

# *Trypanosoma* spp. Pathological to Freshwater Fish\*

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Keywords: *Anguilla*, *Rutilus*, *Tinca*, *Trypanosoma*, spp., eels, fish, pathology (fish).

## ABSTRACT

The observation of lethargic eels from Roath Brook in Cardiff, Wales in 1960 led to an effort to determine the cause of the pathological condition. Blood from such eels and other fish (roach, rudd and tench) showed the presence of trypanosoma. Several trypanosomes lying alongside one or two erythrocytes were seemingly attached as indicated by movement in unison. The possibility that the trypanosome was gaining nourishment from the erythrocyte was inescapable. Added evidence included an appearance of lysis near the point of adhesion after an instance of "breaking apart" was observed. A procedure for taking blood samples from fish without ill effect was developed. The infestation was, in the end, regarded as generally chronic

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\*Presented at INTER/MICRO-89 in Chicago, July 24-28, 1989, originally written in 1960.

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with only mild illness although the effect on some young eels was presumed to be terminal.

## INTRODUCTION

On 5 May 1960 a colleague (J. Michael Bement) reported the presence of noticeably lethargic eels (*Anguilla anguilla*) in Roath Brook, the outflow of an artificial lake in Cardiff, Wales. Although most were swimming normally, their movements were perhaps slower and more deliberate. Evasive action taken by eels we attempted to trap involved sinuous movements of the body associated with the species, but they seemed to find this required more effort than usual and as a result, two were taken.

One was kept for dissection and the other had blood removed by direct cardiac puncture for possible subsequent culture of pathogenic organisms. Small trout and other species, introduced into the lake during restocking, were also taken and, from these, blood smears were taken from specimens obtained by direct cardiac puncture. Numerous young eels (elvers) were also observed, many showed similar symptoms and some were dead. Blood smears were taken from these elvers, each being some 8-10 cm in length.

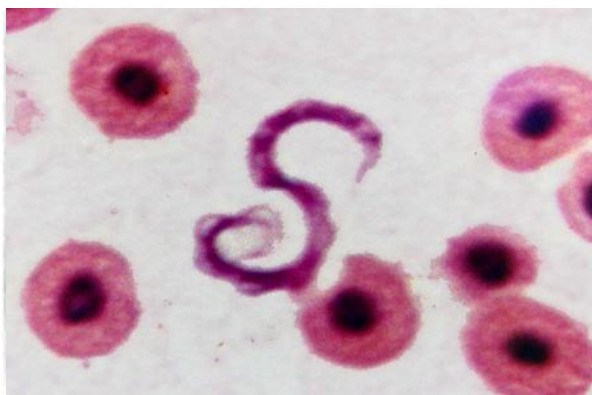
## METHODS

On return to the laboratory the specimen eel was closely examined. There were no external signs apparent which might lead to a diagnosis, and the eel was still active 1/2 hour after its capture, during which time it had been out of contact with water. Intracardiac injection of air soon killed the animal, which was then tied out for dissection.

The abdominal cavity was opened by a median ventral incision from pectoral girdle to vent. The viscera were then displayed. No gross abnormalities were seen, but the spleen was reserved for possible culture later. Blood smears were stained with crystal violet and eosin in Gram's stain, but no signs of any bacteria were noted. Later, a wet preparation of undiluted blood examined by low-power microscopy showed, in about every other field of view, small nematode-like organisms in active movement (Figure 1). Their presence was so unexpected as to draw our attention wholly, and further examination of the eel

(which would have been difficult under the circumstances) was abandoned with a clear conscience.

The slide showing the organisms was heat treated to kill and fix the cells. Following drying in air, the slides were stained with crystal violet and counterstained with eosin. During this time a further fresh blood preparation, examined by oil immersion, showed the organisms to be trypanosoma.



*Figure 1. Erythrocytes from Anguilla blood smear showing cyto-adhesion to trypanosome cell. A zone of lysis is clearly visible, and radiating fibrils may be seen.*

### THE ORGANISMS

Each individual was about 30  $\mu\text{m}$  in length, with the delicate undulating membrane running the entire length of the cell. This terminated in a granule only slightly visible within the endoplasm, the blepharoplast; and at the anterior end the body tapered into an atypically short flagellum. As A. D. Gardner mentions, most trypanosomes have a relatively long flagellum compared to the length of the cell. The majority of the trypanosomes were seen to be in an active state of motility. This consisted of "figure-eight" looping movements like that associated with the Nematoda, and although much of the surrounding medium was disturbed by the process, there was but little locomotion of the organism.

It is assumed that the movement is more to bring about a change in the immediate environment to circulate food material,

rather than to effect locomotion—which might in any case be unnecessary in a parasite living in freely-circulating blood.

The absorption of foodstuffs by simple diffusion through the pellicle is the mode of nutritional up-take normally postulated for these organisms, but several individuals were seen which tend to throw some doubt on this view (Figure 1). These were seen to be lying alongside one or two erythrocytes and, as they occasionally moved, their neighbouring cells moved with them as though attached. One or two were seen to break away from the attached erythrocytes by suddenly recommencing the more energetic "swimming" movements characteristic of the free trypanosomes, and the impression was gained of there being some precise and definite attachment between parasite and host cell, from which they may have been specifically gaining nourishment.

The concept of a trypanosome feeding on an erythrocyte as an ant might gain nourishment from aphids is certainly bizarre to a degree. But this impression was strikingly conveyed on the few occasions when we were privileged to witness an individual break away from a cell to which it had previously appeared to be attached.

The preparation, stained with crystal violet and eosin, was then examined after permanent mounting. One or two trypanosomes were seen to be in the "feeding attitude", some being suitable for photomicrography. Fine fibrils were seen apparently connecting the two organisms in some instances, and a further feature was observed which had not been apparent in the fresh blood studies. The attached erythrocyte appeared to have undergone lysis near the point of cytoadhesion (Figure 1). The lysis was quite clearly delineated and gave the appearance of a bite being taken out of the erythrocyte cytoplasm. It was in this region of lysis that the radiating fibrils were seen to give the impression of connecting host cell to parasite.

It may be that the fibrils represent the dried and stained remains of specific lysis products from the erythrocyte produced by the parasite, so the organisms may be capable of positive association with the host erythrocytes, attacking them with extracellular enzymatic complexes. Confirmation of this could be obtained by the isolation of a water-soluble fraction from a culture of trypanosomes on agar which, in the absence of the parasites, could still produce haemolysis. Attempts were made

to culture organisms from the eel spleen on nutrient agar, but no eel-blood plates could be obtained before the organisms died and these attempts proved ineffective.

It is intended to follow up these observations with further survey work on other fish species. Since no dead—or even moribund—eels were seen, it appears sensible to assume that the infection is of a chronic nature. The sparse number of organisms seen suggests they cause a mild illness so adapted as to cause little incapacity to the host. Since a highly adapted parasite might cause more profound effects in younger and more susceptible individuals, this may account for the presence of dead elvers.

#### FUTHER INVESTIGATIONS

Subsequent investigations led to the demonstration of *Trypanosoma* spp in several species including *Rutilus*, roach; *Scardinius*, rudd (Figures 2 and 3), and *Tinca*, tench; in addition to *Anguilla*. Repeated examinations of blood from *Gasterosteus aculeatus*, the three-spined stickleback, failed to produce any

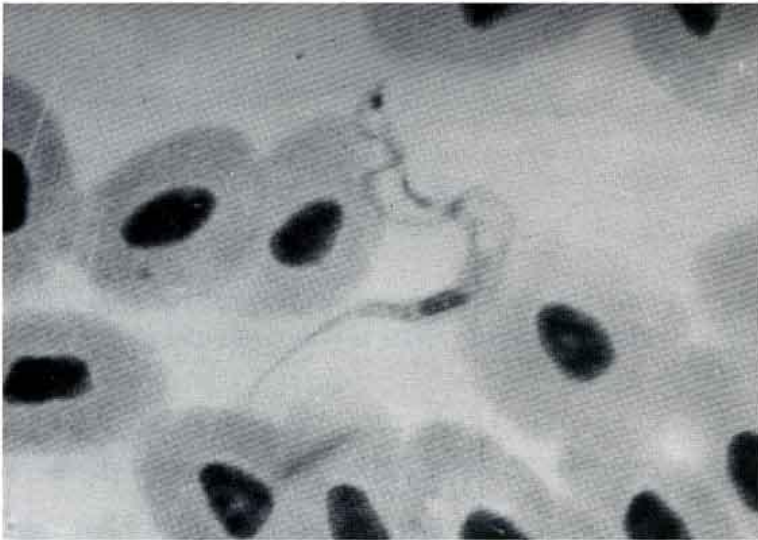


Figure 2. *Trypanosome* in blood smear from *Scardinius*, the rudd, stained with methyl violet and eosin.

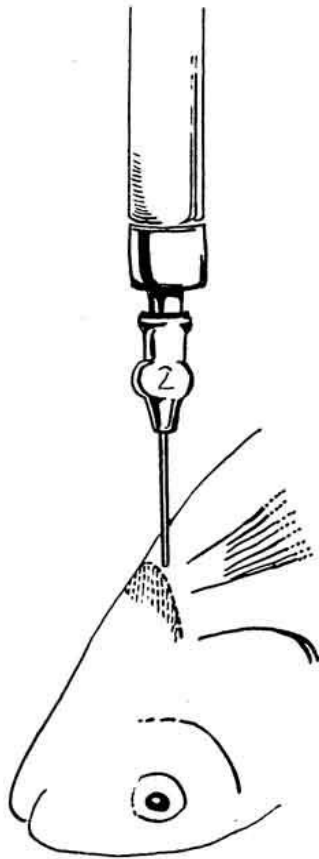


Figure 3. Relationship between pectoral girdle (shaded) and eye-line when inserting hypodermic needle to obtain blood by direct cardiac puncture.

positive result. This was felt to be compatible with a postulated mode of transmission via leeches (sticklebacks are too agile to be accessed by slow-moving leeches).

In September 1960, several fish obtained from the lake were transferred to a tank at the Penarth laboratory. These were used to perfect a technique of cardiac puncture for use in large-scale screening. A specimen of *Rutilus* was killed and laterally dis-

sected, in order to show the angle at which cardiac entry might be obtained with a hypodermic needle. With the superficial landmarking in mind, an attempt to obtain blood by this means from a living adult fish was a complete success. It showed no ill effects whatever, and continued to feed unconcernedly in its tank.

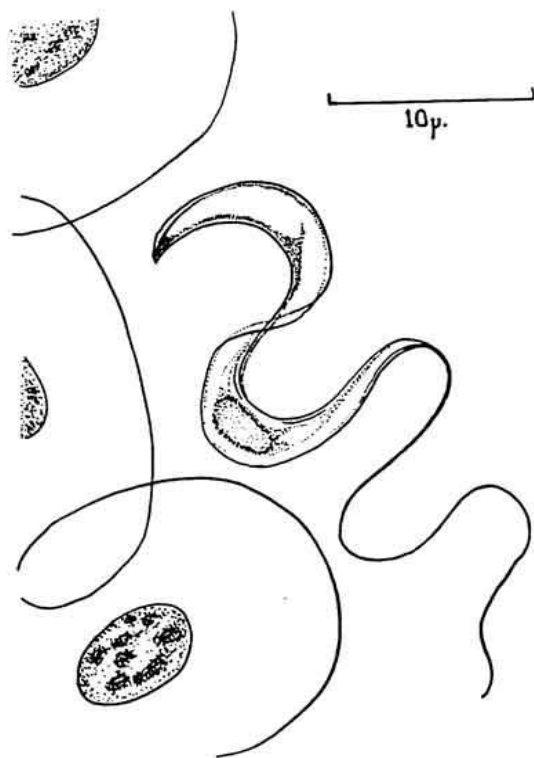


Figure 4. Typical example of *Trypanosoma* with erythrocytes from a blood smear obtained by cardiac puncture.

Details of land-marking are shown in Figure 4. Since the size and position of the pectoral fins, gills and other features are variable from species to species, these were ignored in the calculations. It was found that the safest procedure was to insert the

needle as near as possible to the posterior face of the pectoral girdle as palpated, remembering that it is necessary to avoid striking the girdle itself with the needle. It is driven into the thorax towards a point midway between the eyes. The heart is located relatively near to the surface, and too deep a stroke must be avoided or there is a real risk of penetrating the buccal cavities. With care it is possible to obtain a sample of fresh blood from a range of fish.

Blood smears are obtained in the normal way, taking care to avoid delays as the clotting time seemed very short. The slides were heat-fixed in air or with 3:1 ethanol:acetic acid and stained with methyl or crystal violet, counterstaining with eosin. Routine blood stains give inferior results. Although they are rapid and repeatable, the precision by which minute details of structure may be resolved is not as great as that to be desired with such a small organism. Slides in which trypanosomes have been observed are labelled *Trypanosomes in blood*, whilst those from fish known to be infected (but, in which slides, organisms have not been identified) are labelled trypanosome (or Tr) *positive* (or +).

A thorough search of the organisms has revealed many different types. They may be as small as 25  $\mu\text{m}$  in length, measuring from the extremity of the flagellum to the posterior end of the cell, to as much as 65  $\mu\text{m}$ . The flagellum ranges from 5  $\mu\text{m}$  to 20  $\mu\text{m}$  in length. The breadth of the cell around the mid-point is somewhere in the region of 4  $\mu\text{m}$ , and is subject to but little variation (Figure 6).

#### REFERENCE

1. Gardner, A.D., *Bacteriology for Medical Students*, 182, 1944.

#### ACKNOWLEDGEMENTS

The author is grateful to Mr. W. Nelmes, Director of the Parks Department, for permission to have access to Roath Park Lake and to obtain fish for research, to Mike Bement for assistance, and to Professor J. Brough at the Department of Zoology, Cardiff University, for helpful discussions.