STUDIES ON THE NEUROSECRETORY SYSTEM OF *IPHITA LIMBATA* STAL.

Part V. Probable Endocrine Basis of Oviposition in the Female Insect

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Received October 22, 1957

(Communicated by Dr. C. S. Venkateswaran, F.A.SC.)

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Introduction

The insectan neurosecretory system comprises the neurosecretory cells of the pars intercerebralis of the brain, the axonic bundles proceeding from these cells, the corpora cardiaca and the corpora allata. To these may be added the hypocerebral ganglion also, which generally is closely associated with the corpora cardiaca. In recent years this complex of glandular neurons has been shown to possess endocrine functions and the whole system has come to be regarded as a complicated endocrine system in the insect.

Considerable information is available on the anatomy of the neurosecretory system in the different groups of insects (*vide* reviews by Scharrer and Scharrer, 1954 *a*, *b*). The interrelationships between the parts of the system also have been worked out in many cases (Scharrer and Scharrer, 1954). The corpus allatum, which forms a part of the neurosecretory system, has received attention as early as 1935, and it was immediately recognised as an endocrine centre (Wigglesworth, 1936). Subsequent workers on various insects have confirmed the endocrine nature of the gland, which originally was considered to be a ganglion, and it is now generally accepted that the corpus allatum represents the gland secreting the " juvenile hormone " or the " metabolic hormone " in insects throughout their life (Wigglesworth, 1952, 1954).

A direct relationship between the ovaries and the corpora allata have been observed by several workers (Wigglesworth, 1936; Pfieffer, 1939, 1945; Thomsen, 1942). They have seen that in the female, egg production is controlled by the corpora allata and that the removal of the glands results in the atrophy of eggs due to failure in yolk-deposition. This condition has been reported in *Rhodnius* by Wigglesworth (1936), *Melanoplus* by Pfieffer (1939), *Calliphora* by Thomsen (1942), *Drosophila* by Vogt (1943) and *Leucophœa* by Scharrer (1946). Subsequent work by others in other groups of insects have substantiated the view (Bodenstein, 1953).

The existence of sex hormones in insects has not yet been definitely established. But certain indications have been reported which indirectly suggest the existence of such humoural agents (*vide* Scharrer, 1955, 1956) though the nature of these results have been interpreted in other ways also.

Apart from the corpora allata, no other part of the neurosecretory system has been systematically investigated from the physiological point of view. The brilliant works of Thomsen (1952, 1954) on the neurosecretory cells of *Calliphora* throw considerable light on the functional significance of the cells and the interrelationships of the units of the neurosecretory system. But probably due to the difficulty in handling such minute clusters of cells or due to high mortality rate due to defects in experimentation, no substantial subsequent investigations have been reported on the functional aspects of these cells.

While yolk-deposition and consequent ripening of the egg of insects have been demonstrated to be under the control of the corpora allata in several insects, the problem of oviposition has not been investigated seriously. Dupont-Raabe (1951, 1952) has pointed out that in egg-laying phasmids there is an intense secretion in the neurosecretory cells of females in the reproductive period. Arvy, Bounhiol and Gabe (1953) have suggested that in female *Bombyx*, secretions from the neurosecretory cells get transported along the axons just before oviposition. By transfusion experiments, Mokia showed in 1941 (von Buddenbrock, 1950) that blood from fecundated *Bombyx* can induce oviposition in unfertilised females. Luscher and

Engelmann (1955) have shown in *Leucophæa* a relationship between the egg packet in the uterus and the activity of the corpora allata.

The present investigation owes its origin to a finding made in 1953 (Nayar, 1953) that in histological preparations there is a considerable reduction of neurosecretory colloids in the cells of the pars intercerebralis of the brain soon after oviposition in the common plant bug *Iphita limbata* Stal. This suggested a probable relationship between the neurosecretory matter and egg-laying in the insect. An experimental and histophysiological study was commenced in May 1956 and the present paper embodies the results of the investigations. A preliminary report on the work was presented to the Second International Symposium on Neurosecretion held in Lund, Sweden, in July 1957 (Nayar, 1957). The paper throws light on the functional significance of the neurosecretory cells of the brain in oviposition, and it forms a part of the investigations on the neurosecretory system of *Iphita limbata* Stal., some aspects of which have already been published (Nayar, 1955 *a*, *b*; 1956 *a*, *b*, *c*).

Material and Methods

Nymphs and adults of *Iphita limbata* Stal. (Hemiptera: Pyrrhocoridæ) were collected from the field in various stages of growth and egg development. They were kept in insectary cages in which they thrived feeding on cotton seeds soaked in water, tender shoots and flower-buds of *Plumaria* sp. and raisins.

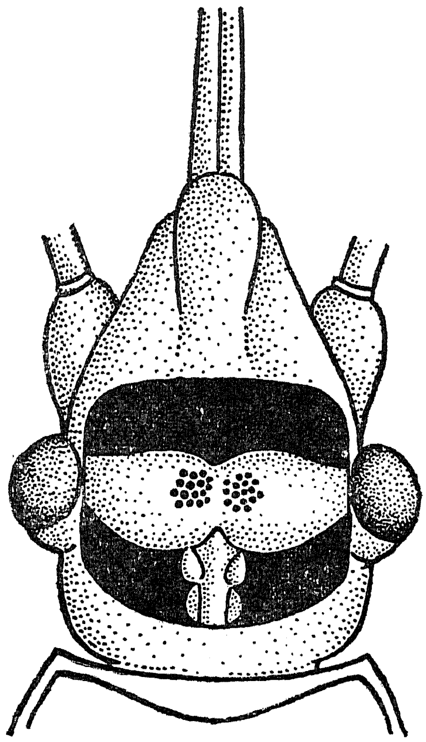
When histological examinations were required, the insect was killed with chloroform and the brain and associated regions of the endocrine, alimentary and nervous systems were dissected out in insect-Ringer and immediately fixed in Bouin's fluid. Sections cut at 5 μ were stained in Gomori's chrome alum-hæmatoxylin-phloxine, which gave clear pictures of the neurosecretory matter, pathways and nature of the secretions.

Ovaries of the different stages were dissected out, fixed in Smith's fluid (Pantin, 1948) for 48 hours and were stored in 5% formalin. Sections cut at 8 μ were stained in Heidenhain's iron alum-hæmatoxylin.

Blood transfusions were done by drawing the blood from an amputated antenna of the donor insect through the needle of an Agla micrometer syringe, and administering measured quantities of it (0·01 ml. or more) into the prothrorax of the etherised recipient. The wound produced on the prothorax in the process was sealed off with paraffin (m.p. 45° C.).

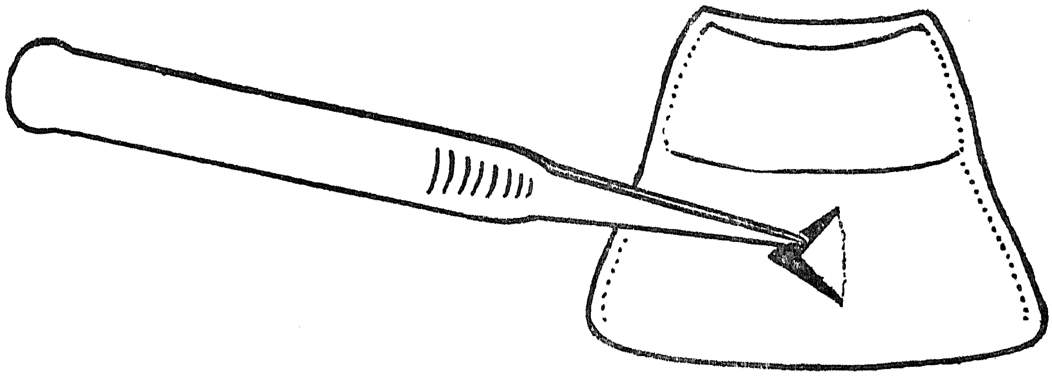
Water extracts of ovary were made as follows: The ovary was dissected out and after a brief and thorough rinse in distilled water, it was placed in a small tube with 1 ml. of distilled water. This was heated in a water-bath for ten minutes near boiling point. After cooling and centrifugation at 5,000 r.p.m., for five minutes, the supernatant was used as extract. Egg extracts were also prepared in the same manner.

Transplantation of the neurosecretory cells from the brain was done as follows: When the dorsum of the head is removed the brain gets exposed over the middle of which lies a stout trachea. When that is cut off and the envelope of the brain dissected off with a needle, the two clusters of neurosecretory cells become visible as bluish white masses (Text-Fig. 1)



TEXT-FIG. 1. Diagram to show the position of the neurosecretory cells of the brain of *Iphita* when the dorsal cranial wall and tracheal trunk are removed.

under the stereoscopic binocular (× 40). With the aid of a watchmaker's forceps, the two clusters could be removed as one clean lump if the cellular masses have been teased around the margin with a sharp needle. The mass of cells is placed in a watch-glass containing a few drops of insect-Ringer. Transplantation is done into the prothorax of the recipient or host. Ether-chloroform has been used as the anæsthetic. The anæsthetised insect was placed flat on a glass plate under the stereoscopic binocular and gently pressed and kept in position with plasticine. With a fine lancet a " < " shaped opening is made on the skin of the pronotum. The narrow end flap is lifted up and with a pair of forceps the neurosecretory clusters are introduced (Text-Fig. 2) deep into the prothorax. Then the wound is closed by leaving the flap down and sealed off with paraffin. Care has to be taken to avoid pressing regions of the insect's body to prevent bleeding, to see that the transplants go deep in and do not come out with the limb of the forceps or the oozing droplet of blood and to avoid air bubbles getting trapped in the process of operation.



TEXT-FIG. 2. Drawing showing the method of transplantation of the neurosecretory cells by means of a pair of forceps.

OBSERVATIONS

Normal Reproductive Cycle

The newly moulted adult female becomes darkened and gets active within a period of about ten to sixty minutes. Generally it takes rest for a few hours and then starts to feed. It feeds intermittently. In the course of a few days it responds to the approaches of the male and mating starts. If left undisturbed this mating generally lasts uninterruptedly for a period of about three weeks. It is during this period that the abdomen of the female begins to swell up as the eggs inside grow by deposition of yolk. Towards the end of this period the female appears 'fully gravid' with considerably swollen abdomen.

Oviposition is marked by a conspicuous "preoviposition behaviour" on the part of the female. With her hindlegs she taps the abdomen and the legs of the male repeatedly and at regular, rapid intervals. An occasional pulling of the abdomen as if to disengage the male also could be seen. This tapping will last from half an hour to three hours. Once the pair separates, the female avoids the male, though he may try to copulate again. On the approach of the male, the female lies low and makes it impossible for him to mount. After separation the female becomes quite restless and begins to move about slowly searching the surrounding regions with the tips of the lowered antennæ. Very frequently the rostrum is projected forwards, and towards the later periods the rostrum is more or less continuously kept so. A fine droplet of saliva, probably indicative of an excessive salivation, is always discernible at the tip of the extended rostrum. The tip of the rostrum is also used in reconnoitering the sites.

This "preoviposition behaviour" lasts for about two to five hours, but the time is highly variable. Towards the end of this phase, the animal seeks a tight corner, often with crevices and debris of leaves and twigs and begins to lay eggs. During this period there is a rapid vibratile movement of the genital plates while the abdomen remains tense and rigid. Pushing the abdomen into the crevice, slowly one by one the eggs are laid. The number of eggs laid varies, usually it is between 160 and 200. Usually egg-laying takes place during night, often towards dawn, but in the laboratory oviposition has occurred both during night and at daytime.

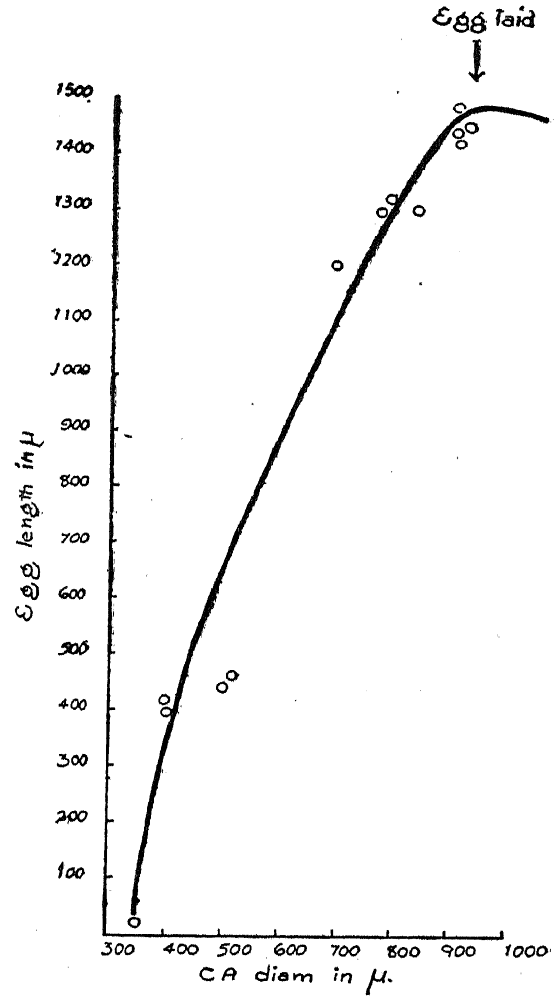
The egg on an average measures 1·45 mm. in length and 1·025 mm. in diameter. It belongs to the typical pyrrhocorid type of egg, bearing a circle of parallel sided processes representing the micropylar structures at the cephalic pole (Southwood, 1956).

After egg-laying the animal remains quiet for a few hours. After this period of rest, the insect gradually begins to get active and it does not try to repel the male if it tries to mate. Mating successfully follows and then the pair moves about and feeds. This mating lasts for about a week or ten days. During this period there is no appreciable swelling of the abdomen. Often mating also gets interrupted. This will also ultimately lead to a "preoviposition behaviour" ending in a laying of about 20 to 70 eggs. After this second oviposition the female gets lethargic and dies in due course.

Histophysiological Observations in the Normal Female Cycle

1. *Endocrine system.*—The neurosecretory cells are conspicuous structures in the pars intercerebralis of the brain. In the newly emerged adult female, the cells show a generally granulated cytoplasm with developing colloids in them. As the animal feeds and becomes active the colloids increase and the cells assume the characteristic appearance which lasts throughout the mating period (Plate XX, A). Most cells are densely filled with dark blue colloids which form the neurosecretory matter (Plate XX, B); a few cells are seen without them (the B-cells described earlier) (*vide* Nayar, 1955 *a*), probably representing cells from which the colloids have flown out.

The relation between the corpus allatum and the ovary is well known from the works of several investigators. So an experimental elucidation of its role in *Iphita* was not attempted. In the newly moulted adult female, the corpus allatum measures about 310 μ in diameter. This gland is connected to the neurosecretory system by the allatic nerve which consists of axons emanating from the neurosecretory cells of the brain. In the young adult which has just emerged, the allatic nerve shows no trace of transport of neurosecretory colloids into the gland. After feeding and when mating has commenced, the sections of allatic nerve stained in Gomori's chrome alum-hæmatoxylin-phloxine, show the presence of neurosecretory colloids along the axons running into the corpus allatum. Thus, probably stimulated by the colloids, the gland becomes active and begins to grow in size. Side by side with this is the growing ovary; here growth is by deposition of yolk in the eggs. The increase in size of the corpus allatum is directly proportional to the increase in size of the egg by yolk deposition (Text-Fig. 3).



TEXT-FIG. 3. Graph showing the relation between the size of the egg (length) and the size of the corpus allatum (diameter). The increase in size is directly proportional.

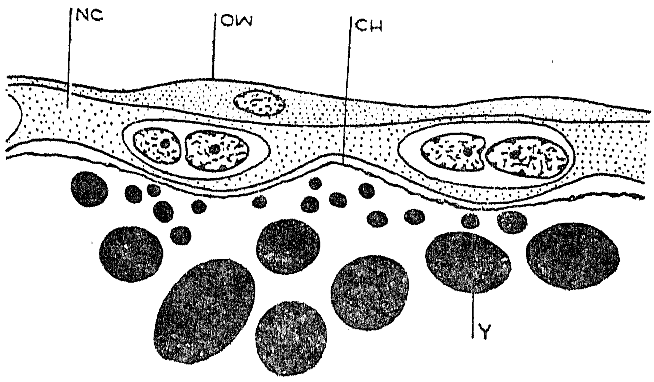
In the fully gravid female, the corpus allatum measures on an average about 910 μ in diameter and is a conspicuous, tumid, yellowish-brown gland. Some details of the cytology of the gland at this stage has already been published (Nayar, 1956 *b*). Throughout the period of egg-growth there is a regular supply of stainable neurosecretory matter to the corpus allatum *via* the allatic nerve (Plate XX, C).

When the insect starts to show the " preoviposition behaviour ", histological pictures appear to change. The allatic nerve shows no visible colloids along the bundle fibres which suggests that neurosecretory supply has been withheld. If at this crucial stage sections are examined, stainable colloids are seen flowing into the anterior end of the aorta close to the corpora cardiaca from the neurosecretory axons. It is also possible that matter from the corpora cardiaca also will be flowing along with this. The neurosecretory cells of the brain get depleted of the stainable colloids in course of time. In the female which has started oviposition, the neurosecretory cells of the brain are mostly devoid of the blue colloids. In many instances the cytoplasm appears to be a homogeneous, poorly granulated structure (Plate XX, D), these granules being generally bluish in Gomori's stain. In other cases the cytoplasm takes up the phloxine component of the stain. The axons which cross and traverse the brain to emerge out near the aorta also appear as reddish streaks, deprived of the blue-stained colloidal components.

In the insect which remains inactive after egg-laying, the neurosecretory cells show a redevelopment of the colloids as granules. When mating has started again, the neurosecretory cells present the earlier picture of copious secretory matter in their cytoplasm.

2. *Reproductive system of the female.*—Each ovary consists of seven ovarioles. In the newly emerged female, the ovary is small and is seen to be almost completely enveloped by the ramifications of two large special bundles of tracheæ from the spiracles of the 2nd and 3rd abdominal segments. Sections of ovarioles taken one hour after the emergence, show the apical part as the best defined region measuring about 700 to 800 μ in length and 200 to 210 μ in diameter. Cells here are numerous and small, measuring on an average 15 μ in diameter. Among these lie larger cells measuring on an average 26 μ in diameter, with vesicular nuclei and finely granulated cytoplasm. Most of these show active stages of division. Towards later stages these cells redivide and become the ova which migrate down retaining protoplasmic connections with the clusters of smaller cells. The rest of the ovariole follicle is a collapsed tube of about 65 μ in diameter.

When mating has started the follicular tube has become filled with eggs, the apical ones retaining connection with the cluster of smaller cells at the extreme apex. In the subapical and distal parts of the follicle, the eggs get surrounded by follicular cells which take up the nutritive function of the egg. Generally eggs over 200 μ in length are without connections to the apical mass; they are nourished by follicular epithelial cells. Towards the final stages the follicular cells become characteristically binucleate. The nuclei themselves appear granulated. When the egg has reached the final size, the follicle appears thin with the paired nuclei lying side by side. The binucleated structure is often in an enclosed vesicular space and remain as loosely packed structures around the egg (Text-Fig. 4). Soon chorion-formation starts.



TEXT-FIG. 4. Camera lucida drawing of the wall of the ovary containing an almost fully grown egg. CH, chorin; OW, ovarian wall; NC, nurse cells; Y, yolk.

Soon after egg-laying, when ovaries are sectioned and examined, it will be seen that the terminal part of the ovarian follicle and oviduct appear collapsed and folded. The lining is raised into folds and kinks and the lumen is narrowed. The egg that is only partly developed and most distal in the partly evacuated ovariole appears to be in a necrotic condition. Masses of clumped coloured granules and disorganised follicular nuclei are seen in this egg. The ones lying proximal to this in each ovariole however are not adversely affected.

Besides histological differences in the various structures mentioned above, histochemical differences also have been noticed in the gravid female. The corpus allatum has been seen to indicate the presence of enzymes in the cytoplasm of the syncytial gland especially acid phosphatase and succinic dehydrogenase. These two enzymes increase considerably as the gland swells up in the gravid female. Of these, succinic dehydrogenase disappears almost totally and abruptly as soon as the female separates and egg-laying starts. This fact has been observed by the author (Nayar, 1955 *c*) earlier and it was confirmed later in repeated tests. It is likely that this change in enzyme activity reflects some change in the secretory activity of the corpus allatum at the critical stage.

Experimental Investigations

1. *Blood transfusions.*—The foregoing histological findings suggested that there probably would be a relation between oviposition and the neurosecretory system of the insect. It was felt that experimental studies should be made to elucidate whether hormonal mechanisms are involved in oviposition.

The first set of experiments consisted of blood transfusion. Blood, drawn from amputated antennæ of females, were injected in measured quantities into the prothorax of experimental insects. 0·05 ml. of blood from a female which has just separated has been seen to induce egg-laying in a female which has not become fully gravid, in about 1 to 3 hours. Blood from laying female and blood from a female just after oviposition can do the same; but blood from earlier stages or from female which has restarted mating cannot bring this effect. Control injections of distilled water showed no effect on the animal. These experiments prove that oviposition is induced by a blood-borne factor, present in the blood of a female which has become " completely gravid " and which has started to show the " preoviposition behaviour".

The results of the experiments are given in Table I.

Histological examinations in these cases showed that the neurosecretory cells of the recipients have reacted in a variable manner to the injections administered to the insects. In some cases a depletion of neurosecretory matter was observed from the cells of the pars intercerebralis.

2. *Injection of ovarian extracts.*—Water extracts of ripe ovary, *i.e.*, ovary containing full-term eggs were injected into the prothorax of experimental insects. In nearly gravid females, the injection of 0·1 to 0·2 ml. of the extract resulted in prompt oviposition. In these cases, egg-laying occurred within a period of 2 to 12 hours after injection. In the early stages of egg development in the female, no oviposition occurred.

This set of experiments, however, yielded interesting features in histophysiology. When the insects were examined in detail, it was seen that the ovary showed the characteristic configuration in the forms which laid eggs, exhibiting the degeneration of the egg which has grown only to half the size (Plate XX, E). In young females the ovary exhibited the same type of eggs

TABLE I

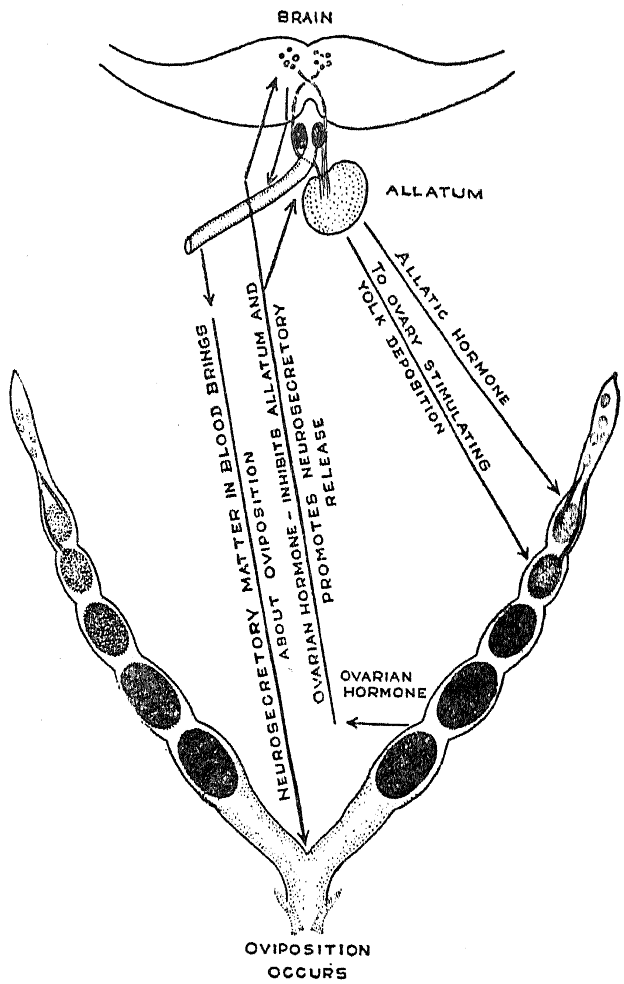
Experiments on blood transfusion in Iphita *and their results*

Source of blood or injected material	Results produced on the recipient after injection	Time under observation
0·05 ml. distilled water	Recipient mating young female. Mating continues. No marked effect	Four days
0·05 ml. distilled water	On mating semi-gravid female with partly swollen abdomen. Mating continues. No marked effect	Four days
0·05 ml. distilled water	On mating gravid female. Mating continues. No marked effect	Four days
0·05 ml. blood from young mating female.	On mating semi-gravid female. Mating continues. No marked effect	Even after 96 hours
0·05 ml. blood from young mating female	On mating nearly gravid female. No marked effect	Even after 96 hours
0·05 ml. blood from female which has started showing "pre-oviposition behaviour"	On mating nearly gravid female. Insect restless. Moving irregular. Separates from male and avoids it. Rostrum extended, seeking sites as if for egg-laying. Of the 20 insects tried, 18 laid eggs	Lays eggs in 1 to 6 hours after injection
0·05 ml. blood as above	On young mating female. Mating interrupted, males are avoided. Shows reactions characteristic of the preoviposition behaviour. No egg-laying	Soon after injection, the reactions appear. Lasts for 3 to 4 hours
0·05 ml. of blood as above	On semi-gravid female, which is mating. Shows preoviposition behaviour. Some laid small-sized eggs	Within 1 to 5 hours after injection
0·05 ml. of blood from laying female	On nearly gravid, mating female. Results positive. Laid eggs.	Laid within 1 to 3 hours.
0·05 ml. of blood from female which has just finished oviposition	On nearly gravid mating female. Results positive. Laid eggs	Laid within 1 to 3 hours
0·05 ml. of blood from female which has re-started mating	On nearly gravid female. Also earlier stages. No marked effect and the recipient remains normal	Even after 96 hours

in necrosis. The histological picture of the neurosecretory system was significant. The neurosecretory cells were depleted of their blue stainable material (Plate XX, F) and the allatic nerve to the corpus allatum was without neurosecretory colloids. This is a picture which resembles that of the laying ordinary female. It is indicative of a probable direct influence of the ovarian extract on the neurosecretory system.

Water extracts of extruded eggs and eggs that were being extruded, showed no effects on oviposition.

These experiments and observations suggest that the ovary containing the ripe eggs liberates some substance, an "*Ovarian Hormone*" into the blood of the animal. Each ovariole is then with a number of ripe eggs, and smaller growing eggs apically. This hormone could be extracted in water



TEXT-FIG. 5. Diagram to show the probable interrelationships between the endocrine organs and the ovary and the mode of action of the "hormones" concerned in oviposition in *Iphita*.

and is heat-stable. When administered into the body of a female, it probably initiates a liberation of neurosecretory matter from the neurosecretory cells into blood and a cutting off of the same to the corpus allatum. The latter is responsible for the failure of further development of eggs which are growing at the apical region as it interrupts the activity of the corpus allatum and thereby interferes with the deposition of yolk. In the normal insect laying eggs this results in the degeneration of eggs just proximal to the ripe series of eggs; this is also what happens when the "*ovarian hormone*" is injected into the female. The interactions of the ovary and the neurosecretory system in the reproductive cycle of the female is given in the diagram (Text-Fig. 5).

3. *Effect of transplantation of neurosecretory cells.*—The above experiments have roughly indicated that a removal of stainable neurosecretory matter into the blood occurs at the time of egg-laying. To test the role of neurosecretory cells on oviposition, two clusters of the same from two adults which were mating and normal (*i.e.*, not gravid), were transplanted into the prothorax through a narrow window. The results of this experiment was indeed striking. In nearly gravid female, it induced immediate oviposition. The genital plates began to quiver and vibrate, and in 5 to 15 minutes egg-laying started. Histological examination showed that these insects suffered no loss of their own neurosecretory content from the cells of the pars intercerebralis. In younger recipients, no egg-laying occurred, but they also exhibited quivering of the genital plates and the general tendencies of the "preoviposition behaviour".

Discussion

In insects, reproductive processes are controlled by several endocrine centres. Among these centres, the influence of corpora allata on the female gonad, is the best known. It has been found in several insects that the maturation of the eggs and functional activities of certain accessory glands depend on the hormone released by the corpora allata. Removal of corpora allata has been seen to prevent yolk-deposition in the developing eggs of *Melanoplus* (Pfieffer, 1939, 1945), of *Rhodnius* (Wigglesworth, 1936), of *Calliphora* (Thomsen, 1942), of beetles (Joly, 1945) and of *Leucophæa* (Scharrer, 1946). Recently Johansson (1954, 1955) has shown in *Oncopeltus* and *Leucophæa*, that in starved females with immature ovaries, egg development could be induced by transplantation of corpora allata from fed donors. It is now generally agreed that the normal development of the eggs in the female is directly dependent on the regular production of the hormone from the corpora allata.

The discovery that the corpus allatum represents a part of the neurosecretory system of the insect has shifted the emphasis to an integrated neuroendocrine system regulating the activities of the animal. Investigations on the histophysiology have revealed that the corpora allata are directly connected to the neurosecretory cells of the pars intercerebralis of the brain through fibres which comprise the allatic nerves. That this is so has been shown in phasmids by Dupont-Raabe (1951, 1952), in *Bombyx* by Arvy, Bounhiol and Gabe (1953), in the blowfly *Calliphora* by Thomsen (1954) and in *Iphita* by the author (Nayar, 1956 *a*, *b*). Further in her experiments, Thomsen (1952) has earlier shown that extirpation of the medial region of the brain containing the neurosecretory cells prevents maturation of eggs, while reimplantation of the cells promotes the same. Thus the gonad-stimulating substances have been described to originate from the brain and act *via* the corpora allata in the blowflies. No other experimental investigation has been done by others to completely corroborate this view. The histological findings, reported in this paper, substantiates the essential point presented by Thomsen. It is the neurosecretory supply to the corpus allatum that keeps the latter active and promotes its activity to guide and complete the deposition of yolk. When growth is completed, yolk production stops probably because the neurosecretory supply to the gland gets cut off.

Some authors have already noticed the depletion of stainable matter from the neurosecretory cells of the brain when the female starts laying eggs. In egg-laying phasmids, Dupont-Raabe (1951, 1952) has observed an intense secretion in the neurosecretory cells in the reproductive period; Arvy, Bounhiol and Gabe (1953) stated that in female *Bombyx*, secretions from the neurosecretory cells get transported along the axons of the cells just before oviposition; and the author (Nayar, 1953) has observed that soon after egg-laying the neurosecretory cells are depleted in *Iphita*. It is further pointed out in this paper that this depletion starts when the insect commences to exhibit the "preoviposition behaviour" and that laying female is also with very little stainable matter in the cells of the pars intercerebralis. This secretory matter actually escapes into the blood.

The "preoviposition behaviour" is a characteristic one exhibited by a gravid insect. It consists of drumming with hindlegs, erection of rostrum and salivation, avoidance of male and searching of sites with feelers. Vaidya (1956) has recently drawn attention to a drumming behaviour in egg-laying butterfly *Papilio*, which however appears to be somewhat different. He doubts whether the drumming and accompanying fluttering of wings indicate momentary uneasiness and pain.

The present report has shown that blood from insects which show preoviposition behaviour, from laying females and from females which have just finished laying, when transfused into nearly gravid females can induce oviposition. It is interesting to recall the experiments of Mokia done on *Bombyx* in 1941 (von Buddenbrock, 1950) where he showed that blood drawn from a fertilised female can induce egg-laying in unfertilised females within a short time.

No direct evidence has yet been reported on the existence of sex hormones in insects (Scharrer, 1955). Recently Altmann (1950, 1952) showed that extracts from ovaries of queen bees when injected into the body of worker bees resulted in the latter developing functional ovaries producing eggs. But whether this substance may be classified as a sex hormone is open to doubt because extracts from other organs of the queen bee like the corpora allata, etc., also have been found to promote ovarian growth in workers. In the present paper, however, the ovarian wall has been shown to produce a substance which when it enters blood brings about neurosecretory release and oviposition. This indicates that the ripe ovary is producing a specific substance calling forth specific reactions on certain organs.

That the ripe ovary has an inhibitory effect on the hypertrophied corpus allatum of *Drosophila* has been noted by Vogt (1941). Luscher and Engelmann (1955) have shown in *Leucophœa* that the function of the corpora allata is steered by the brain and the egg-packet in the uterus. While the corpora allata are needed for egg production, the presence of an egg-packet in the uterus of this cockroach inhibits the activity of the glands. In *Iphita*, a somewhat similar inhibition of the activity of the corpus allatum is probably taking place when the ovary gets filled with ripe eggs and the "*ovarian hormone*" gets released into the blood of the insect.

That changes in the enzyme concentration of tissues occur under the influence of hormones is fairly well known. In the enlarging corpus allatum initiating yolk-deposition, there is a progressive increase of acid phosphatase and succinic dehydrogenase. But there is an abrupt fall and disappearance of succinic dehydrogenase in the corpus allatum of the female which starts to lay. Meyer and McShan (1950) have shown that œstrogens exert an inhibitory effect on oxidative enzymes *in vivo* as well as *in vitro*.

Koller (1954) has indicated in his paper that the oviducal contractions in *Carausius* increase by the addition of extracts of brain. Similarly Enders (1955) has also found an increase in the rhythmicity of the oviduct *in vitro* of *Carausius* following the administration of extracts of brain and corpora cardiaca. This is significant in the light of facts presented in this paper, because here in *Iphita* the neurosecretory cells have been shown to induce expulsion of eggs from the ovarian follicle of the nearly gravid female.

The problem of parturition in mammals is one which has received considerable attention. The hormone called oxytocin gets released in the female mammal as a result of distension of different parts of the reproductive tracts like uterus, vagina and the like exciting the hypothalamus, and also during coition and during suckling (Harris, 1955). As the neural part of the pituitary and the hypothalamus are closely interlinked through neurosecretory pathways, several authors believe that the posterior lobe hormones originate from the neurosecretory clusters of the hypothalamus (Scharrer and Scharrer, 1954 *b*). All the same, a conclusive proof of the necessity of hypophysis in course of normal labour is still needed (Harris, 1955). The exact mechanism of parturition in mammals is still not known and it is believed that it is the result of a complex of factors (Marshall and Moir, 1952).

In *Iphita*, however, it is not merely the distension of the ovarian follicles with ripe eggs that brings about oviposition; but it is possible that such a conditon contributes towards the laying of eggs by releasing the "ovarian substance" into blood eliciting a chain reaction from the neurosecretory system.

SUMMARY

On the basis of histological and experimental studies involving blood transfusions, injection of ovarian extracts, and transplantation of neurosecretory cells, it is concluded that there is an endocrine basis for oviposition in the insect *Iphita limbata* Stal. (Pyrrhocoridæ: Hemiptera), a common plant bug of South India. Oviposition is brought about in the gravid female by a hormone-complex involving the interaction between the ovary and the neurosecretory system. It is tentatively postulated that it probably works out as follows:

Stainable neurosecretory matter from the cells of the pars intercerebralis of the brain passes along the axons of the allatic nerve to the corpus allatum. This stimulates the gland which consequently begins to enlarge and initiates egg-growth by deposition of yolk. When eggs become full grown and ripe, *i.e.*, when the animal becomes gravid, the ovarian wall releases a substance into the blood which may be presumed to exert a dual action on the neurosecretory system. Evidences show that the supply of visible, stainable, neurosecretory colloids to the corpus allatum is reduced or inhibited, which may result in an inactivation of the gland and stops further

elaboration of yolk in the eggs. At the same time, the blood-borne factor influences the cells of the pars intercerebralis to release their colloids into blood. This release of neurosecretory matter may have a role in inducing oviposition by promoting oviducal contractions.

Acknowledgements

The work was done in the Zoological Laboratory of the University College, Trivandrum, and I am thankful to the authorities for all facilities afforded me. I am indebted to Dr. B. Scharrer, Professor of Anatomy, Yeshiva University, New York, and Prof. V. B. Wigglesworth, F.R.S., Quick Professor of Biology, University of Cambridge, for critical discussions and suggestions during the course of the work. I am especially grateful to Dr. B. Scharrer, for editing my advance contribution on the subject and presenting it before the Second International Symposium on Neurosecretion held in Lund, Sweden, in July 1957. I am thankful to Mr. R. Sridharan Pillai for help in the preparation of the figures.

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EXPLANATION OF PLATE XX

FIG. A. Sagittal section through the pars inter cerebralis of a nearly gravid female *Iphita* showing the neurosecretory cells coloured black. Bouin's, Gomori's chrome alum-hæmatoxylin-phloxine; Approx., ×180.

FIG. B. A neurosecretory cell of a mating, nearly gravid female showing the dense cytoplasmic mass of stainable colloids. Bouin's, Gomori's chrome alum-hæmatoxylin-phloxine, ×1,200.

FIG. C. Section to show the allatic nerve (*AL*) entering the corpus allatum (*CA*). In the nerve are neurosecretory colloids (*MA*) flowing into the corpus allatum. Bouin's fluid; Gomori's chrome alum-hæmatoxylin-phloxine. About, ×400.

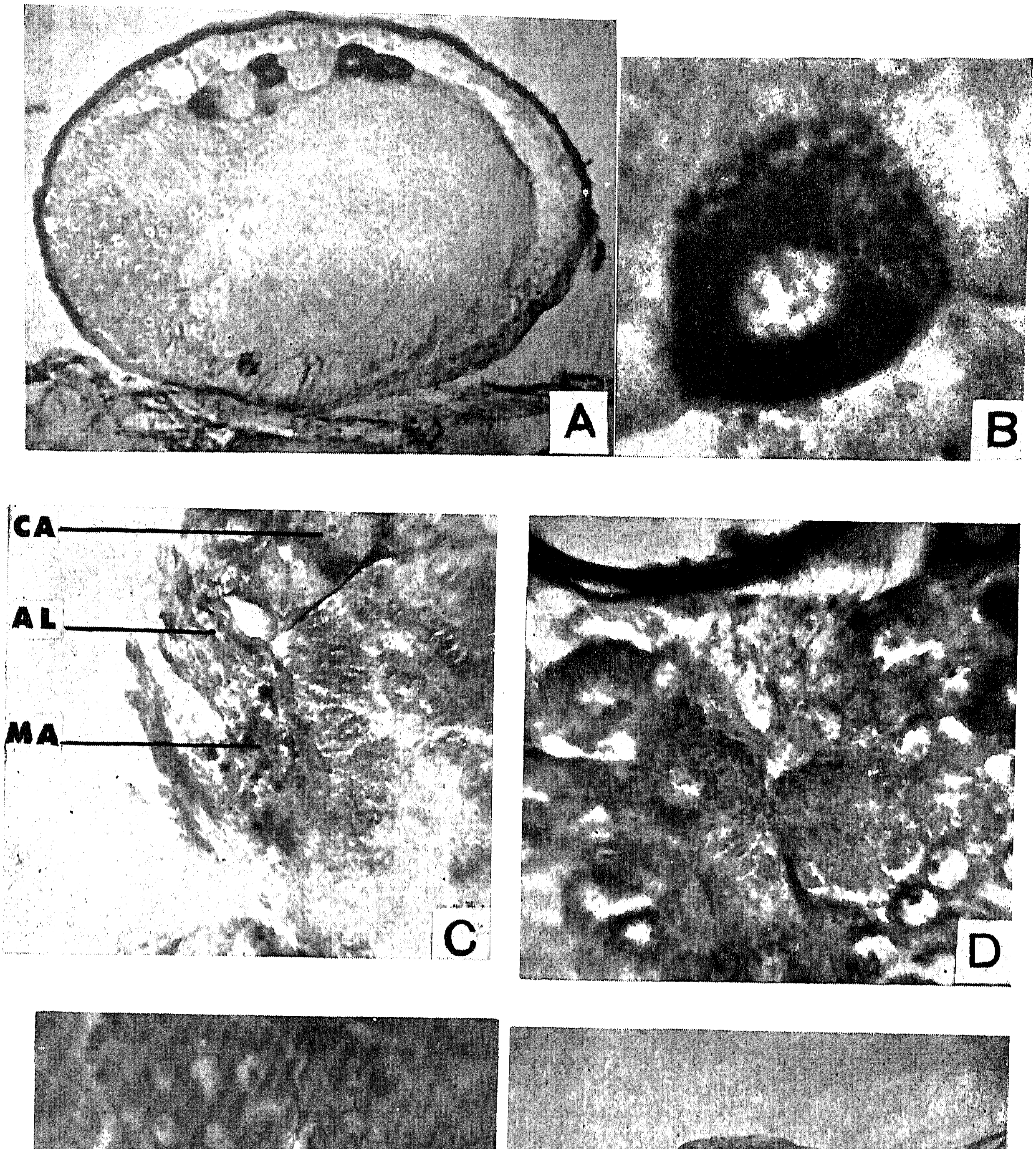
A
B
CA
AL
MA
C
D
F
E

FIG. D. Transverse section of the brain passing through the region of the pars intercerebralis of a female which has just started oviposition. The neurosecretory cells are depleted of their stainable colloids. The cytoplasm appears to be homogeneous. Bouin's, Gomori's chrome alum-hæmatoxylin-phloxine, ×700.

FIG. E. The degenerating egg of the follicle from a female into which an aqueous extract of the ripe ovary has been injected. Smith's, Heidenhain's iron-hæmatoxylin, ×125.

FIG. F. Transverse section through the brain of a mating female injected with ovarian extract. The neurosecretory cells have lost the stainable colloids and some appear reddish (phloxinophilic). Bouin's, Gomori's chrome alum-hæmatoxylin-phloxine, ×480.