CULTIVATION OF THE TRYPANOSOME FOUND IN THE BLOOD OF THE GOLD-FISH.

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Plate III.

Since 1903 when McNeal and Novy first recorded that they had obtained cultures of *T. lewisi*, other trypanosomes found in the blood of mammals, more especially the pathogenic ones, *T. brucei* and *T. evansi*, have been successfully cultivated. Besides those parasitic in the blood of mammals, trypanosomes found in the blood of birds, of amphibians, and lastly those found in the stomachs of mosquitoes and other insects, have been grown on artificial media; but so far there has been no record of the successful cultivation of any species of fish trypanosome.

Brumpt (1906), after having described the evolution of fish trypanosomes, particularly those of the eel, in *Hemiclepsis*, and their behaviour in other species of leeches—*Callobdella punctata, Hirudo troctina, Piscicola geometra*—says that in the medium of McNeal and Novy he was unable to obtain any culture, but that in spite of the difference in the constitution of the medium as compared with that of the blood of the eel he observed some perfectly typical trypanosomes eight or ten days after inoculation. From his various experiments he concludes that the morphological changes which he observed in the trypanosomes were not due to any physical characters of the medium in which they developed, but were the result of a vital reaction of the parasites to a special chemical medium in a particular host.

Lebailly (1906) writes that, contrary to Brumpt's experience, he has found profound changes take place in fish trypanosomes "*in vitro*." The conditions of his experiments were not the same as those of Brumpt's, as Lebailly himself allows. In place of making use of the medium of

McNeal and Novy he simply kept films of blood containing trypanosomes between coverslip and slide by sealing round the edges of the coverslip with paraffin wax. By the end of eight or ten days almost all the trypanosomes in the film had changed into "small granular spherical masses each with flagellum feebly moving."

During the months of May, June and July of 1906, acting on the advice of Dr Dean and helped by him and by Dr Ledingham, I succeeded in obtaining cultures in blood-agar tubes of a trypanosome found in the blood of gold-fishes taken from a pond at Queensbury Lodge, Elstree. The pond is about twenty yards long by about fifteen yards broad. It has a muddy bottom, and contains many weeds and decaying leaves. There is a small inlet and a small outlet so that the water in the pond is slowly changing. In this pond the gold-fishes have been allowed to multiply and to feed naturally for many years. Every fish whose blood I examined harboured trypanosomes. As I examined a great many of all sizes from 1 cm. up to 20 cm. long (the caudal fin which was often damaged being omitted in these measurements), I believe that every fish in the pond from 1 cm. in length upwards is infected. On the other hand I was constantly on the outlook for leeches and although on one occasion (28th May) I had the pond dragged, and also carefully examined with the aid of a hand-lens the gills and the surface of the body between the scales of 178 fishes, I never succeeded in finding a leech. These two facts taken in conjunction —the failure to find leeches during the months of May, June and July, and the constant presence of trypanosomes in every fish examined from 1' cm. in length upwards—suggest that in addition to the known mode of transmission by the leech, there may possibly be some other way or ways by which fish may become infected with trypanosomes.

Description of the trypanosome as it is found in the blood of the gold-fish.

This gold-fish is a species of carp, and the trypanosome found in it (Plate III, Figs. 1 and 2) is, so far as can be judged from its morphology, identical with *T. danilewskyi*, as described by Laveran and Mesnil (1904). As seen in films fixed by methyl alcohol and stained by Giemsa, the body measures 35—45μ long and about 3μ broad; the free flagellum is 15—17μ long; the posterior end of the body is pointed; the kinetonucleus[1], situated about 2·5μ from the posterior extremity, is prominent,

[1] The nomenclature here adopted is that suggested by Woodcock (1906). The smaller body is nuclear in nature, but the term micro-nucleus has already been appropriated for

in dried specimens frequently seen projecting from one side of the body like a short horn: the undulating membrane is large and well folded: the tropho-nucleus is elongated, being about 4 μ long by about 2 μ broad, and lies somewhat nearer to the anterior than to the posterior end of the body: in addition to the tropho- and kineto-nuclei, the body protoplasm contains chromatic granules varying in number and in size.

Cultivation.

The culture medium found successful was that recommended by McNeal and Novy for the culture of *T. brucei*, slightly modified by Nocht and Mayer, viz.,

I. Extract of 125 grammes of ox flesh in 1000 grammes of water.

Agar	25 grammes.
Peptone	20 grammes.
Chloride of Sodium	5 grammes.
Normal carbonate of Soda solution	10 c.c.

II. Defibrinated rabbit's blood.

One volume of I while still fluid at a temperature of 55° to 60° C. is mixed with two volumes of II in test tubes and the mixture allowed to set in the tube kept sloped in the ordinary way. After the medium has set the tubes are placed upright, capped, and incubated for 24 hours to ensure that the contents are sterile.

Tubes prepared as above were inoculated with blood drawn aseptically from the heart of an infected fish immediately after it had been killed, and were kept in a cool chamber at a temperature of about 15° C. With this medium and under these conditions the proportion of successes was one in three.

The chief difficulties encountered were the technical ones of obtaining blood from the infected fish in an aseptic condition and in transferring this blood to the culture tubes. The heart in these small fishes is very friable and the blood is very coagulable. Capillary pipettes were used to withdraw the blood from the heart. In introducing the point of the pipette great care had to be taken to avoid tearing through the heart wall at the point of fixation. The next difficulty arose from the coagulability of the blood. A little citrate of soda solution (1 $^0/_0$) was

the generative nucleus of the Ciliata. Several observers have drawn attention to the fact that the flagellum arises from a small basal granule distinct and separate from the smaller or kinetic nucleus. Professor Minchin referring to these points during the course of his lectures at the Lister Institute defined the blepharoplast as an achromatic body, or centrosome in relation to the flagellum; and was of opinion that the term blepharoplast so often used for the kinetic nucleus should be confined to this basal granule. Whenever the term "Blepharoplast" is used in this paper, it refers strictly to this basal granule.

first drawn into the tube; but even then no time had to be lost in transferring the drawn blood to the culture tube.

Results with rabbit-blood-agar medium.

In tubes that gave successful cultures, developmental forms were first seen on the 6th and 7th days after inoculation, though very probably developmental changes had begun earlier than this. Both living and stained preparations were examined at different stages of the culture. Of living preparations it will suffice to say that small free actively swimming forms were seen at all stages of the culture. These could pass quickly across the field of a 1/12th objective. Large dividing forms, often with several flagella, though they showed very active flagellar movements, had very limited movements of translation. Drawings of stained preparations from a successful culture at different periods after inoculation are reproduced on Plate III, and from these morphological changes can more easily be studied.

Description of stained preparations.

The slides from which these drawings were made were prepared in the following way: a drop taken from the culture tube was spread as a film on a slide, fixed in methyl alcohol, treated with fresh blood serum in the way recommended by Leishman (1904) for sections of tissues to be stained by Romanowsky stains, stained by Giemsa (4 drops to 1 c.c. distilled water for 5 hours), then washed, dried and mounted.

1. *Seventh day of the culture.* Plate III, Figs. 3 to 11, are from a film prepared from culture on the 7th day after inoculation. Fig. 3 represents one of two apparently unaltered forms found in this film. Figs. 4 to 11 have been selected to show how the various cultural forms have been derived from the original trypanosome form as found in the blood of the fish. The initial change seems to consist in a concentration of the endoplasm and a bringing together of endoplasmic structures. The posterior two-thirds of the animal becomes greatly altered in shape and in arrangement of parts. It becomes short and swollen, while the anterior third remains much as it was, so that the whole animal assumes a somewhat tadpole-like appearance as seen in Fig. 4. The kineto-nucleus and the tropho-nucleus, in place of being separated from each other by nearly half the length of the animal, now lie side by side; and the undulating membrane is correspondingly shortened. The tropho-nucleus has become swollen and loose in

structure and its chromatin broken up into chromidia. At this stage apparently division can take place, and Fig. 5 shows division almost completed. The thick swollen end at this stage stains by Giemsa a deep purple. The anterior third unaltered in external form takes no part in the division. The product of division—resembling *Crithidia*—as seen still attached in Fig. 5, and free in Fig. 6, has apparently the power of multiplying freely. This is illustrated by Figs. 7, 8 and 9. Figs. 8 and 9 are probably agglutinations, but the point now is that in this *Crithidia*-like form free multiplication has taken place. Fig. 10 shows a form where the body has become elongated, but where the kineto- and tropho-nuclei are still close together. In Fig. 11 the kineto-nucleus has come to lie at a considerable distance behind the tropho-nucleus. As will be seen below, trypanosome-like forms (with body elongated and kineto-nucleus well behind the tropho-nucleus) become more numerous at later stages of the culture.

2. *Twenty-first day of the culture.* Figs. 12 to 15 inclusive are from a film prepared from a drop taken on the 21st day of the culture. The *Crithidia*-like form (Figs. 12 and 14) is still in evidence. Along with this are other forms with well developed undulating membrane (Fig. 15), and others where the kineto-nucleus is anterior to the tropho-nucleus and where division is going on (Fig. 13).

3. *The twenty-eighth day of the culture.* Plate III, Figs. 16 to 26, are from a film taken on the 28th day of the culture. The preponderance of bulky forms, the signs of considerable nuclear activity, and the presence of so many coarse granules in the body protoplasm are here very striking. The granular condition of the protoplasm may be due to some influence of medium (most probably the result of some accidental change that had taken place in it about this time) and may possibly be analogous to the granular condition of mast cells. Mast cells, as is known, are found in the mesentery more abundantly than in other parts of the body; and in it in greater numbers during digestion or after a meal. Forms with the kineto-nucleus close by the side of the tropho-nucleus—an arrangement seemingly favourable to free multiplication—are still found (Figs. 17 to 20 and Fig. 23). Fig. 16 shows multiple fission. Other forms more trypanosome-like than any hitherto seen in the culture now appear (Figs. 21 and 24). In Figs. 18, 23 and 25 the tropho-nucleus has divided before the kineto-nucleus, while in Figs. 21 and 24 the reverse is the case. In Fig. 21 it is difficult to see how the smaller of the dividing portions was to get its tropho-nucleus; but I have observed an exactly similar condition in a blood film from a rat that had

been inoculated with *T. lewisi*, so that such apparently anomalous forms are not confined to culture tubes. In Fig. 24 the true blepharoplasts or basal granules, from which the flagella arise distinct from the kineto-nuclei, are clearly seen.

4. *Forty-third day of the culture.* Figs. 27 to 35 are from a film taken on the 43rd day of the culture. In all, the body protoplasm is free from granules. It would thus appear that the influence or influences, to which was due the granular condition seen in forms from the 28th day of the culture, were of a temporary nature. Forms that are *Crithidia*-like by the relative position of kineto- and tropho-nuclei are here also seen; and multiplication by division is still actively going on. In Fig. 32 the kineto-nucleus is getting behind the tropho-nucleus; while in Figs. 33, 34 and 35 trypanosome-like forms resembling mammalian trypanosomes more closely than adult fish trypanosomes, are represented. These divide by unequal longitudinal division, and Fig. 33 shows division begun with the division of the blepharoplast. This is often seen to be the case in mammalian types. Division of the kineto-nucleus, whether it takes place before or after division of the tropho-nucleus, is probably always preceded by division of the true blepharoplast or basal granule, and commencing formation of a new flagellum. These trypanosome-like forms were found to be more numerous at this period than at earlier stages in the culture.

How long the culture lived after this I do not know. When I returned from a holiday seven weeks later it had died out. Sub-cultures that I inoculated just before leaving also showed no sign of life on my return.

Summary and Conclusions.

1. The trypanosome of the gold-fish has been successfully cultivated on the medium of McNeal and Novy.

2. Preparatory to division in culture, the original trypanosome as found in the blood of the fish assumes a somewhat tadpole-like appearance, the endoplasm and its contained structures being collected together in the swollen posterior end. The kineto-nucleus now lies close to, and alongside of, the tropho-nucleus, and the latter has become swollen and loose in structure with its chromatin broken up into chromidia. The anterior third or more of the trypanosome undergoes little or no change in form and does not take part in division. It is thus easily seen how the product of the preliminary division comes to have a *Crithidia*-like appearance.

3. The product of this preliminary division—*Crithidia*-like by the relative position of kineto- and tropho-nuclei—is capable of freely multiplying.

According to Brumpt it is in the *Crithidia*-like form that free multiplication of the eel trypanosome first takes place in the stomach of *Hemiclepsis*. It may well be that in this case also the *Crithidia*-like form is arrived at by steps such as are here figured and described.

4. *Crithidia*-like forms are found at all stages of the culture and along with them at various stages other forms where the body is elongated and the kineto-nucleus still close to the tropho-nucleus, and yet other forms where the kineto-nucleus lies at varying distances behind the tropho-nucleus until true trypanosome-like forms are reached.

5. These trypanosome-like forms (resembling mammalian trypanosomes rather than those of the fish from which they are derived) are most numerous in the later stages of the culture.

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DESCRIPTION OF PLATE III.

Figs. 1 and 2. Trypanosomes from film of blood of gold-fish.

Figs. 3—11. Forms found in film prepared from culture on the 7th day after inoculation. Fig. 3, unaltered form. Fig. 4, form showing the change that takes place preparatory to division. Fig. 5, primary division almost completed. The anterior part of the trypanosome which retains its original form takes no part in division, and the product of division is *Crithidia*-like in appearance. Figs. 6—9, *Crithidia*-like forms that multiply freely. Fig. 10, form with body elongated but with kineto-nucleus still close to (here somewhat anterior to) the tropho-nucleus. Fig. 11, form where the kineto-nucleus has come to lie posterior to the tropho-nucleus.

Figs. 12—15. Forms from a film prepared from cultures on the 21st day after inoculation. Figs. 12 and 14, *Crithidia*-like forms. Fig. 13, dividing form with kineto-nucleus anterior to the tropho-nucleus. Fig. 15, form with well-developed undulating

The bulky form of some, the number of dividing forms and the coarse granular appearance of all are conspicuous. In Figs. 18, 23 and 25 the tropho-nucleus has divided before the kineto-nucleus : in Figs. 21 and 24 the reverse is the case : while in Figs. 17 and 20 the true blepharoplasts are the first to divide. Fig. 24 shows clearly the origin of the flagella from small basal granules, or true blepharoplasts, distinct from the kineto-nuclei.

Figs. 25—35. Forms from film prepared from culture on the 43rd day after inoculation. Note the absence of granules in the protoplasm, the *Crithidia*-like forms, dividing forms, and trypanosome-like forms—the latter being more numerous than at earlier stages of the culture.

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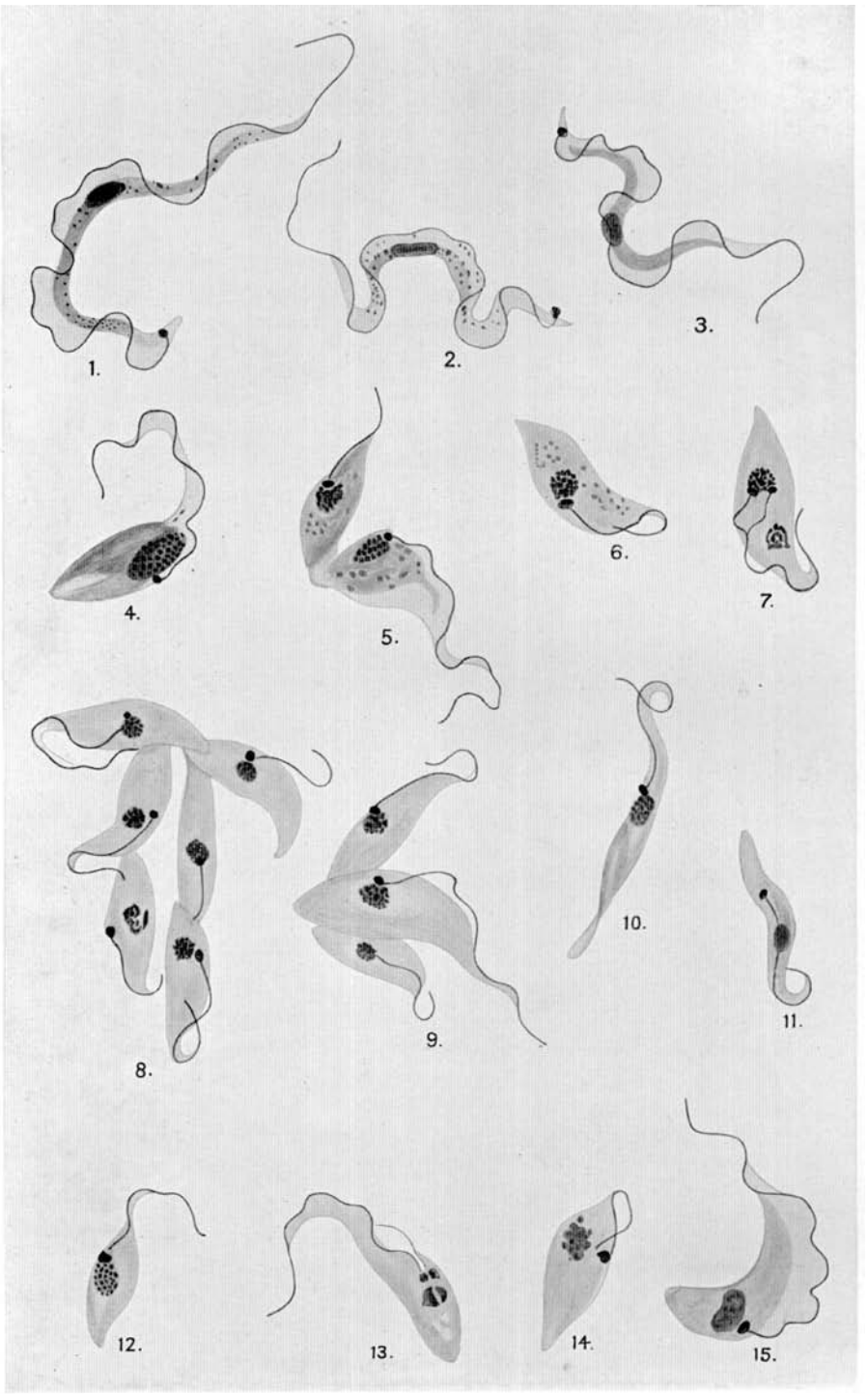

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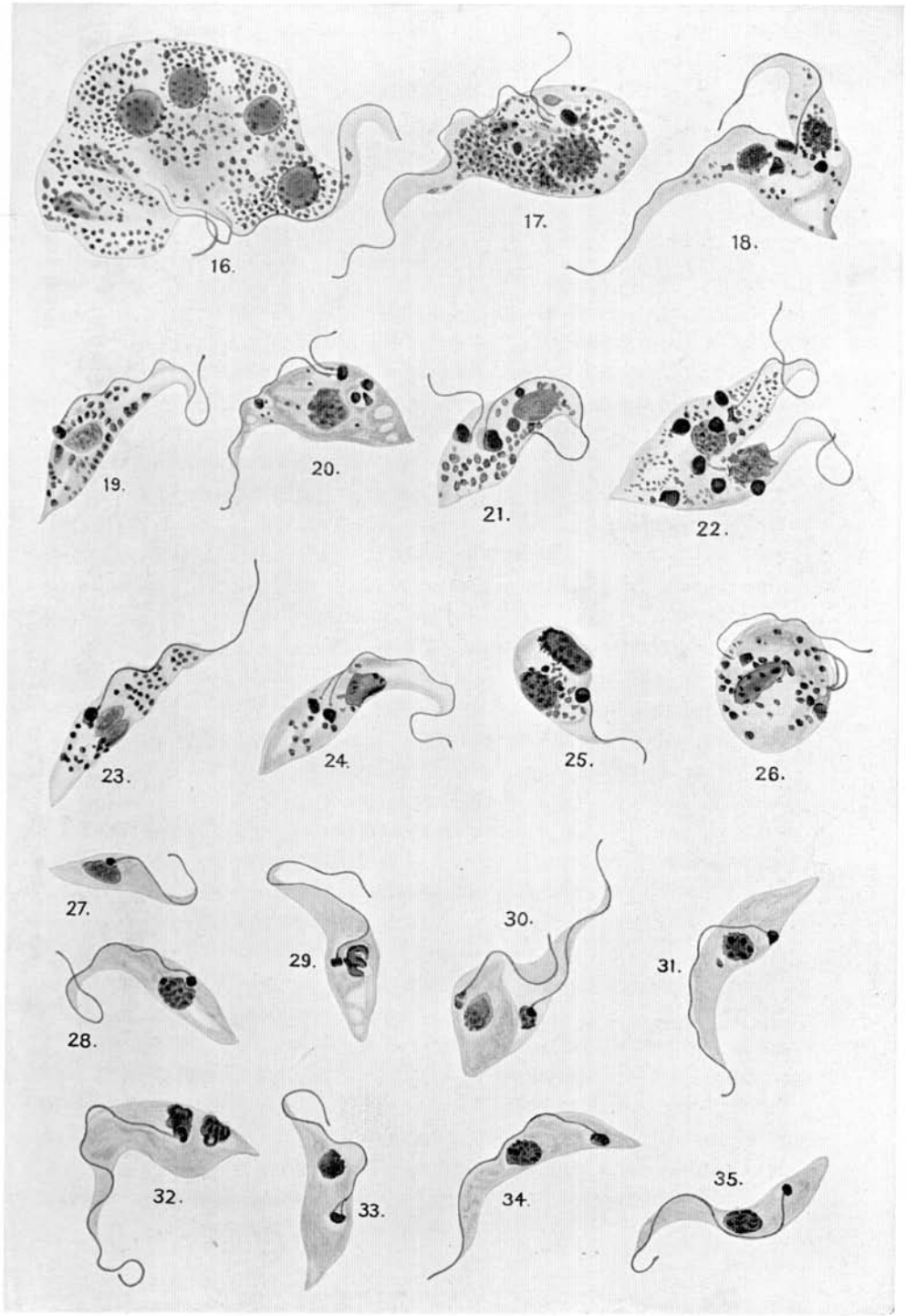
PLATE III



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