophers (1916) found that oaks were most favourable.

The continual association, in the literature, of *A. plumbeus* with forests and woods is natural, in view of the facts now known concerning its habits, since in such places greater opportunities for breeding occur, and the chance of encountering adults is increased. But although this mosquito is always associated with trees it must not be identified solely with forests or woods. We have found larvae in trees situated in isolated spinneys, in meadows, or forming with two or three others a small clump—in situations, in fact, which could not by any stretch of imagination be termed woods. Further, they may and do occur in trees growing near habitations, both in rural districts and in towns, wherever suitable breeding-places exist.

The difficulties attending the search for breeding-places of *A. plumbeus* are many and various. The work is arduous and frequently involves climbing and the use of special apparatus for sampling the water. Ladles and spoons of various sizes, bent at different angles, may be all that is required, but syphons are

TABLE I.

Details of the breeding-places observed.

Locality	Kind of Tree	Height of aperture above ground	Dimensions of rot-hole, in inches (approx.)	Situation	Remarks
ENGLAND LIVERPOOL DISTRICT Hightown, Lancs. ...	Beech (*Fagus silvatica*)	5 ft.	8 × 9 × 18 deep	In small wood with dense vegetation; 200-300 yards from nearest houses.	7.6.1919. Larvae of various sizes and pupae found, also many larvae and pupae of *O. geniculatus*. 26.7.1919. No larvae or pupae found. —.10.1919. No larvae or pupae found.
Fazakerley, Lancs. ...	Elm (*Ulmus campestris*)	8 ft.	19 × 12 × 17 deep	In private grounds; 12 yards from entrance to house	1.2.1920. Numerous small larvae of *A. plumbeus* found.
Knowsley Moss ... (By kind permission of D. Hamilton, Esq.)	Birch (*Betula alba*)	7 ft.	6 × 6 × 12 deep	200 yards from lodge and 10-15 yards from peat cutting	7.2.1920. Many small larvae of *A. plumbeus*. No mosquito larvae found in water of peat cutting.
Roby ... ... (By kind permission of D. Hamilton, Esq.)	Sycamore (*Acer pseudo-platanus*)	3 ft.	14 × 8 × 12 deep	In small wood with dense vegetation; 300-400 yards from nearest house	15.6.1919. Many larvae and pupae of *O. geniculatus*. 27.9.1919. Few larvae of *O. geniculatus* found. Adult ♂ *A. plumbeus* sheltering in rot-hole.
Aigburth Vale, L'pool (By kind permission of R. B. Miller, Esq.)	Beech (*Fagus silvatica*)	12 ft.	16 × 6 × 42 deep	In grounds of house situated near main road	26.7.1919. Numerous larvae of *A. plumbeus*.
Storeton, Ches. ...	Beech (*Fagus silvatica*)	3 ft.	4 × 6 × 12 deep	In small open and wind swept copse with little undergrowth. Nearest houses, 200-300 yards distant	8.6.1919. Many larvae and pupae of *A. plumbeus* and *O. geniculatus* found. —.9.1919. Comparatively few and mostly small larvae of *A. plumbeus* present.
Bidston, Ches. ... No. 1.	Sycamore (*Acer pseudo-platanus*)	4½ ft.	7 × 9 × 9 deep	In waste ground on outskirts of village	14.12.1919. Numerous very small larvae of *A. plumbeus*.
No. 2.	Do.	1½ ft.	10 × 14 × 10 deep	In small plantation bordering road on outskirts of village	14.12.1919. Numerous very small larvae of *A. plumbeus* and a few large larvae of *O. geniculatus*.
No. 3.	Do.	3½ ft.	12 × 8½ × 12 deep	do.	do.
No. 4.	Do.	3 ft.	17 × 17 × 20 deep	In meadow bordering road on outskirts of village	24.1.1920. Many small larvae of *A. plumbeus*.
No. 5.	Do.	5 ft.	12 × 17 × 21 deep	do.	do.
No. 6.	Do.	1½ ft.	11 × 9 × 6 deep	do.	do.

TABLE I—*continued.*

Details of the breeding-places observed.*

Locality	Kind of Tree	Height of aperture above ground	Dimensions of rot-hole, in inches (approx.)	Situation	Remarks
WESTMORLAND. Grasmere ... ... (By kind permission of Sir Francis C. Danson)	Oak† (*Quercus robur*)	5½ ft.	24 × 8 × 42 deep	In private grounds, close to house	6.7.1919. Numerous larvae and pupae of *A. plumbeus.* Aperture almost blocked by a thick layer of soil in which a large fern was growing.
WALES. FLINTSHIRE. Prestatyn	Sycamore (*Acer pseudo-platanus*)	4½ ft.	12 × 12 × 12 deep	In lane 200 yards from hotel	20.12.1919. Many small larvae of *A. plumbeus.*
DENBIGHSHIRE. Abergele. ... ... No. 1.	Sycamore (*Acer pseudo-platanus*)	4 ft.	—	In school grounds; 50 yards from houses	22.12.1919. Small to medium size larvae of *A. plumbeus.*
No. 2	Wych Elm (*Ulmus montana*)	15 ft.	—	Between two cottages and about 10 yards from each	22.12.1919. Small to medium size larvae of *A. plumbeus.* Occupants of cottages* complain of 'bites from buzzing gnats in summer, which raise swellings on arms and legs.'
langollen ... ... No. 1	Horse-chestnut (*Aesculus hippo-castanum*)	20 ft.	10 × 6 × 24 deep	In private grounds, 50 yards from house	3.1.1920. Small larvae of *A. plumbeus* numerous. Altitude 400 ft.
No. 2	Do.	10 ft.	12 × 12 × 24 deep	In private grounds, 10 yards from house	do.
CARNARVONSHIRE nfairfechan ... By kind permission of Mervyn T. Archdale, Esq., M.D.)	Elm (*Ulmus campestris*)	1½ ft.	— × — × 18 deep	In small wood with fairly dense vegetation: bordering cricket ground	—.9.1919. Several pupae and numerous larvae of various sizes of *A. plumbeus.*
IRELAND. Co. DUBLIN. gstown ... ... o. 1	Sycamore (*Acer pseudo-platanus*)	1½ ft.	12 × 12 × 12 deep	In private grounds; several houses within a radius of 50 yards	30.12.1919. Small and medium size larvae of *A. plumbeus* numerous.
o. 2	Do.	13 ft.	16 × 9 × 18 deep	In garden; house within 15 yards	do.
stown District ... y kind permission of M. V. Blacker Douglass, Esq.)	Do.	8 ft.	10 × 10 × 4 deep	In private grounds; 500 yards from house	27.12.1919. Few small larvae of *A. plumbeus.*
ley ... ... bserver Mrs. Stopford Douglas)	Do.	—	—	Nearest house 300-400 yards distant	26.1.1920. Small larvae of *A. plumbeus.*

* Scotland. Breeding-places see footnote, p. 446.

† Also in an Ash tree (*Fraxinus excelsior*) 14.2.1920.

sometimes necessary when the aperture is small. Many rot-holes of course are conspicuous and can be detected several yards away, but the apertures of others are small or at considerable heights above the ground or are so well concealed by rotten pieces of branch or ferns that most careful examination is necessary. The location of breeding-places is distinctly easier in winter. We have been obliged to confine ourselves to the examination of holes situated not more than twenty feet from the ground, but even with such a limit it is extremely difficult to decide that breeding-places, potential and actual, do not exist in any given locality; *and we believe that the proof of their non-existence within a given area will necessitate the closest scrutiny of every tree within that area.*

Larval Habits

The larvae of *A. plumbeus* were, in all probability, first taken by Grassi (1901) in Italy, but it is abundantly clear that this author associated them with *A. bifurcatus* and regarded the peculiar type of breeding-place as occasional for this latter species. Eysell (1912) first described the larvae, his specimens being reared from eggs laid by captive females. Meinert's (1886) description of the larva of *A. nigripes* clearly applies to that of *A. bifurcatus* as may readily be perceived from his figure. To Galli-Valerio and de Jongh (1912[2] and 1913) must be given the credit of finding them in nature and of recognising the great selection shown by the species in its choice of a breeding-place.

Their movements and habit of living mainly at the surface of the water appear, judging by laboratory specimens, to be typically Anopheline. Galli-Valerio and de Jongh (1913), however, state that they require very little air, seldom show themselves at the surface and remain chiefly at the bottom among the débris. We have not observed this to be the case and think it probable that these authors were misled by the rapidity with which the larvae descend when disturbed. On the slightest provocation the larvae move downwards, the small ones wriggling actively, the large ones usually after a few strong jerks of their bodies sinking motionless to the bottom where they lie in any position among the débris. Larvae, particularly older ones, will remain apparently dead at the bottom for considerable periods; we have watched them lie thus for twenty minutes or half-an-hour.

The main food of the larvae is as yet undecided. Christophers (1916) and Edwards (1916) maintain that they are carnivorous or semi-carnivorous, existing largely upon insects which fall into the water. The larvae of *A. barberi* and *A. culiciformis* moreover show strong cannibalistic tendencies and it is probable, therefore, that the above opinion is correct. We have observed that both *A. plumbeus* and *O. geniculatus* larvae are greatly attracted by the dead and floating bodies of 'rat-tailed maggots' of which they ultimately leave only the tips of the syphon-tubes. Eysell (1912) successfully reared adults from the egg by supplying the larvae with bacterial scum from hay infusion.

The stage in which hibernation takes place has been variously surmised, and although Galli-Valerio (1917[1]) found larvae in tree-holes the water of which was covered with ice, no decision on the matter seems to have been reached. Eckstein (1918[1]) discussing this question states that it is unlikely that they over-winter as larvae, as during the winter the tree-holes either dry out or the water is frozen into a solid block; for this reason, also, he has never found hibernating larvae in spite of having repeatedly searched known breeding-places. We fail to follow this author's statements and are convinced that *A. plumbeus* does hibernate in the larval stage. We have obtained young larvae on numerous occasions in December, January and February, and have found no evidence of the holes drying out during these months. That the larvae can revive after the water in which they live has been frozen solid for short periods is shown by the following experiments (Table II).

We can offer no explanation of the results obtained in Experiment 5. In all the experiments the larvae were contained in water taken with them from the rot-holes. In Experiments 3-9 all the larvae after subjection to the low temperature were encased in blocks of ice, and even when the surface layers of water remained liquid, they were embedded in the ice below. This appeared to be due to the paralysing effect of the low temperature (acting from below) causing them to sink to the colder layers of the liquid which shortly after became frozen. In most cases the larvae at the end of the experiments resumed their activities remarkably quickly, and many, particularly the small ones, showed signs of movement directly their bodies, or portions of their bodies, were freed from the ice.

TABLE II.

Freezing Experiments

Experiment	Species	Description of larvae used	Amount of water in larval container	Temperature of water before freezing	Time immersed in freezing mixture (Temp. of mixture −4° C. to −2° C.)	Result of immersion on water containing larvae	Condition of larvae 20 hours after removal of container from freezing mixture	Remarks
1	*A. plumbeus*	Five small and one apparently half-grown	5 c.c.	13° C.	5 minutes	No ice present	All active and apparently unaffected	
2	Do.	do.	do.	do.	10 minutes	do.	do.	
3	Do.	Four small	do.	do.	15 minutes	Lower layers containing larvae frozen solid	do.	One larva remained motionless for at least one hour after removal of container from mixture
4	Do.	Five small and one apparently half-grown	do.	do.	20 minutes	Frozen solid	do.	
5	Do.	do.	do.	do.	25 minutes	do.	One small larva active; rest apparently dead, but showing slight movement under binocular	The small larva regained activity immediately after thawing. Rest dead 40 hours after removal of container from mixture
6	Do.	do.	15 c.c.	do.	30 minutes	do.	All active and apparently unaffected	
7	Do.	Six small	10 c.c. + 5 c.c. débris	do.	do.	do.	do.	
8	*O. geniculatus*	Four half-grown and one apparently mature	15 c.c.	do.	do.	do.	do.	
9	Do.	One half-grown and one nearly mature	15 c.c.	do.	do.	do.	do.	

In connection with the above experiments Howard, Dyar and Knab's (1912) remarks concerning the larvae of the pitcher-plant mosquito, *Wyeomyia smithii*, are of interest; they state that 'the larvae survive complete freezing up of the water in the leaves of the plant and complete their development in the following season.'

The duration of the larval stage in summer appears to be about four weeks. Eysell (1912) gives the length of this stage as 28 days and the cycle from egg to adult as 33-35 days, and Christophers (1916) obtained the full evolution in about four weeks. The larvae observed by both these authors received an ample supply of food. Galli-Valerio and de Jongh (1913) give the minimum time for the completion of the cycle as 30 days and state that the larval period is often prolonged, so that the metamorphosis from egg to adult may occupy as long as eleven months. The factor of hibernation entered into the last named period, but in cases where it did not act and the larval stage was prolonged the cause may have been a deficient or unsuitable food supply. These observers make no particular reference to the food provided, and the inference therefore is that the larvae were merely placed in jars with water and débris from the rot-hole, and such conditions, according to Edwards (1916) do not favour rapid development.

The larvae of *A. plumbeus* are often found associated with those of the Aëdine mosquito, *Ochlerotatus geniculatus*, Ol., with which they appear to live in amity. Recently Macgregor (1919[1 & 2]) has found them in Epping Forest with the larvae of a previously undescribed species of *Orthopodomyia* and apparently also with those of *Stegomyia fasciata*, Fabr.* Other dipterous larvae not infrequently occurring in the rot-holes are those of various Chironomids and the 'rat-tailed maggots' of *Myiatropa florea*, L.†, and *Helophilus pendulus*, L.

DISTRIBUTION OF *A. plumbeus* IN THE BRITISH ISLES

The known distribution of this mosquito in this country is shown in Map 2. At present records from about fifty localities only are available, and most of these are isolated and due to the capture of

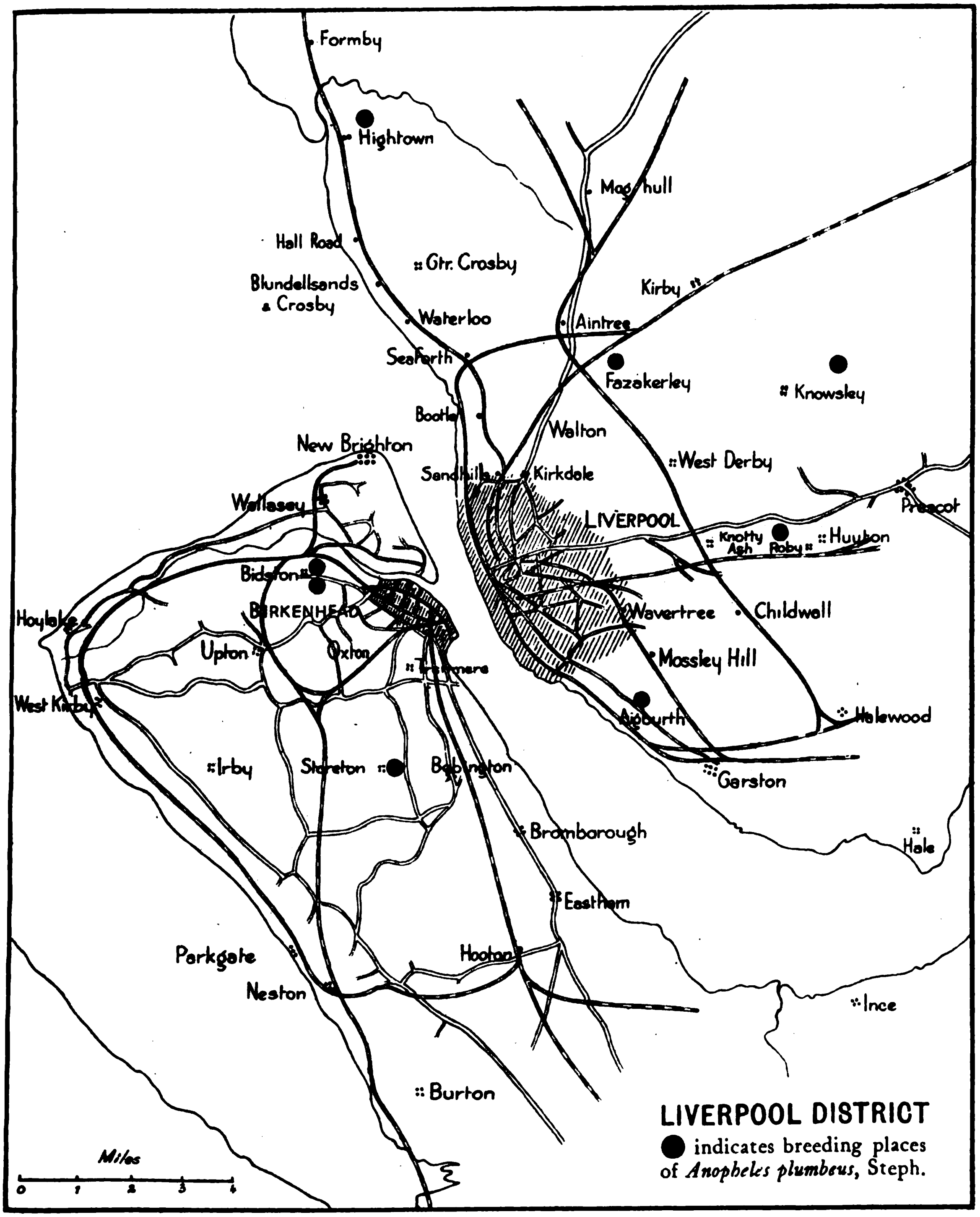
* The larvae of this mosquito were not actually observed by Macgregor, but two males emerged from the tank in which the tree-hole species were kept.

† Identification kindly confirmed by Major E. E. Austen of the British Museum.

occasional blood-seeking females. Such records spread over a long period of time, and give no clue to the abundance of the species. Much attention has been given to our other indigenous Anophelines—*A. maculipennis* and *A. bifurcatus*—and valuable information has been obtained regarding their bionomics and distribution, but *A. plumbeus*, on account of its apparent rarity, has been greatly neglected. Even in places where it was known to occur, no serious attempts to discover its habits appear to have been made, and it was taken for granted that in these it resembled the other two species. Edwards (1916), following Galli-Valerio and de Jongh's (1912 and 1913) work in Switzerland, discovered the breeding-places of *A. plumbeus* at Burnham Beeches in Buckinghamshire, but until last year not more than three or four additional records of this nature had been made. Our investigations began in June, 1919, and the direct results, so far as this line of research is concerned, are shown in the Maps 1 and 2 and in Table I. We have found breeding-places in every locality which we have searched, and although no attempts at intensive surveys have been made, and no idea of the numerical distribution of the mosquito in any locality obtained, we are convinced that the species is widely and commonly distributed throughout the country.

Occurrence of *A. plumbeus* in the Liverpool District

With reference to the occurrence of the adults of this species in the Liverpool district, Newstead informed Scott (quoted by Lang (1918)) that *A. plumbeus* was relatively scarce. Beyond this, the only records from Lancashire are those of Theobald (1910, and in Lang (1918)) from Manchester. Map 1 shows that we have discovered a number of breeding-places of this species at varying distances from the centre of the city and at approximately equal distances from each other. But it must not be supposed that no breeding-places exist in the intervals. In all probability they do, but time did not allow of a more extensive survey, and we were obliged to limit ourselves to particular localities. At the commencement of the investigations we naturally selected spots which we thought, from the nature of the country, would be most likely to prove satisfactory. But as success attended our search and the diffuse nature of the distribution of this mosquito was realised, we only


Formby
Hightown
Maghull
Hall Road
Gtr. Crosby
Blundellsands
& Crosby
Waterloo
Kirby
Aintree
Seaforth
Fazakerley
Knowsley
Bootle
Walton
New Brighton
West Derby
Sandhills
Kirkdale
Wallasey
Prescot
LIVERPOOL
Knotty
Ash
Roby
Huyton
Bidston
BIRKENHEAD
Hoylake
Wavertree
Childwall
Upton
Oxton
Mossley Hill
West Kirby
Aigburth
Halewood
Irby
Storeton
Bebington
Garston
Bromborough
Hale
Eastham
Parkgate
Hooton
Neston
Ince
Burton
LIVERPOOL DISTRICT
indicates breeding places
of *Anopheles plumbeus*, Steph.
Miles
0
1
2
3
4

MAP I

chose such places as would, by their direction, enable us to complete as far as possible the cordon of breeding-places round the town. In one of these districts the outlook was most unpromising, as trees were by no means numerous; yet the result was successful, and a breeding-place (several likely rot-holes too high for us to examine were also seen) was found within two hours. At first we examined places situated from five to eight miles away, but subsequently we reduced the distance. Within the four-mile limit the work was more difficult, as most of the trees are enclosed in private property; nevertheless on several occasions breeding-places were found.

From the above remarks it will be seen that *A. plumbeus* is not uncommon in the Liverpool area, that it occurs widely distributed round the city and within the four-mile limit.

Possible connection of *A. plumbeus* with the Spread of Malaria

The importance of *A. plumbeus* as a natural carrier of malaria cannot yet be estimated. Far more detailed information regarding its bionomics and distribution is required before any definite statement can be made. But in our opinion the matter is not to be dismissed so lightly as is being done; on the contrary, *A. plumbeus* is worthy of serious consideration in this respect.

In country districts or woodlands much frequented by the public the possibility of the dissemination of the disease by this mosquito can be readily appreciated; for when old trees or trees whose preservation has been neglected are numerous the opportunities for breeding will be greater, and the output of adult mosquitoes increased. But in towns or villages not situated in the immediate neighbourhood of large woods the question is more complex. It will be urged that *A. plumbeus* is a 'wild' species, and that in such places it is extremely rare and consequently of little or no importance. This may be so, but neither the degree of its domestication nor its numerical distribution can be measured solely by the frequency with which adults are observed in man's dwellings. The extent to which it occurs in and around the larger towns can only be decided by careful intensive surveys for breeding-places. The adult habit of resting in the rot-holes renders any conclusions drawn from an adult census elsewhere entirely misleading. From 1910 to 1916 a few isolated specimens only of *A. plumbeus* had been found in Northern India, but Christophers (1916), after his discovery of the breeding-places at Simla and his subsequent observations, stated that 'there can be no doubt that in the particular area investigated *Anopheles* (in the form of *A. barianensis**) must have been quite numerous.' It seems to us not improbable that the highly selective and unusual habits of a mosquito such as *A. plumbeus* may, at least in some cases, have a direct bearing upon the condition known as 'Malaria *sine* Paludism,' where malaria may occur in a locality which appears relatively free from Anophelines. It has been repeatedly observed that marshiness and the abundance of Anophelines are not always in proportion to the extent and severity of a malaria epidemic; Celli (1903), in fact, states that in Basilicata in Italy, and in Sicily, this proportion is inverse, the area of marsh being very small and sometimes limited to the beds of torrents or streams and Anophelines scanty, while malaria is common and of a severe type.

It has been shown (cf. p. 429) that the breeding-places of *A. plumbeus* may occur in more or less isolated trees, and when such trees are situated within a few yards of a house (cf. Table I, Fazakerley, Grasmere, Abergele No. 2, Llangollen No. 2, and Kingstown Nos. 1 and 2) it is practically certain that the females in their search for blood will frequent this house with considerable regularity.

These considerations make it desirable to investigate fully the immediate surroundings of any houses to which the occurrence of cases of malaria seems peculiarly restricted, with a view to discovering the breeding-places of *A. plumbeus*.

ACKNOWLEDGMENTS

We are indebted to Professor W. Ramsden for analysing two samples of rot-hole water submitted to him. Our thanks are also due to Mrs. Stopford Douglas for obtaining and sending larvae from Killiney, Co. Dublin, and to those gentlemen, named in the table of breeding-places, who have courteously granted us permission to examine the trees in their private grounds.

* *A. barianensis* is synonymous with *A. plumbeus* (see p. 422).

PRELIMINARY REPORT BY PROFESSOR W. RAMSDEN ON LIQUIDS FROM CAVITIES OF HOLLOW TREES

SPECIMEN 1. *Filtrate of mixed samples of liquids in all of which* A. PLUMBEUS *was breeding.*

Inodorous. No froth when shaken. Brown (porter) colour with slight greenish fluorescence; spectroscopically, general absorption of the blue end, but no definite band. Specific gravity: 1004.

Total solids from 100 c.c. evaporated to dryness on water-bath 0·960 grams.

Total Nitrogen by Kjeldahl method: 0·0053 grams in 100 c.c.

Reaction: markedly alkaline to litmus.

Gives off CO_2 with mild effervescence when acidified, therefore contains Carbonates.

Dry residue chars strongly when heated sufficiently; smell does not suggest either proteins or carbohydrates.

Ash obtained rich in Calcium, Potassium and Phosphates, and contains also Sodium, Iron, Magnesium, Chlorides and Sulphates.

Surface-tension: markedly less than water.

The enzyme 'catalase' is absent.

A doubtful Molisch's reaction is obtainable, although a small quantity of a Furfural is given off when the liquid is boiled with 60 per cent. H_2SO_4 (anilin acetate turned pink): therefore some carbohydrate grouping in small quantity is presumably present.

No fermentation with yeast. No colour with Iodine solution. No reduction of Fehling's or Nylander's solutions.

The brown pigmented organic matter is precipitated on adding any strong mineral acid or oxalic acid, but not by acetic acid. It is also salted out by saturation with ammonium sulphate. It gives a strong 'xanthoproteic' reaction. It is not a protein.

SPECIMEN 2.* *Filtrate of sample in which no larvae were found.*

Specific gravity was greater: 1009, but in every qualitative respect the statements made above for the first liquid are equally true for the second.

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* See footnote on p. 428.

LITERATURE

ALCOCK, A. (1911). Entomology for Medical Officers, London, p. 70

AUSTEN, E. E. (1906). Illustrations of British Blood-sucking Flies. London. p. 18.

BACOT, A. (1918). Mosquitoes and the danger of Malaria in England. *Essex Naturalist.* Vol. 18, pp. 241-263.

BASTIANELLI, G., BIGNAMI, A., and GRASSI, B. (1898). Zoologia medica—Coltivazione delle semilune malariche dell' uomo nell' *Anopheles claviger,* Fabr. (sinonimo : *Anopheles maculipennis, Meig.*) *Atti della Reale Accad. dei Lincei.* Vol. 7, pp. 313-314.

BEZZI, M. (1892). Contribuzione alla fauna Ditterologica della provincia di Pavia. *Bull. Soc. Ent. Ital.* Anno. 24, p. 133.

BLACKLOCK, B., and CARTER, H. F. (1919). The Experimental Infection of *Anopheles plumbeus,* Hal., with *Plasmodium vivax.* (Sporozoites in Salivary Glands). Preliminary Note. *Ann. Trop. Med. & Parasitol.* Vol. 13, pp. 187-188.

BLANCHARD, C. (1918). Sur un nouveau type larvaire du groupe des Anophélinés. *Bull. Soc. Path. Exot.* T. 10, pp. 669-677.

BLANCHARD, R. (1905). Les Moustiques. Histoire naturelle et médicale Paris, pp. 164 and 166.

——— (1918). *Bull. de l'Académie de Médicine.* August. (Note regarding the identity of the larvae obtained by C. Blanchard, *vide supra*). 3e Sér. T. 80, p. 146.

CELLI, A. (1903). La malaria in Italia durante il 1902. *Atti della Soc., per gli Studi della malaria.* Vol. 4, p. 551.

CHRISTOPHERS, S. R. (1916). An Indian tree-hole breeding *Anopheles, A. barianensis,* James= *A.* (*Coelodiazesis*) *plumbeus,* Haliday. *Ind. Journ. Med. Res.* Vol. 3, pp. 489-496.

CHRISTOPHERS, S. R., and KHAZAN CHAND (1916). A tree-hole breeding *Anopheles* from Southern India : *A.* (*Coelodiazesis*) *culiciformis,* Cogill. *Ind. Journ. Med. Res.* Vol. 3, pp. 638-645.

COQUILLETT, D. W. (1903). A new Anopheles with unspotted wings. *Canad. Ent.* Vol. 35, p. 310.

CORDIER, E. (1918). Capture en Argonne d'*Anopheles nigripes,* Staeger 1839, espèce nouvelle pour la faune française. *Bull. Soc. Path. Exot.* Tome 11, pp. 726-727.

DONOVAN, C. (1901). Anopheles in Ireland. *B. M. J.* Vol. 2, p. 14.

DYAR, H. G. (1918). Notes on American Anopheles. *Insecutor Inscitiae Menstruies.* Vol. 6, pp. 141-151.

DYAR, H. G., and KNAB, F. (1906). The larvae of Culicidae classified as independent organisms. *Journ. N.Y. Ent. Soc.* Vol. 14, p. 177.

ECKSTEIN, F. (1918[1]). Die Ueberwinterung unserer Stechmücken. *Biol. Central.* Bd. 38, pp. 530-536

——— (1918[2]). Zur systematik der einheimischen Stechmücken. 1. Vorläufige Mitteilung : Die Weibchen. *Cent. f. Bakt. Parasit. u. Infektionskr.* 1te Abt. Orig. Bd. 82, pp. 57-68.

——— (1919). Zur systematik der einheimischen Stechmücken. 2. Vor. Mitt : Die Larven. *Ibid.* Bd. 83, pp. 281-294.

EDWARDS, F. W. (1912). Notes on the British Mosquitos (Culicinae). *The Entomologist.* Vol. 45, pp. 193-194.

——— (1916). Notes on Culicidae, with descriptions of new species. *Bull. Ent. Res.* Vol. 7, p. 201.

EYSELL, A. (1912). *Cyclophorus* (*Anopheles*) *nigripes,* Staeger (nov. gen.) *Arch. f. Schiffs u. Tropen Hyg.* Bd. 16, pp. 421-431.

——— (1913). Die Stechmücken. *Handbuch der Tropenkrankheiten. Leipzig.* Bd. 2, pp. 44-94.

FICALBI, E. (1896). Revisione sistematica della famiglia delle Culicidae Europee. Firenze, pp. 99, 109 and 217.

——— (1899). Venti specie di Zanzare (Culicidae) italiane classate, descritte e indicate secondo la loro distribuzione corologica. *Bull. Soc. Ent. Ital.* Anno. 31, pp. 46-234.

GALLI-VALERIO, (1915[1]). Studi e ricerche sui Culicidi. *11a Mem. La Malariologia.* Vol. 8, pp. 141-143.

——— (1915[2]). Beobachtungen über Culiciden. *Cent. f. Bakt. Parasit. u. Infektionskr.* 1te. Abt. Orig. Bd. 76, pp. 260-261.

——— (1916). Beobachtungen über Culiciden. *Cent. f. Bakt. Parasit. u. Infektionskr.* 1te. Abt. Orig. Bd. 78, pp. 90-96.

——— (1917[1]). Beobachtungen über Culiciden. *Ibid.* Bd. 79, pp. 139-143.

——— (1917[2]). La distribution géographique des Anophélines en Suisse au point de vue du danger du formation de foyers de Malaria. *Bull. Office Internat. d'Hyg. Publique*, Paris. T. 9, pp. 1566-1582.

GALLI-VALERIO, B., and ROCHAZ DE JONGH, J. (1903). Studi e ricerche sui Culicidi dei generi Culex e anopheles. *Atti della Soc. per gli Studi della malaria.* Vol. 4, p. 44.

——— (1912[1]). Beobachtungen über Culiciden und Mitteilung über das Vorkommen von *Phlebotomus papatasi*, Scop. im Kanton Waadt (Schweiz). *Cent. f. Bakt. Parasit. u. Infektionskr.* 1te. Abt. Orig. Bd. 63, pp. 222-227.

——— (1912[2]). Studi e ricerche sui Culicidi. 8a Mem. *Atti della Soc. per gli Studi della malaria.* Vol. 13, pp. 1-5.

——— (1913). Beobachtungen über Culiciden. *Cent. f. Bakt. Parasit. u. Infektionskr.* 1te. Abt. Orig. Bd. 67, pp. 472-478.

——— (1914[1]). Beobachtungen über Culiciden. *Ibid.* Bd. 72, pp. 529-531.

——— (1914[2]). Studi e ricerche sui Culicidi. 9a Mem. *Atti della Soc. per gli Studi della Malaria.* Vol. 14, pp. 1-5.

——— (1915). Studi e ricerche sui Culicidi. 10a *Mem. La Malariologia.* Vol. 8, pp. 9-11.

GILES, G. M. (1900). A Handbook of Gnats or Mosquitoes. London. 1st ed., p. 175.

——— (1902). *Ibid.* 2nd ed., p. 330.

——— (1904). A revision of the Anophelinae being a first supplement to the second edition of 'A Handbook of Gnats or Mosquitoes.' London. p. 23.

GRASSI, B. (1898[1]). Zoologica medica—Rapporti tra la malaria e peculiari insetti (zanzaroni e zanzare palustri). *Atti della Reale Accad. dei Lincei.* Vol. 7, pp. 163-172.

——— (1898[2]). Zoologica medica—La malaria propagata per mezzo di peculiari insetti. *Ibid*, pp. 234-240.

——— (1898[3]). Zoologica medica—Rapporti tra la malaria e gli artropodi. *Ibid*, pp. 314-315.

——— (1899). Ancora sulla malaria. *Ibid.* Vol. 8, p. 559.

——— (1901) Die Malaria. *Studien eines Zoologen. 2te vermehrte Auflage.* Jena, pp. various.

——— (1903). Documenti riguardanti la storia della scoperta del modo di trasmissione della malaria umana. Milano, p. 96.

GRASSI, B., BIGNAMI, A., and BASTIANELLI, G. (1899[1]). Ciclo evolutivo delle semilune nell'. *Anopheles claviger* ed. altri studi sulla malaria dall' Ottobre 1898 al maggio 1899. *Atti della Soc. per gli Studi della Malaria.* Vol. 1, pp. 14-27.

——— (1899[2]). Resoconto degli studi fatti sulla malaria durante il mese di gennaio. *Rendiconti della Reale Accad. dei Lincei.* Vol. 8, Feb. 5.

——— (1899[3]). Ulteriori ricerche sulla malaria. *Ibid.* Vol. 8., May 7.

GRUNBERG, K. (1910). Diptera Zweiflügler. Brauer: Die Süsswasserfauna Deutschlands. Heft. 2 A., p. 83.

HALIDAY, A. H. (1828). Notice of Insects taken in the North of Ireland. *Zool. Journ.* Vol. 3

——— (1833). Catalogue of Diptera occurring about Holywood in Downshire. *Ent. Mag.* Vol. 1, pp. 148 and 151.

HERMES, W. B. (1915). Medical and Veterinary Entomology. New York, p. 99.

HESSE, E. (1918). Sur la presence dans le Dauphiné de l' *Anopheles nigripes*, Staeger. *Arch. Zool. Expér. Notes et Revue.* Paris, T. 57, pp. 32-35.

HINDLE, E. (1914). Flies in Relation to Disease. Blood-sucking Flies. Cambridge, pp. 81 and 102.

HOWARD, L. O., DYAR, H. G., and KNAB, F. (1912-1917). The Mosquitos of North and Central America and the West Indies. Vol. 1, p. 417. Vol. 4, pp. 1035-1038.

JAMES, S. P. (1911). *Anopheles barianensis*, James. A monograph of the Anopheline Mosquitoes of India. 2nd edition. Calcutta, pp. 55 and 76.

——— (1917). Note recording the proof that *Anopheles maculipennis* is an efficient host of the Benign Tertian parasite in England, *Journ. R.A.M.C.* Vol. 29, p. 615.

JARVIS, F. E. (1919). On the occurrence of the immature stages of Anopheles in London. *Ann. Applied Biology*. Vol. 6, pp. 40-47.

LANG, W. D. (1918). A map showing the known distribution in England and Wales of the Anopheline Mosquitoes with explanatory text and notes. *British Museum* (*Nat. Hist.*) London, pp. 48-52 and 63.

LOEW, H. (1845). Dipterologische Beiträge. Posen. p. 4.

LANGERON, M. (1918). Presence de l'*Anopheles nigripes*, Staeger, 1839 dans la region parisienne. *Bull. Soc. Path. Exot.* T. 11, p. 728.

MACGREGOR, M. E. (1919[1]). On the occurrence of *Stegomyia fasciata* in a hole in a beech tree in Epping Forest. *Bull. Ent. Res.* Vol. 10, p. 91.

——— (1919[2]). A new mosquito of the genus *Orthopodomyia* from a beech tree-hole in England. *Journ. R.A.M.C.* Vol. 33, pp. 451-454.

MARTINI, E. (1915). Über drei weniger bekannte deutsche Kuliziden: *Aëdes ornatus*, Mg. *Mansonia richiardii*, Fic. und *Anopheles* (*Coelodiazesis*) *nigripes*, Staeger. *Arch. f. Schiffs. u. Tropen. Hyg.* Bd. 19, pp. 585-607.

MEINERT, F. (1886). De eucephale Myggelarver. Sur les larves eucéphales des Dipteres. Leurs moeurs et metamorphoses. Danske videnskaberne Selsk. Skrift. Bd. 3, pp. 373-493.

MITZMAIN, M. B. (1916). Anopheles infectivity experiments. *U. S. P. H. S. Pub. Health Rep.* Washington. Vol. 31, p. 2325.

NUTTALL, G. H. F. (1899-1900). Neuere Forschungen über die Rolle de Mosquitos bei der Verbreitung der Malaria. *Cent. f. Bakt., Parasit. u. Infektionskr.* 1te Abt. Bd. 25, pp. 877-881. Bd. 26, pp. 140-147 and Bd. 27, pp. 218-225.

NUTTALL, G. H. F., COBBETT, L., and STRANGEWAYS-PIGG, T. (1901). Studies in Relation to Malaria, I. The geographical distribution of Anopheles in Relation to the former distribution of ague in England. *Journ. Hyg.* Vol. 1, pp. 4-44.

NUTTALL, G. H. F., and SHIPLEY, E. (1901). Studies in Relation to Malaria II. The Structure and Biology of Anopheles (*Anopheles maculipennis*). The Egg and Larva. *Ibid.* Vol. 1, pp. 45-73.

OSTEN-SACKEN, C. R. (1878). Cat. Dipt. N. America. 2nd ed., p. 19.

PARSONS, A. C., and BROOK, G. R. (1919). The mosquito problem in Britain: Suggestions for a winter campaign against the important mosquitoes, with notes on Insecticides. *Journ. R.A.M.C.* Vol. 32, pp. 1-23.

PATTON, W. S., and CRAGG, F. W. (1913). A Text-book of Medical Entomology. Calcutta, p. 241.

PEJU, G., and CORDIER, E. (1918). Epidémie palustre et conditions de l'Anophélisme en Argonne. *C. R. Soc. Biol.*, Paris. T. 81, pp. 1039-1041.

——— (1919. Paludisme et Topographie Anophélienne en Argonne a propos d'une épidemie de paludisme autochtone. *Bull. Soc. Path. Exot.*, Paris. T. 12, pp. 23-24.

SCHINER, J. R. (1864). Fauna Austriaca. Die Fliegen (Diptera). Wien. Bd. 2, p. 625.

SCHNEIDER, P. (1914). Beitrag zur Kenntnis der Culiciden in der Umgebung de Bonn. Verhand. Naturhist. Ver Preuss. Rheinlande u. Westfalens, Bonn. Bd. 70, pp. 1-54.

SCHOO, H. J. M. (1902). La Malaria in Olanda. *Atti della Soc. per gli studi della Malaria.* Vol. 3, pp. 189-208.

SMITH, J. B. (1906). Report of the mosquito investigation in 1905. *Rep. Ent. Dept., New Jersey Agric. Expt. Sta., for the year* 1905. pp. 671-675.

STAEGER, C. (1839). Systematik Fortegnelse over de hidtil i Danmark fundne Diptera. Krojer: Naturhistorisk Tidskrift. Bd. 2, p. 552.

STEPHENS, J. F. (1828). Note on the foregoing paper (Haliday, *ut supra*) with a description of a new species of Anopheles (*Anopheles grisescens*). *Zool. Journ.* Vol. 3.

——— (1829). A systematic catalogue of British Insects. London.

STEPHENS, J. W. W., and CHRISTOPHERS, S. R. (1908). The practical study of Malaria. London. 3rd. ed., p. 158.

STROBL, G. (1894). Die Dipteren von Steiermark. *Mittheil. Natur. ver. f. Steiermark.* Bd. 31, p. 200.

Theobald, F. V. (1901). A Monograph of the Culicidae of the World. Vol. 1, p. 202.

——— (1905). Culicidae. Wytsman: Genera Insectorum. Bruxelles 26me Fasc., p. 7.

——— (1908). Mosquitoes and Peat. *Nature*, Oct. 15, pp. 607-608.

——— (1910). A Monograph of the Culicidae of the World. Vol. 5, pp. 12 and 13.

WALKER, F. (1856). Insecta Brittannica. Diptera. Vol. 3, p. 249.

WINGATE, W. J. (1906). A preliminary list of Durham Diptera, with analytical tables. *Trans. Nat. Hist. Soc. of Northumberland, Durham and Newcastle-upon-Tyne.* Vol. 2, p. 62.

WRIGHT, M. J. (1901). The resistance of the larval Mosquito to cold. Notes on the habits and life-history of mosquitoes in Aberdeenshire. *B. M. J.* Vol. 1, pp. 882-883.

WULP, F. M. VAN DER (1877). Diptera neerlandica. De Tweevleugelige Insecten van Neder land. 's Gravenhague. Deel. 1, p. 331.

ZETTERSTEDT, J. G. (1850). Diptera Scandinaviae disposita et descripta (Lundae). Insecta lapponica. Lipsia. Tomus 9, p. 3467.

(1918). Reports to the Local Government Board on Public Health and Medical Subjects. (New series, No. 119).
Reports and papers on Malaria contracted in England in 1917. London.

(1919). *Ibid.* (New series, No. 123). Reports and papers on Malaria contracted in England in 1918. London.

NOTE.—(cf. p. 422)—A paper by B. Prashad on 'The description and life-history of a new species of *Anopheles* that breeds in holes in trees' (*Rec. Ind. Mus.*, Vol. 15, iii, 1918, pp. 123-127) has come to our notice too late for reference in the text. This author describes *Anopheles annandalei*, from a male, bred from a larva collected from a tree-hole in the Eastern Himalayas. The adult possesses spotted wings and a conspicuous tuft of scales on each of the hind femora and thus superficially resembles *A. asiatica*, Leic. Prashad indeed groups it with this species and with *A. barbirostris*, Wulp, and *A. wellingtonianus*, Alcock, but the excellent description and figure of the larva of *A. annandalei* given by this writer demonstrate conclusively that it is a member of the sub-genus *Coelodiazesis*.

REFERENCES TO LOCALITY RECORDS SHOWN IN MAP II.*

England.

1. Penzance and Helford, R., Cornwall.
2. Ugbrooke, Devonshire.
3. Sidmouth, Devonshire.
4. Lyndhurst, Hampshire.
5. New Forest, Hampshire.
6. Wye, Kent.
7. †Boxhill, Surrey (F. W. Edwards, Esq., private communication)
8. Merton, Surrey.
9. Acton, Middlesex.
10. Bushey Heath, Hertfordshire.
11. †Cassiobury Park, Watford, Hertfordshire
12. †Epping Forest, Essex
13. Rochford and Gt. Wakering, Essex.
14. †Burnham Beeches, Buckinghamshire.
15. †Hitchen, Hertfordshire (F. W. Edwards, Esq., private communication).
16. Radwell, Hertfordshire.
17. †Cambridge (F. W. Edwards, Esq., private communication).
18. Newmarket, Suffolk.
19. Butley, Suffolk.
20. Oxford.
21. Lamport, Northamptonshire.
22. †Longner Hall, S.E. of Shrewsbury, (R. F. L. Burton, Esq., private communication).
23. Attingham Park, Shropshire.
24. Shrewsbury.
25. Wood Eaton Manor, Staffordshire.
26. Bakewell, Derbyshire.
27. Poynton, Cheshire.
28. Manchester.
29. †Roby and Knowsley Moss, Lancashire.
30. †Aigburth Vale, Liverpool.
31. {†Hightown, Lancashire
†Fazakerley, Liverpool
32. †Storeton, Cheshire
33. †Bidston, Cheshire
34. †Grasmere, Westmorland

Wales.‡

35. †Prestatyn, Flintshire
36. †Abergele, Denbighshire
37. Colwyn Bay, Denbighshire.
38. †Llanfairfechan, Carnarvonshire.
39. Bettws-y-Coed, Carnarvonshire.
40. Beddgelert, Carnarvonshire.
41. †Llangollen, Denbighshire
42. Llangammarch Wells, Brecknockshire.

Scotland.‡

43. Edinburgh.
44. Culross, Fifeshire.
45. Aberdown Woods, Fifeshire
46. Fife.
47. Blairgowrie, Perthshire.
48. Torphins, Aberdeenshire.

Ireland.

49. Holywood, Co. Down.
50. Harold's Cross, Co. Dublin.
51. †Kingstown, Co. Dublin
52. †Killiney, Co. Dublin (Mrs. Stopford Douglas).
53. Courtmacsherry, Co. Cork.

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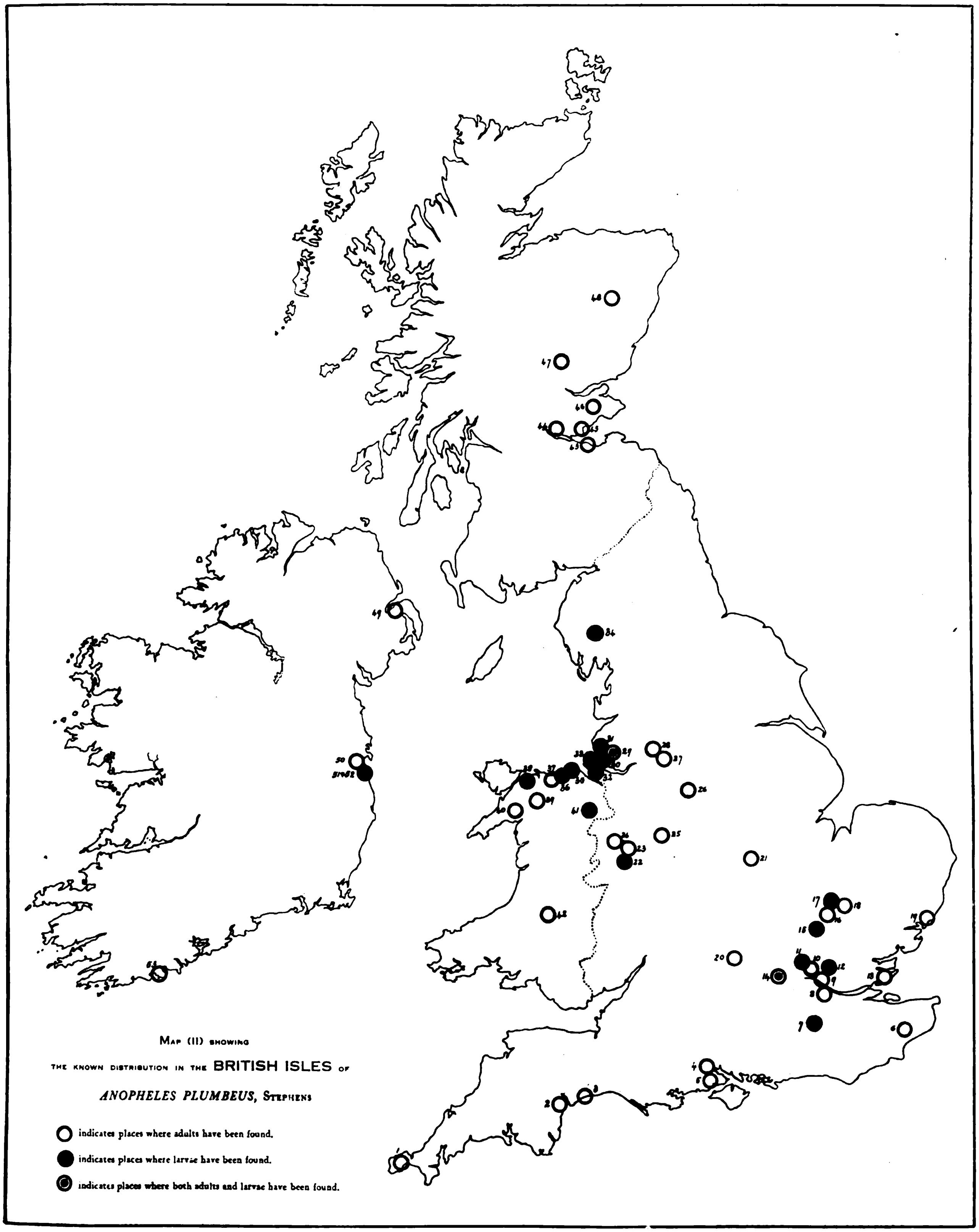
* This map is based upon that recently published by Lang, and most of the records of adult captures have been obtained from his work.

† Indicates breeding place found.

‡ Additional localities:—Trawsmawr, 4 miles north of Carmarthen, September, 1907. Prof. J. W. W. Stephens. Three females captured in garden when attempting to bite. Scotland: Skibo Castle, Sutherland, 29.2.1920. Breeding place in sycamore tree. Fearn, Ross and Cromarty, 1.3.1920. Breeding places in beech and scyamore trees, nearest houses 300-500 yards distant.



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EXPLANATION OF PLATES

Plate X

Breeding-places of *Anopheles plumbeus*, Steph., in the Liverpool district.

Fig. 1. Rot-hole in sycamore tree (*vide* record Bidston No. 4 in Table I).

Fig. 2. Rot-hole in sycamore tree (*vide* Bidston No. 3).

Fig. 3. Rot-hole in sycamore tree (*vide* Bidston No. 5).

Fig. 4. Rot-hole in sycamore tree (*vide* Bidston No. 2).

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Photographs by Miss M. Brown

C. Tinling & Co., Ltd., Imp

Plate XI

Breeding-places of *Anopheles plumbeus*, Steph.

Fig. 1. Rot-hole in tree (*vide* record Kingstown No. 2 in Table I). (Photograph by Commander Stopford Douglas, R.N.)

Fig. 2. Rot-hole in beech tree (*vide* Aigburth Vale record).

Fig. 3. Hole formed in the exposed root of a beech tree, Burnham Beeches, Bucks. (Photograph by Miss M. Carter.)

Fig. 4. Closer view of fig. 3. (Photograph by Miss M. Carter.)

NOTE.—In all the figures the black arrows are directed towards the orifices of the rot-holes; in fig. 2 ~~1~~ the white line indicates approximately the size of the cavity.

Fig. 1

Fig. 2

Fig. 3

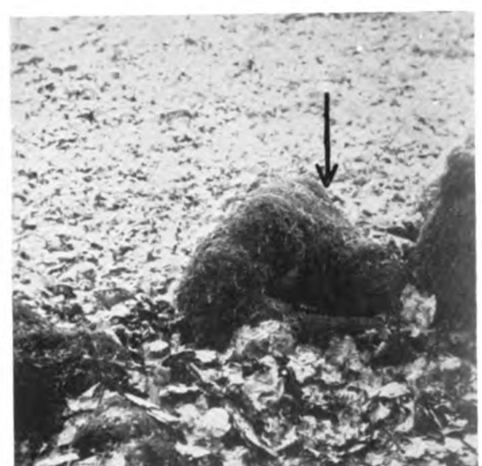

Fig. 4

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Plate XII

Larvae of the commoner English tree-hole breeding mosquitoes.

Fig. 1. *Anopheles plumbeus*, Steph. Larva in normal resting position at surface. (× 18 *circa.*)

Fig. 2. Do. do. (× 6 *circa.*)

Fig. 3. *Anopheles plumbeus*, Steph. Larva in position not uncommonly assumed at surface. (× 18 *circa.*)

Fig. 4. Do. do. (× 6 *circa.*)

Fig. 5. *Ochlerotatus geniculatus*, Ol. Larvae resting at surface. (× 6 *circa.*)

Fig. 6. Do. Larva. (× 18 *circa.*)

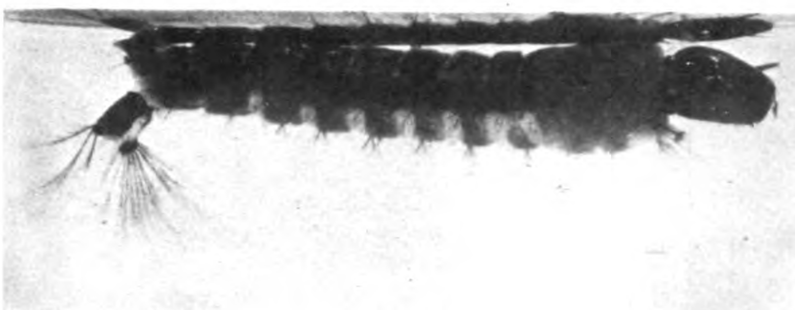

FIG. 1

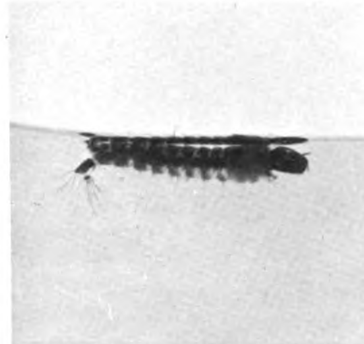

FIG. 2

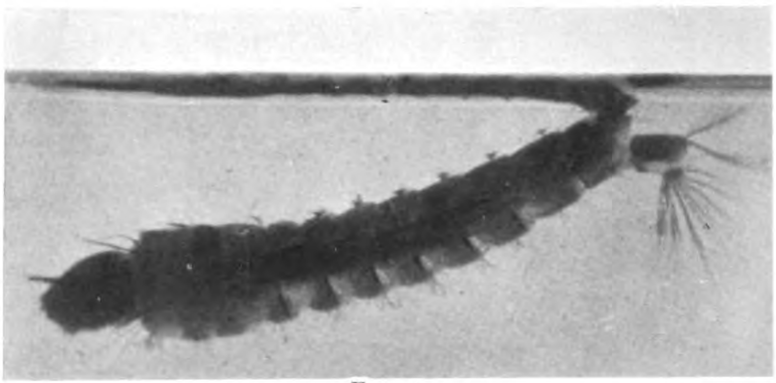

FIG. 3

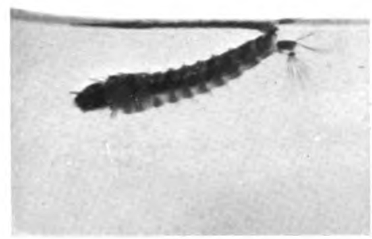

FIG. 4

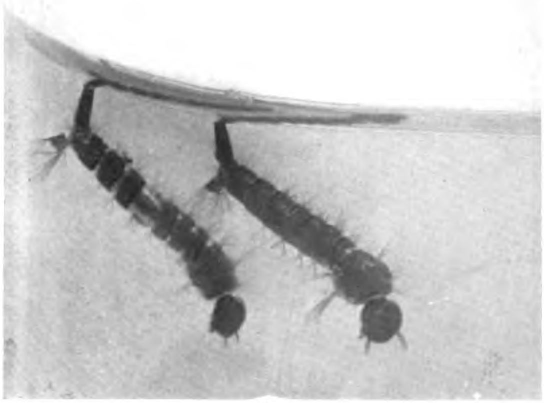

FIG. 5

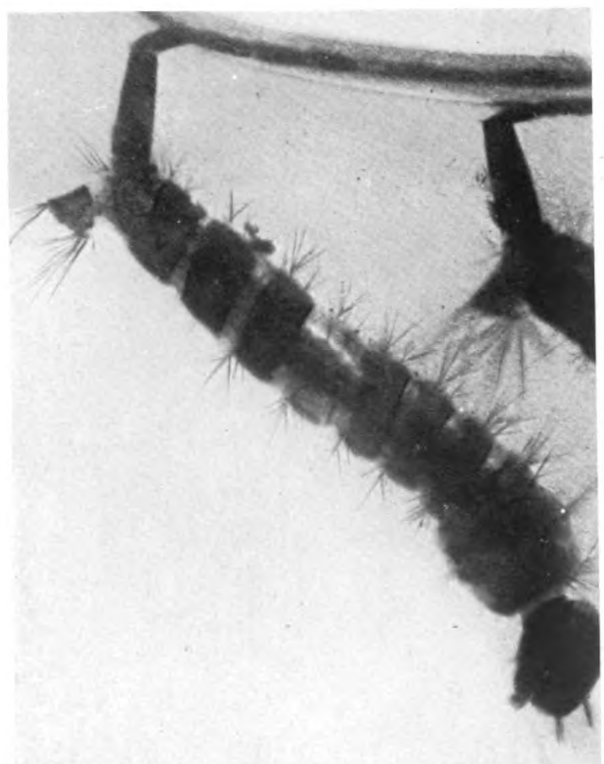

FIG. 6

Photographs by Miss M. Brown

C. Tinling & Co., Ltd., Imp

DESCRIPTIONS OF THE MALE GENITAL ARMATURES OF THE BRITISH ANOPHELINE MOSQUITOES

BY

HENRY F. CARTER

(*Received for publication* 16 *February*, 1920)

GENUS ANOPHELES, Meig.

Anopheles maculipennis, Meig.

(Figs. 1 and 2)

Basal portion of clasper more than twice as long as wide, broad and gently rounded distally. Dorsal surface clothed with hairs which are of moderate length on the outer side and at the apex. Ventral surface with scattered hairs on the sides and apical half, and two stout tuberculate spines at the base. Inner margin with a stout recurved bristle arising just beyond the middle. Terminal portion of clasper rather stout, about one and a quarter times as long as the basal portion, with very fine lateral hairs on the inner side, and a patch of very minute ones at the base. Apical tooth relatively short and broad.

Basal lobes (harpagones) relatively small, each composed of two portions; the larger inner portion clothed with short hairs and bearing on its apical margin a short stout hair or spine, a shorter, finer hair and two short pointed spines or bristles; the smaller portion, a tubercle, lying above the outer side of the main area, carrying one or two short blunt spines.

Penis sheath very long and narrow, with three almost straight leaflets on each side at its extremity; the apical leaflet is very long and extends as far as the tips of the spines arising from the basal lobes. Ninth segment with two conspicuous, slightly curved, rod-like, chitinous processes projecting backwards from the ventral surface. Anal membrane hairy.

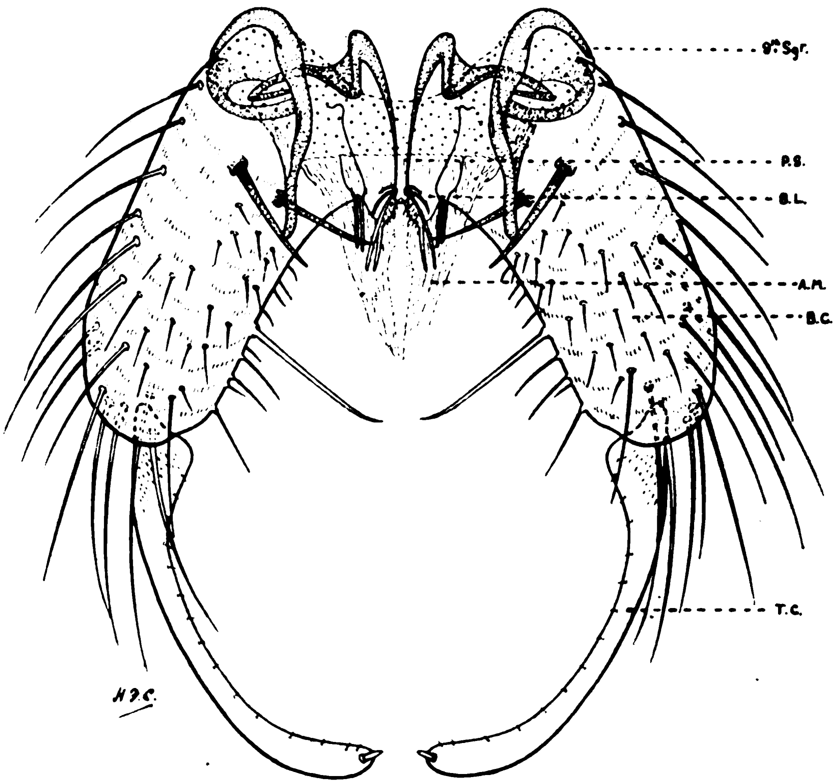

FIG. 1 *Anopheles maculipennis*, Meig. Male genitalia (× 160 circa). P.S., penis sheath; B.L., basal lobe (harpagone); A.M. anal membrane; B.C., basal portion of clasper; T.C., terminal portion of clasper.

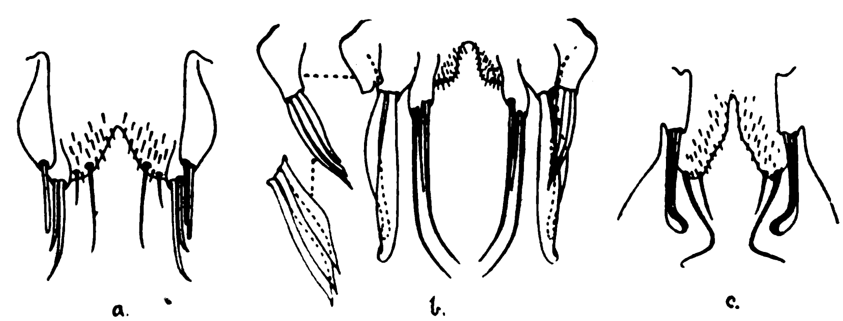

FIG. 2. Basal lobes (harpagones) of (*a.*) *A. maculipennis*, Meig.; (*b.*) *A. bifurcatus*, L. and (*c.*) *A. plumbeus*, Steph.

Anopheles bifurcatus, L.

(Figs. 2 and 3)

Basal portion of clasper about two and a half times as long as broad, sub-conical; apex broadly rounded. Dorsal surface with numerous short hairs on the inner half and many very long coarse hairs on the outer half and near the apex. Ventral surface with sparsely arranged hairs on the distal half (on the outer side they are of considerable length), a strong slightly recurved tuberculate spine and two vertical dendriform bristles at the base (see fig. 3). Inner

FIG. 3. *Anopheles bifurcatus*, L. Male genitalia (× 160 circa).

margin with a strong bristle, recurved at the tip, arising shortly before the apex. Terminal portion of clasper relatively stout, rather longer than the basal portion and bearing beside the usual hairs a group of fine ones near the base. Apical tooth short and stout.

Basal lobes large and complex, each consisting of three divisions; the innermost division is clothed with minute hairs and bears a large tubercle externally from which arise two moderately stout curved bristles and, between the latter, a short hair; the second portion lies below the third and bears two closely apposed unequal chitinous processes, the tips of which are composed of much thinner chitin and appear recurved when mounted without pressure; third division with three flattened spear-shaped spines.

Penis sheath elongate with eight short, slender terminal leaflets. Ninth segment without ventral processes. Anal membrane hairy.

Anopheles (*Coelodiazesis*) *plumbeus*, Stephens.

(Figs. 2 and 4)

Basal portion of clasper rather more than twice as long as broad, sub-conical with the apex broadly rounded. Dorsal surface with scattered hairs which are longer on the outer side and near the apex. Ventral surface with short scattered hairs, confined chiefly to the

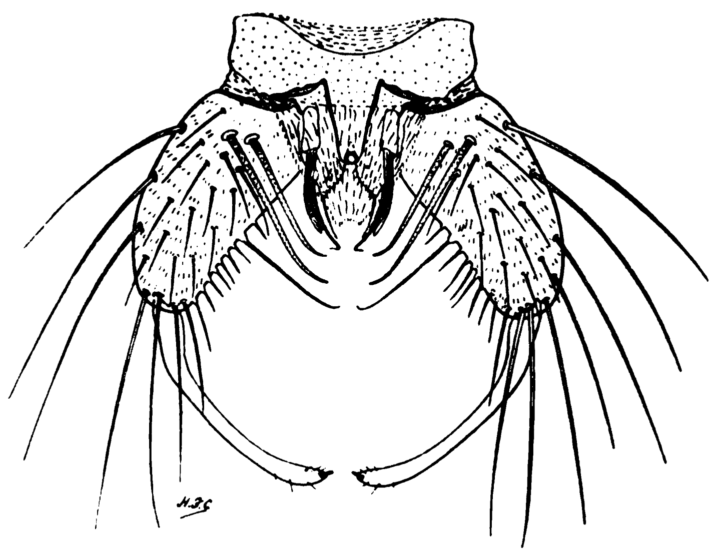

FIG. 4. ***Anopheles plumbeus***, **Steph. Male genitalia (× 160 circa.)**

outer side and apical half, and two strong basal spines with recurved tips arising from adjacent tubercles. Inner margin with a strong bristle, recurved at the apex, situated slightly beyond the middle.

Terminal portion of clasper moderately stout, about one and a quarter times the length of the basal portion with several fine lateral hairs on the inner side and three on the outer side near the apex. Apical tooth slender.

Basal lobes moderately large, the inner portion partly clothed with minute hairs and with a conspicuous hair and a long somewhat flattened curved bristle projecting from the apical margin; outer and ventral portion in the form of a small lobe or tubercle, bearing three or four curved, flattened, distally expanding spines or bristles.

Penis sheath short and broad; without terminal leaflets. Ninth segment without ventral processes. Anal membrane covered with small hairs.

ON HUMAN TRYPANOSOMIASIS IN PERU

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In December last, Escomel (1919) recorded that he had discovered trypanosomes in the blood of a patient coming from the tropical forests in the eastern portion of Peru bordering on Brazil and Bolivia. As this is the first recorded case of human trypanosomiasis in Peru, the observation is not without interest. The following is a summary of the clinical account of the case given by Escomel:—

'The patient stated that he had, for a long time, suffered from forest fevers of variable duration and intensity. At the time he was seen by Escomel the condition was sub-febrile, the pulse was feeble, and there was general pallor with the greenish tint typical of the inhabitants of the region in the centre of equatorial America. The eyelids, the limbs, and the rest of the body exhibited a condition of solid oedema, which, as there was neither valvular disease of the heart nor albuminuria, was really a myxoedema. The patient complained of great lassitude and extreme prostration, and stated that for some time he had suffered from uncontrollable desire to sleep, which overcame him no matter where he was. The reflexes were slightly decreased, and the appetite was very poor; the spleen and liver were hypertrophied.'

In view of the locality where the disease was contracted—the region bordering on Brazil where *Triatoma megista* and other vectors of trypanosomiasis exist—and of the symptoms exhibited, viz., myxoedema, previous febrile attacks followed by the sub-febrile condition, the great prostration and uncontrollable desire to sleep, Escomel considered it necessary to examine the blood of the patient. His research was rewarded by the discovery of a trypanosome, which, he states, is probably *Schizotrypanosom cruzi*, and adds that the existence of this trypanosome is not astonishing in the region where the disease was contracted.

The following is Escomel's account of the parasite based on examination of dehaemoglobinised thick films stained with Giemsa or Leishman:—

'Avec le Giemsa, ils se présentèrent teints en bleu clair avec le flagelle, le noyau et le blépharoplaste en rouge pourpre. Le protoplasma des leucocytes est bleu clair, leurs noyaux et les hématoblastes pourpre foncé. Le fond de la préparation est rose très pâle en raison des restes des hématies hémolysées.'

Les parasites se montraient très nets; onduleux, serpigineux, avec une longeur variant entre 20 et 40 μ, flagelle compris; largeur maxima, 3 à 4 μ. Le flagelle était toujours plus long que le corps protoplasmique.

Celui-ci est granuleux sur toute son étendue. La membrane ondulante a un aspect variable, suivant la position dans laquelle le parasite a été fixé.

Le noyau violet-pourpre au Giemsa, siège presque toujours à la hauteur de la moitié du corps.

Le blépharoplaste, petit, à peine visible, mais toujours bien différencié, se continue par le flagelle.'

Examination of this description reveals certain peculiarities which lead one to doubt whether Escomel's parasite is really *Schiz. cruzi*; unfortunately, the text figure illustrating the trypanosome is not sufficiently good to afford any aid in forming an opinion.

Two points in the description of the parasite call for special attention. In the first place, the size of the Peruvian trypanosome, viz., 20-40μ in length and 3-4μ in breadth, is very much greater than that of *Schiz-cruzi,* the length of which in the blood averages about 20μ, certainly never approaching 40μ, and the breadth of which is not more than 2-3μ; and, in the second place, the 'small, hardly visible blepharoplast' in no way resembles this structure in *Schiz. cruzi* where it is always voluminous and assumes either an elongate or, more usually, an ovoid form.

In these two respects, then, Escomel's trypanosome presents striking differences from *Schiz. cruzi.* Of course, it must be borne in mind that Escomel based his description on the examination of thick blood films, but this fact can hardly explain the great size of the Peruvian parasite and the minuteness of its blepharoplast..

An interesting feature in the symtomatology of the case, on which considerable stress is laid by Escomel, is the overpowering somnolence from which the patient suffered. So far as I am aware this symptom has not been noted in Chagas' disease, although, of course, it is a striking feature of African trypanosomiasis of man.

Assuming Escomel's description to be correct, one is compelled to conclude that the trypanosome found by him in the inhabitant of the eastern Peruvian forests differs from *Schiz. cruzi* and is in reality a new species. As the parasite in question is capable of infecting man, the question is important and one which requires further investigation. In the meantime, I propose for this Peruvian parasite of man the name of *Trypanosoma escomeli* in recognition of its discoverer.

REFERENCE

Escomel, E. (1919). *Bull. Soc. Path. Exot.*, Vol. XXI, p. 723.

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