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MEASUREMENTS OF *TRYPANOSOMA DIEMYCTYLI* FROM DIFFERENT HOSTS AND THEIR RELATION TO SPECIFIC IDENTIFICATION, HEREDITY AND ENVIRONMENT

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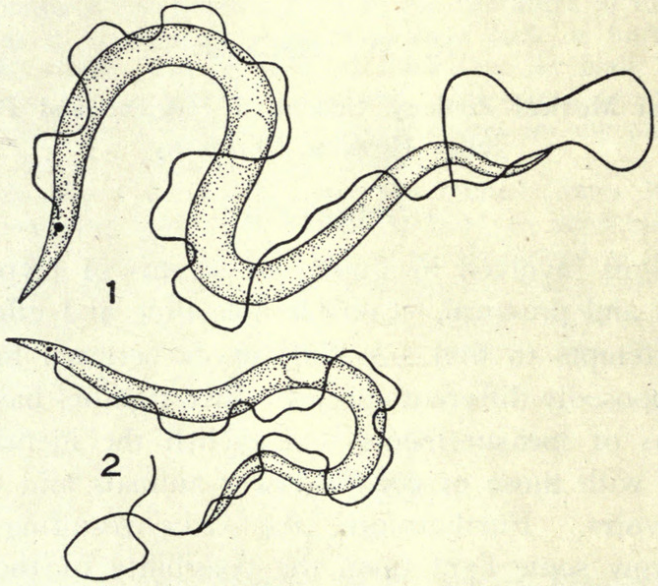
INTRODUCTION

The problems involved in studies of strains of trypanosomes are both scientific and practical. Considerable time and effort have been devoted to attempts to find size differences between human trypanosomes of supposedly different species. Investigators have endeavored also by means of measurements to establish the identity of human trypanosomes with those of certain lower animals and thus to locate animal reservoirs. Furthermore, the data resulting from these researches throw some light upon the possibility of the existence of heritably diverse strains within a species, and upon the effects of the environment, *i. e.* the blood stream, of different species of hosts and of different hosts of the same species on a single strain. It is this last problem that is considered in the following pages.

Trypanosoma diemyctyli (Figs. 1 and 2) was found by Tobey in 1906 to be present in all of a number of newts purchased in an animal store in Boston. During the spring months of 1919 the writer examined the blood of six different species of salamanders. In none of these were any organisms found except in *Diemyctylus viridescens.* All of seventy-eight aquatic specimens of these were infected and two out of seven land specimens. The negative animals were 46 specimens of *Necturus maculosus,* six of *Plethodon glutinosus,* five of *P. cinereus,* 11 of *Desmognathus fusca,* and six of *Spelerpes bilineatus.*

The difference between the aquatic and land forms of *Diemyctylus viridescens* as regards infection with trypanosomes presents an interesting problem. The life cycle of these amphibia includes a year in the water, then a second year on land, and finally a return to the water for mating in the third year. Evidently while on land the infection with the trypanosomes is much decreased. The small numbers of

organisms found in these terrestrial specimens may be due to the absence on land of the transmitting agent, which is unknown. The universal abundance of the trypanosomes in the aquatic specimens may be due to their continued inoculation with young stages that have developed in the intermediate host; and the lesser numbers in the land forms, to a gradual dying out of older trypanosomes. It is of interest in this connection to note that of 34 "land" frogs examined during the spring and summer of 1919 only two were infected with trypanosomes whereas of 41 "water" frogs 28 were infected.



EXPLANATION OF FIGURES

Fig. 1.—Typical specimen of *Trypanosoma diemyctyli* from newt 19. × 1600.
Fig. 2.—Typical specimen of *Trypanosoma diemyctyli* from newt 15. × 1600.

METHODS OF MEASURING TRYPANOSOMES

In any investigation involving measurements the accuracy of the results depends primarily on the accuracy of the measurements. Trypanosomes are difficult to measure precisely, since their bodies are almost always thrown into curves when fixed. This is especially true of long slender forms. Several methods of obtaining accurate measurements have been employed by investigators.

The method adopted by Bruce, Hamerton and Bateman in 1909 was to draw an outline of each specimen with a camera lucida at a magnification of 2000 diameters "and then to measure along the middle line of the body by means of a pair of fine compasses, the points of which are separated 2 mm. Each step the compass takes is therefore equal to 1 micron."

A modification of this method was employed by Stephens and Fantham (1912). They projected the trypanosomes on a screen with

a microprojection apparatus and then traced their outlines with a sharp pencil. A magnification of 2500 diameters was adopted. The drawings were then measured by placing over them semitransparent tracing paper on which a straight line was drawn in ink. One end of the ink line was placed on one end of the drawing and rotated whenever the axis of the trypanosome curved. When the end of the drawing was reached the distance was measured with a millimeter scale.

The method used by the writer seems more desirable than those described above. The trypanosomes were projected with a camera lucida upon a drawing card at a magnification of 1600 diameters. The anterior and posterior ends and kinetonucleus were then indicated with a dot; the width of the body at the nucleus was recorded by two short parallel lines; and the nucleus was drawn. A single line was then drawn down the center of the body from the posterior end to the anterior end. With a chartometer or "map measurer" the distances were easily and accurately obtained.

INVESTIGATIONS INVOLVING TRYPANOSOME MEASUREMENTS

Bruce and his colleagues have measured thousands of trypanosomes of various species in their endeavor to distinguish by size characteristics between the species pathogenic in man and those that occur in the lower animals. The organisms measured were derived from various strains and were taken from a number of species of both wild and laboratory animals. Bruce finally decided that this method of specific identification could not be depended on.

Data regarding the effects of different hosts on the size of the specimens have been provided by various investigators. Thus Laveran and Mesnil (1912) noted a difference between the length of specimens of *T. brucei* grown in the horse and those grown in rodents. Duke (1912) has suggested that strains of numerous varieties exist among trypanosomes of any species, and that alterations in the morphology of a strain may follow continued passage through laboratory animals.

It seems probable from the work of Miss Robertson (1912) on *T. gambiense* that one difficulty in biometric studies of such trypanosomes, is the presence of an endogenous cycle in mammals which results in changes in the types at intervals that cannot be determined by the date of infection. Even if diversities in size were noted in specimens from different hosts the results would not be conclusive. It seems, therefore, that measurements of these polymorphic species are of doubtful value and that better results may be expected when monomorphic forms are studied.

Pearson (1914) has made a biometrical study of many of the measurements published by other investigators and concludes that actual statistical analysis does not in any way confirm the bulk of the

conclusions reached by Sir David Bruce and his collaborators. He points out the fact that the data available do not provide material for an analysis of the relative influence of the various environmental factors and hence one cannot determine whether divergences indicate different strains or merely modifications due to different environments.

Most of the quantitative studies of trypanosomes deal with attempts to secure data that will provide means of specific diagnosis. Those data that might furnish evidence of diversities due to the character of the host in which the specimens were grown are of doubtful value because most of the species studied have been dimorphic or polymorphic and hence have exhibited great variations even from a single host. Furthermore, many of the strains measured had received dissimilar treatment; some were taken directly from wild animals, whereas others had been passed through series of laboratory animals of different species during periods of varying length. This treatment may have had an influence on the morphological characteristics of the strains used. The available measurements of monomorphic trypanosomes do not exhibit variations that make possible any definite conclusion as regards diversities when grown in different hosts.

A review of the literature, especially the paper by Pearson, emphasizes the importance of more careful studies of the relations between trypanosomes and their environment represented by different species of hosts and by different individuals of one host species. It is possible to isolate single trypanosomes and to obtain in one host animal a supply of specimens that can be compared with the descendants of other single specimens in host animals of the same or other species. Work of this character is now in progress in this laboratory.

TRYPANOSOMA DIEMYCTYLI FROM DIFFERENT HOSTS

Measurements were made of 100 specimens of *T. diemyctyli* that were taken at random, ten from each of ten individuals of *Diemyctylus viridescens*. Some of these measurements are presented in Tables 1 and 2. The preparations were all made on the same day and stained with Wright's stain. No selection was made either of the newts from which the trypanosomes were obtained or of the trypanosomes on the slides. The first ten trypanosomes that were found in the preparation from each newt were drawn at a magnification of 1,600 diameters and then measured with a map measurer.

Table 1 includes the variations in the distances from the anterior end of the body to the center of the nucleus, from the center of the nucleus to the kinetonucleus,* and from the kinetonucleus to the posterior end. The width of the body at the point where the nucleus is

* By the kinetonucleus is meant the body called by the French centrosome, by the Germans blepharoplast, and by certain Americans parabasal.

situated is also given as well as average distances. The data are arranged in a series with those of the largest set of ten trypanosomes at the top of the table and those of the smallest set of ten at the bottom. The final averages differ somewhat from those given by Tobey. Tobey's specimens were from 45 to 50μ in length with a flagellum 24μ long. My specimens ranged from 42.5 to 75.3μ in length with a flagellum 32μ long. The measurements of the trypanosomes from newts 19 and 15 are particularly interesting. The data show that in *every one* of the trypanosomes from newt 19 the distance from the anterior end to the center of the nucleus is considerably greater than it is in *any* of the specimens from newt 15. This distance in newt 19 ranges from 31 to 44μ, whereas in newt 15 it ranges from 20 to 23μ. The average in the ten specimens from newt 19 is 36.3μ and in newt 15, 21.4μ, a difference of over 50 per cent. The differences between the distances from the center of the nucleus to the kinetonucleus are similar but not so great, those of the trypanosomes from newt 19 being only 25 per cent. greater than from newt 15. The distances from kinetonucleus to posterior end show differences averaging over 50 per cent., and the average difference in total length exclusive of the flagellum is approximately 50 per cent.

TABLE 1.—VARIATION IN LENGTH AND WIDTH OF 100 SPECIMENS OF *T. diemyctyli* TAKEN AT RANDOM, TEN FROM EACH OF TEN NEWTS, AND THE AVERAGE LENGTH AND WIDTH OF THE SAME GROUPS OF TEN. ALL MEASUREMENTS IN MICRONS

Number of Newt	Anterior End of Body to Center of Nucleus		Center of Nucleus to Kinetonucleus		Kinetonucleus to Posterior End		Total Length Exclusive of Flagellum		Width of Body at Nucleus	
	Variation	Average	Variation	Average	Variation	Average	Variation	Average	Variation	Average
19	31–44	36.3	23–32	25.6	5.6–6.9	6.1	61.6–75.3	68.0	3.1–3.8	3.3
20	32–42	35.5	23–29	26.1	5.0–6.9	5.9	58.6–72.9	66.5	2.5–4.4	3.3
14	28–33	30.5	26–29	27.7	3.1–5.6	4.3	59.4–66.6	62.5	2.5–3.1	2.9
18	23–36	27.9	22–27	23.9	3.8–5.0	4.3	50.8–65.4	56.1	2.5–3.8	2.9
16	22–31	27.2	20–30	23.9	3.1–5.6	3.9	45.1–65.4	55.0	2.5–3.1	2.6
10	23–31	26.4	21–26	23.9	3.8–4.4	4.3	48.8–60.0	54.6	3.1–4.4	3.4
17	21–27	24.1	22–25	24.0	3.1–3.8	3.5	48.1–55.8	51.6	2.5–3.1	2.7
12	23–29	25.1	21–25	22.9	2.5–3.8	3.3	38.1–57.1	51.3	2.5–3.8	3.0
9	19–29	24.4	18–27	21.8	3.1–4.4	3.6	45.0–60.3	49.7	1.9–3.1	2.6
15	29–23	21.4	19–22	20.6	2.2–3.4	2.7	42.5–48.4	44.7	1.9–2.5	2.2

The striking fact brought out by a comparison of these data is the large and constant difference in length between the two sets of trypanosomes taken from two different hosts of the same species. That this difference in length is not due to methods of preparation causing the elongation of one set and the contraction of the other is evident when a comparison is made of the diameters of the body in the region of the nucleus. The long specimens from newt 19 were also thicker than the short specimens from newt 15, the former averaging 3.3μ in diameter, and the latter only 2.2μ. The trypanosomes in newt 19 were therefore uniformly larger in all dimensions than those in newt 15.

Further study of Table 1 shows that the sets of 10 trypanosomes from each of the 10 newts were comparatively constant in their measurements. On the whole, the longest specimens are also the thickest, and length and width decrease together as one proceeds down the table.

In every set the average distance from the anterior end to the center of the nucleus is greater than that from the center of the nucleus to the kinetonucleus. When these distances are compared in the different sets, however, considerable variation becomes evident. For example, in specimens from newt 19 the difference between these two distances averages 10.7μ whereas in those from newt 17 the average difference is only 0.1μ and in those from newt 15 only 0.8μ. These distances are more uniform also in trypanosomes from newt 15 than in those from the other newts. The variations in the distances from the kinetonucleus to the posterior end were slight in the trypanosomes of each set, but the average distance is greatest in those of the longest set and becomes gradually less as the total length decreases.

After the work just described was completed it seemed desirable to obtain a more accurate measure of the differences between the trypanosomes in newts 19 and 15. Ninety more specimens from each newt were therefore measured by my assistant and the results are indicated in Table 2; this gives the averages of the various distances for the first ten trypanosomes measured from newts 19 and 15, for the succeeding ninety and for the entire one hundred. It is interesting to note that the averages for the first ten are very nearly the same as for the succeeding ninety. This indicates that the data obtained by measuring ten specimens from each newt as presented in Table 1 give fairly accurate averages.

Among the specimens from newt 15 six were found that were very much larger than any of the others. Measurements of these six are given in Table 2 and were omitted from those used for getting the averages of the 100 specimens given in this table. These six specimens resemble very closely those taken from newt 19 and apparently represent a type differing widely from the other more abundant trypanosomes from newt 15. Several explanations suggest themselves to account for the diversity between these two types found in a single host. Most probably there are here two size races of one species or there may be two distinct species living in a single host. The two types may possibly represent sexual stages of one species and although from what is known of the life cycles of other blood-inhabiting protozoa one would expect to find the sexual stages in the invertebrate host, still, as in the malarial organism, gametocytes may be developed in the vertebrate and remain dormant until stimulated to further activity within the invertebrate host. It is also possible that the two

types of trypanosomes from a single host may be due to different stages of growth. A final suggestion is that *T. diemyctyli* is dimorphic.

DISCUSSION

No one has succeeded in classifying satisfactorily the trypanosomes and their allies, a condition due in part to the difficulty of determining morphologic differences of diagnostic value and to the fact that many species are polymorphic. *T. diemyctyli* is a favorable form for study because it is apparently monomorphic. Its life history, however, is unknown. The account Tobey gives of this species and the experience of the writer indicate that types other than the long, slender form do not occur in the blood of the newt, at least at the time of year when the examinations were made (May). No specimens were found that showed any signs of division and hence it seems safe to assume that all of the organisms measured represented "adult" forms.

TABLE 2.—AVERAGE LENGTH AND WIDTH IN MICRONS OF SPECIMENS OF *T. diemyctyli* FROM NEWTS 19 AND 15

	Anterior End of Body to Center of Nucleus	Center of Nucleus to Kineto-nucleus	Kineto-nucleus to Posterior End	Total Length Exclusive of Flagellum	Width of Body at Nucleus
Specimens from newt 19:					
Average of specimens 1-10.....	36.3	25.6	6.1	68.0	3.3
Average of specimens 11-100...	35.1	28.0	6.5	69.6	3.6
Average of specimens 1-100....	35.7	26.8	6.3	68.8	3.5
Specimens from newt 15:					
Average of specimens 1-10.....	21.4	20.6	2.7	44.7	2.2
Average of specimens 11-100...	21.9	21.4	2.3	45.7	2.9
Average of specimens 1-100....	21.7	21.0	2.5	45.2	2.6
Average of 6 largest specimens	33.5	26.3	4.8	64.6	3.5

Two hypotheses suggest themselves to account for the constant diversities in the total length and the length of portions of the trypanosomes from the different individual newts; (1) the observations may deal with pure lines, and (2) the organisms in one newt may be derived from various lines but may have become comparatively uniform in size due to life in one environment. The differences between groups of trypanosomes from different hosts might be accounted for by differences in the environment.

Pure Lines in Protozoa.—It has been shown by many investigators that "wild" specimens of free-living protozoa differ from one another in their heritable characteristics and that the descendants derived by vegetative reproduction from one "wild" individual may be uniformly different from those descended from another "wild" individual. The number of these pure lines that may exist in nature seems almost infinite.

Considerable interest has recently been created by the discovery of different strains of cysts of *Entamoeba histolytica* and *E. coli.* Mathis and Mecier (1916, 1917) recognize cysts of two sizes from *E. histolytica* which they consider indicates a sort of sexual dimorphism. Various strains of cysts as regards size have also been noted in *E. histolytica* by Wenyon and O'Connor (1917), Dobell and Jepps (1917), Matthews (1918), Mackinnon (1918), Smith (1918, 1919) and Kofoid, Kornhauser, and Swezy (1919). The evidence indicates the existence in these parasitic protozoa of heritably diverse races similar to those that have been described in a number of free-living protozoa. What influence environmental factors may have on the size of the cysts can be determined in several ways; for example, single specimens could be isolated from cultures and pure lines obtained from these also in culture. The effects of changes in environment could then be observed by modifying the culture medium or by inoculating specimens from the same pure line into different laboratory animals.

The habitat of the intestinal amoebae resembles that of the trypanosomes in certain respects although it may be more or less varied because of the many different kinds of food taken into the alimentary canal. The composition of the blood differs in different species of animals and to a lesser degree in different individuals of the same species. This means that trypanosomes also are subjected to differences in their environment. No one knows what effect these different environments may have on trypanosomes belonging to the same pure line, but as noted above a method of determining this point is available and is being put to the test in this laboratory.

SUMMARY

(1) Every one of 78 aquatic specimens and 2 of 7 land specimens of the newt, *Diemyctylus viridescens,* collected in Pennsylvania were found to be infected with *Trypanosoma diemyctyli* Tobey. No trypanosomes were found in 72 specimens of 5 other species of salamanders. Inoculation experiments with *T. diemyctyli* on 6 species of salamanders and 2 species of frogs were unsuccessful.

(2) Measurements were made of trypanosomes from 10 newts. These groups of trypanosomes differed from one another in their range of variation in total length exclusive of the flagellum, in the length of portions, and in the width of the body, in the average length of the entire body exclusive of the flagellum, in the average length of portions and in the average width.

(3) Length and width show a positive correlation and on an average the longer the specimen the wider it is.

(4) Of 106 trypanosomes from one newt, 100 were uniformly small and the remaining 6 were much larger, indicating 2 different types in a single host.

(5) The different types of trypanosomes obtained from the different newts are probably races of one species that are heritably diverse in size. They may, however, belong to different species or may be sexual phases of a single species, or may differ because of changes due to the environment.

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References to other literature on the specific identification of trypanosomes may be found in the above papers, especially that by Pearson.