Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 26 (1969)
Heft: 1

Artikel: Esterases and acid phosphatases in sporogonic stages of plasmodia
Autor: Freyvogel, Thierry A. / Hunter, Robert L.
DOI: https://doi.org/10.5169/seals-311608

Swiss Tropical Institute, Basel, Switzerland, and Department of Human Anatomy, School of Medicine, University of California, Davis, California, USA

Esterases and Acid Phosphatases in Sporogonic Stages of Plasmodia

THIERRY A. FREYVOGEL and ROBERT L. HUNTER

GARNHAM, in his classic book on "Malaria Parasites" (6), has reviewed the subject of cytochemistry of Plasmodia. Most of the work carried out so far was done with *P. (H.) gallinaceum*, *P. cynomolgi*, *P. knowlesi* and *Hepatocystis kochi*. All these species are said to react with considerable uniformity. In the ookinetes, DNA and RNA were shown to occur. The tests for alkaline phosphatase yielded negative results. In the oocysts, DNA is present in quantities increasing with development. Lipids and alkaline phosphatase were found as well. The occurrence of mucopolysaccharides is probable. In sporozoites the Feulgen reaction is strong. More recently, VANDERBERG *et al.* (11) have examined oocysts and developing sporozoites of *P. berghei* for DNA and RNA. They showed DNA to be present in the nuclei moving into the budding sporozoites, and RNA to occur in large quantities in the sporoblastoid body.

Following our preceding investigation on mosquitoes (5), in the present study we undertook to examine ookinetes, oocysts and sporozoites of various *Plasmodium* species for esterases. Knowing about the occurrence of phosphatases in other Protozoa (2, 3), we also included tests for acid phosphatases.

Materials and Methods

We examined *P. cynomolgi* (RO/PMR strain), *P. cynomolgi bastianellii* (E strain) and *P. fieldi* (N 3 strain) in *Anopheles freeborni*, all obtained from the National Center for Primate Biology in Davis, as well as *P. (H.) gallinaceum* (7 A strain), obtained from the National Institutes of Health, Bethesda, in *Aedes aegypti*. The infected Anophelines were kept at a temperature of about 26°C. Due to the prevailing weather conditions the infected *Aedes* were maintained, in a first experiment, at temperatures varying from 26 to 29°C, and in a second experiment, varying from 28 to 32°C.

The methods used were described in previous papers (4, 5, 10). Mainly, the whole mount technique was applied. Infected mosquitoes were briefly anaesthetised with ether and, either the midgut or the salivary glands dissected in insect Ringer solution (8), buffered at pH 7.0. For oocysts, the guts were fixed in cold (4°C) Ca-acetate formalin (9) for approximately 5 minutes, rinsed in deionized water for about 1 minute and immersed in the mixture of diazonium salt and substrate solution. In this solution they were left at room temperature, for 20 minutes, rinsed in water again, and mounted in glycerol gelatin. In order to obtain better contrast between the oocysts and the underlying midgut tissues some of the guts were partly lysed in water prior to formalin fixation. For ookinetes, the mosquitoes were dissected about 24 hours after the infectious blood meal. The peritrophic membrane and the ookinetes adhering to it were attached to the slide and allowed to dry in the refrigerator. In some instances, cold acetone fixation was then applied, which, however, showed to have no

detectable effect on the subsequent enzyme reaction. After this reaction, the preparations were covered with glycerol gelatin and a cover slip. For immature sporozoites, growing oocysts, and for mature sporozoites, salivary glands were squashed under a cover slip, the cover slip removed and the preparation dried in the refrigerator. It was then treated as ookinete preparations.

In an attempt to separate the esterase isozymes of oocysts from the isozymes of the mosquito's midgut, disc-electrophoresis was applied. Polyacrylamide gels of 5 mm diameter were used. The samples consisted of 20 microliters aqueous homogenates, containing either 20 or 45 midguts each. For control, non-infected midguts were run concurrently with the infected ones.

A few sections of oocyst bearing stomachs were prepared with the use of the paraffin embedding technique, utilizing vacuum and as brief an exposure as possible to the heated paraffin (56°C), not exceeding 15 minutes.

In all experiments, "Blue RR" was the diazonium salt used. The substrates were α-naphthyl acetate, naphthol-AS-D acetate, naphthol-AS acetate for esterases, and sodium α-naphthyl phosphate in 0.1 M sodium acetate acetic acid, pH 5.17, for acid phosphatase. For control, preparations were made with diazonium salt solution, without substrate.

Results

The results obtained in our experiments are similar for all *Plasmodium* species examined. They will, therefore, be presented together.

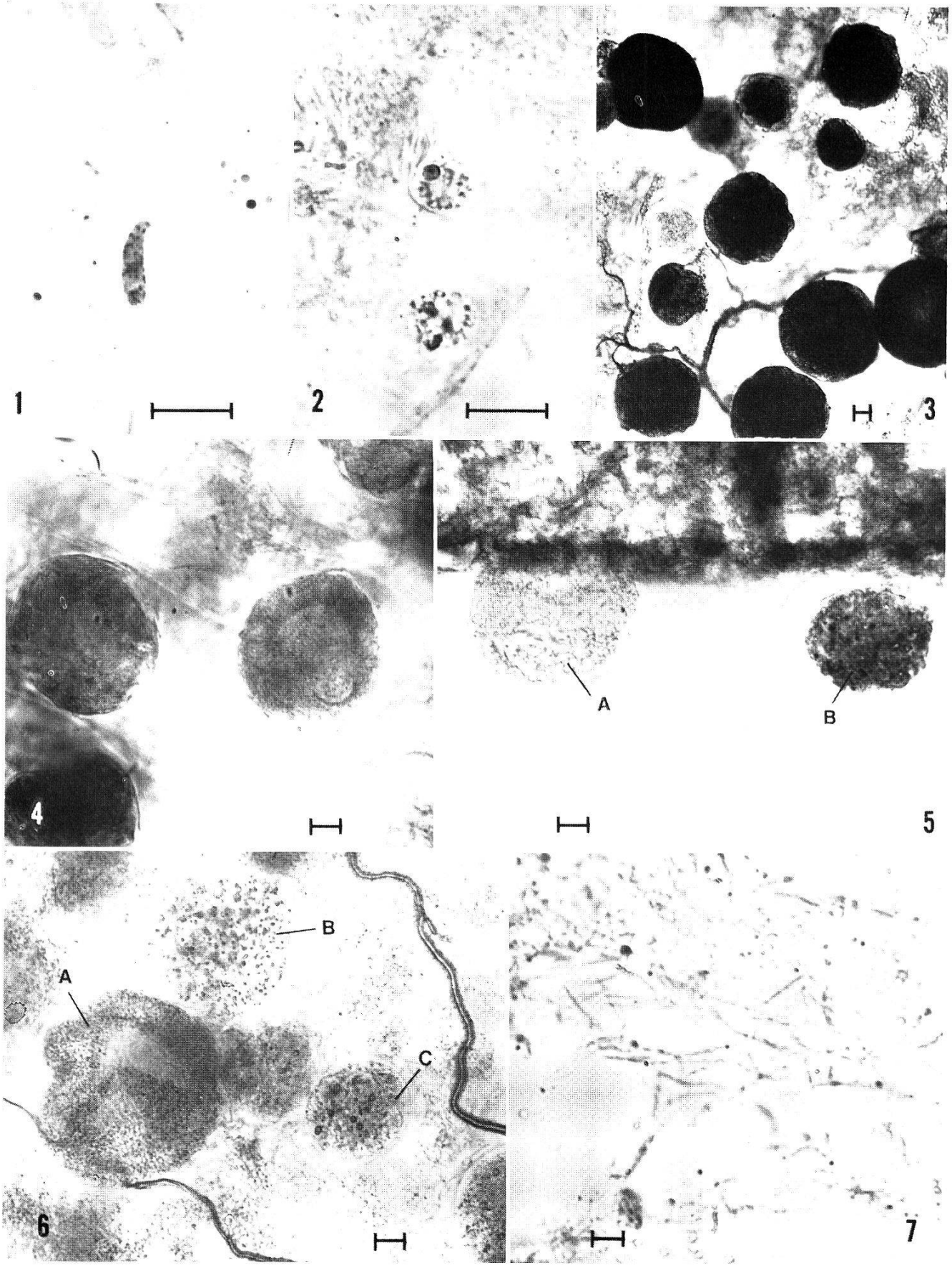
Ookinetes

Upon treatment with non-substrate solution, the ookinetes of *P. cynomolgi*, *P. cynomolgi bastianellii* and *P. fieldi* exhibit a fine brown granulation, distributed all over the organism (Fig. 1). Near the anterior pole these granules are found in greater density. Finer granules, of light greyish color, can sometimes be observed all along the periphery. The same pattern of granulation may be seen in unstained ookinetes, fixed only with formaline, although it is much less conspicuous than after treatment with diazonium salt solution. No difference can be found between preparations "stained" with non-substrate solution and preparations subjected to either one of the substrates applied in this investigation.

The ookinetes of *P. gallinaceum* show the same type of reaction. However, the color of the granules is deep black, which makes them very conspicuous (Fig. 8).

Oocysts

Non-substrate solution control. With non-substrate solution the oocysts of all species examined stain light yellowish as do the underlying midgut tissues. There is no positive reaction in the sense of a conspicuous coloration as is found in ookinetes.



Figs. 1–7. Sporogonic stages of *P. fieldi.* Scale 10 μ. *1:* Ookinete, diazonium salt, without substrate. *2:* Oocysts, 3rd day, α-naphthyl phosphate. *3:* Oocysts, 8th day, α-naphthyl acetate. *4:* Oocysts, 8th day, naphthol-AS-D acetate. *5:* Oocysts, 10th day, naphthol-AS-D acetate. *6:* Oocysts, 8th day, α-naphthyl phosphate. *7:* Sporozoites, from mature oocysts, α-naphthyl phosphate.

Pigment can be seen in the earlier stages of oocyst development only, i.e. from the third or fourth day after the infectious blood meal – the earliest oocyst stages detected in the course of this investigation – till the sixth or seventh day. From this day until maturation of the oocysts, on the tenth or eleventh day, no pigment can be observed, except in small oocysts, the growth of which apparently is either interrupted or delayed. The pigment bodies appear centrally located in the early stages of oocyst development, while, later on, they seem scattered throughout the cytoplasm of the oocysts.

Alpha-naphthyl acetate substrate. With this substrate, in all *Plasmodium* species examined, the oocysts assume a dark brown, almost black coloration (Fig. 3). This coloration is observed in the earliest stages which were detected. No further details can be seen in oocysts located on guts with intact epithelium, which itself reacts to the substrate. Where the guts were partly lysed in order to permit even more critical examination of the attached oocysts, two types of brown or black granulations become apparent, a very fine one, quite dense and apparently evenly distributed throughout the oocysts, and a sparser one, consisting of somewhat larger, spherical bodies. In some preparations, in more advanced oocysts, it appears as if the color was most dense in the sporoblastoid body. Once an oocyst is ruptured its wall remains attached to the stomach's surface. The wall shows only weak or no reaction to the substrate.

Disc-electrophoresis was performed with homogenates of midguts from *Anopheles freeborni*, infected with an average of approximately 30 oocysts of *P. cynomolgi bastianellii*, 10 days after the infectious blood meal. Sporozoite formation was under way, but the oocysts were still immature. The gels (Fig. 12, A–D) show mainly bands from area 73–100, known to occur in the midguts of *A. freeborni* (5). In gels B and D, run with homogenates of infected guts, band 73 seems more intense than its counterpart in the controls A and C. In addition, gels B and D exhibit very faint bands in locations 43, 47 and 57, which are not seen in the controls[1].

Naphthol-AS-D and -AS acetate substrates. The majority of experiments were carried out with naphthol-AS-D acetate. As no difference was found, however, between the results with either one of these two substrates, they will be presented together.

[1] For the nomenclature of isozymes see reference (5).

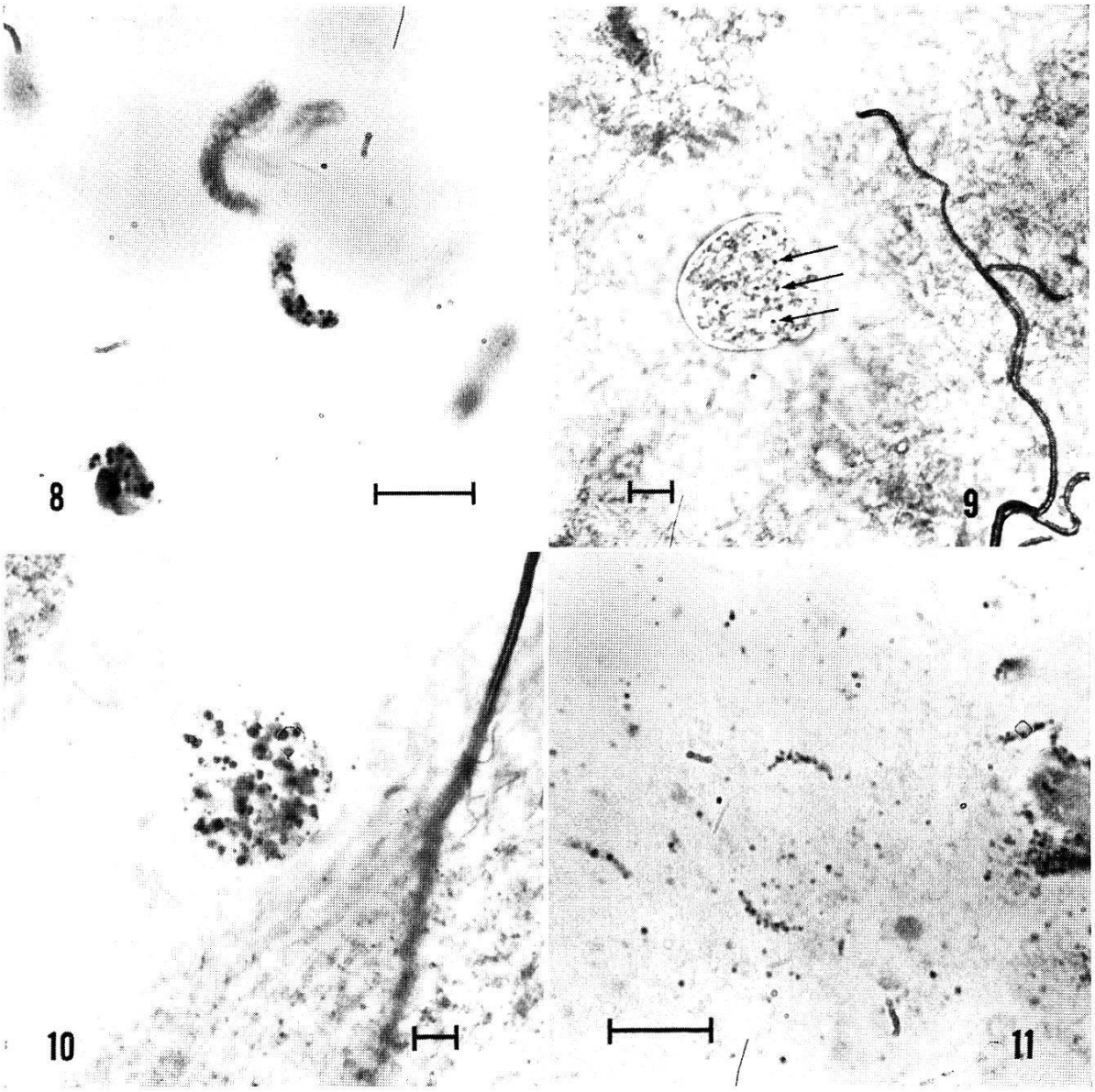


Fig. 8–11. Sporogonic stages of *P. gallinaceum*. Scale 10 μ. *8:* Ookinetes, diazonium salt, without substrate. *9:* Young oocyst. Dark dots represent sites of reaction with naphthol-AS-D acetate (arrows). *10:* Growing oocyst. α-naphthyl phosphate. *11:* Sporozoites, from salivary glands, α-naphthyl acetate.

In the mammalian *Plasmodium* species, on the fourth day after the infectious blood meal, either no or very few, little blue dots may be found in the oocysts' cytoplasm. Their number increases with development until, between the eighth and tenth day, i.e. when sporozoite formation is under way, the oocysts look entirely blue (Fig. 4). This is all the more remarkable in lysed preparations where the gut tissues remain virtually unstained, while the oocysts appear vividly blue. At this stage of development, sections reveal that the bulk of the reacting enzymes is located peripherally, between the oocyst wall and the outer layer of forming sporozoites. Upon complete maturation, however, the oocysts no longer react

with the substrate (Fig. 5 A). After rupture, the cyst wall, too, remains unstained.

Electrophoresis was carried out with the same material as mentioned above. In both gels (Fig. 12 E and F) the same seven bands are seen which are known to occur in the midgut (5). Both gels exhibit one additional band in location 57. As opposed to control gel E, gel F still displays three other bands, in locations 43, 47 and 51.

In *P. gallinaceum*, the blue dots seen in the oocysts, four days after the infectious blood meal, are more conspicuous than in the *Plasmodium* species discussed above (Fig. 9). During the oocysts' development, the dots increase in number and size and, gradually, assume a slightly irregular shape. Because of the similarity of the reaction with the substrate, at this stage, the oocysts resemble somehow small fragments of fat body, as they frequently remain attached to the gut's surface. However, the "catalysomes" (13) in the fat body appear rather star shaped and a little larger than the blue dots in the oocysts and may, therefore, accurately be distinguished. Blue speckles may still be seen in the oocysts when the sporozoite formation is well under way, but the oocysts of *P. gallinaceum* were never seen to stain entirely blue.

Alpha-naphthyl phosphate substrate. With this substrate, oocysts of *P. fieldi* may be detected already three days after the infectious blood meal. In the other three *Plasmodium* species investigated, the oocysts are seen from the fourth day on. In all species in the early stages, two types of granulation can be observed, one of fine to intermediate size and the other of rather coarse, droplet like character (Fig. 2). After some development the fine granulation gives the oocysts their golden-brown background coloration, while the coarse granula remain comparatively scarce. From the eighth and ninth day onwards, these larger granules seem to disintegrate (Fig. 6 B), until, from the tenth day on, the oocysts appear almost uniformly brown (Fig. 6 A). Thereafter, it is the sporozoites which stain brown and become visible, inside the oocysts. The wall of the oocysts remains practically colorless.

Sections of immature oocysts show the cytoplasm of the sporoblastoid to stain darker than the underlying midgut tissues. When forming sporozoites are seen, they appear light. Mature oocysts, on the contrary, display coloration in sporozoites only. Neither the residual parts of the sporoblastoid nor the cyst wall are stained.

In *P. gallinaceum*, the coarse type of granules can be seen until the sporozoites in the oocysts are well under formation. It, presumably, persists up to full maturation of the oocysts (Fig. 10).

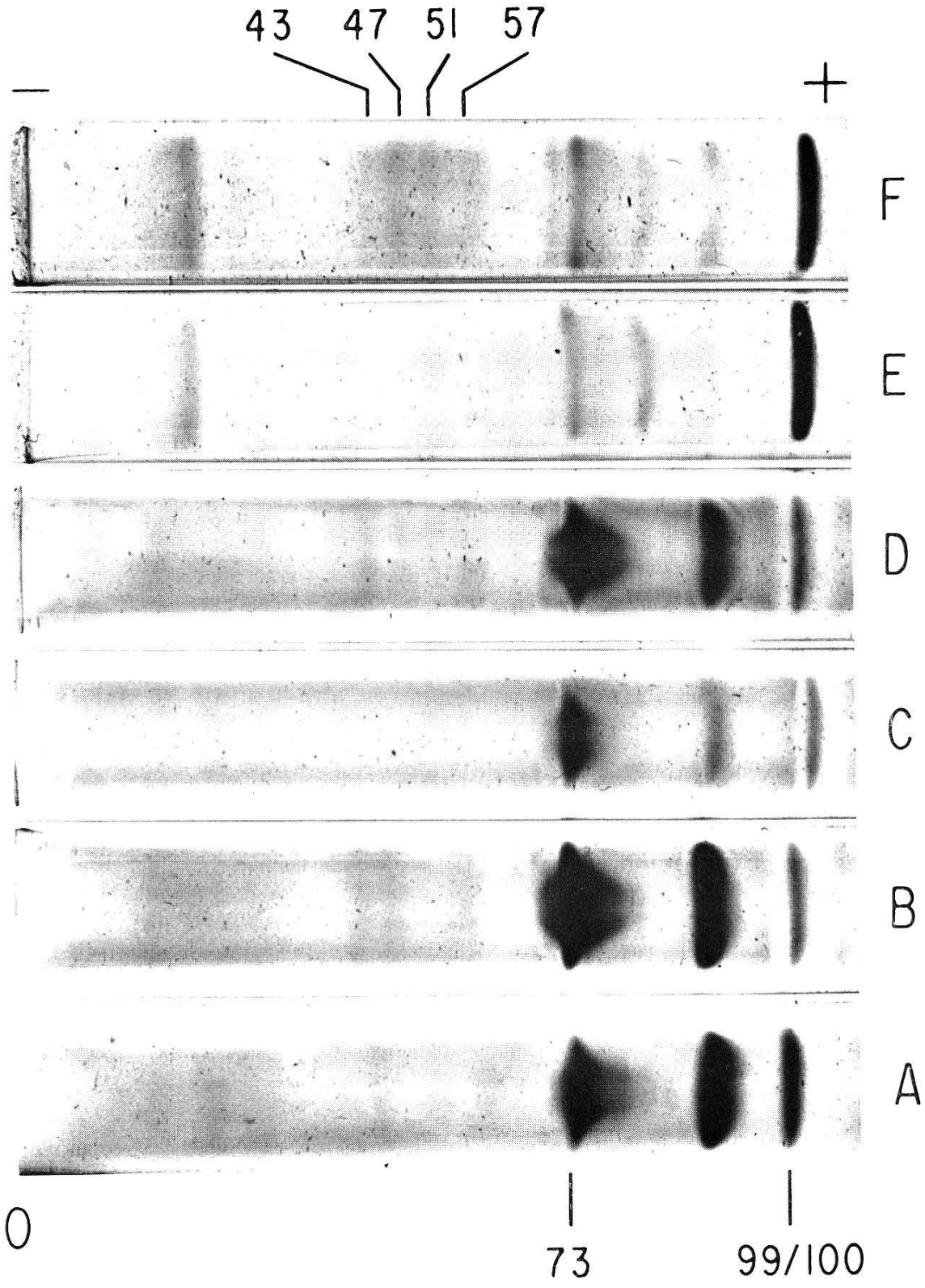


Fig. 12. Electrophoretic separation of esterase isozymes from midguts of *Anopheles freeborni* females. A, C, E: non-infected controls; B, D, F: infected with oocysts of *P. cynomolgi bastianellii*. A–D: α-naphthyl acetate; E, F: naphthol-AS-D acetate. A, B: samples containing homogenates of 45 midguts each; C–F: samples containing homogenates of 20 midguts each. For further explanation see text.

Retarded and non-viable oocysts. What had been said in the foregoing four sections refers to the fastest developing oocysts, considered to display the "normal" growth rate. Although sporogony is known to be fairly synchronous in general (6), in most instances some oocysts are found which develop more slowly or which even stop growing. In our experiments, the number of oocysts per stomach, on the average, amounted to 100 for *P. cynomolgi*, 30 for *P. cynomolgi bastianellii* and 250 for *P. fieldi*. In all three infections, there was a substantial number of oocysts, which grew either slowly or which underwent no more than the initial stages of development. Although no exact figures were taken, an estimate seems to indicate that the percentage of slow or non-viable oocysts was higher, the larger the total number of oocysts.

No difference can be seen between "normal" oocysts and either slow or non-viable ones, when treated with non-substrate control solution. Pigment, however, is usually seen in the smaller oocysts after the seventh day and remains visible there throughout the experiment. No difference is found, either, after application of α-naphthyl acetate. Irrespective of their size, most oocysts stain dark brown (Fig. 3). With naphthol-AS-D and naphthol-AS acetate, on the other hand, it becomes evident that the production of the reacting enzyme(s) does not go beyond the initial stage of the appearance of blue dots in non-viable oocysts and is significantly delayed in retarded oocysts. In these retarded oocysts, the size of the granules sometimes varies appreciably (Fig. 5 B). A difference is also found with regard to acid phosphatase. In non-viable oocysts, the granules of the coarse type appear to remain intact throughout the experiment's duration (Fig. 6 C), while, as has been stated above, they gradually disappear in oocysts developing "normally". The disintegration process is merely slowed down in retarded oocysts (Fig. 6 B).

Sporozoites

"Immature" sporozoites were obtained by squeezing nearly mature oocysts. "Mature" sporozoites were obtained from salivary glands and, occasionally, from mature oocysts. No sporozoites of *P. cynomolgi* and no immature sporozoites of *P. gallinaceum* were examined. Neither with immature nor with mature sporozoites is any reaction observed with the non-substrate control solution.

Immature sporozoites. They appear exteriorly covered with black granules when treated with α-naphthyl acetate. They appear

bluish when treated with naphthol-AS-D acetate. Possibly, this, too, is a surface bound reaction. The sporozoites may be seen staining blue, also, while they are still contained in nearly mature oocysts. When examined for acid phosphatase, the sporozoites obtained from immature oocysts remain unstained. When obtained from nearly mature oocysts, however, they display a light brown color.

Mature sporozoites. In *P. cynomolgi bastianellii* and *P. fieldi,* the sporozoites appear almost unstained when treated with α-naphthyl acetate. Some few black granules may be observed, apparently located on the outer surface of the sporozoites. The sporozoites remain similarly unstained when treated with naphthol-AS-D acetate. Exceptionally, some two to three blue dots may be found on their outside. The sporozoites do, however, react with α-naphthyl phosphate in that they show a light brown coloration and, in addition, some two to six, comparatively conspicuous brown granules. It is difficult to say whether these are located in or outside the sporozoites (Fig. 7).

As to the mature sporozoites of *P. gallinaceum,* they look densely covered with black or grey granules when subjected to α-naphthyl acetate treatment (Fig. 11). With naphthol-AS-D acetate, most sporozoites display some blue granules, apparently on the outside. Occasional sporozoites show a bluish coloration of the cytoplasm, with the exception of the zone containing the nuclear material. With α-naphthyl phosphate, most sporozoites appear almost imperceptibly stained grey. Only a few sporozoites show some brown granules on their outside.

Discussion

To our knowledge, only WARREN & WHARTON (12) reported an attempt of cyclical transmission of *P. fieldi* with *A. freeborni.* They found sporozoites produced in this mosquito species, but were unable to pass on the infection to new monkeys. Cyclical transmission has, since, been achieved with mosquitoes other than the natural vectors, at the National Center for Primate Biology in Davis, California (Dr. R. N. ROSSAN, personal communication). Thereby, *A. freeborni* and *A. stephensi* were utilized. However, as the two species were allowed to feed simultaneously on the same Rhesus monkey, it is unknown whether both species, or only one, successfully transmitted the infection, and in the latter case, which

of the two species was the efficient vector[2]. Although details were given no special consideration, it may be stated that the sporogony of *P. fieldi* very closely resembles the sporogony of *P. cynomolgi* and *P. cynomolgi bastianellii*.

With respect to the cytochemical reactivity, GARNHAM (6) states that different species are of considerable uniformity. This seems to apply equally to the results obtained in the course of this investigation. The whole mount technique, as applied by us, reveals no differences among *P. cynomolgi*, *P. cynomolgi bastianellii* and *P. fieldi*. As to *P. gallinaceum*, the work was carried out under high temperature conditions and the results must, therefore, be viewed with care. They seem to indicate minor differences between this species and the other three Plasmodia. The possible significance of these differences cannot be evaluated at the present stage of our understanding of the functional role of esterases and acid phosphatases. At any rate, it is interesting to note that in all *Plasmodium* species examined esterase(s) is present in the oocysts, which are known to contain comparatively large quantities of lipid material (6). The simultaneous presence, in the same tissues, of lipid material and esterase(s) has been reported repeatedly (5, 10, 13).

In the ookinetes, non-substrate solution causes a positive diazonium reaction. Thus, our results are inconclusive with respect to the presence of esterase(s) and acid phosphatase(s). The occurrence of a diazonium reaction, however, is interesting in itself. It could be considered to be a "false-positive" reaction caused by some artifact (1). To us, it would rather seem to be connected with the production of staining known to occur normally in enterochromaffin cells (1). In mosquitoes it was shown to obtain with material thought to be of lipoid nature (5). In ookinetes, it appears difficult to correlate the reacting granules with any of the known organelles as described by GARNHAM *et al.* (7). In *P. gallinaceum*, the distribution of the reacting granules looks quite similar to the distribution of the pigment. Pigment shows no diazonium reaction in the early stages of oocysts. However, in ookinetes, the pigment bodies were found to be contained in vacuoles limited by a membrane (7). Thus, the diazonium reaction possibly takes place within these vacuoles, with material supposedly surrounding the pigment bodies. Admittedly, the same does not seem to apply to the ookinetes of e.g. *P. cynomolgi bastianellii*, where no such vacuoles

[2] Since the present paper was submitted to the editor, W. E. COLLINS and his collaborators reported the successful cyclical transmission of *P. fieldi* with *Anopheles maculatus*, *A. stephensi*, and *A. balabacensis balabacensis* (J. Parasit. 54, 1968, 376).

could be demonstrated. Also, in this species, the pigment is said to be localized almost exclusively near the ookinete's anterior pole. In this species, the reacting granules would rather seem to be related to some other organelles, widely distributed throughout the ookinetes. No definite answer may be given, at this point, with respect to the precise site of the diazonium reaction in ookinetes. The final answer may well prove to be different from one *Plasmodium* species to another.

With the use of α-naphthyl acetate, oocysts show an intense reaction. This reaction is observed from the earliest stages detected throughout the oocysts' development. Electrophoretic separation indicates, with the exclusion of band 57, the presence of at least two esterase isozymes in the case of *P. cynomolgi bastianellii,* shortly before maturation of the oocysts. These isozymes are different from the ones found in non-infected midguts of the vector species. However, whether they originate from the oocysts or rather from the midgut, is not really known. It could be that the presence of oocysts induces production of new isozymes by some midgut tissues. It could also be that oocysts cause merely an increase in the production of isozymes, otherwise present in undetectable quantities. Also, whether the observed increase of the intensity of midgut band 73 in infected mosquitoes is significant, remains to be studied with quantitative methods. Immature sporozoites of *P. cynomolgi bastianellii* and *P. fieldi* appear to be covered by esterase(s), while mature sporozoites of the same species seem to be devoid of it. This would suggest that esterase(s) assumes some function in the formation of sporozoites. The mature sporozoites of *P. gallinaceum* differ from the former *Plasmodium* species in that they apparently remain covered with esterase(s) and, thus, rather resemble immature sporozoites of the two other Plasmodia.

With naphthol-AS-D and naphthol-AS acetate, also, esterase(s) is demonstrated in oocysts. Electrophoretic analysis indicates the presence of at least three isozymes. Of these, two are found in locations identical to the ones seen with α-naphthyl acetate. In *P. cynomolgi, P. cynomolgi bastianellii* and *P. fieldi,* the esterase quantity is found to increase until shortly before the oocysts achieve maturation. Sporozoites are seen to react with the substrates during the period of formation. Mature sporozoites no longer display a reaction. Again, esterase(s) seem to be taking part in the processes leading to the formation of sporozoites. This view finds support in the observation that oocysts, further development of which is interrupted, display no increase in their esterase(s) activity. Whether the interruption of esterase production is a reason or

rather a consequence of the interrupted development, is open to question. In *P. gallinaceum,* the increase of esterase quantity in oocysts is not as marked as in the other *Plasmodium* species examined. Also, mature sporozoites of *P. gallinaceum* resemble immature sporozoites of the other two species observed.

No electrophoretic separation of the acid phosphatases was undertaken. However, two distinct types of granulation are observed in the growing oocysts. It may, perhaps, be assumed that at least two phosphatase isozymes are present. In *P. cynomolgi, P. cynomolgi bastianellii* and *P. fieldi,* the coarse type of granulation gradually disintegrates during the oocysts' development. Immature sporozoites display no acid phosphatase activity, while mature sporozoites do. It is as if the required phosphatase(s) was first produced in the oocysts' cytoplasma and, then, distributed to the maturing sporozoites. In non-growing oocysts, the coarse granules are not being broken down. Besides the size of the oocysts, the appearance of their acid phosphatase granulation could possibly be considered an indicator of the stage of development attained. The observations made with *P. gallinaceum* yielded results less informative and necessitate further clarification.

For mosquitoes it has been demonstrated that esterase zymograms were species and even strain specific (5). Conceivably, the same could apply to Plasmodia. In this work, electrophoretic separation of esterase isozymes was undertaken with one *Plasmodium* species only. Comparative zymogram studies are suggested. We feel, however, that to this aim, more precise and sensitive electrophoretic methods need to be devised.

Acknowledgements

We wish to express our gratitude to Dr. L. H. Schmidt, Director, National Center for Primate Biology, University of California, Davis, California, and to his collaborators for kindly supplying us with *Anopheles freeborni, Plasmodium cynomolgi, P. cynomolgi bastianellii* and *P. fieldi.* We also thank Dr. G. M. Jeffery, Chief Laboratory of Parasite Chemotherapy, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, for providing us with *P. gallinaceum.* Our sincere thanks are due to Drs. C. L. Judson and G. A. H. McClelland, Department of Entomology, University of California, Davis, for keeping *Aedes aegypti* at our disposition. We further thank Dr. H. E. Adler and his co-workers for allowing us to use their facilities in the Avian Respiratory Disease Building.

References

1. Barka, T. & Anderson, P. J. (1963). Histochemistry, theory, practice and bibliography. — New York: Hoeber Medical Division, Harper and Row, Publishers, 660 pp.

2. DEMBITZER, H. M. (1968). Digestion and the distribution of acid phosphatase in *Blepharisma*. — J. Cell Biol. *37*, 329-344.
3. ELLIOTT, A. M. & HUNTER, R. L. (1951). Phosphatase activity in Tetrahymena. — Biol. Bull. *100*, 165-172.
4. FREYVOGEL, T. A. (1966). Shape, movement in situ and locomotion of Plasmodial ookinetes. — Acta trop. *23*, 201-222.
5. FREYVOGEL, T. A., HUNTER, R. L. & SMITH, E. M. (1968). Nonspecific esterases in mosquitoes. — J. Histochem. Cytochem. *16*, 765-790.
6. GARNHAM, P. C. C. (1966). Malaria parasites and other Haemosporidia. — Oxford: Blackwell Scientific Publications, 1114 pp.
7. GARNHAM, P. C. C., BAKER, J. R. & BIRD, R. G. (1962). Electron microscope studies of motile stages of malaria parasites. III. The ookinete of *Haemamoeba* and *Plasmodium*. — Trans. roy. Soc. trop. Med. Hyg. *56*, 116-120.
8. HAYES, R. O. (1953). Determination of a physiological saline solution for *Aedes aegypti* (L.). — J. econ. Entomol. *46*, 624-627.
9. LILLIE, R. D. (1965). Histopathologic technic and practical histochemistry. 3rd ed. — New York (etc.): McGraw-Hill Book Company, 715 pp.
10. THOMSEN, E. (1966). Esterase in the cells of the hind-midgut of the *Calliphora* female and its possible dependence on the medial neurosecretory cells of the brain. — Z. Zellforsch. *75*, 281-301.
11. VANDERBERG, J., RHODIN, J. & YOELI, M. (1967). Electron microscopic studies of sporozoite formation in *Plasmodium berghei*. — J. Protozool. *14*, 82-103.
12. WARREN, McW. & WHARTON, R. H. (1963). The vectors of simian malaria: identity, biology and geographical distribution. — J. Parasit. *49*, 892-904.
13. WIGGLESWORTH, V. B. (1966). "Catalysomes", or enzyme caps on lipid droplets: an intracellular organelle. — Nature *210*, 759.

Zusammenfassung

Ookineten, Oozysten und Sporozoiten von *Plasmodium cynomolgi, P. cynomolgi bastianellii, P. fieldi* und *P. gallinaceum* wurden auf Esterasen und saure Phosphatasen untersucht. Dabei gelangten als Substrate zur Anwendung: α-naphthyl-azetat, Naphthol-AS-D- und Naphthol-AS-azetat sowie Natrium α-naphthylphosphat. Als Diazonium-Salz diente «Blue RR salt».

Ookineten ergeben schon mit dem Diazonium-Salz, ohne Substrat, eine positive Reaktion. Infolgedessen kann über das Vorhandensein von Esterasen und sauren Phosphatasen nichts ausgesagt werden. Alpha-naphthyl-azetat-positive Esterasen können bei den drei untersuchten Säugetier-Plasmodien in den Oozysten während deren ganzer Entwicklung nachgewiesen werden. Unreife Sporozoiten ergeben eine positive Oberflächen-Reaktion. In oder an reifen Sporozoiten lassen sich keine solchen Esterasen finden. Mit Naphthol-AS-D- und -AS-azetat zeigt sich, daß Esterasen quantitativ im Verlaufe der Oozysten-Entwicklung zunehmen, daß sie jedoch unmittelbar vor deren Reifung wieder verschwinden. Mit den Sporozoiten verhält es sich ähnlich wie nach Behandlung mit α-naphthylazetat. Aus diesen Befunden und aus Beobachtungen an in der Entwicklung stehengebliebenen Oozysten wird allgemein auf eine aktive Beteiligung von Esterasen bei der Bildung von Sporozoiten geschlossen. Ein Versuch zur elektrophoretischen Auftrennung der festgestellten Esterasen bei einer Plasmodien-Art ergibt, daß mindestens drei Isozyme vorhanden sein müssen. Dabei bleibt allerdings die Frage offen, ob die gefundenen Isozyme den Oozysten oder dem Mitteldarm-Gewebe des Vektors entstammen. Es wäre denkbar, daß Oozysten den Wirt zur Bildung neuer Isozyme veranlassen. Saure Phosphatasen lassen sich in

den Oozysten in zweierlei Granulationsformen erkennen. Die gröbere der beiden wird gegen die Reifung der Oozysten hin abgebaut und ihr Material anscheinend in den Sporozoiten eingelagert. Die Ergebnisse mit *P. gallinaceum* sind im ganzen denjenigen der untersuchten Säuger-Plasmodien ähnlich, obgleich Abweichungen in einzelnen Punkten festgehalten werden.

Résumé

Dans les ookinètes, les oocystes et les sporozoïtes de *Plasmodium cynomolgi, P. cynomolgi bastianellii, P. fieldi* et *P. gallinaceum,* on a recherché les estérases et les phosphatases acides. On utilisa les substrats suivants : « α-naphthyl-acetate » (acide α-naphtylacétique), « naphthol-AS-D acetate » (O-méthylanide de l'acide 2-hydroxynaphtalique), « naphthol-AS acetate » et « sodium α-naphthyl phosphate ». Le « Blue RR salt » servit de sel de diazonium.

Les ookinètes montrèrent déjà une réaction positive avec le sel de diazonium, sans substrat. Par conséquent, on ne peut tirer aucune conclusion sur la présence d'estérases et de phosphatases acides dans cette forme de parasites. Des estérases positives sur acide α-naphtylacétique furent trouvées dans les oocystes des trois plasmodies de mammifères étudiées, et cela pendant toute la durée de leur développement. Les sporozoïtes immatures donnèrent une réaction positive de surface. Sur et dans les sporozoïtes mûrs, il nous fut impossible de trouver de telles estérases. Comme on l'a montré avec l'O-méthylanide de l'acide 2-hydroxy-naphtalique et le « naphtol-AS-acetate » la quantité de certaines estérases augmente au cours du développement des oocystes pour disparaître juste avant leur maturité. Après traitement avec le même substrat, les sporozoïtes donnent des résultats comparables à ceux obtenus avec l'acide α-naphtylacétique. On peut conclure de ces expériences, comme aussi d'observations sur des oocystes dont le développement s'est arrêté, que les estérases prennent une part active à la formation des sporozoïtes. Chez une espèce de plasmodie, un essai de séparation des estérases par electrophorèse a montré qu'au moins trois isozymes sont présents. Il reste cependant à savoir si les isozymes répertoriés appartiennent aux oocystes ou au tissu de l'intestin moyen du vecteur. On pourrait admettre que les oocystes induisent l'hôte à former de nouveaux isozymes. Les phosphatases acides sont présentes sous deux formes granulaires dans les oocystes. La plus grossière est détruite vers la fin de la maturation des oocystes et vraisemblablement son matériel est alors inclus dans les sporozoïtes. A part quelques différences de détails, les résultats obtenus avec *P. gallinaceum* sont les mêmes que ceux décrits pour les plasmodies des mammifères.