Neurosecretion in the insect embryo

August DORN

Institut für Zoologie der Johannes Gutenberg-Universität Mainz
Saarstrasse 21, D-6500 Mainz
Federal Republic of Germany

1 INTRODUCTION

In insects neurosecretory cells (NSCs) are scattered across the whole central nervous system but are concentrated in the pars intercerebralis region of the brain and the intrinsic part of the corpora cardiaca (CC). Also neurons of the stomatogastric ganglia may contain NSCs in some species (Borg *et al.*, 1973; Yin and Chippendale, 1975). Finlayson and Osborne (1968) described peripheral NSCs. The classical neurohormones (NHs) represent peptides which are released into haemolymph and reach their target via the bloodstream; i. e., they are effective across long distances. Neuronal secretions which are effective across short distances (transmitters) were thought to be non-peptidergic and called neurohumours. In recent years it has become apparent that this classification is not strict. Direct innervation of target tissue by peptidergic axons has been observed and neurohumours may act in some cases over long distances. Many of the observations on neurosecretion (NS) were made by electron microscopy. Peptidergic neurosecretory granules are well preserved by fixatives and are easily detectable. But aminergic neurosecretory granules are also often preserved and exhibit a dense core withdrawn from the surrounding membrane (Agricola *et al.*, 1975). A clear cut distinction is not possible and in histological studies they are dealt with together.

On grounds of histochemical and fine structural studies in the brain alone up to seven different types of NSCs could be demonstrated (Herman and Gilbert, 1965; Fletcher, 1969; Schooneveld, 1974). A correlation of a specific NSC with a defined NH was only achieved in *Manduca sexta* pupae in the case of the prothoracicotropic hormone (PTTH) producing cells (Agui *et al.*, 1979). There are two groups of NHs in insects, those which regulate morphogenesis and those which regulate metabolism. Morphogenetic NHs are PTTH (activation of prothoracic glands), allatotropin and antiallatotropin (activation and inhibition of corpora allata), eclosion hormone (direction of ecdysial processes), bursicon and pupariation hormone (direction of sclerotization process after ecdysis and at pupariation of blow flies respectively) and diapause hormone of *Bombyx mori* (for reference see Dorn, 1978a). According to their function in metabolism the following NHs are known: hyperglycemic hormone, adipokinetic hormone, diuretic and antidiuretic hormone, and such that regulate protein level of haemolymph, protein secretion in fat body, production of digestive enzymes a. o. . Also respiration and heartbeat are probably controlled by NHs. Many other NHs are certainly still undetected (for references see Dorn, 1978a).

For our review on embryonic NSs those which regulate morphogenetic processes deem of special interest. Haemolymph is already present in later stages of embryogenesis and hormones regulating its composition should probably be expected. There is, however, absolutely no information on such NHs in the embryo. Therefore we have to concentrate on morphogenetic NHs.

2 Histological Demonstration of NSCs

2.1 Identification of NSCs by "cytological appearance" and special staining methods

Jones (1956 a, b) was the first to report neurosecretory activity in the brain of an insect embryo, *Locustana pardalina*, beginning at the 3rd day of post-diapause development, which is 2 days before katatrepsis. An embryonic moult takes place in *Locustana* on day 6, just after completion of dorsal closure, and the neurosecretory processes were brought into connection with these embryonic moults. They supposedly stimulate the ventral (=moulting) glands (Jones, 1953). Since Jones' observations on embryonic NS laid the foundation for subsequent investigations – however not unchallenged – a more detailed quotation of the relevant findings is given in the footnote[1].

The presence of NSs was subsequently described in embryos of *Periplaneta americana* and *Blatta orientalis* (Füller, 1960), *Dysdercus cingulatus* (Sharan and Sahni, 1960) and *Periplaneta americana* (Khan and Fraser, 1962). The latter were the first to demonstrate unequivocally PAF-positive cells in the embryonic but fully differentiated brain. These authors questioned the findings on *Locustana pardalina* and *Dysdercus cingulatus*, since NS was reported from developmental stages in which nerve cells were not fully formed, i. e., neurites had not yet sprouted. Recent studies on embryonic NS were carried out in *Schistocerca gregaria* (Küçükeksi, 1969) and *Morimus funereus* (Stanipć *et al.*, 1978).

Considerable efforts were made in *Oncopeltus fasciatus* to depict embryonic NSCs by staining with PAF, chrome-haematoxylin, pseudoisocyanin and other histochemical procedures (Dorn, 1972). However none of them showed positive results. Only during early development of the central nervous system did some cells in the head lobes include granules which stained red with azan, a non-specific reagent for aminergic NSs. A neurosecretory activity was considered, but as in *Locustana pardalina*, this would be prior to neurite formation. PAF-staining in *Carausius morosus* had also negative results (Dorn, unpublished). The few convincing micrographs showing PAF-positiv cells stem from Khan and Fraser (1962) (*Periplaneta americana*), Polivanova (1976) (*Eurygaster*, but only after experimental manipulations), Mitsuhashi (1963) and Loeb and Hayes (1980) (*Lymantria dispar*).

Although concentrated in the brain, NSCs occur in all parts of the postembryonic central nervous system of insects, i. e., the suboesophageal ganglion and the thoracic and abdominal ventral ganglia. Another neurosecretory center is the corpus cardiacum (CC) which exists of intrinsic parts comprising NSCs and glial cells and extrinsic parts consisting of conventional and neurosecretory nerve fibres. The perikarya of these nerve fibres are (mostly) located in the brain and reach the CC via nervus corporis cardiacum (NCC). Only in the case of *Oncopeltus fasciatus* (Dorn, 1972; 1978a) and *Carausius morosus* (Dorn, 1978a) attempts were made to demonstrate neurosecretory material in the CC of embryos. In *Oncopeltus fasciatus* and *Carausius morosus* the total retrocerebral complex – CC, corpora allata and aortal wall – showed no reaction with PAF and chrome-haematoxylin.

[1] Jones (1956 a): "On the 3rd day, in the same region of the protocerebrum, there were to be seen relatively large cells which had developed the histological appearance of neurosecretory cells. The nuclei of these cells were indistinguishable from the normal nerve-cell nuclei, but, on the other hand, the cytoplasm contained material which stained blue with chrome-haematoxylin. However, on the 4th day these cells showed all the histological signs of being neurosecretory cells. For instance, by this time they had also acquired large vacuoles. It was interesting that by the end of the following day it was most difficult to distinguish neurosecretory cells from other nerve cells, in particular, the neuroblasts."

2.2 Identification of NSCs and neurosecretory nerve fibres by electron microscopy.

Being proteinacious or peptidergic in nature, the "classical" NSs are well preserved by fixatives used for electron microscopy and can be readily demonstrated on ultrathin sections. Nevertheless, there are only two insect embryos, to our knowledge, which were investigated by this method. Several questions of interest can be resolved by this method: a) Do embryonic nerve cells produce NSs? b) Are there different types of NSCs? c) When does NS start? d) Are NSs released into the haemolymph during embryogenesis? e) What is the biological function of NSs, if present?

The last question cannot be resolved by a histological approach beyond possible correlations with other events (see below). We tried to answer at least some of these questions in the embryos of *Oncopeltus fasciatus* and *Carausius morosus*.

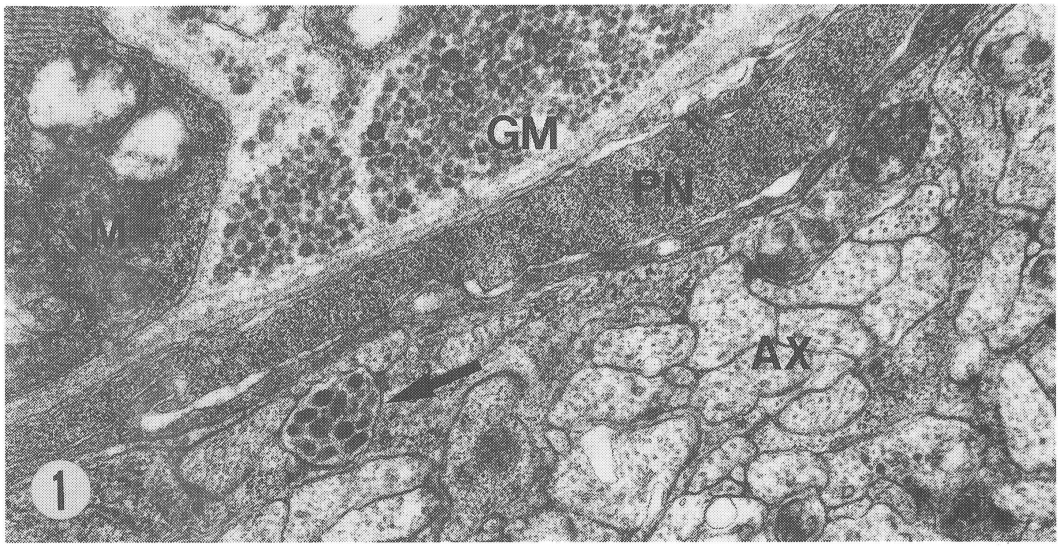


Fig. 1 Neurosecretory axon in the neuropil of the embryonic brain of *Oncopeltus fasciatus* shortly before hatching; arrow points to neurosecretory axon. **AX** conventional axons, **GM** unknown granular material located above the brain, **M** muscle, **PN** perineurium. 20,000 x.

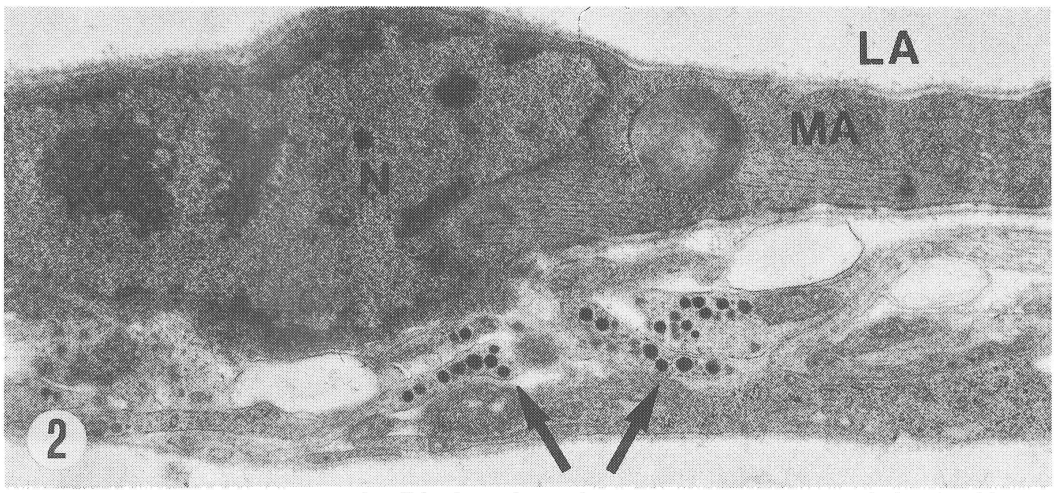


Fig. 2 Longitudinal section of aortal wall in the embryo of *Oncopeltus fasciatus* shortly before hatching; small neurosecretory nerve fibres (presumably nerve endings) can be recognized (arrows). **LA** lumen of the aorta, **MA** muscle cell of the aorta, **N** nucleus. 15,000 x.

2.2.1 NS in the brain

In *Oncopeltus fasciatus* the electron microscopical studies confirmed the histochemical findings: No perikarya contained accumulations of granules proving to be neurosecretory (Dorn, 1975a). However, a few nerve fibres in the neuropil of the brain contained neurosecretory granules (Fig. 1). These nerve fibres left the brain in the NCC and branched in the CC and the aortal wall, the neurohaemal organ of the hemipterans. Although the origin of these axons could not be traced, it seems highly probable that the appertaining perikarya are located in the brain. All profiles of neurosecretory nerve fibres in the brain and retrocerebral complex contained granules of the same type, electron dense, diameter up to 1300 Å. The morphological appearance is different from all types observed in the imago (Unnithan *et al.*, 1971; Unnithan and Nair, 1977; Dorn, 1978a). Neurosecretory fibres were first observed 96 hrs after oviposition – which is approximately at onset of secretion of larval (= 3rd embryonic) cuticle – in nerve endings of NCC in the aortal wall (Fig. 2). NS seemed to increase toward hatching time. Signs of release were never observed.

Comparative studies were carried out on embryos of *Carausius morosus* approaching hatching, i. e., during synthesis of larval (= 3rd embryonic) cuticle (Dorn, 1978a). In this species six to eight perikarya in the pars intercerebralis region of the brain enclosed scattered secretion products (Figs. 3, 4). The granules are formed by the Golgi complex and travel via NCC to the retrocerebral complex. Morphological features and pathway of the granules prove them as neurosecretory products, presumably peptidergic in nature. All neurosecretory perikarya of the pars intercerebralis in which granules accumulate have the same morphological features and in parti-

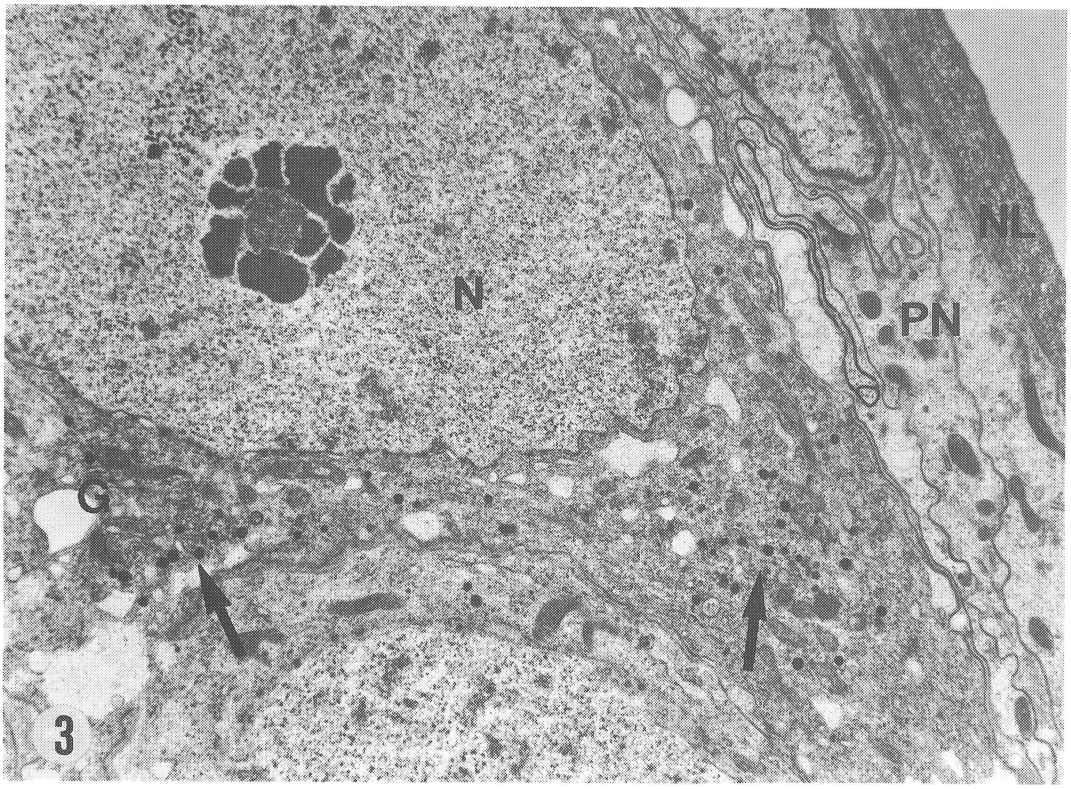


Fig. 3 Neurosecretory cell in the pars intercerebralis of the embryo of *Carausius morosus* shortly before hatching; arrows point to small accumulations of neurosecretory granules. G. Golgi complex, **N** nucleus with nucleolus, **NL** neural lamella, **PN** perineurium. 8,000 X.

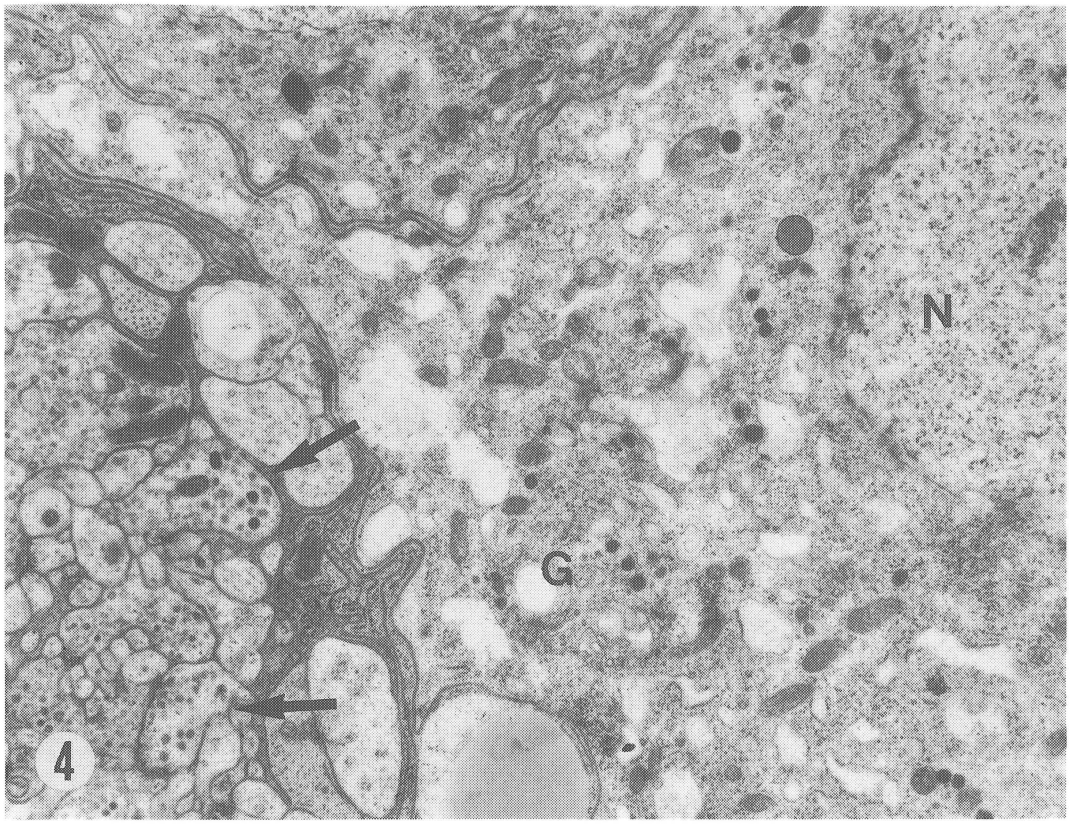


Fig. 4 Part of neurosecretory cell with scattered neurosecretory granules and part of neuropil (same embryo as in Fig. 3); the neuropil contains neurosecretory axons (arrows) with a different type of granules than seen in the perikaryon. **G** Golgi complex, **N** nucleus. 14,000 x.

cular, all granules have the same expression: they are round in profile, membrane bound with an electron dense content and a maximal diameter of about 1800 Å. Axons projecting fron NCC into the CC (Fig. 5) and corpora allata (Figs. 7, 8) comprise granules of the same type (see below).

The neuropil of the pars intercerebralis region and the extrinsic parts of the CC include axons with still another type of NSs (Figs. 4, 5). The granules of these axons are also round of profile but much smaller than the others: up to 900 Å in diameter. Their content is somewhat withdrawn from the limiting membrane. They are reminiscent of aminergic granules. The perikarya of these axons could not be detected, but are presumably located within the brain. This type of neurosecretory axon makes many synaptoid contacts with conventional nerve fibres. They are also found in the corpora allata (Fig. 7).

Like in *Oncopeltus fasciatus* no signs of granule release could be detected in extrinsic nerve fibres in the CC. But the release processes are presumably very rapid and nerve endings rather rare in the embryonic neurohaemal organs, which may account for the failure to detect exocytosis figures or synaptoids (Scharrer, 1983).

2.2.2 NS in the CC

Another neurosecretory center are the CC which are believed to represent transformed visceral ganglia. They differentiate before katatrepsis (Dorn, 1972; 1975b). In the imago of *Oncopeltus fasciatus* they include 3 different NSC types, in *Carausius morosus* at least 2. In the embryo of *Oncopeltus fasciatus* first tangible signs of NS were observed 110 hrs after oviposition,

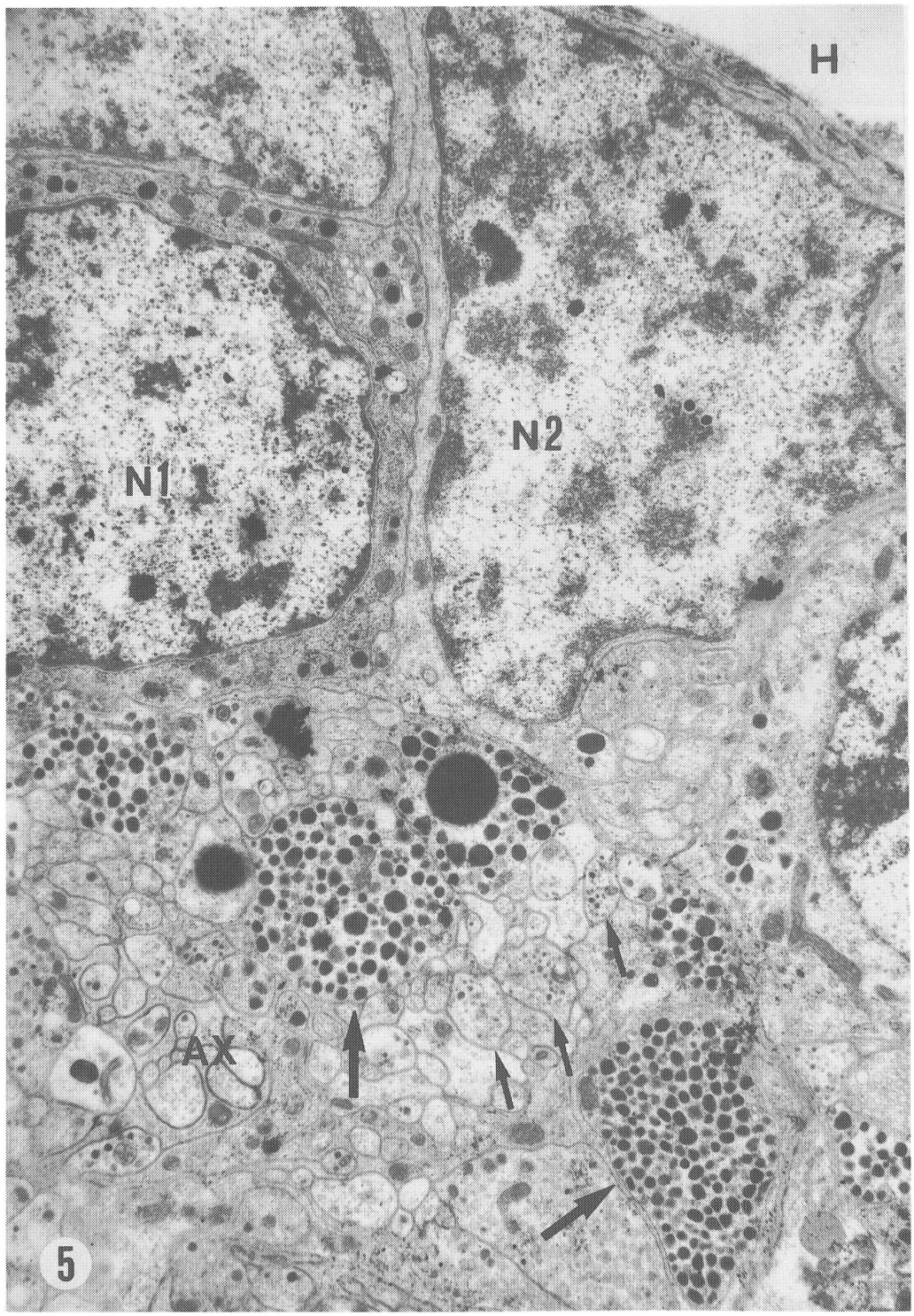


Fig. 5 Embryonic corpora cardiaca of *Carausius morosus* a few days before hatching; the intrinsic part consists of glandular cells (N1 nucleus of glandular cell) and glial cells (N2 nucleus of glial cell). The extrinsic parts contain 3 types of nerve fibres: conventional axons (**AX**), axons with neurosecretory granules which presumably stem from neurosecretory cells in the pars intercerebralis (big arrows), and axons with small neurosecretory granules the perikarya of which are not identified (small arrows). **H** haemocoel. 15,000 x.

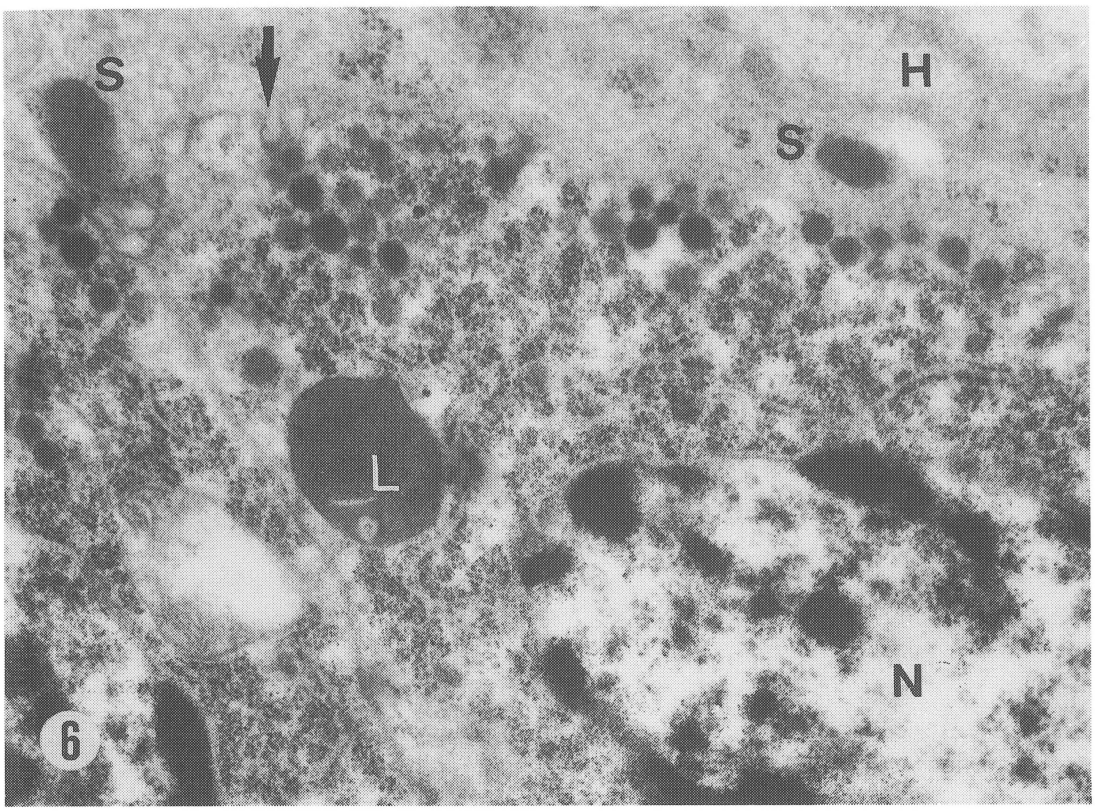
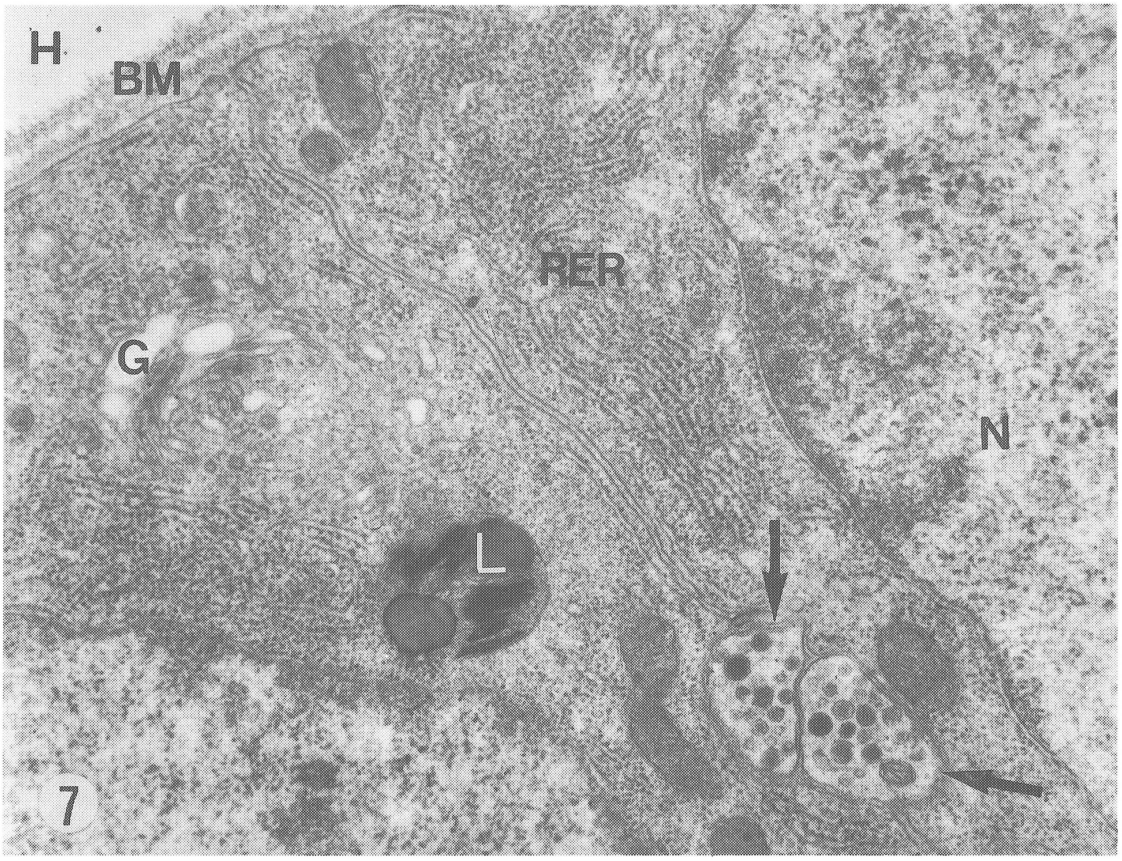


Fig. 6 Part of an intrinsic glandular corpus cardiacum cell of *Oncopeltus fasciatus* in an embryo immediately before hatching; secretory granules accumulate below the cell membrane facing the haemocoel (**H**). Omega-shaped cell membrane indentations indicate granulum release (arrow) and electron dense substance in the stroma surrounding the gland may represent aggregations of released secretions (**S**); note abundance of rough endoplasmatic reticulum. **L** lysosome, **N** nucleus. 20,000 x.

i. e., after the 2nd embryonic moult and 13 hrs before hatching (Fig. 6). The neurosecretory granules are formed by the Golgi complex and accumulate shortly before hatching. Immediately before hatching omega-shaped indentations of the cell membrane indicate release of the granules (Fig. 6). All secretory cells are of the same type; the granules are round in profile and electron dense. At the beginning of synthesis (110 hrs-embryo) they are smaller (diameter about 700 Å) than in older embryos (diameter up to 1500 Å at hatching time). The granulum type does not correspond with any of those found in imaginal CC.

Quite similar observations have been made in the CC of *Carausius morosus* shortly before hatching. The intrinsic cells, besides the glial cells, produce large granules (up to 2500 Å in diameter) which are pinched off from the Golgi complex. The secretions are apparently synthesized in cysternae of rough ER, like in the CC of *Oncopeltus fasicatus*. The nuclei of glandular cells are large and cytoplasm is scant, in contrast with the situation in imagines (Dorn, 1978a). Cell projections, typical for CC cells in adult insects, are not yet formed. The intrinsic cells surround extensions of the NCC (extrinsic part of CC) which form a compact mass of 3 types of nerve fibres: Conventional axons, axons with neurosecretory granules presumably originating in the pars intercerebralis and axons which carry small granules (up to 900 Å in diameter) intermingled with vesicles of the size of synaptic vesicles. The origin of the latter axons is obscure. Similar nerve fibres are seen in the neuropil of the brain (Fig. 4).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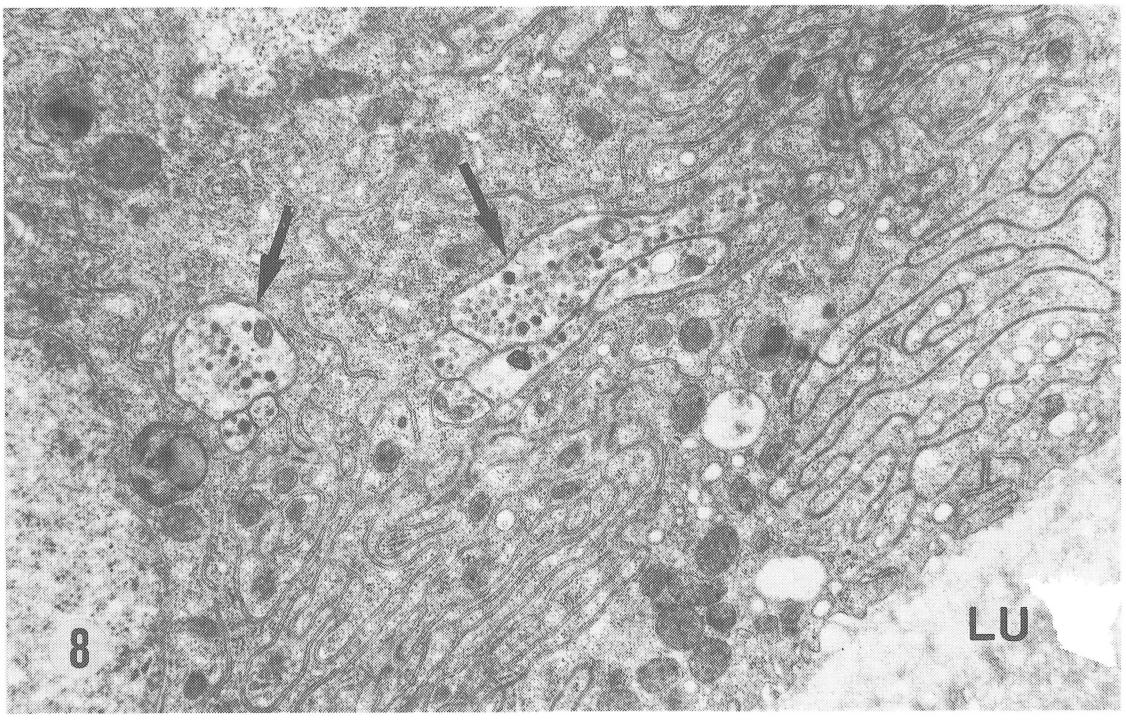
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No indication for release has been found in the neurosecretory nerve endings in the CC, the neurohaemal organ of *Carausius morosus*, or intrinsic CC cells. But again, this is no proof against granulum discharge (see above).

2.2.3 NS in the corpora allata (CA)

In many species the CA are innervated by neurosecretory nerve fibres, branches of the NCC. In *Manduca sexta* they even represent a neurohaemal organ (Agui *et al.*, 1980). In the imago of *Oncopeltus fasciatus* different types of neurosecretory nerve endings are in close contact with the gland cells and form synaptoid structures (Dorn, 1973). The role of this innervation is not yet clear. An allatotropic function of one or several of the NSs is probable. The CA in embryos of *Carausius morosus* contain neurosecretory axons during deposition of the larval cuticle (Figs. 7, 8). Two types of axons can be seen, identical with the two types described in the NCC. The fine structure of the CA cells indicates a glandular activity (see Haget *et al.*, 1981) which is also expected from embryonic CA in *Oncopeltus fasciatus* (Dorn, 1975c). Synthetic activity of embryonic CA have previously been proven by biochemical methods in the case of *Nauphoeta cinerea* (Lanzrein *et al.*, 1984).

2.2.4 NS in the stomatogastric nervous system

The occurrence of NSCs in the stomatogastric nervous system is reported in Lepidoptera (Borg *et al.*, 1973; Yin and Chippendale, 1975) and Diptera (Tombes and Malone, 1977). In other orders there are apparently no NSCs but the neuropil of the ganglia contains numerous neurosecretory axons (Dorn, 1978b; Ude *et al.*, 1978) the origin of which is largely unknown. In the case of the frontal ganglion of *Carausius morosus* such axons may come from the brain via the frontal connectives (Dorn, 1978b). Three types of neurosecretory axons can be distinguished in the imago. The embryonic frontal ganglion in this species also shows many neurosecretory fibres (Figs. 9, 10). These, however, are all of the same type. The fine structural characteristics of the granules are identical with those observed in the neuropil of the brain and in the NCC (Figs. 4, 5). Most conspicuous are the numerous syaptoid structures facing intercellular spaces, glial elements, nerve fibres of the conventional type, or other neurosecretory fibres (Figs. 11, 12). It cannot be decided whether the NSs can be considered as neurohormones, neurotransmitters or neuromodulators.

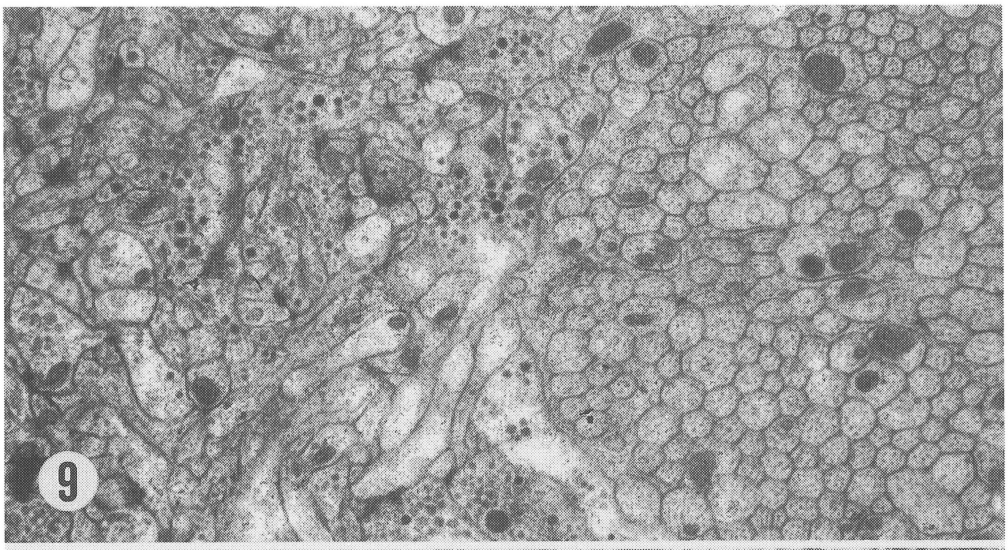
2.3 Comparative view of histological studies

The imaginal brain of insects, in particular the pars intercerebralis, and the CC contain numerous NSC types which reflect the multiplicity of its function: regulation of metabolic processes, ecdysis, metamorphosis, reproduction etc.. The morphological diversity of these cell types

Figs. 7 and 8 Embryonic corpus allatum cells of *Carausius morosus* during deposition of larval (= 3rd embryonic) cuticle; the paired gland is vesicle-like in this species; the epithelium is monolayered, and the cells exhibit a polarity in subcellular structure along the surface-lumen axis; the organelle equipment indicates glandular activity.

Fig. 7: Parts of two cells facing the haemocoel (**H**); note the profiles of two neurosecretory axons (arrows) the granules of which resemble those of neurosecretory cells in the pars intercerebralis. **BM** basement membrane, **G** Golgi complex, **L** lysosome, **N** nucleus, **RER** rough endoplasmatic reticulum. 36,000 x.

Fig. 8: Parts of several cells facing the lumen (**LU**) of the gland; note the profiles of 7 neurosecretory axons (arrows) the granules of which resemble those found in the brain, NCC and CC with the small granulum type; the location of the appertaining perikarya is not known; neighbouring cell membranes interdigitate; small electron lucent vesicles are apparently released into the lumen. 20,000 x.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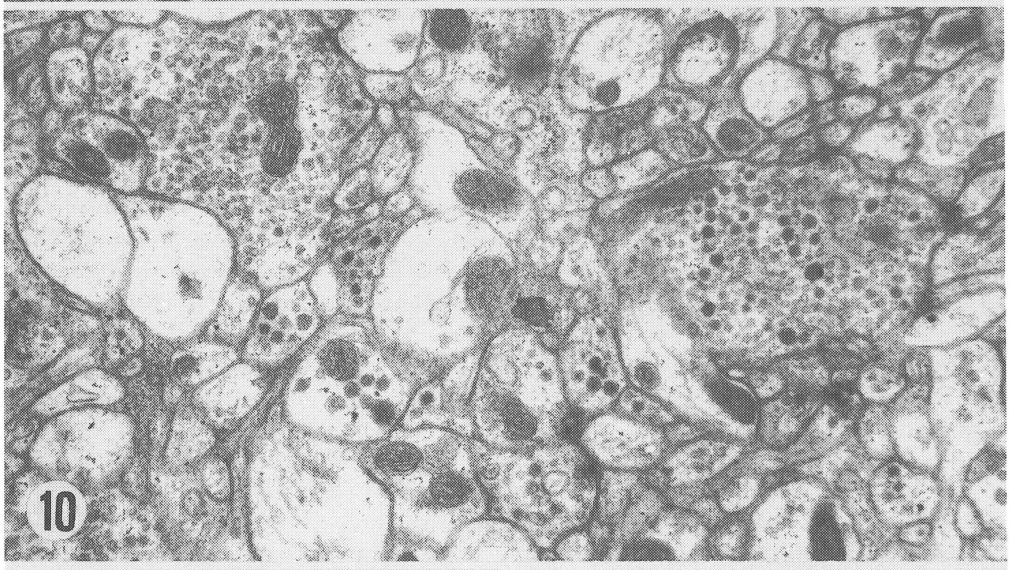
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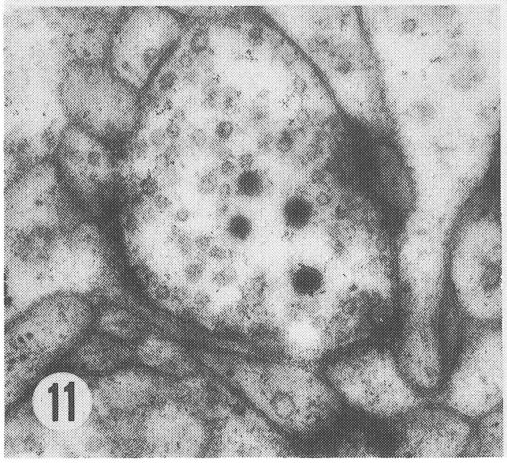
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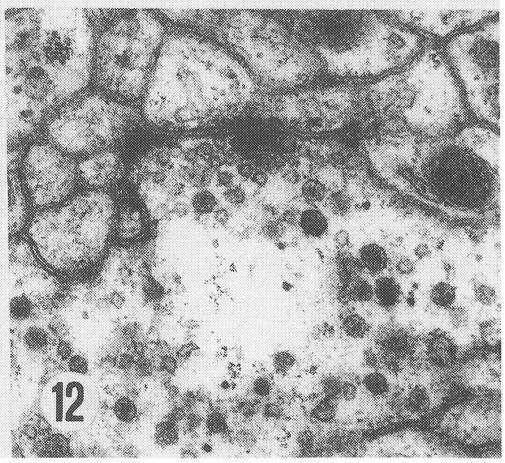
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was first demonstrated by the classical staining methods and later by electron microscopy and immunological methods. Size, shape and electron density of the granular neurosecretory products appeared to be suitable criteria for classification. Although there are only a few cases where a distinct neurosecretory cell type could be equated with a distinct function, it was expected that a morphologically defined cell type produces hormonal factor(s) with a specific role.

In sharp contrast, in the entire embryonic brain and retrocerebral complex only two types of morphologically distinguishable neurosecretory nerve fibres could be identified other than the intrinsic NSCs of the CC. One type contains larger electron dense granules which are manufactured in perikarya of the pars interecerebralis and travel via NCC to CC and CA and in the hemipteran *Oncopeltus* to the aortal wall. The second type with smaller granules occurs in the neuropil of the brain, in the retrocerebral complex and frontal ganglion. The morphological uniformity of the brain NSCs in the embryo and the smaller number of NSCs provoke several questions: Has the embryo so much fewer NHs than the older stages? Or is it merely the morphological differentiation of the granules that occurs later, i. e., various NHs are synthesized in the embryonic brain, but the granules are of the same type?! Can an individual NSC produce several hormonal factors, and if so, simultaneously or successively?

The onset of NS remains largely unclear. First granules are apparently readily transported to the neurohaemal organ and not stored in the perikaryon. Consequently, at this point the cells cannot be recognized as neurosecretory. In rapidly developing embryos like that of *Onocpeltus fasicatus*, the granules never accumulate in NSCs, but only at nerve endings. Only in slowly developing embryos like that of *Carausius morosus* is there a chance to locate NSCs.

No release signs have been found for brain NS in the neurohaemal organ which is not surprising since discharge is apparently a very quick process (Scharrer, 1983).

Little can be said about the neurosecretory fibres with the small granules. The location of their perikarya is unknown. Further it must be questioned if all the fibres seen in brain-retrocerebral complex and frontal ganglion are really uniform. In the frontal ganglion the granules are obviously not only stored but also discharged via synaptoids indicating a physiological function.

The glandular cells of the CC produce secretory granules shortly before hatching and releases them by exocytosis into the haemocoel. Since these cells have no large projections in the embryo the granules are crowded at the cell membrane and easily detected. This might be the reason that by phloxin staining the CC of *Periplaneta americana* was found to be secretory active (Füller, 1960).

3 Functional Significance of NSs

3.1 Prothoracicotropic activity

Already Jones (1953) suspected in his studies on embryonic moult that the activation of the moulting gland is regulated by NSs of the brain (i. e., PTTH). Also in *Oncopeltus fasciatus* an involvement of NSCs in the regulation of embryonic moults was implied. However, even today the

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Figs. 9 — 12 Neuropil of the embryonic frontal ganglion of *Carausius morosus* during deposition of larval (= 3rd embryonic) cuticle; many nerve fibres contain small-sized neurosecretory granules; morphologically similar granules are seen in axons of the brain neuropil and in the entire retrocerebral complex; the site of synthesis of these granules is obscure.
Fig. 9: The neuropil is rather distinctly divided into areas with conventional nerve fibres (right half of the micrograph) and such with neurosecretory nerve fibres (left half of the micrograph). 10,000 x.
Fig. 10: Neurosecretory nerve fibres at higher magnification. 30,000 x.
Figs. 11 and 12: Neurosecretory nerve fibres with synaptoid formations. 50,000 x.

role of the prothoracic gland in the embryo is not clear; in *Oncopeltus fasciatus* it appears inactive throughout embryogenesis on grounds of fine structural examinations (Dorn and Romer, 1976). Since large amounts of ecdysteroids are supplied by the mother, activation and inactivation of this supply during embryogenesis could account for titre fluctuations which are often observed (see Dorn, 1983; Hoffmann and Lagueux, 1985). PTTH would be dispensible in this case. Ecdysteroid synthesis could also occur in other tissues, probably without regulation by the brain. According to Haget *et al.* (1982) the embryonic ventral glands of *Carausius morosus* exhibit two activity cycles as judged by fine structural features. However, embryos deprived of their future ventral glands can produce considerable amounts of immunoreactive ecdysteroids. Similar observations are reported from *Clitumus extradentatus* (Cavallin and Fournier, 1981). Brain and CC seem to have little effect on the expression of ecdysteroid peaks; the authors even consider some inhibitory influence of this complex on ecdysteroid production in old phasmid embryos. On the other hand, cerebral NSCs and CC seem to be indispensable in very late embryonic stages; a putative function is not discussed.

A sensitive *in vitro* bioassay has been developed for PTTH (Bollenbacher *et al.*, 1979), and its titre was determined in pupal brain and haemolymph extracts of *Manduca sexta* (Gilbert *et al.*, 1981). This bioassay has been extended to embryonic brain extracts of *Manduca sexta* (Dorn, Gilbert and Bollenbacher, unpublished). It was shown that these extracts are able to activate pupal and larval inactive prothoracic glands. But still more biochemical evidence is needed to establish the identity of this factor with pupal PTTH. Hoffmann and Lagueux (1985) mention in their review on endocrine aspects of embryonic development that PTTH-like activity was also found in embryonic brains of *Locusta migratoria*.

3.2 Allatotropic activity

There is fine structural and biochemical evidence that CA become active during embryogenesis (Dorn, 1972, 1975c, 1983; Haget *et al.*, 1981; Lanzrein *et al.*, 1984). The micrographs on embryonic CA of *Carausius morosus* shown here (Figs. 7, 8) prove a direct neurosecretory innervation by two types of neurosecretory nerve fibres. The functional significance of these nerve fibres is obscure, since directed experiments are lacking. Although in many insects a chemically not further characterized allatotropin is demonstrated by bioassay (e. g. Granger *et al.*, 1981), it is not clear whether the NH reaches its target via haemolymph or direct innervation as could be expected by histological observations.

3.3 Embryonic diapause and neurosecretion

Embryonic diapause may occur during early or late embryonic development. In the latter case, endocrine glands are differentiated, and a diapause regulation similar to that in pupae seems possible (pupal diapause is initiated in the absence of ecdysteroids – prothoracic glands remain inactive since PTTH although stored in NSCs is not released into the haemolymph). Thus in *Aulocara elliotti* the obligatory diapause is thought to be the consequence of hormonal deficiency which probably results from the lack of neurosecretory stimulus form the brain-CC complex (Neumann-Visscher, 1976). In *Lymantria dispar* brain NSCs become increasingly stainable with PAF when diapausing embryos are chilled (Loeb and Hayes, 1980). After 90 days of chilling NSCs appeared packed with NS which are apparently transported along the axons. Return of chilled eggs to 24°C after 120 days at 4°C induced increased transport and consequent loss of PAF-positive substances, so just prior to hatching little PAF-positive material remained in the brains (Loeb and Hayes, 1980). The authors point out that similar neurosecretory processes take place in lepidopterans which diapause at more mature stages. It remains to be seen, however, if the NSs accumulated during embryonic diapause in deed represent PTTH, which is implied.

Diapause in *Bombyx mori* occurs at an early embryonic stage and is regulated by the

mother's endocrine system (for review see Yamashita and Hasegawa, 1985). The suboesophageal ganglion produces a diapause hormone which programs the maturing eggs. Although eggs are programmed at oviposition, it was recently demonstrated that diapause hormone also enters the egg (Kai, 1977)! Its action during embryogenesis, if any, is not investigated.

3.4 Eclosion hormone

Eclosion hormone is a neurosecretory peptide which was first found in Lepidoptera. It triggers adult ecdysis by acting on the central nervous system to elicit the ecdysial motor programmes and also causes other physiological changes associated with ecdysis (Truman and Riddiford, 1970). Recently Truman and coworkers (1981) showed that eclosion hormone may control all ecdyses in insects including embryonic moults; eclosion hormone was demonstrated in the *Hyalophora cecropia* embryo by bioassay. Preliminary experiments indicate that eclosion hormone is present mainly in the head region and appears on day 5 of embryonic development. The titre remains high for the next 2 days but then drops sharply between days 7 and 8 and remains low until the time of hatching of day 10. This drop in stored hormone coincides with the ecdysis of an embryonic cuticle by the developing 1st instar larva within the egg (Truman *et al.*, 1981). Histological demonstration of the eclosion hormone producing cells was not yet attempted.

4 Conclusion

The histological data show clearly that NSs are manufactured in cells of the brain and CC. Neurosecretory fibres are present in the neuropil of the brain, the extrinsic part of the CC, the CA and the aortal wall of the hemipterans. They are also found in the stomatogastric ganglia, e. g., frontal ganglion. The onset of NS is difficult to determine in the brain. Neurosecretory granules are apparently rapidly transported along the axons to the neurohaemal organ, where they accumulate first. In rapidly developing embryos they are never stored in appreciable amounts in the perikarya. Only in slowly developing embryos is there a chance to demonstrate them by histochemical methods or electron microscopy. It is therefore obvious that NS may begin some time before it can be visualized by the mentioned methods. Signs of NS are reported from "immature neurons", i. e., before the outgrowth of neurites and dendrites which usually develop shortly before katatrepsis. It remains to be seen if this is the case.

Secretory granules in intrinsic CC cells are visible shortly before hatching but after embryonic moults. Signs of release can be observed at hatching. Although direct histological indications for release of the brain NS are lacking, the studies on embryonic diapause in *Lymantria dispar* and on the eclosion hormone in *Hyalophora cecropia* are in favour of a discharge and biological function. In the frontal ganglion synaptoid formations of neurosecretory fibres are frequent.

In the species studied only 3 different types of neurosecretory granules have been found: one type synthesized by neurons of the pars intercerebralis (and probably other parts of the brain) and another type by intrinsic CC cells. The 3rd type was seen in nerve fibres of the brain, the whole retrocerebral complex and the stomatogastric ganglia. The multiplicity of NSC and granule types found in post-embryonic stages is thus in striking contrast to the far reaching morphological uniformity in the embryo.

The neuroendocrinology of the insect embryo has become a most interesting topic in developmental biology. Numerous studies on occurrence and function of ecdysteroids have provided new and unexpected results. The role of JH in morphogenesis is presently under investigation. Our knowledge of NHs during embryogenesis, however, is still rudimentary, but undoubtedly will experience a dramatic progress in the near future.

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5 References

Agricola, H., Baumann, E., Eckert, M., Gersch, M., Hentschel, E., Hertel, W., Kusch, T., Langbein, L., Richter, K., Ude, J. (1975): Prinzipien neurohormonaler und neurohumoraler Steuerung physiologischer Prozesse. *Wissenschaftliche Beiträge der Friedrich-Schiller-Universität Jena.* Magnus Poser, Jena.

Agui, N., Bollenbacher, W. E., Granger, N. A., Gilbert, L. I. (1980): Corpus allatum is release site for insect prothoracicotropic hormone. *Nature (Lond.)*, **285**, 669–670.

Agui, N., Granger, N. A., Gilbert, L. I., Bollenbacher, W. E. (1979): Cellular localization of the insect prothoracicotropic hormone: In vitro assay of a single neurosecretory cell. *Proc. Natl. Acad. Sci. U. S. A.*, **76**, 5694–5698.

Bollenbacher, W. E., Agui, N., Granger, N. A., Gilbert, L. I. (1979): In vitro activation of insect prothoracic glands by the prothoracicotropic hormone. *Proc. Natl. Acad. Sci. U. S. A.*, **76**, 5148–5152.

Borg, T. K., Bell, R. A., Picard, D. J. (1973): Ultrastructure of neurosecretory cells in the frontal ganglion of the tobacco hornworm, *Manduca sexta* (L.). *Tissue & Cell*, **5**, 259–267.

Cavallin, M., Fournier, B. (1981): Characteristics of development and variations in ecdysteroid levels in *Clitumnus* embryos deprived of their cephalic endocrine glands. *J. Insect Physiol.*, **27**, 527–534.

Dorn, A. (1972): Die endokrinen Drüsen im Embryo von *Oncopeltus fasciatus* Dallas (Insecta, Heteroptera). Morphogenese, Funktionsaufnahme, Beeinflussung des Gewebewachstums und Beziehungen zu den embryonalen Häutungen. *Z. Morph. Tiere*, **71**, 52–104.

Dorn, A. (1973): Electron microscopic study on the larval and adult corpus allatum of *Oncopeltus fasciatus* Dallas (Insecta, Heteroptera) *Z. Zellforsch.*, **145**, 447–458.

Dorn, A. (1975a): Elektronenmikroskopische Studien über das neurosekretorische System von *Oncopeltus fasciatus* Dallas (Insecta, Heteroptera) während der Embryonalentwicklung. *Cytobiol.*, **10**, 227–234.

Dorn, A. (1975b): Elektronenmikroskopische Studien über Differenzierung und Funktionsaufnahme der Corpora cardiaca im Embryo von *Oncopeltus fasciatus* Dallas (Insecta, Heteroptera). *Cytobiol.*, **10**, 235–248.

Dorn, A. (1975c): Struktur und Funktion des embryonalen Corpus allatum von *Oncopeltus fasciatus* Dallas (Insecta, Heteroptera). *Verh. Dtsch. Zool. Ges.*, **67**, 85–89.

Dorn, A. (1978a): Ontogenese des endokrinen Systems der Insekten. Untersuchungen an *Oncopeltus fasciatus* (Heteroptera) und *Carausius morosus* (Phasmatodea). *Habilitationsschrift, Universität Mainz.*

Dorn, A. (1978b): Neurosecretion in the frontal ganglion of the stick insect, *Carausius morosus*. *In*: W. Bargmann, A. Oksche, A. Polenov, B. Scharrer (eds.) "*Neurosecretion and Neuroendocrine Activity. Evolution, Structure and Function*", pp. 361–364, Springer-Verlag, Berlin, Heidelberg.

Dorn, A. (1983): Hormones during embryogenesis of the milkweed bug, *Oncopeltus fasciatus*. *Entomol. Gener.*, **8**, 193–214.

Dorn, A., Romer, F. (1976): Structure and function of prothoracic glands and oenocytes in embryos and last larval instars of *Oncopeltus fasciatus* Dallas (Insecta, Heteroptera). *Cell Tiss. Res.*, **171**, 331–350.

Finlayson, L. H., Osborne, M. P. (1968): Peripheral neurosecretory cells in the stick insect

(*Carausius morosus*) and the blow-fly larva (*Phormia terrae-novae*). *J. Insect Physiol.*, **14**, 1793–1801.

Fletcher, B. S. (1969): The diversity of cell types in the neurosecretory system of the beetle *Blaps mucronata*. *J. Insect Physiol.*, **15**, 119–134.

Füller, H. (1960): Morphologische und experimentelle Untersuchungen über die neurosekretorischen Verhältnisse im Zentralnervensystem von Blattiden und Culiciden. *Zool. Jb. Physiol.*, **69**, 223–250.

Gilbert, L. I., Bollenbacher, W. E., Agui, N., Granger, N. A., Sedlak, B. J., Gibbs, L., Buys, C.M. (1981): The prothoracicotropes: Source of the prothoracicotropic hormone. *Amer. Zool.*, **21**, 641–653.

Granger, N. A., Bollenbacher, W. E., Gilbert, L. I. (1981): An in vitro approach for investigating the regulation of the corpora allata during larval-pupal metamorphosis. *In*: G. Bhaskaran, S. Friedman, J. Rodiguez (eds.) "*Current Topics in Insect Endocrinology and Nutrition*", pp. 83–105, Plenum, New York.

Haget, A., Ressouches, A., Rogueda, J. (1981): Chronological observations on the activities of the embryonic corpus allatum in *Carausius morosus* Br. (Phasmida, Lonchodidae). *Int. J. Insect Morph. Embryol.*, **10**, 65–81.

Haget, A., Ressouches, A., Rogueda, J. (1982): Ultrastructural detection of endocrine activities in the ventral glands of the head of *Carausius* (Phasmida: Lonchodidae) during embryonic development. *Int. J. Insect Morph. Embryol.*, **11**, 99–108.

Herman, W. S., Gilbert, L. I. (1965): Multiplicity of neurosecretory cell types and groups in the brain of the Saturniid moth *Hyalophora cecropia* (L.). *Nature* (*Lond.*), **205**, 926–927.

Hoffman, J. A., Lagueux, M. (1985): Endocrinological aspects in embryogenesis. *In*:G. A. Kerkut, L. I. Gilbert (eds.) "*Comprehensive Insect Physiology, Biochemistry and Pharmacology*", Vol. 1, Embryogenesis and reproduction, pp. 435–460, Pergamon Press, Oxford.

Jones, B. M. (1953): Activity in the incretory centres of *Locustana pardalina* during embryogenesis: function of the prothoracic glands. *Nature* (*Lond.*), **172**, 551.

Jones, B. M. (1956a): Endocrine activity during insect embryogenesis. Function of the ventral head glands in locust embryos (*Locustana pardalina* and *Locusta migratoria*, Orthoptera). *J. Exp. Biol.*, **33**, 174–185.

Jones, B. M. (1956b): Endocrine activity during insect embryogenesis. Control of events in development following the embryonic moult (*Locusta migratoria* and *Locustana pardalina, Orthoptera*) *J. Exp. Biol.*, **33**, 685–696.

Kai, H. (1977): The existence of diapause hormone in adult ovaries and mature eggs of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Japan*, **46**, 239–245.

Khan, T. R., Fraser, A. (1962): Neurosecretion in the embryos and later stages of the cockroach (*Periplaneta americana* L.). *Mem. Soc. Endocr.*, **12**, 349–369.

Küçükeksi, S. (1969): Some observations on the endocrine systems in the embryos of the gregarious and solitary phases of *Schistocerca gregaria* (Forsk.) (Orthoptera: Acrididae). II. The neurosecretory cells and the corpora cardiaca and the corpora allata. *Comm. Fac. Sci. Univ. Ankara, ser. C*, **14**, 25–41.

Lanzrein, B., Imboden, H., Bürgin, C., Brüning, E., Gfeller, H. (1984): On titers, origin, and functions of juvenile hormone III, methylfarnesoate, and ecdysteroids in embryonic development of the ovoviviparous cockroach *Nauphoeta cinerea*. *In*: J. Hoffmann, M. Porchet (eds.) "*Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones*", pp. 454–465, Springer-Verlag, Berlin, Heidelberg.

Loeb, M. J., Hayes, D. K. (1980): Neurosecretion during diapause and diapause development in brains of mature embryos of the gypsy moth, *Lymantria dispar*. *Ann. Entomol. Soc. Amer.*, **73**, 432–436.

Mitsuhashi, J. (1963): Histological studies on the neurosecretory cells of the brain and on the corpus allatum during diapause in some lepidopterous insects. *Tokyo Bull. Nat. Inst. Agric. Sci. Ser. C*, no. **16**, 67–118.

Neumann-Visscher, S. (1976): The embryonic diapause of *Aulocara elliotti* (Orthoptera, Acrididiae). Histological and morphometric changes during diapause development and following experimental termination with juvenile hormone analogue. *Cell Tiss. Res.*, **174**, 433–452.

Polivanova, E. N. (1976): Development, embryonisation and differentiation of the neurosecretory system in the course of insect embryogenesis. Data presented on the VII Int. Symposium on Neurosecretion, Leningrad.

Scharrer, B. (1983): Current views on the mechanism of release of neurosecretory products. *In*: A. P. Gupta (ed.) *"Neurohemal Organs of Arthropods"*, pp. 602–607, Charles C. Thomas Publisher, Springfield.

Schooneveld, H. (1974): Ultrastructure of the neurosecretory system of the Colorado potatoe beetle, *Leptinotarsa decemlineata* (Say) I. Characterization of the protocerebral neurosecretory cells. *Cell Tiss. Res.*, **154**, 275–288.

Sharan, R. K., Sahni, S. L. (1960): Provisional embryonic cuticles of *Dysdercus cingulatus* (Hemiptera, Pyrrhocoridae). *Ann. Entomol. Soc. Amer.*, **53**, 538–541.

Stanipć, V., Ivanivić, J., Janković-Hladni, M., Milanović, M. (1978): Differentiation of the neurosecretory system during development of *Morimus funereus* (Insecta, Coleoptera). *Gen. Comp. Endocr.*, **34**, 107.

Tombes, A. S., Malone, T. A. (1977): Ultrastructure of the frontal ganglion of *Chaoborus* and observations on neurosecretory cells during the annual cycle. *J. Insect Physiol.*, **23**, 639–648.

Truman, J. W., Riddiford, L. M. (1970): Neuroendocrine control of ecdysis in silkmoths. *Science*, **167**, 1624–1626.

Truman, J. W., Taghert, P. H., Copenhaver, P. F., Tublitz, N. J., Schwartz, L. M. (1981): Eclosion hormone may control all ecdyses in insects. *Nature (Lond.)*, **291**, 70–71.

Ude, J., Eckert, M., Penzlin, H. (1978): The frontal ganglion of *Periplaneta americana* L. (Insecta). An electron microscopic and immunohistochemical study. *Cell Tiss. Res.*, **191**, 171–182.

Unnithan, G. C., Bern, H. A., Nayar, K. K. (1971): Ultrastructural analysis of the neuroendocrine apparatus of *Oncopeltus fasciatus* (Heteroptera). *Acta Zool.*, **52**, 117–143.

Unnithan, G. C., Nair, K. K. (1977): Fine structure of the A cells of the pars intercerebralis of normal and gamma-irradiated female milkweed bug, *Oncopeltus fasciatus* (Heteroptera, Lygaeidae). *J. Morph.*, **154**, 59–82.

Yin, C.-M., Chippendale, G. M. (1975): Insect frontal ganglion: fine structure of its neurosecretory cells in diapause and nondiapause larvae of *Diatraea grandiosella*. *Can. J. Zool.*, **53**, 1093–1100.

Yamashita, O., Hasegawa, K. (1985): Embryonic diapause. *In*: G. A. Kerkut, L. I. Gilbert (eds.) *"Comprehensive Insect Physiology, Biochemistry and Pharmacology"*, Vol. 1, Embryogenesis and reproduction, pp. 407–434, Pergamon Press, Oxford.