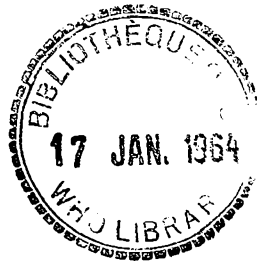


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FORMATION OF THE PERITROPHIC MEMBRANE IN SOME CULICINAE,  
AND CYTOLOGICAL PROCESSES CONNECTED THEREWITH<sup>1</sup>  
(Preliminary communication)

by

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INTRODUCTION

The studies reported below were based on the working hypothesis that species of Anopheles possessing a peritrophic membrane (P.M.) which hardened rapidly would not be very suitable for the transmission of Plasmodium, but that varieties with a P.M. which hardened slowly, if at all, would be well suited for this purpose. As a first step towards testing this assumption we set ourselves the task of finding out as much as possible about the structure of the P.M., the time of its formation following the mosquito's blood meal, and the manner of its formation. In principle, three methods were employed: dissection of the intestine, preparation of histological specimens, and electron-microscope examination - all at various intervals of time after the mosquitos had ingested a blood meal.

1. MATERIAL AND METHODS

The material studied comprised Anopheles gambiae, A. stephensi, A. maculipennis atroparvus, and, for comparison purposes, Aedes aegypti, all of which had been bred in the Swiss Tropical Institute (26°C and 85% relative humidity). These mosquitos were allowed to feed chiefly on guinea-pigs and Rhesus monkeys, but also, in a few instances, on chicks and humans.

<sup>1</sup> This investigation was assisted, in part, by the World Health Organization.

The posterior, extended portion of the middle intestine ("stomach") was dissected in physiological saline or Ringer's solution after the mosquito had been quickly killed with ether or chloroform. The intestinal wall and, where present, the P.M. were separated from the blood content with the aid of watchmaker's tweezers and fine glass needles, and were then studied in the phase-contrast microscope.

Cross-sections and sagittal sections were prepared. The fresh material was fixed in Carnoy's fluid for the preparation of paraffin sections and staining with azan.

#### Electron microscopy

Cold, buffered 2% osmium tetroxide solution was injected into the thorax of mosquitos killed with chloroform. After 15 minutes the intestine was dissected free and fixed for a further hour in the same fixing fluid. The intestines were then embedded in Araldite or Vestopal, the thin slices rendered opaque with potassium permanganate or lead hydroxide, and pictures taken with a Siemens Elmiskop I.

## 2. RESULTS

Two points should be mentioned right away: firstly, the blood meal appears to be digested in the same manner, irrespective of the type of host. Secondly, the age of the female mosquitos does not seem to have any bearing on the process of blood digestion. One experiment showed that Aedes aegypti formed membranes of a similar type in the same period of time following the first, second and third blood meal.

### 2.1 Macroscopic findings

#### Aedes aegypti

Two types of findings were obtained in a number of test groups fed on guinea-pigs. The first type tallied with the description of Stohler (1957): four to seven hours after the blood meal a layer of adhesive, viscous material was observed between the intestinal epithelium and the coagulated blood; after nine hours a cohesive P.M. of gradually increasing strength could be detached. Towards the end of the digestive process (after 42 to 54 hours) the P.M. lost its strength again. Finally, the remains of the P.M. were excreted anally in a fluid state together with the residue of the blood.

The second type of finding was obtained on several occasions, and in each case it occurred in all the mosquitos used simultaneously. Only five hours after the blood meal the P.M. could be detached in the form of a thin, transparent, colourless and somewhat adhesive skin. After nine hours it took on a brownish tint and was less adhesive, while after 12 hours it was comparatively resistant to tearing and flexible. After 24 hours, only remnants of the P.M. could still be observed on the blood coagulum, although digestion continued for about 48 hours.

It must be pointed out in this connexion that the guinea-pigs used also served on the same day to feed Anopheles mosquitos which did not display any deviations in blood digestion. It appears therefore that the chronological pattern of P.M. formation and degradation in Aedes aegypti may vary.

The P.M. of Aedes aegypti, irrespective of age, can be preserved intact for a matter of days in physiological saline.

#### Anopheles gambiae

The earliest time at which the P.M. could be detached was 12 hours after the blood meal. The P.M. appeared to become thicker towards the end of the digestive process (after between 46 and 70 hours). It persisted for several hours longer than the blood. None the less, it lost some of its strength while still in the "stomach" and was apparently likewise broken down there after the blood had been digested. The P.M. of Anopheles gambiae, however, was always thinner than that of Aedes aegypti. Moreover, it invariably dissolved within two to three hours in physiological saline.

#### Anopheles stephensi

The P.M. did not appear until 32 hours after the blood meal. Its posterior segment still seemed to be weak and open at the caudal end after 48 hours. It attained its greatest strength towards the end of the digestive process (after 56 to 60 hours), but was then likewise digested together with the last remnants of blood. Like the P.M. of Anopheles gambiae, it dissolved after a short time in an aqueous solution.

Anopheles maculipennis atroparvus

Although macroscopically visible amounts of a yellowish, transparent, slightly viscous fluid were superimposed rostrally to the agglutinated blood in the very first minutes following the blood meal, no P.M. was formed throughout the entire duration of the digestive process. The phase-contrast microscope occasionally revealed streaks of a material reminiscent of that observed in the P.M. of the other two species of Anopheles studied. However, a membrane surrounding the whole of the blood, or a large proportion of it, was never observed. This finding is in contrast to that of Yagujinskaia (1940) who, however, worked with A. maculipennis messeae.

2.2 Microscopic and submicroscopic findings

Starved mosquitos

By "starved" mosquitos we mean female mosquitos which have not ingested any blood for more than three days. In most cases these females had had at least one previous blood meal. Their intestine is, as far as can be judged, empty; at least, it does not contain any remnants either of a previous blood meal or of a P.M. formed during the digestion thereof.

Aedes aegypti

The intestinal epithelium was found to consist of typical columnar cells measuring 44-50  $\mu$  in height and about 10  $\mu$  in width. The nuclei were clearly distinguishable and had a diameter of up to 7-8  $\mu$ . The apical cytoplasm contained granules which stained a weak orange colour. In the electron-microscope picture the resting epithelial cell was marked by the presence of large, more or less strictly arranged complexes of granular endoplasmic reticulum (E.R.), which probably corresponded to the orange granules seen in the light microscope. These complexes frequently displayed a concentric arrangement of E.R. cisterns and reminded one of finger-prints. The basement membrane of the cell, with its invaginations protruding a considerable distance into the cytoplasm, formed an actual basal labyrinth.

Anopheles gambiae, A. stephensi, and A. maculipennis atroparvus

The slight differences that exist in the intestinal epithelium of these three species of Anopheles appear to be unimportant in the present context. We therefore considered all these three species under a single heading and compared them with Aedes aegypti. The epithelial cells measured 20-30  $\mu$  in height, and about 7  $\mu$  in width. The nuclei attained roughly the same size as in the case of Aedes aegypti. The microvilli formed a regular layer 2-4  $\mu$  thick and covered all the epithelial cells. At both the apical and the basal pole the protoplasm appeared to be arranged in fine longitudinal "threads". Between and beneath these "threads" there were small "vacuoles", i.e. colourless round spots which, in Anopheles maculipennis atroparvus, attained approximately the same size as the nucleus, exhibited relatively sharp contours, and contained, as electron-microscope examination showed, glycogen in particulate form. The E.R. was substantially smaller in extent in all the varieties of Anopheles. "Finger-prints" could seldom be demonstrated, and even when they were found, they consisted simply of a few concentrically arranged granular cisterns. In Anopheles, in contrast to Aedes aegypti, many granules measuring 220-430  $\mu$  in diameter were observed in the apical portion of the cells. Owing to their morphological similarities to the secretion granules of the pancreas, they were considered to be zymogen granules. We suppose that digestive enzymes or their precursors are stored in these bodies. In Anopheles, too, a basal and a lateral labyrinth were found, which were apparently responsible for the "thread-like" structure observed in the light microscope.

Freshly fed mosquitos

The ingestion of blood was associated with marked changes in the intestinal wall, which was stretched to a very considerable extent. The height of the cells, measured in the middle portions of the "stomach", diminished to about 4  $\mu$  in the case of Aedes aegypti and to about 2  $\mu$  in that of the various species of Anopheles. Cell width was difficult to ascertain because the cell boundaries were hardly visible any longer, but it would seem to be in the region of 20  $\mu$ . The nucleus had adapted itself to the changed shape of the cell and appeared as a flattened oval body.

In the case of Aedes aegypti, no orange granules were found any longer shortly after the blood had entered the intestine. This observation is matched by the electron-microscope findings. Only seven minutes at the most after the blood meal, the highly organized E.R. complexes had given way to a population of vesicles. Furthermore, a blue-staining border appeared between the intestinal wall and the blood coagulum, being particularly pronounced in a costal direction. In chronological correlation with the destruction of the finger-prints, a granular material, which probably corresponds to the "blue border" seen in the light microscope, appeared between the microvilli of the brush border. We consider this blue border to be wholly or in part a secretion product of the epithelial cells.

In the Anopheles mosquitos, the "vacuoles", which we regard as being identical with zymogen granules, disappeared shortly after the blood meal. In the electron-microscope picture, no zymogen granules were observed any longer in the apical portion of the cytoplasm at the most 90 minutes after the blood had entered the intestine.

Findings obtained in the various types of mosquito at the time when the peritrophic membrane was fully developed

Aedes aegypti (24 hours after the blood meal)

In the section, the epithelial cells appeared to be cubical. Vacuoles were seen in the protoplasm on the basal side of the nucleus.

The "blue border" could already be observed at a certain distance from the brush border. It appeared to be clearly marked off from the epithelial cells; on the other hand, it seemed as if there was a fluid transition from the "blue border" to the adjacent, half-digested erythrocytes. In this stage of blood digestion, the "blue border" doubtless represented the peritrophic membrane. It displayed a certain longitudinal structure in parts and was not more than 10  $\mu$  thick. Occasionally it contained inclusion bodies which could not be more closely defined.

Anopheles gambiae (24-48 hours after the blood meal)

As in the case of Aedes aegypti, the intestinal wall was somewhat less taut. The epithelial cells were covered all over with a vertical brush border. In contrast to the conditions observed in Aedes aegypti, there was a continuous transition from the centre of the blood coagulum containing barely digested erythrocytes to the brush border outside; next to the erythrocytes in the centre were semi-degraded erythrocytes which, in turn, faded into a zone of empty erythrocyte membranes; this zone also contained to some extent material from the "blue border". It must be assumed that in this case, too, the "blue border" represented the P.M. A granular blue material of very irregular shape and thickness surrounded the blood meal; in some places it was directly connected with the brush border, while in others it was clearly separated from this border; some portions of its periphery were thicker than others, giving the impression of a longitudinal structure, while elsewhere the material appeared as a loose, unconnected conglomerate.

Anopheles stephensi

The findings for A. stephensi were similar, except of course that, as already mentioned, the peritrophic membrane did not appear until later and the character of the membrane was more clearly recognizable in sections prepared 48 hours after the blood meal. Although the material of the "blue border" likewise appeared granular, it condensed round the blood meal, so that in the section it became a clearly delimited band. A kind of longitudinal structure was observed in places. The membrane became thinner towards its caudal end and passed over into the structureless intestinal contents.

Anopheles maculipennis atroparvus

The secretion of blue-staining material - particularly in a costal direction and also, to a somewhat lesser extent, caudally - would seem to have passed its peak only a few minutes after the blood meal. After half an hour no further secretion appeared to take place. After only one hour the original secretion no longer

entirely covered the permanent contents of the middle portion of the "stomach". On the other hand, after two hours a very bright red layer appeared beneath the costal "blue cap"; no further details can be given at present about this red layer. In all other respects, the digestive processes seemed to follow the same pattern as in A. gambiae and A. stephensi.

These observations tally with those recorded in fresh material. A. maculipennis atroparvus does not develop a P.M. (even after a blood meal).

In all species investigated electron-microscope pictures failed to reveal the existence of a membrane constituting a clearly delimited layer between brush border and blood coagulum in any of the varieties of mosquito studied. Only in a few cases did we observe a high concentration of material such as was described by Bertram & Bird (1961) as a P.M. In addition, erythrocytes in the process of degradation frequently advanced as far as the brush border or even came to rest directly on the microvilli. Both these findings seem to indicate that no continuous P.M. surrounding the blood coagulum exists.

### 3. SUMMARY AND TENTATIVE CONCLUSIONS

(a) There are fundamental differences between the epithelial cells of the middle intestine of Aedes aegypti on the one hand and those of the Anopheles varieties studied on the other. An essential difference is to be found in the intracellular preparation of digestive enzymes. In the case of Aedes aegypti, highly developed, concentrically arranged complexes of endoplasmic reticulum are encountered. In Anopheles, these complexes are insignificant, but on the other hand zymogen granules are seen.

(b) (i) As with Aedes aegypti, a "peritrophic membrane" is also observed macroscopically in the middle intestine of Anopheles gambiae and A. stephensi after every blood meal.

(ii) No P.M. is found in the case of Anopheles maculipennis atroparvus.

(iii) The formation, consolidation, and degradation of the P.M. in Aedes aegypti may follow various chronological patterns.

(iv) The P.M. of Aedes aegypti is insoluble in water, while those of Anopheles gambiae and A. stephensi are soluble in water. This indicates that the membranes have different chemical structures.

(c) Under the microscope, the P.M. does not usually appear to be clearly delimited, and, to that extent, it does not necessarily correspond to what is generally meant by the term "membrane". This applies to a still greater degree to the photographs produced with the electron-microscope, in which it is often impossible to demonstrate a P.M. at all. The question thus arises as to what the real nature of the P.M. is in the various species of Culicini. In this connexion, the possibility of a handling artefact - e.g. polymerization during dissection - or of a methodological shortcoming connected with the electronic process must be borne in mind.

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