Vol. 133, No. 2 October, 1967

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

HEMOLYMPH PROTEINS AND REPRODUCTION IN PERIPLANETA AMERICANA: THE NATURE OF CONJUGATED PROTEINS AND THE EFFECT OF CARDIAC-ALLATECTOMY ON PROTEIN METABOLISM[1]

K. G. ADIYODI AND K. K. NAYAR

Department of Zoology, University of Kerala, Trivandrum-7, S. India

That cardiacectomy results in a loss of urates from the fat bodies (Bodenstein, 1953) of the American cockroach is indicative of a role for the corpora cardiaca in protein metabolism, and it is quite possible that this effect may be related to the neurosecretory factors abundantly stored in this organ (Scharrer, 1955). The corpora cardiaca are also known to play a role in carbohydrate metabolism: administration of semi-purified extracts of the cardiaca results in this species in increased blood trehalose levels at the expense of fat body glycogen, and this has been found to be the result of increased phosphorylase activity (Steele, 1961, 1963). Evidence has accumulated to suggest that the corpus allatum plays a role in lipid and protein metabolism in female *Periplaneta americana:* allatectomy induces fat body hypertrophy (Bodenstein, 1953; Mills, Greenslade and Couch, 1966) and slows the turnover of phospholipid and triglyceride fractions (Vroman, Kaplanis and Robbins, 1965); it affects RNA synthesis (Thomas and Nation, 1966b) and results in adult females in increased blood protein concentration due to non-utilization (Mills, Greenslade and Couch, 1966). The corpora allata are known particularly to influence the formation and utilization of a sex-specific protein in adult females of *Periplaneta americana* (Menon, 1963, 1965; Adiyodi and Nayar, 1966; Thomas and Nation, 1966a). The nature of the conjugated proteins of this species in relation to the molting and clotting processes has been described in some detail by Siakotos (1960a, 1960b), using paper electrophoresis and various staining procedures. Very little information, however, is available regarding the behavior of the conjugated proteins in relation to reproduction and the effect of the total removal of the cardiacum-allatum complex on the biosynthesis, chemical composition and mobilization of plasma protein fractions in adult females of *Periplaneta.*

[1] Supported by grants from the Ford Foundation. We are thankful to Dr. K. K. Thomas, Northwestern University, Evanston, Illinois, for reading the proofs for us.

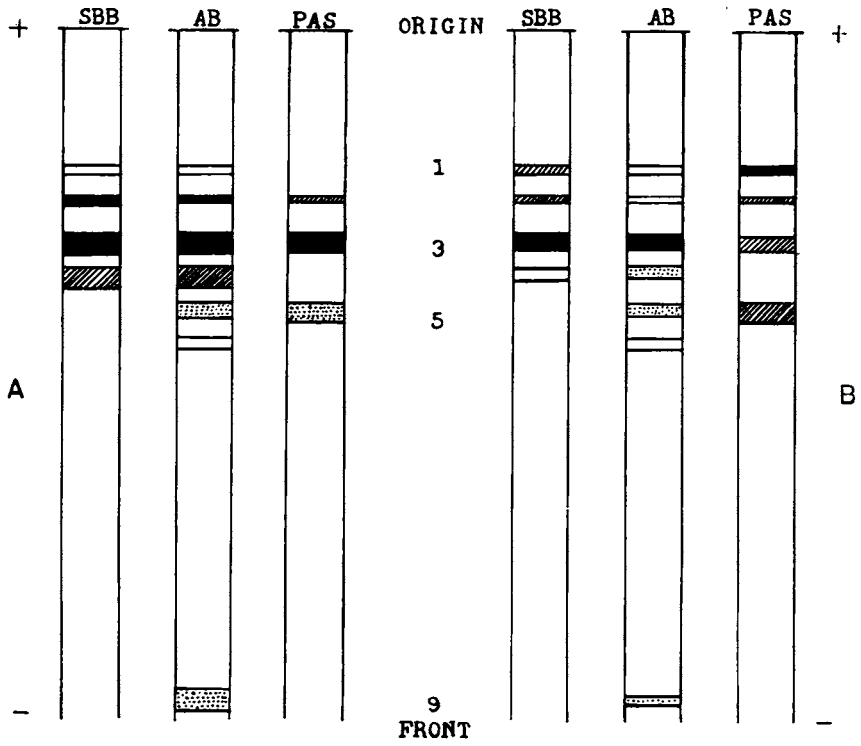

FIGURE 1. Electrophoretogram showing the nature of hemolymph proteins during the pre-ovipositional period in adult female *Periplaneta americana*. Length of basal oocytes (A) 2.70 mm. and (B) 3.36 mm. AB, Amido Black; PAS, Periodic acid-Schiff; SBB, Sudan Black B.

MATERIAL AND METHODS

For cardiac-allatectomy only adult females of the American cockroach *Periplaneta americana* (L.) bearing oothecae were used. The cardiacum-allatum complex was carefully removed after ether anaesthesia through a slit made on the dorsum of the head behind the brain and between the compound eyes, taking care to see that the frontal ganglion and the oesophageal nerve were left intact. A few crystals of AMBISTRYN were placed in the wound, before it was closed with the original flap of the cuticle, and sealed with molten paraffin. Post-operative mortality was about 20% during the first week, but much less during the subsequent period. The animals were maintained on biscuits and water was provided *ad libitum*.

The neck was ligatured in a few animals bearing the ootheca, as this process is likely to induce a condition similar to that of cardiac-allatectomy. Their blood samples were analyzed after 4 days. Such animals showed some decrease in blood volume and, therefore, adequate amounts of hemolymph were obtained by centrifuging whole animals.

The hemolymph has been chosen for our investigations as it is a medium that is likely to reflect changes, if any, in protein metabolism. Blood samples were

TABLE I

Composition of hemolymph proteins in normal adult females of Periplaneta americana in relation to the ovarian cycle

Nature of the ovary or stage of reproduction	Protein fractions	Proteins (% of total distribution)	Lipids (% of total bound lipids)	Carbohydrates (% of total bound carbohydrates)
Average length of basal oocytes 2.70 mm.	1	5.38	6.77	—
	2	7.60	16.15	23.27
	3	52.94	72.92	46.82
	4	16.54	4.16	—
	5	6.18	—	29.91
	6	1.90	—	—
	9	9.46	—	—
Ootheca formed and tanned	1	5.55	13.19	12.50
	2	2.78	14.65	52.08
	3	35.42	72.16	—
	4	48.61	—	—
	5	3.12	—	35.42
	6	1.74	—	—
	9	2.78	—	—

taken from females 4, 7, 18, 30 and 60 days after the operation, and fractionated for proteins by disc electrophoresis in polyacrylamide gel columns as already described (Adiyodi and Nayar, 1966) using Amido Black (AB) as the staining dye. The success of the operation was tested by autopsy after bleeding, and the nature of the ovary noted in each case.

For the demonstration of glycoproteins the samples were electrophoresed as usual for 25 minutes and the gel columns then immersed in 7½% glacial acetic acid for 1 hour at room temperature. The columns were immersed in a 0.2% solution of periodic acid and placed in a refrigerator for an equal duration. Periodic acid was later removed electrophoretically for 1 hour using 7½% glacial acetic acid. The gel columns were placed in Schiff reagent and incubated in a refrigerator until the red bands became distinct. They were then removed and kept at room temperature in stoppered tubes.

Lipoproteins of the hemolymph were identified by staining the gel columns with Sudan Black B (SBB) in the manner described by Whittaker and West (1962).

Blood samples and tissue homogenates of fat bodies of normal adult females in different stages of vitellogenesis, homogenates of ovaries (average length of basal oocytes: 3.456 mm.) and blood samples of adult males were analyzed for information on conjugated proteins similarly by staining in periodic acid-Schiff reagent (PAS) and SBB. As far as possible electrophoresed blood or homogenate samples of the same individual were variously stained with AB, PAS, and SBB in one lot and this made easy the comparison of the hemolymph protein patterns, particularly of the conjugated proteins. The protein fractions in the stained gel columns have been numbered from the "origin," represented by the top of the small pore gel, to the "front," and their quantitation was done with a Canalco Model E Microdensitometer.

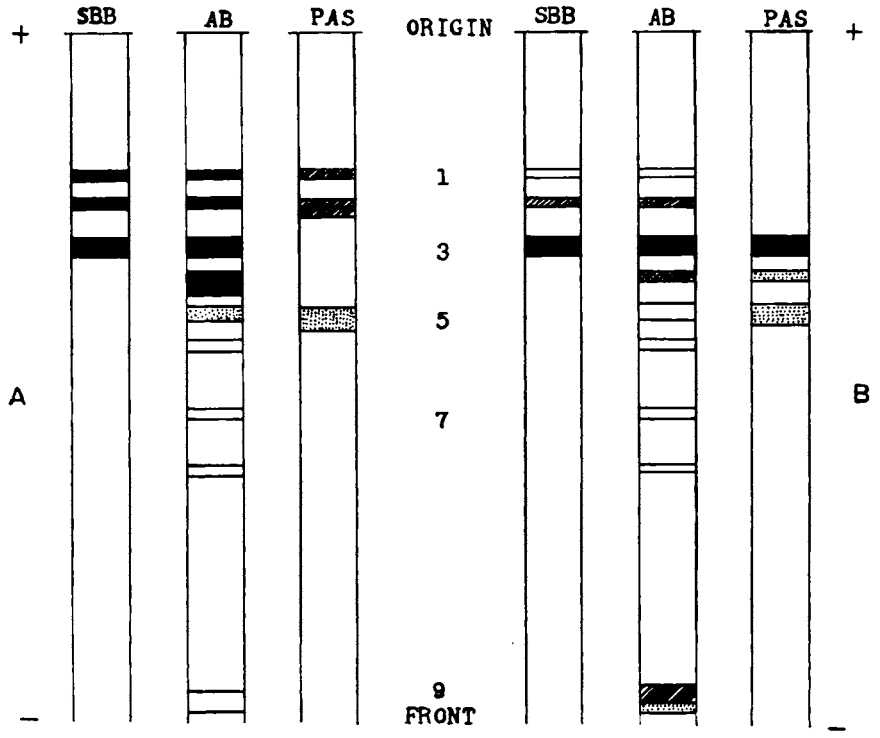

FIGURE 2. *Periplaneta americana*. Schematic representation of hemolymph proteins of (A) adult female with ootheca fully protruded and tanned, and (B) adult male. AB, Amido Black; PAS, Periodic acid-Schiff; SBB, Sudan Black B.

RESULTS

Nature of conjugated proteins in normal animals

In normal adult female cockroaches there are in all 9 detectable protein fractions stainable with AB in the hemolymph, 3 and 4 being the major proteins (Adiyodi and Nayar, 1966). In animals with protruding ootheca and also in their

TABLE II

Composition of hemolymph proteins in adult males of Periplaneta americana

Protein fractions	% of total distribution	% of bound lipids	% of bound carbohydrates
1	2.61	9.32	—
2	8.00	24.16	—
3	49.22	66.52	69.64
4	11.76	—	7.14
5	2.60	—	23.22
6	5.12	—	—
7	2.08	—	—
9	18.61	—	—

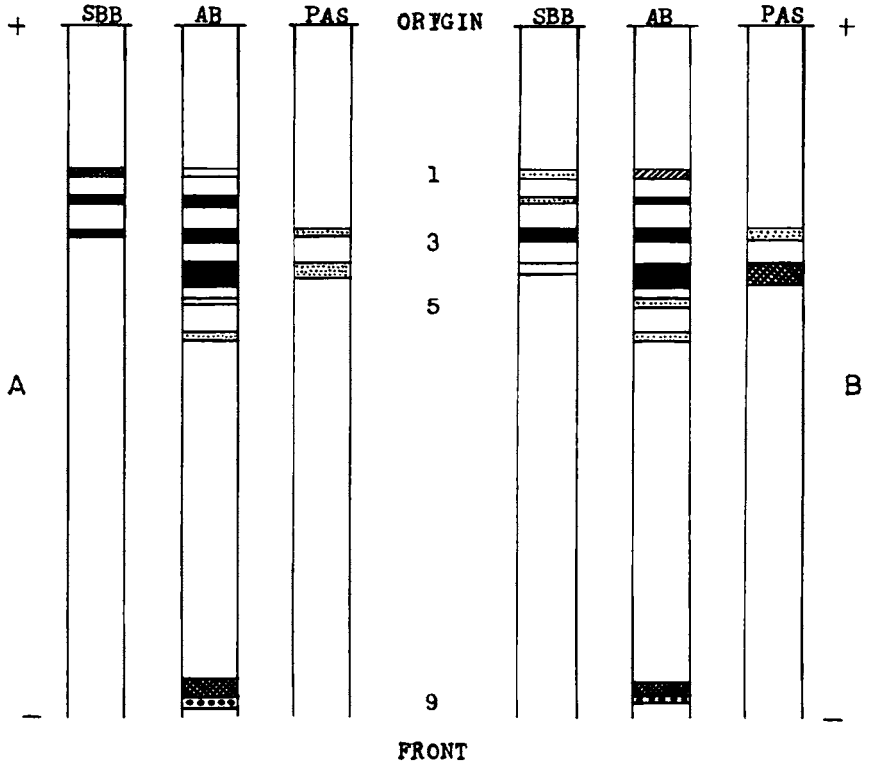

FIGURE 3. Pattern of hemolymph proteins of adult female *Periplaneta americana* 4 days after (A) Cardiac-allatectomy and (B) neck-ligaturing. AB, Amido Black; PAS, Periodic acid-Schiff; SBB, Sudan Black B.

pre-oviposition period, fractions 1 and 2 stain as lipo- and 2, 5 and 1 as glycoproteins (Figs. 1, 2). Fraction 2 generally and 1 often may, therefore, be said to occur as glycolipoprotein complexes.

Fraction 3 is a strongly indicated lipoprotein and contains, in the case of cockroaches constructing and bearing the oothecae, almost three-fourths of the bound lipids (Table I); during pre-ovipositional ovarian growth this appears to assume the characteristics of a glycolipoprotein complex, as it has been found to stain positively with PAS and SBB. The other fractions, including the female blood protein represented by fraction 4, reacted negatively with both the selective biochemical staining agents during periods of accumulation as while carrying the ootheca, but in the pre-ovipositional stages fraction 4 was found to show a mild reaction for lipids. Fraction 9, when present, was often bipartite and appears to represent some chromoprotein. The fat bodies of normal adult females taken at different stages of vitellogenesis gave uniformly a negative reaction with PAS and SBB; so did the homogenates of the ovary with yolk-laden eggs.

In males fraction 3 stains strongly positively with PAS and SBB, but contrary to the situation in females, fraction 4, which is present only in small amounts in

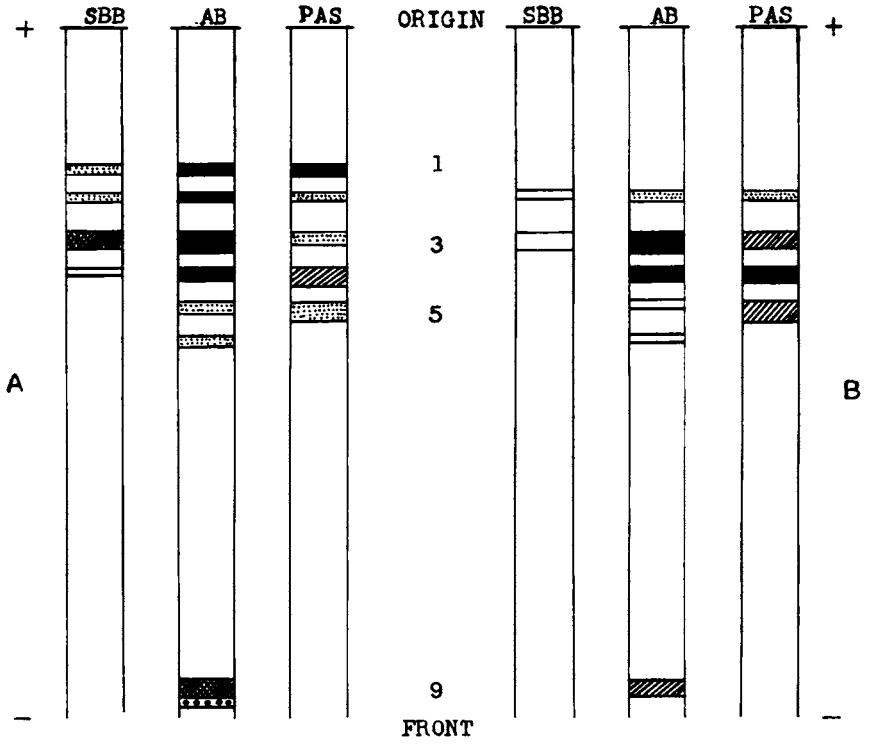

FIGURE 4. Electrophoretogram of hemolymph proteins of adult female *Periplaneta americana* (A) 7 days and (B) 18 days after cardiac-allatectomy. AB, Amido Black; PAS, Periodic acid-Schiff; SBB, Sudan Black B.

the male cockroach plasma, is non-sudanophilic, and reacts often with PAS (Fig. 2). Fraction 5 stains preferentially with PAS and has been found to contain more than one-fifth the net protein-bound carbohydrates (Table II) in the male hemolymph.

Pattern of hemolymph proteins in cardiac-allatectomized females

Cardiac-allatectomy in regular laying adult females suppresses ovarian activity by inhibiting vitellogenesis normally in about 7–12 days after the operation. Such females may produce either no ootheca at all, or deposit one or very rarely two of them before terminating the reproductive cycle. Rarely individuals have been found to deposit oothecae even 3–5 weeks after the operation; the number of oothecae deposited during this postoperative period, being few, in the order of 2 or 3. Sham-operated females did not differ from the normal either in the frequency of ootheca production or in their hemolymph protein patterns.

Figures 3–6 give a schematic representation of the hemolymph proteins of adult females 4, 7, 18, 30 and 60 days after cardiac-allatectomy. The experimental females exhibited uniformly an accumulation in fraction 4, and this tendency was quite evident even in animals examined four days after cardiac-allatectomy (Fig.

TABLE III

Effect of cardiac-allatectomy and neck-ligaturing on hemolymph proteins in adult female Periplaneta americana

Protein fractions	% concentration of the proteins					
	Neck-ligatured	Cardiac-allatectomized				
	4 days	4 days	7 days	18 days	30 days	60 days
1	1.79	1.83	3.67	—	4.82	9.86
2	6.74	7.83	4.42	7.54	7.27	9.42
3	7.49	9.46	52.84	46.54	45.94	47.98
4	65.87	56.75	27.74	33.62	33.57	26.01
5	4.49	0.78	2.27	1.80	3.36	3.59
6	2.69	2.61	2.27	1.50	3.91	2.25
9	10.93	20.74	6.79	9.00	1.13	0.89

3). Fraction 3, on the contrary, showed a marked decline in the amount of stainable material during the initial stage, much as in starved females (Adiyodi and Nayar, 1966). Thus in 4-day post-cardiac-allatectomized animals fraction 3 was nearly as extensive and strong as the minor band representing fraction 2. In 4-day experimentals fractions 1–3 stained for lipids and 3–4 for carbohydrates. Fraction 3 in these animals thus appears to be in the nature of a glycolipoprotein; it stained at this stage preferentially and strongly with SBB and gave only a comparatively mild reaction with PAS.

Seven-day post-cardiac-allatectomized females showed a conspicuous accumulation of stainable material in all fractions from 1–4 (Fig. 4). A bipartite "front" fraction (9) was conspicuous in gel columns stained with AB, much in the same form as in 4-day experimentals. Fraction 3 was stronger and more extensive and came to comprise as much as half the soluble proteins in the plasma, and this was attended with a corresponding decrease in the amount of stainable material in fraction 4. Fraction 3 had come to contain nearly two-thirds of the protein-bound lipid present in the hemolymph (Table IV). Fraction 4 also reacted positively though mildly with SBB. More striking, however, was the fact that all protein fractions from 1 to 5 gave a positive reaction for carbohydrates. If staining

TABLE IV

Distribution of protein-bound lipids in the hemolymph of cardiac-allatectomized adult female Periplaneta americana

Protein fractions	% concentration of protein bound lipids				
	4 days	7 days	18 days	30 days*	60 days
1	13.71	16.14	—	—	17.95
2	22.75	15.69	7.32	—	15.38
3	63.54	63.23	92.68	100.00	66.67
4	—	4.94	—	—	—

* Variable; for explanation see text.

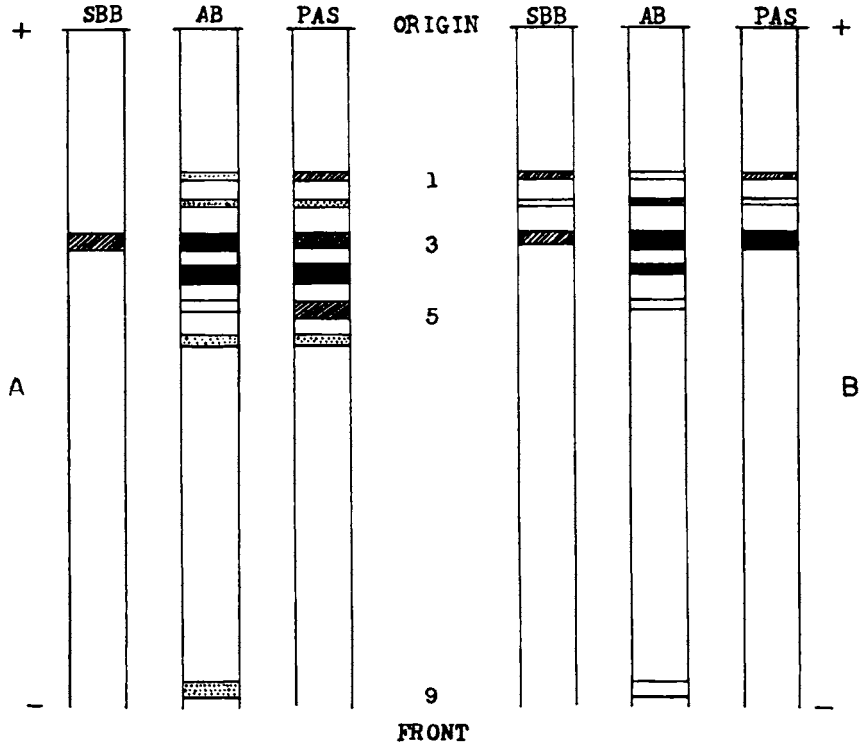

FIGURE 5. Disc electrophoresis of hemolymph proteins in 30-day post-cardiac-allatectomized adult females of *Periplaneta americana*. (A) with atrophied ovaries and (B) with basal oocytes 3.12 mm. in length. AB, Amido Black; PAS, Periodic acid-Schiff; SBB, Sudan Black B.

intensity with PAS and SBB is any criterion, respectively, of the concentration of the carbohydrate and lipid prosthetic groups within conjugated proteins, it may be said that the glycolipoprotein fraction 3 of 7-day post-cardiac-allatectomized females contained more lipid and less carbohydrate and conversely its fraction 4 more carbohydrate and much less lipid. There has thus been not only an increase in the number of bands reacting positively with PAS, but also a rise in the amount of material stainable with it.

The hemolymph pattern of 18-day post-cardiac-allatectomized females (Fig. 4) resembled that of 7-day animals in that there was nearly as much accumulation of AB-stainable material in fractions 3 and 4 (Table III) and also in that fraction 3 was likewise in the form of a glycolipoprotein complex. The slow moving fraction 1 was feeble or indistinct in AB, SBB and PAS preparations. Fractions 2–5 reacted with PAS, the most intense accumulation of stainable material being in fraction 4. Fractions 2 and 3 were sudanophilic, but only very mildly so, and contained, respectively, roughly only one-thirteenth and one-sixth the lipids normally bound in these fractions in ootheca-bearing phases.

In 30-day experimental animals all fractions from 1 to 6 have been seen to give

TABLE V

Distribution of protein-bound carbohydrates in the hemolymph of cardiac-allatectomized adult females of Periplaneta americana

Protein fractions	% concentration of protein bound carbohydrates				
	4 days	7 days	18 days	30 days	60 days
1	—	18.42	—	8.07	15.46
2	—	2.11	5.31	5.19	19.32
3	44.29	2.11	21.24	9.51	28.99
4	55.71	37.89	48.67	59.37	36.23
5	—	39.47	24.78	8.93	—
6	—	—	—	8.93	—

a positive and preferential reaction for glycoproteins, and fraction 3 and less frequently 1 and 2 reacted with SBB (Fig. 5). Judged by the intensity of staining with SBB, there appears to have occurred a slight accumulation of the lipid prosthetic groups in fraction 3 compared to 18-day animals. There was a considerable increase in the total amount of material stainable with PAS in the hemolymph, and fraction 4 came to contain as much as 59.37% of the total bound carbohydrates (Table V). This female fraction stained often as a strong glycoprotein with PAS even in those animals in which cardiac-allatectomy had been incomplete, with the anterior portions of the cardiaca remaining inadvertently unremoved during the operation. In a few of the females that showed varying degrees of oocyte growth 30 days after the removal of the cardiacum-allatum complex, fraction 4 was sometimes found to occur in the form of an unconjugated or lipo-protein and only fractions 1–3 stained for carbohydrates.

Experimental animals 60 days after the removal of the cardiacum-allatum complex (Fig. 6) still showed some retention of material in fraction 4, but only in nearly the same amounts as in 7-day post-cardiac-allatectomized animals. The fast moving "front" fraction (9) was almost inconspicuous. Fractions 1–3 reacted with SBB for lipids, 3 rather strongly so, and 1–4 with PAS for carbohydrates. Fraction 3 appeared bipartite with a proximal comparatively extensive staining area containing carbohydrates only in medium amounts and a narrow but densely PAS-positive disc-like part distally confluent with the former. In the electric field the mobility of this bipartite PAS-positive fraction has, however, been found to correspond well with that of fraction 3 in AB-stained gel columns.

Hemolymph proteins in neck-ligatured animals

Adult females with their necks ligatured and bled after 4 days showed slightly more protein in fraction 4 than 4-day post-cardiac-allatectomized females (Table III). This fraction in the former stained positively with SBB unlike that of the cardiac-allatectomized female. Neck-ligatured animals experienced difficulty in depositing ootheca and some of them even showed beginnings of ovarian malfunction. Accumulation in fraction 4 in the hemolymph of these animals is particularly interesting, because in the controls the stainable material in this fraction is low. In gel columns stained with AB, fraction 1 occurred as a weak band, but

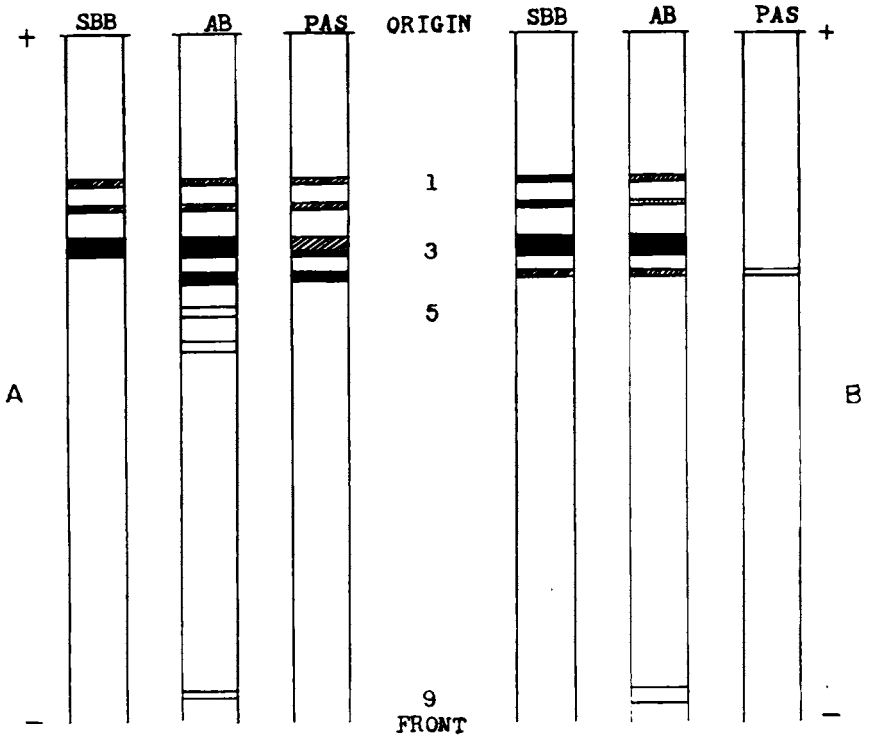

FIGURE 6. *Periplancta americana.* Electrophoretogram of hemolymph proteins of (A) 60 lays post-cardiac-allatectomized adult female and (B) 21 days post-cardiac-allatectomized female, 3 days after implantation of cardiacum-allatum complex. AB, Amido Black; PAS, Periodic acid-Schiff; SBB, Sudan Black B.

the concentration in fraction 3 was almost comparable to that of 4-day post-cardiac-allatectomized animals. In both the cases there was also a strong "front" band representing fraction 9. Further, fraction 3 in both the cases was a glycolipoprotein complex; but fraction 4 reacted positively with PAS and SBB in neck-ligatured animals, whereas in cardiac-allatectomized females it was generally non-sudanophilic (Fig. 3). Fractions 3 and 4, the major proteins, together constituted only 66.21% of the total hemolymph proteins in 4-day post-cardiac-allatectomized females, but 73.36% in 4-day neck-ligatured animals. This increase may perhaps be related to inanition, the consequent general reduction in blood volume and the concentration of metabolites.

Effect of implantation of cardiacum-allatum complex in cardiac-allatectomized females

Though the reproductive ability of the female is impaired on removal of the cardiacum-allatum complex, such experimental females responded readily to the implantation of these organs and matured eggs in about 3–4 days, the normal time taken for a gonadal cycle by regular laying females. Thus 18-day post-cardiac-

allatectomized females implanted with 3 cardiacum-allatum complexes, each taken from adult females, had basal oocytes measuring on the average 3.636 mm. in length when sacrificed 60–66 hours after the implantations.

Twenty-one days after the operation another set of animals were implanted similarly with 3 cardiacum-allatum complexes each and the blood fractionated for proteins 3 days afterwards. All the fractions from 1 to 4 were sudanophilic and fraction 4 alone showed any indications of carbohydrates. Fraction 4 occurred only in small concentrations, judged from its staining intensity in AB, PAS and SBB preparations (Fig. 6).

Discussion

The observations reported here point to the fact that correlated with different phases of ovarian activity there may be not only a change in the relative concentration of the different protein fractions, as already reported by us (Adiyodi and Nayar, 1966), but also differences in the chemical composition of the major proteins. Thus during ovarian growth fraction 3 is in the form of a glycolipoprotein complex, but when the ootheca has been fully formed and tanned, the fraction appears to be freed from the bound carbohydrates and gives a positive reaction for only the lipids. Similarly in the pre-oviposition period fraction 4 stains as a mild lipoprotein, but in females carrying the ootheca, and having this fraction accumulated in the hemolymph it appears to contain only protein and no stainable bound carbohydrates or lipids, as borne out by the negative reaction with SBB and PAS. The minor fractions, *viz.*, 1, 2 and 5, are a little variable in their occurrence, but when present are relatively stable as far as the staining affinities with the selective biochemical agents are concerned through the different stages of the ovarian cycle except perhaps fraction 1. Fraction 2, which is almost uniformly in the nature of a glycolipoprotein, and also 1 show a tendency for accumulation in the ootheca-bearing phase and stain more readily and intensely with SBB rather than PAS at nearly all the stages investigated. Fraction 5 is a monotonous and rather diffuse glycoproteinaceous band in both males and females, with its maximum concentration in ootheca-bearing females.

In 4-day post-cardiac-allatectomized females fractions 3 and 4 together constitute only 66.21% of the total hemolymph proteins, but in 7- and 18-day experimentals it is as much as 80% (Table III). This shows that in cardiac-allatectomized animals there is almost an immediate increase in the amount of proteins in these two electrophoretic components. Initially (*cf.* 4-day experimentals) there occurs a decrease in fraction 3 and a pronounced accumulation in fraction 4, but by a week after the operation the table is turned. But in 18-day animals the content of fraction 3 is again on a slight decline and 4 on the increase, compared to 7-day animals; with still further aging there occurs a reduction in the amount of AB-stainable material in fraction 4 (Table III).

Cardiac-allatectomy results not only in a pronounced accumulation of proteins in the blood, but also in changes in their chemical composition. In normal reproductive cycles, accumulation of the different protein fractions occurs during the ootheca-bearing phase, when fraction 3 stains as a lipoprotein and 4 reacts negatively with both SBB and PAS. In cardiac-allatectomized animals accumulation in 3 and 4 is nearly as pronounced as in ootheca-bearing animals (Tables I, III),

and there is a tendency for both the fractions in the beginning stages to be in the nature of glycolipoprotein complexes (Fig. 4). With aging these two major proteins and also the other fractions stain preferentially for carbohydrates. The relatively slow-moving and usually dense-staining fraction 3 in our electrophoreograms seems to represent the "common insect protein" described by Whittaker and West (1962). A similar alteration in the composition of this common protein and the sex-specific protein has been reported in 14-day post-ovariectomized females of the same species by Thomas and Nation (1966a). They ascribed this phenomenon to the inability of the animal to maintain normal activity of the corpus allatum in the absence of the ovaries. In ovariectomized adult female cockroaches the corpus cardiacum and corpus allatum, in the absence of the normal feed-back from the ovary, become filled with large quantities of secretory material of extrinsic and intrinsic origin, respectively, and release of such materials appears to be considerably restricted (unpublished observations), a condition analogous in some way to cardiac-allatectomy, and whatever proteins synthesized under the influence of these factors may remain in the blood unutilized. Under conditions of allatectomy (Adiyodi and Nayar, 1966), ovariectomy (Menon, 1963, 1965; Thomas and Nation, 1966a) and cardiac-allatectomy there occurs an accumulation in the female blood protein (our fraction 4). Further, this fraction is present in homogenates of enlarged ovary (Adiyodi and Nayar, 1966) and shows variations in the hemolymph which could be clearly correlated with the reproductive cycle. Implantation of cardiacum-allatum complex in cardiac-allatectomized females results in a depletion of this protein already accumulated in the hemolymph and lifts the restraint on ovarian function. All these suggest strongly the possibility that the female blood protein is under the control of the cardiacum-allatum complex, and that it is normally removed from the blood by the ovary. The very similar biochemical behavior of the two major proteins in ovariectomized and cardiac-allatectomized females also suggests it as possible that in *Periplaneta americana* ovariectomy perhaps induces a state in the internal *milieu* physiologically comparable to that of cardiac-allatectomy.

The fast-moving fraction 9, generally found to occur in the blood as a strong bicolored band in cardiac-allatectomized adult females in their early stages and also in 4-day neck-ligatured females, reacts negatively with PAS and SBB, and seems to represent some chromoprotein. What probably looks like the pigment part of this protein(s) is inconsistent in its appearance in normal adult females, and we have not been able to correlate the presence or concentration of this fraction with any event in the reproductive cycle. However, under conditions of stress such as starvation (Adiyodi and Nayar, 1966) and cardiac-allatectomy it has been found that there occurs initially some accumulation of material in this conjugated fraction.

Mills, Greenslade and Couch (1966) observed some sort of a cyclicity in the RNA content of the fat body in the adult female American cockroach and suggest that the fat body may be contributing to hemolymph protein level in the early as well as the last phase of each gonadal cycle. But our fractionation studies on fat body homogenates of females in different stages of vitellogenesis seem to suggest, on the contrary, that none of the soluble lipo- or glycoproteins, including fraction 4, is probably as such synthesized or even stored in the fat body. No fractions other than 9, which appears to be only a tissue protein, could be detected by electro-

phoresing homogenates of this tissue of normal adult females and staining them with either of these three techniques.

Clark and Ball (1956) observed in *Periplaneta americana* lipid associated with each of the six protein fractions tested by them by paper electrophoresis, Stephen and Steinhauer (1957) only in two, and Siakotos (1960a) in 4 out of 5 fractions in the nymph. We have been able to detect lipid only in a maximum of 4 among the 9 fractions at any time during the reproductive cycle. In the case of the lipoproteins the lipid moiety appears to be contributed by the fat bodies to the proteins synthesized elsewhere, probably in the hemolymph itself. This view is in keeping with the suggestion of Gilbert, Chino and Domroese (1965) that triglycerides are synthesized and stored in the fat bodies and liberated into the blood as diglycerides, in which state they become bound to proteins for transport. Menon (1963, 1965) found a conspicuous fall in serum fats in allatectomized females, and it is now well known that the turnover of the triglycerides and phospholipids becomes affected in such animals (Vroman, Kaplanis and Robbins, 1965). Vroman, Kaplanis and Robbins (1965) further observed that 70% of the ovarian lipid is triglyceride and maintained on this ground that the allata regulate the metabolism of phospholipid and triglyceride in *Periplaneta americana* by exerting a control over the utilization mechanisms of these lipids. They ascribed the greater accumulation of the triglyceride fraction in allatectomized females to ovarian failure. We have at present no data regarding the nature of the lipid prosthetic groups of the different lipoprotein complexes in the cockroach plasma. However, the fact that the female blood protein (fraction 4) assumes the biochemical characteristics of a lipoprotein during ovarian growth in normal and also cardiacum-allatum complex re-implanted experimental females suggests the possibility that this fraction and also perhaps fraction 3 may serve normally as carrier proteins in the transport of the diglycerides in addition to the proteinaceous vitellogenic precursors to the ovary. Further, such a view will be also in agreement with the suggestion of Siakotos (1960a, 1960b) that the hemolymph proteins in the American cockroach act as carriers of nutrients such as carbohydrates and lipids in the molting process. It is also interesting to observe that the female sex-specific protein described by Siakotos (1960a) in the cockroach nymphs (his fraction III) is in the nature of a lipoprotein. In *Hyalophora cecropia* and *Antheraea polyphemus* the female protein is likewise sudanophilic (Telfer, 1965).

Fractions 3 and 4 found in the enlarged ovary of the American cockroach may represent vitellogenic blood proteins, as they appear in the ovaries only when laden with yolk (Adiyodi and Nayar, 1966). In females in advanced stages of vitellogenesis fraction 3 in the blood is in the nature of a glycolipoprotein complex and fraction 4 a lipoprotein, but in the homogenates of the ovaries of such animals these fractions are represented only as PAS- and SBB-negative simple proteins. In case the electrophoretic components 3 and 4 present in the enlarged ovary of the American cockroach owe their origin to similar fractions in the hemolymph rather than to independent synthesis [the ultrastructure of the ovarian surface is suggestive of one that could adsorb blood proteins (Anderson, 1964)], it may be said that the bound lipids and sugars become freed from these major proteins at entry.

Cardiacectomy interferes with protein metabolism (Bodenstein, 1953) and allatectomy with the synthesis and mobilization of proteins (Menon, 1963, 1965;

Adiyodi and Nayar, 1966; Thomas and Nation, 1966a) as well as lipids (Bodenstein, 1953; Menon, 1963, 1965; Vroman, Kaplanis and Robbins, 1965). It appears to be more than probable that the neurosecretory factors may reach the hemolymph through the cut ends of the cardiac nerves or other means in cardiacectomized or cardiac-allatectomized females, and that at least during the initial periods some protein may be synthesized under the influence of these factors as well as the allatal hormone already present. As most of the reserve fat occurs in the fat body in insects, analyses of whole-body fat as by Vroman, Kaplanis and Robbins (1965) may be considered as sufficiently indicative of the composition of fat in the fat body itself (see Kilby, 1963; Gilby, 1965). The accumulation of triglycerides (Vroman, Kaplanis and Robbins, 1965), therefore, in the fat body and the proteins in the hemolymph in allatectomized females is indicative of a mobilization failure. Serum lipid follows more or less the same cycle as that of the proteins (Menon, 1963, 1965), and the little available evidence seems to favor the view that lipid metabolism may be almost exclusively governed by allatal factors.

Information is scanty regarding the mechanism whereby the lipids combine with the lipoprotein protein: in mammals it has been suggested as probable that the lack in the synthesis of one moiety in this conjugated protein automatically affects the other (Robinson and Seakins, 1963). Robinson and Seakins found that treatment with chemical agents like ethionine, carbon tetrachloride and puromycin resulted in fatty livers in the rat and reduction in plasma lipid concentration, and they are of opinion that these changes in turn are caused by the inability of the liver to synthesize plasma lipoprotein protein. The relative loss of the lipid prosthetic group from hemolymph proteins in cardiac-allatectomized female cockroaches appears to be related to the bilateral arrest imposed on lipid and protein metabolism rather than on any one alone. An upset in the metabolism of carbohydrates may only be readily expected in our experimental animals as the operation involves removal of the corpora cardiaca. The reason for the preferential conjugation of several of the hemolymph protein fractions, particularly 4 to carbohydrate moieties and the consequent increase in the quantity of protein-bound PAS-stainable material in the hemolymph of cardiac-allatectomized animals, however, remains obscure. It is possible that in cardiac-allatectomized and allatectomized females the hydrolysis by active lipase of the triglycerides reported to be synthesized and stored in the fat body into diglycerides for release into the hemolymph becomes affected in the absence of the source of the allatal hormone. This may eventually upset the dynamic equilibrium between the fat bodies and the hemolymph. Such a view would also account for the increased storage of triglycerides (Vroman, Kaplanis and Robbins, 1965) in the fat body and the paucity of lipid fractions in the plasma proteins in the experimental animals. The marked loss in protein-bound lipids in the hemolymph of cardiac-allatectomized females may perhaps be related to their preferential utilization as an energy source, and the inability of the fat bodies to replenish the same due to arrest at some stage in their intermediary metabolism. Yet another possibility is that the vitellogenic blood proteins already synthesized, but not utilized, by the ovary become transformed into, or could probably be stored only as glycoproteins in the cockroach. Female American cockroaches administered crab eyestalk extract and

having the ovarian growth retarded due to some ovary-inhibiting principle contained therein, showed a similar conversion of the unutilized female protein into a glycoprotein (Adiyodi and Adiyodi, unpublished). In this connection it may also be noted that the relatively small fraction 4, which constitutes about 11.76% of the total hemolymph protein of adult male cockroaches (Table II; Fig. 2), stains often as a glycoprotein. The larger conjugation to lipid prosthetic groups of fractions 1–3 which occurs in some females 30 and also 60 days after cardiac-allatectomy, compared to 18-day animals, may perhaps be related to some functional readjustment in lipid metabolism with further aging, but even in such animals fraction 4 usually remains a glycoprotein, when not utilized for oocyte growth. Starvation in the American cockroach is known to affect fraction 3, the "common insect protein" (Adiyodi and Nayar, 1966). Whittaker and West (1962) found similarly in last-instar *Malacosoma americanum* that this fraction almost disappeared on 48 hours of starvation. The conspicuous decrease in the concentration of fraction 3 in starved, neck-ligatured and cardiac-allatectomized females in the initial stages perhaps suggests that this "common insect protein" is in the nature of a nitrogen reserve hydrolyzed to maintain amino acids, and thus the osmotic equilibrium under stress conditions (see Wyatt, 1961; Loughton and West, 1965). Restitution of this fraction takes place probably by conversion of fraction 4 into proteins of lower electrophoretic mobility, as borne out by a decline in the concentration of fraction 4 with aging in starved as well as cardiac-allatectomized females.

Summary

1. The nature and behavior of the conjugated proteins in the hemolymph of adult female *Periplaneta americana* (L.) have been studied in relation to the ovarian cycle, using disc electrophoresis in polyacrylamide gel and various staining procedures. It is shown that there may be not only a change in the relative concentration of the different protein fractions with oocyte growth, but also differences in their chemical composition. Fraction 3 and the female fraction 4, which represent the major proteins in the blood, appear to serve as carriers of lipids to the ovaries, besides most likely providing proteinaceous yolk precursors to the same. Fractionations made with ovarian homogenates seem to indicate that the lipids and sugars bound to such proteins may become freed at entry into the ovary.

2. Cardiac-allatectomy has been found to result in a pronounced accumulation of proteins in the blood, and also in changes in their chemical composition. In such animals there is a tendency with aging for the loss of lipid prosthetic groups and for several of the fractions including the major proteins to stain preferentially for carbohydrates. This effect could be reversed by the implantation of fresh cardiacum-allatum complexes taken from adult females. It is suggested that in cardiac-allatectomized females there is probably a bilateral arrest of lipid and protein metabolism, and that the vitellogenic proteins already synthesized, but not utilized by the ovary, become converted into glycoproteins.

3. Fractionation studies on fat body homogenates of females in different stages of ovarian activity appear to indicate that in *Periplaneta americana* none of the soluble lipo- or glycoproteins, including the female fraction, is as such synthesized or stored in the fat body.

LITERATURE CITED

ADIYODI, K. G., AND K. K. NAYAR, 1966. Haemolymph proteins and reproduction in *Periplaneta americana*. *Curr. Sci.*, **35**: 587–588.

ANDERSON, E., 1964. Oocyte differentiation and vitellogenesis in the cockroach *Periplaneta americana*. *J. Cell Biol.*, **20**: 131–155.

BODENSTEIN, D., 1953. Studies on humoral mechanisms in growth and metamorphosis of the cockroach *Periplaneta americana* III. Humoral effects on metabolism. *J. Exp. Zool.*, **124**: 105–115.

CLARK, E. W., AND G. H. BALL, 1956. Preliminary microelectrophoretic studies of insect proteins. *Physiol. Zoöl.*, **29**: 206–212.

GILBERT, L. I., H. CHINO AND K. A. DOMROESE, 1965. Lipolytic activity of insect tissues and its significance in lipid transport. *J. Insect Physiol.*, **11**: 1057–1070.

GILBY, A. R., 1965. Lipids and their metabolism in insects. *Ann. Rev. Entomol.*, **10**: 141–160.

KILBY, B. A., 1963. The biochemistry of the insect fat body. *In*: Advances in Insect Psysiology (J. W. L. Beament, J. E. Treherne and V. B. Wigglesworth, eds.), **I**: 111–174. New York, Academic Press.

LOUGHTON, B. G., AND A. S. WEST, 1965. The development and distribution of haemolymph proteins in Lepidoptera. *J. Insect Physiol.*, **11**: 919–932.

MENON, M., 1963. Endocrine influences on protein and fat in the hemolymph of the cockroach *Periplaneta americana*. *Proc. 16th Internatl. Cong. Zool.*, **1**: 297.

MENON, M., 1965. Endocrine influences on yolk deposition in insects. *J. Anim. Morph. Physiol.*, **12**: 76–80.

MILLS, R., F. C. GREENSLADE AND E. F. COUCH, 1966. Studies on vitellogenesis in the American cockroach. *J. Insect Physiol.*, **12**: 767–779.

ROBINSON, D. S., AND A. SEAKINS, 1963. Reduced plasma lipoprotein production as a factor in the development of fatty livers. *In*: Biochemical Problems of Lipids (A. C. Frazer, ed.), pp. 359–366. Amsterdam, Elsevier Publishing Company.

SCHARRER, B., 1955. Hormones in invertebrates. *In*: The Hormones (G. Pincus, and K. V. Thimann, eds.), **3**: 57–95. New York, Academic Press.

SIAKOTOS, A. N., 1960a. The conjugated plasma proteins of the American cockroach. I. The normal state. *J. Gen. Physiol.*, **43**: 999–1013.

SIAKOTOS, A. N., 1960b. The conjugated plasma proteins of the American cockroach. II. Changes during the molting and clotting process. *J. Gen. Physiol.*, **43**: 1015–1030.

STEELE, J. E., 1961. Occurrence of a hyperglycaemic factor in the corpus cardiacum of an insect. *Nature*, **192**: 680–681.

STEELE, J. E., 1963. The site of action of insect hyperglycaemic hormone. *Gen. Comp. Endocrinol.*, **3**: 45–62.

STEPHEN, W. P., AND A. L. STEINHAUER, 1957. Sexual and developmental differences in insect blood proteins. *Physiol. Zoöl.*, **30**: 114–120.

TELFER, W. H., 1965. The mechanism and control of yolk formation. *Ann. Rev. Entomol.*, **10**: 161–184.

THOMAS, K. K., AND J. L. NATION, 1966a. Control of a sex-limited hemolymph protein by corpora allata during ovarian development in *Periplaneta americana* (L.). *Biol. Bull.*, **130**: 254–264.

THOMAS, K. K., AND J. L. NATION, 1966b. RNA, protein and uric acid content of the body tissues of *Periplaneta americana* (L.) as influenced by corpora allata during ovarian development. *Biol. Bull.*, **130**: 442–449.

VROMAN, H. E., J. N. KAPLANIS AND W. E. ROBBINS, 1965. Effect of allatectomy on lipid biosynthesis and turnover in the female American cockroach, *Periplaneta americana* (L.). *J. Insect Physiol.*, **11**: 897–904.

WHITTAKER, J. R., AND A. S. WEST, 1962. A starch-gel electrophoretic study of insect hemolymph proteins. *Canad. J. Zool.*, **40**: 655–671.

WYATT, G. R., 1961. The biochemistry of insect hemolymph. *Ann. Rev., Entomol.*, **6**: 75–102.