in Palæozoic Monticuliporas the same relation holds, for in the case of fossils encrusting shells, a thin graphite-like layer of alga is sometimes found between the Monticulipora and the shell.

At present I am not prepared to say to what genus the Zooxanthella monad of Merlia belongs. I propose to name the species "noronhæ" in honour of Senhor A. C. Noronha, who rendered invaluable assistance during the dredging operations off Madeira and Porto Santo Island. The name Merlia is a synonym of Monticulipora, the name of the sponge being *Monticulipora normani*, Kirkp.

A Camel Trypanosome, with some Remarks on the Biometric Method of Diagnosing Trypanosomes.

By Dr. H. L. Duke.

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The trypanosome which forms the subject of the following experiments was kindly forwarded to Mpumu by Mr. E. Montgomery, M.R.C.V.S., from the veterinary pathological laboratory, Nairobi. The organism was originally obtained from the blood of a camel from Boran. Experiments were undertaken to see whether the trypanosome was transmissible by laboratory-bred *G. palpalis*, and a few sub-inoculations were performed.

Morphology.—Length: 400 trypanosomes taken at random were measured, and the results are given in Table I. As is there shown, the length varies between 18 μ and 34 μ.

Shape: The great majority of the trypanosomes seen are slender; a few forms occur which are markedly broader. The flagellar end may be very much drawn out, the kinetic nucleus being sometimes from 4 μ to 4·5 μ from this extremity.

Undulating membrane: Well developed.

Flagellum: In the slides examined, only one single specimen was observed in which there could be any doubt as to the presence of a free flagellum. In the majority, the free portion of the flagellum is very well marked.

Kinetic nucleus: Always clearly discernible; small and round, situated

either almost in contact with the posterior end, or at varying distances from this extremity up to 4·5 μ.

Nucleus: Situated near the middle of the body.

The following Table I gives the measurements from four experimental animals. 100 examples were drawn from each experiment:—

Table I.*

Expt.	18 μ.	19 μ.	20 μ.	21 μ.	22 μ.	23 μ.	24 μ.	25 μ.	26 μ.	27 μ.	28 μ.	29 μ.	30 μ.	31 μ.	32 μ.	33 μ.	34 μ.	Average.
Rat 780.....			6	7	11	11	7	6	7	10	8	8	10	6	3			25·1
Rat 696.....			1	3	4	7	9	5	5	15	15	9	13	6	5	2	1	27·0
Monkey 646.....			5	4	8	8	6	11	18	13	16	2	7	2				25·5
Dog 671	1	1	6	6	12	11	11	17	12	15	2	2	4					24·4
Totals	1	1	18	20	35	37	33	39	42	53	41	21	34	14	8	2	1	
Percentages ...	0·2	0·2	4·5	5	8·7	9·2	8·2	9·7	10·5	13·2	10·2	5·2	8·5	3·5	2	0·5	0·2	

* In the table of measurements of the antelope trypanosomes as printed in my paper earlier in this volume (p. 166), two numbers are omitted, viz., in column 18 μ on line (2) 511, monkey, the figure 1 is omitted, and in the line of totals the figure 7 is omitted.

The fly experiments may now be briefly summarised. On no occasion was a successful transmission obtained and no flagellates were found in the flies employed, either in proboscis or gut:—

Table II.

Expt. No.	Period on infected animal.	Number of flies. 1st day.	30th day.	Dissected.	Containing flagellates.	Duration of experiment (days).	Result.
680	Mar. 27—30 on Monkey 676	48	35	48	0	33	—
681	„ 28—30 „ 676	42	29	42	0	32	—
683	„ 31—Apr. 4 on Dog 671	82	56	82	0	30	—
684	Apr. 1— 6 „ 671	47	26	47	0	29	—
687	„ 2— 6 „ 671	62	43	62	0	52	—
693	„ 8—15 „ 671	79	47	79	0	45	—
698	„ 10—15 on Monkey 676	73	40	73	0	41	—
	Totals	433	276	433	0		

Animals Susceptible to this Trypanosome.—In the following table those experiments marked * were performed by Mr. Montgomery, and I wish to express my indebtedness to him for the permission to quote them. It will be

noticed that the disease is rapidly fatal to rats and monkeys, while in dogs it is relatively slow:—

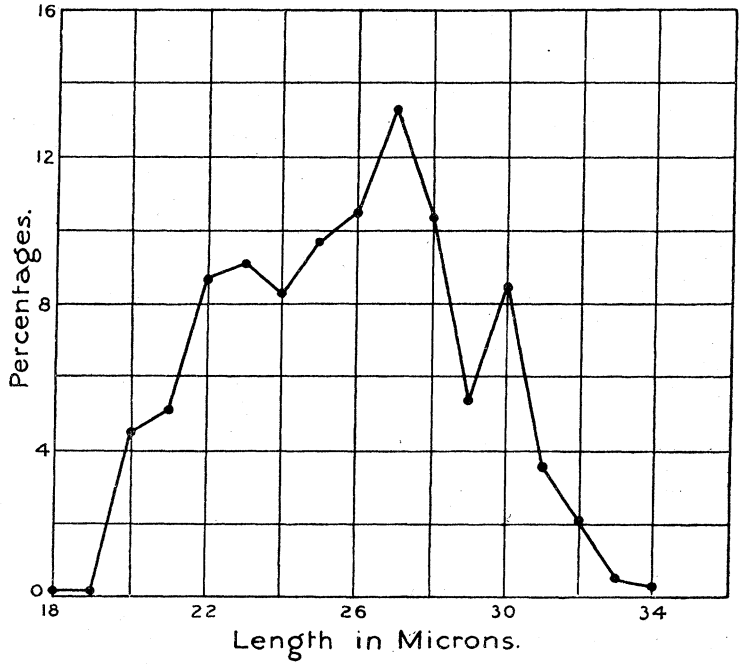
Table III.

Expt. No.	Source of virus.	Incubation period.	Duration of disease.	Remarks.
		days.	days.	
*Ox 123	Camel 8	?	112	Trypanosomes never seen in peripheral blood; proved by subinoculation.
*Mule 6	Dog 3	5	—	Alive after 130 days; not showing by direct observation.
* ,, 25	Donkey 22	?	136	
*Donkey 13	Dog 3	8	99	
* ,, 22	Donkey 13	8	128	
*Dog 3	Camel 8	11	68	
* ,, 5	Ox 123	10	40	
* ,, 6	Dog 3	8	21	
* ,, 14	Mule 6	7	34	
* ,, 21 }	,,	?	—	Alive after 150 days; some emaciation; no corneal changes.
* ,, 671 }				
* ,, 29	,,	10	62	
* ,, 30	Donkey 22	7	83	
Monkey 676	Dog 671	4	26	Old monkey at commencement of the experiment.
White rat 696 ...	Monkey 676	4	10	
,, 697 ...	,,	4	10	
,, 674 ...	Dog 671	3	10	
,, 675 ...	,,	3	5	
,, 780 ...	Goat 677	?	14	
Goat 677.....	Dog 671	?	—	Trypanosomes never seen in peripheral blood. Alive and in good condition after 110 days.
Guinea-pig	,,	4	85	
,, ...	,,	4	70	

Identity of the Trypanosome.—The diagnosis appears to rest between the trypanosomes of the surra-nagana group, *e.g.*, *T. brucei, evansi, equiperdum,* and *equinum.* The well marked kinetic nucleus seen in stained films excludes *T. equinum,* and the absence of any of the characteristic plaque lesions of dourine is against *T. equiperdum.*

There remain *T. brucei* and *T. evansi* to be considered. The following curve constructed from Table I corresponds roughly to Bruce's curve for *T. evansi,* and the absence of any short stumpy forms from all the experimental animals examined is against *T. brucei.* Thus out of 400 specimens measured in the present instance no trypanosomes were seen measuring less than 18 μ in length, while Bruce describes a considerable number of examples of *T. brucei* between 13 μ and 17 μ. Turning to the table of animal reactions little or no assistance is forthcoming in deciding between *T. brucei* and *T. evansi.* In

both species the disease is typically a very rapid one in white rats, and the number of trypanosomes in the peripheral blood shortly before death is enormous. The course of the disease in dogs in the above table is not characteristic of either *T. brucei* or *T. evansi*, which are rapidly fatal to these



animals. In the case of surra, however, the disease appears less rapid in its course than with nagana. Thus Laveran quotes experiments of Lingard's in which dogs lived 27½, 29, 34, 36, 47, 97 days after inoculation with *T. evansi*, the last having been inoculated from a naturally infected bovine. In the case of *T. brucei*, however, the same authority gives 26 days as the maximum duration of nagana in dogs.

Though by no means a typical *T. evansi* it is plain that the above experiments suggest this trypanosome rather than *T. brucei*, and this conclusion is supported by the evidence of morphology.

I shall take this opportunity to consider briefly the question of the biometric method of diagnosing trypanosomes from the point of view of a worker dealing with the problem in the field. Of late a great deal of attention has been devoted to this method, with the result that there appears to be some danger of its attaining undue importance. In certain cases it is of undoubted value, as in discriminating between two such species as *T. vivax* and *T. uniforme*, where the difference in size is one of the most important

points of distinction. Here, however, it will not be necessary to measure a great number of trypanosomes. In the case of other mammalian trypanosomes, *e.g.*, *T. brucei*, *gambiense*, *pecaudi*, *rhodesiense*, *evansi*, *equiperdum*, *equinum*, *nanum*, and *congolense* exhaustive measurements are of little use in diagnosis.

The following example will illustrate very well both the uses and the limitations of the measurement method. In papers dealing respectively with the morphology of *T. gambiense* and *T. evansi* Bruce gives three curves of *T. brucei* for purposes of comparison. The apices of the curves are at 18 μ, 20 μ, and 24 μ respectively, a very considerable variation. Further, in dealing with *T. evansi*, he claims that from a comparison of the two curves it is possible to distinguish the two species from one another. In the case of *T. brucei* the bulk of the curve will be between 13 μ and 35 μ, with *T. evansi* between 18 μ and 30 μ. A glance at the curves, however, will reveal the fact that the real difference between the two trypanosomes as determined by the biometric method is that *T. evansi* shows no forms of less than 18 μ in length. In other words, if in the course of examining a slide a trypanosome is found below say 15 μ in length, the diagnosis cannot be *T. evansi*. Thus the diagnosis has in reality turned upon the minimum measurements of the two species.

The measurements quoted for the different species of trypanosomes vary with the observer, even although the staining and fixing methods are the same in every case. This variation is due to a number of factors, the least important of which is probably the technique employed in the actual measurement.

It would appear from Miss Robertson's recent work on *T. gambiense** that an endogenous cycle exists in the mammal, the duration of which is variable and incalculable, the result being that the type of trypanosome predominating in the peripheral blood is constantly changing. The date of the infection has no constant connection with the course of the phenomenon. In each cycle the change would appear to be from short to long forms, returning to the short forms again before the temporary disappearance from the blood.

To obtain an accurate conception of the dimensions of any trypanosome it is plain that all the stages of such a cycle must be followed out, from its commencement to its conclusion. Slides taken haphazard from time to time are useless for comparative purposes, as practically any type of curve may be obtained according to the stage of the cycle. The three curves of *T. brucei* quoted above from Bruce's paper are an instance of this. In a recent report summarised in 'Sleeping Sickness Bulletin 36' (vol. 4, p. 145), Dr. Stephens lays great stress on the minute technique of measurement, and

* 'Roy. Soc. Proc.,' B, vol. 85 (No. 582).

points out that Bruce's method is open to criticism. The contention advanced here is that such a criticism, though doubtless true as regards geometrical exactitude, is irrelevant as regards its practical application. The errors involved are so small that they can have no serious effect in the matter of diagnosis, when all the other aspects of the question are considered. Dr. Stephens does not apparently criticise the method of preparing the slide adopted by Bruce, he merely asserts that the actual estimate may err within fractions of a micron. Surely such a source of error cannot be very important in plotting out a curve whose unit is 1 μ, considering, for example, that all values between 5·5 μ and 6·5 μ will necessarily be registered as 6 μ.

Again, there are other considerations which tend to make the biometric method of diagnosis at best inconstant:—

(1) Fixation will vary in different parts of the same slide and in the preparation of slides by different observers.

(2) Numerous varieties of strain exist among trypanosomes of any species.

(3) The great similarity between many so-called species as regards their length variation.

(4) Probably continued maintenance in laboratory animals leads to slight alterations in the morphology of a strain, which to be kept true should from time to time be passed through its insect host. Thus the age of a strain after its last passage through the appropriate intermediate host may be a very important factor in determining its morphology.

In the face of such objections it would appear that, although the biometric method may have a certain value in diagnosis, such refinements as suggested by Dr. Stephens are by no means essential. What is required is that measurements be made in as constant a manner as possible. Dr. Stephens' method, involving as it does a complicated projection apparatus, is quite impossible for workers in the field. This in itself is an objection which would seriously neutralise undoubted advantages. It is, however, difficult to believe that the ultimate results of the application of both methods to the same slide would differ to any appreciable extent. Granting, however, for the sake of argument Dr. Stephens' contention, it must still be remembered that its occasional employment will introduce yet another variable factor into this method of diagnosing trypanosomes.