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MORPHOLOGICAL AND CYTOCHEMICAL OBSERVATIONS ON THE PHAGOTROPHY OF *PLASMODIUM (SAURAMOEBA) TROPIDURI* AND TWO OTHER SPECIES OF MALARIA PARASITE

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SUMMARY

Studies of fine structure demonstrate that the phagotrophy of *Plasmodium tropiduri* is similar to that of *P. elongatum*. Cytochemical studies of the intraerythrocytic stages of *P. tropiduri*, *P. berghei* and *P. gallinaceum* demonstrate the existence of two hydrolases with different distribution in the cytoplasm. The activity of amino acid naphthylamidase suggests strongly that this enzyme exists in the food vacuoles of these parasites. Arylacetylesterase activity has been observed in the schizonts and the segmenters of the three species studied; the possibility of its being localized in the paired organelles is discussed.

INTRODUCTION

When RUDZINSKA & TRAGER [20] described the mechanism of intracellular phagotrophy in *Plasmodium berghei* they confirmed, from an ultrastructural point of view, the long-held idea that haemoglobin is ingested by malaria parasites.

The presence of malarial pigment inside the parasites led investigators to suppose from the beginning that haemoglobin must be digested within the parasite. BROWN [5] suggested that an enzyme within the parasite might split off the proteid from the haemoglobin molecule, leaving the haemin to deposit as malarial pigment.

This idea, supported also by MOULDER & EVANS [15], was challenged by DEEGAN & MAEGRAITH [9], who pointed out that malarial pigment is not exactly haematin, but a complex of haematin and denatured proteinoid.

Proteolytic enzyme activity capable of decomposing haemoglobin was demonstrated by MOULDER & EVANS [15] in *P. berghei*, *P. gallinaceum* and *P. knowlesi*. More recently, COOK et al. [8] isolated and characterized a partially purified proteolytic enzyme from homogenates of *P. berghei* and *P. knowlesi*.

Even though digestive enzymes are supposed to exist in the food vacuoles of the intraerythrocytic stages of malaria parasites, hydrolase activity has not yet been demonstrated cytochemically in these vacuoles. The precise localization of enzymatic activity in the intracellular stages of the parasites can be carried out unequivocally only by means of electron microscope techniques. However, we believe that a cytochemical study of this activity could throw some light upon the phonomena of digestion and would contribute toward resolving certain contradictions in the interpretation of phagotrophy in the malarial parasites of birds and mammals. RUDZINSKA & TRAGER [20] supposed that the digestive activity in *P. berghei* took place in the pinocytic vesicles pinched off from the large vacuoles into which this parasite ingests haemoglobin.

AIKAWA et al. [1] maintain that in bird malaria parasites, host-cell cytoplasm is digested

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in the food vacuoles formed solely by the action of the cytostome. RUDZINSKA & TRAGER [21] have ratified their point of view in their studies of pinocytic uptake of haemoglobin by several species of malaria parasites. THEAKSTON et al. [25] maintain that both types of ingestion-pinocytosis and cytostomal-phagothophy may occur in the same species of parasite. They cite *P. vinckei*, in which species cytostomal activity appears to predominate in the young trophozoite, while pinocytosis may be more active in the more developed stages. SCALZI & BARR [22] support the idea of the two processes in one species, but suppose cytostomal activity to be more prevalent in the advanced stages of the parasite.

AIKAWA et al. [3], comparing the fine structure of intracellular and host-cell free trophozoites of *P. knowlesi*, have produced evidence that denis the existence of the large food vacuoles reported from the malaria parasites of mammals, considering them to be intrusions of host-cell cytoplasm that deform the parasite.

We believe that a cytochemical study of hydrolase activity in malaria parasites, known to differ in their mechanisms of ingestion, might establish whether digestive activity demonstrable by the available cytochemical techniques occurs in the large vacuoles, or whether it takes place only in the pinocytic vesicles and the vacuoles formed by the cytostome. Therefore, we have investigated three species of *Plasmodium*: *P. berghei*, with large food vacuoles and small vesicles; *P. gallinaceum*, with large food vacuoles and no pinocytic vesicles; and *P. tropiduri*, with small food vacuoles.

In order to study two different hydrolytic enzymes, we have selected one technique for the study of aminopeptidase and two more techniques for the study of esterases, taking into account the exaggerated quantities of amino acids and free fatty acids produced by intraerythrocytic malaria parasites from the haemoglobin and the lipids of the erythrocyte. Recent reports on these phenomena have been published by SIDDIQUI & TRAGER [24], SCHNELL et al. [23], and by CENEDELLA et al. [7].

MATERIALS AND METHODS

The strain of *P. tropiduri* used was obtained from Venezuela; it was maintained by bimonthly intraperitoneal subinoculations into the lizard *Tropidurus torquatus*. *P. berghei yoelii*, kindly provided by Mr. Killick-Kendrick, was maintained by subinoculations every 12 days, into 21-day old mice. *P. gallinaceum*, obtained from Dr. M. Anwar of the Imperial College Field Station, was sub-inoculated into 3-day old chicks every twelve days.

Amino acid naphthylamidase activity was investigated by the technique of NACHLAS et al. [16], in fresh smears of blood fixed in cold acetone and incubated in a medium containing L-leucyl-4-methoxy-naphthylamide and Fast Garnet GBC salt; the smears were counterstained with methyl green, according to the technique of BANCROFT [4]. The optimal pH for aminopeptidase activity was determined by using incubation media buffered at pH 3.8, 4.04, 4.99, 6.21 and 6.5 with 0.1 M acetate buffer, and at pH 7.2, 8.5 and 9.1 with TRIS-HCL 0.2 M buffer. Control smears were made in all cases by incubation in media containing no substrate.

Esterase activity was investigated by the methods of PEARSE [17] and of HOLT & WITHERS [12]. In the first method, smears fixed for five minutes with formaldehyde vapour were incubated 45 minutes at room temperature in a medium containing napthol AS acetate and Fast Bleu BB salt at pH 6.8. In the second method, smears were fixed in acetone at 4°C for 10 minutes, or in Formol-Calcium for five minutes; the smears were then incubated for up to two hours in a medium containing 5-Bromo-4-Chloro-indoxyl acetate with an oxidation-reduction mixture of potassium ferrocyanide-ferricyanide at pH 7.2. Controls were incubated in a medium without substrate. Naphthyl acetate esterase activity was exposed to the effects of 10^{-3} and 10^{-5} M E-600 (diethyl-p-nitrophenyl phosphate), in 0.2 M phosphate buffer at pH 6.8, and also of eserine at 10^{-4} M.

RESULTS

Aminonaphthylamidase (leucine aminopeptidase, LAP) activity was seen in all of

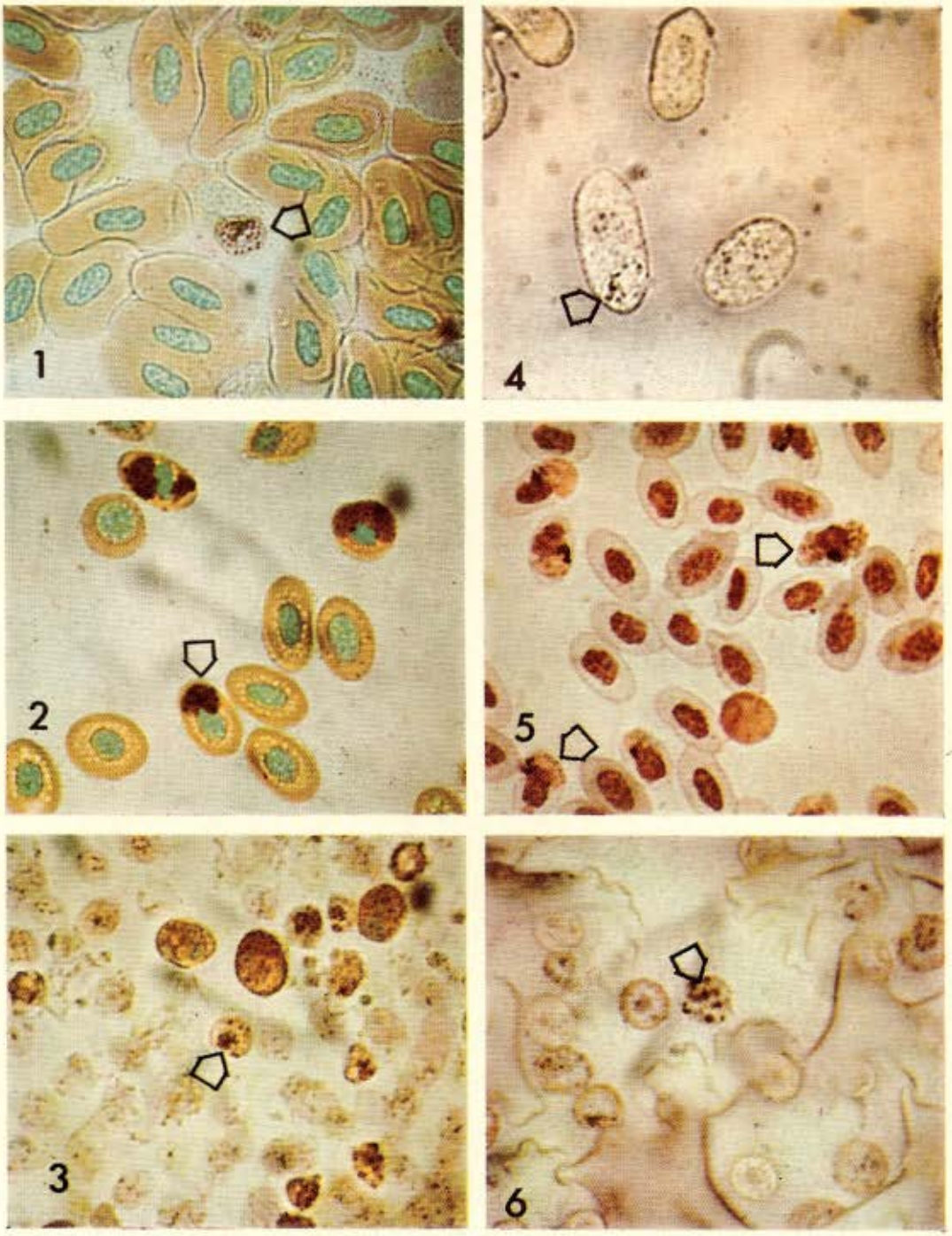


Fig. 1 — Amino acid naphthylamidase activity in a gametocyte of *P. tropiduri*. Numerous small round vacuoles appear dispersed throughout the whole cytoplasm of the parasite. No reaction is visible in the *Tropidurus torquatus* erythrocyte. 900 ×. Fig. 2 — Strong positive reaction of amino acid naphthylamidase activity in trophozoites and schizonts of *P. gallinaceum*. Schizont at bottom clearly shows LAP reaction, appearing as round vacuole. 900 ×. Fig. 3 — *P. berghei* trophozoites showing LAP reaction. In the centre of one trophozoite, four ovoid vacuoles are visible. In addition to other parasitized erythrocytes, there may be seen polymorphonuclear neutrophils and a monocyte showing different degrees of reaction in the cytoplasm. A diffuse reaction is seen in unparasitized erythrocytes. 900 X. Fig. 4 — Holt-Withers arylesterase activity in a half-grown schizont of *P. tropiduri*. Blue, dot-like reaction appears in the outer cytoplasm of the parasite. Minute granules of erythrocyte nuclear and cytoplasmic esterase also visible. 900 ×. Fig. 5 — Schizonts and segmenters of *P. gallinaceum* showing minute dot-like positive reaction of Holt-Withers esterase in outer cytoplasm of the parasite. In some of the merozoites of the segmenter at the right it is possible to observe the reaction. 900 ×. Fig. 6 — *P. berghei* schizont. Intense blue dots show sites of Pearse arylacetyl esterase. 900 ×.

the intraerythrocytic stages of all the parasites studied; it was observed as spots of regular shape, having a vacuolar appearance, with well-defined limits, and sometimes surrounded by a pink or yellowish area.

Differences in pH of the incubation media gave different results; between pH 3.08 and 4.99, the reaction was negative, the parasites being uniformly stained yellow, like the controls without substrate. This effect may be due to the Fast Garnet. A brick-red colour, characteristic of the positive reaction, began to develop at pH 6.5 and reached a peak of intensity between pH 7.2 and 8.5. At pH 9.1, the reaction was clearly negative.

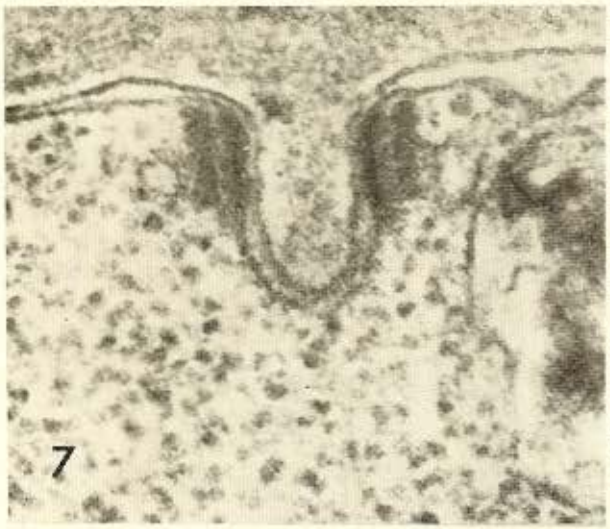
In the trophozoites and schizonts of *P. berghei*, the reaction site appeared as elongate oval vacuoles, rarely circular, of 0.2 — 0.3 micra in lenght (Fig. 3). In the same stages in *P. gallinaceum*, rounded vacuoles 0.6-1.2 micra in diameter are seen (Fig. 2). The trophozoites, segmenters, and gametocytes of *P. tropiduri* show very small circular vacuoles 0.2-0.4 micra in diameter (Fig. 1). Up to 21 small vacuoles were seen in the schizonts and gametocytes of *P. tropiduri*, about 10 rounded vacuoles were observed in the similar stages of *P. gallinaceum*, and no more than six were seen in the trophozoites and schizonts of *P. berghei*.

Esterase activity (arylacetylesterase, AAE) upon naphtholic or indoxylic ester substrates was observed ni the segmenters of *P. gallinaceum* and *P. tropiduri* and, with less certainty, in the comparable stages of *P. berghei*. The reaction appeared as a blue dot in the periphery of the schizonts or in each merozoite of a segmenter, apparently located outside the nucleus of the merozoite. The reaction was not intense in he segmenters of *P. tropiduri*, where it appeared as minute oval masses with their major axes at right angles to the axis of the merozoite (Fig. 4). In the segmenters of *P. gallinaceum*, the reaction was weaker and punctiform (Fig. 5). The diffuse nuclear staining by Mayer's carmalum in the schizonts of *P. berghei* made it difficult to locate the esterase activity precisely; the reaction in this parasite was intense, well-defined, and irregularly distributed in the form of 6-12 granules (Fig. 6). A similar reaction, appearing as much smaller granules, was seen in the erythrocytes of *T. torquatus* and mice, but it was so characteristic in appearance that it could not be confused with the reaction in the parasites.

The esterase activity observed in the erythrocytes and parasites, according to the technique of PEARSE [17], using naphthol AS acetate, was not inhibited by Eserine, but was totally eliminated by 10^{-3} M and 10^{-4} M E-600.

The fine structure of intraerythrocytic stages of *P. tropiduri* has been studied by us and will be published elsewhere. We now describe some additional details of the digestive process. The cytostome of *P. tropiduri* is similar to those of other malaria parasites. In the inactive state, that is, when it is not forming a bolus, it has an internal diameter of some 100 millimicra (Fig. 7). This dimension does not increase during the act of cytophagy; instead, the bottom of the cavity of the cytostome widens and deepens to permit the entry of a portion of the host-cell cytoplasm, thus forming a bolus which separates itself from the cystostome (Fig. 8). The bolus is limited by a double mem-

Fig. 7 — Cytostome of trophozoite of *P. tropiduri* during the initial phase of ingestion; the internal membrane of the trophozoite, at the bottom of the cytostome shows some ribosome-like particles. 120,000 ×. Fig. 8 — Cytostome of thophozoite in active phase of ingestion; the internal bars of the cytostome are less evident. 120,000 ×. Fig. 9 — Phagosome recently detached from cytostome. External membrane appears discontinuous and to be formed by granules similar in size to ribosomes. 90.000 ×. Fig. 10 — Section of a gametocyte of *P. tropiduri;* a bolus, limited by two membranes, is being pinched off from the cytostome. Two phagosomes (a, b) show two limiting membranes and different degrees of electron desity of the contents. Telephagosomes, os collector visicles, limited by a single membrane, containing remnants of phagosomes (c, d, e). Telephagosomes showing clear areas from which malarial pigment has been dissolved during staining (f). 40,000 ×. Fig. 11 — Recently formed phagosome. In the zone of contact with the endoplasmic reticulum a group of ribosomes (arrow). 80,000 ×. Fig. 12 — Phagosome within which digestion is far advanced; the external membrane (arrow) shows a dense structure similar to that of the phagosome of the preceding figure. 100,000 ×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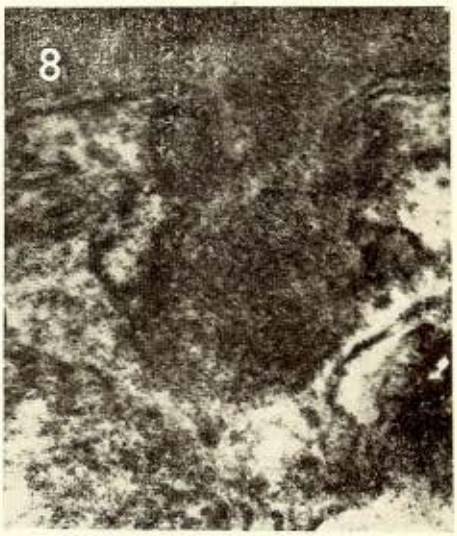
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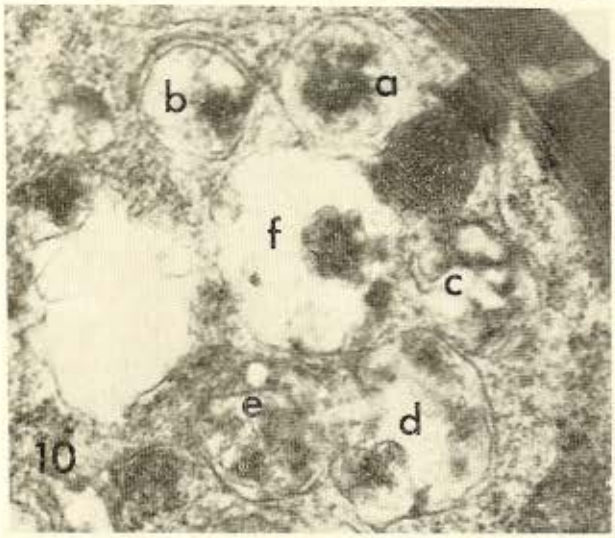
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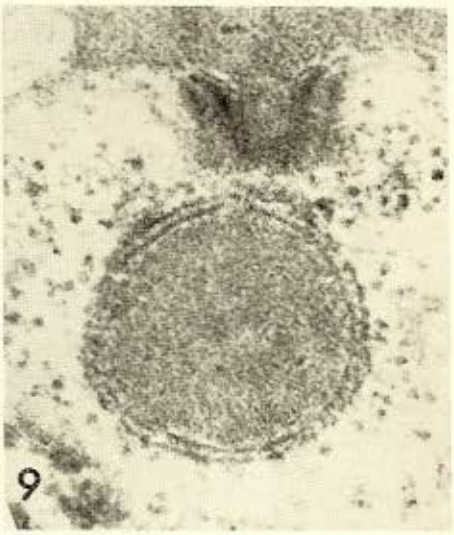
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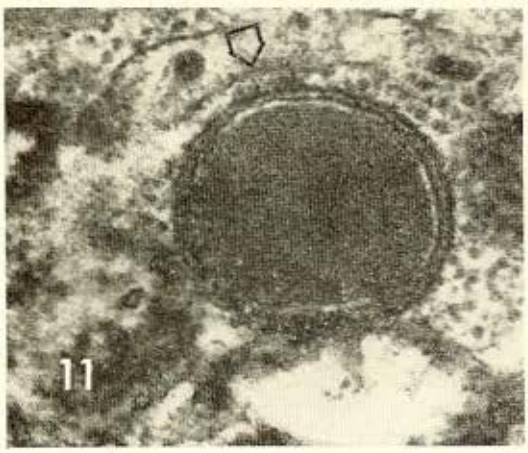
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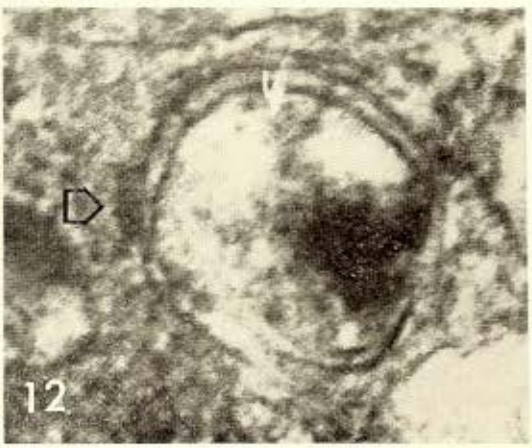
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brane, of which the outer one corresponds to the membrane proper of the trophozoite. This membrane appears to be discontinuous at the beginning of ingestion, suggesting that it is being actively synthesized, rather than passively stretched. Actually, in the recently formed vacuole, the outer membrane seems to be a layer of granules, of density and size similar to the ribosome granules, instead of being continuous. The integrity of the inner membrane is greater; it is an extension of the membrane of the host cell (Fig. 9).

The cytostomal phagotrophy of the parasite appears to be incessant. In addition to the forming bolus, others may be seen, surrounded by a double membrane and containing material with different degrees of electron density, which indicates that the material within the boluses is undergoing change (Fig. 10, A-B). The boluses not yet separated from the cytostome have a diameter of 200-300 millimicra; free boluses are 350-450 millimicra in diameter. There is no reason to think that the free boluses enlarge during digestion. On the contrary, study of the photographs suggests that, once the contained material has lost a great part of its electron density, the bolus diminishes in size. This is probably a consequence of absorption which leaves the remnants — mainly membranes and amorphous opaque material — to be collected in larger vacuoles where digestion is carried further (Fig. 10, C, D and E). This is to say that the larger vacuoles contain particular residues, appearing as concentric folded membranes, which surround opaque material. It is probable that the final phase of digestion occurs within these vacuoles limited by one single membrane and that, finally, the malarial pigment accumulates at these sites. Some of the large vacuoles have a content which is transparent or of low electron density, as a consequence of the malarial pigment of this species dissolving rapidly in the pH 12 solution of lead citrate used in staining. Finally, we should like to call attention to certain structures apparently associated with the externad membrane of some boluses; these are regular aggregates of granules, similar to polyribosomes but with lower electron density. We shall discuss their possible function further on.

DISCUSSION

There seems to be at least one aminonaphthylamidase in the digestive vacuoles of all three species of parasite studied. The form and dimensions of the areas of maximal enzymatic activity are different in all the three species of *Plasmodium*. The greatest number of vacuoles with LAP activity was observed in the schizonts and gametocytes of *P. tropiduri*, measuring 0.2 — 0.4 micra in diameter. The number, size, and form of the vacuoles correspond to those of the boluses we have described in the study of the fine structure of the parasite. LAP activity sites in the trophozoites and schizonts of *P. gallinaceum* had the aspect of rounded vacuoles, 0.6 — 1.2 micra in diameter. Study of the photographic material published by AIKAWA et al. [1] on the ultrastructure of *P. gallinaceum* indicates that the food vacuoles are circular and measure 0.8 — 1.1 micra in diameter; our results therefore suggest that LAP activity in this species occurs within the food vacuoles.

The results on *P. berghei* look contradictory; the illustrations published by RUDZINSKA & TRAGER [20] and by JERUSALEM & HEINEN [14] give the impression that the food vacuoles in *P. berghei* are oval or elongated, rarely circular; they measure 0.7 — 1.25 x 0.3 — 0.65 micra. LAP activity sites in *P. berghei* rarely appears as circular areas, but rather as oval or elongate spots of much smaller size, 0.2 — 0.4 micra long. Thus the LAP activity would seem to occur within small vacuoles, whose dimensions are comparable to those of the pinocytic vesicles described by RUDZINSKA et al. [21] and considered by these Authors to be the site where haemoglobin is really digested. It may be inferred, therefore, that aminopeptidase activity may be absent in the large food vacuoles observed in *P. berghei*, and that, as suggested by AIKAWA et al. [3] the large vacuoles are not structures produced by the parasite. In agreement with this point of view, SCALZI & BAHR [22] considered these large vacuoles as large vesicles which might be, topologically speaking, either "inside" or "outside" the young trophozoite, increasing the area available for pinocytosis. THEAKSTON et al. [25] present a similar point of view. In any case, our observations indicate

clearly that aminopeptidase activity occurs solely in the small vacuoles formed by the cytostomes or by pinocytosis, and that the large food vacuoles of *P. berghei* do not seem to exhibit this enzyme activity.

Arylacetylesterase activity has been demonstrated in the schizonts and segmenters of *P. berghei, P. gallinaceum* and *P. tropiduri*; a dot-like reaction site has been observed at the external margin of large schizonts and segmenters. This location corresponds to the anterior poles of the merozoites. The structure of the polar rings, the paired organelles, are prominent structures in this region. GARNHAM et al. [10] have postulated a secretory function for *Toxoplasma gondii* structures which are similar to the paired organelles. In *T. gondii*, in addition to the paired organelles, convoluted tubules are found, which GARNHAM et al. [10] considered to be synonymous with lysosomes. HANSON & SOURANDER [11] have demonstrated aryl sulfatase and acid phosphatase activity in *T. gondii*, and have established that these enzymes are located in small subpellicular vesicles and not in the toxonemas or paired organelles. This eliminates the possibility that the latter, at any rate, have lysosomal activity.

The merozoites of *Plasmodium* are cells to specialized for passing through the membranes of erythrocytes and, instead of a permeabilizing substance, their organelles might secrete esterases for the digestion of membranes rich in lipoprotein. The esterase activity which we have found is not likely to have any relationship to the esterase activity responsible for the production of free fatty acids in parasitized erythrocytes. However, it is possible that, in the merozoites, this secretion contributes toward rupturing the membrane of the host cells when the parasites are liberated, since, in addition to the extraordinary increase in the free fatty acid contents in erythrocytes parasitized by *P. berghei*, CENEDELLA et al. [7] have demonstrated a phospholipase A in homogenates of parasites freed from their host cells. The non-specific esterase activity detected by us is not an acetylcholine esterase, since it is not inhibited by Eserine; neither is it a lysosomal esterase, as it is inhibited by E-600. Thus, it must be a non-specific acetylesterase or arylesterase.

Finally, we should like to discuss the phagothophic activity of *P. tropiduri*. The fact that this parasite ingests haemoglobin in relatively large boluses shows a relationship to *P. elongatum*, which ingests erythrocytic cytoplasm in a similar manner, according to AIKAWA et al. [2]. However, while in *P. elongatum* the boluses are introduced into digestive vacuoles where digestion occurs, in *P. tropiduri* digestion begins in the bolus and terminates in the collector vacuoles.

Recently, HOWELLS et al. [13] have proposed the name of phagosomes for the pinocytic vesicles pinched off from the large vacuoles produced during the endocytotic activity of *P. berghei*, considering that the said phagosomes are the true sites of haemoglobin digestion. These Authors suggest that the name might be extended to the intracellular particles formed by ingestion at the base of the cytostomal pocket. We are partisans of this name, and our observations appear to establish certain relationships between the origin of the membranes that limit the phagosome of *P. tropiduri* and the origin of similar membranes observed in *Amoeba proteus* by CASLEY-SMITH & SAVANAT [6]. These Authors have suggested that, in the synthesis of the membranes of the phagosomes, the reticulosome plays an active part. The reticulosome is a microsomal fraction isolated by POLLAK & SHOREY [18]; it consists of ribosome-like particles that organize themselves around intracellularly injected foreign bodies to form digestive vacuoles, upon whose membranes, the primary lysosomes are aggregated. Study of our photopraphic material suggests that similar particles intervene in the formation of the phagosome (bolus) in *P. tropiduri*. In effect, it is possible to observe that, in recently formed digestive vacuoles like those here illustrated, granules similar in size and density to the ribosomes are regularly disposed to form the exterior membrane of the bolus. Furthermore, groups of granules similar to the polyribosomes are visible in some vacuoles that have begun digestive activity, suggesting that they may be centres of enzyme synthesis, or else sites where absorption by pinocytic vesicles is going on, as ROTH [25] has suggested (Figs. 11 and 12).

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CONCLUSIONS

Cytochemical studies demonstrate the existence of amino naphthylamidase and arylacetylesterase activity in the intraerythrocytic stages of *Plasmodium berghei, P. gallinaceum* and *P. tropiduri.* The sites of amino acid naphthylamidase activity appear to correspond, in form and size, to the food vacuoles observed in the fine structure of *P. gallinaceum* and *P. tropiduri.* In *P. berghei,* the zone of enzymatic activity is smaller than the vacuoles formed by phagotrophy, but corresponds to that of the pinocytic vesicles, where it is believed that the digestion of haemoglobin occurs.

Activity of arylacetylesterases has been observed only in the large schizonts and segmenters. The size, form, and distribution of the areas of enzymatic activity suggest that they may correspond to the paired organelles.

The process of phagotrophy in *P. tropiduri* is described, and compared with that of *P. elongatum.* The probable mechanism of formation of the membranes of the phagosome is discussed.

RESUMEN

Observaciones morfológicas y citoquímicas sobre la pagotrofia de Plasmodium (Sauramoeba) tropiduri *y de otras dos especies de parásitos maláricos*

Estudios ultrastructurales parecen demostrar que la fagotrofia de *Plasmodium tropiduri* es similar a la de *P. elongatum.* Estudios citoquímicos en los estadios sanguíneos de *Plasmodium tropiduri, P. berghei* y *P. gallinaceum* demuestran la presencia de dos hidrolasas con diferente distribución citoplasmática: actividad de naftilamidasa que parece occurrir en las vacuolas digestivas y de arilacetilesterase que parece corresponder a los organelos pareados que se observan en los esquizontes y segmentados.

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REFERENCES

1. AIKAWA, M.; HUFF, C. G. & SPRINZ, H. — Comparative feeding mechanisms of avian and primate malarial parasites. *Milit. Med.* **13** (Suppl.):969-983, 1966.

2. AIKAWA, M.; HUFF, C. G. & SPRINZ, H. — Fine structure of the asexual stages of *Plasmodium elongatum, J. Cell Biol.* **34**: 229-249, 1967.

3. AIKAWA, M.; HUFF, C. G. & SPRINZ, H. — Fine structure of erythrocytic stages of *Plasmodium knowlesi. Z. Zellforsch.* **100**: 271-284, 1967.

4. BANCROFT, J. D. — *An Introduction to Histochemical Technique.* London, Butterworths, 1967.

5. BROWN, W. H. — Malarial pigment (So-called Melanin): its nature and mode of production. *J. Exper. Med.* **13**:290-298, 1911.

6. CASLEY-SMITH, J. R. & SAVANAT, T. — The formation of membranes around microorganisms and particles injected into amoebae: support of the reticulosome concepto. *Austral. J. Exper. Biol. Med. Sci.* **44**:111-122, 1966.

7. CENEDELLA, R. J.; JARRELL, J. J. & SAXE, L. H. — *Plasmodium berghei*: production *in vitro* of free fatty acids. *Exp. Parasit.* **24**:130-136, 1969.

8. COOK, L.; GRANT, P. T. & KERMACK, W. D. — Proteolytic enzymes of the erythrocytic forms of rodent and simian species of malarial Plasmodia. *Exp. Parasit.* **11**: 372-379, 1961.

9. DEEGAN, T. & MAEGRAITH, B. G. — Studies on the nature of malarial pigment (Haemozoin). I and II. *Ann. Trop. Med. Parasit.* **50**:194-211, 212-222, 1956.

10. GARNHAM, P. C. C.; BAKER, J. R. & BIRD, R. G. — Fine structure of the cystic form of *Toxoplasma gondii. Brit. Med. J.* **1**:83-84, 1962.

11. HANSON, H. A. & SOURANDER, P. — Ultrastructural demonstration of lysosomes in *Toxoplasma gondii. Acta Path. Microbiol. Scand.* **74**:431-444, 1968.

12. HOLT, S. J. & WITHERS, R. F. — Studies in enzyme histochemistry V. An appraisal of indigogenic reaction for esterase locali-

SCORZA, J. V. — Morphological and cytochemical observations on the phagotrophy of *Plasmodium (Sauramoeba) tropiduri* and two other species of malaria parasite. *Rev. Inst. Med. trop. São Paulo* **14**:97-105, 1972.

zation. *Proc. Roy. Soc. (B)* **148**:520-532, 1958.

13. HOWELLS, R. E.; PETERS, W. & THOMAS, E. A. — The chemotherapy of rodent malaria. IV — Host-parasite relationships, part. 4. The relationships between haemozoin formation and host-cell age in chloroquine- and primaquine-resistant strains of *Plasmodium berghei*. *Ann. Trop. Med. Parasit.* **62**:271-276, 1968.

14. JERUSALEM, Ch. & HEINEN, U. — Elektronenmikroskopische Untersuchungen am Malariaerreger (Plasmodium berghei) in polychromatischen un oxyphilen Erythrocyten. *Z. Tropenmed. Parasit.* **16**:377-394, 1967.

15. MOULDER, J. W. & EVANS, E. A. — The biochemistry of the malaria parasites. VI — Studies on the nitrogen metabolism of the malaria parasite. *J. Biol. Chem.* **164**: 145-157, 1946.

16. NACHLAS, M. M.; CRAWFORD, D. J. & SELIGMAN, A. M. — The histochemical demonstration of leucine aminopeptidase. *J. Histochem. Cytochem.* **5**:264-278, 1967.

17. PEARSE, A. G. E. — Azo dye methods in enzyme histochemistry. *Intern. Rev. Cytol.* **3**:344, 1954.

18. POLLAK, J. K. & SHOREY, C. D. — The isolation and characterization of a new cytoplasmic component, the reticulosome. *Biochem. J.* **93**:36-38, 1964.

19. ROTH, L. E. — Electron microscopy of pinocytosis and food vacuoles in *Pelomyxa*. *J. Protozool.* **7**:176-185, 1960.

20. RUDZINSKA, M. A. & TRAGER, W. — Phagotrophy and two new structures in the malaria parasite *Plasmodium berghei*. *J. Biophys. Biochem. Cytol.* **6**:103-112, 1959.

21. RUDZINSKA, M. A.; TRAGER, W. & BRAY, R. S. — Pinocytotic uptake and the digestion of hemoglobin in malaria parasites. *J. Protozool.* **12**:563-576, 1965.

22. SCALZI, H. A. & BAHR, G. F. — An electron microscopic examination of erythrocytic stages of two rodent malaria parasites; *Plasmodium chabaudi* and *Plasmodium vinckei*. *J. Ultrastruc. Res.* **24**:116-133, 1968.

23. SCHNELL, J. V.; SIDDIQUI, W. A. & GEIMAN, Q. M. — Free amino acid pools in blood of monkeys infected with malarial parasites. *Fed. Proc.* **27**:478, 1968.

24. SIDDIQUI, W. A. & TRAGER, W. — Free aminoacids of blood plasma and erythrocytes of normal ducks infected with malarial parasite, *Plasmodium lophurae*. *Nature* **214**:1046-1048, 1967.

25. THEAKSTON, R. D.; FLETSCHER; K. A. & MAEGRAITH, B. G. — The fine structure of *Plasmodium vinckei*, malaria parasite of rodents. *Ann. Trop. Med. Parasit.* **62**:122-134, 1968.

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