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Filaments of *Trypanosoma brucei:* Some Notes on Differences in Origin and Structure in two Strains of *Trypanosoma (Trypanozoon) brucei rhodesiense*

D. S. Ellis, W. E. Ormerod and W. H. R. Lumsden

Abstract

Filaments attached to trypanosomes of two strains of *T. (T.) brucei* were studied by electron microscopy and two distinct types identified: short-thick and long-thin. The former are associated with stumpy trypanosomes and are secretions, via the flagellar pocket, which originate in the area of the Golgi complex, during the infection of the host. They are referred to as 'secretory filaments'. Their diameter is 0.09 to 0.14 μm. The long-thin filaments are associated with slender forms of trypanosome in various artificial situations; those shown by negative staining are believed to be cytoplasmic extrusions from the anatomically weak extremities of the parasite and are referred to as 'plasmanemes'. Their diameter is 0.06 μm. Both types appear to maintain their structure without the aid of the normal type of unit membrane as myelin formations.

Introduction

Filaments which arise from the extremities of trypanosomes were first seen by Schelipewsky (1912) who used dark ground illumination and described them as 'thread-like appendages' attached to the anterior and posterior ends of blood trypomastigotes. Molloy and Ormerod (1974) demonstrated 'fibrils' by phase contrast which were similar structures, up to 100 μm in length, attached to the posterior end of individual trypomastigotes of *Trypanosoma (T.) brucei.* Fine structural observations were first made by Babudieri and Tomasini (1962). These workers used the technique of metal shadowing and demonstrated filamentous appendages attached to the flagellum of *T. brucei.* Filaments were also demonstrated by Wright, Lumsden and Hales (1970). They described filaments of fixed and sectioned material, as well as threads, which they referred to as 'filopodium-like processes', and demonstrated them at both ends of trypanosomes which had been fixed with glutaraldehyde prior to negative staining; filaments, presumed to be of an analogous structure, were seen under phase contrast protruding from trypanosomes suspended in solutions of methyl cellulose. These filaments, described by various authors as thread-like appendages, fibrils, filopodia and plasmanemes (Vickerman & Luckins, 1969), have tended to be regarded as artifacts; it has also been suggested that they might be myelin formations (Gicklhorn, 1932, as quoted by Frey-Wyssling, 1953) or even simply

the contents of ruptured parasites. All observations have hitherto been made of organisms outside their host and so in an abnormal environment.

In this paper we describe various filaments associated with parasites fixed within the capillaries of the host and are able to correlate them with the different periods in the life-cycle of the trypomastigotes of *T. brucei*. Using a synchronous strain (see below) we were able to distinguish structures of differing origin, and to relate them to different structures found in other strains and to other similar phenomena.

Materials and Methods

Trypanosomes

Two strains were studied: 1. *Trypanosoma rhodesiense* strain LSHTM 180. This strain was isolated from man (Dumelo) in Botswana in 1960 by inoculation of blood into a rat. Blood was frozen as a stabilate 26 days later. Three successive stabilates were made, each from a 72-hour infection in rats; the last of these (passage 3) was used for these experiments. Observations were also made on infections serially derived from that stabilate at passage 4–9 from the original isolation. The strain has the particular advantage that it is synchronous in showing only agranular (slender) trypomastigotes at 72 hours and heavily granular (stumpy) trypomastigotes at 120 hours. 2. *T. rhodesiense* Liverpool monomorphic (York, Adams & Murgatroyd, 1929). This strain, originally from man, has been passaged serially in mice and rats for nearly 50 years in laboratories.

Animals

Wistar rats of 300 g (approx.) were infected with 1×10^6 (approx.) blood trypomastigotes intraperitoneally and maintained at room temperature and at 37 °C.

Cultures

Cultures of strain 180, carried out by the method of Brown, Evans and Vickerman (1973), were kindly supplied by Dr D. A. Evans.

Materials from rats

Blood was examined on slides and from centrifuged pellets of trypanosomes separated by the method of Lanham (1968). Meninges and choroid plexuses were dissected from the brains of rats immediately they had been killed by exsanguination under ether anaesthetic.

Fixation and Embedding

Material was immediately placed in 3% glutaraldehyde with cacodylate buffer at pH 7.4 and left for 8 hours, washed overnight in the same buffer, post-fixed with 1% osmium tetroxide for 2 hr and, after dehydration, the majority of blocks stained with 1% phosphotungstic acid. Both methanol and ethanol

Table 1. The origin and embedding procedure of the fixed material examined of Strain 180 Botswana. Specimens showing filaments are marked in bold face

Duration of infection	Number of rats examined	Origin of specimens	Number of specimens	Embedding procedure		
				Araldite via toluene	Araldite via propylene oxide	Spurr via alcohol
2½ days (62 hrs)	3	Choroid plexus	5	1	–	4
		Peripheral blood [1]	5	2	–	3
3 days (72 hrs)	15	Choroid plexus	10	2	–	8
		Meninges	4	2	–	2
		Peripheral blood [1]	10	2	–	8
		Blood on agar [2]	3	–	–	3
		Blood on polythene [2]	2	–	–	2
4 days (96 hrs)	2	Choroid plexus	4			4
		Peripheral blood [1]	2			2
5 days (120 hrs)	14	Choroid plexus	14	2	**2**	**10**
		Meninges	6	2	–	**4**
		Peripheral blood [1]	7	2	–	**5**
		Blood on agar [2]	3	–	–	**3**
		Blood on polythene [2]	2	–	–	**2**
6 days (144 hrs)	2	Choroid plexus	5	2	–	**3**
		Meninges	2	1	–	**1**
		Peripheral blood [1]	2	1	–	**1**
10 days (240 hrs)	6	Choroid plexus	14	6	**2**	**6**
		Meninges	7	3	**1**	**3**
13 days (312 hrs)	5	Choroid plexus	9	9	–	–
		Meninges	5	5	–	–
14 days (336 hrs)	3	Choroid plexus	5	5	–	–
21 days (504 hrs)	2	Choroid plexus	4	2	–	**2**
		Peripheral blood [1]	4	2	–	**2**

[1] Fixed in a variety of ways including, as smears on slides, centrifuged pellets, or pellets following separation by Lanham column.

[2] Fixed under coverslips on slides.

were used for dehydration, and in all cases the graded series used was 30%, 60%, 90% and 100%, each for 5 minutes' duration. Some material was stained with uranyl acetate and lead citrate after sectioning. Whenever toluene was used for Araldite embedding, two changes of this solvent were employed for 5 minutes each.

Table I lists the times of infection at which various numbers of specimes of rats were examined using Strain 180.

Some observations were made from sections cut from Araldite blocks prepared by the standard method of GLAUERT and GLAUERT (1958), but the majority of observations were made on sections cut from blocks embedded in 'Spurr' (SPURR, 1969), which proved the best method to preserve the filaments. (See Table 1.)

Blocks were prepared of trypomastigotes, at 72 and 120 hours after inoculation, which had already been studied on agar; this was done by introducing buffered glutaraldehyde under the coverslip to which the trypanosomes and their filaments adhered. A block for sectioning was then prepared by removing the resin plug incorporating the trypanosomes from the coverslip (ELLIS, 1971).

Sections were cut with a Cambridge Huxley Ultra-Microtome and the majority of observations were made with a Zeiss EM9 electron microscope.

Negative staining

Negative staining was carried out by the method described by BIRD et al. (1971), although some trypanosomes were prefixed with 4% glutaraldehyde buffered to pH 7.2 (WRIGHT, LUMSDEN & HALES, 1970).

Results

Embedded Material (I)

The series of choroid plexuses fixed at intervals of from 62 to 504 hours following infection is shown in Table 1. On sectioning, these specimens containing trypanosomes within the capillaries at varying stages of development. After 120 hours, some of the capillaries were

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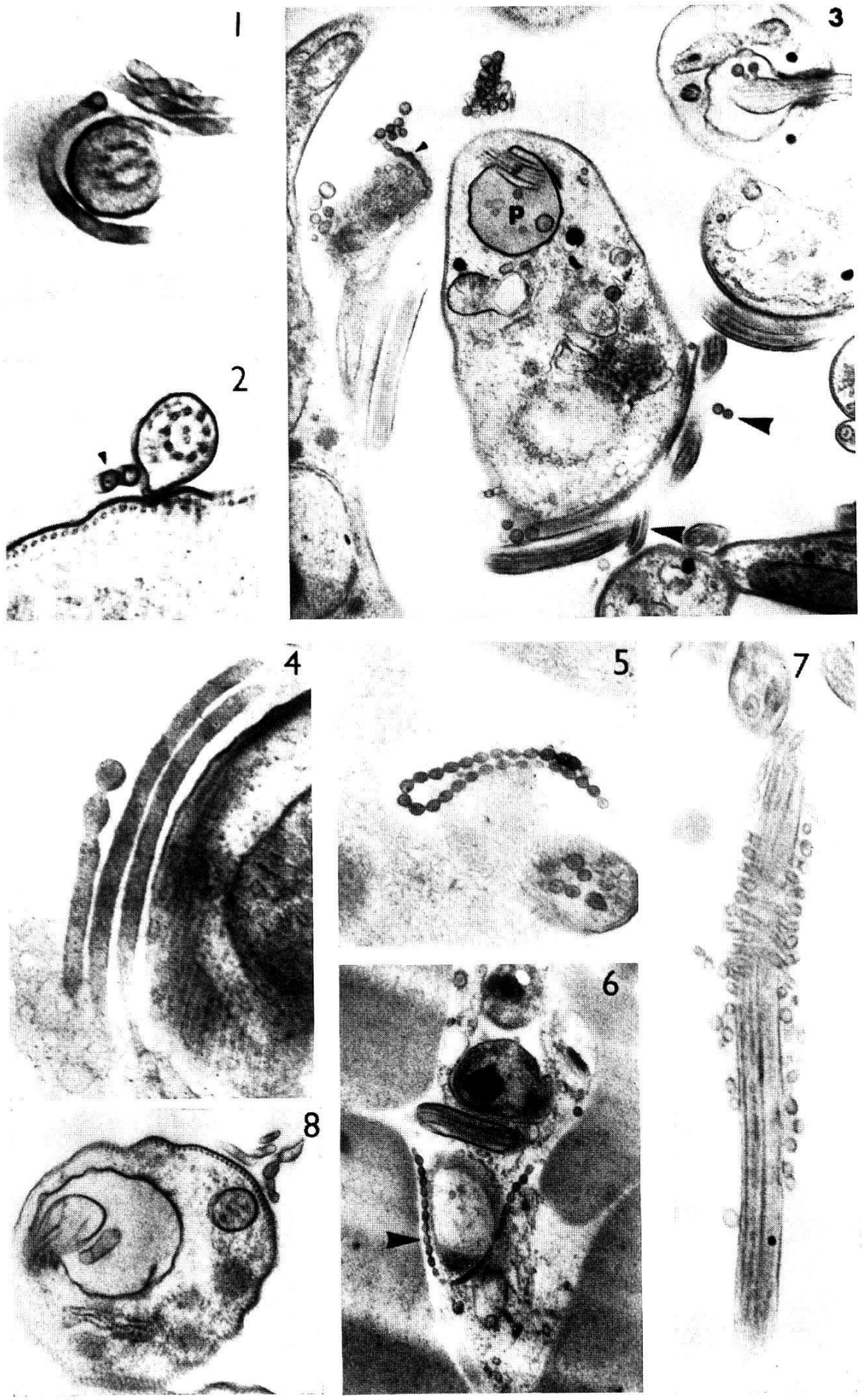
Botswana Strain No. 180 is illustrated except where otherwise stated.

Plate I

1 & 2: Filaments associated with stumpy form trypomastigotes; flagella sectioned *in situ* in the rat choroid plexus, after 10 days' infection (× 32,000). 3: Section of choroid plexus capillary. Filaments are seen as grouped spheres and rods (arrowed). Note enlarged flagellar pockets (P) containing spheres. 120 hours' infection (× 9,000). 4: Section of tail blood film after 120 hours' infection, with filaments presenting as rods and 'popper beads' (× 32,000). 5 & 6: Sections of choroid plexus clot with filaments seen as 'popper beads' among debris after 10 days' infection (Fig. 5 × 16,000; Fig. 16 × 9,000). 7: Unusually long filament wrapped round flagellum (× 14,000). 8: Rods inside and outside flagellar pocket after 120 hours' infection (× 14,000).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blocked with clots, and parasites were increasingly found in the perivascular spaces.

All 42 specimens taken from rats infected with the Botswana strain after periods longer than 120 hours and embedded in Spurr resin showed filaments which were associated with the body of the trypanosomes, particularly the flagellum (Table 1; Figs. 1, 2 and 3). No filaments were found before 100 hours. Blood taken from the tail at the time of sacrifice contained parasites at the same stage of development as those found in the choroid plexus, and these always had associated filaments after 120 hours, but never before about 100 hours (Fig. 4). Within the choroid plexus these filaments seemed stable, surviving in clots and debris (Figs. 5 and 6). Their width was 0.09 to 0.12 μm and they appeared as 'rods' and 'spheres' or as an intermediate 'popper bead' formation (Figs. 3, 4 and 5). If presenting as rods their length did not exceed that of the parasite; occasionally long spiral forms were found wrapped round the flagellum (Fig. 7).

All the specimens prepared by fixation and embedding of the Liverpool strain failed to show these filaments unless the animal host had been maintained at a raised temperature. Under these conditions, however, as long as toluene was not used during preparation, the short-thick type filaments could be found both inside the flagellar pocket and outside associated with the flagellum. These filaments appeared to us to be indistinguishable from those found associated with the stumpy trypomastigotes of Strain 180.

Serial observations during the first 6 days of infection by the synchronous Botswana strain made it clear that these filaments were associated only with stumpy-form trypomastigotes (which, like the filaments, only appeared around 120 hours) whether these were within the choroid plexus or in the peripheral blood. We never found the filaments with slender-form populations (Table 1).

Similar rods and spheres were found in the large flagellar pockets (Figs. 3, 8 and 9) of the stumpy forms. These appeared to us to be

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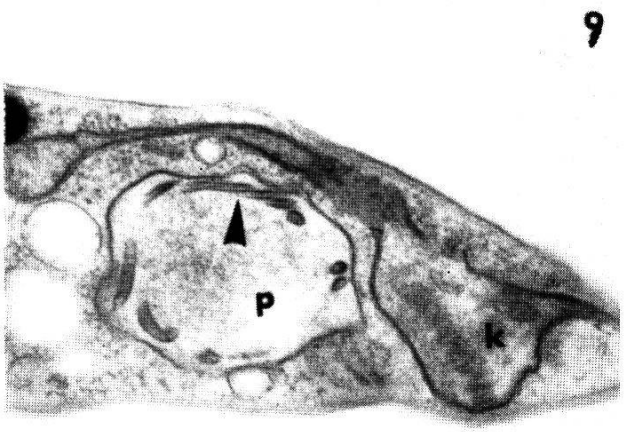
Plate II

9: Rods within the enlarged flagellar pocket (P) of the stumpy form trypanosome after 120 hours' infection. Cristae can be seen in the kinetoplast (K) (× 16,000). 10: From the same material as 9, showing a 'spinous vesicle' outside the pocket (arrowed) and another fusing with the pocket membrane (× 24,000). 11, 12 & 13: Negatively-stained preparations showing 'rods' and 'popper beads' on the surface of 120-hour stumpy trypomastigotes. These filaments, though sometimes seen near the extremities of the parasite (e.g. Fig. 13) are not continuous with, nor derived from these areas (Fig. 11 × 16,000; Figs. 12 & 13 × 9,000). 14: The flagellar pocket (P) of a negatively-stained 'Liverpool' strain trypanosome, whose host had been kept at a raised temperature, showing similar spheres to those found in stumpy forms of Botswana Strain 180 (× 24,000).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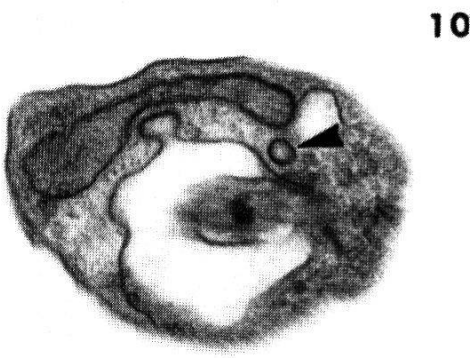
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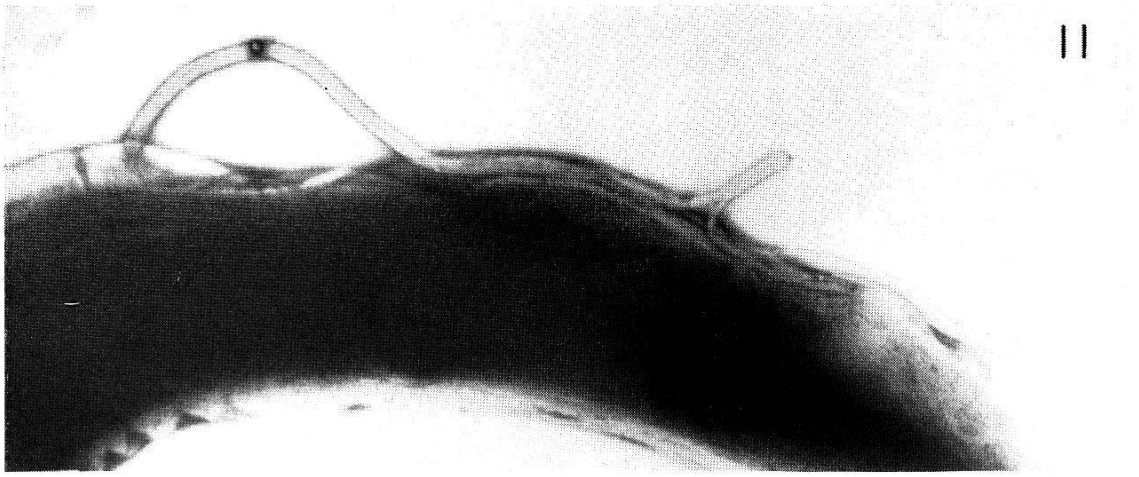
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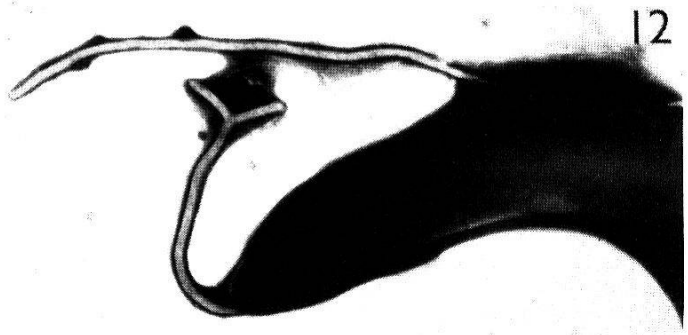
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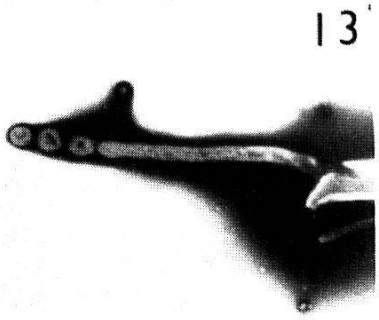
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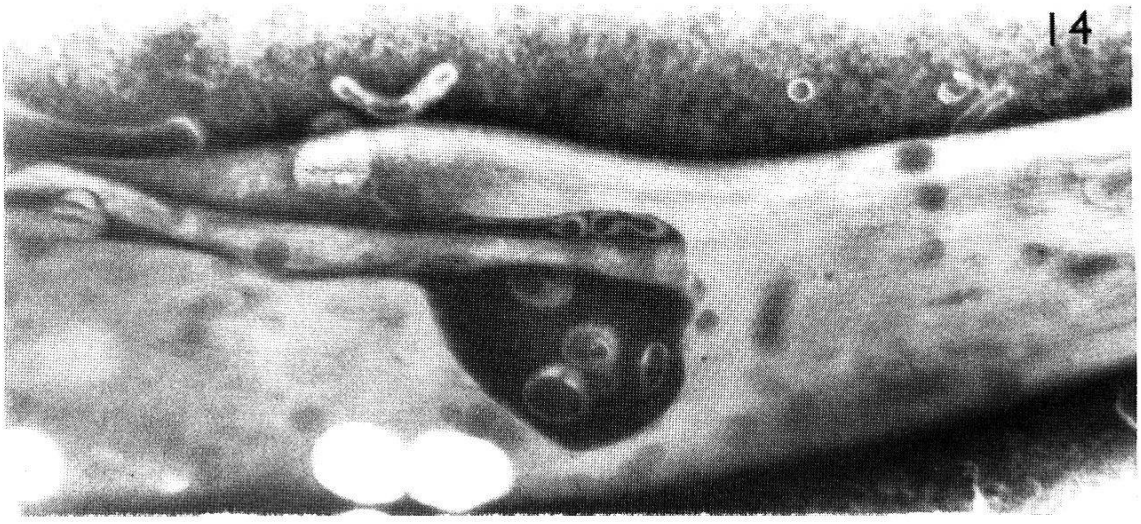
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formed by the fusion of special vacuoles with the pocket membrane (Fig. 10). These vacuoles, described by VICKERMAN (1969) as 'spinous vesicles and caveolae' arose in association with the Golgi apparatus and passed posteriorly to the flagellar pocket (see below).

We noted throughout that if toluene was used during embedding with Araldite, none of these filaments were preserved outside the body of the trypanosome. As shown in Table 1, filaments were seen when Spurr was used as the embedding material, although they could be demonstrated in Araldite blocks if propylene oxide was substituted for toluene during preparation.

Negative Stained Material (I)

Botswana strain stumpy forms showed short-thick and popper-bead type filaments on negative staining as long as the pellicle remained intact (Figs. 11, 12 and 13). Prefixing with glutaraldehyde greatly increased the number of these unburst organisms presenting this type of filament. Their width was 0.10 to 0.14 μm. Only if the parasite burst did long-thin streamers appear. The monomorphic 'Liverpool' strain does not produce this short-thick type of filament except when the host is artificially kept at raised temperatures by the method of OTIENO (1973). Under these conditions typical stumpy forms appear, and these are associated with the thick type of filament which is seen particularly in the flagellar pocket (Fig. 14). These short-thick filaments remain associated with the main body of the trypanosome rather than with the extremities. They never swelled up to form large globules.

Negative Stained Material (II)

If either the Liverpool strain or the slender (72-hour) form of the Botswana strain are negatively stained (Fig. 15), long-thin streamers are formed, 0.05 to 0.07 μm in diameter and up to 100 μm in length. They always arise from the extremities of the parasite and often they

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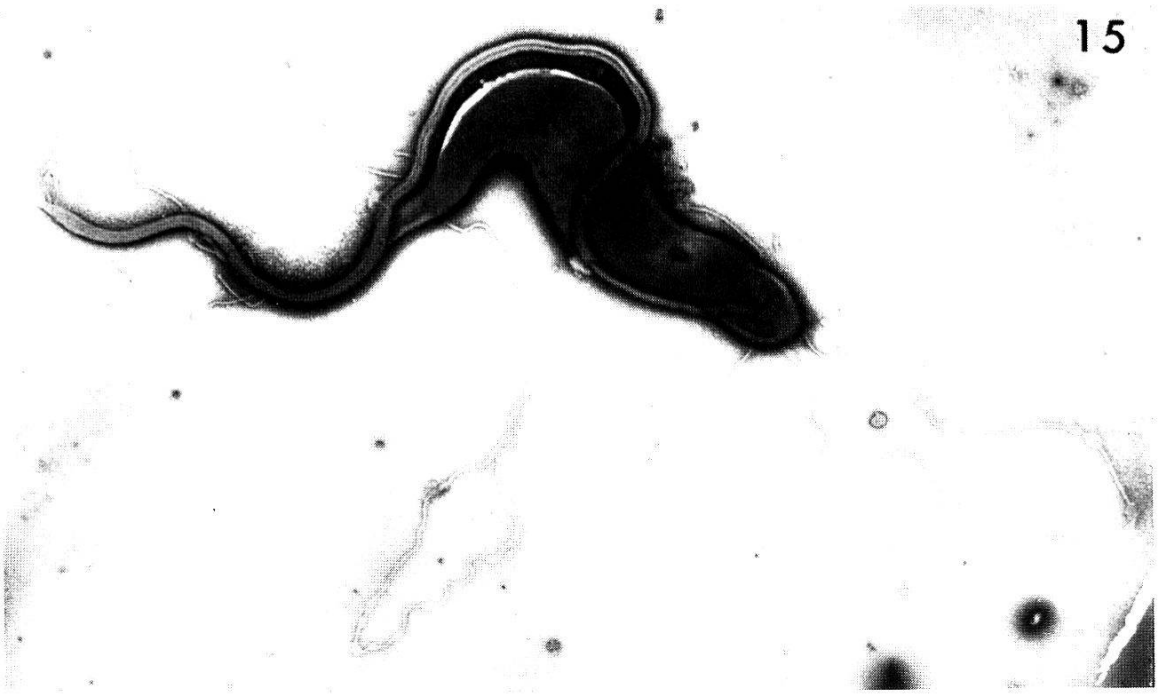
Plate III

15: Typical long-thin streamers from the extremities of a negatively-stained 72-hour trypomastigote (× 3,500). 16, 17, 18 & 19: A series of negatively-stained 72-hour slender forms showing progressive protrusion of cytoplasm from the blunt posterior end. Note 'surface coat' (arrowed) covering these protrusions, continuous with that over the body of the trypanosome. 20: Similar protrusion from the posterior end of a cultured form of Strain 180. Note absence of 'surface coat' (Figs. 16–20 × 35,000).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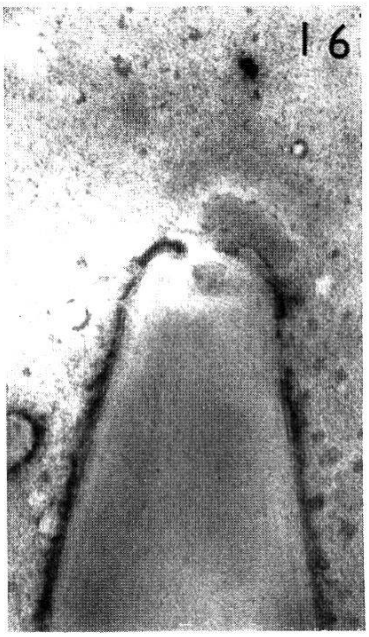
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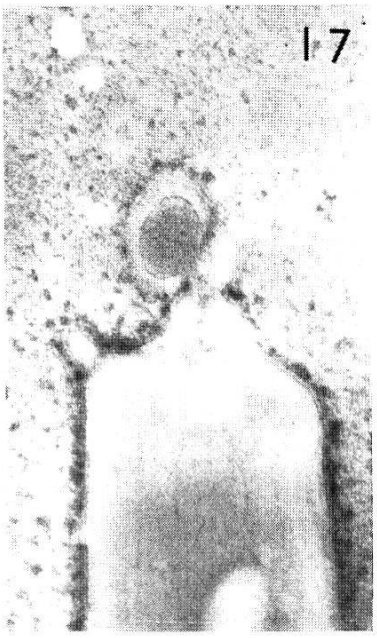
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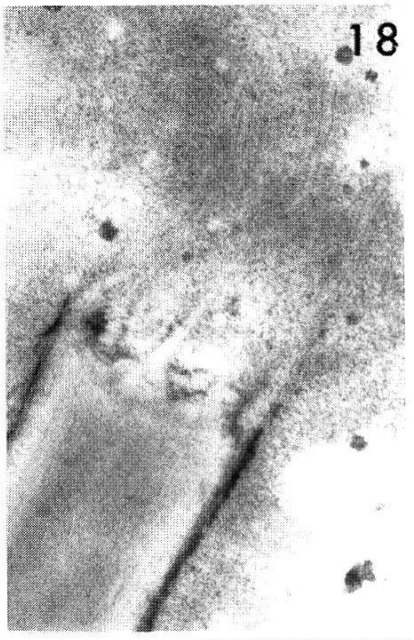
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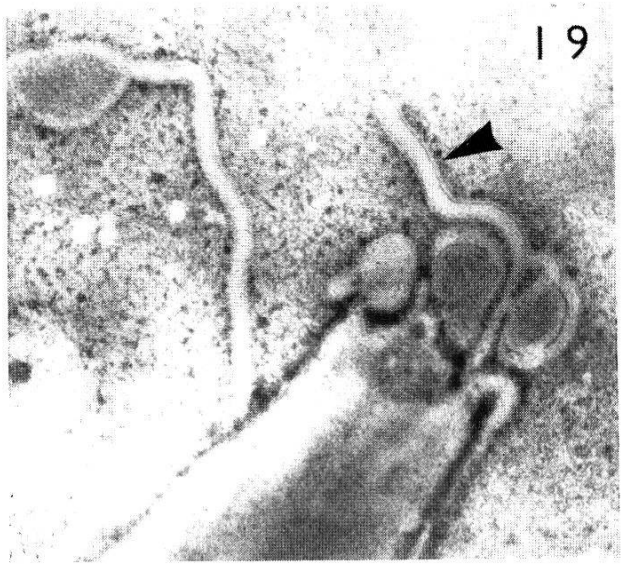
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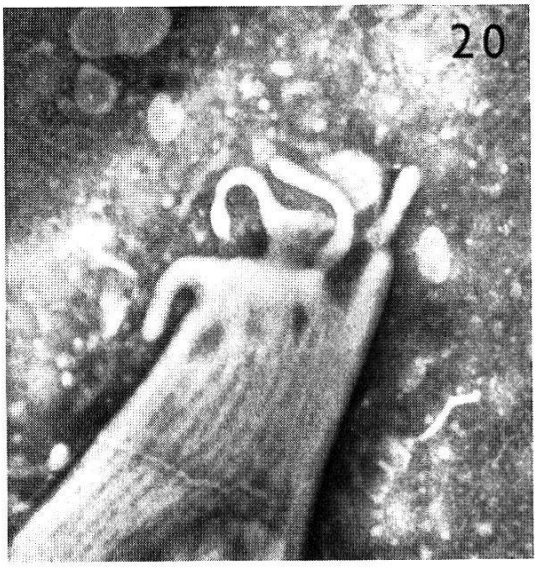
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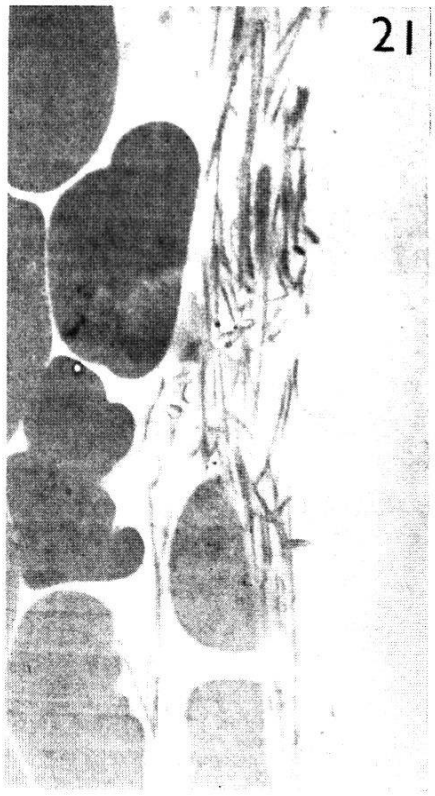
Table 2. Filaments of *Trypanosoma rhodesiense*

	Long-thin type (plasmaneme)	Short-thick type (secretory filament)
Occurrence	Only seen by negative staining or on special media such as agar or methyl cellulose, etc.	Found in host tissue specimens and blood in *in vivo* situations, provided no toluene is used in the processing.
Type of associated trypanosomes	From long-thin forms found in blood, or cultured strains.	Only from short stumpy forms in infected tissue, blood, in culture.
Dimensions	0.05 μm to 0.07 μm in diameter and up to 100 μm in length.	0.09 μm to 0.14 μm in diameter and less than length of the trypanosome. (*i.e.* 20 μm).
Shape	Long streamers with occasional bulbous swellings.	Spheres, rods or 'popper beads'.
Origin	From the extremities of the organism.	From the Golgi complex via the flagellar pocket.
Contents	Extruded cytoplasm of the trypanosome.	Secretion containing active acid phosphatase.
Causation	Stress at weak points in the pellicle.	Biological secretion via the flagellar pocket.

Plate IV

21: The edge of an uninfected blood drop phalanx on agar with fibrin-like filaments at the outer edge (× 3,000). 22: A 120-hour trypanosome (with short-thick filaments (arrowed) coming from the flagellar pocket) entangled in the fibrinlike filaments at the edge of the agar phalanx (× 8,000). 23: A section through the posterior end of a typical slender (72-hour) trypanosome. Note the clear termination of the subpellicular tubules (arrowed) which do not fuse, leaving wide unsupported area (× 40,000). 24: Photograph of the model illustrating the anatomy of the posterior end of a slender form with its 'drum-skin' construction. 25: Negatively-stained posterior end of a slender (72-hour) form with the 'drum skin' ripped off (arrowed) exposing the subpellicular tubules round the rim (× 35,000). 26: Negatively-stained tip of flagellum of a slender-form trypomastigote with long-thin streamers and globule extruded from it, both covered with 'surface coat' (× 18,000). 27: Myelin forms (arrowed) arising when the pellicle of the trypanosome has been damaged during negative staining (× 32,000).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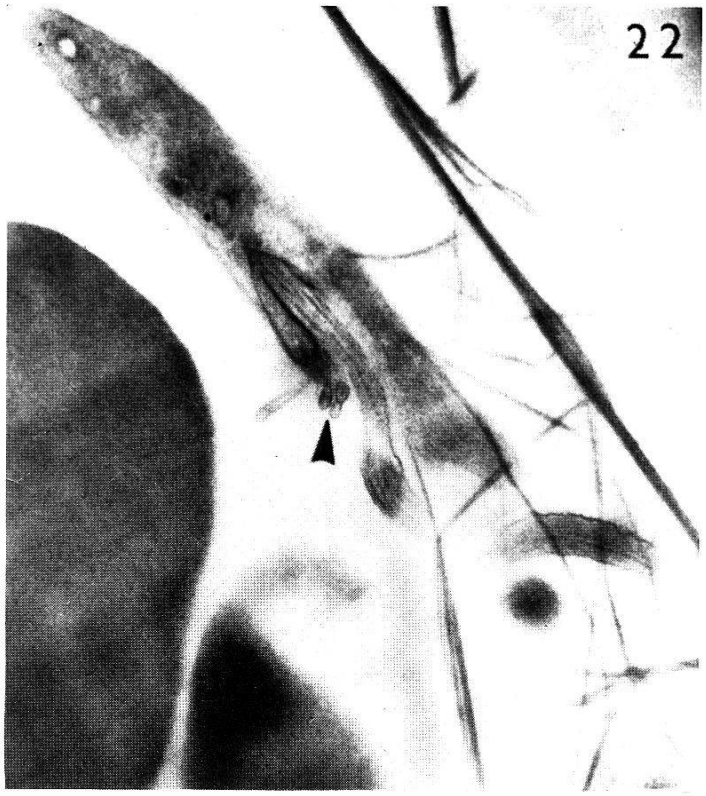
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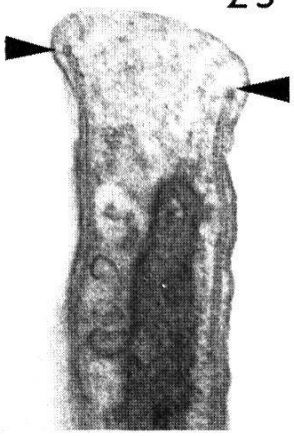
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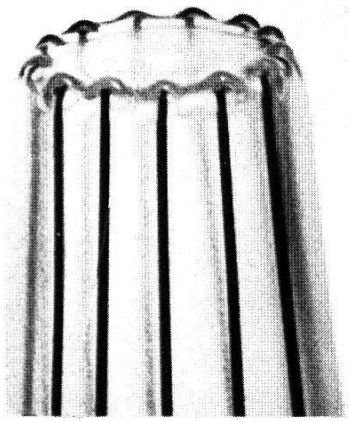
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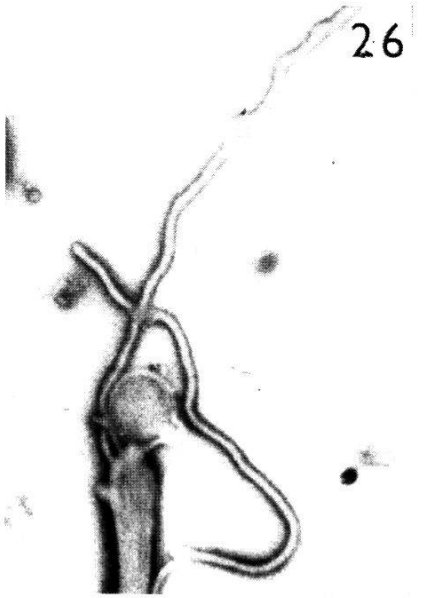
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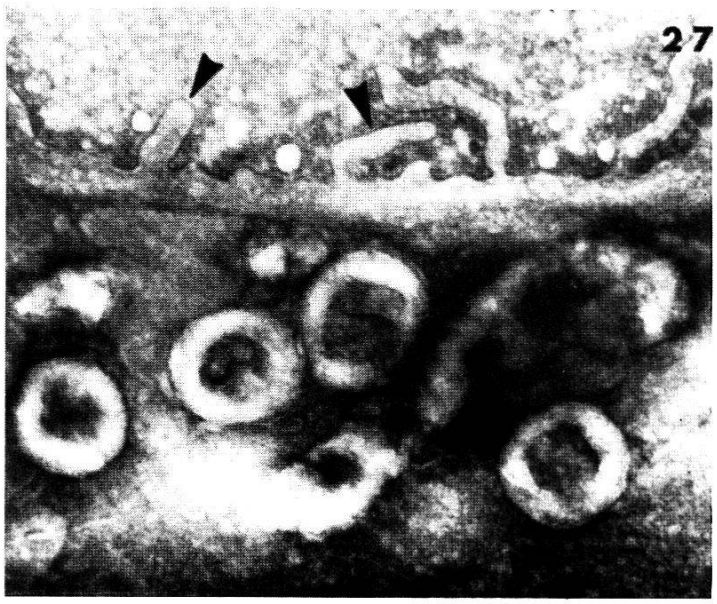
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show globular swellings (Fig. 26). The short-thick filament described above is never found. The series of figures (16, 17, 18 and 19) show what we believe to be the process involved in the long streamer type of filament formation, while Fig. 20 shows the same process in a cultured (and therefore without surface coat) strain of *T. brucei*.

Embedded Material (II)

Selected trypanosomes of 72- and 120-hour rat infections were fixed under coverslips on agar. The phalanx (ORMEROD, 1958) was found to be always surrounded by what appeared to be fibrin (Fig. 21). The marked parasites were always found to be entangled in this (Fig. 22) and, if over 120 hours old, had their short-thick filaments similarly enmeshed.

When long-thin filaments, visible under phase contrast by light microscopy, were fixed on the agar slide and sectioned, nothing was seen of a direct attachment to the trypanosome; only long strands which resembled fibrin. We were never able to establish an independent long-thin type of filament which alone could account for the appearance found using dark ground or phase contrast, and described by SCHEPILEWSKY (1912), MOLLOY and ORMEROD (1964) and WRIGHT, LUMSDEN and HALES (1970).

Table 2 summarises the characteristics of these two distinct types of filament.

Discussion

We would seem to be dealing with at least two types of filament: the short-thick filament which we have demonstrated here as an *in vivo* phenomenon associated with the stumpy form of trypanosome; and the long-thin filament which, as an entity, is less well defined, being associated with various special preparations of slender forms of trypanosomes (Table 2).

Short-thick type of filament

This type of filament was observed in sections of parasites fixed either *in situ* in the tissues, in the blood, or isolated and negatively stained. These filaments appeared as spheres and rods in the flagellar pocket, or secreted outside where they remained, usually in groups associated with the body of the trypanosome, often coming to lie alongside the flagellum.

From 60 to 96 hours after the rat was first infected with this strain (LSHTM 180), special globules (Vickerman's 'spinous vesicles') could be seen associated with the Golgi apparatus. These could be traced towards the flagellar pocket. They could never be found anterior to the nucleus, or anywhere except between the Golgi apparatus and the flagellar pocket. These globules appeared to fuse with the flagellar pocket membrane, and their contents to be found as both rods and spheres within the pocket (Figs. 3, 8 and 9). Outside the pocket the filaments are found covered with 'surface coat' (VICKERMAN, 1969) and so must have existed before fixation to have acquired this. VENKATESAN, BIRD and ORMEROD (1975) found the contents of this type of filament had acid phosphatase activity, both when excreted outside the trypanosome and as globules passing from the Golgi apparatus to the flagellar pocket. Finally, the appearance and dimensions of this type of filament are identical whether or not prefixation is used for negative staining.

Summing up, this short-thick type of filament has the following characteristics:

1) We found these filaments in infected tissue sections, in blood, and by negative staining, but only in association with stumpy forms of the trypanosome.

2) This type has a diameter of 0.09 to 0.14 μm.

3) When outside the trypanosome, these filaments are covered with surface coat, whenever this is present over the trypanosome itself.

4) These filaments leave the body of the trypanosome via the flagellar pocket, in which they can be observed. They are found mainly alongside the body of the parasite and its flagellum. Their origin is not associated with the extremities *in vivo* or under negative staining (unless the trypanosome is ruptured, when many bizarre forms are seen).

5) This short-thick type of filament is not found in monomorphic strains, but can be produced if the strain is rendered 'pleomorphic' by the host being maintained at a raised temperature.

6) They appear to contain acid phosphatase activity.

7) These thick filaments have the same size and conformation whether they are fixed and sectioned or visualised by negative staining of the stumpy form of the parasite.

8) We have never been able to find any form of filament from fixed and sectioned material containing only slender forms of trypanosome; and negative staining of these forms never produces this type of short-thick filament, but only the thin type of streamer (see below).

It would thus seem that these short-thick filaments are natural biological phenomena associated with the stumpy form of trypanosomes. They are excreted via the flagellar pocket, and during extrusion pick up the surface coat. They are associated with globules arising in

the region of the Golgi apparatus complex which discharge into the flagellar pocket. Both globules and filaments contain acid phosphatase activity. In view of these observations, we suggest that this type of filament be referred to as a 'secretory' filament.

Long-thin type of filament

This type is associated with the long-slender form of blood trypomastigote, and can be seen typically when these forms are either negatively stained or observed on agar or methyl cellulose. Their diameter is about half that of the thick type (0.05 to 0.07 μm), but their length may reach many times that of their associated trypanosomes. Filaments of this type have never been found in material fixed *in situ,* though they are clearly visible in the living parasite, e.g. in methyl cellulose preparations. Every worker has noted that they originate from the ends of the trypanosome and flagellum, and not from the pocket, as does the thick type. Although normally associated with the slender forms, they can with difficulty be elicited from the stumpy forms when these have been damaged or burst; and when this happens this type of filament does not originate from the extremities but from the damaged area.

Whenever long filaments have been identified on agar, their subsequent fixation and examination by electron microscopy reveals only a fibrin-trypanosome conglomerate, in which the surface coat may play some part. This process cannot account for the negatively-stained streamers, since these can be produced just as well from carefully washed trypanosomes where there is no fibrin or serum present. These streamers can be seen to be continuous with the trypanosome body (VICKERMAN & LUCKINS, 1969) and so, in contrast to the thick secreted type, are in direct connection with the parasite's cytoplasm (Figs. 16 to 19) at its extremities. The name 'plasmaneme', as suggested by VICKERMAN and LUCKINS (1969), would therefore be appropriate for this type of filament. If 'surface coat' covers the body of the trypanosome, it also extends out over these plasmanemes as a continuous structure.

The slender blood trypomastigote has a posterior end which is structurally different from that of the stumpy form: it is characteristically blunt, while the latter is pointed. The subpellicular tubules stop abruptly (Fig. 23) forming the edge of a drum-shaped end as opposed to a cone. In neither form do the subpellicular tubules fuse. This blunt end is the largest area of trypanosome pellicle unsupported by the tubules, the only other such area, though very much smaller, being the anterior end and the tip of the flagellum. Only the pellicle and surface

coat cover the cytoplasm at these positions. The model (Fig. 24) illustrates this construction and the large unsupported area of the posterior end of the long-slender form of the trypomastigote.

It is known that the end of the flagellum of trypanosomatids can swell out for purposes of attachment, as shown by VICKERMAN (1973) and KILLICK-KENDRICK et al. (1974). We believe that the much weaker posterior end of the slender form can behave similarly under stress, and the series of figures (16 to 19) shows increasing protrusion of the cytoplasm on negative staining. The continuity of cytoplasm and coat can be clearly seen. Fig. 20 is of a cultured long form which does not possess a surface coat, but nevertheless produced similar streamer-type plasmanemes (*sic*), showing that the coat is not essential to the process of plasmaneme formation. Fig. 25 illustrates the effects of more extreme stress when the unsupported 'drum skin' of the posterior end of a slender form has been ripped off, leaving the circle of subpellicular tubules exposed. Fig. 26 shows streamers coming from the end of the flagellum, the other site of origin of these types of plasmanemes.

Summing up the characteristics of this long-thin form of filament (plasmaneme) we found that:

1) they are found in special preparations of slender-form trypanosomes, but never *in situ* in the host tissues or in the blood fixed direct from the host;

2) their diameter varies from 0.05 to 0.07 μm, their length up to 100 μm;

3) they are probably extrusion artifacts from the weak points of the trypanosome pellicle as a result of stress;

4) they always arise from the extremities of the parasite, not from the flagellar pocket;

5) on agar they are associated with filaments of fibrin-like material; the agar system exerts a containment effect on the trypanosomes and this may be the stress factor responsible for their formation;

6) by negative staining, itself the stress here, the plasmanemes are covered by surface coat if blood forms are examined, but this coat is incidental to their formation.

It should be emphasised that the phenomenon of the long-thin filaments cannot be so clearly defined as that of the short-thick form. The long-thin filaments arise in complex and varying circumstances, and, while easily demonstrated as extrusion phenomena (plasmanemes) on negative staining, no fully satisfactory explanation can be found for the very long forms, on agar for example, where only fibrin conglomerates are seen by electron microscopy. There remains the possibility that fixation itself may remove some underlying structures here, in the same fashion as toluene was found to do in the case of the thick secretory filaments of stumpy-form trypanosomes.

General structure of filaments

Neither type of filament appears to possess a normal unit membrane, and this applies to the short-thick type whether it is inside the flagellar pocket or outside the body of the organism. During passage from the Golgi area the globules have a distinctive spinous or 'basket-work' appearance (VICKERMAN & LUCKINS, 1969). This is not the usual vacuole or lysosome-type membrane, but nevertheless this structure, either whole or in part, certainly appears to fuse with the flagellar pocket membrane for the release of the contents into the pocket (Fig. 10).

Although we have fixed such vesicles at the moment of their discharge of filament contents into the flagellar pocket, we feel that we should mention at this point that we do not claim either that these are the only sort of spinous vesicles found in trypanosomes, or that this secretory activity is their only function. Recently LANGRETH and BALBER (1975) have suggested that a larger type of spinous vesicle is used for the uptake and transport to phagolysosomes of ferritin-marked material from the flagellar pocket. But they also noted that culture forms which do not secrete filaments do not have these spinous vesicles, although they can still take up ferritin and transport it to the phagolysosomes. It is possible that some of the invaginations seen by these authors in their blood forms as developing from the pocket membrane may perhaps have been vesicles that had just fused with this membrane and discharged their filament contents. They would then contain some ferritin if this was present in the contents of the pocket. Possibly this spinous vesicle phenomenon may be a dual-purpose transport system of material both to and from the trypanosome flagellar pocket.

Once the 'filament' has been excreted into the flagellar pocket we are left with the problem of explaining how it maintains its clearly defined structure without a membrane; and in the case of the short-thick type, also how it manages to coexist in sphere, rod and popper-bead form, apparently with equal stability. We believe that both forms of filament are essentially myelin formations; that is, their structure is created by lipid/water interfaces and is maintained by surface charges. These formations were first described by GICKLHORN in 1932 and were observed by light microscopy in lipid-containing materials such as homogenates of nervous tissue or of impermeable plant epithelial cells. Fig. 27 shows myelin formations coming from a damaged trypanosome pellicle. We suggest that the short-thick filaments are biological secretions containing lipids that are given their form by myelin phenomena. The popper-bead effect would seem to be a reversible reaction of the fusion of the spheres into tubes and their subsequent breakdown into

spheres as a result of local changes in charge potentials. Such a phenomenon would be very susceptible to a strong lipid solvent, and this would account for our inability to find these filaments if toluene was used during the processing of these specimen blocks.

Different lipids are likely to give myelin forms with different dimensions. This may explain why the long-thin type of plasmaneme has a diameter approximately half that of the short-thick type. If, in fact, the thick form is excreted via the flagellar pocket from Golgi-associated vacuoles, it is not unreasonable to assume it to have a different lipid cover from the long-thin type formed by extrusions of cytoplasm from the extremities of the trypanosome body.

If we are correct in regarding the long streamer-type filaments (associated with long-thin forms of trypanosomes) as being a stress-induced extrusion from the posterior end, we cannot, of course, exclude the possibility that these structures may contain elements of pellicle membrane on their surfaces, perhaps concentrated in areas nearest to their point of origin. Such a possibility may explain certain apparent filaments in the literature that are clearly surrounded by a membrane.

Both types of filament are covered with surface coat if this is present on the parent trypanosome. But the surface coat seems to play no essential part in the formation of either type, since similar types are produced from cultured strains which do not possess such a coat.

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