Chapter 8

HISTOCHEMICAL STUDY OF RIBONUCLEIC ACID CONTENT OF THE FAT BODY OF POICELOCERA PICTA DURING OVARIAN DEVELOPMENT AND VITELLOGENESIS

The fat body of insects is known to provide reserve food material for the production of eggs. The transport of fat is believed to be in the form of lipoprotein which is believed to be carried through blood to the growing oocyte (Telfer, 1958). The blood proteins are known to be synthesized by the fat body in the silk worm, Bombyx mori (Shigematsu, 1958). Since protein yolk forms one of the major forms of reserves in the mature egg and is progressively added during oocyte development, changes may be expected in the blood and fat body of the animal during ovarian development. Cellular systems engaged in protein synthetic activity invariably show a concommitant level of ribonucleic acid turnover. In consideration of this possible functional nature of the fat body cells, the present study dealing with a general histochemical investigation of the relative concentration of ribonucleic acid, maintained in the cells of the fat body at different times during the period of ovarian development and vitellogenesis in Poicelocera picta, has been undertaken.

MATERIALS AND METHODS

Nymphs of Poicelocera picta in various stages of

development were collected during the months of July-August from the fields where they are found on their food plant Calotropis sp. They were reared in laboratory cages on fresh leaves of Calotropis and were exposed to sunlight during day time. Newly emerged adults were separated and reared in mixed cultures (containing both males and females) in cages containing at the bottom a thick layer (about 9") of loose soil to enable the insects to oviposite.

The insects were dissected after decapitation and immersed in the fixative mentioned below. Sheets of perivisceral fat body tissue mostly from the dorsal side of the alimentary canal and gonads were dissected out of the animal and separated from the intervening tracheae. The material was spread on clean slides and blotted free of excess fixative. After ensuring adherence of the tissue to the slide, the slides were treated with Carnoy fluid (3:1) for two houfs.

Nucleic acids were studied by the methyl-green-pyronin technique. The stain was standardised according to the method of Kurnick as outlined by Pearse (1960). A 2% solution of methyl green and pyronin Y were separately treated with chloroform by constant shaking in separatory funnels before use.

Slides were brought to water after passing through

various grades of alcohol, stained for 6 minutes in a mixture of methyl-green and pyronin Y (12.5 ml pyronin, 7.5 ml methylgreen and 30 ml distilled water), excess stain blotted with a filter paper and differentiated in two changes of n-butyl alcohol, 5 minutes in each. Slides were mounted in DPX after clearing in xylene for 5 minutes.

Total nucleic acid content and ribonucleic fraction were selectively extracted from two different sets of slides prior to staining with methylgreen-pyronin in order to characterize nucleic acid basophilia and to check staining specificity. Total nucleic acid was extracted by treating thee slides with 5% trichloracetic acid at 90°C for 10 minutes. Ribonucleic acid removal was accomplished by treating the slides for 3 hours at 37°C with 0.4% ribonuclease in glass distilled water. Ribonuclease was boiled prior to use for removal of proteolytic enzymes present if any.

Fat body was taken from animals of various ages and the development of the ovarioles was determined by measuring the length of the terminal oocytes as described in chapter 7.

RESULTS

Since in gauging the relative nucleic acid content, the staining reaction is of initial concern, attention was given to the slides treated with trichloracetic acid and ribonuclease prior to staining with methylgreen-pyronin.

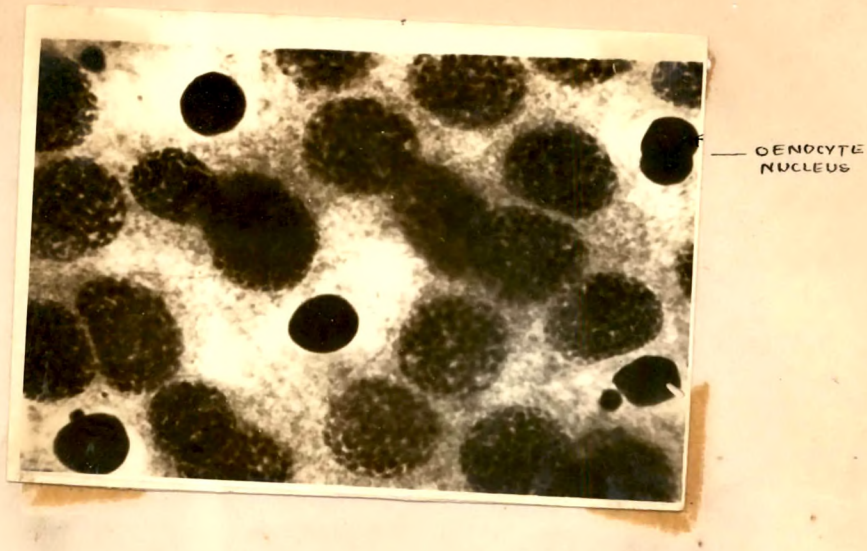

FIG. 1

Photomicrograph showing the granular distribution of RNA basophilia in the fat body cells. The cytoplasm of the oenocytes is free of any pyronin staining. DNA staining in the nuclei of the fat body cells is granular and green while that in the nuclei of the oenocytes, homogeneous and deep pink.

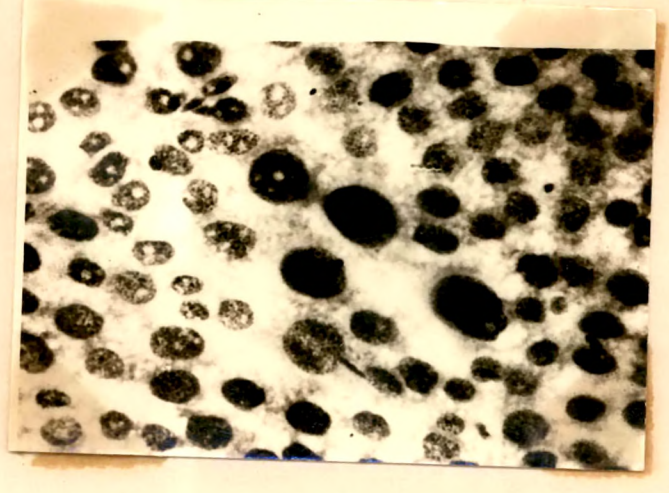

FIG. 2

Photomicrograph showing the fat body cells with nuclei of different sizes. Note a group of five gaint nuclei in the centre.

In all cases ribonuclease treatment completely abolished staining in cytoplasmic and nucleolar areas (fig. 5). Also a distinct reduction in the general basophilia of the nucleus was noted. Observation were made on the normal fat body cells as well as oenocyte cells which are found interspersed in the fat body.

With methlgreen- pyronin staining the nuclei of the fat body cells stained green while the cytoplasm and nucleoli stained bright pale pink.

The fat body at a particular time of observation always contained a few cells which were at different phases of activity but the majority of the cells showed same activity. The fat body contained mainly two types of cells, one with gaint nuclei and the other with small nuclei. Cells with intermediate forms of nuclei were also observed (fig. 1 and 2).

Changes could be observed in the frequency of the occurrence of giant nuclei during the various phases of ovarian development. The frequency of their occurrence increased during oocyte development (terminal oocyte length 4.0 mm to 7.0 mm). They were most common about 72 hours before oviposition. These giant nuclei at this stage were found often to divide into giant daughter nuclei (Fig.3). The nuclei of the cells contained varying number of nucleoli.

In the early stage of oocyte development, the nucleus contained one or two nucleoli. The nucleolar number tended to increase (fig. 6) during the period of development of the terminal oocyte and the animals with mature oocytes contained often 4-6 nucleoli in the nuclei of their fat body cells. Some of the nucleoli at a late stage of oocyte development showed negative reaction for RNA. At the same time vacuolization of the nuclei was discernible, probably owing to the fusion of the nucleoli (fig. 6 and 7).

FIG. 3

Photomicrograph showing the division of gaint nuclei of the fat body cells.
Length of terminal oocyte = 1.5 mm.
24 hours after oviposition.

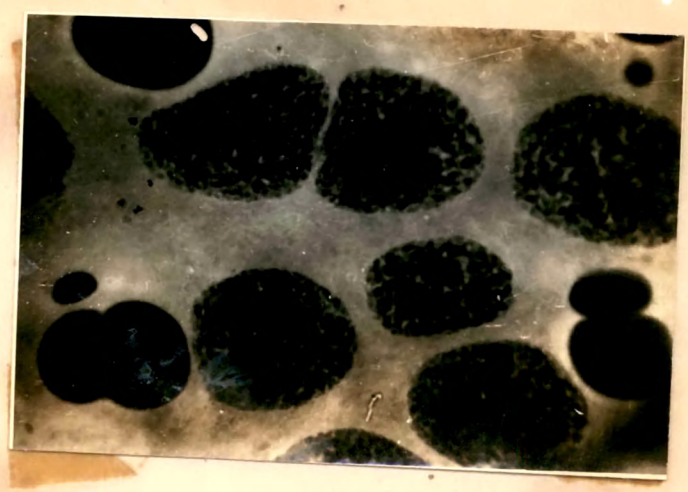

FIG. 4

Photomicrograph showing the division of gaint nuclei of fat body cells as well as that of nuclei of oenocytes. Length of terminal oocyte=9 mm.

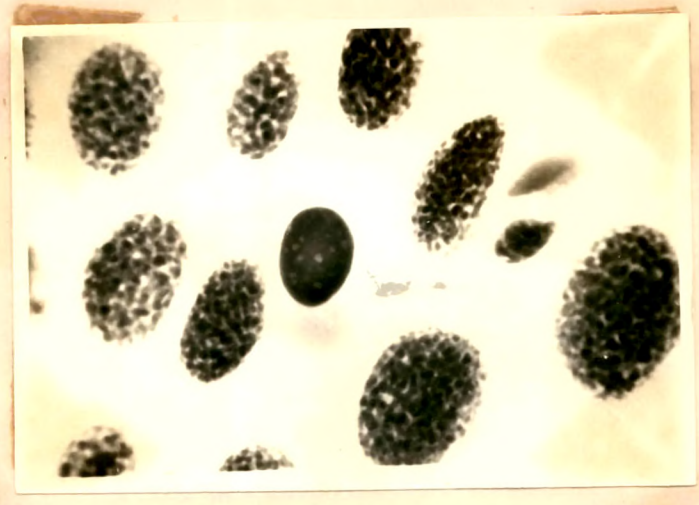

FIG. 5

Photomicrograph showing the control for ribonucleic acid. Treated with a solution of 0.4% ribonuclease at 37°C for 3 hours.

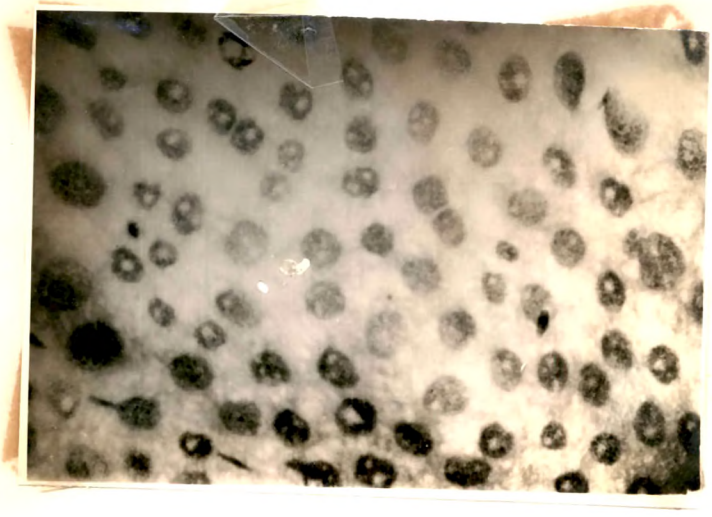

FIG. 6

Photomicrograph showing the predominence of nucleoli in the nuclei of the fat body cells. Vacuolization and sinking of the perinuclear cytoplasm is seen. Length of terminal oocyte=7.7 mm. (48 hours before oviposition).

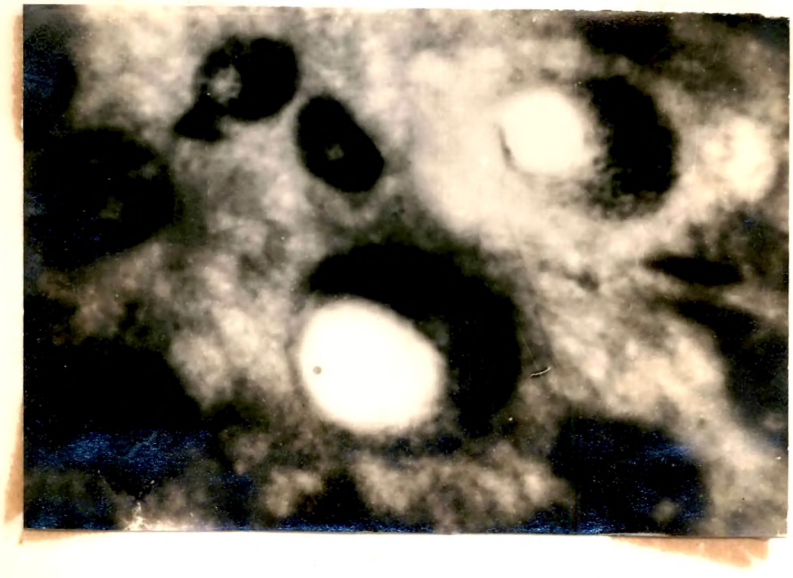

FIG. 7

Photomicrograph showing a portion of figure 6 enlarged.

Throughout most part of the ovarian development a high level of RNA basophilia in the cytoplasm of the fat body cells was observed. It tended to increase appreciably during the time the terminal oocyte increased from 4.0 mm to 7-8 mm in length. After this there was a progressive decrease in the density of the perinuclear cytoplasmic RNA basophilia and the vacuolization of the general cytoplasm was evident. After 24 hours of oviposition, though the condition of the fat body was similar to that of a mature animal, a significant level of RNA was maintained in it.

The dividing nuclei always had dense RNA basophilia in their cell cytoplasm.

An interesting observation was regarding the oenocyte cells which occur interspersed among the fat body cells. Their cytoplasm did not show any RNA basophila at any stage while their nuclei stained deep pink (fig. 1) unlike the nuclei of the fat body cells, which stained green. The nuclei of the oenocytes stained light pink in control slides treated with ribonuclease.

DISCUSSION

The present state of knowledge indicates that nucleic acids of insects are similar in their monomeric composition to the nucleic acids of other organisms (Wyatt, 1959). The

main constituents of insect ribonucleic acid are the four usual bases plus a trace of 2-methyladenine (Wyatt, 1959). So any objection to the application of the methylgreen-pyronin technique to study nucleic acids in insects may be ruled out.

Most authors believe that protein synthesis is intimately connected with the synthesis of ribonucleic acid and that ribonucleic acid participates directly in protein synthesis. Insect fat body has been shown to synthesize blood protein (Shigematsu, 1958). Using the fat body of adult leafhoppers, Littau (1960) showed that the cytoplasmic sites of protein synthesis to be virtually identical with those of ribonucleic acid. She further showed that by using azur B at pH 5.0 the protein was located in almost all the structures where ribonucleic acid is present i.e. cytoplasm, nuclei and nucleoli.

Changes in nucleic acid levels has been observed by some workers during embryogenesis, metamorphosis and adult development of insects. Chino (1956) observed that during the early phase of growth and cell multiplication in Bombyx, both ribonucleic acid and deoxyribonucleic acid increased several fold. During larval life there was a reduction of both RNA and DNA in relation to body weight as most of the food intake was channeled into nutrient reserves for metamorphosis. During adult development

RNA and DNA both increase in relation to body weight as adult structures are formed and food reserves diminish (Niemierko et al, 1956). During ovarian development the the food reserve of the fat body is known to diminish (chapter, 7).

During the period of oocyte development the fat body cells undergo fluctuations in ribonucleic acid basophilia which presumably reflects associated changes in RNA metabolism. The high content of RNA observed in the fat body of Poicelocera picta undoubtedly suggested the high level of protein build-up going on in these cells. Preceeding the growth of terminal oocytes there was an initial build-up in RNA concentration in the fat body cells. A significantly high level of cellular RNA content was maintained throughout the period when yolk deposition in the oocyte took place. Just before oviposition (about 48 hours) and after egg laying, the RNA level was diminished.

Bonhag (1959), who made a detailed study of nucleic acids during oogenesis of Periplaneta americana found that of the three growth periods, the cytoplasmic RNA in the oocyte reached its highest concentration durig the first part of the second growth period. He related the large amount of RNA present to the high level of protein synthesis

going on in the oocyte during the second growth period. During the third growth period which is characterised by the formation and deposition of protein yolk, a low level of RNA was seen. He further observed that the cytoplasm of the follicular epithelium was rich in RNA at all times. Bier (1954) and Colombo (1955) working on the honey bees and the grasshoppers respectively, concluded that the low level of RNA in the yolk forming oocytes meant that protein was no longer being synthesized by the oocyte, only the RNA rich follicular cells were actively carrying on protein synthesis and that these materials were merely being deposited as protein yolk. However Telfer (1958) demonstrated the route of transfer of lipoproteins from the blood to the growing oocyte of Cecropia moth. So it is possible that the RNA rich fat body cells, at the time of yolk formation in the oocyte, may play a definite part in supplementing the protein material required by the blood to transport it to the developing oocyte. The large number nucleoli in the nuclei of the fat body cells also indicates intense activity of the cells at this time.

The lack of any ribonucleic acid basophilia in the oenocytes may exclude these cells of the function of protein synthesis in the fat body of Poicelocera picta. However, the deep pink staining of the nuclei of the

oenocyte cells must be interpreted with caution. Kurnick (1947, 1949, 1950 a,b) considered that differences in stainability of chromosomal and cytoplasmic nucleoproteins by methylgreen- pyronin might be a matter of degree of polymerization. Using the same two dyes, together and separately to stain DNA and RNA in vitro, he found that the highly polymerized DNA stained with pyronin about 1/5 as intensely as RNA and the DNA histone stained about 1/6 as intensely. However, the depolymerized DNA stained as strongly as RNA with pyronin. Using the two dyes together he found that RNA stained pale pink, depolymerized DNA to a similar colour and polymerized DNA green. It is possible that the DNA in the fat body cell nuclei and that in the oenocyte nuclei may differ in its degree of polymerization.