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Dedicated to Dr. Ellen Thomsen and to Prof. Mathias Thomsen, pioneers in the field of insect neuroendocrinology, on the occasion in 1976 of their seventieth and eightieth birthdays, respectively.

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I. Introduction

In this article neurosecretion is considered cytologically as the process by which neurosecretory cells secrete their products.

The mechanism of secretion has been the subject of much speculation and controversy, particularly during the past decade. Biochemical evidence indicating exocytosis of neurosecretory material was first obtained by Douglas and Poisner (1966) and Kirschner *et al.* (1966; see also reviews by Douglas, 1966, 1968). Unequivocal electron microscope evidence for exocytosis of neurosecretory granules was first found in insects (Normann, 1965; also independently by Smith and Smith, 1966). Before secretion, neurosecretory substances are sequestered in so-called (elementary) neurosecretory granules measuring 100–300 nm, each of which is surrounded by a membrane (Bargmann and Knoop, 1957; Palay, 1957). When a granule membrane fuses with the axolemma so as to void its content into the extracellular medium (exocytosis), Ω-like figures can be found in thin sections for electron microscopy, provided that (1) this process occurs at the moment of fixation, (2) the fixative acts fast enough to "freeze" it, and (3) the plane of the section happens to pass through this membrane configuration. Such a coincidence is *rare*.

The failure of many investigators to observe omega figures with electron microscopy, together with other observations that seemed incompatible with a generalized concept of neurosecretion by exocytosis, have sustained confusion and dispute. At present, however, an extended exocytosis theory is gaining wide acceptance (see, e.g., A. D. Smith, 1971; Dreifuss *et al.*, 1973; Maddrell, 1974), and attention is turning toward a closer analysis of details of the process. This is probably a useful case for the study of controlled membrane interactions.

It should be recognized that the controversy has at least served to keep minds open. Investigators—often of different viewpoints—have contributed by bringing to our attention phenomena that are just as relevant for our understanding of neurosecretory cell function as are, for example, omega profiles. Whereas much attention was focused on omega figures in neurosecretory axon endings in the blowfly *Calliphora erythrocephala*, Normann (1965) failed to attach significance to clusters of tiny vesicles in the secretory axon endings. Johnson (1966), in a simultaneous study of the blowfly *Calliphora stygia*, described possible release sites in the form of clusters of small, pale vesicles. Johnson also pointed out the transient nature of secretory phenomena. Still, the enigma—to be dealt further with in this article—remained unsolved until it was realized that omega figures and vesicle clusters could most simply be interpreted as consecutive phases of exocytosis,

viewed as a dynamic sequence of membrane phenomena (Normann, 1969, 1970; Bunt, 1969; Smith, 1970).

A few words of caution and conjecture are perhaps better placed here than in the following more detailed analysis of the most relevant literature of the past. In several cell fractionation studies possible artifacts such as the risk of leakage of substances from cytoplasmic particles into the medium have been more or less ignored, and substances present in the supernatant have subsequently been claimed to exist in a genuine cytoplasmic pool. Likewise, electron microscopists have in many cases underestimated the limitations of the technique (such as the rapidity and effectiveness of fixation), as well as the hazards involved in the interpretation of static two-dimensional pictures in terms of dynamic cellular processes. Even more amazing is the value attributed to negative evidence.

As Douglas *et al.* (1971) noted: "All this suggested to us that the failure to demonstrate exocytosis in the neurohypophysis (or indeed in any vertebrate nervous material)—a failure repeatedly emphasized in the literature . . . —might simply reflect the difficulty of capturing such doubtless fleeting events with electron microscopic procedures and encouraged us to undertake a careful search of electron microscopic sections obtained from actively secreting glands. The results, here reported, provide unequivocal evidence of secretion by exocytosis in the neurosecretory terminals of posterior pituitary glands of rats and hamsters."

Our remarks on conjecture do not imply a general adverse criticism of evidence that has been interpreted as favoring alternative mechanisms of neurohormone release. They are, however, considered, when we venture to state our unawareness of any evidence in the literature that effectively rules out the view that in general neurosecretory cells secrete by exocytosis.

II. The Concept of Neurosecretion

The classic concept that certain nerve centers can specialize in neurosecretory activity has gradually evolved from a combination of cytological and physiological investigations. [For reviews, see Scharrer and Scharrer (1963), Bern (1966), Bern and Knowles (1966), Bargmann (1966), and Scharrer (1970).]

Originally, the neurosecretion theory was based on the selective staining of secretory substances within some neurons of the central nervous system. Later these neurons became associated with the production of hormones (such as oxytocin and vasopressin in vertebrates,

hormones regulating growth, development, metabolism, and a host of other functions in invertebrates (see, e.g., Scharrer and Weitzman, 1970).

Later came the neurohemal concept (Knowles and Carlisle, 1956). Neurohemal organs, exemplified by the neurohypophysis of vertebrates, the sinus glands of crustaceans, and the corpus cardiacum of insects, contain neurosecretory axon terminals with stored material ready to enter the circulation. Neurohemal organs, however, have a complex structure and contain elements in addition to blood vessels and neurosecretory axon terminals, the cell bodies of which are situated outside the neurohemal organ. In insects, for example, the corpora cardiaca contain not only *extrinsic* neurosecretory axons coming from the brain, but also *intrinsic* neurosecretory cells (Scharrer, 1963; Normann, 1965; and others). Still, the neurohemal concept seemed useful as a criterion for assessing whether a neuron was truly neurosecretory. The concept implied that all neurosecretory cells liberate their products at neurohemal organs in contrast to neurons engaged in transmitter activity.

It appears, however, that some neurosecretory axon terminals make direct contacts with other endocrine cells, such as the corpora allata and the thoracic glands of insects (Scharrer, 1964a,b; Normann, 1965; Thomsen and Thomsen, 1970), and in the pars intermedia of the vertebrate pituitary (Bargmann and Knoop, 1960). The term "neurosecretomotor junctions" was suggested by Bern (1963, 1966), and this term may also be appropriate in cases in which other effectors are innervated by neurosecretory fibers, such as heart muscle (Normann, 1972) and Malpighian tubules of insects (Maddrell, 1969). More examples can be found in the review on insect neurosecretion by Maddrell (1974).

A further complication involving the concept of neurosecretion is the problem of its identification by means of the classic staining techniques such as the Gomori chrome alum–hematoxylin (CAH) method and the Gabe paraldehyde–fuchsin method. Neurosecretory cells that can be stained by these methods are present in some of the best known neuroendocrine systems, such as (1) the hypothalamoneurohypophysis of higher vertebrates, (2) the X organ–sinus gland system of crustaceans, and (3) the brain-corpus cardiacum–aorta wall system of insects. However, the intrinsic neurosecretory neurons of the insect corpus cardiacum are not stainable with PAF, neither are they Gomori-positive (Thomsen, 1969). The same applies to neurosecretory cells in the brain of other invertebrates, the caudal neurosecretory system of some fishes (Bern *et al.*, 1965), and neurosecretory

neurons in the median eminence of tetrapods (for survey, see Knowles and Bern, 1966). Besides, it can be mentioned that, for example, PAF stains some nonneurosecretory cells such as the insulin-producing β cells in the pancreatic islets. Therefore such staining methods are insufficient for the identification of neurosecretory cells.

Ultrastructurally, the presence of granules like the above-mentioned "elementary" granules (100–300 nm) provides an important aid in assessing whether a particular cell is neurosecretory or not. Here again, their value (when taken alone) is insufficient, partly because several types of granules exist (see Section II,B), and partly because morphologically similar granules exist in many other cell types present, for example, in the anterior pituitary, pancreatic islets, and chromaffin cells of the adrenal medulla. Moreover, similar granules—although not very abundant—may be present in conventional neurons.

In many cases, morphological signs of cyclic activity of neurosecretory cells have been observable, and this has been used as an additional criterion.

Physiological establishment of their endocrine function and the demonstration that particular neurons secrete particular hormones is of central importance in the concept of neurosecretion, but considering the chemical messages and their effects on target organs is beyond the scope of this review. When trying to distinguish between conventional neurons and neurosecretory neurons, one difference is that transmitters have to travel only about 20 nm across the synaptic cleft, whereas neurohormones may act on much more distant receptors.

Another—physiologically most significant—difference is in the time course of their action. The nervous system conveys signals rapidly from one part to another (or to an effector), transmitter function occurring within milliseconds. Neurohormones may act over minutes or even hours. Some of them regulate other endocrine glands and, since they provide the link between the nervous and endocrine systems, they have been characterized as "the final common pathway" for neuroendocrine regulation (Scharrer, 1965).

A. The Neurosecretory Cell

1. *Defining a Neurosecretory Cell*

It is apparent from the above considerations that defining a neurosecretory cell and distinguishing between an endocrine and a conventional neuron are problematical and should perhaps be avoided. In his

review on neurosecretion, Bargmann (1966), however, accepted the definition of Yagi *et al.* (1963), who described an endocrine neuron as "a neuron that also possesses glandular activity." This definition is adequate enough for this article, in which secretion of bona fide neurosecretory granules is the main theme.

Still, most, if not all, neurons secrete. This was observed by Scott (1905), who discovered Nissl substance in nonnervous tissue and who was struck by the similarities between the cytoplasm of exocrine pancreas cells and of nerve cells. Scott regarded the nervous system as something more than a mere system of conducting paths, and he suggested that nerve cells are true secretory cells that "act upon one another or upon cells of other organs by the passage of chemical substances from the first cell to the second." According to Scott, the discharge of such substances from the nerve endings depended on the arrival of impulses (Scott, 1905).

After 70 years this view is remarkedly unaltered and, in view of some comparative aspects (see Section II,C), one might in fact consider neurons long, thin, sometimes branching, gland cells with additional excitatory properties (spike activity). Thus the nerve cell body is comparable to the basal region of a pancreatic acinar cell, and the axon and its terminal comparable to the apical (luminal) region of the pancreas cell, where granules are secreted by exocytosis.

2. *Criteria*

Most of the evidence concerning the release mechanism to be dealt with in the following has been obtainted from studies of bona fide neurosecretory cells.

For example, the corpus cardiacum neurosecretory cells (c.n.c.) of the insect *Calliphora* are regarded as true neurosecretory neurons because of: (1) a shape similar to that of unipolar neurons (see Fig. 1); (2) the ganglionlike structure of the corpus cardiacum (Fig. 2) and its functional and anatomical connection with the central nervous system; (3) the ontogenetic development of the corpus cardiacum together with the hypocerebral ganglion from the stomodeal ectoderm; (4) the synaptic innervation of the c.n.c. (Figs. 3 and 4); (5) the electrical excitability of c.n.c. axons (see Section V); (6) the production and secretion of material packed in elementary granules (size 150–300 nm); and (7) the fact that the secretory product is a hormone (hyperglycemic) (Normann and Duve, 1969; Vejbjerg and Normann, 1974).

At least criteria such as the three latter (5 to 7) should be met for any cell to qualify as a neurosecretory neuron *sensu strictu*.

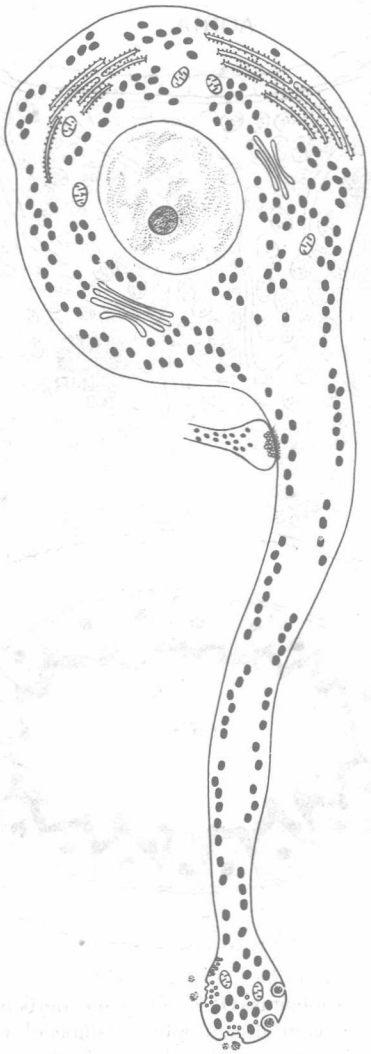

FIG. 1. Schematic drawing of a c.n.c. (see text). The granules are produced in the perikaryon (packed in membranes by the Golgi apparatus). Granules are transported down the axon in rows or trains running parallel with microtubules (not shown). An axo-axonic synapse is indicated near the cell body (see Fig. 3). In the axon terminal different stages of exocytosis are indicated (see Fig. 16); omega profiles are on the right, consecutive phases proceeding clockwise around the terminal. (From Normann, 1973b.)

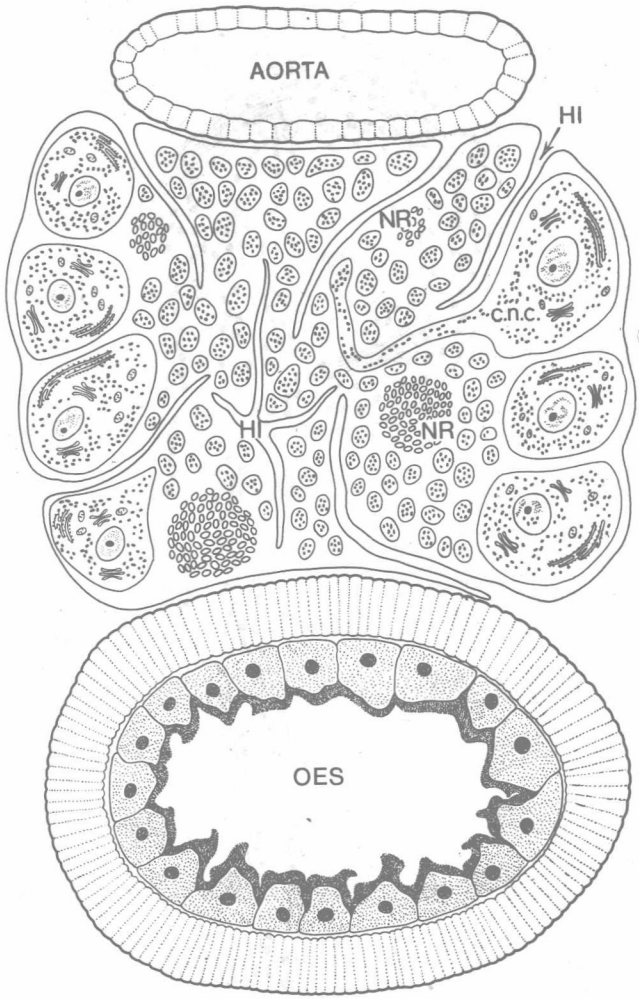

FIG. 2. Cross section (simplified diagram) of the *Calliphora* corpus cardiacum between the aorta and the esophagus (OES). The c.n.c. perikarya in the periphery send axonal projections into the central neuropile. Hemocoel indentations (HI) ramify deeply into the neuropile, insuring rapid exchange of substances between neurosecretory terminals and the blood. Thinner dorsal and thicker ventral branches of the recurrent nerve (NR) are shown. Other extrinsic axon types or glial cells are not indicated. (From Normann, 1973b.)

3. *Ultrastructure of Neurosecretory Cells*

Some morphological features have already been mentioned (criteria 1 and 6; see also Fig. 1). As regards criterion 4, synapses on neurosecretory neurons, only a few well-documented cases have so far been

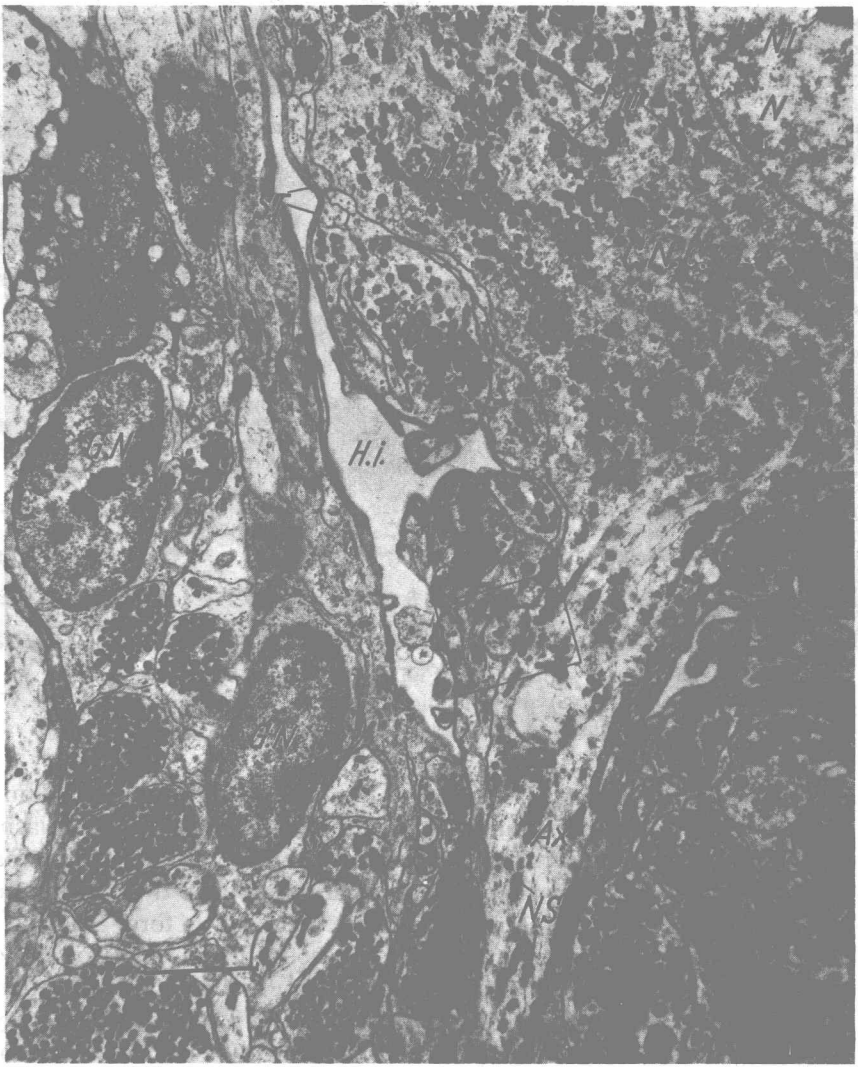

FIG. 3. Part of a c.n.c. cell body with an axonal projection (Ax). N, Nucleus with nucleolus (NL); Mi, mitochondria; NS, neurosecretory granules; H.i., hemocoel indentations. The axo-axonic synapse (in rectangle) is shown at higher magnification in Fig. 4. Glial cell nuclei (G.N.) in neuropile are dense and compact. Bar: 2 μm. (From Normann, 1965.)

found by morphological methods (Normann, 1965, 1970) (Figs. 4 and 5; the latter is from the rat hypothalamus, by courtesy of J. Morris).

The neurosecretory material is synthesized in the cell body and packed there as membrane-bounded granules or vesicles by the Golgi apparatus (Scharrer and Brown, 1961; Normann, 1965; Zambrano and

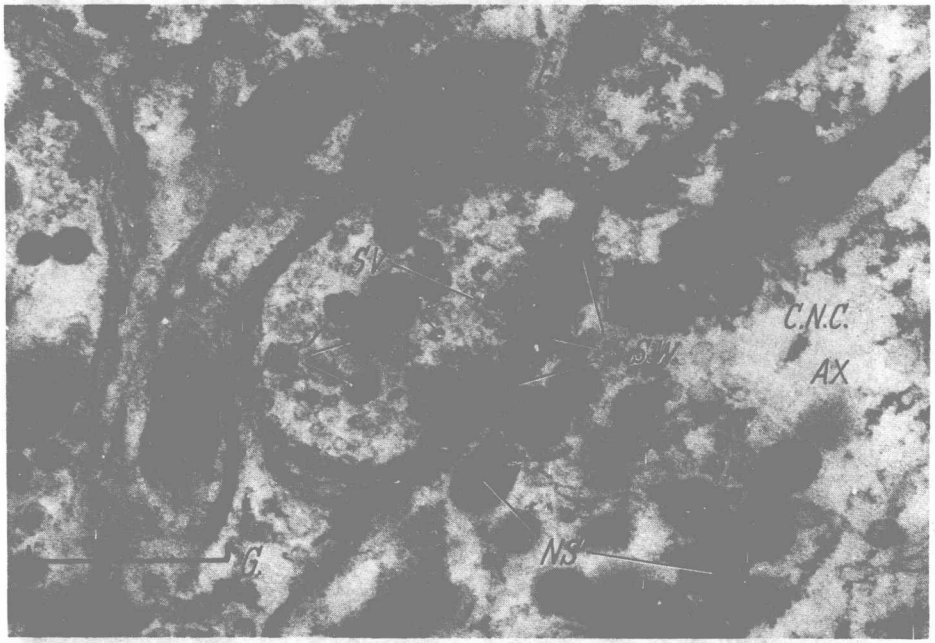

FIG. 4. Detail of area indicated in Fig. 3. Presynaptic axoplasm contains small neurosecretory granules (NS) and synaptic vesicles (SV). Subsynaptic web (S.S.W.) shown in the c.n.c. axoplasm. G, Glioplasm. Bar: 0.5 μm. (From Normann, 1965.)

DeRobertis, 1966; Bassurmanova and Panov, 1967; and others). Thus the dense neurosecretory material is sequestered from the cytoplasm by a single membrane with a special ability to fuse with the axolemma at release sites. In view of the particular importance of the membrane in this respect, it might seem preferable to choose the term "vesicle" rather than "granule." However, in many cases the contents appear to have a certain rigidity, several types of granules being nonglobular, and some even showing a substructure such as a paracrystalline array of rodlike, tubular subunits (Figs. 6, 7 and 19) (see also Normann, 1970, 1974; Donev, 1970). Other patterns of substructure have been described by Knowles (1960), Bargmann and von Gaudecker (1969), and by Livingston and Lederis (1971). This may justify the continued use of the term "neurosecretory granules" to denote entities (100–300 nm) consisting of a secretory package (or quantum) enveloped by a limiting membrane.

Neurosecretory granules are transported down the axon by a process that is so far poorly understood. Granules often occur in rows close to and parallel with microtubules. Structural connections

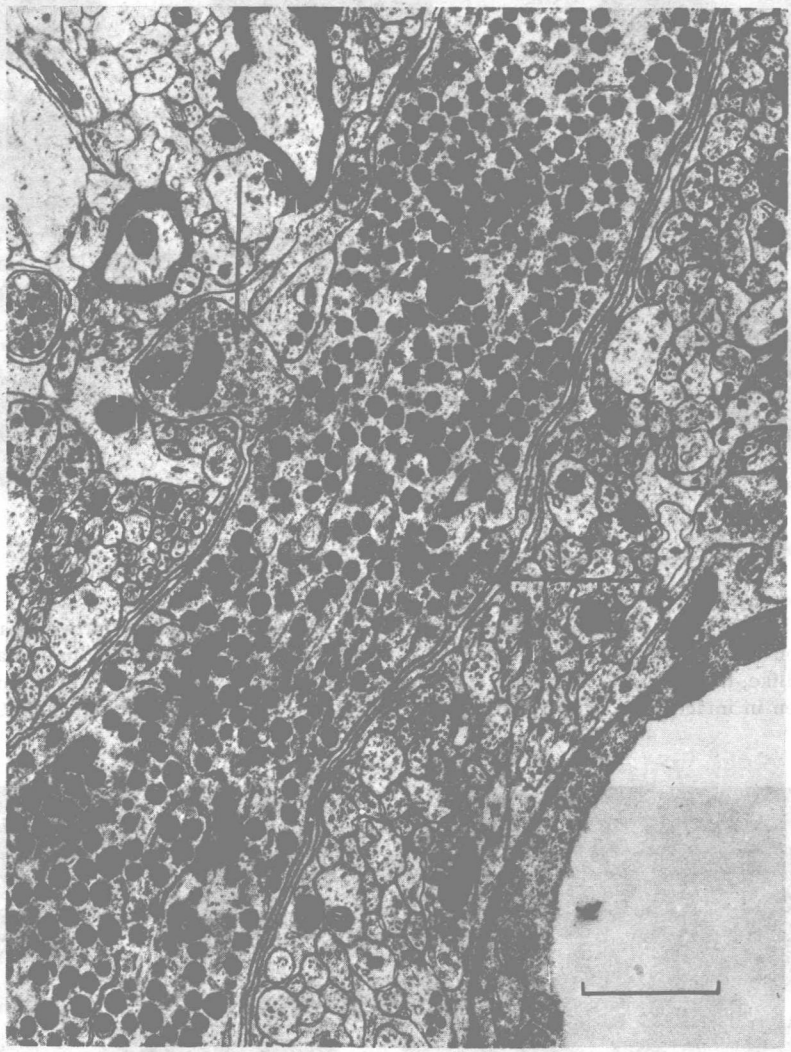

FIG. 5. Longitudinal section of neurosecretory axon in the hypothalamus of a rat. Note the two axo-axonal synapses (arrows). Bar: 1.0 μm. (Courtesy of J. Morris.)

linking granules together in "trains" have recently been described by McLaughlin and Howes (1973). [For reviews on axonal transport, see Dahlström (1971) and Heslop (1974).]

Although neurosecretory neurons may possess not only dendrites but also axon collaterals, an appreciable part of the secretory material is stored in bulbous axon terminals, from which controlled release can occur.