Blood Protein Concentration in Relation to Vitellogenesis in *Dysdercus cingulatus*

Blood proteins in the female insects generally increased in concentration during the earlier part of vitellogenesis[1–4], and the growing oocytes took up at least some of the blood proteins unaltered through the follicle epithelium into the oocytes by pinocytosis[5,6]. The mechanism involved in the control of build-up of the blood proteins and their entry into oocytes was different in different insects[7–9]. In most insects the median neurosecretory cells of the brain stimulated synthesis of the proteins and their subsequent release into blood, whereas the corpus allatum facilitated their entry into the oocytes[2,8,10,11]. However, in *Leucophaea* the corpus allatum was concerned with both these functions[7,9]. The present study was undertaken in an attempt to elucidate the mechanism of vitellogenesis in the red cotton bug *Dysdercus cingulatus*.

Material and methods. Animals were reared in the laboratory on soaked cotton seeds and water. Females were studied on each day of adult growth for 6 days, those at emergence being labelled 0-day-old. Blood from females was collected by cutting the antennae and its protein concentration was studied by the method of GORNALL et al.[12] using a Bausch and Lomb Spectronic 20 spectrophotometer and microcell accessories. Bovine serum albumin (Sigma) was used as the standard. The protein yolk in the oocytes was studied histochemically by staining with Millon's reagent and by mercury bromphenol blue method as described by PEARSE[13].

Results and discussion. Results of the studies on blood proteins are shown in Figure 1. It may be seen that blood proteins almost doubled on day 2, inspite of which, there was no protein yolk in the oocytes of these females (Figure 2a). However, plenty of protein yolk was present in oocytes from ovaries of day 3 animals (Figure 2b). Yolk deposition continued in day 4 animals also (Figure 2c). Evidently, vitellogenesis took place only some time after blood proteins considerably increased in concentration, as in the desert locust[1]. The fact that by day 4, protein concentration in the blood fell to the level

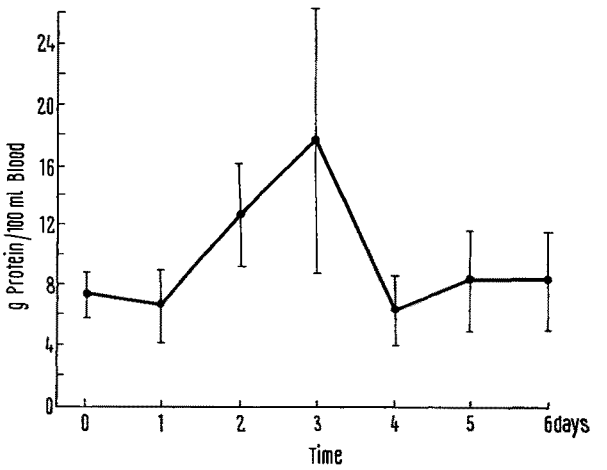

Fig. 1. Mean haemolymph protein concentration in g/100 ml blood, of 0- to 6-day-old females of *Dysdercus cingulatus*, bovine serum albumin taken as standard. Vertical bars represent standard deviation of the mean.

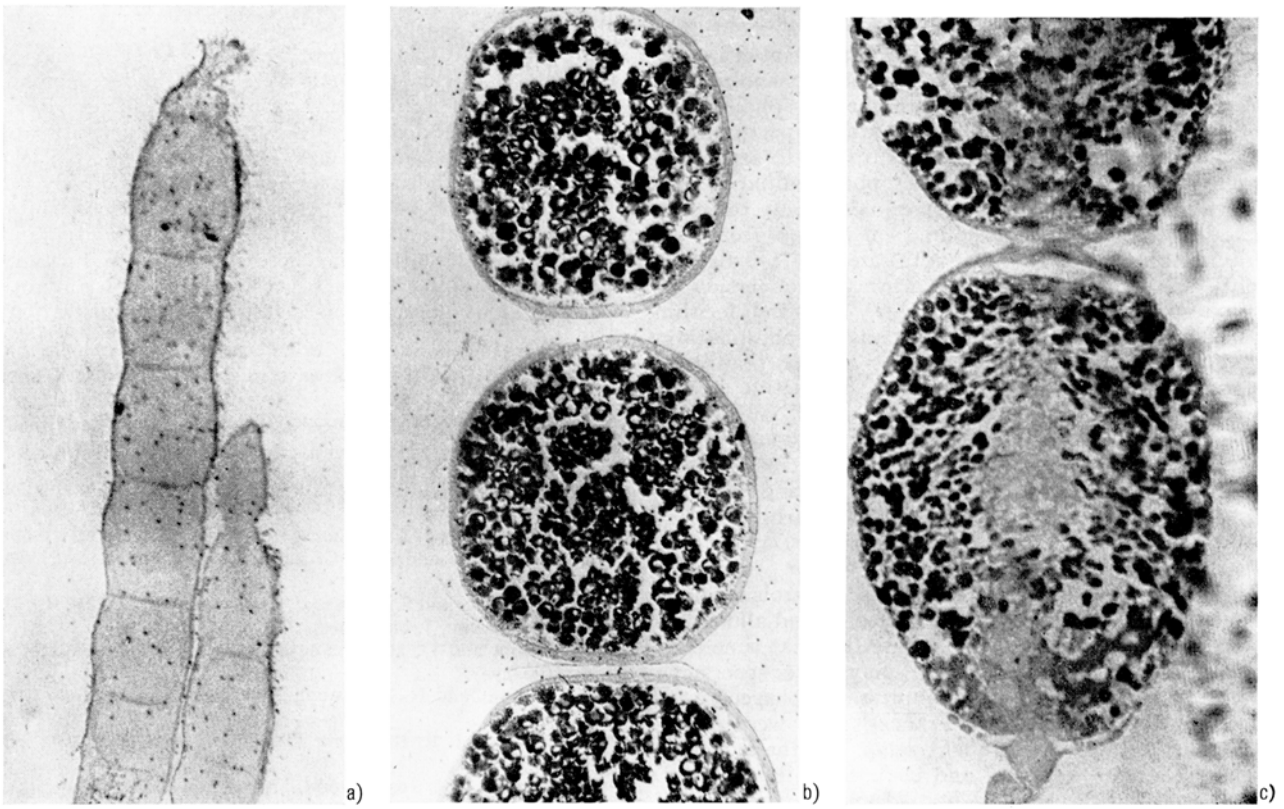

Fig. 2. Sections of ovarioles of: a) 2-day-old, b) 3-day-old and c) 4-day-old *Dysdercus cingulatus* under identical magnification. Millon's reaction. No protein yolk in 2-day-old oocytes whereas abundant protein yolk in 3-day-old oocytes, and still more in 4-day-old oocytes.

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of day 1 showed that all the excess proteins were apparently utilized by the growing oocytes. Inspite of low blood protein concentration, vitellogenesis continued on day 4. Vitellogenesis failed to occur till day 2 not because of the absence of any vitellogenic proteins in the blood. Thus it appeared that protein build-up in the blood on the one hand, and its deposition as yolk in the oocytes on the other, were controlled by 2 factors in *Dysdercus cingulatus*: the former probably by neurosecretion from the brain and the latter by the corpus allatum hormone, as in *Schistocerca*[2,8,10,11].

Zusammenfassung. Nachweis, dass Blutproteine bei der Wanze *Dysdercus cingulatus* die Konzentration am 2. Tag nach der Eiabstossung verdoppeln, ohne dass Eiweissdotter in den Oozyten auftritt.

M. JALAJA and V. K. K. PRABHU[14]

Department of Zoology, Kerala University, Kariavattom, Trivandrum (India), 10 July 1970.

Microsomal Phospholipid Biosynthesis after Phenobarbital Administration

Hypertrophy of the endoplasmic smooth membranes in the hepatocyte is a common response to the microsomal enzyme induction after administration of a large number of drugs and pesticides[1,2].

The mechanism of the phospholipid-rich lipoprotein matrix overgrowth is unclear. Previous reports on the microsomal phospholipid synthesis, an indicator of the lipoprotein turnover, are conflicting. ORRENIUS et al.[3,4] found an increase of phospholipid synthesis in the liver microsomal membranes of rats treated with phenobarbital. Using the same ^{32}P-labelling technique. HOLTZMAN et al.[5] recently drew the conclusion that after phenobarbital treatment the synthesis of membrane phospholipids is not increased and thus the membrane hypertrophy is due to a slower catabolism. It is difficult to settle the problem because the label of tissue phospholipid in vivo after a pulse of radiophosphorus measures only the complete synthesis of phospholipid molecules from the terminal phosphate group of ATP and the 1,2-diglyceride[6]. Two other metabolic pathways, conversion of lysophospholipids to phospholipids[7] and the lecithin synthesis by successive methylations of phosphatidyl ethanolamine[8] are not evaluated by the ^{32}P phosphate incorporation. These two pathways are active in the endoplasmic reticulum.

The present experiment studies the behaviour of these different pathways in the rat liver microsomes by simultaneous labelling with ^{32}P-phosphate and ^{14}C-fatty acid after a single administration of phenobarbital.

Material and methods. 12 male Sprague Dawley rats (Charles River, C.D.), 200–210 g body weight, were given 80 mg/kg of phenobarbital (Merck) i.p. Controls received 1 ml of saline. All the animals were fasted and allowed to drink 20% (W/V) glucose for 24 h. After that time each animal received i.v. 0.5 mc of ^{32}P-phosphate, spec. act.: 20 μci/μg and 2 μci of ^{14}C-palmitic acid, spec. act.: 50 mci/m*M* (C.E.A. Saclay, France). Rats were exsanguinated 1 h later under slight ether anesthesia. Blood was collected on heparine and centrifuged in the cold. Livers were rapidly scissed, blotted and weighed. 1 g of tissue was homogeneized with 10 ml of chilled 5% trichloroacetic acid. After centrifugation, soluble phosphorus[9] and radioactivity were measured in the supernatant. Aliquots were used for P determination[9] and radioactivity counting. Microsomal membranes were isolated[10] and aliquots were used for anilinhydroxylase[10] and protein[11] determinations. Lipids were extracted by the FOLCH's technic[12] and lipid phosphorus was measured[9].

Phospholipids were separated by thin layer chromatography (Silicagel G Merck). The spots of individual phospholipids marked after exposure to iodine vapours were scraped off into counting vials. After 10 min at 100 °C in the oven, the vials were dried under vacuum, then 10 ml of scintillating solution (Toluene, PPO, POPOP) were added; the ^{14}C and ^{32}P radioactivity was counted 3 h later in a Mark II spectrometer (Nuclear Chicago) in channels set up for ^{32}P and ^{14}C respectively.

Results and discussion. After a single phenobarbital dose the protein and the total phospholipid contents of the microsomal fraction were increased (Figure 1). Furthermore anilin hydroxylase activity increases in accordance with previous observations[5]. Repeated administrations

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