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Shape, Movement in situ and Locomotion of Plasmodial Ookinetes*

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Introduction

It is generally accepted by malariologists that the plasmodial zygote very soon assumes a vermicule-like shape in the midgut of a vector mosquito and thus becomes an ookinete. The latter—as its name suggests—is motile and migrates from the lumen to the outside of the gut, where an oocyst subsequently forms. In 1962, this assumption was seriously questioned by HOWARD (11) in the light of experimental studies involving the feeding of mosquitoes with plastic pellets. HOWARD suggested that the actual zygote was spherical and non-motile and was passively conveyed through the wall of the midgut during the reorganisation of the gut epithelium in the course of blood digestion. He did admittedly observe ookinetes, but he denied that they were in any way motile, and he appears to have considered them as degenerative forms of gametocytes. HOWARD supported his hypothesis with a review of the literature (10), which revealed that no one had in fact seen plasmodial ookinetes in motion and that their motility had simply been assumed by analogy with the conditions obtaining in the case of *Haemoproteus*. Not very long afterwards, GARNHAM et al. (8), with the aid of electron-microscope pictures, confirmed the presence of ookinetes in the brush border and in gut epithelial cells of mosquitoes. This finding cast considerable doubt on the correctness of HOWARD's theory, but it did not invalidate his most important argument which was that the ookinetes were non-motile. We therefore set ourselves the task of demonstrating the motility of ookinetes and of showing how they are able to pass through the peritrophic membrane and into the epithelial layer.

Material and Methods

The material for our investigation was selected in such a way as to make it possible, firstly, to provide a connecting link with previous findings (8, 16, 18) and, secondly, to draw conclusions as to the conditions presumably obtaining in plasmodia pathogenic to man. We therefore worked with four plasmodial species:

Plasmodia (Haemamoeba) gallinaceum, strain kept at the Swiss Tropical Institute (4) since 1948; in *Aedes aegypti.*

Plasmodium (Haemamoeba) matutinum, isolated by LOVRICS [1] from blackbirds in Switzerland in 1964; in *Culex pipiens.*

Plasmodium berghei [1], strain S.P. 11 Antwerp; in *Anopheles stephensi.*

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* An abridged version of this paper was read in conjunction with the film "The motility of plasmodial ookinetes and sporozoites" at the "Second International Conference on Protozoology", London, July/August 1965 (5, 22).

Plasmodium cynomolgi bastianellii [1], from the London School of Hygiene and Tropical Medicine; in *Anopheles maculipennis atroparvus.*

As we were dealing with live, unstained organisms, we used a phase-contrast microscope. The movements of ookinetes can as a rule hardly be perceived by direct observation, and we therefore had recourse to time-lapse films, taken at 30″, 15″ or 6.6″ intervals. At varying times after an infective blood meal [16–32 hours for *P. (H.) gallinaceum,* 42–43 hours for *P. (H.) matutinum,* 16–19 hours for *P. berghei,* and 26–29 hours for *P. cynomolgi bastianellii*] the mosquitoes were lightly anaesthetised with ether and the midgut dissected free. The physiological medium we used for this purpose was TC 199 10×, Code No. 0696 (3) with a few salt and sugar supplements as indicated in the extensive data published by CHAO & BALL (2). In this medium the ookinetes remained motile for up to 21 hours after dissection. Depending on the particular aspect of the problem being studied, the gut epithelium was then either simply removed and the peritrophic membrane—if present (6)—left intact, or else the peritrophic membrane was removed in whole or in part from the blood coagulum. About a quarter of an hour after removal of the epithelium, large numbers of ookinetes left the blood coagulum, gradually sank to the bottom of the preparation, and attached themselves to the microscope slide. Their movements were filmed both when they left the blood coagulum—and thus, in the case of *Aedes aegypti,* also when they passed through the peritrophic membrane—and on the microscope slide. When the peritrophic membrane was removed from the blood coagulum, numerous ookinetes remained attached to it as a rule, provided care was observed in washing off the remnants of blood. When the membrane was laid down flat, the locomotion of the ookinetes and, occasionally, their passage through folds in the membrane, could be easily followed. In order to study the behaviour of the ookinetes on the inside of the epithelium, we spread out the opened gut wall, brush border uppermost, on the slide and placed a peritrophic membrane laden with ookinetes, inside downwards, on top of it. When the membrane was removed, a few ookinetes invariably remained on the gut wall, and some of them penetrated into the epithelium.

In order to analyse the films, individual frames were selected in the viewer and photographed. A single composite drawing of consecutive situations was then prepared on tracing paper. Care was taken to find at least two fixed reference points in each frame. To determine the speed of migration, the distance travelled by the ookinetes on the composite picture was measured with a curvimeter or a ruler and correlated with the time calculated from the number of pictures taken.

Results

A. Shape, movement in situ and mode of locomotion

Plasmodium (Haemamoeba) gallinaceum

Morphology. As regards its outer contours, the shape of the ookinete is often best compared with that of a leech. The body of

[1] Our sincere thanks are due to Prof. P. C. C. GARNHAM, Miss V. LOVRICS, Mrs. M. SCHEEPERS-BIVA, and Prof. I. H. VINCKE for kindly letting us have the strains.

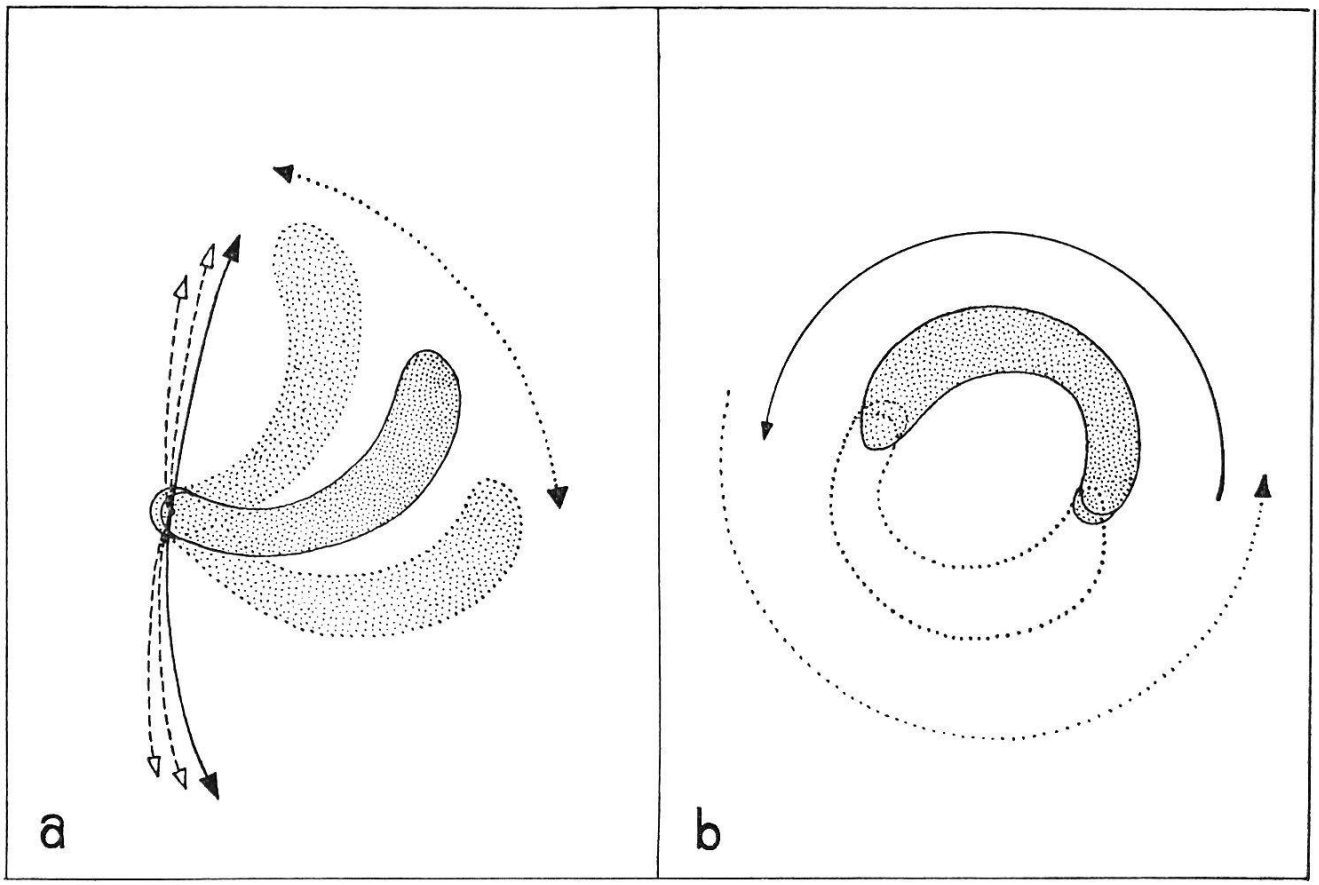

Fig. 1. P. (H.) gallinaceum. Schematic drawing of the ookinete's movement *in situ* a) with firmly fixed posterior end, b) with occasional detachment of the posterior pole.

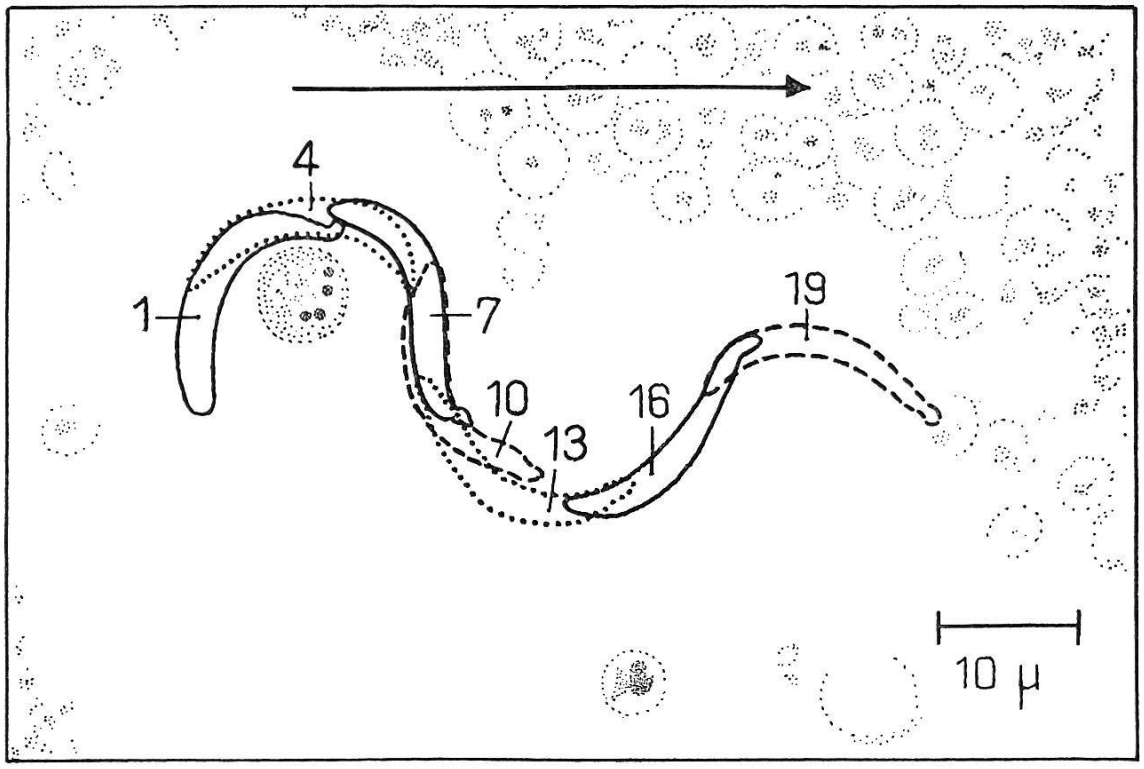

Fig. 2. P. (H.) gallinaceum. Snake-like, gliding locomotion (25 h after ingestion of an infective blood meal by the mosquito).

the cell is elongated and narrowed a little at the front. The posterior end is rounded, and the anterior pole exhibits a thickened area, frequently not very clearly marked, known as the 'pole cap' (cf. Fig. 2). The narrowed portion and the pole cap may be completely invisible. The ookinete is between 15 and 19 μ long, while in width it measures about 1 μ at its narrowest point, and up to 2.7 μ at its thickest.

Movement in situ. When living ookinetes from the blood coagulum in a mosquito's gut come into contact with a microscope slide, their posterior end adheres to the latter's surface. The anterior, free portions of their bodies perform swivelling movements to the sides and upwards. The speed of these movements varies widely; one swivel through a 90° arc may take minutes or it may be accomplished by a series of jerks in only a few seconds (Fig. 1a). An ookinete may relax its hold temporarily, carry out a semicircular movement, and then anchor itself again in its new position (Fig. 1b). In both cases, the ookinete displays a clear-cut automotility which, however, does not involve any locomotion.

Locomotion. On the inside of a peritrophic membrane that has been spread out flat, ookinetes can move (i.e. change their location) in two different ways. Firstly, they may display a snake-like, gliding motion, during which they appear, just like a snake, to make use of the unevenness of the surface beneath them. In Fig. 2 seven out of 19 consecutive film frames have been combined to show the path travelled by the ookinete. In this case, the ookinete covered 65 μ of the substrate in five minutes (13 μ/min) —or 42.5 μ measured in a bee-line (8.5 μ/min).

The second mode of locomotion is illustrated in Fig. 3, which reproduces eight out of fifty consecutive film frames. We took as our main reference point a solid object (top right in the frames) filmed by chance, and as auxiliary reference points two crossed threads which can be seen in the centre of the frames. In frame 1 the ookinete appears markedly constricted at the level of the lower crossed thread, and this constriction persists right up to frame 20. In frame 5, the anterior pole of the ookinete exhibits a dark pole cap; a further constriction develops at the upper crossed thread and can be observed up to frame 48. The nucleus is visible at times; between frame 20 and frame 25 it appears to be forced forwards through the anterior constriction. When viewed in terms of the ookinete, these constrictions arise successively at the anterior pole and move varying distances into the posterior half of the cell where they 'fade out'. In terms of the substrate, however, the annular constrictions remain where they are; the cell contents pass through the constrictions to the front,

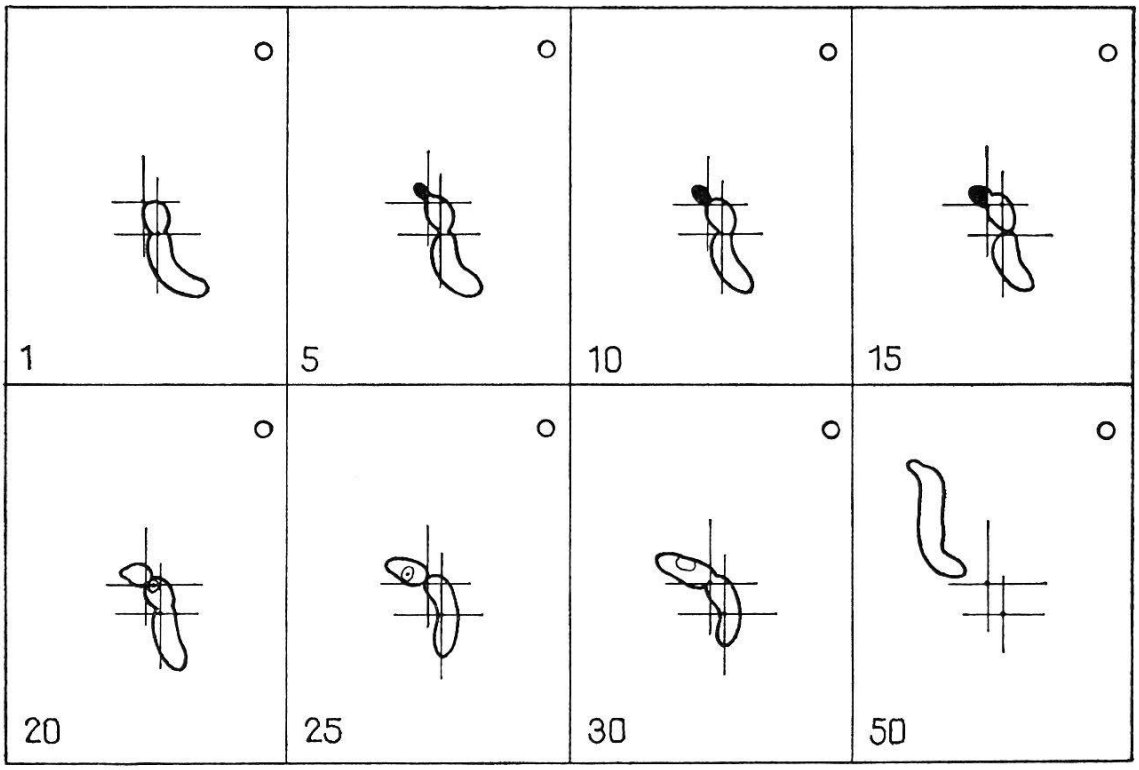

Fig. 3. P. (H.) gallinaceum. Locomotion by means of annular waves of contraction (16 h after an infective blood meal).

thus giving rise to a slow forward movement. In the films studied no evidence could be found that the development of the annular constrictions might be due to the ookinete passing through an excessively small hole in the peritrophic membrane. In the example reproduced in Fig. 3, the distance covered amounted to 20 μ in 25 minutes (0.8 μ/min). In this instance, the locomotion was produced by annular waves of contraction, combined with internal plasma currents.

Plasmodium (Haemamoeba) matutinum

Ookinetes of this species were only filmed on two occasions. In shape and size they very closely resemble those of *P. (H.) gallinaceum* described above; however, neither a narrowed portion nor an anterior thickening was observed. Movement *in situ* (as illustrated in Fig. 1b) and snake-like, gliding locomotion were recorded. The movement *in situ* consisted essentially of two complete clockwise revolutions, each of which took 4-5 minutes to complete. The locomotion took the form of a 120 μ long journey from the margin to the centre of the field of vision and back again along a similar path; the speed of travel varied, and the ookinete occasionally lay quiet for a while. The journey took altogether 167 minutes (0.7 μ/min); part of the distance (17 μ), however, was

covered in only 6 minutes (3 μ/min). As far as can be established on the basis of the scanty data available, no essential differences from *P.(H.) gallinaceum* exist in either type or speed of locomotion. Some of the ookinetes that exhibited a snake-like, gliding form of locomotion revealed signs of an annular constriction, but this was never so clear-cut as in *P.(H.) gallinaceum*—a finding which is all the more remarkable as in this case the ookinetes on several occasions travelled through the flattened peritrophic membrane, as could be seen from the variations in the definition of their contours.

Plasmodium berghei

The ookinetes of this species are much smaller than those of the *Haemamoeba* species already mentioned; they are only about 8.5 μ long and, measured at the middle of the cell, some 2.5 μ wide. In addition, they taper off towards the posterior pole and often appear to be curved like a sickle (Fig. 4a). The motility of these ookinetes is clearly visible from the few photographs we managed to take of live specimens. The contours of the cell seem sometimes to change in a manner reminiscent of the annular

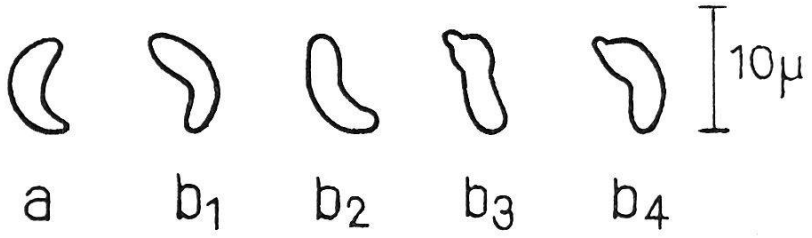

Fig. 4. P. berghei. Two ookinetes; ookinete b in various successive forms (16 h after a blood meal).

waves of contraction seen in *P.(H.) gallinaceum* (Fig. 4b_3). In the main, however, the locomotion of these ookinetes consists of a gliding or helical movement, as illustrated in Fig. 5. It is impossible to decide on the basis of our film material whether the movement is a gliding one, combined with a slight wriggling of the body in only two dimensions, or whether it is a spiral movement performed by the ookinete turning about its axis of locomotion. The ookinete in Fig. 5 moved at varying speeds, covering 15.5 μ in 16 1/2 minutes (0.9 μ/min).

Plasmodium cynomolgi bastianellii

Morphology. The shape of the ookinetes varies to some extent. In addition, the contours of the individual ookinete change as it

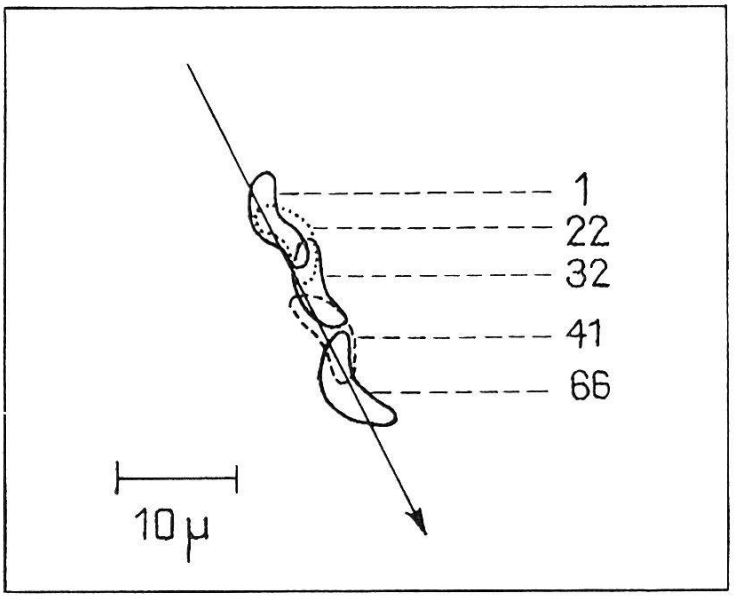

Fig. 5. P. berghei. Ookinete in the process of locomotion (16 h after a blood meal).

moves along. In Fig. 6 the ookinetes d and e (from Fig. 8) and the various forms they take are illustrated side by side. Their length is relatively constant, amounting to between 10 and 13 μ. By contrast, their width is much more variable, ranging from 2 to 4 μ. In view of these variations in shape and of the results of the movement analysis given below, an attempt was made to prepare a plastic model of an ookinete of *P. cynomolgi,* three different aspects of which are reproduced in the forms of drawings in Fig. 7. This ookinete is rhomboid in shape, with a short anterior portion and a somewhat longer posterior part which ends in a slightly bent, blunt 'tail'. The rhomboid is flattened, and appears to be twisted along its longitudinal axis into a gentle, counter-clockwise spiral.

Annular constrictions, as described above in connection with *P.(H.) gallinaceum,* were observed only in dying ookinetes of *P. cynomolgi* (Fig. 6 h and i).

Movement in situ. The ookinete of *P. cynomolgi,* like that of *P.(H.) gallinaceum,* is able to anchor itself with its tapered pos-

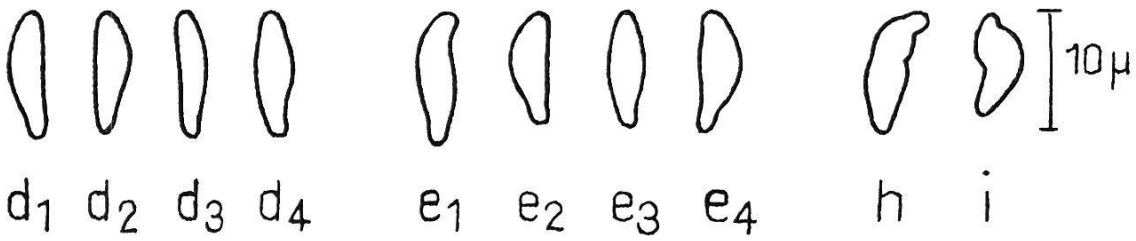

Fig. 6. P. cynomolgi bastianellii. Various forms of ookinetes (28–29 h after a blood meal). As regards d and c, cf. Fig. 8. h and i are degenerating forms.

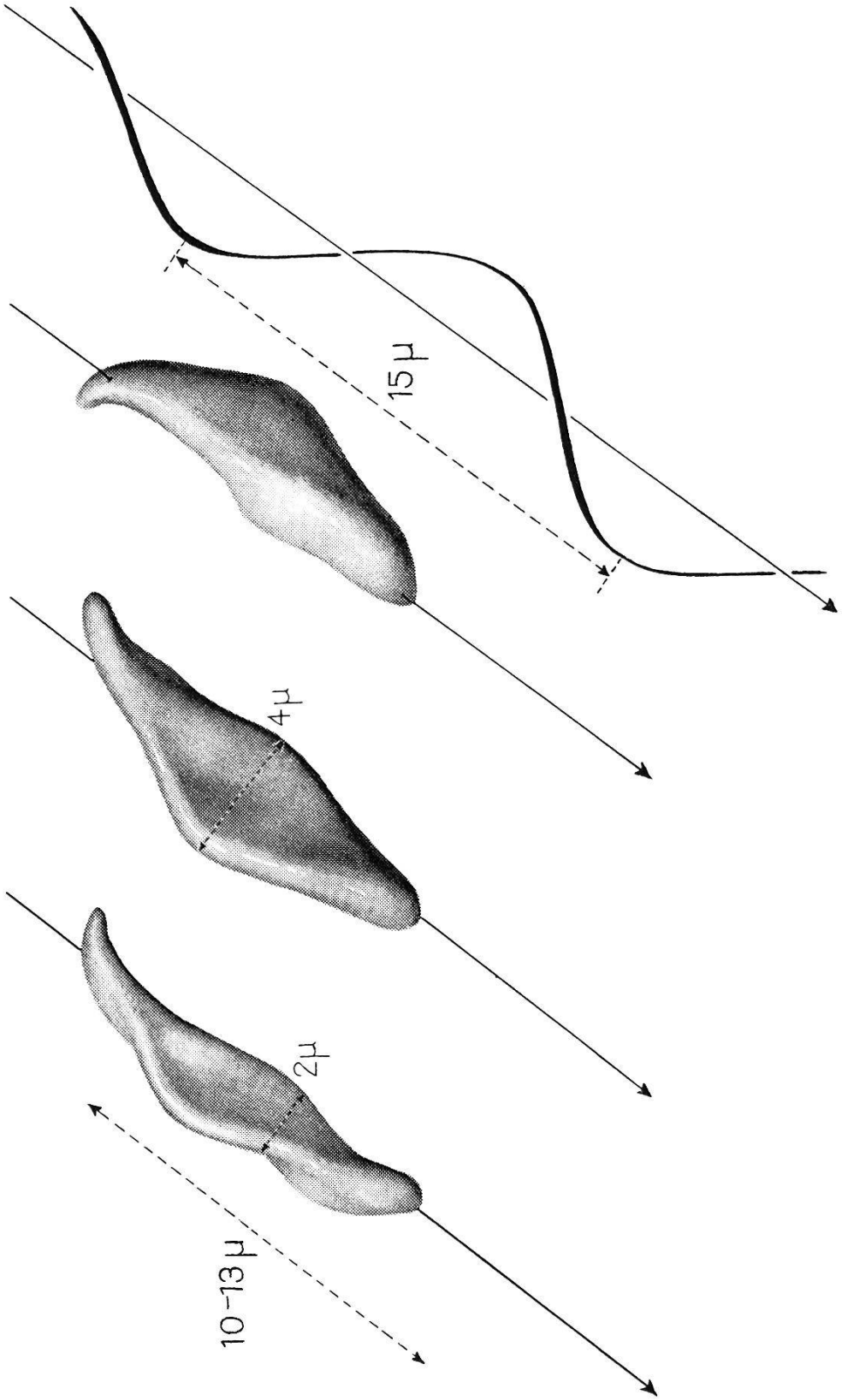

Fig. 7. P. cynomolgi bastianellii. Model of ookinete in three different positions relative to the axis of locomotion (for further explanation, see text).

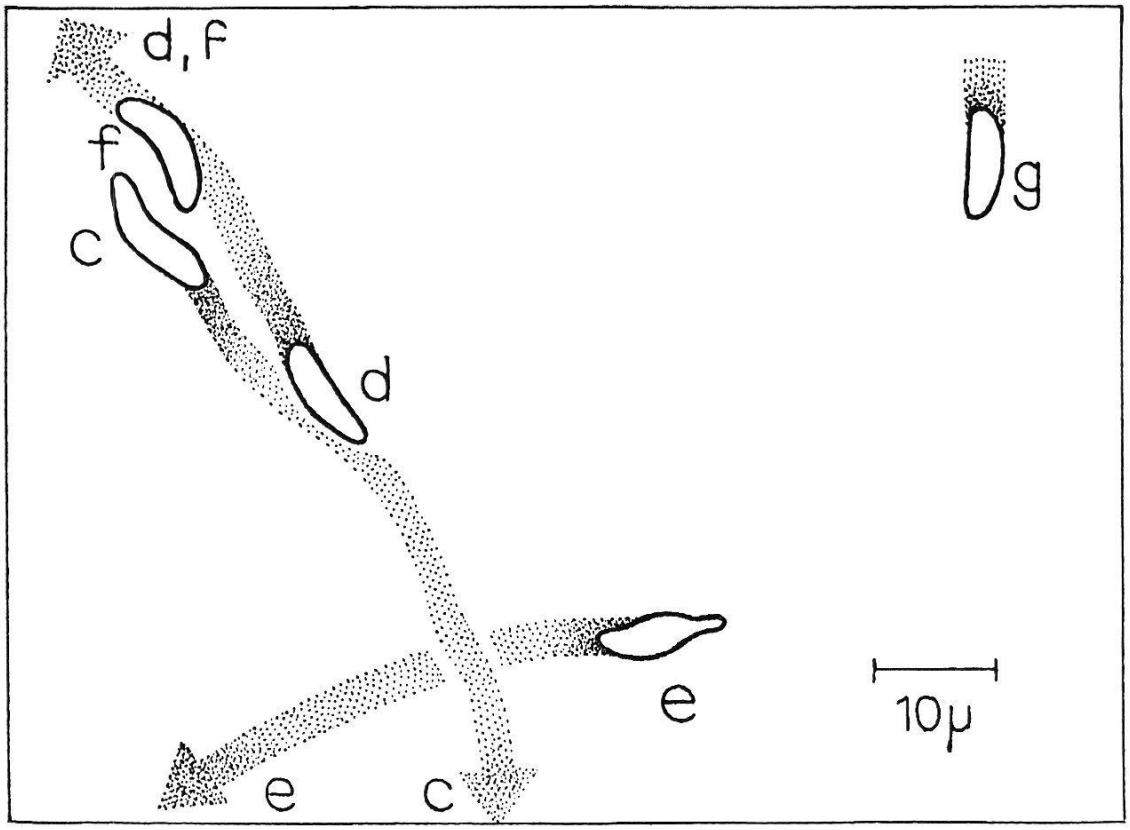

Fig. 8. P. cynomolgi bastianellii. Five ookinetes moving simultaneously in three different directions (29 h after a blood meal).

terior end to a surface and even to resist a certain current for a time. With the free part of its body it likewise makes irregular movements to the sides and upwards (cf. Fig. 1a).

Locomotion. Fig. 8 is based on a film sequence in which 5 ookinetes were observed simultaneously. Four of them (c, d, e, and f) are actively moving out of the field of vision in various directions, while the fifth (g) moves only a very short distance and then disintegrates. In the field of vision the ookinetes covered the following distances: c 60 μ in 8 minutes (7.5 μ/min), d 47 μ in a little over 9 minutes (5.2 μ/min), e 54 μ in a little less than 3 minutes (18 μ/min), and f 21 μ in $3^1/_2$ minutes (6 μ/min). If the various pictures of a single ookinete are put together, as was done for c, d, and e in Fig. 9, the most striking feature in comparison with *P. berghei* and, particularly, with the *Haemamoeba* species is the straightness of the path travelled. If such a composite drawing is based solely on one type of ookinete shape (e.g. d_4 in Fig. 6), the shape selected appears at regular intervals (Fig. 9c′). The film frames in which ookinete c, d, or e was the same in shape or resembled most closely the type d_3 or d_4 in Fig. 6 have been evaluated in Table 1. As the individual frames were taken at intervals of 6.6″, the time that elapsed can be calculated from the number of frames in between. The distance travelled can then be measured

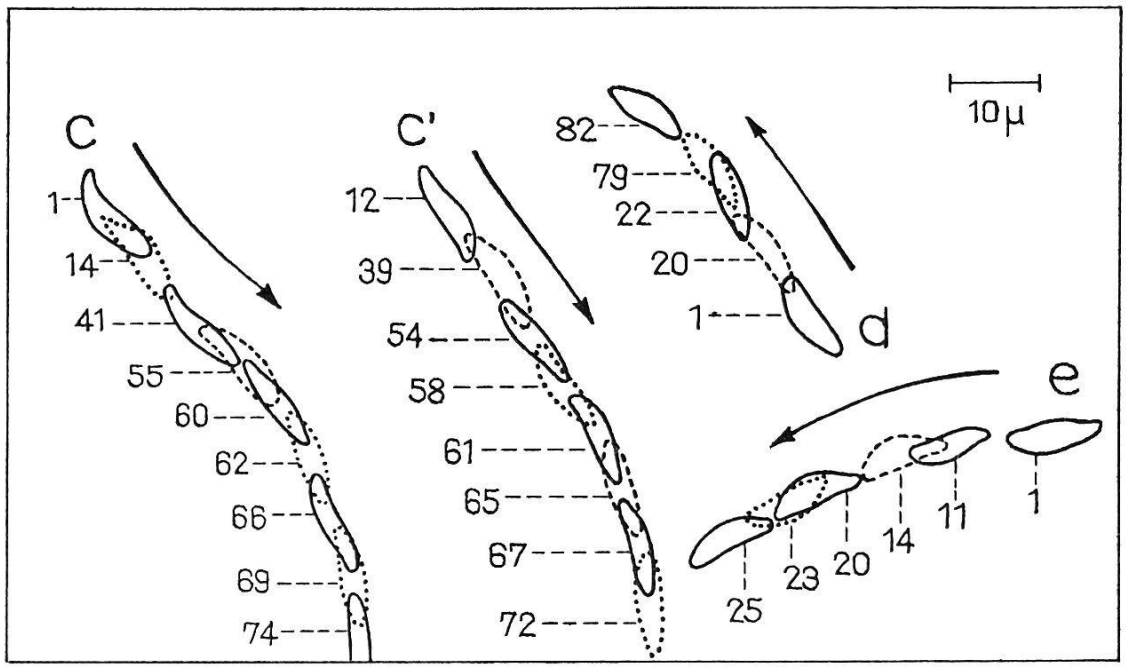

Fig. 9. P. cynomolgi bastianellii. The locomotion of ookinetes c, d, and e from Fig. 8.

TABLE 1

P. cynomolgi bastianellii
Time taken and distance travelled by ookinetes rotating 180° about their axis of locomotion

Ookinete c			Ookinete d			Ookinete e		
Frame No.	Time	Distance	Frame No.	Time	Distance	Frame No.	Time	Distance
12			12			6		
	3′	10.3 μ		53″	8.4 μ		33″	13.1 μ
39			20			11		
	1½′	7.5 μ		13″	6.5 μ		13″	2.8 μ
54			22			13		
	26″	5.6 μ		6¼′	5.6 μ		33″	9.3 μ
58			79			18		
	20″	7.5 μ		10″	5.1 μ		33″	9.3 μ
61			80–81			23		
	26″	7.0 μ		10″	5.1 μ		27″	10.3 μ
65			82			27		
	13″	6.5 μ						
67								
	26″	7.0 μ						
72								
Mean values		7.3 μ			6.1 μ			9.0 μ

in a corresponding composite drawing (Fig. 9c′). It was found that the time lapses between the appearance of the shape types d_3 and d_4 varied greatly (from 10″ to over 6′). The reason for this is that when travelling 'long' distances the ookinetes repeatedly stop and remain motionless for a while. The distances covered, however, are much more uniform; they averaged 7.3 μ for c, 6.1 μ for d, and 9.0 μ for e, making an overall average of about 7.5 μ. Any irregularities including especially those affecting ookinete e, might be due to currents in the preparation, vibration of the photographic equipment, uneven density of the medium, or other disturbances. The 'maximum speed' of an ookinete can also be deduced from Table 1: for ookinetes c and d this speed was 6.5 μ in 13″ or approx. 5.1 μ in 10 seconds, which is equivalent to 0.5 μ/sec (30 μ/min).

In the composite drawings (e.g. Fig. 9c′) the types d_1, d_2, and d_3 are missing between the shape types d_4, into which they should be inserted alternately. It should be pointed out that d_1 and d_2 are mirror-images of each other (cf. Fig. 6). There are two possible reasons for this striking change in the contours of the ookinete: an actual modification in shape or else rotation about a longitudinal axis. The fact that the width varies between 2 and 4 μ, the straight path of travel, the regular replacement of one type of shape by another of the same kind, and above all the mirror-image similarity of types d_1 and d_2 suggest that no essential change in shape takes place but that a rotational movement occurs. This rotation might be continuous in a single direction or it might consist of pendular movements to either side. In the case of a pendular movement, only three types of shape would be conceivable. On the other hand, if the above-mentioned model of an ookinete is continuously rotated about its axis of locomotion and if the resultant contours—roughly four in number—are compared, they will be found to tally well with the single frames of the film. In addition, the film gives a definite impression of a continuous, counterclockwise rotation. Hence, the locomotion of *P. cynomolgi bastianellii* would seem to be based essentially on a straight, counterclockwise spiral movement with a half-convolution length of 7.5 μ.

B. Migration into the gut epithelium

Depending on the species of mosquito serving as host to the plasmodium, an ookinete has to overcome either one or two mechanical obstacles on its journey to the underside of the gut epithelium's basement membrane. These two obstacles are, firstly, the steadily hardening peritrophic membrane (6, 16) and, secondly,

the single-layered gut epithelium. The observations reported below relate solely to *P.(H.) gallinaceum* in *Aedes aegypti,* in which the passage through the peritrophic membrane is particularly clearly visible. It is far more difficult to follow the entry of an ookinete in the gut epithelium, but this was nevertheless successfully filmed on one occasion.

The passage through the peritrophic membrane can be studied *in vitro* on a membrane laid flat and cleaned of blood remnants or on an intact membrane enveloping the entire blood coagulum. An example of the first alternative is illustrated in Fig. 10 in which an ookinete is forcing its way through a fold in the membrane. In doing so, it appears to employ the method of locomotion by means of annular waves of contraction described above. The time that elapsed between frame 1 and frame 49—i.e. from the beginning to the end of the passage through the membrane fold—was 12 minutes; the distance covered by the ookinete in this time was 24 μ (2 μ/min). (In the case illustrated, the ookinete is dragging with it some indeterminate object which is brushed off during the passage through the membrane. As this is the only observation of this type that we made, it should probably be regarded as simply acciden-

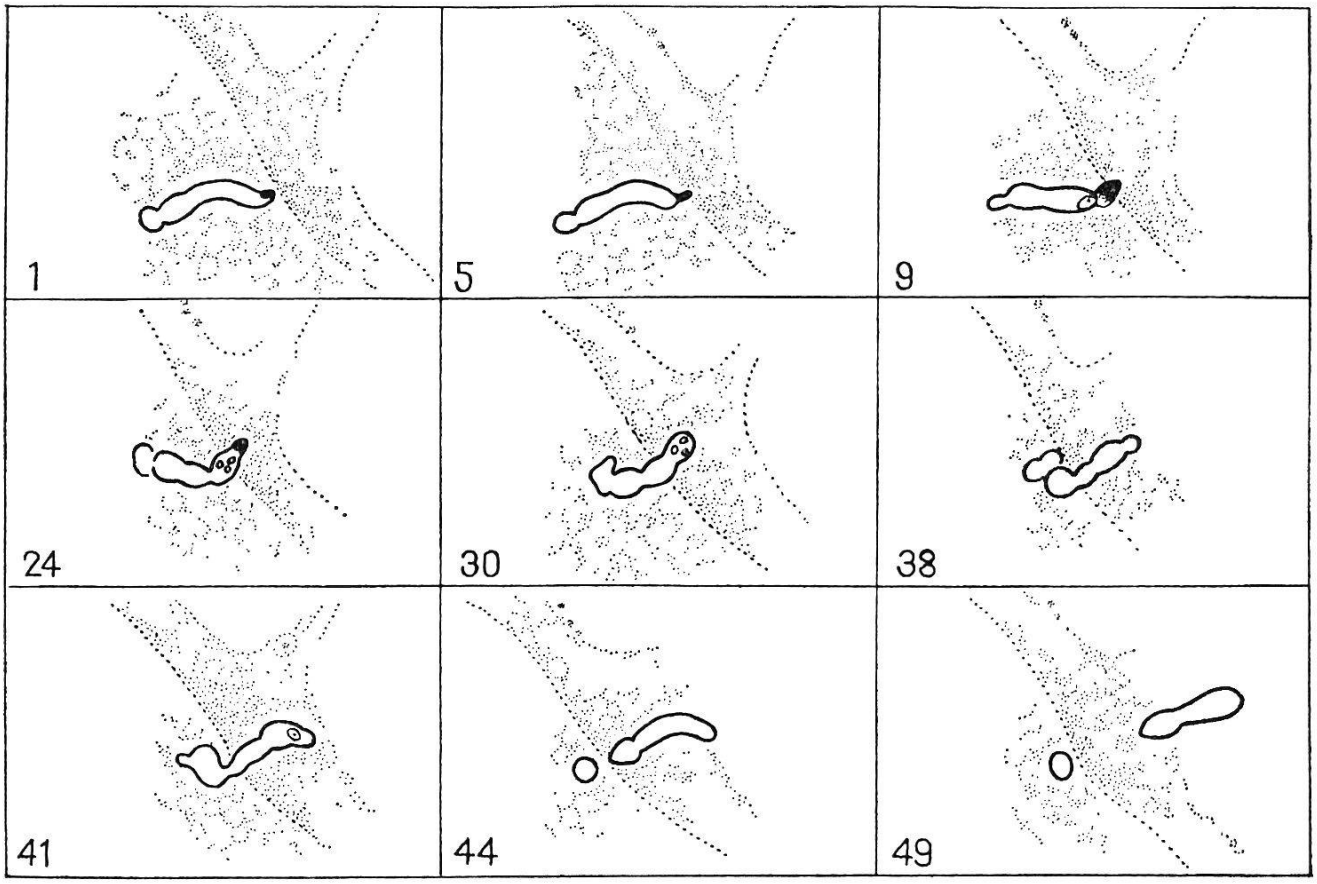

Fig. 10. P.(H.) gallinaceum. Ookinete passing through a peritrophic membrane spread out flat *(Aedes aegypti)* (32 h after a blood meal).

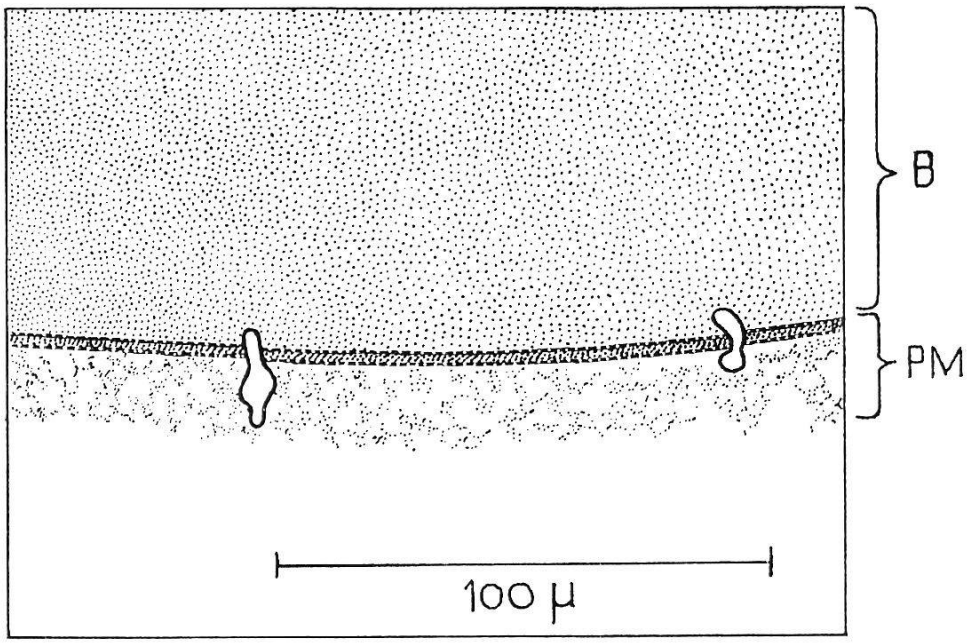

Fig. 11. P. (H.) gallinaceum. Change in shape occurring in two ookinetes when passing through the PM = peritrophic membrane *(Aedes aegypti)* round the B = blood coagulum ($27^1/_2$ h after blood meal).

tal.) The same result was obtained with the aid of a low-magnification film of an entire blood coagulum surrounded by an intact membrane. Fig. 11 shows two ookinetes on the point of passing through the membrane. Their contours correspond, on the one hand, to those shown in our Fig. 10 and, on the other, to those illustrated in STOHLER's section pictures (Ill. 10 and 11). Another sequence was filmed under higher magnification (Fig. 12). Owing to the comparatively poor depth definition of the lens no details of shape or movement can be seen here, but the chronological course of the passage through the membrane can be established to some extent. At the time X three ookinetes are lying just inside the membrane. 17 minutes later (X + 17′) the ookinete m has its posterior portion adhering to the outside of the membrane and is performing swivelling movements *in situ* with the free part of its body. Ookinete l is still in the membrane. Two minutes later (X + 19′) ookinete l, too, is adhering to the outside of the membrane. Half an hour after the commencement of the film (X + 29′) the ookinetes l and m have detached themselves from the outside of the membrane and are drifting away in the liquid medium. Ookinete k seems to be incapable of passing through the membrane and to be dying just inside the latter. Our pictures do not show how long the ookinetes k, l, and m had already been lying against the inside of the membrane when the film was started; it is therefore not possible to give any exact details about the duration of the entire process of penetration.

If these and numerous other microscopic observations are examined together, it appears that the ookinetes are temporarily held up by the peritrophic membrane on their journey to the outside of the gut. They travel a short distance along the peritrophic membrane or else lie right up against it for some time until finally they work their way through comparatively rapidly with the aid of their annular waves of contraction. Once they reach the outside, their posterior end remains fixed to the membrane while they perform 'searching' movements with the free part of their bodies. Under natural conditions these movements will very quickly bring them into contact with the gut epithelium. For the individual ookinete the whole process probably takes not more than 30 minutes.

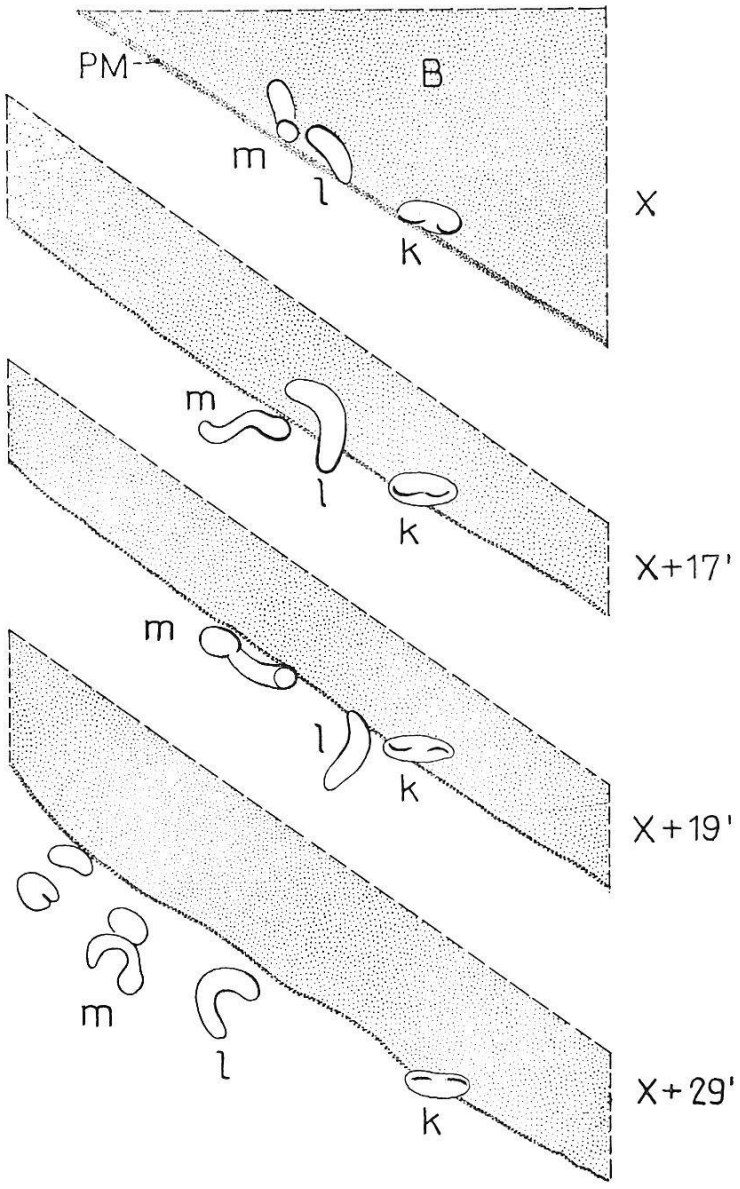

Fig. 12. P. (H.) gallinaceum. Ookinetes passing through the intact PM = peritrophic membrane *(Aedes aegypti)* (32½ h after a B = blood meal).

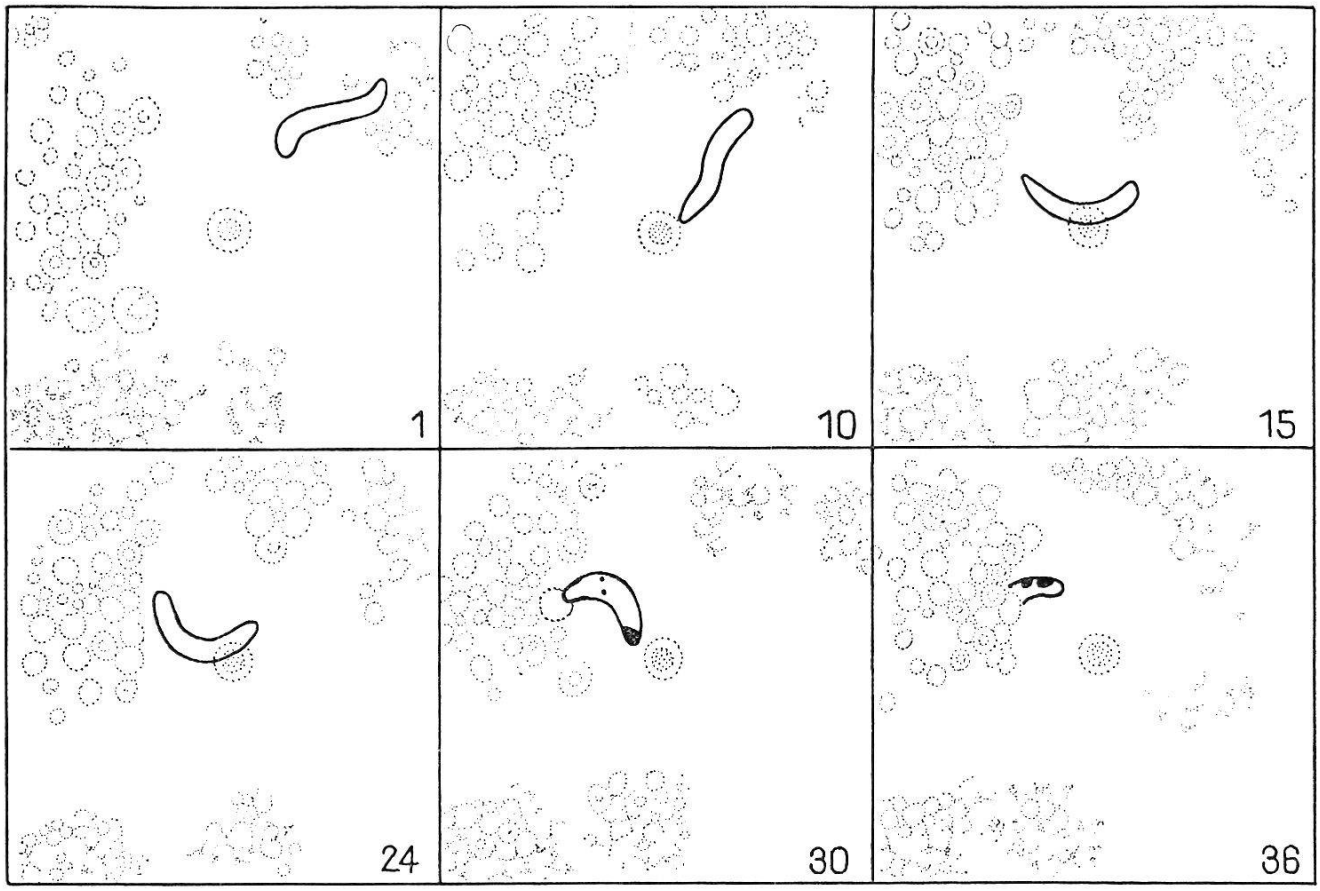

Fig. 13. P. (H.) gallinaceum. Ookinetes wandering over the brush border and penetrating into the gut epithelium *(Aedes aegypti)* (30 h after a blood meal).

The gut epithelium might conceivably exert a chemical attraction on the ookinetes. For this reason, a portion of gut wall was repeatedly placed in the immediate vicinity of the intact blood coagulum, from which the ookinetes were just emerging through the peritrophic membrane. The result was invariably negative. The ookinetes could only be brought on to the gut wall by laying a peritrophic membrane 'charged' with ookinetes directly on the wall. From this finding we would conclude that the ookinetes pass from the peritrophic membrane to the gut epithelium only when they come into direct contact with the latter.

Entry into the gut epithelium, successfully filmed on one occasion, is illustrated in Fig. 13. To begin with, the ookinete wandered about on the inside of the flattened epithelium, over a 'carpet' of microvilli, employing a snake-like, gliding form of locomotion. In the example illustrated it covered 42 μ during the observation period of 8 minutes (5 μ/min)—measured in a bee-line, 34 μ (4 μ/min). It unexpectedly disappeared, anterior pole foremost, from the plane of the picture within one minute. A subsequent check in this and a few similar cases showed that after their travels over the microvilli the ookinetes can in fact be found

inside the epithelium. This would seem to prove that the 'travelling vermicules' actively penetrate the epithelial cell layer.

The subsequent journey of the ookinetes to beneath the basement membrane could not be followed with the methods used.

Discussion

A. Morphology, movement in situ and locomotion of the ookinetes

Plasmodium (Haemamoeba) gallinaceum

GARNHAM et al. (8) describe the mature ookinete, 18 hours after the mosquito has ingested an infective blood meal, as a longitudinal body measuring some 15 μ in length. These authors' electron-microscope section pictures (Figs 6, 13 and 14) show that the cross-section of the ookinete must be more or less round. They also mention that the ookinete has a mouth-like slit at its anterior pole, as well as at least 55 peripheral longitudinal fibrils, which they interpret as being organelles connected with movement.

Our own findings, obtained in living ookinetes (cf. p. 202) are in good agreement with those of GARNHAM et al., although the specimens we observed were as a rule a little older. In addition, the film shots demonstrate the ability of the ookinete to attach itself by means of its posterior pole, to carry out active movements *in situ*, and to move from one place to another. The investigations of GARNHAM et al. do not provide any evidence to show that the posterior pole of the ookinete cell possesses special attachment organelles. The ookinete's ability to attach itself must therefore probably be ascribed to the cell surface and is perhaps not confined solely to the posterior pole. The ookinetes are in fact also able to position themselves lengthwise against, for instance, the peritrophic membrane; in two film shots (not previously mentioned) it even appeared that the ookinetes might be able to attach themselves briefly to a surface with their anterior poles. Nevertheless, the approximately 50 film sequences taken clearly show the polarity of the ookinetes; no examples of any 'backward' sliding ookinetes were ever seen.

Insofar as movement *in situ* and locomotion are due to curving of the body of the cell, they may well be accounted for by contractions of the longitudinal fibrils demonstrated by GARNHAM et al. This curvature alone, however, is not sufficient to produce locomotion. It must be accompanied by a sliding mechanism, as

postulated by JAHN (13, 14) for adult gregarines and other micro-organisms, but about which nothing further is yet known. The gliding of an ookinete over a surface might be connected with rotation about its longitudinal axis, but film sequences do not furnish any evidence for this, so that it is no doubt correct to assume the existence of a simple gliding motion without rotation.

Nor can the locomotion observed be accounted for at the moment by annular waves of contraction. GARNHAM et al. did not record the presence of any annular or transverse fibrils, or of any other suitable organelles. Perhaps a parallel for this form of motion, too, is to be found in monocystid gregarines, as would appear from GRASSÉ's (9) review (Fig. 471 A).

Plasmodium (Haemamoeba) matutinum

Since the ookinetes may undergo minor changes in shape, our observations are too scanty for us to pinpoint the insignificant morphological differences between this plasmodium and *P.(H.) gallinaceum*. All the indications are that *P.(H.) matutinum* closely resembles *P.(H.) gallinaceum* in respect of shape and mode of locomotion.

Plasmodium berghei

So far as we are aware, the only exact indication of the length of this ookinete is to be found in GARNHAM's paper (7). This author states that 18 hours after ingestion of the blood meal, the ookinetes were 10-12 μ long. A drawing, with scale, published in an earlier paper by other authors (12) indicates that the size of the ookinetes may vary between 7 and 18 μ. Our own measurements (8.5 μ) suggest that the ookinetes of *P. berghei* tend to be smaller than those of the other species. As regards their shape, our findings tally for the most part with those of a number of authors (12, 19, 20, 21): as a rule the ookinetes—in the live state, too—are curved in the form of a sickle or banana; the anterior pole is broader than the posterior pole and exhibits in some cases a 'pole cap' (17, 19, 20). In place of this 'pole cap' JADIN et al. (12) drew and GARNHAM (7) described a 4 μ long filament which they had seen in some cases. This filament, however, could not be found in any of our film sequences.

GARNHAM refers to this filament in connection with the ookinetes' locomotion. VANDERBERG & YOELI (18) state that the ookinetes attach themselves to the inside of the gut wall and pass actively through the latter; these authors, however, do not give any

further details about the mode of locomotion and the way in which the ookinetes pass through the gut wall. Our own observations relate solely to locomotion on a spread-out peritrophic membrane *(Anopheles stephensi)*. They confirm the locomotory ability of the ookinetes and show that this locomotion, in contrast to that of the *Haemamoebae* investigated, tends to take place in a straight line, although it is not clear whether it involves a snake-like wriggling of the body, as in the *Haemamoebae,* or a spiral movement, as in *P. cynomolgi.* Since the ookinetes of *P. berghei* bear a certain morphological resemblance to those of *P. cynomolgi,* we are inclined to consider a spiral movement to be more likely.

Plasmodium cynomolgi bastianellii

Little information about the ookinetes of this plasmodium is to be found in the literature. SHORTT (15) merely hints at their morphology in a schematic illustration of the life cycle of *P. cynomolgi;* in his drawings, he depicts one end of the ookinete as being markedly pointed and the other as rather blunt. GARNHAM et al. (8) describe informative electron-microscope sections of this species as well. A particularly striking feature in their Figs 3, 4, and 5 is the concave pattern of the contour lines on one side. This cannot be explained by the fact that the cells were sectioned tangentially. However, if these section pictures are viewed in conjunction with our own model (Fig. 7)—i.e. if one side of the rhomboid is imagined as being more flattened than the other—and if one imagines cross-sections through the cell at varying distances from the anterior pole, the pictures published by GARNHAM et al. correspond to sections that could have been made through our model. The fact that GARNHAM's Figs 17 and 18 show ovoid section pictures instead of ones that are concave on one side is immaterial; these pictures probably come from zones near the posterior or anterior pole, which also appear approximately round in our model.

In a manner similar to that observed in the case of *P. (H.) gallinaceum,* the ookinete of *P. cynomolgi bastianellii* is able to anchor itself to a surface with its posterior end (its 'blunt tail'); it is also capable of movement *in situ* and of locomotion. In contrast to the ookinetes of *P. (H.) gallinaceum,* however, those of *P. cynomolgi bastianellii* display a spiral-type motion which corresponds well to their asymmetrical shape. Here, again, the 65 longitudinal fibrils demonstrated by GARNHAM et al. do not suffice to explain the mode of locomotion; an additional mechanism, as postulated by JAHN (13), must be required, and the most likely one is the

'helical waves' mechanism which is said to apply also to sporozoan sporozoites and merozoites.

On comparing ookinetes of *P.(H.)gallinaceum* with those of *P. cynomolgi bastianellii,* GARNHAM et al. (8) found a marked similarity in the fine structure of the two cells. The pronounced —and hitherto barely noticed—differences in external configuration and mode of locomotion thus appear all the more striking. It is conceivable that these differences might serve as additional means of distinguishing between the subgenera *Plasmodium* and *Haemamoeba;* the findings obtained in *P.(H.) matutinum* and *P. berghei*—incomplete though they are—appear to support this view. In future perhaps studies on the origin of recent species (e.g. l) should also take account of details of the behaviour of the plasmodial forms in the vector.

B. The migration of the ookinetes in the vector

It has proved possible to observe and film the various phases in the migration of the ookinetes of *Plasmodium (Haemamoeba) gallinaceum* up until the moment they enter the gut epithelium. HOWARD (11) worked with the same species of *Plasmodium.* His assumption that the ookinetes are non-motile is thus refuted and his hypothesis that the ookinetes are passively absorbed through the epithelium during the latter's process of reorganisation in the course of blood digestion has in our opinion become untenable. Now that three additional species of *Plasmodium* have also been shown to be capable of locomotion, and in view of the findings of other authors (8, 18), it is safe to assume that plasmodial ookinetes in general actively penetrate the mosquito's gut epithelium, and, where present, the peritrophic membrane as well.

We have carried out too few measurements of the ookinetes' speed of travel to be able to reach any definite conclusions on this point. Nevertheless, it is worth noting that the speeds ascertained in all four species investigated were of the same order of magnitude. It is important in this connection to draw a distinction between the maximum speed (30 μ/min) and the average speed, because it was found that the ookinetes travel at varying rates and often also remain motionless for a time. In addition, we do not know whether or in what way the speed of travel was influenced by our experimental conditions. If, despite this objection, the average speed is taken to be 1 μ/min and assuming that an ookinete arises in the centre of the blood coagulum and has to cover a distance of 250-1,000 μ between its site of origin and the nearest point on the epithelium, it would take between 4 and 17 hours to

make the journey. This value is theoretical, but nevertheless fits in well with existing ideas on the subject since the majority of ookinetes must have reached the basement membrane 24-30 hours after ingestion of an infective blood meal by the mosquito.

On the basis of sections, GARNHAM et al. (8) and STOHLER (16) point out that the ookinetes, before passing through the peritrophic membrane or gut epithelium, position themselves parallel to the obstacle. The film shows that in reality the ookinetes by no means simply lie quietly up against the membrane or gut wall, but tend rather to wander along it. As the peritrophic membrane appears to be of fairly irregular structure (6), it is not inconceivable that the ookinetes may move along it in search of a thinner point through which they can pass more easily. This hypothesis would not exclude the possibility that they also secrete enzyme-like substances from their 'mouth slit'. It is impossible to say at the moment to what extent passage of the ookinetes through the gut wall is subject to similar conditions.

Apart from this tendency for the ookinetes to wander along obstacles and thus to deviate from their original direction of travel towards the outside of the gut, the considerations mentioned above on the subject of the time of travel seem nevertheless to indicate that while the distance covered may not be the mathematically shortest distance possible, it cannot involve very extensive deviations. This would suggest that the ookinete possesses an orientation mechanism which enables it to keep moving in the right direction. The simple attraction experiments carried out with a piece of gut wall (cf. p. 215) yielded negative results, which tallies with the finding that the ookinetes also left the blood coagulum when the gut wall was removed. It thus appears unlikely that the gut epithelium exerts a positive chemotactic effect on the ookinetes.

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Zusammenfassung

1. Mit Hilfe eines Zeitrafferfilms wurden die Ookineten von *Plasmodium (Haemamoeba) gallinaceum, P. (H.) matutinum, P. berghei* und *P. cynomolgi bastianellii* auf ihre Gestalt, Beweglichkeit und Lokomotionsweise untersucht.

2. Die Ookineten aller vier genannten Plasmodien-Arten sind beweglich und der aktiven Fortbewegung fähig.

3. Bei *P. (H). gallinaceum* erfolgt die Lokomotion entweder mit gleitend-schlängelnder Bewegung oder mit Hilfe ringförmiger, von vorn nach hinten ziehender Kontraktionswellen. Die Gestalt ist länglich und im Querschnitt rund.

4. Die Gestalt von *P. cynomolgi bastianellii* ist einseitig abgeflacht und der Länge nach zu einer leichten Spirale verdreht. Die Lokomotion erfolgt in einer gegen den Uhrzeigersinn laufenden Schraubenbewegung.

5. Für *P. (H.) gallinaceum* wird der Durchtritt der Ookineten durch die peritrophische Membran von *Aedes aegypti* und der Eintritt in das Mitteldarmepithel auf Grund weiterer Filmaufnahmen beschrieben.

6. In der Diskussion werden die Ergebnisse im Lichte der neueren Literatur besprochen. Die herkömmliche Auffassung der aktiven Wanderung der Ookineten im Mitteldarm der übertragenden Stechmücken wird bestätigt.

Résumé

1o A l'aide de l'accéléré, on a observé la forme, les mouvements et la locomotion des oocinètes de *Plasmodium (Haemamoeba) gallinaceum, P. (H.) matutinum, P. berghei* and *P. cynomolgi bastianellii.*

2o Les oocinètes de ces 4 espèces de plasmodes sont mobiles.

3o La locomotion de *P. (H.) gallinaceum* se fait soit par des mouvements rampants et glissants, soit par vagues de contractions circulaires du corps se suivant d'avant en arrière. La forme de l'oocinète est allongée et sa coupe transversale est ronde.

4o *P. cynomolgi bastianellii* est de forme aplatie d'un côté et légèrement spiralisée sur toute sa longueur. La locomotion s'effectue par un mouvement en spiral dans le sens opposé à celui des aiguilles d'une montre.

5o Le passage de *P. (H.) gallinaceum* à travers la membrane péritrophique d'*Aedes aegypti* et l'entrée dans l'épithélium intestinal est décrit à l'aide d'autres bandes filmées.

6o Les résultats sont discutés à la lumière de la littérature récente. L'opinion habituelle qui veut que les oocinètes pénètrent de manière active à travers l'intestin du moustique transmetteur est confirmée.