No. 23
Dec. 5, 1966

HAEMOLYMPH PROTEINS AND REPRODUCTION IN *PERIPLANETA AMERICANA**

K. G. ADIYODI AND K. K. NAYAR
Department of Zoology, University of Kerala, Trivandrum-7

INVESTIGATION of the hæmolymph proteins of the larva and adult of the cockroach *Periplaneta americana* by paper electrophoresis and starch gel electrophoresis has been attempted by several investigators, and it has been found that the pattern changes with the stages in development and moulting (*see* Wyatt[1] for review). Menon[2,3] studied by paper electrophoresis the pattern of hæmolymph proteins in adult females and recognized four distinct negatively charged fractions. She found that the allatum initiates oocyte growth by making available to the oocytes serum proteins especially the negatively charged fraction 2.

The hæmolymph from newly emerged adult females, and the hæmolymph, ovary, left colleterial glands and fat bodies of regular laying adult females and starved females were analyzed by disc electrophoresis in the manner described by Reisfeld *et al.*,[4] to study the pattern of distribution of proteins in the cockroach in relation to the ovarian cycle and to assess the role of the allata in the production and/or mobilization of the soluble type of proteins. For purpose of comparison the electrophoretic pattern of the blood of normal male and allatectomized and virgin females was also studied.

Hæmolymph was collected from animals by making a small puncture on the membrane connecting the coxa of the first leg with the body. The ovary, fat bodies, and colleterials were carefully washed in minute quantities of glass distilled water and homogenised. Hæmolymph and the tissue fluids were centrifuged before spotting. The electrophoresis was run in a Canalco model 6 Disc Electrophoretic Unit on polyacrylamide gel for 25 m. using β alanine acetate as the buffer at pH 4·5.

In adult female *Periplaneta* approximately nine different protein fractions could be detected. The fractions have been numbered here from the 'origin' to the 'front'. The females in general showed a higher concentration of hæmolymph proteins than males judged from the intensity of the stained bands. Two slow moving proteins, represented by fractions 3 and 4, appear to be the major components in the blood in newly emerged and adult males and females, but in males fraction 4 occurs only in comparatively low amounts. The other fractions are variable. Fraction 4 appears to be synthesized under the influence of the corpus allatum and its concentration shows fluctuations which may be clearly correlated with the reproductive cycle. When the oocytes are fast growing this fraction is present only in medium amounts, and by the time the oocytes have reached their maximum size it becomes weak. During the period of ovulation and ootheca protrusion, there is an increase in the amount of this fraction, and in cockroaches with ootheca fully protruded, the accumulation of this fraction reaches its maximum. In allatectomized females there is a marked increase in the amount of hæmolymph compared to the normal females. Some accumulation occurs in the protein fraction 4 following allatectomy, and this may be probably because of the protein formed under the influence of the allatum hormone already present. By about a month this fraction becomes weak; fraction 5 becomes strong in these animals, whereas the other fractions disappear. In just emerged adult females there is much less hæmolymph compared to the adult female. In just emerged adult females, virgin females, and in adult females starved for a considerably long period fraction 4 occurs only in small concentrations, in some cases much less than in males (Fig. 1).

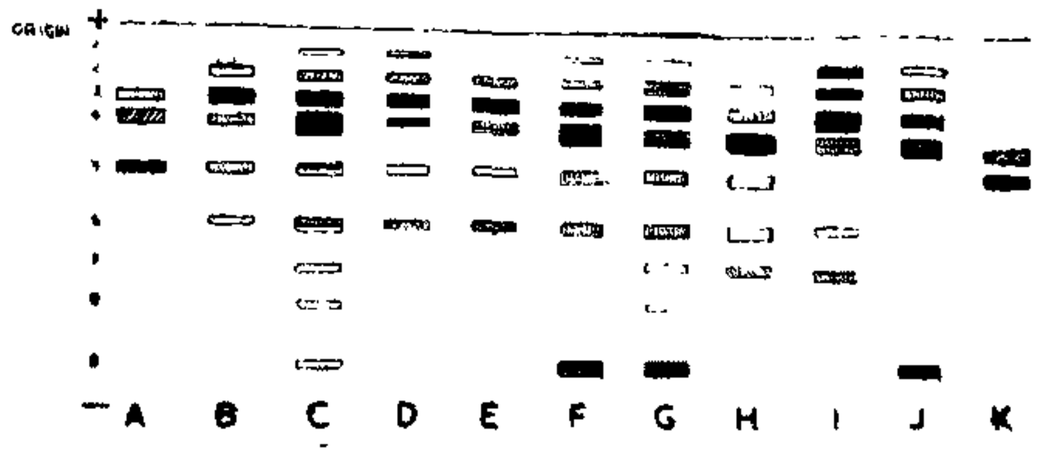

FIG. 1. Hæmolymph proteins of *Periplaneta*. A. Normal adult female with basal oocytes 2·64 mm. (eggs are usually laid when they reach an average length of 3·84 mm.). B. Normal adult female with basal oocytes in the range = 3·6 mm.–3·84 mm. C. Normal adult female with ootheca fully protruded. D. Normal adult male. E. Just emerged female. F. Just emerged adult female starved for 10 days. G. Just emerged adult female, fed and kept as virgin., sacrificed on the 9th day. H. Adult female starved for 2 weeks. I. Starved adult female with resorbing ovaries. J. Adult female starved for 3 weeks. K. Allatectomized adult female sacrificed after a month.

* The research work has been supported by a generous grant from the Ford Foundation.

In young adult females starved from the time of their emergence and sacrificed on the 10th day there is accumulation in fraction 4. Regular laying adult females subjected to starvation for 2-2½ weeks showed a similar general retention of fraction 4 and a corresponding decrease in the amount of fraction 3. Accumulation of fraction 4 may be taken as due to its non-utilization for oocyte growth under conditions of inanition whereas the paucity of fraction 3 may be related to the selective utilization of this fraction for maintenance during starvation. In starved females as time progresses and when the ovary undergoes resorption the accumulated fraction 4 becomes depleted, and there is a corresponding increase in fraction 3, suggestive of a conversion of fraction 4 to fraction 3 probably in an attempt to restore the deteriorating basic functions of the body.

In newly emerged females maintained as virgins or starved right from the time of their emergence and in starved adult females a 'front' fraction (fraction 9) becomes conspicuous. In normal males and females no such phenomenon has been observed.

Ovary with full-grown oocytes shows four protein fractions, 3, 4, 6 and 9, all common to the corresponding fractions in blood. The most conspicuous one is fraction 9, 6 being in traces. This suggests a probability that hæmolymph proteins may pass into the ovary. Fractions 3 and 4, however, occur only as mild bands compared to blood. In the ovary of females starved for a long time and with vitellogenesis nearly completely inhibited fractions 3 and 4 fail to appear, fraction 9 alone being present. This may be taken as indicating that the relatively slow moving fractions 3 and 4 represent the "yolk proteins" in the oocytes of the cockroach. In spite of the accumulation in fraction 4 during the initial periods and fraction 3 at a later stage in the blood of starved females, these fractions fail to appear in their ovaries, and only fraction 9 occurs in moderate concentrations. This is consistent with the view that corpus allatum provides a mobilizing hormone making available serum proteins to the oocytes (Highnam *et al.*[5]); the failure of the ovary to incorporate yolk proteins in starved adult females may be due to the low titre of the allatal hormone in blood.

The fat bodies of males, females with nearly full-grown oocytes, females with ootheca fully protruded, and females starved for 33 days showed only fraction 9 in nearly the same concentrations.

The secretion of the left colleterial glands has been seen to consist of four protein fractions, the most conspicuous ones being fractions 4 and 5. There are thus two fractions common to the left colleterial gland and ovary, *viz.*, fractions 4 and 9. These glands in the starved females show, like their ovary, only one band corresponding to fraction 9 (Fig. 2).

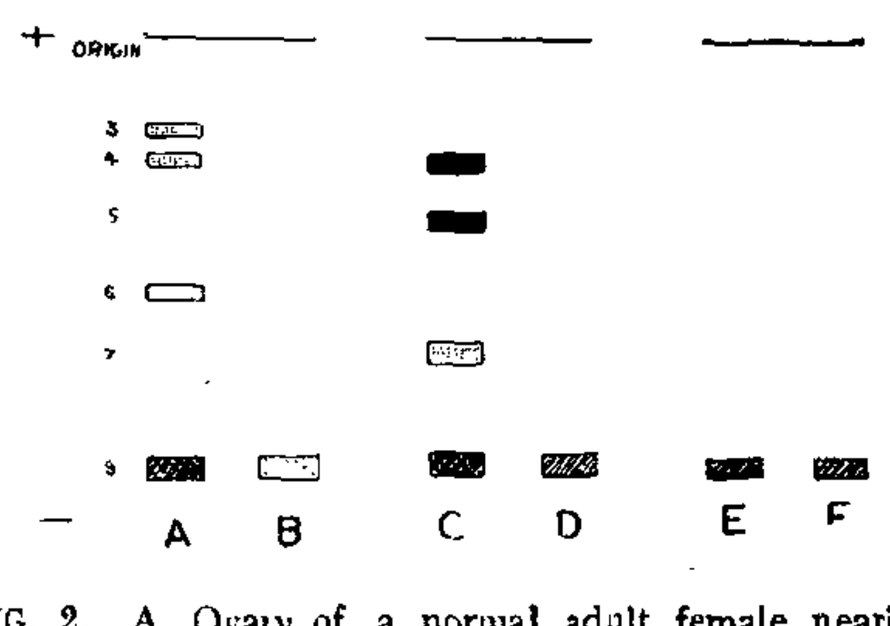

FIG. 2. A. Ovary of a normal adult female nearing oviposition (basal oocytes: 3·70 mm.). B. Spent ovary of an adult female starved for 2 weeks C. Left colleterial of an adult female about to oviposit (basal oocyte: 2·84 mm.). D. Left colleterial of a starved adult female E. Fat bodies of a normal female with basal oocytes 3·26 mm. F. Fat bodies of an adult female starved for 33 days.

The evidence presented here thus seems to suggest that the corpus allatum influences not only the synthesis of proteins especially fraction 4, but also that it takes part in the mobilization of this and other fractions like 3 and 6 to the ovary, and 5 and 7 to the colleterials for utilization.

1. Wyatt, G. R., *Ann. Rev. Ent.*, 1961, **6**, 75.
3 Menon, M., *Proc. XVI Internat. Cong. Zool.*, 1963, 1, 297.
3. —. *J. Anim. Morph. Physiol.*, 1965, **12**, 76.
4 Reisfeld, R. A., Lewis, V. J. and Williams, D. E., *Nature*, 1962, **195**, 281.
5. Highnam, K. C., Lusis, O and Hill, L. *J. inst Physiol.*, 1963, **9**, 587.