THE MEASUREMENTS OF A THOUSAND EXAMPLES OF *TRYPANOSOMA VIVAX*

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The trypanosomes which form the basis of this communication are derived from a strain kept up in goats, which strain was obtained from a horse naturally infected in the Gambia. In a previous paper dealing with the trypanosomes found in this horse, Yorke and Blacklock (1911) acknowledged their indebtedness to Professor Todd for his kindness in sending the animal (*Horse A*) to Runcorn. As was stated in the above paper, two forms of parasites were present in the blood of *Horse A*, viz., a comparatively long, free-flagellated trypanosome, possessed of great activity of movement, and a short non-flagellated form, of sluggish movement. It is with the former of these trypanosomes, the long form, that this present paper deals, and the name *T. vivax* is used to include *T. cazalboui*, which it will be recalled Bruce (1910) says is probably the same species.

THE SPECIES OF ANIMAL HOST CHOSEN

In making drawings and measurements of a particular trypanosome, it appears desirable that all observers should, if possible, adhere to the same species of animal host in order to establish a ready standard for purposes of comparison. For this reason it would have been of advantage if one could have utilised one of the smaller laboratory animals, for example the white rat, which has proved itself so easily susceptible to many forms of trypanosome infection, and by means of which ready comparisons can be made between *T. gambiense*, *T. brucei*, *T. rhodesiense* and many other trypanosomes. But as regards the long parasite with which we are dealing, it was found to be a matter of difficulty to produce infection in small laboratory animals. In fact, a few

rabbits and white rats alone, of a large number of experimentally inoculated animals, became infected, and the majority even of those few recovered. So great was the difficulty encountered in this respect that it was thought better to make use of goats, in which animals infection was produced with great ease and certainty, and in which after the first few passages, the long parasite alone persisted. The short non-flagellated parasite of *Horse A* had died out in the goats, as shown not only by microscopic examination of the blood, but also by repeated inoculations (with negative results) into a large number of small laboratory animals.

GENERAL PLAN OF MEASUREMENT

The trypanosomes were drawn and measured in small groups, each containing twenty specimens. The number of goats from which parasites were measured was four, which, for convenience, are called A, B, C, D. The number of days of the disease represented is twenty-two. There are thus fifty groups, each of which contains twenty trypanosomes, drawn and measured from four goats, on twenty-two days. The earliest day of the disease represented among the goats is the seventeenth, and the latest is the forty-fifth.

The arrangement of the groups is made as follows:—

(1) 400 trypanosomes (twenty groups of twenty each) were drawn on twenty separate days of the disease from three of the goats (A, B, C).

(2) 400 trypanosomes (twenty groups of twenty each) were drawn on one day of the disease from one goat (D).

(3) 100 trypanosomes (five groups of twenty each) were drawn on one day of the disease from one goat (C.)

(4) 100 trypanosomes (five groups of twenty each) were drawn on one day of the disease from one goat (D).

The reasons for adopting this plan are these:—

(1) By spreading out the first 400 trypanosomes over three goats and twenty days of disease, and confining the second 400 trypanosomes to one goat and one day of the disease, one can compare two large sets of parasites drawn and measured under widely different conditions.

(2) By drawing and measuring two sets, each of 100 trypanosomes, drawn on a single day of the disease

from separate goats, one can form comparisons between small numbers on a somewhat different basis.

(3) Finally, one can collect for comparison other sets of 100 or less spread over various animals and various days and compare them with those given above, and can form tables and charts to illustrate the comparative relation of any one set to another, larger or smaller.

METHOD OF FIXING, STAINING AND DRAWING

Thin films, made from the blood of the ear, were dried, fixed for five minutes in absolute alcohol, and stained with Giemsa's stain for twenty minutes*. Non-dividing parasites (taken in order as they were found) were drawn in clear outline with the help of the Abbé camera lucida, using a No. 18 Zeiss compensating ocular with a 2 mm. apochromatic objective.

METHOD OF MEASURING

Measurements were carried out by Stephens's method, which is briefly as follows: Along the middle of a narrow strip of smooth transparent paper a straight line is drawn. It is convenient to have this line considerably longer than the longest trypanosome to be measured, and terminating short of the margin of the paper. At one extremity of the line a mark is made for identification. A sharp pin or mounted needle is then taken, and the marked end of the straight line is made to coincide with one extremity of the outlined trypanosome to be measured. Transfix the end of the line to the extremity of the subjacent trypanosome, with the needle held perpendicularly. Rotate the paper until the straight line lies along the long axis of the trypanosome. Hold the paper in position with one hand and with the other take out the needle and pass it through the tissue paper again at the first point at which the axis of the trypanosome begins to deviate from the straight line. Repeat this process, following carefully every bend of the trypanosome and keeping in the long axis of it until the opposite extremity is reached. Hold the needle steady and place against it

* Two drops of Giemsa's solution added to each cubic centimetre of distilled water. A subsequent rapid wash with 10 per cent. orange tannin solution gave good results for drawing purposes.

a millimetre scale and read off the distance from the needle to the marked starting point of the straight line. By this simple method a very accurate measurement of the drawn parasite is obtained, and from it, by calculation, the actual length of the trypanosome.

CONSIDERATION OF THE RESULTS OBTAINED

An analysis of the 1,000 trypanosomes and of the component sets is given in Table I. From this table it will be seen that the average measurement of the 1,000 dealt with is 21·7 μ, the range being from a maximum trypanosome of 26·7 μ long to a minimum of 15·5 μ long. The trypanosome is monomorphic, all forms found being provided with a well-marked free flagellum.

Between the averages of the two sets of 400 each, there is a difference of only 0·9 μ, the first set averaging 21·4 μ and the second 22·3 μ.

Between the averages of the two sets of 100 each a smaller difference is observed, the first averaging 20·9 μ, the second 21·5 μ. When one comes down to the groups of twenty each, larger variations are naturally observable.

In Table II the trypanosomes are tabulated according to their percentage in microns under three heads, viz., those measuring less than 20 μ, those between 20 μ and 23 μ, and those measuring 23 μ and over.

It will be seen from this table that in each set dealt with, whether 1,000, 400, or 100, the largest number of trypanosomes constantly lies between 20 μ and 23 μ.

In Charts I, II, III, which give, for the various groups, a graphic representation of the percentages in length, this same fact is clearly shown.

CONCLUSIONS

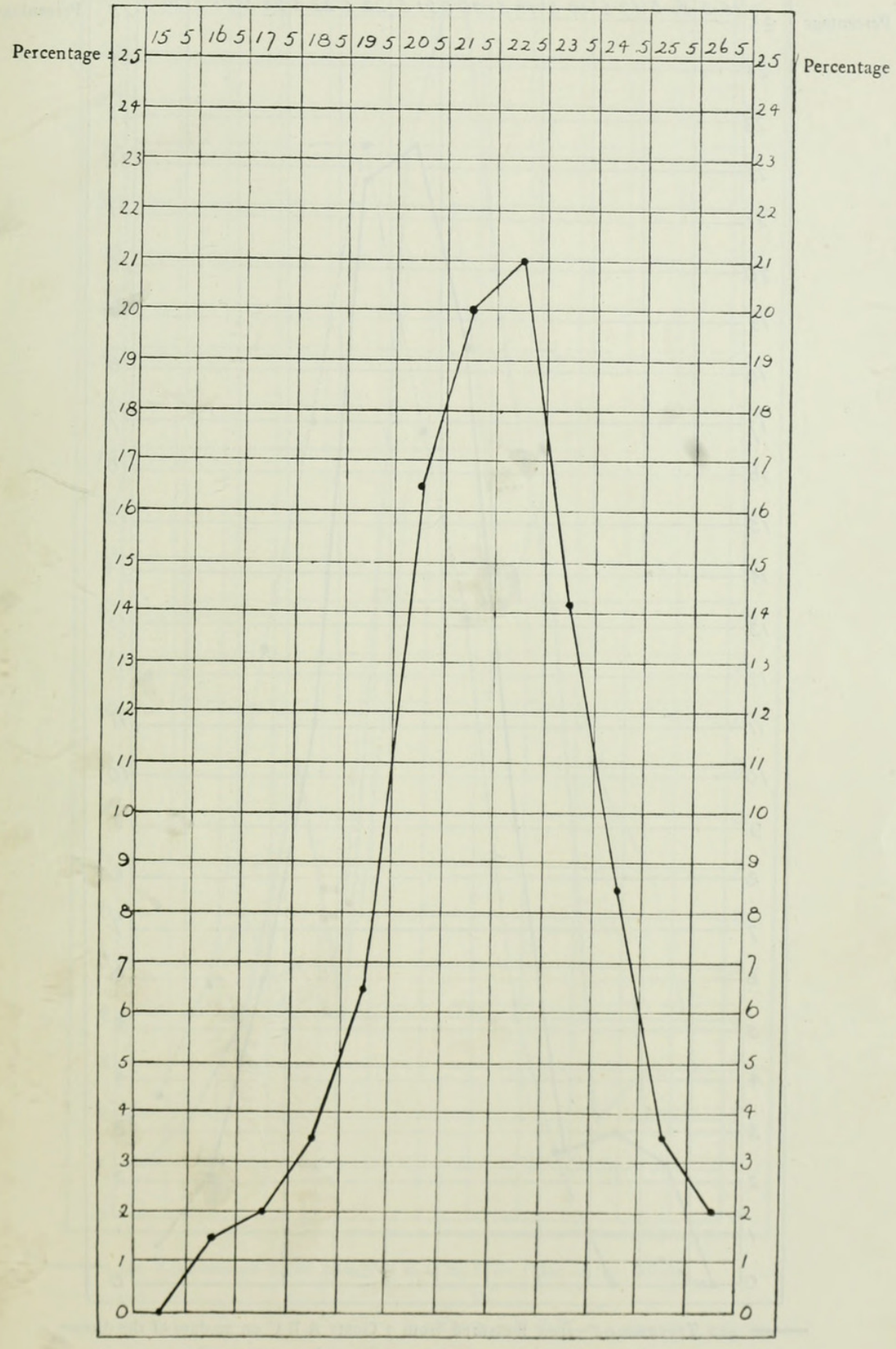
(1) This *T. vivax* from the Gambia (*Horse A*) is a free flagellated monomorphic trypanosome of an average length of 21·7 μ.

(2) The range of its extreme measurements is comparatively small.

(3) The curve of percentage length is remarkably constant, whether large or small numbers are dealt with.

CHART I

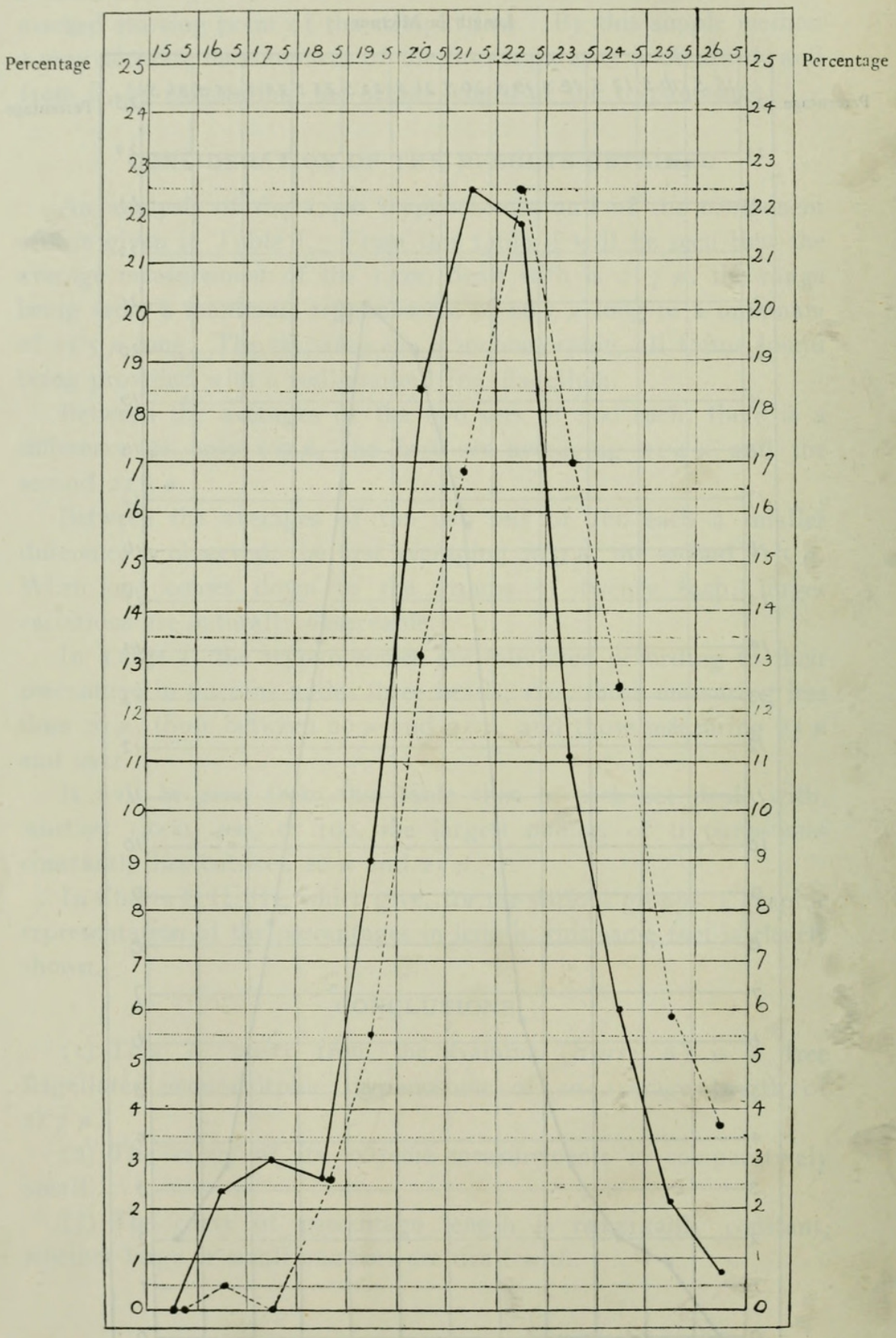
Length in Microns



1000 *Trypanosoma vivax* measured on 22 days in 4 Goats.

CHART II

Length in Microns

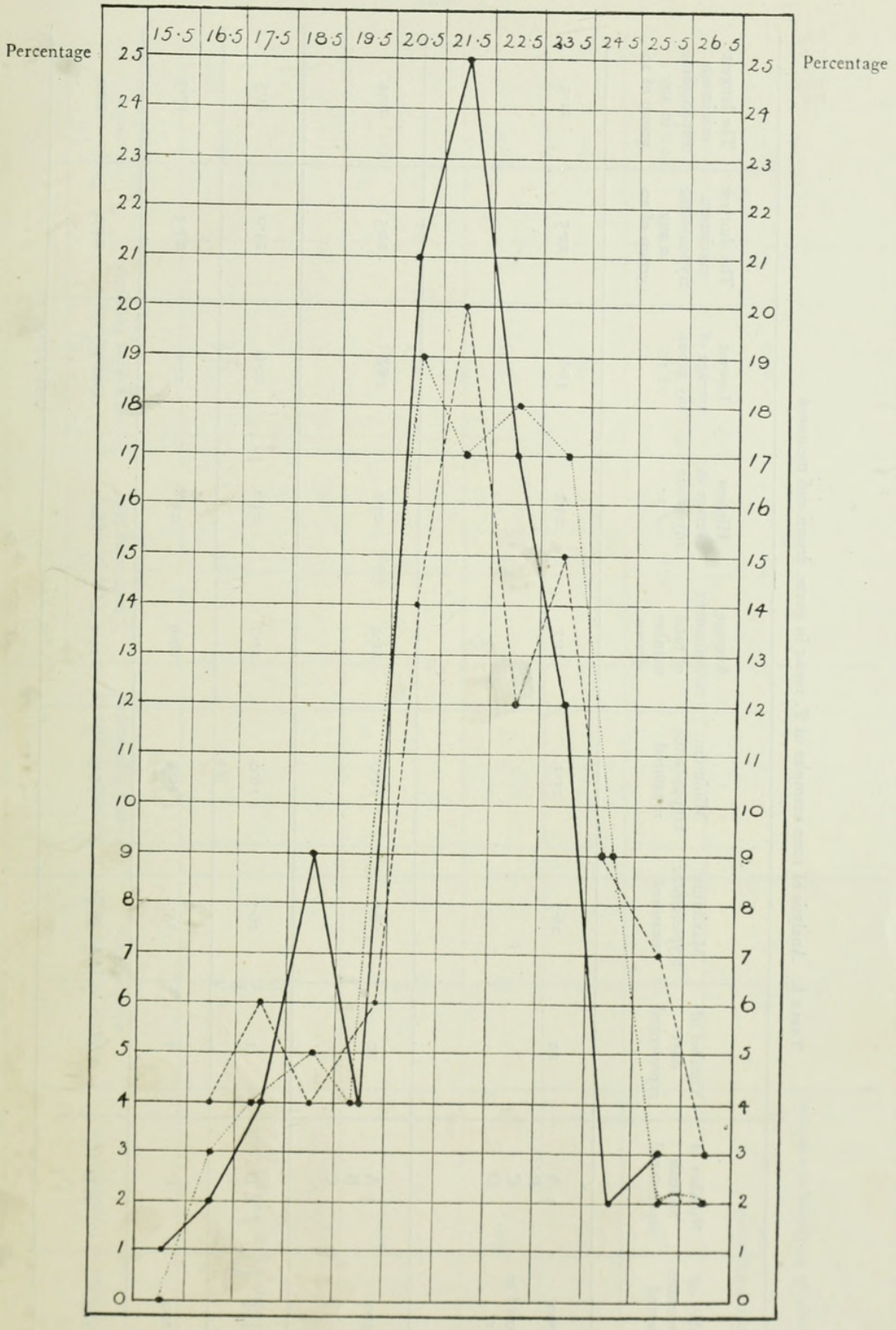


400 *Trypanosoma vivax* measured from 3 Goats A B C on 20 days of the disease.

400 ,, ,, ,, ,, 1 Goat D on 1 day ,, ,,

CHART III

Length in Microns



——— 100 *Trypanosoma vivax* measured in Goat C on 1 day of the disease.

..... 100 ,, ,, ,, ,, D ,, ,, ,,

- - - - - 100 ,, ,, ,, in 4 Goats A B C D on 5 days of the disease (chosen at random)

TABLE I. Analysis of 1000 examples of *T. vivax* in goats, drawn and measured

Number of trypanosomes measured	Number of animals represented	Number of days represented	Maximum trypanosome measured	Minimum trypanosome measured	Average measurement of total number drawn	Highest average in any group of 20	Lowest average of any group of 20	The shortest maximum trypanosome in any group of 20	The longest minimum trypanosome in any group of 20
1000 composed as under	4 A B C D	22	26·7	15·5	21·7	23·2	18·8	22·5	21·5
400	3 A B C	20	26·6	16·0	21·4	23·0	18·8	22·5	20·4
400	1 D	1	26·7	16·0	22·3	23·2	20·6	24·0	21·5
100	1 C	1	25·2	15·5	20·9	21·8	20·6	23·5	17·5
100	1 D	1	26·5	16·0	21·5	22·1	20·9	24·2	20·0

Table II. Showing percentage incidence according to length in microns of 1000 *Trypanosoma vivax*, and of the groups composing the total.

Number of trypanosomes	Number of goats from which drawn	Number of days of disease represented	Percentage of		
			(1) Trypanosomes measuring less than 20μ	(2) Trypanosomes measuring between 20 and 23μ	(3) Trypanosomes measuring 23μ and over
1000 composed as under	4 A B C D	22	13·8	57·8	28·4
400	3 A B C	20	17·2	62·7	20·1
400	1 D	1	8·5	52·5	39·0
100	1 C	1	20·0	63·0	17·0
100	1 D	1	16·0	54·0	30·0
For comparison 100 5 groups of 20 (taken at random)	4 A B C D	5	20·0	46·0	34·0

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