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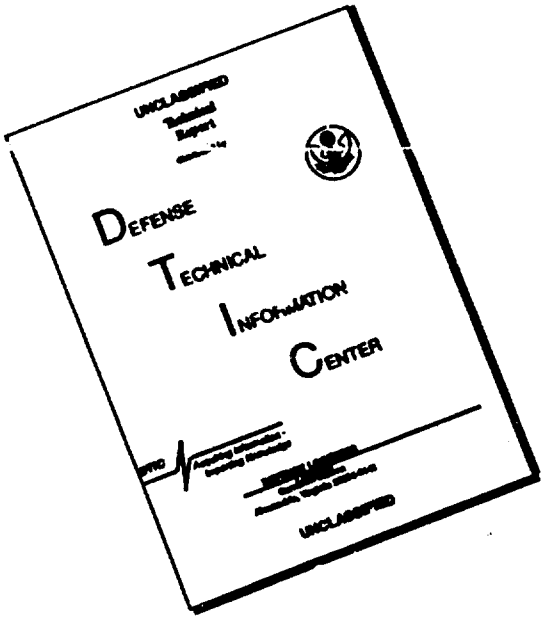

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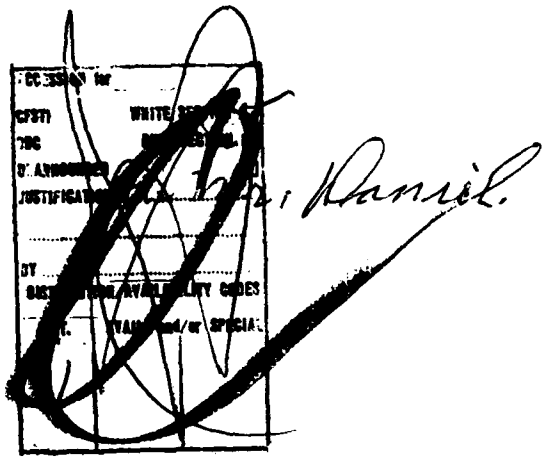

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FORMATION OF A PERITROPHIC MEMBRANE IN AEDES AEGYPTI L. MOSQUITOES

Following is the translation of an article by D. P. Zhuzhukov, published in the Russian-language periodical Nauchnyye Doklady ysshey Shkoly, Biologicheskiye Nauki (Scientific Reports of the Higher Schools, Biological Sciences) No 4, 1962, pages 25--27. It was submitted on 29 Dec 1961. Translation performed by Sp/7 Charles T. Ostertag, Jr.

A peritrophic membrane in adult mosquitoes of the genus Aedes was detected for the first time by T. S. Detinova (1942). Waterhouse, (1953) established that in A. aegypti, just as in other species of blood-sucking mosquitoes, the peritrophic membrane is formed periodically in connection with blood-sucking and only in the females. On histological cross-sections of mosquitoes' mid-guts, made at various stages in digestion, he was able to detect a formed peritrophic membrane only after 24 hours following the taking of blood, when it was already digested to a significant degree. Blood in the early stages of digestion, in the opinion of Waterhouse, is surrounded by a "gelatinous" capsule, and the actual peritrophic membrane is absent.

It has been established by us that in the higher flies the peritrophic membrane carries the enzymes of the mid-gut to the food and the products of digestion to the epithelium, especially with a protein diet. The presence of this membrane only in female mosquitoes and its formation only following the entry of blood into the mid-gut (protein nourishment) suggest its similar functions in flies and mosquitoes. However, in such a case with the mosquitoes the peritrophic membrane should already be formed in the very beginning of the digestion of blood. In connection with this, we decided to clarify the period and the nature of its formation.

We used laboratory reared mosquitoes for the investigation. Immediately after feeding on white mice the female mosquitoes were placed separately in chemical test tubes. After a specific time the abdomens were severed and fixed in Bouins fluid. In this manner the following series of fixed mid-guts were obtained: Empty mid-guts, immediately following blood-sucking, in 15 and 30 minutes, and 1, 2, 4, and 24 hours following the taking of blood. Paraffin microscopic sections with a thickness of 5 microns were prepared from the fixed material. The sections were stained with Delafild's hematoxylin.

Examination of the preparations yielded the following results.

In hungry females (figure 1, A) the mid-gut is empty, its walls are congregated in numerous longitudinal folds. The epithelim is quite high and columnar. The cells with a granular plasma have vacuoles between the nucleus and the edge which is facing the gut cavity. The nuclei are large and oval shaped and sharply stained. Easily visible on the inner borders of the cells was a rod-shaped layer with a secretion coming out into the gut cavity.

Immediately after taking blood (figure 1, B) the mid-gut is greatly distended. The epithelium becomes very thin and flat, and even the cell nuclei are depressed. Formed elements of blood are easily seen in the gut cavity. At the border of the epithelium, especially in the posterior portion of the mid-gut a band of secretion of epithelium cells is noticeable. There are no signs of secretion in the epithelium cells themselves.

After 15 and especially after 30 minutes following feeding (figure 1, C), the blood in the mid-gut of the mosquitoe is coagulated, and on the border of the blood clot a thin homogeneous membrane is formed. It is best discernible in the posterior portion of the mid-gut. In the course of the first 4 hours following blood-sucking, dark fine granules accumulate gradually on the inner edge of this membrane. These are the products of blood digestion, which by the end of the fourth hour form a clear line along the edge of the blood clot (figure 1, D, p.k.).

After 24 hours, at the site of this line the products of digestion of blood hemoglobin are accumulated. As a result a wide black band is formed (figure 1, E, k.). In preparations of this series the peritrophic membrane is not seen since it is saturated with the digestion remnants of blood.

In examining the preparations it is impossible to accurately establish the moment of formation of the peritrophic membrane in the stomach of the mosquitoe, since on the cross-sections it is not always possible to distinguish the secretion from the formed dense membrane. Upon dissection we were not able to detect the peritrophic membrane, since it is impossible to separate it from the blood clots in the early stages of digestion. In connection with this, we selected a method for the artificial feeding of mosquitoes with a protein-less substrate with the subsequent dissection of the mid-guts.

For feeding the mosquitoes we made up the following nutrient solution, which is close to that described by Liles, J., Meolan R., Jones, W., DeLong, D., 1960: Sodium chloride -- 750 mg, sugar -- 200 mg, agar-agar -- 100 mg, arginine -- 10 mg, phenylalinine, -- 10 mg, histidine --

10 mg, leucine -- 10 mg, lysine -- 10 mg, methionine -- 10 mg, threonine -- 10 mg, tryptophan -- 10 mg, water -- up to 100 ml.

Eight-day old females, which had never had a blood-meal, were placed in a gauze box with the dimensions 100 x 100 x 100 mm (figure 2). One lateral side was made out of transparent X-ray film. In the cardboard bottom of the box a small beaker with nutrient solution is placed. It is drawn closed on top with the skin of a white mouse. The small beaker is submerged in a dish of water. The temperature of the water is maintained at around 40°C. The mosquitoes pierced the mouse skin very actively and drank the solution. The mosquitoes which flew off of the skin were immediately transferred to chemical test tubes. The mid-guts were dissected in 10, 20, 30, 40, 50, 60, and 90 minutes following feeding in series of ten each. All told 70 mid-guts were dissected.

In the mid-guts, which were filled with the transparent liquid solution, it was very easy to detect and distinguish the peritrophic membrane. In several females small sectors of peritrophic membrane in the form of very thin tenacious film were found in the posterior portion of the mid-gut already in 10 minutes after feeding, and after 20 minutes the film was in all the females. After this the peritrophic membrane gradually thickened and grew in an oral direction. After 30--40 minutes it had taken position along the length of about half of the gut and in approximately an hour it completely covered all the food which had entered the mid-gut.

On the basis of the observations carried out the process of the formation of the peritrophic membrane in female A. aegypti amounts to the following in our opinion. Already prior to blood-sucking, a liquid secretion is being prepared in the epithelium of the mid-gut of a hungry female. It serves as the future material for constructing the peritrophic membrane. At the time of feeding, the gut of the mosquitoe is stretched out by the blood. The epithelial cells are strongly contracted and their secretion enters the gut cavity, surrounding the blood which has arrived there. After 15--20 minutes the blood in the mosquitoe's gut coagulates and at this time in the posterior portion of the mid-gut the formation of the peritrophic membrane begins. It gradually encompasses all the blood clots. In the first hour, according to the data of J. Boorman, 1960, the absorption of water from the blood is taking place intensively in the gut. Probably the digestion of blood begins in the second hour, when the peritrophic membrane is already completely formed.

In this manner, the digestion of protein food (blood) in the mid-gut of the female A. aegypti takes place, just as with flies, under a peritrophic membrane. This supports our assumption on the analogous functions of the peritrophic membrane in flies and mosquitoes. Experimental support of this problem emerges beyond the framework of the present article.

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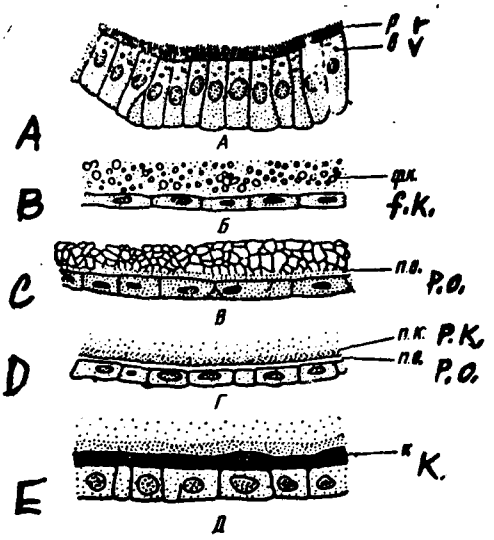

Figure 1. Transverse sections of the stomach of female Aedes aegypti L.
A -- empty stomach; B -- immediately after blood-sucking; C -- after 30 min.;
D -- after 4 hours; E -- 24 hours following blood-sucking.
r -- rhabdom; v - vacuoles of secretion; f.k. -- formed elements of blood;
p.o. -- peritrophic membrane; p.k. -- products from the digestion of blood;
k -- dark band from the products of blood digestion.

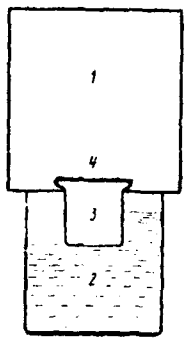

Figure 2. Breeding place for the artificial feeding of mosquitoes:
1 -- gauze breeding place with mosquitoes; 2 -- small beaker with warm water;
3 -- small beaker with nutrient solution; 4 -- pelt from a white mouse.