Structure and Movements of Tick Spermatozoa (*Arachnida, Acari*)

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With five plates (figs. 2 to 6)

Summary

1. The spermatozoa of the tick, *Ornithodoros moubata*, which until now have been reported to move without any apparent means of propulsion, have been examined under the light and electron microscopes.

2. The surface of the spermatozoon is covered with ridges, running parallel to the long axis of the body, 0·30 to 0·47 μ long and 500 to 1,000 Å wide.

3. In longitudinal section approximately every other ridge exhibits wave-like deformations.

4. On the basis of these sub-microscopic structures and the behaviour of polystyrene balls stuck to the surface of live spermatozoa, the hypothesis is put forward that tick spermatozoa move by propagating lateral bending waves along the ridges in an antero-posterior direction.

5. No '9 + 2' or '9 + 9 + 2' arrangement of fibrils has been observed anywhere in tick spermatozoa.

6. There is a striking though not complete resemblance between the sub-microscopic surface structure of tick spermatozoa and that of gregarines.

Introduction

TICK spermatozoa are some of the most unusual in the animal kingdom. They may be just under a millimetre in length whereas the average spermatozoon, such as that of man or a sea-urchin, is about 60 μ long; during spermatogenesis, the head and acrosome are said to move from the 'front' to the 'back' end of the spermatozoon; ripe spermatozoa are only found in the female genital tract, where they are twice as long as in the male; the spermatozoa swim backwards (if the anterior end is defined as that where the head is located (fig. 1)); finally, the spermatozoa have, until now, been described as swimming or moving without any visible means of propulsion.

The general morphology of tick spermatozoa has been described by a number of scientists such as Christophers (1906, *Ornithodoros savignyi*, *Rhipicephalus annulatus*, and *Hyalomma aegyptium*); Casteel (1917, *Argas miniatus*); Nordenskiöld (1909, *Ixodes reduvius*); Oppermann (1935, *A. columbarum*); Tuzet and Millot (1937, various species of *Ixodes*); and Sharma (1944, *H. aegyptium*, *R. sanguineus*, and *A. persicus*). The spermatozoon is shaped like an Indian club (fig. 2, A, B), though the body is very flexible and often bent or twisted. The head is a long, thin structure inside the cytoplasm, at the posterior end. Mitochondrial granules are distributed throughout the cytoplasm, but they are especially dense at the anterior end, at the front of

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which there is said to be a ring-shaped centriole (fig. 2, B). Tick spermatogenesis has been described in detail by Sharma (1944), who also explained, as did Christophers in 1906, how ripe spermatozoa in the female genital tract come to be twice as long as those removed from the male.

The means by which tick spermatozoa move has puzzled previous investigators. Christophers (1906, p. 42) said they 'may exhibit a steady gliding

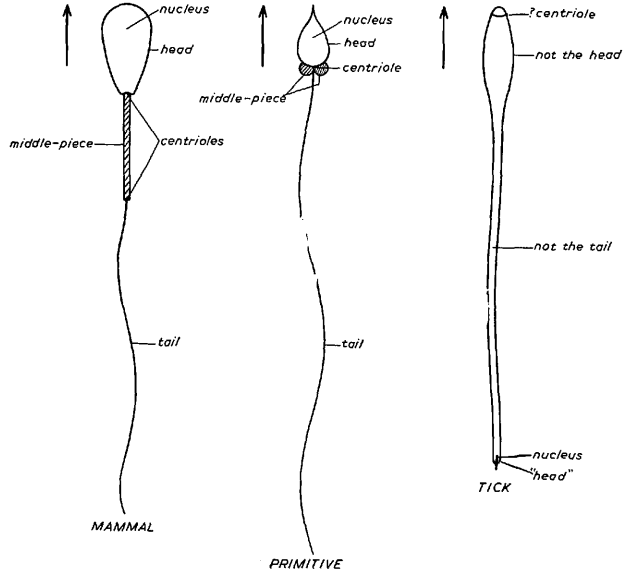

FIG. 1. Comparison of two common types of animal spermatozoa with that of the tick, *O. moubata*; not to scale. The arrows show the directions of motion.

movement, together with marked vermicular contortions of the anterior portion of their substance'. Sharma (1944, p. 313) said that 'The movement of the ripe spermatozoa takes place by the peculiar rotatory movement of the centrosome', but it is far from clear how a rotating centrosome or centriole could propel the spermatozoon. Nor have I seen this rotating centriole, though irregular, writhing movements of the anterior end can often be discerned, whether the spermatozoon is moving or not. Sharma also said that 'When the ripe spermatozoon is moving the mitochondria seem to be in a state of commotion. . . . It may be that these mitochondrial granules help in the peculiar movement of the spermatozoon.' It might be inferred from these statements that tick spermatozoa are neither ciliated nor flagellated and this is correct. (Sharma (1944) uses the word 'flagellum' to describe a long thin

structure in the spermatozoon of the fowl tick, *A. persicus*. The structure in question is not a flagellum in the normal sense of the word.) So far as I am aware, all spermatozoa which move do so either by the amoeboid method, like immature *Ascaris* spermatozoa (Panijel, 1951), or by propagating bending waves along their tails, which invariably contain the '9 + 2' or '9 + 9 + 2' system of fibrils. The body of a moving tick spermatozoon is not amoeboid and it does not bend; no '9 + 2' or '9 + 9 + 2' system has been seen; nor is there any evidence of 'jet propulsion' as in dragonfly nymphs (Hughes, 1958).

The object of the experiments described in this paper was to identify the structures concerned in tick sperm-movement and to try and find out how they work. Some progress is believed to have been made, though the structures themselves and their presumed mode of action are unusual.

Material and Methods

Spermatozoa of *O. moubata* Murray were obtained from spermatophores in female ticks. The spermatozoa were suspended in arachnid Ringer whose composition was 114 ml 67 mM KH_2PO_4, 144 ml 67 mM Na_2HPO_4, 347 ml 540 mM NaCl, 5·6 ml 360 mM $CaCl_2$. $6H_2O$, 5·6 ml 360 mM $MgCl_2$. $6H_2O$, 544·4 ml H_2O (compare Parry and Brown, 1959). The pH was 6·30 and the freezing-point depression Δ 0·67, which is the same as that of tick blood. A Zeiss 'planachromat' objective, × 100, with variable aperture, was used for examination by dark-ground illumination, with a mercury arc lamp.

For electron microscopy, the spermatozoa were fixed and prepared in the following ways: (1) 123 mM Na_2HPO_4, pH 7·4, Δ, 0·53, containing 1% OsO_4 for 1h; washed in 123 mM Na_2HPO_4, pH 7·4; alcohols; methacrylate (85% butyl, 15% methyl methacrylate); sections, 350–500 Å thick; grids floated on saturated solution of uranyl acetate in 50% alcohol for 2 h; washed in water. This way of preparing the material was unsatisfactory. (2) The same as (1), but with arachnid Ringer substituted for 123 mM Na_2HPO_4. This was even more unsatisfactory. (3) The same as (2), but with acetone-araldite substituted for alcohol-methacrylate. (4) By a method similar to that of Kellenberger, Sechaud, and Ryter (1959). The procedure was as follows: 3 ml of sperm suspension in arachnid Ringer containing 1% Bacto Tryptone (Difco) + 0·3 ml Michaelis acetate-veronal buffer, containing 1% OsO_4, pH 6·1, Δ, 0·67, 5 min; spermatozoa transferred to 1 ml acetate-veronal buffer, containing 1% OsO_4 + 0·1 ml arachnid Ringer + 1% BactoTryptone, pH 6·10, Δ, 0·67, and left overnight at room temperature; 8 ml acetate-veronal buffer added, 5 min; spermatozoa transferred to acetate-veronal buffer containing 0·5% uranyl acetate, 2 h; alcohols; methacrylate; sections. (5) The same as (4) but with acetone-araldite substituted for alcohol-methacrylate.

Sections were examined without removal of methacrylate or araldite on a Siemens-Elmiskop I electron microscope, with the assistance of Mr. R. W. Horne, the Cavendish Laboratory, Cambridge, and on a Philips 75-kV instrument.

Results

Observations with the light microscope

Morphology. The general form of the spermatozoon is shown in fig. 2, A (dark ground) and B (phase contrast). According to Sharma (1944), a ring-shaped centriole can be seen in stained specimens at the anterior end. A rim can just be seen in fig. 2, B, in the right place. The site of this structure is invariably visible in sections (fig. 2, D), though it is not certain that the rim is a centriole. The cytoplasm at the anterior end is packed with granules (fig. 2, B), which, under the electron microscope, are seen to have a typical mitochondrial structure (fig. 4, A). Contrary to previous reports, these granules are not 'in a state of commotion' when the spermatozoon is moving. It is only when a spermatozoon has stopped moving and is moribund, as evidenced by subsequent disintegration, that intense Brownian movement of the granules is evident. The nucleus, which is at the distal end, cannot be clearly seen in fig. 2, A, B; the tail-like structure at the posterior end (fig. 2, A) is the 'head'.

Speed. The mean speed of 11 spermatozoa was 17·1 μsec^{-1}, standard error of mean, 4·1 μsec^{-1}, range 2·3 to 22·9 μsec^{-1}. The inclusion of 0·01 M ATP in the suspending medium had no effect on the movements of motile or motionless spermatozoa. This high concentration was deliberate, because the ATP was applied externally.

Tick spermatozoa sink to the bottom of a drop of arachnid Ringer. With normal methods of viewing, therefore, they move over a solid surface. They also move in a hanging drop, though they naturally sink to the bottom of it. These observations do not answer the question whether tick spermatozoa would move or swim in a medium whose density was such that they did not sink in it. Experiments to examine this point present difficulties and, for reasons discussed later, might not be of much help in elucidating the locomotory mechanism.

Flagella'. When examined by dark-ground illumination, the spermatozoa were found to have fine filaments, as much as 10 μ long and reminiscent of bacterial flagella, on some parts of their surface. No reliable estimate of the diameter of the filaments could be made from examination by dark ground illumination, because, in such circumstances, there is no theoretical lower limit to the thickness of a line which can be detected, as there is in the case of direct illumination. (If structure *a* is wider than structure *b*, the amount of light scattered from *a* will be greater than from *b* and there will, therefore, be an increase in the amplitude of the diffraction pattern; but the width of the pattern may be unchanged.) Bearing these points in mind, the diameter of the

FIG. 2 (plate). A, live tick spermatozoa; dark-ground illumination. The head is at the extreme 'posterior' end.

B, live tick spermatozoon; phase contrast.

C, surface of a tick spermatozoon, showing fibrillar structure; dark-ground illumination.

D, anterior end of tick spermatozoon in longitudinal section; araldite.

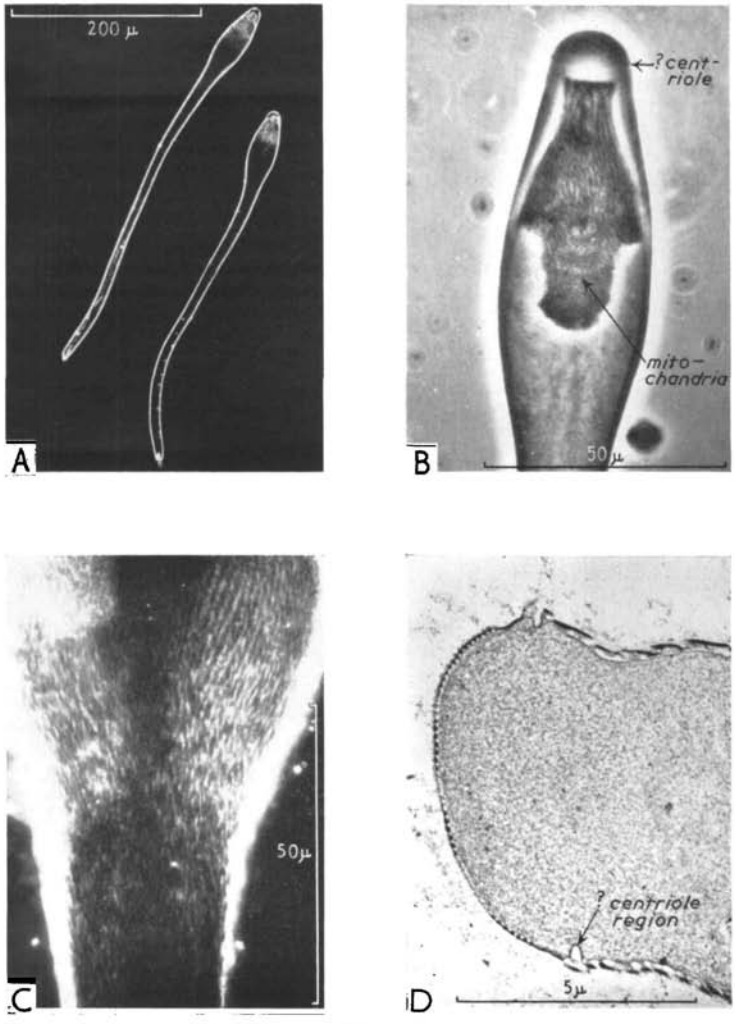

FIG. 2

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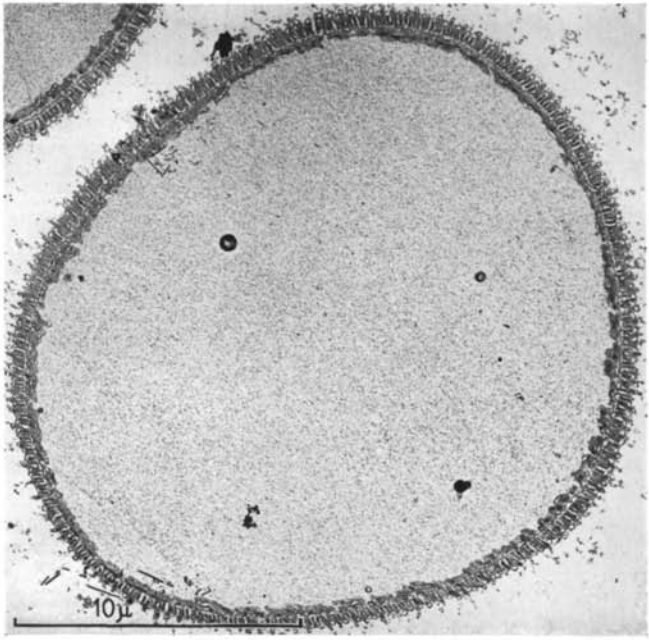

FIG. 3

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filaments was believed to be less than 5,000 Å when examined by dark-ground illumination. The filaments are invisible by phase contrast. Though theoretically possible, it was difficult to photograph the filaments because the amount of light they scattered was exceedingly small; moreover, they exhibited intense Brownian movement which precluded long exposures. The amount of light scattered during a few 30-μsec 50-joule flashes from a high-speed multiple flash-lamp (Brown and Popple, 1955) was insufficient to photograph them. The Brownian movement of the filaments could be reduced by suspending the spermatozoa in a viscous medium; but as a number of people in this Department have seen the filaments, little time was wasted in trying to photograph them, though one is visible on the original positive from which fig. 2, c was made.

The filaments have been seen on dead tick spermatozoa and on those which were not moving but which were believed to be alive. They were said above to be in a state of Brownian movement, because no cause for their movements other than thermal agitation is available in the case of dead spermatozoa. This does not, however, mean that the filaments move or vibrate in a random way. They assume a characteristic undulatory form, as if, because of anisotropic mechanical properties, they can only respond to Brownian forces in a particular geometrical way. No differences were discernible between the form of the undulations along filaments on live and dead spermatozoa.

Normally, the filaments seem to lie along the long axis of the body of the spermatozoon and, every now and then, to get out of this position and assume one in which their long axes are more or less at right angles to the surface of the spermatozoon, when they are visible if maximum magnification and dark-ground illumination are used. The distribution of visible filaments on the surface of the spermatozoon is unpredictable and there is no certainty that they will be seen on any particular spermatozoon. This suggests that normally, the filaments may be part of a membrane of continuous structure on the sperm surface which itself has a fibrous or filamentous appearance (fig. 2, c). The spermatozoon is, therefore, unlikely to be covered with separate 'bacterial flagella' and it follows that tick sperm-movement is not caused by bending waves in separate filaments, if that is how multiflagellate bacteria move. This system of propulsion is improbable for other reasons, discussed later. The fibrous membrane on the surface of the spermatozoon must be assumed to be so fragile that, occasionally, filamentous elements in it become detached except at their proximal ends.

Movements of spheres on the sperm surface. The surface of the spermatozoon is sticky, so that if polystyrene spheres, diameter 1·2 μ, are suspended in the medium, those which are free in the medium exhibit Brownian movement, whereas those on the sperm surface do not. When, however, a spermatozoon is motionless but alive (as evidenced by lack of Brownian movement of the mitochondrial granules), polystyrene spheres on the sperm surface can

FIG. 3 (plate). Transverse section of tick spermatozoon; methacrylate.

often be seen being transported along the surface in an antero-posterior direction, though occasionally they move forward. The movement of the spheres is jerky but sometimes sufficiently dramatic to merit the description 'snapping backwards', as if some barrier to movement (mucus?) were suddenly overcome. The simplest hypothesis to explain the predominatingly backward movement of the polystyrene spheres and the forward movement of the spermatozoon is that deformations in some structure at the sperm surface pass from the front to the back end of the spermatozoon. This statement implies the existence of a contractile system at or associated with the sperm surface. A striated or ridged structure can be seen in light micrographs (fig. 2, c). There is little possibility of finding out any more about these structures with the light microscope, or of determining the plane of the (so far) hypothetical deformations relative to the axes of the spermatozoon.

Observations with the electron microscope

Transverse sections. Figs. 3 and 4 show transverse sections of a tick spermatozoon. The surface is covered with processes, hitherto undescribed, of different lengths, every fourth process usually being a long one. The average lengths of the shorter and longer types are 0·30 μ and 0·47 μ. The width of the processes is 500 to 1,000 Å, depending on the region of the spermatozoon in which the section is cut. This width is somewhat less in araldite-embedded specimens (fig. 4, B). Wave-like deformations of the processes are often observed. There is a membrane 80 Å wide at the surface of each process; it is composed of two electron-opaque layers, each 30 Å wide, with an electron-transparent layer, 20 Å wide, between them. A peculiar and ubiquitous feature of Kellenberger-methacrylate specimens is the presence of a vacuole at the end of almost each process (figs. 3; 4, A). These vacuoles are not always present in Kellenberger-araldite specimens (figs. 3; 4, A).

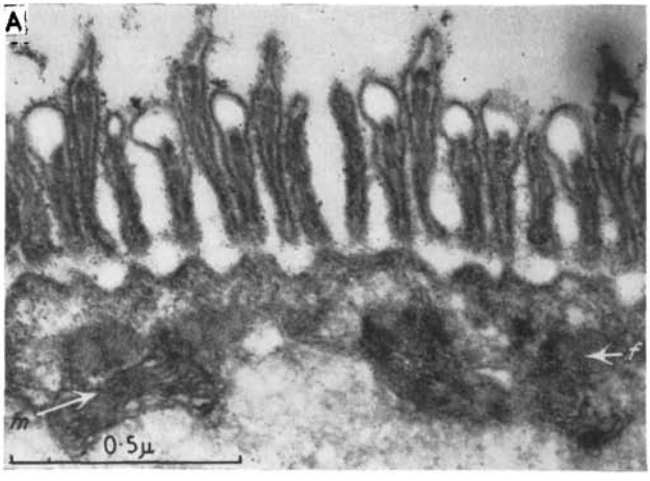
The processes do not appear to be continuous with the body of the spermatozoon, at the surface of which there is a plasma membrane. Presumably, the material round and at the bases of the processes is mucus. As already mentioned, the surface of the spermatozoon is sticky.

There is a dense layer of mitochondria immediately inside the body of the spermatozoon (figs. 3; 4, A), while sets of regularly spaced 'fibrils', 70 Å in diameter and 36 Å apart, run parallel to the long axis of the spermatozoon (figs. 4, A; 5).

Longitudinal sections. If a structure appears as a finger-like process in transverse section it may be a ridge, and this was found to be the case by examination of longitudinal sections (fig. 5, A, B). The ridges run roughly parallel with the long axis of the spermatozoon. The wavy shape of the ridges is very plain in longitudinal sections, which also reveal a difference between

FIG. 4 (plate). A, transverse section of tick spermatozoa, showing ridges on surface; methacrylate. *m*, mitochondria; *f*, longitudinal fibrils in transverse section.

B, transverse section of tick spermatozoon, showing ridges on surface; araldite.



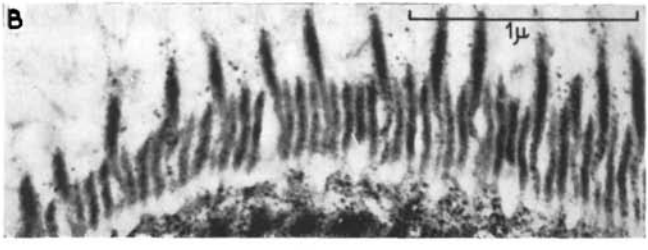


FIG. 4

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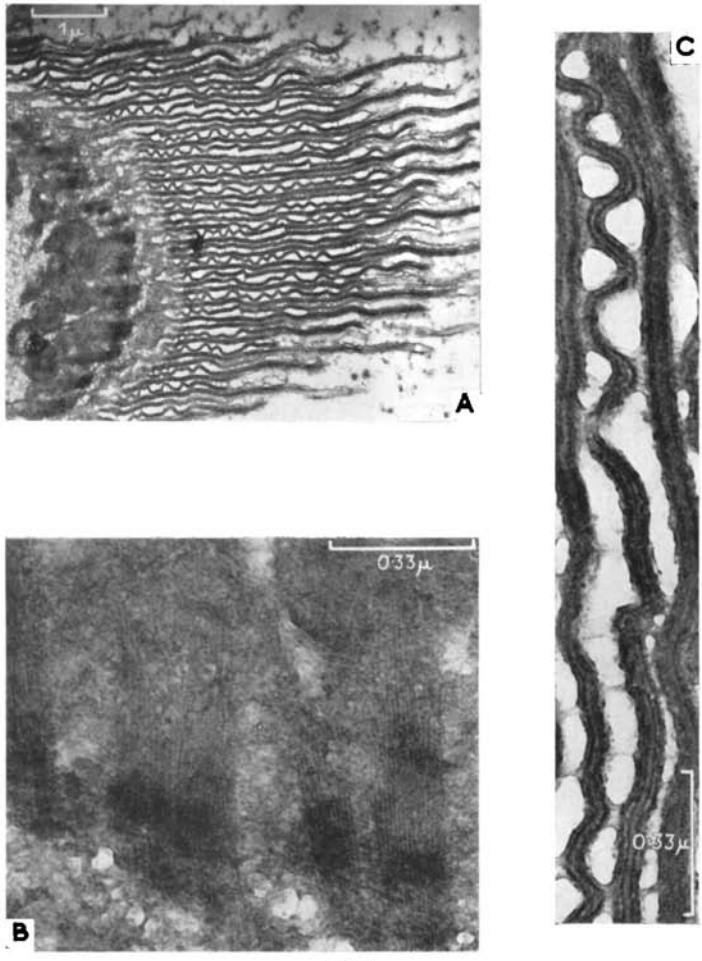

FIG. 5

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ridges, as some have waves in them whereas others do not. The periodicity of straight and wavy ridges is not completely regular, though there is an obvious tendency for a wavy ridge to have a straight one on each side of it. The ridges appear to have fibrils 80 Å in diameter within them. The word 'appear' is used because the fibrils were only visible when an 'unsatisfactory' fixative, e.g. 123 mM Na_2HPO_4 + 1 % OsO_4, was used.

Inexplicable structures. (*a*) In several sections believed to be in the region of the head, structures have been observed which cannot be identified with certainty (fig. 6, B). Outside the familiar finger-shaped processes there is a set of transverse sections of filamentous structures whose diameter varies from 650 to 1,200 Å. Some appear to contain sub-fibrils; others have cartwheel-shaped structures inside. As mentioned earlier, there are reasons for thinking that in certain circumstances, the ridges which run longitudinally along the body of the spermatozoon may get displaced from their normal positions. The fact that, at any rate for part of their length, they are not connected to the body of the spermatozoon (fig. 4, A), makes the possibility of their becoming detached more likely. The structures in fig. 6, A outside the finger-shaped processes may therefore be transverse sections of detached ridges. Their diameters are consistent with this possibility.

(*b*) In one, but only one, unshadowed whole preparation, 'flagella' were observed at one end (fig. 6, A). These are 4,300 Å in diameter. Although it would be convenient to ignore this electron micrograph on the grounds that it may be of some other organism which accidentally got into the preparation, I decided not to do this. Other shadowed and unshadowed whole preparations showed the ridges identified in transverse and longitudinal sections, though they were never displaced from the surface of the spermatozoon. The 'flagella' in this electron micrograph are not believed to be the same as those visible with the light microscope. The latter are probably displaced ridges.

Discussion

There seems little doubt that tick spermatozoa move by propagating bending waves along some, but possibly not all, of the processes revealed on their surfaces by the electron microscope. It has not so far been possible to decide whether deformations in these processes only propel the spermatozoon when it is in contact with a solid surface or whether they can do so when the spermatozoon is freely suspended in an aqueous medium. There is little hope of making further progress by observations on living tick spermatozoa because the processes are too small to be resolved with the light microscope.

Fig. 5 (plate). A, longitudinal section through ridges on surface of tick spermatozoon; methacrylate. This section is slightly oblique, the left-hand side passing through the body of the spermatozoon and the right-hand side through the ridges.

B, enlarged view of left-hand region of A, showing longitudinal fibrils seen in transverse section in Fig. 3, B.

C, part of a ridge in longitudinal section; methacrylate.

The irregular writhing movements which occur from time to time at the 'anterior' end of tick spermatozoa are unlikely to be connected with their translatory movements, though it is possible that the bending waves in the processes are initiated at the 'anterior' end, where mitochondria are particularly dense and where the processes may be connected to the body of the spermatozoon.

The processes bear a striking, but not complete, resemblance to those described by Kümmel (1958) in the gregarines *Gregarina polymorpha* and, particularly, *Beloides*; and it may, therefore, be that gregarines and tick spermatozoa move by the same mechanism.

The structure of the processes and the way they connect with the mitochondria underlying them will doubtless be further elucidated, if only because the electron micrographs reproduced in this paper are not entirely satisfactory. It is not at present certain, for example, whether the vacuoles regularly observed in specimens embedded in methacrylate are artifacts, or whether they reflect some secretory activity (the surface of a tick spermatozoon is very sticky). These vacuoles are not always seen in specimens embedded in araldite, but the Kellenberger method of fixation is believed to give as 'good' results with methacrylate as with araldite embedding. If more was known about the composition of tick body fluid or such liquid as there may be within the spermatophore, better results might be obtained.

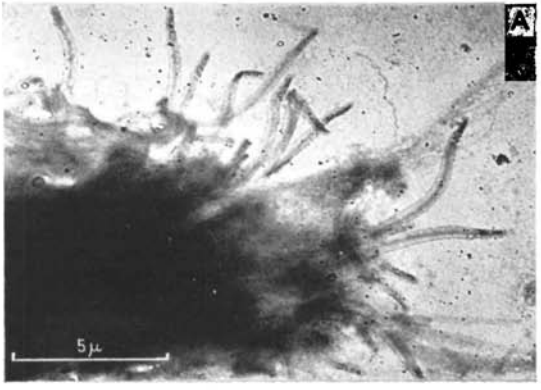
As mentioned earlier, the 'flagella' observed with dark-ground illumination are unlikely to be separate organs of propulsion. It is far from clear how structures sticking out more or less at right angles to the surface of a spermatozoon and in a state of intense Brownian agitation could propel it forward, even if bending waves were propagated along them as is postulated in bacterial flagella. As Gray (1951) suggested in the latter case, the disorientated fibrils of tick spermatozoa are more likely to be part of a membranous sheath composed of a series of parallel fibrils which, under certain circumstances, become torn out of the sheath.

From what has been said above, it is evident that more work is needed to obtain a true picture of the structures responsible for tick sperm-movement; and the same applies in regard to the normal location of the fibrils which are occasionally seen in abnormal positions. But some progress has been made in the main objective of the experiments, to identify the organ of propulsion and suggest a method by which it works.

I am indebted to Sir James Gray, F.R.S., and Dr. A. V. Grimstone for commenting on the typescript of this paper. This work is supported by the Agricultural Research Council.

FIG. 6 (plate). A, unshadowed electron micrograph of one end of a tick spermatozoon, showing 'flagella'.

B, structures occasionally seen at anterior end of tick spermatozoon; methacrylate. For further details, see text.



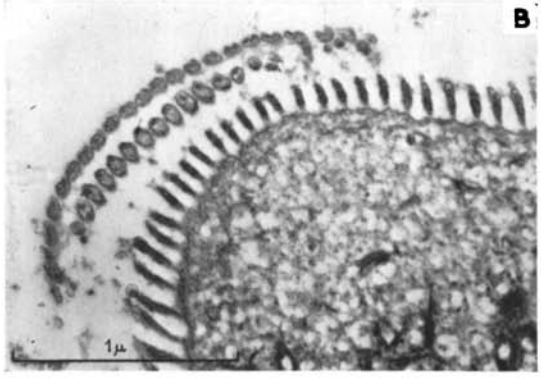


FIG. 6

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