

The Histology of the Neurosecretory System of the Adult Female Desert Locust, *Schistocerca gregaria*

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With one plate (fig. 1)

SUMMARY

The pars intercerebralis of the brain of the desert locust contains about 2,400 cells in two groups, which stain with chrome-haematoxylin-phloxin and with paraldehyde-fuchsin. On the basis of differences in size and staining reactions, four types of cell, called A-, B-, C-, and D-cells may be differentiated. The A- and B-cells produce different kinds of material; they are not thought to be stages in a secretory cycle. The C- and D-cells are probably not neurosecretory. The corpora cardiaca are divided into two regions. One part stores neurosecretory material from the pars intercerebralis and the other is glandular in appearance. Material discharged from the A- and B-cells in the immature female passes along the nervi corporis cardiaci I to the anterior parts of the corpora cardiaca. The mature female is characterized by the presence of very much larger amounts of material in the corpora cardiaca, in the nervi corporis cardiaci I, and in the A-cells of the pars intercerebralis. The significance of this larger amount of material with respect to neurosecretory cell activity is discussed.

INTRODUCTION

NEUROSECRETORY cells in the central nervous system control many major physiological events in the post-embryonic life of insects. The part played by neurosecretory cells in the pars intercerebralis during moulting and metamorphosis is well known (Wigglesworth, 1954), and in some insects these cells are also involved in ovarian development and oviposition (E. Thomsen, 1952; Nayar, 1958).

Differences in their histological appearance at different times have often been used as evidence that neurosecretory cells are involved in developmental events (Dupont-Raabe, 1952, 1956; Arvy & Gabe, 1952, 1953 *a, b, c, d*; Arvy, Bounhiol, & Gabe, 1953; Lhoste, 1952; Junqua, 1956; Fraser, 1957, 1959; Kobayashi, 1957; Highnam, 1958). This method of investigation has much to commend it, since the effect of slight variations in activity of the cells can be studied, as opposed to the effect of gross disturbances of the hormonal balance in the body engendered by the experimental removal or implantation of the neurosecretory cells.

In general, two kinds of neurosecretory cell can be differentiated in the pars intercerebralis by chrome-haematoxylin-phloxin staining: those that stain blue-black with the chrome-haematoxylin component, and those that stain red with phloxin (M. Thomsen, 1954 *a, b*; Nayar, 1955; de Lerma, 1956; Formigoni, 1956; Kopf, 1957). The two kinds of cell are often called A- and B-cells respectively (Nayar, 1955; Kopf, 1957; Kobayashi, 1957; Johansson, [Quarterly Journal of Microscopical Science, Vol. 102, part 1, pp. 27-38, 1961.]

1958). Further, with paraldehyde-fuchsin, the A-cells may stain deep purple, and the B-cells red or bluish-green (Johannson, 1958; Fraser, 1959). But Nayar (1955) reports that there is no selective staining of A- and B-cells with paraldehyde-fuchsin in *Iphita limbata*, and de Lerma (1956) states that B-cells may contain purple granules after paraldehyde-fuchsin staining. Dupont-Raabe (1956) has shown that the staining reactions of the different cells in phasmids may depend on the fixative used. It would seem that strict uniformity of technique is necessary for the exact comparison of the cell-types present in different insect species.

The corpora cardiaca have been shown experimentally to play some part in the control of developmental events (E. Thomsen, 1952, 1954; Highnam, 1958). It is thought that this control is effected by the release of material which originates in the neurosecretory cells in the brain, and migrates through the nervi corporis cardiaci to the corpora cardiaca, where it accumulates (Scharrer, 1952; E. Thomsen, 1954). The volume of the corpora cardiaca increases during the course of an instar (Brisson, 1949) and this may be due to the accumulation of neurosecretory material within the glands (Arvy & Gabe, 1952; Highnam, 1958). In *Oncopeltus fasciatus* neurosecretory material accumulates in the wall of the aorta (Johannson, 1958). The release of material from the corpora cardiaca may be correlated with the moulting cycle (Arvy & Gabe, 1953 d).

It is possible that developmental events may be controlled either by variations in the rate of production of neurosecretory material by the brain, or by variations in the amount of material released from the corpora cardiaca. These mechanisms need not be self-exclusive, and a combination of the two processes might be responsible for the regulation of post-embryonic development. This problem is being investigated in the desert locust, *Schistocerca gregaria* Forskål. The present paper describes the histology of the neurosecretory system in the mature female locust and certain changes which take place in it during the development of the ovaries.

MATERIAL AND METHODS

Newly emerged females were reared in glass-fronted cages $10 \times 10 \times 12$ ins., 25 to a cage, at a temperature of $30 \pm 2^\circ\text{C}$ and a relative humidity of 60%. They were fed upon freshly cut grass and ground rat cake. A few mature males were kept in the same cages. Under these conditions, the females matured in 3 to 4 weeks. Owing to the rather large individual variation in maturation rates, it was found impossible to predict maturation times from dates of emergence alone. The description of the histology of the neurosecretory system of the mature female which follows is therefore based upon material taken from adults which had terminal oocytes 5 to 7 mm long. These animals varied in age from 19 to 29 days, and had laid no eggs. The immature adults examined were fixed 4 to 6 days after emergence and all had terminal oocytes 1.0 mm or less in length.

Heads were fixed in Bouin's fluid under reduced pressure to collapse the

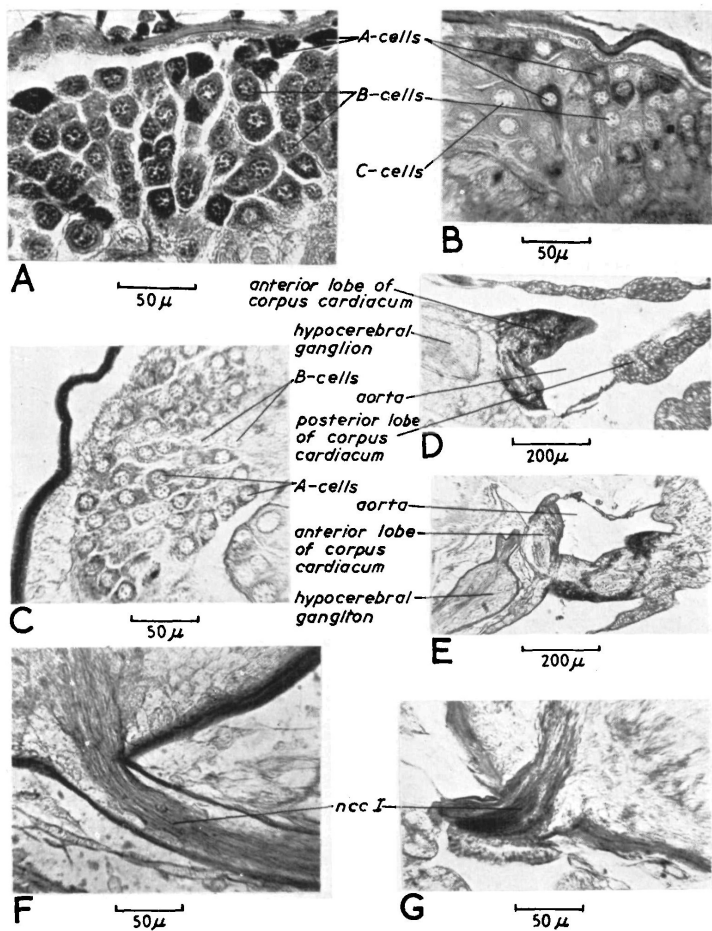


FIG. 1
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large air-sacs in the head and allow rapid and unrestricted fixation of the brain and endocrine system. The cuticle of the head capsule was dissected away before impregnation with paraffin wax. Serial sections were cut at $10\ \mu$ and stained with Gomori's chrome-haematoxylin-phloxin (CHP) or Gomori's paraldehyde-fuchsin (PF) after the methods of Halmi (1952) or Dawson (1953). The volumes of the neurosecretory cells and corpora cardiaca were calculated from their mean areas, which were measured with the 'Allbrit' (Stanley) disk planimeter. Percentages of different cell-types in the neurosecretory cell groups were estimated by counting the numbers of cells in every fifth section of a series.

RESULTS

Neurosecretory cells. Two closely apposed groups of neurosecretory cells are present in the dorsal part of the pars intercerebralis region of the protocerebrum. Each group is about $0.35\ \text{mm}$ long by $0.22\ \text{mm}$ wide, and extends about $0.12\ \text{mm}$ into the brain near the mid-line. The neurosecretory cells are covered by a layer of vacuolated cells beneath the brain membrane. About 1,200 cells may be counted in each group: some 90% of them typically contain inclusions stainable with CHP and PF.

In the mature female, four types of cell may be identified on the basis of differences in size and staining reactions (table 1). About 68% of the cells stain blue-black with CHP. These are the so-called A-cells. Varying amounts of inclusions are found both in the cell-bodies and along their axons (figs. 1, A, G; 2, A). Where the cells are large, the inclusions are also large and completely fill the cell-bodies (figs. 1, A; 2, A). A few of the cells are vacuolated (fig. 2, A). When stained with PF, a similar proportion (about 68%) of the cells contain deep purple inclusions (figs. 1, B; 2, A). The inference that these are also A-cells is supported by the examination of alternate sections of the same series stained with CHP and PF. Many A-cells appear in adjacent sections and the reaction of the same cell to the two stains can be compared.

FIG. 1 (plate). Photomicrographs of sections of various parts of the neurosecretory system of adult female *S. gregaria*. All fixed in Bouin's fluid; A stained with chrome-haematoxylin-phloxin, the rest with paraldehyde-fuchsin.

A, vertical section through the pars intercerebralis region of the brain of a mature female. Note particularly the large A- and B-cells; the A-cells packed with large, deeply staining inclusions.

B, as A, but in addition a few C-cells may be seen.

C, vertical section through the pars intercerebralis of the brain of an immature female. Note the absence of large, deeply staining A-cells (compare A and B). The inclusions are small, and aggregated closely around the nucleus.

D, section through the anterior parts of the corpora cardiaca of a mature female. Note the amount and distribution of the deeply staining neurosecretory material.

E, as D, except that the corpora cardiaca are those of an immature female. The neurosecretory material is smaller in amount than in the mature female, and has a more peripheral distribution.

F, section through one of the two nervi corporis cardiaci I at the point where it leaves the brain. Immature female. Note the absence of accumulated material compared with C.

G, as F, but from a mature female. Note the difference in appearance of the nerve compared with F, caused by the presence of accumulated neurosecretory material.

PF appears to stain the inclusions without colouring the ground cytoplasm of the cell, and consequently the constitution of the inclusions can be more easily seen than with CHP. It is clear that the large inclusions are actually aggregates of smaller particles (fig. 2, A). Vacuoles cannot be seen after PF staining.

About 17% of the cells stain red with CHP. These are the B-cells. They contain phloxinophil inclusions of small size, never as large as the inclusions within the A-cells. The B-cells vary in size (figs. 1, A, B; 2, B) and some are vacuolated. Their inclusions can be traced along the axons leaving the cells.

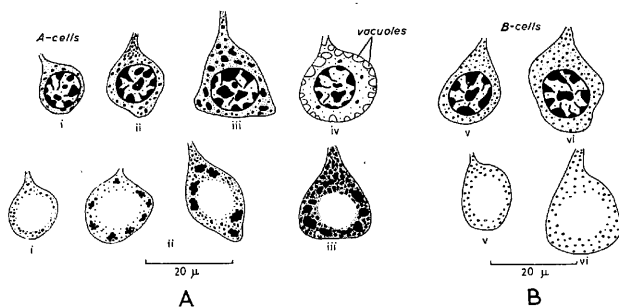


FIG. 2. Camera lucida drawings of A- and B-cells in the pars intercerebralis of adult female *S. gregaria*. Upper row, stained with chrome-haematoxylin-phloxin. Lower row, stained with paraldehyde-fuchsin. A, A-cells, showing differences in size, and differences in the size and the number of inclusions. i and ii are typical of the immature female; iii and iv, in addition, are typically found in the mature female. B, B-cells. Typically found in both mature and immature females.

With PF, the B-cells stain a faint pink, or sometimes take on a greenish tinge; the inclusions are reddish-purple.

Large cells with large nuclei are present near the periphery of each neurosecretory cell group (fig. 1, B). They stain a faint purple with CHP and reddish with PF. Inclusions staining blue-black with CHP and red-purple with PF are scattered sparsely through the cytoplasm of the cells, but have not been seen along the axons. These cells, called C-cells here, make up about 12% of the total number of cells in each group.

Cells very like the C-cells in their staining reactions, but very much larger (table 1), are found particularly towards the lateral borders of the neurosecretory cell groups. These are designated D-cells. Their inclusions, well seen after PF staining, are arranged regularly through the cytoplasm of the cell-bodies, but cannot be seen along the axons. D-cells make up about 5% of the total number of cells in a group. They are very similar in appearance to large motor neurones present in this part of the brain. Neither the C-cells nor the D-cells, in the absence of inclusions along the nerve axons, satisfy the cytomorphological criteria established by Scharrer & Scharrer (1954 b)

for the identification of neurosecretory cells. They are included in this account only because their axons run through the brain in company with the axons of the A- and B-cells. A similar association of neurosecretory and non-neurosecretory cells has been found in *Platysamia cecropia* (Stumm-Zollinger, 1957) and *O. fasciatus* (Johannson, 1958).

In each half of the brain a further group of neurosecretory cells is present between the pars intercerebralis and the mushroom body, about 0.5 mm from the mid-line. Each group comprises about 10 cells which have the staining characteristics of A-cells but are larger.

TABLE I

Characteristics of cells present in the pars intercerebralis of the brain of adult female S. gregaria

Cell type	Nuclear diameter	Cell volume	Staining reaction		Stages present (see fig. 2)	
			CHP	PF	immature	mature
A	10-14 μ	1.5-5.6 $\times 10^3 \mu^3$	blue-black	deep purple	i, ii	i, ii, iii, iv
B	10-14 μ	1.5-6.0 $\times 10^3 \mu^3$	red	pink (sometimes faint green)	v, vi	v, vi
C	20 μ	33.5 \pm 2.5 $\times 10^3 \mu^3$	faint purple	pink, reddish-purple inclusions		
D	23 μ	47.7 \pm 4.0 $\times 10^3 \mu^3$	faint purple	pink, reddish-purple inclusions		

The nervi corporis cardiaci. The combined axons of the neurosecretory cells in each pars intercerebralis group form a nerve (NCC I) whose path through the brain can be easily traced owing to the presence of stainable material along its length (fig. 1, G). The two nerves pass vertically downwards into the brain for a short distance and then cross one another, all the axons in each nerve apparently crossing to the opposite side. Each nerve then continues in a curving path forwards and downwards, running more or less parallel with the anterior face of the pars intercerebralis but at some distance from it, and finally emerges from the ventral surface of the brain just to one side of the mid-line. Immediately after leaving the ventral surface of the brain, the NCC I bend sharply forwards and pass to the anterior parts of the corpora cardiaca.

The material along the lengths of the NCC I is identical in its staining properties with the neurosecretory material within the cell-bodies of the pars intercerebralis. In material stained with CHP, both blue-black and red inclusions can be identified. The blue-black A-material forms aggregates of very much larger size than the similar material within the cell-bodies (fig. 1, G). These large masses are only seen distal to the point of decussation. The red B-material, on the contrary, occurs in small aggregates only a little larger

than the inclusions within the B-cells, and only rarely forms large clumps. These observations suggest that the two kinds of material differ in physical as well as chemical properties. With PF staining, in sections 10μ thick, individual axons may be followed for a considerable distance through the brain, and it can be seen that the A-material may occur at rather regular intervals along the axon. This may indicate a phasic discharge of the material from the cell-body, although it may be a fixation artefact.

It has not been found possible to trace through the brain the paths of the nervi corporis cardiaci II from their origin in the lateral groups of neurosecretory cells, owing to the absence of stainable material along the axons at this time. This implies that these neurosecretory cells are not discharging material in the mature female locust. The NCC II can be followed as distinct nerves from the ventral surface of the brain to the anterior parts of the corpora cardiaca.

The corpora cardiaca. These are elongated paired bodies lying behind the brain, dorsal to the hypocerebral ganglion (fig. 3, A). They are intimately associated with the walls of the aorta, anterior to the point where the vessel bends downwards beneath the brain, following the course of the oesophagus.

The anatomy of the corpora cardiaca (oesophageal ganglia) and their relation with the aorta and sympathetic nervous system has been described for *Locusta migratoria* by Albrecht (1953). His description differs in several details from that given below for *S. gregaria*. Albrecht based his description upon dissected material alone, whereas the following description is amplified and supported by a detailed examination of histological material. *L. migratoria* heads have also been examined histologically, and the structure of the endocrine system has been found to differ very little from that of *S. gregaria*.

With the insect in its normal posture, the long axes of the corpora cardiaca run almost vertically, owing to the hypognathous structure of the locust head. The 'posterior' parts of the corpora cardiaca are therefore actually dorsal to the 'anterior' parts. However, the terms anterior and posterior are retained in this description in preference to ventral and dorsal, to allow clearer comparison with the glands of other insects.

The posterior ends of the glands are delimited by a transverse strip of tissue originating from the dorsal wall of the aorta (fig. 3, A). This divides into median and lateral strands (Albrecht, 1953), which are inserted into the dorsum of the brain and the anterior horns of the tentorium. A small pouch is formed dorsally to the aorta, where this tissue originates, and the posterior ends of the corpora cardiaca follow the walls of the pouch rather than the main course of the vessel. The corpora cardiaca constitute the lateral walls and the greater part of the ventral wall of the aorta anterior to the region of bending of the vessel. Anteriorly, the corpora cardiaca form two large lobes, which project into the lumen of the aorta (fig. 3, A, B), and fuse ventrally to form an unpaired lobe which makes up the whole of the ventral wall of the aorta in this region (fig. 3, B). This ventral unpaired lobe projects a short distance in front of and behind the dorsal paired lobes; it lies almost imme-

diately above the hypocerebral ganglion, to which it is connected by a pair of short nerves. The NCC I and II enter the paired anterior lobes of the corpora cardiaca (fig. 3, A), and the nerves to the corpora allata leave the anterior unpaired lobe (fig. 3, A). The posterior parts of the corpora cardiaca in the lateral

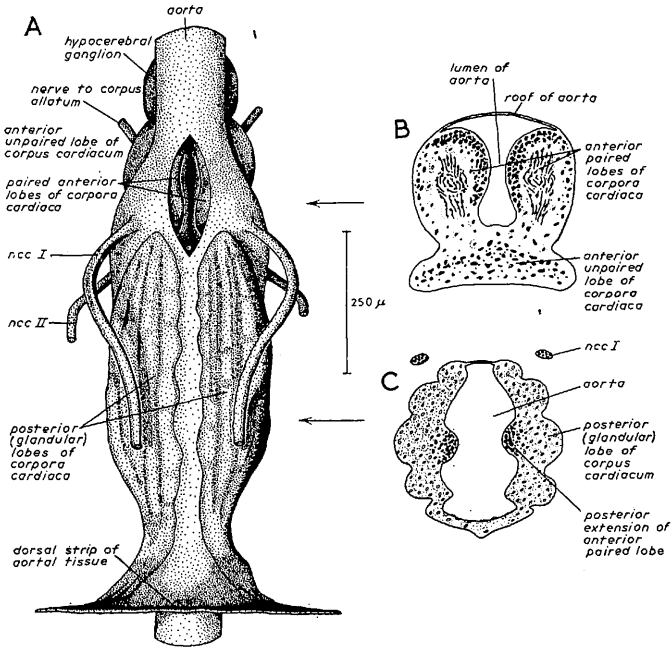


FIG. 3. A, corpora cardiaca and associated structures of adult female *S. gregaria*. Semi-diagrammatic. Part of the dorsal wall of the aorta is shown cut away to reveal the anterior paired lobes of the glands beneath. Explanation in the text. B and C, drawings of sections through the corpora cardiaca at the levels marked by the arrows. Note the clear differentiation between anterior and posterior parts of the glands.

aorta walls do not project into the lumen of the vessel (fig. 3, C); their outer surfaces are folded and lobed (fig. 3, A, C). These posterior parts of the glands are most easily seen in fresh dissections, and seem to be the parts figured as complete corpora cardiaca by Albrecht (1953) in *L. migratoria*.

Histologically, the anterior and posterior parts of the corpora cardiaca are strikingly differentiated by the distribution of stainable material from the neurosecretory cells in the brain (figs. 1, D; 3, B, C). With CHP, the anterior paired and unpaired lobes contain large amounts of A-material stained

blue-black; this is particularly abundant where the NCC I enter, and also around the periphery of the glands (fig. 3, B). A-material is found also in two finger-like processes of the paired anterior lobes which project backwards along the inner sides of the posterior parts of the glands (fig. 3, C). Other than these narrow extensions, the posterior parts of the corpora cardiaca contain no A-material. *L. migratoria* has also been examined and exhibits a similar distribution of A-material in the corpora cardiaca of the adult female. This contrasts with the report of Nayar (1954) that this species gives a negative reaction with chrome-haematoxylin. After PF staining, a deep purple colour is distributed in the same way in the corpora cardiaca of both *S. gregaria* and *L. migratoria*.

In addition to the A-material, phloxinophil material is present in the anterior lobes of the glands after CHP. It is most obvious in the central parts of the anterior lobes, but its presence in the more peripheral regions may be obscured by the deeply staining A-material. The phloxinophil material is thought to be derived from the B-cells in the pars intercerebralis, since it is also present along the lengths of the NCC I.

Phloxinophil material is found in addition in the posterior parts of the corpora cardiaca, but here it is intracellular and is consequently thought to be different in origin from the similarly staining material in the anterior lobes of the glands: it is probably an intrinsic secretion of the corpora cardiaca. The rather sharp demarcation between those parts of the corpora cardiaca of *S. gregaria* which store material from the neurosecretory cells in the brain, and those parts which produce the intrinsic secretion of the glands should make it easier to determine the physiological functions of the two kinds of secretion in this species, than in those insects where the glandular cells of the corpora cardiaca are very closely associated with the storage regions, as in *Mimas tiliae* (Highnam, 1958). This possibility is at present being investigated.

With CHP, A-material can be traced for a short distance along the nerves to the corpora allata, but never into the glands themselves. With PF, deep purple material can be traced for a similar distance along the nerves, but this then gives way to a diffusely staining reddish-purple material which can be followed into the corpora allata. This reddish-purple staining may indicate the transformation of the deep purple-staining material into one with different chemical properties, or it may indicate the presence of material from the B-cells in the pars intercerebralis, the presence of which in the proximal parts of the nerves to the corpora allata is obscured by the deeply staining A-material. Similar observations have been made in other insects (Stutinsky, 1952; Arvy, Bounhiol, & Gabe, 1953 *b, c*; M. Thomsen, 1954 *a, b*). Neurosecretory material can also be traced along the lengths of the short nerves connecting the anterior unpaired lobe of the corpora cardiaca with the hypocerebral ganglion.

The neurosecretory system in the immature female. Changes in the histological appearance of the neurosecretory cells in the brain during the development of the ovaries have previously been described in other insects (Dupont-Raabe, 1952; Arvy, Bounhiol, & Gabe, 1953; Formigoni, 1956). In *S. gregaria*, cautery of the neurosecretory cells in the pars intercerebralis prevents ovarian

development (Highnam, unpublished). The neurosecretory system of immature females was consequently examined in order to compare it with that of the mature female and determine whether any histological differences could be related to the neurosecretory control of ovarian development. Making due allowance for the individual variation in maturation times, one can see that the progress of ovarian development is accompanied by a gradual change in the histological appearance of the system in the immature form to that described for the mature form.

In the newly emerged female, the neurosecretory cells in the pars intercerebralis are not so obvious as in the mature female (fig. 1, c), but this is only a matter of degree. A-cells may be identified with certainty with both CHP and PF staining. They constitute about the same proportion (68%) of the total number of cells as they do in the mature female. But no large cells packed with large inclusions can be seen at this time. All the cells are relatively small, with small inclusions arranged mainly around the nucleus (fig. 1, c). This arrangement is best seen with PF staining. The cells are indistinguishable from the smaller A-cells in the mature female (fig. 2, A). B-cells also occur in the same proportion in the pars intercerebralis of the immature female as in the mature form. Their size, the number and appearance of their inclusions, &c., are very similar to those of the B-cells in the mature female. C- and D-cells are identical in number and appearance with those of the mature female. The only obvious histological differences between the neurosecretory cells of mature and immature females occur in the A-cells of the pars intercerebralis.

The NCC I in the immature female present a further difference in appearance compared with those in the mature form. The large accumulations of A-material along the lengths of the NCC I, so typical of the mature female (fig. 1, c), are not found in the immature (fig. 1, f). Close examination of the nerves in the immature female reveals that A-material is present, but always in the form of *small* droplets or particles. This difference is also reflected in the corpora cardiaca: they are smaller in volume than those of the mature female, and the neurosecretory material is less concentrated and aggregated into smaller masses (fig. 1, e; compare fig. 1, d). B-material appears to be present in larger amount, both along the NCC I and in the corpora cardiaca of the immature female, but this appearance is probably due to a reduction in the masking effect of the A-material which is present in smaller amount. The implication of this observation is that the B-cells of the pars intercerebralis show little change in activity during ovarian development compared with the A-cells. With PF staining, diffuse reddish-purple material can be traced into the corpora allata in the immature female, as in the mature form.

DISCUSSION

The pars intercerebralis region of the protocerebrum of different insects may contain a large number of small, or a small number of large, neurosecretory cells. There is probably a fairly constant relationship between total neurosecretory cell volume and body-size. This variation in the number and

size of neurosecretory cells in different insects makes a strict comparison of the nature and function of various cell-types rather difficult. *S. gregaria* possesses a very large number of small neurosecretory cells, being similar in this respect to various phasmid (Dupont-Raabe, 1952) and hymenopteran (M. Thomsen, 1954 b) species.

In *S. gregaria*, A- and B-cells are clearly differentiated with CHP. In addition, the A-cells stain purple with PF, and the B-cells red, sometimes with a greenish tinge. The cells are therefore very similar to the A- and B-cells in the pars intercerebralis of *O. fasciatus* (Johannson, 1958), *Drosophila* (Kopf, 1957), and *Bombyx* (Kobayashi, 1957). Two additional kinds of cell, C- and D-cells, are also present in this part of the brain in *S. gregaria*. The reaction of these cells to PF is rather different from that of the C- and D-cells described by Johannson (1958) in *Oncopeltus*. It is doubtful whether these cells are neurosecretory in *S. gregaria* (p. 30). Johannson (1958) similarly considers that the D-cells of *Oncopeltus* may not be neurosecretory. It is possible that the C- and D-cells are concerned with some mechanism which releases stored neurosecretory material from the corpora cardiaca, since in *S. gregaria* the axons of these cells accompany those of the A- and B-cells to the glands.

The B-cells have been thought to be a stage in the secretory cycle of the A-cells (M. Thomsen, 1954 b; de Lerma, 1956; Herlant-Meewis & Pacquet, 1956; Kopf, 1956). Nayar (1955), who examined the reaction of the neurosecretory cells of *I. limbata* to CHP after various experimental treatments, has suggested that the B-cells are actually A-cells deprived of the bulk of their stainable inclusions. This view is supported by the observation of M. Thomsen (1954 b) that both red and blue inclusions may be present in the same cell after CHP staining in some Hymenoptera. In *S. gregaria*, both A- and B-cells vary in size, and the different sizes of each cell-type may well represent stages in the elaboration of its own particular inclusion. Further, after CHP staining, red as well as blue-black material can be traced along the axons of the NCC I; and this suggests that both A- and B-cells are discharging their respective inclusions. In mature female *S. gregaria*, where the activity of the A-cells appears to be very different from that in the immature form, no difference in the ratio of A-cells to B- is found. These observations indicate that in *S. gregaria* the B-cells are different in kind from the A-, and are not simply a stage in the secretory cycle of the latter. This conclusion is supported by Johannson (1958), who subjected *O. fasciatus* to a variety of treatments but was unable to detect any transformation from B-cells to A-.

The presence of many large inclusions in the A-cells has usually been thought to indicate that the cells are more active than when the inclusions are few and small (Dupont-Raabe, 1952; Arvy & Gabe, 1952; Formigoni, 1956; Herlant-Meewis & Pacquet, 1956). On this interpretation the difference in appearance of the A-cells in *S. gregaria* in immature and mature females would seem to indicate that the cells are secreting more actively in the mature insects, and that more material is transported along the NCC I to the corpora cardiaca. This interpretation, however, needs to be carefully examined.

The amount of material contained in a neurosecretory cell at any time depends both upon its rate of synthesis and its rate of discharge. The observation that at times some neurosecretory cells contain larger amounts of material than at others may therefore indicate not an increased rate of synthesis, but a decreased rate of discharge, with a consequent accumulation of material within the cell-bodies. In mature *S. gregaria*, this accumulation of material within the A-cells may be a reflection of accumulation in the corpora cardiaca and along the NCC I. In effect, the amount of material in the cell-body is no indication of its *rate* of secretion. Further, material from the neurosecretory cells is discharged along the NCC I to the corpora cardiaca in the immature female locust, as well as in the mature female, so that the large cells packed with inclusions found in the mature female are not necessarily an essential stage in the secretory cycle of the A-cells.

Another factor that may still further complicate the picture is the duration of the secretory cycle. If this changes, by being speeded up or retarded, the output of the neurosecretory cells (in a given time) could increase or decrease without in any way affecting the histological picture. It is significant that no *histological* differences exist between the neurosecretory cells of *Rhodnius prolixus* before and at the height of secretion of the brain hormone, the times of which had been precisely determined experimentally (Wigglesworth, 1940).

It is concluded, therefore, that the histological differences which exist between the neurosecretory cells in the pars intercerebralis of immature and mature *S. gregaria* do not provide sufficient evidence for either increased or decreased activity at these different stages in development. However, the observations presented here do suggest that neurosecretory material accumulates in the corpora cardiaca of the mature female, and there is a strong possibility that the material accumulates also along the NCC I and within the A-cell-bodies. Further information concerning the importance of this material for oviposition and the subsequent development of more oocytes will be presented in a later paper.

It should be remembered that the stainable material within the neurosecretory cells and the corpora cardiaca is not necessarily identifiable with any hormonally active principle (Scharrer & Scharrer, 1954 *a*). The stainable material is thought to be a 'carrier protein' for the actual brain hormone (or hormones). The assumption is made that when the stainable material is present, so also is the hormone.

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