Notes on Zululand Trypanosomes.

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The historic investigations of Major—now General Sir David—Bruce, into the aetiology of Nagana in Zululand in 1895-6 proved conclusively the relationship of a trypanosome to the disease and the rôle of the tsetse fly as the transmitting agent, but at that time only one other pathogenic trypanosome was known, and the possibility of more than one species existing in the country was not taken into consideration. As time went on, however, other varieties of trypanosomes were discovered in various parts of Africa and when, in 1908 Dr. Theiler described a small variety of trypanosome which had been found in the blood of cattle in Portuguese East Africa, the possibility of this organism occurring also in Zululand had to be considered. That such was actually the case was proved by Dr. Theiler a few months later, when in the blood of two horses that had been exposed in Zululand he found a small trypanosome differing in no respect from that met with in Portuguese East Africa.

All the experimental and epidemiological facts in connection with Nagana go to prove that Trypanosoma Brucei is transmitted only by one or other variety of Glossina (tsetse fly), but the disease produced by the small trypanosome had been found to occur in districts where tsetse flies did not exist, and it became of the utmost importance to ascertain whether other biting flies were capable of transmitting this small trypanosome.

At the Pan-African Veterinary Conference held at Pretoria in 1909 the question of the relation of biting flies other than Glossinae in the spread of trypanosomiasis was discussed, and, as a result of the evidence submitted, a resolution was proposed and carried, expressing the imperative necessity for an extended enquiry into the trypanosome diseases of animals in Central and East Africa, especially with respect to the transmitting agent. With this object an expedition was despatched in May, 1910, to Portuguese East Africa, and from the investigations subsequently undertaken by Jowett, it was shown that the species of trypanosome found in the majority of cases examined could be transmitted by flies other than Glossinae. In the experiments carried out, Haematopota and Stomoxys were used, and of these the former was said to be probably responsible for the transmission.

This trypanosome from Portuguese East Africa, while resembling the Zululand trypanosome described by Dr. Theiler in its morphology, differed from the latter in that it was highly pathogenic for guinea pigs, while the Zululand trypanosome was non-pathogenic for this animal. In an addendum to the Report on *T. Pecorum*, however, the members of the Sleeping Sickness Commission in Uganda show that while *T. Pecorum* was originally non-pathogenic for guinea pigs, after a series of inoculations into rats and other animals, guinea pigs could be readily infected with it; the question of virulence to

Dr. A. Theiler—Bulletin de la Societe de Pathologie Exotique.

guinea pigs would not, therefore, seem sufficient to differentiate two otherwise similar species of trypanosome from each other.

When it is realised that biting flies are widely distributed throughout South Africa, some of which have been shown to be capable of transmitting a trypanosomiasis at present existing on the border of Zululand, and that at least a nearly related trypanosome is known to exist in Zululand, the vital necessity of a thorough investigation into the whole question of the animal trypanosomiasis of that country is evident.

This need was recognised by the late Natal Government, and in 1909 an additional professional assistant was obtained for the Bacteriological Division with the object of conducting an enquiry in Zululand, but owing to changes in the Department it was found impossible to carry out the investigation.

During the last few years there has been a marked increase of big game in Zululand, and, probably as a result, considerable extension of the fly belts in the low-lying parts of that country; in equines and the cattle that escaped or survived the devastating march of East Coast Fever, cases of trypanosomiasis have been frequent and, bearing in mind the possibility of the presence of trypanosomes of the dimorphon or pecorum type, a sharp look-out has been kept for small forms of trypanosome in all films received from Zululand.

Towards the end of 1911, slides were received, which had been taken from a beast at Entonjaneni, and on examination, these were found to contain small trypanosomes, none of which showed free flagella (see Plate 38, fig. 3, and Plate 39, fig 4); later, similar trypanosomes were observed in slides from a donkey at Somkele. Unfortunately in both these cases the animal died before arrangements could be made to get an experimental animal inoculated from them, but shortly afterwards, in the hope of obtaining a strain of this trypanosome, the Stock Inspector at Somkele, acting on instructions from the Principal Veterinary Officer, Natal, inoculated a dog with blood from a sick mule, and forwarded it to the Laboratory. This dog (No. 1) showed trypanosomes in its blood six days after inoculation, and supplied the strain of organism with which this note is chiefly concerned.

The observations were merely carried out with the object of arriving, if possible, at a diagnosis as to the species of trypanosome present, but they are recorded as a preliminary note on the Zululand trypanosomes, and as having a bearing on the question of trypanosome classification.

Examination of the parasite, as it first appeared in blood films from Dog No. 1, at once showed that we were not dealing with an organism of the dimorphon or pecorum type, since a considerable number possessed a well-marked free flagellum, and it seemed probable that this was a case of Nagana.

In determining this point one would imagine that no doubt existed as to what constituted the chief characteristics of probably the best known and most widely distributed of the pathogenic trypanosomes, yet a comparison of the descriptions of *Trypanosoma Brucei*, as given by various authorities, shows that these differ to a marked degree.

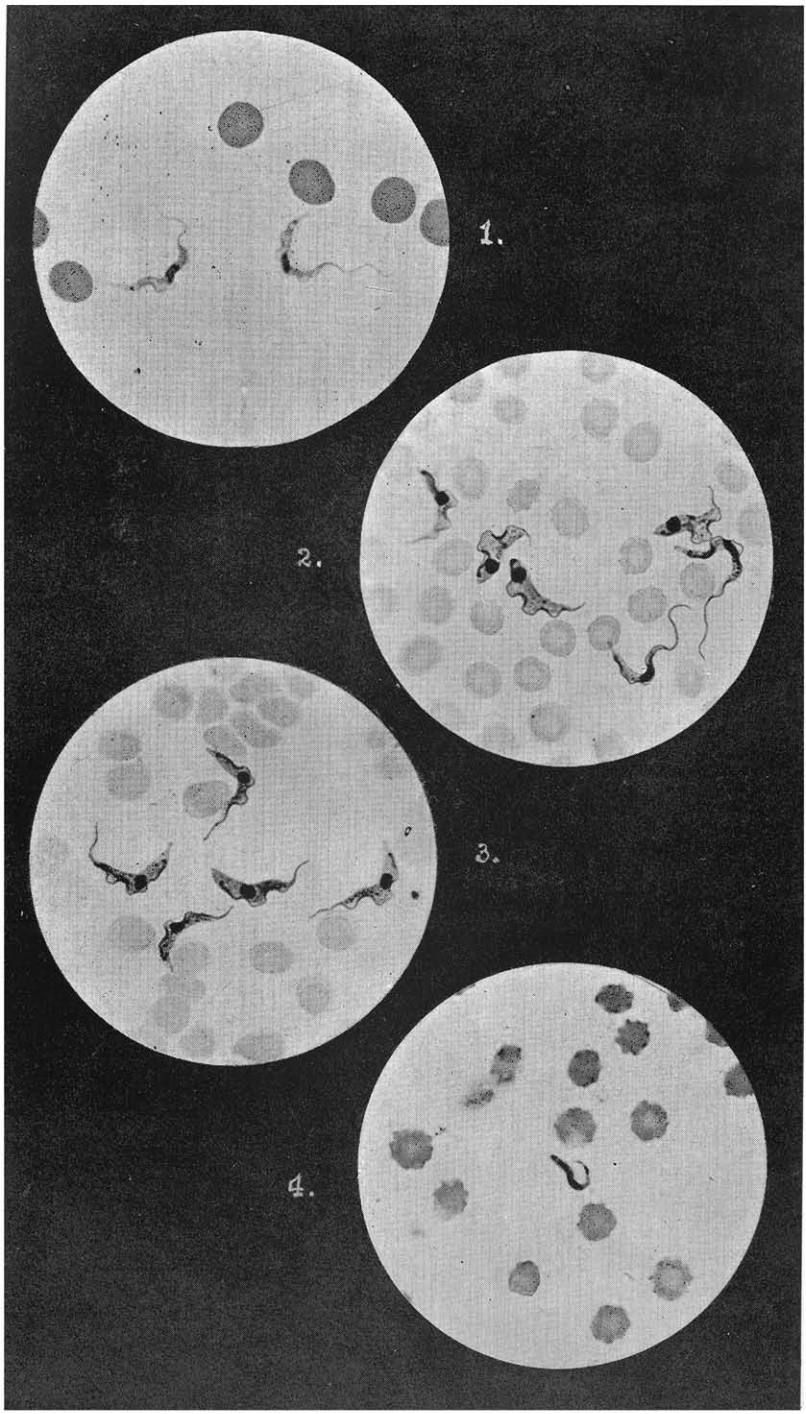

Plate No. 39.] *Zululand Trypanosomes.*

Fig. 1.—T. Brucei—1896 strain. (Royal Veterinary College, London.) Stained Giemsa. X800.
Fig. 2.—T. Brucei—1912, Zululand—blood of Guinea Pig, 70th day. Stained Giemsa. X800.
Fig. 3.—T. Brucei—1912, Zululand—blood of Horse. Stained Giemsa. X800.
Fig. 4.—T. Pecorum ?—Zululand—blood of Ox. Stained Giemsa. X800.

All the earlier accounts of this parasite, both English and Continental, refer to a strain of trypanosome brought to England from Zululand in 1896, and until recently this has been accepted as the type strain of *T. Brucei*.

Laveran and Mesnil (1), in their description, state: "All the trypanosomes are approximately of the same length in the blood of any particular animal; in the rat, mouse, guinea-pig, rabbit and dog 26 to 27 microns long by 1.5 to 2.5 microns wide. In the horse and donkey the parasite is longer, varying from 28 to 33 microns." These authors also describe the constant presence of a free portion of the flagellum.

Also Laveran (2), in his "Classification of trypanosomes," places *T. Brucei* under the heading of "Trypanosomes which have always a free flagellum."

In the Report of the Sleeping Sickness Commission of the Royal Society No. XI., Bruce and the other members of the Commission describe *T. Brucei* of Uganda as follows:—"This species is interesting on account of its well-marked dimorphism, in which *T. Gambiense* resembles it. There are two varieties, the long and slender with free flagellum, and the short and stumpy without free flagellum. The shortest only 13 and the longest 35 microns in length."

A comparison is made between this organism and *T. Brucei* of Zululand, as present in the original slides taken by Bruce in 1895-6, and by means of graphic curves showing the percentage distribution in respect to length, the similarity of the parasites in the two cases is demonstrated. The Commission conclude that the Uganda strain is identical with that of Zululand.

It has been suggested that discrepancies in the various descriptions of particular trypanosomes may be due to personal temperament or differences of technique and in endeavouring to reconcile minor points of disagreement in two otherwise corresponding accounts, these factors must be taken into consideration; but in the present case such an explanation is quite inadequate since the question lies between an organism of uniform type and one constantly showing wide variations of form and structure, a distinction which must constitute the basis of any classification according to morphological characters.

†Montgomery and Kinghorn and Bevan and Macgregor record the appearance of trypanosomes with a free flagellum, in experimental animals when inoculated with strains in which no long forms occurred and the transition of these long forms into short, stumpy ones; similar observations were made and are recorded later in the case of the trypanosome from Zululand under consideration, but so far as the writer is aware, no evidence has been brought forward to show that a strain of trypanosome exhibiting these variations of form may, under certain conditions, become of uniform type and remain so during repeated sub-inoculation.

Unless some such explanation is offered with reference to the difference in the two descriptions of *T. Brucei*, from which extracts are quoted above, the only possible conclusion would appear to be that two distinct species of trypanosome have been described under the same name.

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1. Trypanosomes & Trypanosomiasis—Laveran & Mesnil—page 151.
2. A. Laveran—Annales de l'Institute Pasteur, 1911—Juillet XXV.

†Veterinary Journal, January, 1910.

Applying Bruces mensuration method of comparing strains of trypanosome, charts have been prepared from slides of what may be called the European strain of *T. Brucei* and for comparison, of *T. Brucei* as found in the Sudan, shown to be identical with *T. Brucei* of the Uganda Commission, and of the Zululand trypanosome. These charts, and the other measurements and illustrations, will, I think, be sufficient to demonstrate that the European *T. Brucei* is a totally different type of organism from that described by Bruce. If this be so then there is no reason why the strain sent to England in 1896 should not still exist in Zululand, but at present there is no evidence to show that such is the case; since, however, with the exception of Dr. Theiler's observation already referred to, no investigation has been made from the time of Bruce's original research, it is quite possible that a strain of trypanosome having the characters of the European strain of *T. Brucei*, may yet be found when a full and complete study is made of the trypanosomiases of Zululand.

This question has been discussed somewhat fully as it is one which seems to have received little notice by recent writers, the tendency of whom has been to accept the dimorphic trypanosome as *T. Brucei* without attempting to explain how it is that the earlier accounts of the organism differ so materially from those of later date.

MULE TRYPANOSOME FROM ZULULAND.

MORPHOLOGY.

Living and Unstained.—In this condition the variations in size and shape of the organism can be observed, the long slender flagellated forms being readily distinguished from the short, stumpy forms, while all gradations between these two occur. The circular vacuole close to the micro-nucleus is very distinct. Although the organisms are actively motile, their progression is not rapid, frequently they simply travel in a small circle.

Fixed and Stained.—Films were fixed by Osmic Acid vapour, whilst moist, followed by Alcohol, or in Alcohol alone, and stained by Giemsa's stain.

Length.—A large number of measurements of this organism have been made, as present in various species of animal, and at various stages of the disease, and charts prepared according to the method described by Sir David Bruce.

In making the actual measurements a modification of Dr. Stevens "tangent line method" was adopted*; in this method, which is said to be more accurate than that of Sir David Bruce, who employs compasses, a piece of tissue or tracing paper is used; on this a straight line is ruled, and by rotating the paper and keeping it in position with needles, the line is made to follow the main axis of the trypanosome from end to end; the length of line used is then measured.

Since the tissue paper has to be frequently renewed, the writer used instead thin photographic film, from which the negative had been removed, and to avoid measuring the line after each estimation, a scale was scratched on the film and holes pricked along the line, one

* Dr. J. W. Stevens—Report Tropical Disease Research Fund, 1911, p. 100.

millimetre apart. By using this film, as in Dr. Stevens method, steadying it with the left hand, and moving the needle from hole to hole as the curves of the figure are followed, the exact length of the trypanosome can be at once read on the scale. If the magnification employed is 1,000 or 2,000 diameters, each millimetre on the scale represents 1μ or 0.5μ as the case may be.

It is essential that the scale be accurately drawn, and this takes a little time, but once made, its use will speedily repay the time lost in its preparation should many figures have to be measured.

In the dog (No. 1) inoculated from the natural case in a Mule in Zululand the trypanosomes were found to vary in length, from 14 to 30 microns, the average being 19 microns.

The following chart shows the percentage distribution in respect of length of the trypanosomes in this animal at the 13th day of the disease.

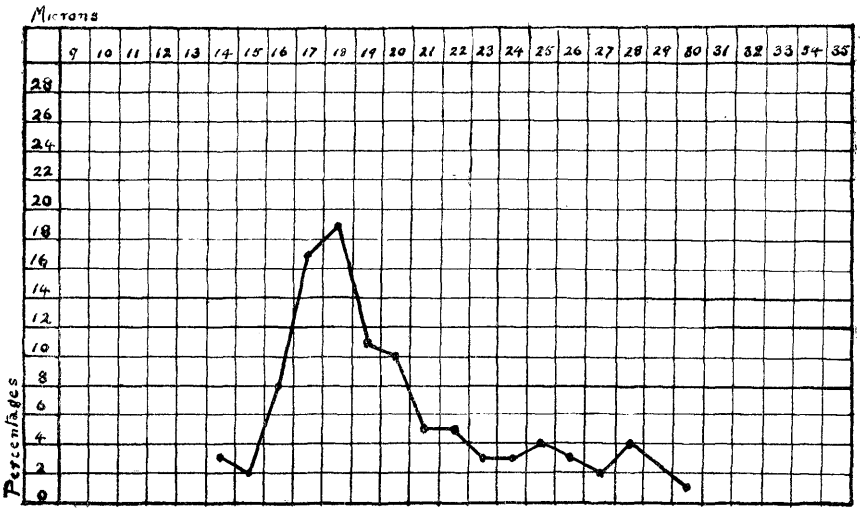


CHART I.—Zululand Trypanosome. Dog No. 1. 13th day of disease.

Captain Fry,* in discussing Bruce's method, points out that fallacies may arise if comparisons are made with trypanosomes from different animal sources (compare Charts I. and III.), but an even greater variation is shown in Charts prepared from a single animal at various stages of the disease (compare Charts III., IV., V. and VI.).

The charts published in the Reports of the Sleeping Sickness Commission are each prepared from strains of the particular trypanosome as they occur in various animals, and at different periods of the infection.

The following chart gives the percentage in respect of length of 400 organisms, occurring in Dog No. 1, Mule No. 37, and guinea pig No. 149, the measurements being made at varying periods of the disease.

*Capt Fry—Animal Trypanosomes of the Sudan—4th Report Wellcome Research Laboratory.

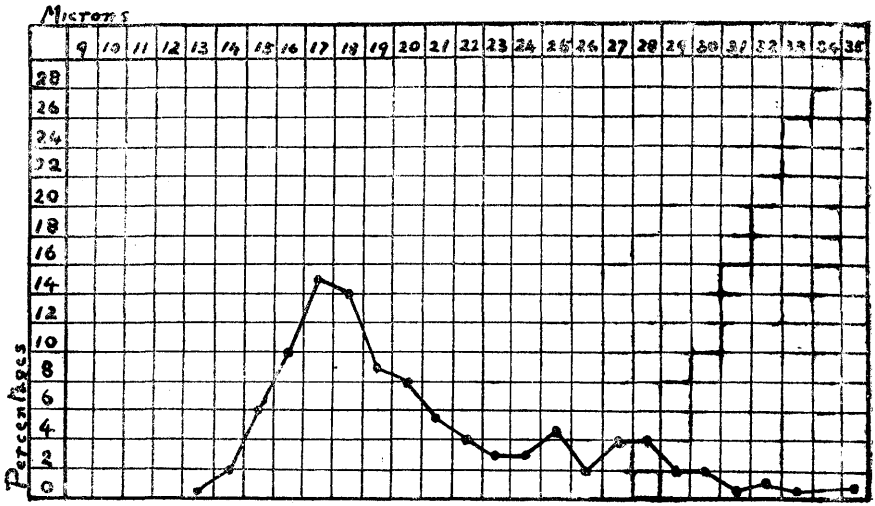


CHART II.—Zululand Trypanosome, various animals.

This chart bears a general resemblance to that published by Sir David Bruce, prepared from slides of the original Zululand *Trypanosoma Brucei*, in that the bulk of the organisms lie between 15 and 20 microns; the more regular character of the curve in the above chart is probably due to the greater number of organisms measured, while the smaller percentage of long forms is accounted for by the fact that the measurements were mostly made at periods of the disease when parasites were very numerous in the blood, and at such times the short forms are present in great excess.

Breadth.—The longest forms are about 1.5 to 2 microns in breadth, while the short forms, including the undulating membrane may be 4 microns, but average 3 microns in breadth.

The general character of the various forms is seen in Plate 38, fig. 2, and Plate 39, figs. 2 and 3. The anterior portion of the cell body contains numerous chromatin staining granules, frequently arranged in two parallel lines; these are present in all forms of the parasite.

In the long forms the nucleus is oval, 3 to 4 microns in length, and situated about the middle of the body; in the intermediate forms the nucleus is rounder, and in the short forms usually round, and placed nearer the posterior extremity.

The micro-nucleus is small and round, situated 1 to 3 microns from the posterior extremity in the long forms, while in the short forms it is usually 1 micron from the end, but may be practically terminal.

The undulating membrane is well developed and thrown into deep folds, especially in the short forms.

The long forms have a free portion of flagellum 5 to 10 microns in length, the short forms have no free flagellum.

The following table gives measurements of the various parts of the trypanosome body from parasites in the blood of Dog No. I.

TABLE I.—MEASUREMENTS OF THE VARIOUS PARTS OF ZULULAND TRYPANOSOME. DOG NO. I.

Posterior extremity to micro-nucleus.	Micro-necleus to nucleus.	Length of nucleus.	Nncleus to anterior extremity.	Free flagellum.	Total length.
3	6	3	13	10	35
3	5	3·5	11·5	8	31
3	8	3	8	8	30
3	5	4	9	9	30
2	7	3	10	8	30
3	4	3	12	7	29
2	5	3	9	9	28
2	7	3	9	7	28
1·5	5·5	3	8	9	27
1	6	4	11	4	26
1	5	2·5	11·5	6	26
1	5	3	11	5	25
1·5	5·5	2·5	7·5	8	25
1	6	2·5	7·5	7	24
1	5	2	11	3	22
1	4	2·5	8·5	6	22
1	5	2	10	3	21
1	5·5	3	11·5	—	21
1	5	2	10	2	20
1	5	3	11	—	20
1	5	2·5	10·5	—	19
·5	4	3	11·5	—	19
1	4	3	11	—	19
1	4	3	10	—	18
1·5	4	2·5	10	—	18
·5	3·5	3	11	—	18
1	4	2·5	10·5	—	18
1	5	2	9	—	17
·5	4	3	9·5	—	17
·5	4·5	2·5	8·5	—	16
1	4	2·5	8·5	—	16
·5	3·5	2	9	—	15
·5	3·5	2·5	7·5	—	14
—	4	2·5	7·5	—	14

In the Report on *T. Brucei*, Uganda, the great variation in the proportion of long and short forms of the parasite, on different days, is noted; this is clearly shown in the case of the present strain by the following four charts.

These were prepared from Mule No. 37, from slides taken on the 5th, 7th, 19th, and 29th days after inoculation. Mule No. 37 received 4 c.c. of blood containing numerous parasites, from Dog No. 4, intravenously, and trypanosomes were detected on the 3rd day after injection.

On the 5th day the parasites were present in fair numbers, while on the 7th and 19th days they were very numerous, 4 to 5 in each field, although during the interval they were very scarce, by the 29th day the organisms became less numerous. The animal died on the 35th day.

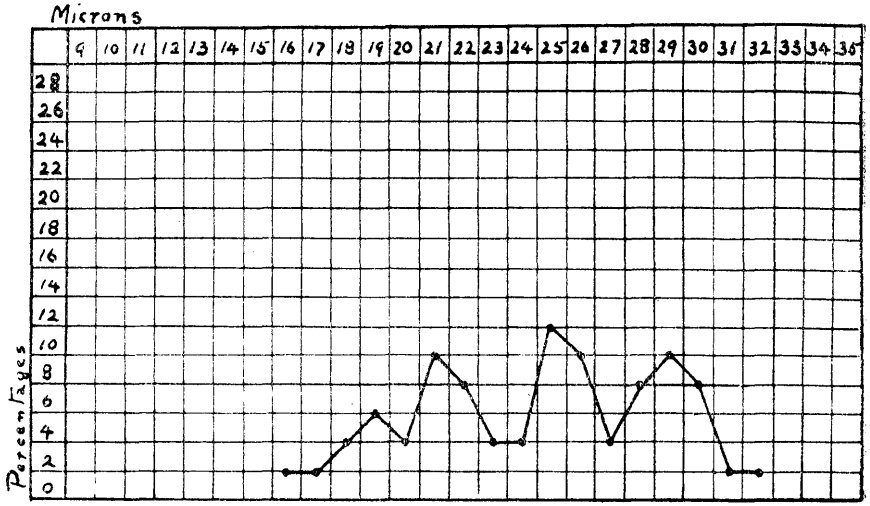


CHART III.—Zululand Trypanosome. Mule No. 37. 5th day of disease.

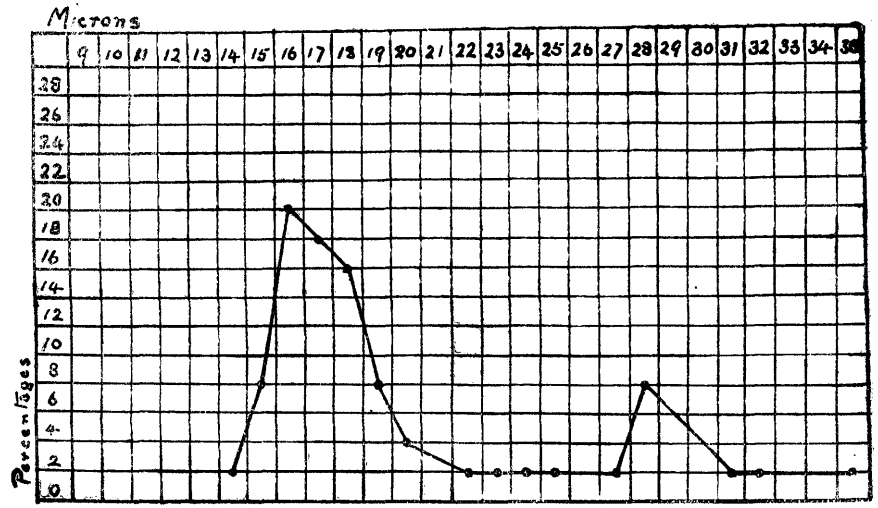


CHART IV.—Zululand Trypanosome. - Mule No. 37. 7th day of disease.

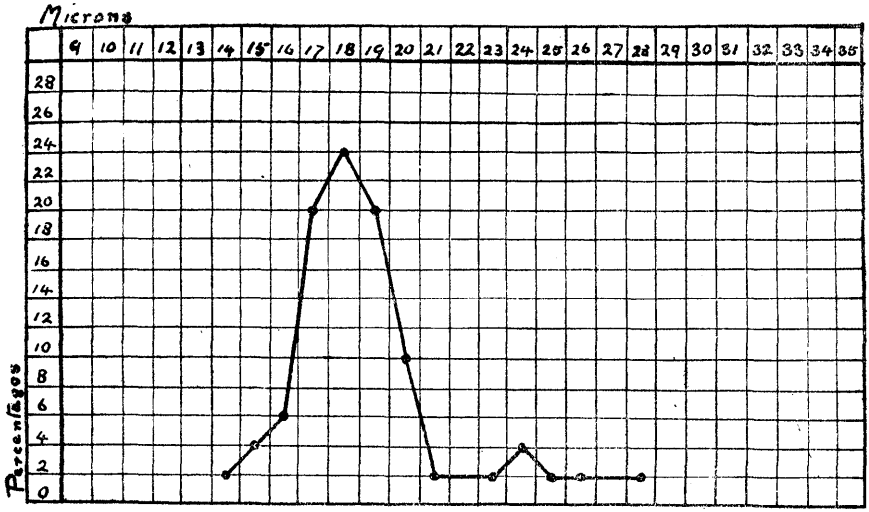


CHART V.—Zululand Trypanosome. Mule No. 37. 19th day of disease.

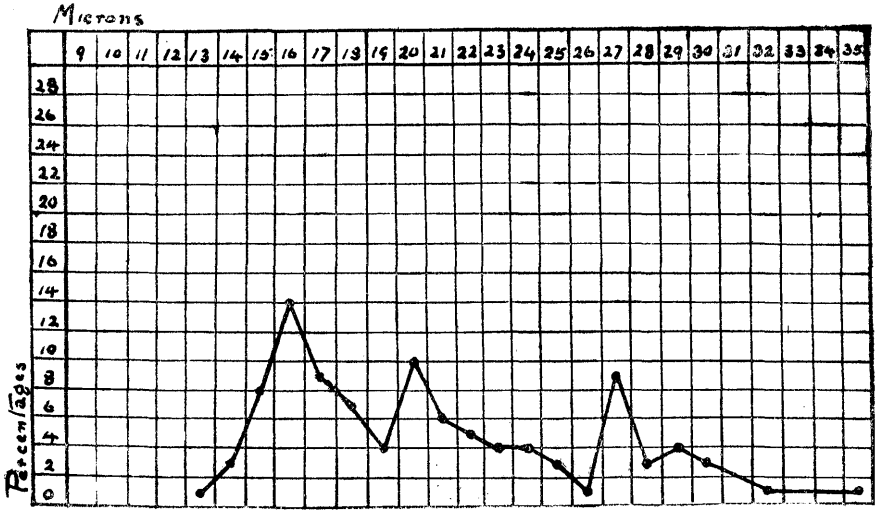


CHART VI.—Zululand Trypanosome. Mule No. 37. 29th day of disease.

The almost constant appearance of long forms at the beginning of infection, led to a suspicion that we might be dealing with a double infection, the short form of trypanosome having a longer period of incubation than the long slender form.

To settle this point resort was made to Bourri's method for isolating trypanosomes, in which a single organism is isolated under the microscope and inoculated into a Laboratory animal; should infection follow it must therefore be due to one trypanosome, and the characters of the resulting organisms are those of a single species.

In all cases in which infection occurred, both forms of the parasite appeared in the blood, proving that the strain of trypanosome in question is truly dimorphic, and not a mixture of two types.

COMPARISON WITH T. BRUCEI EUROPEAN STRAIN.

For purposes of comparison, measurements were made from stained films of *T. Brucei*, obtained from Sir John McFadyean, Royal Veterinary College, London, and the *Inst. f. Schiffs v. Tropenkrankheiten*, Hamburg.

The following table shows the measurements of various parts of twenty parasites of each strain; these were taken as they appeared under the microscope, only dividing forms being passed over:—

TABLE II.—MEASUREMENTS OF VARIOUS PARTS OF T. BRUCEI., EUROPEAN STRAIN.

(*a*). Royal Veterinary College, London, slide.

Posterior extremity to micro nucleus.	Micro-nucleus to nucleus.	Length of nucleus.	Nucleus to anterior extremity.	Free flagellum.	Total length.
5	6	3	8	9	31
4	6·5	3	7·5	10	31
4	7	3	6	10	30
3	6	3	8	10	30
3	5	3	8	10	29
2	7	3	7	10	29
4	5	3	7	10	29
4	5	3	6	11	29
3	5	2·5	8·5	10	29
3	6	3	7	10	29
5	6	3	5	9	28
2	6	3	7	10	28
3·5	6·5	3	5·5	9·5	28
3	7	3	5	10	28
2	6	3	7	9	27
3	6	4	5	9	27
3	6	3	6	9	27
4	5	3	5	9	26
2	6	3	5	9	25
3	7	3	6	6	25
Average 3·27	6	3	6·47	9·47	28·25

TABLE II—(continued).

(*b*). Inst. f. Schiffs v. Tropenkronkheiten, Hamburg, slide.

Posterior extremity to micro-nucleus.	Micro-nucleus to nucleus.	Length of nucleus.	Nucleus to anterior extremity.	Free flagellum.	Total length.
4	6	3	11	8	32
3	6	3	12	8	32
4	6	3·5	11·5	7	32
4	5	3	12	7	31
2·5	7	3	9·5	9	31
2·5	7	3·5	8·5	8·5	30
2·5	5	3	11·5	8	30
3	6	3	10	7	29
3	6	3	10	7	29
3	6	3·5	9	7·5	29
2·5	5·5	3	12	6	29
3	5	3	11	7	29
2	5	3	10	7	27
2	5	4	10	6	27
2·5	5·5	3	10	6	27
2	6	4	7	7	26
2	5	3	10	6	26
2	5	3	10	6	26
3·5	5	3	6·5	7	25
2·5	5	3·5	7	7	25
Average 2·7	5·6	3·2	9·9	7·1	28

	Average length.	Maximum length.	Minimum length.
Royal Veterinary College, London.	28·25	31	25
Inst. f. Schiffs v. Tropenkronkheiten, Hamburg.	28·6	32	25

The above measurements show very clearly the great similarity of the parasites in the two strains, and also the very narrow limits within which the trypanosomes vary in respect to length.

The shape and character of the various parts of the trypanosomes are also seen to be strikingly uniform; all have a tapering posterior extremity, narrow body, much folded undulating membrane, elongated nucleus and free flagellum, while the metachromatic granules in the anterior portion of the body are fairly constant in number and distributed (see Plate 38, fig. 1, and Plate 39, fig. 1).

The following chart, prepared from 100 measurements of *T. Brucei*, Royal Veterinary College strain, shows the percentage distribution in respect of length of this trypanosome:—

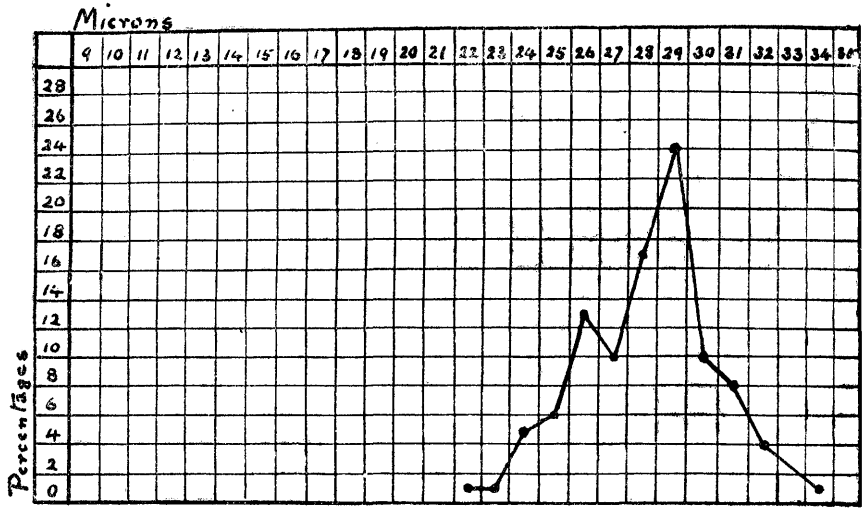


CHART VII.—T. Brucei. European strain.

It is evident from a consideration of the morphological characters of this strain of trypanosome, that it corresponds very closely to the description given by Laveran and Mesnil and other authorities, of *Trypanosoma Brucei*, while it is equally certain that it differs in many important points from *T. Brucei*, as described by Sir David Bruce and others.

Through the courtesy of Dr. Andrew Balfour, in forwarding slides of various trypanosomes occurring in the Sudan, to the Pietermaritzburg Laboratory, the writer has been able to make measurements of the type described as *Trypanosoma Brucei or Pecaudi*, in the fourth report of the Wellcome Research Laboratory; the following chart shows the percentage distribution in the case of this trypanosome: —

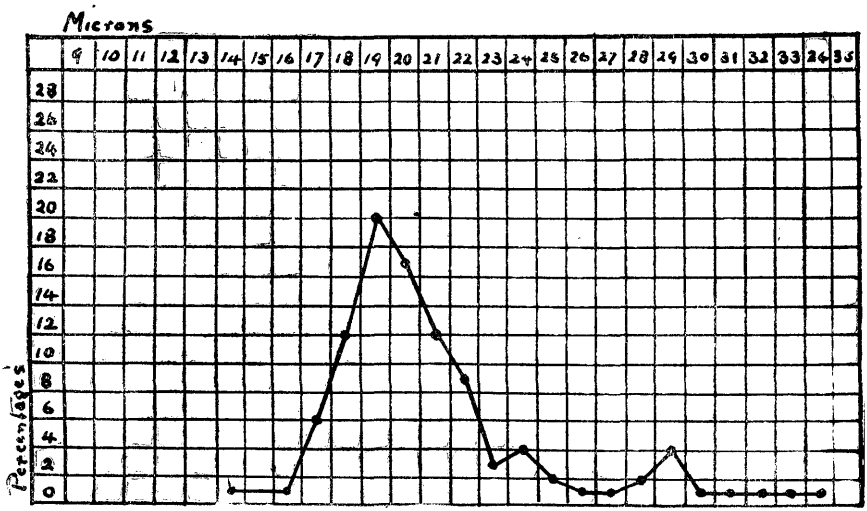
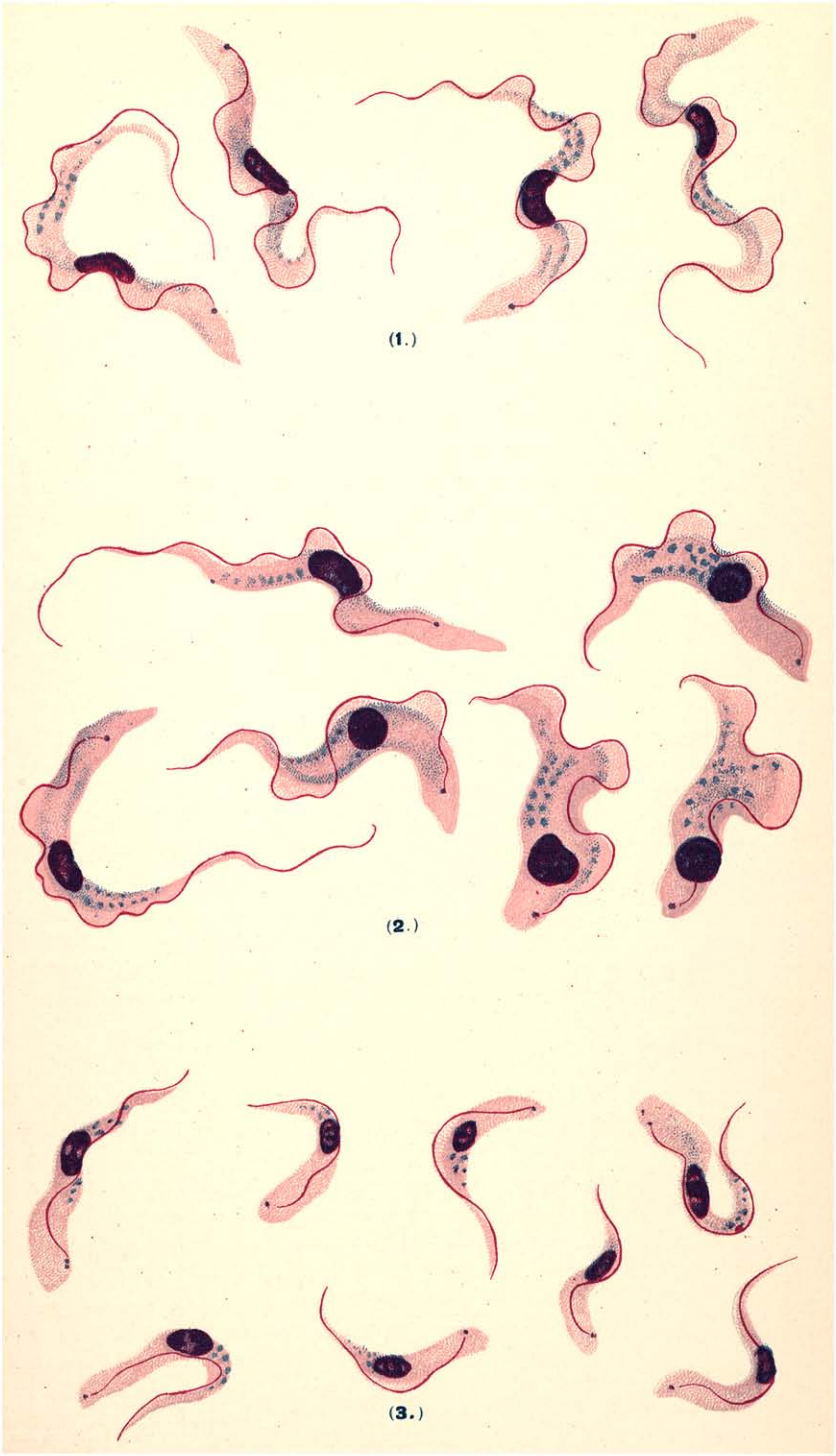


CHART VIII.—T. Brucei or Pecaudi, Sudan.



x2750

ZULULAND TRYPANOSOMES.

A. W. Shilston, del.

1. T. Brucei—Inst. f. Schiffs u Tropenkrank.
2. Zululand Trypanosome.

The above chart differs somewhat from those of the same type of organism published by Capt. Fry in his article on the Animal Trypanosomiasis of the Anglo-Egyptian Sudan, but this is probably owing to its having been prepared from a single slide in which the parasites are very numerous; the average length of the parasites is 21 microns.

Dr. Balfour studied this trypanosome and describes and figures it in the Second and Third Reports of the Wellcome Research Laboratory; he compares it with *T. Dimorphon* and *T. Pecaudi*, and agrees with Laveran in his opinion that it closely resembles the latter organism. In the light of Sir David Bruce's description of *T. Brucei*, however, Capt. Fry in the Fourth Report describes this trypanosome under the name of *T. Brucei* or *Pecaudi*, and shows by means of measurements and charts, how closely it resembles Bruce's account of the former.

We may adopt the same methods in the case of the trypanosome from Zululand under consideration, and in doing so one is at once struck by the great similarity it shows both to *T. Brucei*, of Uganda, and of the Sudan.

Chart VIII. above and charts I. and V. are very much alike in the general characters of the curves.

The only point in which the Zululand trypanosome differs from *T. Brucei*, of Uganda, is with regard to the greater percentage of short forms present in most cases, and consequent smaller number of long forms; in this respect it would seem more to resemble *T. Pecaudi*, but there appears to be considerable doubt as to whether this species is distinct from that now described as *T. Brucei*. As has already been pointed out, the stage of the disease at which the measurements are made may influence considerably the relative proportion of short and long forms.

On the other hand, a comparison of chart VII. with those of the Zululand trypanosome and *T. Brucei*, of the Sudan, and of table II. with table I. demonstrates very clearly how great is the difference between the strain of trypanosome originally described as *T. Brucei* by European investigators and that which is now held by Sir David Bruce to have been the parasite he discovered and worked with in Zululand, and which, apparently, still exists in that country.

ANIMAL REACTIONS OF THE ZULULAND TRYPANOSOME.

The following table shows that equines, cattle, sheep, dogs and the smaller Laboratory animals are susceptible to this trypanosome:—

TABLE III.—ANIMALS INOCULATED WITH ZULULAND TRYPANOSOME.

Animal.	No.	Source of Virus.	Period of Incubation.	Duration of Disease.	Remarks.
Mule ...	37	Dog 4 ...	3 days...	35 days...	Very old animal, typical Nagana.
Ox ...	22	Dog 4 ...	6 „ ...	...	Still alive and blood infective after 120 days.
Sheep ...	1	Dog 4 ...	12 „ ...	...	Still alive and blood infective after 120 days.
Dog ...	1	Mule ... Zululand	6 „ ...	19 days...	Spleen and liver enlarged.
	2	Rabbit 146	22 „ ...	22 „ ...	Spleen and liver enlarged.
	3	Rabbit 150	...	18 „ ...	Spleen and liver enlarged.
	4	Rat 4 ...	5 days...	16 „ ...	Spleen and liver enlarged.
					Average duration—19 days.
Rabbit ...	145	Dog 1 ...	...	33 „ ...	Spleen enlarged.
	146	Dog 1 ...	...	39 „ ...	Spleen enlarged.
	150	Dog 2 ...	...	31 „ ...	Spleen enlarged, ulceration of stomach.
	157	Rat 4A ...	13 days...	34 „ ...	Spleen enlarged.
	158	Mule 37 ...	3 „ ...	27 „ ...	Spleen enlarged and stomach ulcerated.
	165	Rabbit 158	5 „ ...	27 „ ...	Spleen and liver enlarged and stomach ulcerated.
					Average duration—32 days.
Guinea Pig	147	Rabbit 145	30 „ ...	78 „ ...	—
	149	Rabbit 146	15 „ ...	48 „ ...	Spleen enlarged.
	152	Rat 2 ...	20 „ ...	57 „ ...	Spleen enlarged, stomach ulcerated.
	153	Dog 3 ...	14 „ ...	28 „ ...	Spleen enlarged, in advanced stage of pregnancy.
	154	Dog 3 ...	8 „ ...	21 „ ...	Spleen enlarged.
	179	Mule 37 ...	12 „ ...	32 „ ...	Spleen enlarged, stomach ulcerated.
	184	Rabbit 165	9 „ ...	46 „ ...	Spleen enlarged, stomach ulcerated.
					Average duration—44 days.
White Rat	4	Rabbit 150	4 „ ...	45 „ ...	Spleen enlarged.
	4A	Rabbit 150	4 „ ...	23 „ ...	—
	8	Rabbit 157	6 „ ...	16 „ ...	Spleen enlarged, stomach ulcerated.
	9	Guinea Pig 179	5 „ ...	12 „ ...	Spleen enlarged, stomach ulcerated.
					Average duration—24 days.
Wild Rat	1	Rabbit 145	...	9 „ ...	—
	2	Rabbit 145	...	58 „ ...	—
	6	Mule 37 ...	4 days...	21 „ ...	Spleen enlarged, stomach ulcerated.
	7	Rabbit 157	7 „ ...	14 „ ...	Spleen enlarged, stomach ulcerated.
	11	Guinea Pig 184	8 „ ...	...	Still alive 47 days after inoculation.
					Average duration—30 days.

In comparing the above results with those published by Laveran and Mesnil for *T. Brucei*, the most notable difference is seen in the case of rats; these authors found that rats died in $3\frac{1}{2}$ to $5\frac{1}{2}$ days after inoculation, while with the Zululand trypanosome the shortest time in which death occurred was 9 days and the average duration of the disease in nine cases was $27\frac{1}{2}$ days. It is possible that the extreme virulence in the one case was brought about by continuous sub-inoculation through rats, but the increase would seem to be too great to have been produced by this means, and during seven months of passage through laboratory animals, the present strain does not appear to have increased in virulence to any degree.

In the case of *T. Brucei*, Uganda, the two rats inoculated are reported to have died in 22 and 23 days respectively.

Cultivation.—Only one attempt has been made to cultivate this trypanosome, and owing to bacterial contamination this was not successful.

In this connection, however, the fact that at the Wellcome Research Laboratory, cultivation of the Sudan *T. Brucei* was found comparatively easy, while others working with the European strain of *T. Brucei* report that growths are only obtained exceptionally, is another indication of the difference existing between these two organisms.

Conclusions.

1. That a trypanosome occurs in animals in Zululand, which is markedly dimorphic, and in this and other respects closely resembles the trypanosome described by Sir David Bruce and others as *Trypanosoma Brucei.*

2. That the trypanosome introduced into Europe in 1896 and described by various authorities as *Trypanosoma Brucei*, differs to a marked degree from that described under the same name by Bruce, and from the Zululand trypanosome described above.

3. That a careful study of the trypanosomiasis of Zululand is necessary to determine, among other things, whether a trypanosome having the characters of *T. Brucei* as formerly described, also exists in that country.

NOTES ON SMALL TRYPANOSOME FROM ZULULAND.

In relating the circumstances which led to the study of the trypanosome described in the preceding note reference is made to the occurrence of small trypanosomes in blood slides forwarded to the Laboratory, Pietermaritzburg, for examination; since then similar trypanosomes have been repeatedly observed in slides from supposed cases of Nagana in Zululand.

Up to the present it has not been possible to obtain a strain of this trypanosome for accurate study, and until that is done any description or opinion as to its probable identity must of necessity be made with considerable reserve.

Owing, however, to the frequency with which it has been found and in slides from widely separated sources, it seems advisable to place these few observations on record at the present time if only to show the need there is for fuller investigation.

Morphology.—All the films were fixed in Alcohol and stained by Giemsa.

Length.—The following table gives the distribution in respect of length of 100 parasites from slides from various sources:—

TABLE IV.

SOURCE.	MICRONS.								
	9	10	11	12	13	14	15	16	Average.
Ox Entonjaneni ...	—	3	5	3	6	3	3	2	12·7
Ox Ubombo	1	6	3	5	5	3	2	—	11·9
Donkey Somkele ...	—	2	4	5	9	3	2	—	12·5
Ox Somkele	—	3	5	3	—	2	—	—	11·4
Donkey Somkele ...	—	2	1	3	3	1	—	1	12·3
Ox Inguavuma ...	—	—	—	—	—	—	1	—	15
TOTAL ...	1	16	18	19	23	12	8	3	12·3

The distribution is shown graphically in the following chart:—

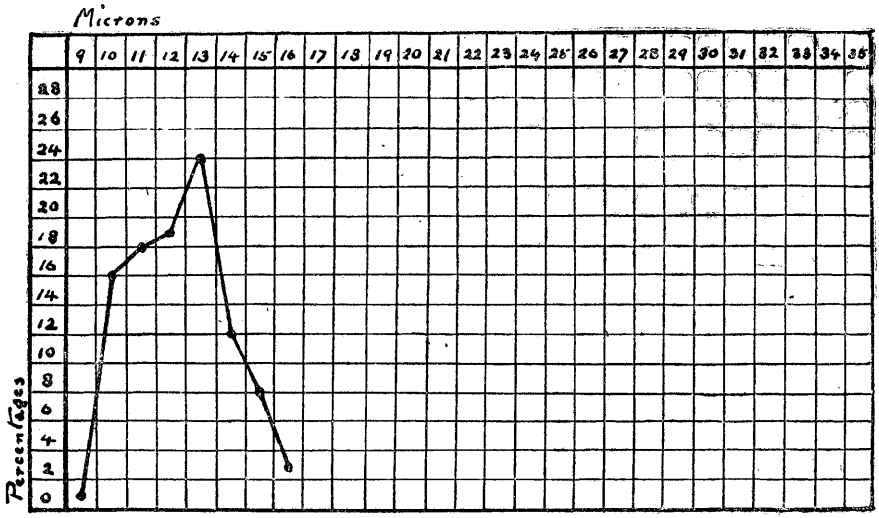


CHART IX.—T. Pecorum? Zululand.

Breadth.—1.5. to 2.5 microns.

Shape.—The bodies are short and stout, with rounded posterior extremities, and gradually tapering to the anterior end; a few chromatin granules may be present in the anterior part, but frequently the whole body stains a uniform pale blue.

Nucleus is oval, rather elongated in shape, and placed about the centre of the body.

Micronucleus, round and situated close to the posterior extremity.

Undulating Membrane, not very well developed.

There is no free flagellum.

While in the absence of animal inoculations and more reliable films for examination no definite opinion can be expressed as to the identity of the parasite, a comparison of the above measurements and chart, and the illustrations—Plate 38, fig. 3, and Plate 39, fig. 4, with those published of *T. Pecorum* will show a very close resemblance between the two organisms, and for the present this is all that can be said on the point. The fact that the trypanosome was found in slides from a donkey, proves that, in this case at least, we are not dealing with *T. Nanum; T. Uniforme* (Bruce), would seem to be excluded by reason of its greater average length, 16 microns.