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Growth, morphology and division of flagellates of the genus *Trypanoplasma* (Protozoa, Kinetoplastida) in vitro

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Abstract. Nine strains of trypanoplasms were grown in axenic culture. Cultures of *Trypanoplasma borreli* Laveran and Mesnil, 1901 from fish hosts *Blicca bjoerkna, Cyprinus carpio, Scardinius erythrophthalmus* and *Tinca tinca* and of *T. guerneyorum* Minchin, 1909 from *Esox lucius* and *Trypanoplasma* spp. from the leech *Piscicola geometra* were maintained in biphasic blood-agar medium SNB-9 supplemented with vitamins and antibiotics. In culture, the flagellates transformed into smaller, elongated stages with a little-developed undulating membrane and into short flagella that were morphologically similar to stages in the leech vector. The cultures were passaged weekly at 17–20° C, but they also grew at 4° C. The flagellates divided by binary fission, which was initiated by the formation of two new anterior flagella. The original anterior flagellum of the mother individual was gradually apposed to the cell surface and became the recurrent flagellum of one of the daughter individuals. In the meantime, nuclear division took place, followed by transverse cleavage of the kinetoplast. The division was completed by longitudinal fission of the mother individual into two offspring. Multiple fission that resulted in rosettes, which then cleaved into several daughter cells, was also observed, as well as some dyskinetoplastic and other anomalous forms. In cultures isolated from tenches with high parasitaemia, non-dividing, long filiform stages were observed. Culture stages were not infective for susceptible fishes.

Since their initial discovery in the blood of rudd (*Scardinius erythropthalmus*), kinetoplastid flagellates of the genus *Trypanoplasma* Laveran and Mesnil, 1901 have been found in the blood of many freshwater and marine fish hosts. Due to their pathogenicity and, hence, their importance in pisciculture, they presently attract increasing attention (e.g., Woo 1987; Kruse et al. 1989b). However, at variance with those on trypanosomes in fishes, data on the successful cultivation of trypanoplasms have been extremely scarce, although in vitro culture is one of the prerequisites for studies of the biology and cytology of these culture forms.

The first to cultivate trypanoplasms was Ponselle (1913), who grew *T. varium* from *Noemacheilus barbatulus* in a simple blood medium, without recording incubation times or the number of passages. Tanabe (1924) grew *Trypanoplasma* spp. from *Misgurnus anguillicaudatus* in Ponselles's medium and in NNN blood agar; however, he did not mention subcultures in his report. Nowicki (1940) briefly mentioned culturing *T. cyprini* but gave no details. Qadri (1962) cultivated *T. willoughbii* from *Salvelinus alpinus* (=*willoughbii*) in NNN blood agar and was the first to describe in detail the morphology and division of culture forms.

The attempts of Putz (1972) to cultivate *T. cataractae* in three types of biphasic media, in tissue-culture medium M 199 and in bovine fetal fluid resulted in a survival rate that was limited to only 7–16 days. Woo (1979) grew *T. salmositica* in Hanks' tissue-culture solution supplemented with 10% thermally inactivated fetal calf serum at 5° C, and later at 10° C. Burreson (personal communication to Woo 1987) maintained *T. bullocki* at 15° C for about 4 years in MEM medium supplemented with 20% fetal calf serum and other ingredients. In biphasic medium SNB-9, Hajdú and Matskási (1984) maintained continuous cultures of one *Trypanoplasma* strain isolated from *Esox lucius* and one isolated from the leech vector *Piscicola geometra*. The same medium was used by Nohýnková (1984) for the successful cultivation of a strain of *T. borelli* isolated from a common carp (*Cyprinus carpio*) fingerling.

We succeeded in cultivating nine strains of trypanoplasms; this report describes the growth, morphology, division and some other properties of the culture forms.

Materials and methods

Between 1986 and 1987, the blood of 275 fishes belonging to 23 species was taken aseptically by cardial puncture. one or two drops of blood were inoculated into the culture tubes, and the rest was

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used for examination in fresh mounts and for making blood smears. In addition, 25 specimens of the leech *P. geometra* were examined. The leeches were rinsed in antibiotic solution, their suckers were cut off, and the gut contents were squeezed out and transferred by Pasteur pipettes into the medium.

The medium used for isolation was biphasic blood-agar medium SNB-9 (Diamond and Herman 1954) which was prepared from samples of sterile human blood for transfusion whose expiration date had elapsed, supplemented with vitamins (0.1 mg choline chloride, 0.2 mg I-inositol, 0.1 mg folic acid, 0.1 mg nicotinamide, 0.1 mg D-calcium panthotenate, 0.1 mg chloride pyridoxal, 0.01 mg riboflavin and 0.1 mg thiamine chloride to 100 ml medium) and antibiotics (3,000 IU penicillin, 1000 µg kanamycin and 500 µg streptomycin to 1 ml medium). The volume of the liquid overlay in each culture tube was 0.5 ml. The maintenance medium contained lower antibiotic concentrations: 250 IU penicillin and 150 µg streptomycin per 1 ml medium.

Monophasic medium L4NHS after Evans (1978) consists of the stock solution [1.5 g proteose peptone (Difco), 0.25 g liver digest oxoid, 0.5 g yeast extract (Difco) and 0.5 g NaCl in 100 ml distilled water], 5 ml rabbit serum that had been inactivated at 50° C for 30 min and 10 ml lysate prepared by lysing rabbit erythrocytes (from defibrinated blood) in 9 ml sterile distilled water. The medium was also enriched by the vitamins mentioned above and sterilized by filtration.

For experimental infections, goldfishes (*Carassius auratus auratus*) and pikes (*E. lucius*) that had been reared parasite-free were used. When inoculated intraperitoneally with bloodstream trypanoplasms from common carp (*C. carpio*) and tench (*Tinca tinca*) the goldfishes develop high parasitaemia and can be used for in vivo culture of the flagellates. The other host species was used for inoculation of homologous strains from pikes. The fishes were incoulated intraperitoneally with 1000–2000 trypanoplasms.

Blood from clipped-off pieces of gill filaments was examined for flagellates at weekly intervals. After 3–6 weeks post-infection (p.i.), a re-isolation in the culture medium was attempted using blood taken by cardial puncture. Flagellate morphology was studied in blood smears stained with Giemsa's stain according to Wallace (1962). Cryopreservation was carried out according to the method of Dar et al. (1972), using 10% dimethylsulfoxide as a cryoprotectant.

Results

Cultivation

Of the 275 fish examined, 30 proved to be positive by microscopical examination of fresh blood, including the asp (*Aspius aspius*), bream (*Abramis brama*), common carp (*C. carpio*), crucian carp (*Carassius carassius*), pike (*E. lucius*), roach (*Rutilus rutilus*), rudd (*Scardinius erythrophtalmus*), stone loach (*Noemacheilus barbatulus*), tench (*Tinca tinca*) and white bream (*Blicca bjoerkna*).

Six fishes – carp, crucian carp, bream, stone loach and two tenches – harboured mixed infections with trypanosomes and trypanoplasms. The attempt to cultivate flagellates from the blood of bream and stone loach failed. The trypanoplasms from the remaining hosts grew for only two to three subcultures, then trypanosomes completely prevailed in the culture.

Of the remaining 24 fishes, 14 had low and 10, high parasitaemia. Of the 14 fishes with low parasitaemia, no culture could be isolated from 1 asp, 2 roaches, 2 breams or 3 tenches. Continuous culture could be established from 1 carp, 1 tench, 1 rudd, 1 white bream and 2 pikes. The fresh isolates yielded axenic cultures in the SNB-9 medium in the presence of antibiotics at 17°–20° C. Within 2–3 weeks, the trypanoplasms in the primary culture transformed into culture forms capable of continuous in vitro growth and multiplied enough for subculturing.

The fishes with severe parasitaemia included one crucian carp, four common carps and five tenches. The culture from crucian carp failed, and only one of the isolates from common carp grew for some time – not longer than three subcultures – but did not transform into culture forms. Among the five tenches, only two of the isolates obtained grew well in culture. One isolate did not grow at 17°–20° C, but did so at 4° C. After 3 weeks of growth at this temperature, these trypanoplasms were transferred into fresh medium at 17°–20° C, where they multiplied intensively. Trypanoplasms from the other tench grew successfully at 17°–20° C from the moment of their isolation. One successfully growing strain was isolated from the leech *P. geometra*.

All strains were maintained at 17°–20° C by weekly subinoculations, since by day 7 postinoculation they had reached a stationary phase of growth. The two strains of *T. guerneyorum* from pike and the *T. borreli* strains from tenches with high parasitaemia yielded $3–5 \cdot 10^6$ flagellates/ml. The remaining four strains grew better, peaking at $4–6 \cdot 10^7$ cells/ml (Fig. 1).

Since the flagellates tolerated lower temperatures, they could also be grown at 4° C. The growth was slow, such that they could be subcultured at monthly intervals,

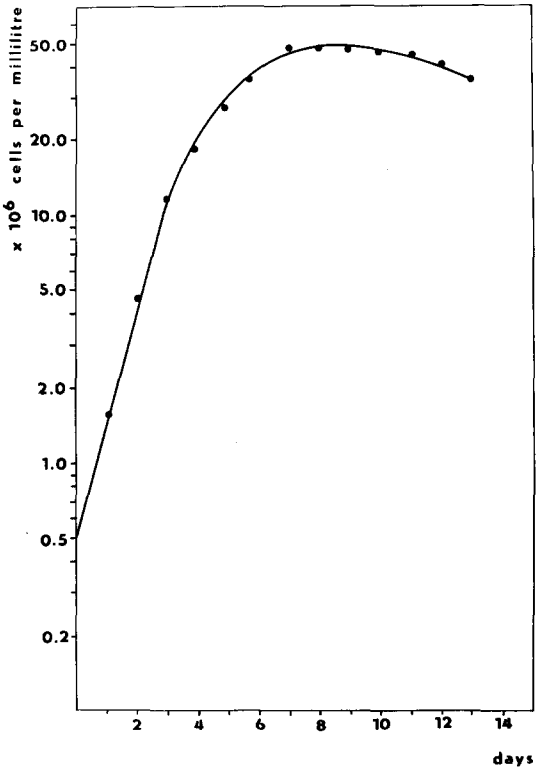


Fig. 1. Growth curve of *T. borreli* isolated from a rudd with low parasitaemia

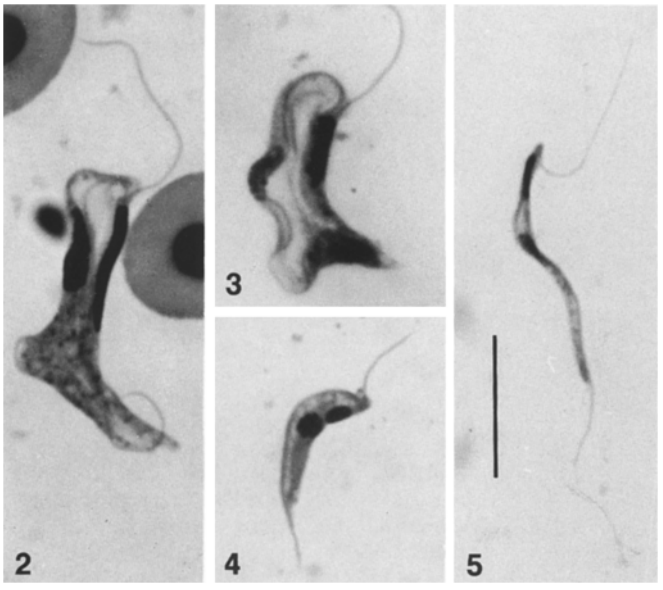


Fig. 2. An "adult" *T. borreli* from a chronically infected common carp. **Fig. 3.** An "adult" *T. guyerneyorum* from a chronically infected pike; the recurrent flagellum has almost no free end. This form looks different from the specimen in Fig. 2; however, each represents just one sample in the wide range of morphological variation of these stages. **Fig. 4.** Commonly occurring small form of *T. borreli*. **Fig. 5.** A long filiform culture from of *T. borreli* isolated form a tench. Bar (Figs. 2–5), 10 μm

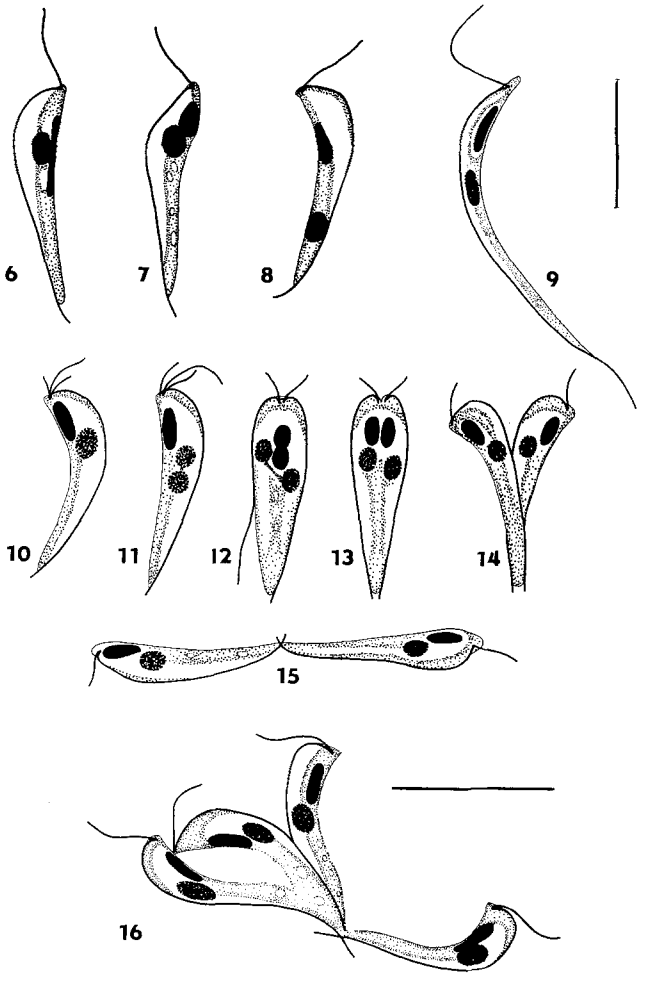
but the final concentration was comparable with that obtained in cultures grown at 20° C.

The monophasic L4NHS medium also supported the growth of the flagellates, but since the final concentration only reached $1–2 \cdot 10^6$ cells/ml, this medium was not used for strain maintenance. The trypanoplasms were also grown in petri dishes with only the solid phase of the SNB-9 medium; they produced colonies in the form of either small droplets or a thin layer on the surface of the plate. All strains were successfully cryopreserved for over 36 months at a survival rate sufficient for the initiation of new growth.

Morphology of the culture forms

Chronic-phase bloodstream forms ("adult" forms) of *T. borreli* (Fig. 2) and *T. guerneyorum* (Fig. 3) showed only slight morphological differences. No differences at all were found between the culture forms of these two species or any of the other strains isolated. They were elongated, with a little-developed undulating membrane. Two types of culture forms could be distinguished.

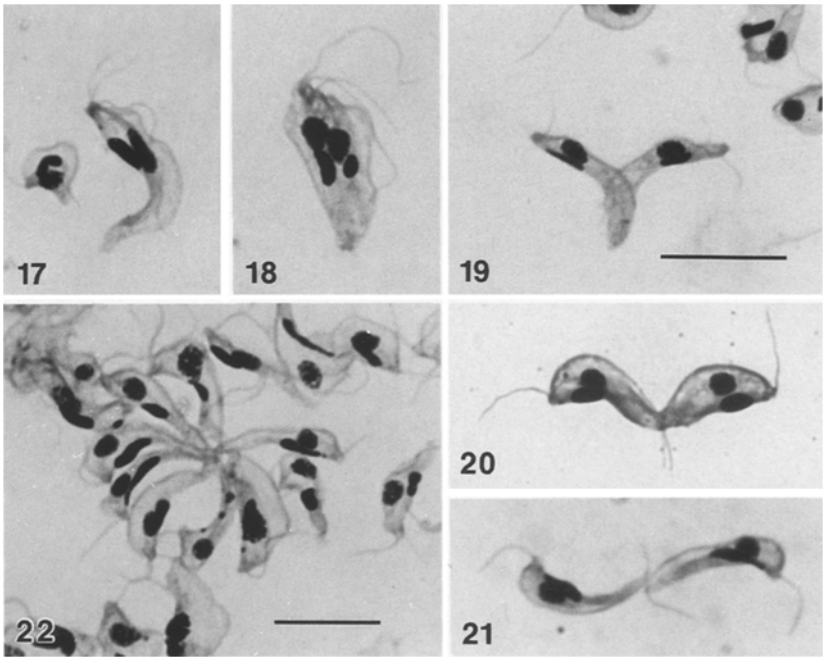
Small forms (Figs. 4, 6–8) were observed that had a tapering posterior end that was 12.5 (10–15) × 3.2 (2.5–4.2) μm in size, with the kinetoplast varying considerably in shape (elongated, tear-shaped, ellipsoidal or spherical) and size [3.4 (2.3–6.1) × 1 (0.6–1.4) μm]. An ellipsoidal or spherical nucleus that was 2.4 (1.7–3.5) × 1.7 (1.2–2.8) μm in size was situated at the same level as the kinetoplast or directly posterior to it, sometimes even occurring in the posterior end of the body (Figs. 6–8). The anterior



Figs. 6–8. Small culture forms of *T. borreli* with different mutual positions of the nucleus and kinetoplast: **Fig. 6** side by side; **Fig. 7** with the nucleus directly behind the kinetoplast; **Fig. 8** separated by distance. **Fig. 9.** Long, almost filiform culture form of *T. borreli* isolated from a tench. **Figs. 10–15.** Successive stages of binary fission of culture forms of *T. borreli* and *T. guerneyorum* (diagrammatically): **Fig. 10** formation of two new flagella; **Fig. 11** the old anterior flagellum grows longer, nuclei are connected by a chromatic strand after division; **Fig. 12** the old anterior flagellum is apposed to the cell and becomes the new recurrent flagellum, the kinetoplast divides, nuclei are still connected by a chromatic strand; **Fig. 13** two karyomastigonts just prior to cell fission; **Fig. 14** the fission starts from the anterior end; **Fig. 15** two daughter individuals prior to separation. **Fig. 16.** A rosette of individuals produced by multiple division are still connected at their posterior ends. Bar, 10 μm (applies to all drawings except Fig. 9, which has its own scale)

flagellum was 4.6 (3.5–6) μm long and the free end of the recurrent flagellum was quite short [2 (0.9–2.6) μm]. Flagellates differing in kinetoplast and nucleus morphology and in the mutual position of the former bodies occurred simultaneously in each phase of the culture growth. A regular alternation of distinctly different forms – at variance with fish trypanosomes in culture – could not be observed.

Long, filiform forms (Figs. 5,9) that were 21.9 (17.8–27.1) × 2 (1.5–2.8) μm in size were identified, with a thin, elongated kinetoplast measuring 4.2 (2.8–6.4) × 0.6 (0.5–0.9) μm being situated at the anterior end of the cell.



Figs. 17–22. Division stages of *T. borreli*; Giemsa stain. **Fig. 17** New flagella have been formed; **Fig. 18** kinetoplast and nuclei have completed their division; **Fig. 19** daughter cells cleaving from the anterior end; **Fig. 20** daughter cells still connected at their posterior ends; **Fig. 21** cells just prior to separation; **Fig. 22** a rosette of cells produced by multiple division. Bar in **Fig. 19**, 10 μm (applies to all figures except 22, which has its own scale)

An ellipsoidal nucleus that was 3.2 (2.4–4.2) × 1.4 (1.1–1.7) μm in size lay in the first half of the body at a certain distance behind the kinetoplast. The flagella were much longer than those in the small forms: the anterior flagellum was 16.5 (12.8–20.1) μm long and the free end of the recurrent flagellum measured 8.4 (5.7–12) μm in length. These long, slender forms were abundant only in primary cultures and in a few subsequent subcultures of the strains isolated from tenches with high parasitaemia; in other strains they were seldom seen.

Anomalous forms – dyskinetoplastic, or with several kinetoplasts or nuclei, or even anuclear. probably resulting from disorders in cell division – could also be found in some cultures. One *T. borreli* isolate from carp that could not be continuously cultivated retained its original bloodstream forms.

Division of culture forms

This process was most thoroughly studied in strains isolated from the carp and the rudd; however, no differences could be detected in strains from other hosts. Only small forms divided; long, slender forms were never observed to do so.

Most common was longitudinal binary fission. The first sign of division was the formation of two new, short anterior flagella (Figs. 10, 17). The original anterior flagellum of the mother individual then gradually lengthened, was apposed to the cell surface and became the recurrent flagellum of one of the two offspring. In the meantime, division of the nucleus took place (Fig. 12), followed by cleavage of the kinetoplast (Figs. 13, 18). The nuclei remained connected by a thin chromatic strand until the kinetoplast had completed its division (Figs. 11, 12). The two new kinetoplasts were often of quite unequal size. Eventually, the mother cell containing two sets of new organelles was cleaved longitudinally, starting at the anterior end of the cell (Figs. 14, 15, 19–21).

Multiple division could also be observed; it resulted in the formation of rosettes comprising from several (Fig. 16) to many daughter cells (Fig. 22) that remained connected at their posterior ends for some time.

Infectivity of the culture forms for fish

Infectivity tests using culture forms proved that the trypanoplasms growing in an established culture failed to produce infection in fish; i.e., *T. borreli* from carp and tench did not infect goldfish and *T. guerneyorum* failed to infect pikes. The infectivity was lost after 10–14 days in the primary culture. The only strain infective for goldfish was a *T. borreli* strain isolated from carp. The culture did not last longer than three subinoculations because of poor growth, and the flagellates did not transform from their bloodstream shape into culture forms.

Discussion

At variance with the rather scarce data available on *Trypanoplasma* cultivation, based either on short-lived cultures or on observations in one or a few strains only, we gathered information using continuous cultivation of nine strains derived from several host species. In view of the lack of a definitive identification of the species of trypanoplasms in European fish, in the present study we referred to the strains isolated from cyprinids as being *T. borreli*, in agreement with their morphology and host

range (see Lom 1979; Kruse et al. 1989a). We continued to designate the flagellates from pike as being *T. guerneyorum* because of slight morphological differences – the free end of the recurrent flagellum tended to be shorter and the nucleus seemed to be shifted more posteriorly – and due to the failure of previous cross-infection experiments between cyprinids and the pike that used flagellates derived from both groups of hosts (Lom, unpublished data).

Not taking in account fishes infected with a mixture of trypanoplasms and trypanosomes, the 8 successfully established culture strains represent about 33% of the 24 parasitaemic fishes. This relatively low percentage suggests either that although the final flagellate concentration achieved was rather high, the culture medium was not optimal or that various stages of infection encountered in the fish examined were represented by flagellates with differing adaptability to culture environment. Although both alternatives may be true, the second seemed to be supported by differences in successful isolations between the two groups of fishes. Fishes with high (probably near-peak) parasitaemia yielded 2 strains from 10 isolates (i.e., 20%), whereas hosts with low parasitaemia yielded 6 strains from 14 isolations (i.e., about 43%). These fishes probably suffered from chronic infection, with few trypanoplasms surviving in the bloodstream. This phase is long-lasting as compared with the initial, latent phase of infection and is the stage most commonly encountered in infected fishes. Thus, the large, "adult" forms representing chronic infection are probably more likely to give rise to an established culture than are the smaller, more slender forms representing near-peak parasitaemia. In this regard, it is interesting to consider the stumpy forms of mammalian trypanosomes that result in the forms of the arthropod vector.

The short forms with litte-developed undulating membranes (to some extent resembling the ecto- or endocommensal *Cryptobia* species) that multiplied in the culture were morphologically similar to forms that proliferate in the crop of leeches. Although the leech forms eventually transform into slender metacyclic forms that migrate into the proboscis and are infective for the fish, the formation of such infective forms was not detected in our cultures. As a rule, only short forms were observed, which were non-infective for fish. The speed at which the infectivity to fish is lost in the culture may be strain-dependent; whereas in the present study it was lost as early as in the primary culture, the single strain isolated by Nohýnková (1984) lost its infectivity after as many as six in vitro passages.

The loss of infectivity is also associated with the complete transition to the morphological culture form. The aforementioned isolate of *T. borreli* from common carp that could not establish a continuous culture did not transform in vitro but was infective for the goldfish. Similarly, *T. salmositica*, which grows in culture while retaining its bloodstream form, kept its infectivity to rainbow trouts (Woo 1979). Nevertheless, the infectivity of the culture form does not match that of the bloodstream forms; if the former are treated with heat-inactivated immune blood plasma, they cease to be infectious, whereas this treatment does not affect the infectivity of the latter (Jones and Woo 1987).

The long, slender forms (the above-mentioned type II) recorded by Keysselitz (1906) and Robertson (1911) in leeches were abundant only in primary cultures isolated from tenches with high parasitaemia. During subinoculation they gradually vanished from the cultures, since they could not divide in vitro. The transformation of long, filiform flagellates into stubby ones (and back again) that was previously described by Hajdú and Matskási (1984) was not observed in the present study. Ponselle (1913) also failed to find any long, slender trypanoplasms in his culture of *T. varium*; he described the culture forms as being very similar to the bloodstream forms. Our observations and previous data suggest that the artificial medium of in vitro culture does not fully substitute for conditions found in the digestive tract of the leech. Although the flagellate multiplies successfully, some forms are not produced at all; moreover the natural sequence of forms that is found to some extent in fish trypanosome culture is also non-existent in culture.

Although the bloodstream forms were observed to divide only by binary fission (Bower and Woo 1977; Woo 1978; Pecková 1989), the culture forms underwent not only binary fission but also multiple fission, which confirms previous findings by Qadri (1962), Hajdú and Matskási (1984) and Nohýnková (1984). Most likely, however, multiple division is an anomalous phenomenon induced by culture conditions, such as the presence of forms with many nuclei, kinetoplasts or flagella.

There are few data on the division of *Trypanoplasma* culture forms. Ponselle (1913) reported that nuclear division preceded or ran parallel to kinetoplast division in *T. varium*. In *T. willoughbii*, Qadri (1962) observed the formation of a new anterior flagellum, immediately followed by the development of a new recurrent flagellum, then the constriction of the kinetoplast and subsequent nuclear division and, eventually, the longitudinal fission of the flagellates.

The essential features of the division process observed in culture forms of *T. borreli, T. guerneyorum* and *Trypanoplasma* sp. from the leech correspond to the division of bloodstream forms previously observed in *T. catostomi* (Bower and Woo 1977), *T. salmositica* (Woo 1978), *T. bullocki* (Burreson 1982) and *T. borreli* (Pecková 1989). In these species as well, the formation of two new flagella precedes nuclear division. The two daughter nuclei also remain connected by a thin strand until the constriction and division of the kinetoplast is complete. Then, the nuclei finally separate and the cell cleaves longitudinally. A similar course of binary fission was observed by Shavanas et al. (1990) in *T. omoki* stages from the leech *Hemiclepsis marginata*.

Although the division process observed in our *Trypanoplasma* cultures is essentially similar to that of bloodstream forms, there are some important differences in the course of division in bloodstream forms, which separate *T. salmositica* from the second group comprising *T. borreli, T. bullocki* and *T. catostomi*. During the course of division, *T. salmositica* retains its elongated

cell shape and one of the two offsprings receives both of the newly formed flagella, whereas in the second group the flagellates round up prior to division and each daughter individual receives one of the new flagella. The second difference lies in the cell fission; it is unequal and starts from the posterior cell end in *T. salmositica*, whereas in the second group the division is equal and starts from the anterior end of the cell. Finally, in *T. salmositica* the kinetosomes and kinetoplasts remain in the anterior part of the cell after division, whereas in the second group they are situated in apposing ends of dividing individuals. The dividing culture forms of *T. borreli, T. guerneyorum* and *Trypanoplasma* sp. show some features of both groups; they retain an elongated body shape but cleave equally from the anterior end of the cell.

Further comparative studies on other trypanoplasms will show whether the special features of *T. salmositica*, along with the presence of a contractile vacuole in bloodstream forms (Paterson and Woo 1983) and with the infectivity of culture forms to the fish host (Woo 1987), can constitute a basis for taxonomic separation at the level of subgenus or genus.

The division process in most trypanoplasms is similar to that in some other bodonids. Essentially the same process was described in *Cryptobia congeri* obtained from the stomach of conger eels by Martin (1913) – although he erroneously assumed that the new flagella originated by fission from the old ones – and in *C. helicis* obtained from seminal vesicles of terrestrial snails (Kozloff 1948). In the latter species, however, kinetoplast division was completed prior to the start of nuclear division in some individuals. Nevertheless, in both of these species the thin chromatin strand that connects the two daughter nuclei for some time also exists.

A similar course of division was observed in *Bodo caudatus* (Bělař 1929; Robertson 1927; Hollande 1952). Apart from observations of the initial, four-flagellated division stage (Joyon and Lom 1969; Becker 1977), no data are available on the division of *Ichtyobodo necator*.

References

Becker CD (1977) Flagellate parasites of fish. In: Kreier JP (ed) Parasitic Protozoa, vol 1. Academic Press, New York, pp 357–416

Bělař K (1926) Der Formwechsel der Protistenkerne. G. Fischer Verlag, Jena, p 420

Bower SM, Woo PTK (1977) Division and morphogenesis of *Cryptobia catostomi* (Protozoa: Kinetoplastida) in the blood of white sucker (*Catostomus commersoni*). Can J Zool 55:1093–1099

Burreson EM (1982) The life cycle of *Trypanoplasma bullocki* (Zoomoastigophorea: Kinetoplastida). J Protozool 29:72–77

Dar FK, Lighthart GS, Wilson AJ (1972) Cryopreservation of pathogenic African trypanosomes in situ: metacyclic and bloodstream forms. J Protozool 19:494–497

Diamond LS, Herman CM (1954) Incidence of trypanosomes in the Canada goose as revealed by bone marrow culture. J Parasitol 40:195–202

Evans DA (1978) Kinetoplastida. In: Taylor AER, Baker JR (eds) Methods of cultivating parasites in vitro. Academic Press, London, pp 55–88

Hajdú E, Matskási I (1984) In vitro cultivation of *Trypanoplasma* strains isolated from pike and leech (preliminary report). Acta Vet Hung 32:79–81

Hollande A (1952) Ordre des Bodonides (Bodonidea ord. nov.). In: Grassé PP (ed) Traité de zoologie. Anatomie, systématique, biologie. Masson, Paris, pp 669–693

Jones SRM, Woo PTK (1987) The immune response of rainbow trout, *Salmo gairdneri* Richardson, to the haemoflagellate, *Cryptobia salmositica* Katz, 1951. J Fish Dis 10:395–402

Joyon L, Lom J (1969) Etude cytologique, systématique et pathologique d'*Ichthyobodo necator* (Henneguy, 1883) Pinto, 1928 (Zooflagellé). J Protozool 16:703–719

Keysselitz G (1906) Generations- und Wirtwechsel von *Trypanoplasma borreli* Laveran und Mesnil. Arch Protistenkd 7:1–74

Kozloff EN (1948) The morphology of *Cryptobia helicis* Leidy, with an account of the fate of the extranuclear organelles in division. J Morphol 83:253–273

Kruse P, Steinhagen D, Körting W, Friedhoff KT (1989a) Morphometrics and redescription of *Trypanoplasma borreli* Laveran & Mesnil, 1901 (Mastigophora, Kinetoplastida) from experimentally infected common carp (*Cyprinus carpio* L.). J Protozool 36:408–411

Kruse P, Steinhagen D, Körting W (1989b) Development of *Trypanoplasma borreli* (Mastigophora, Kinetoplastida) in the leech vector *Piscicola geometra* and its infectivity for the common carp, *Cyprinus carpio*. J Parasitol 75:527–531

Laveran A, Mesnil F (1901) Sur les Flagelles à membrane ondulante des poissons (genre *Trypanosoma* et *Trypanoplasma* n. gen.). C R Acad Sci (Paris) 133:670–675

Lom J (1979) Biology of the trypanosomes and trypanoplasms of fish. In: Lumsden WHR, Evans DA (eds) Biology of the Kinetoplastida, vol 2. Academic Press, London, pp 270–328

Martin C (1913) Further observations on the intestinal trypanoplasms of fishes, with a note on the division of *Trypanoplasma cyprini* in the crop of a leech. Q J Microsc Sci 59:175–195

Nohýnková E (1984) In vitro cultivation of the bodonid flagellate *Trypanoplasma borreli*. J Protozool 32:52A

Nowicki E (1940) Zur Pathogenität der *Trypanoplasma cyprini*. Z Parasitenkd 11:468–473

Paterson WB, Woo PTK (1983) Electron microscopic observations of the bloodstream form of *Cryptobia salmositica* Katz 1951 (Kinetoplastida: Bodonina). J Protozool 30:431–437

Pecková H (1989) Division of *Trypanoplasma borreli* in the blood of experimentally infected goldfish. Vestn Cesk Spol Zool 53:76 [A]

Ponselle A (1913) Culture in vitro du *Trypanoplasma varium* Léger. C R Soc Biol (Paris) 74:685–688

Putz RE (1972) Biological studies on the hemoflagellates *Cryptobia cataractae* and *Cryptobia salmositica*. Bur Sport Fish Wildl (U.S.) Res Rep 63:3–25

Qadri SS (1962) *Trypanoplasma willoughbii* n.sp. from British freshwater fish, *Salvelinus willoughbii*. Riv Parassitol 23:1–9

Robertson M (1911) Transmission of flagellates living in the blood of certain freshwater fishes. Philos Trans R Soc Lond [Biol] 202:29–50

Robertson M (1927) Notes on certain points in the cytology of *Trypanoplasma raiae* and *Bodo caudatus*. Parasitology 29:375–393

Shavanas KR, Ramachandran P, Janardanan KP (1990) *Trypanoplasma ompoki* sp.n. from freshwater fishes in Kerala, India, with observations on its vector-phase development and transmission. Acta Protozool (in press)

Tanabe M (1924) Studies on the hemoflagellata of the loach, *Misgurnus anguillicaudataus*. Kitasato Arch Exp Med 6:121–138

Wallace FG (1962) Trypanosomatid parasites of horse flies with the description of *Crithidia rileyi* n.sp. J Protozool 9:53–58

Woo PTK (1978) The division process of *Cryptobia salmositica* in experimentally infected rainbow trout (*Salmo gairneri*). Can J Zool 56:1514–1518

Woo PTK (1979) *Trypanoplasma salmositica*: experimental infections in rainbow trout, *Salmo gairdneri*. Exp Parasitol 47:36–48

Woo PTK (1987) *Cryptobia* and cryptobiasis in fishes. Adv Parasitol 26:199–237