Some Observations on the Fine Structure of the Sinus Gland of a Land Crab, *Gecarcinus lateralis*

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PLATE 267

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ABSTRACT

The dilated axon endings of the sinus glands of the brachyuran crab, *Gecarcinus lateralis*, are filled with homogeneously dense granules, each granule being bounded by a delicate membrane. The granules are of two orders of magnitude: 0.05 to 0.1 μ and 0.15 to 0.2 μ in diameter. Each axon ending contains granules of a nearly uniform size. Endings with granules of the larger size range predominate.

Non-nervous cells endogenous to the sinus gland are scattered among the nerve endings. The cell contours are irregular, and cytoplasmic processes ramify between endings.

The axons are unmyelinated, having only thin limiting membranes, and they possess many neurofibrils. Granules in preterminal portions of the axons tend to lie at the periphery of the fiber, and in some cases in chains at the core of the fiber.

The granules appear to be storage and release centers for neurosecretory substances or their precursors.

In neurons capable of producing and releasing regulatory substances into the circulatory system, granules are found which are presumed to contain these neurosecretory substances or their precursors. The existence of such neurosecretory material has been demonstrated histologically with Gomori's chrome-alum-hematoxylin and other methods (20). Recent observations with the electron microscope on the avian and mammalian neurohypophyses (1, 8, 11, 15) have revealed the presence of spherical granules, 0.1 to 0.2 μ in diameter, both in the nerve endings of the central lobular portions of the pars nervosa and in the hypothalamic-hypophysial tract. They correspond to the "Gomori-positive" material found there. In the neurohypophysis of dehydrated rats the dense secretory material has disappeared, leaving only empty circular profiles (15), indicating that an anti-diuretic hormone or its precursor is stored in granules.

In the adrenal medulla, a non-nervous tissue of neuroblastic origin, granules of 0.1 to 0.6 μ in diameter have been reported as the sites of the catechol amines in the chromaffin cells (7, 12, 21). In addition, non-neurosecretory neurons have been observed to have vesicular components at their synaptic terminals. These vesicles are 200 to 500 A in diameter (4–6, 14).

Among the invertebrates, neurosecretory systems also occur: notably, the intercerebralis-cardiacum-allatum system of the insects and X-organ-sinus gland complex of the crustaceans. In the crustacean eyestalk, the so called sinus gland is an aggregation of swollen nerve endings impinging on a blood sinus. This structure serves as a storage and release center for substances which are formed by neurosecretory cell bodies in the X-organ and in other parts of the nervous system. These substances are transported to the endings in the sinus gland *via* the sinus gland nerve. The presence of the neurosecretory materials has been adequately demonstrated with chrome-alum-hematoxylin and other stains (3, 9, 19). With phase microscopy, granules estimated to be 0.1 to 0.3 μ in diameter have been seen in the brachyuran sinus gland and its nerve (17, 19). The aim of this present study is not only elucidation of the fine structure of the brachyuran sinus gland but

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also confirmation of the presence of neurosecretory granules.

Materials and Methods

The material used was obtained from the red land crab of Bermuda and the West Indies, *Gecarcinus lateralis* (Fréminville), namely from intermolt male crabs of about 3.5 cm. width of carapace. The eyestalks were severed, their lateral aspects were cut with fine scissors, and the two halves separated, exposing the ganglia and eye muscles. The sinus gland, a bluish-white structure on the dorsal aspect of the medulla interna, was carefully dissected out under *Carcinides* perfusion fluid (16, 23). The tissues were fixed 1 hour at 10°C. in 1 per cent osmium tetroxide, made up with perfusion fluid and buffered with acetate-veronal to pH 7.7 (14). They were then washed in perfusion fluid, dehydrated by passage through an alcohol series, and were finally embedded in gelatin capsules in a mixture of three parts normal butyl methacrylate to one part ethyl methacrylate polymerized at 70°C. in the presence of 1.5 per cent luperco CDB. Ultrathin sections were cut with an experimental model of a thermal expansion microtome (13). Sections were studied and micrographed on an RCA EMU-2D electron microscope equipped with a 50 μ objective aperture.

OBSERVATIONS AND DISCUSSION

The disorderly array of unmyelinated axons of the sinus gland of *Gecarcinus* is very apparent. These nerve fibers twist around one another and often appear to cross at right angles. They lie very close together, one fiber membrane directly apposed to the next (Fig. 1). Interdigitations, occurring as villus-like processes, extend from one fiber to indent the limiting membrane of an adjacent fiber. The limiting membranes of the axons are from 85 to 115 A in thickness. Numerous neurofibrils, which run parallel to the axes of the nerve fibers, are very conspicuous in the preterminal portions of the endings (Fig. 2). They are from 150 to 200 A thick and appear to be solid structures. Mitochondria are seen along the nerve fibers and at the endings, usually lying near the periphery of a fiber. They have double limiting membranes and internal cristae. They vary from 0.2 to 0.7 μ in diameter.

Branches of the blood sinus ramify through the gland. The lining of the blood sinus is about 0.3 μ thick. It is continuous and appears to be composed of parallel lamellae which are invisible with the light microscope. At loci where endings meet the blood sinus, limiting membranes of both the nerve terminals and the sinus separate the axoplasm from the blood.

Occasionally, large cells, 3 to 4 μ in diameter, can be seen (Fig. 1). Presumably, these are the "endogenous" cells or "neuroglia" described in the sinus gland of the blue crab (19). Their cytoplasmic processes, 2 μ or more long, ramify among the axons and endings. Their limiting membranes, 80 A thick, are continuous and of irregular outline. So intimate is the contact between an endogenous cell and its adjacent nerve fibers that only a low density region of 100 A or less separates their limiting membranes. Profiles of tubular, membrane-limited elements of the endoplasmic reticulum, as well as profiles of a Golgi region, are seen in some sections of endogenous cells. The relatively few mitochondria vary from 0.3 to 0.7 μ along their greatest axes. The nuclei have irregular contours outlined by a double-membraned envelope which is 260 A thick, each membrane being 50 A thick with a low density area of 160 A separating them. Since these large nuclei are 1.5 to 3 μ in diameter, they fill much of the cell.

Such endogenous or glial cells have been compared to the pituicytes of the vertebrate neurohypophysis seen in histological preparations (19). The comparison was mainly based on staining properties and on the fact that in both neurosecretory structures these cells send long processes among the nerve endings. Recent studies with the electron microscope reveal even greater similarities. In the glial cell of the sinus gland, the irregular outlines of limiting membrane and nucleus, the close relationship between neuroplasm and glial cytoplasm, and the definite lack of granules within the cell, all resemble closely the structure of the pituicyte described by Bargmann and Knoop (1). This absence of granules in the glial cell indicates that it cannot supplement the secretory material of the axons; nor has it a granular component to release independently. However, the presence of a structurally undetectable substance which may add to or modify the neurosecretory material cannot be ruled out. The controversial issue of whether there occurs in the sinus gland only neurosecretion or neurosecretion associated with some endogenous secretion remains to be settled (20). The present observations appear to support the former view. Yet, the intimate contact between glia and axons could suggest a glial influence on secretion. The relative scarcity of glial cells among nerve fibers

suggests that the glia do not act as a sheath or supporting element, unless their cytoplasmic processes can in some manner perform this function.

The most conspicuous elements in the sinus gland are the extremely abundant neurosecretory granules; that is, granules which appear to be identical with the granular substance stained with chrome-alum-hematoxylin. Axon endings are almost completely filled with these granules, to the point of obscuring the neurofibrils (Figs. 1 and 2). The spherical granules are dense, and homogeneous, and are surrounded by a delicate limiting membrane (Fig. 3). Their contours are regular. Thus far, two distinct size ranges have been seen: 0.15 to 0.2 μ and 0.05 to 0.1 μ in diameter. That each ending contains granules of a uniform size is consistent with the histological observations. Endings with the granules of the smaller size are seen rather infrequently. Granules of 0.15 to 0.2 μ in diameter are by far the more abundant.

Preterminal portions of the axons show fewer granules (Fig. 2). In these cases, the granules appear in close association with the neurofibrils. The relatively few granules along the axons tend to lie at the periphery, though in some cases they form a chain in the central core of the fiber. Earlier observations on the sinus gland and sinus gland nerve with light microscopy have revealed granular material mainly in the periphery of the axoplasm (3, 17, 19). Rows or chains of the granules are presumably comparable to the "moniliform strings" of secretory material in living and in stained axons in the corpora cardiaca and allata, and in the esophageal nerve of adult *Calliphora* (22).

Histologically, with Heidenhain's azan and with aldehyde fuchsin after Bouin's fixation, six distinct tinctorial types of axon endings are displayed in the sinus gland of the blue crab, the red type (staining with azocarmine) being the most numerous (19). The sinus gland of *G. lateralis* shows only four tinctorial types of endings. When the tissue is fixed with osmium tetroxide, only two tinctorial types appear after staining with azan, again the predominant type staining with azocarmine (19). The presence of two size ranges of granules seen with the electron microscope after osmium fixation suggests that there are at least two types of neurosecretory endings in the sinus gland which differ in their structural contents. Since the large granules, 0.15 to 0.2 μ, are by far the more numerous, it is possible that the endings containing such granules correspond to the azocarmine-stained endings which predominate in histological preparations. Interestingly, Duncan (8) reports two orders of granule size in the avian neurohypophysis, with the smaller droplets (0.05 to 0.1 μ) being half the size of the larger ones (0.1 to 0.2 μ).

Physiological experiments on the sinus gland of the fiddler crab, *Uca pugilator*, further substantiate the belief that the neurosecretory material is contained within granules (18). Homogenates of sinus glands have a low activity in inducing chromatophore expansion in isolated *Uca pugnax* legs as long as they are kept in sucrose or a sucrose-sea water medium isotonic with crab blood. Upon dilution of the homogenate with distilled water, an active chromatophorotropic hormone is rapidly released. Freezing and thawing or addition of detergents or digitonin also induce hormone release. Such observations strongly suggest that the hormone is contained within granules possessing a semipermeable membrane. Earlier physiological studies, upon which these experiments were based, have shown that cytoplasmic granules, 0.1 to 0.6 μ in diameter, in the chromaffin cells of the adrenal medulla possess semipermeable membranes and are the sites of the catechol amines (2). The presence of these granules, which are osmiophilic and argentophilic, has been confirmed by observations with the electron microscope (12, 21).

Thus, it appears that dense, cytoplasmic granules are a component of neurosecretory nerve fibers in all vertebrates and invertebrates so far investigated. Even cells of neural origin but nonneural function in the adrenal medulla, which contain humoral agents, possess such a granular component. Neurosecretory granules, then, can be demonstrated physiologically and structurally. They appear to be the storage and release centers for neurosecretory substances or their precursors which ultimately enter the blood stream and regulate a variety of metabolic processes.

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Explanation of Plate 267

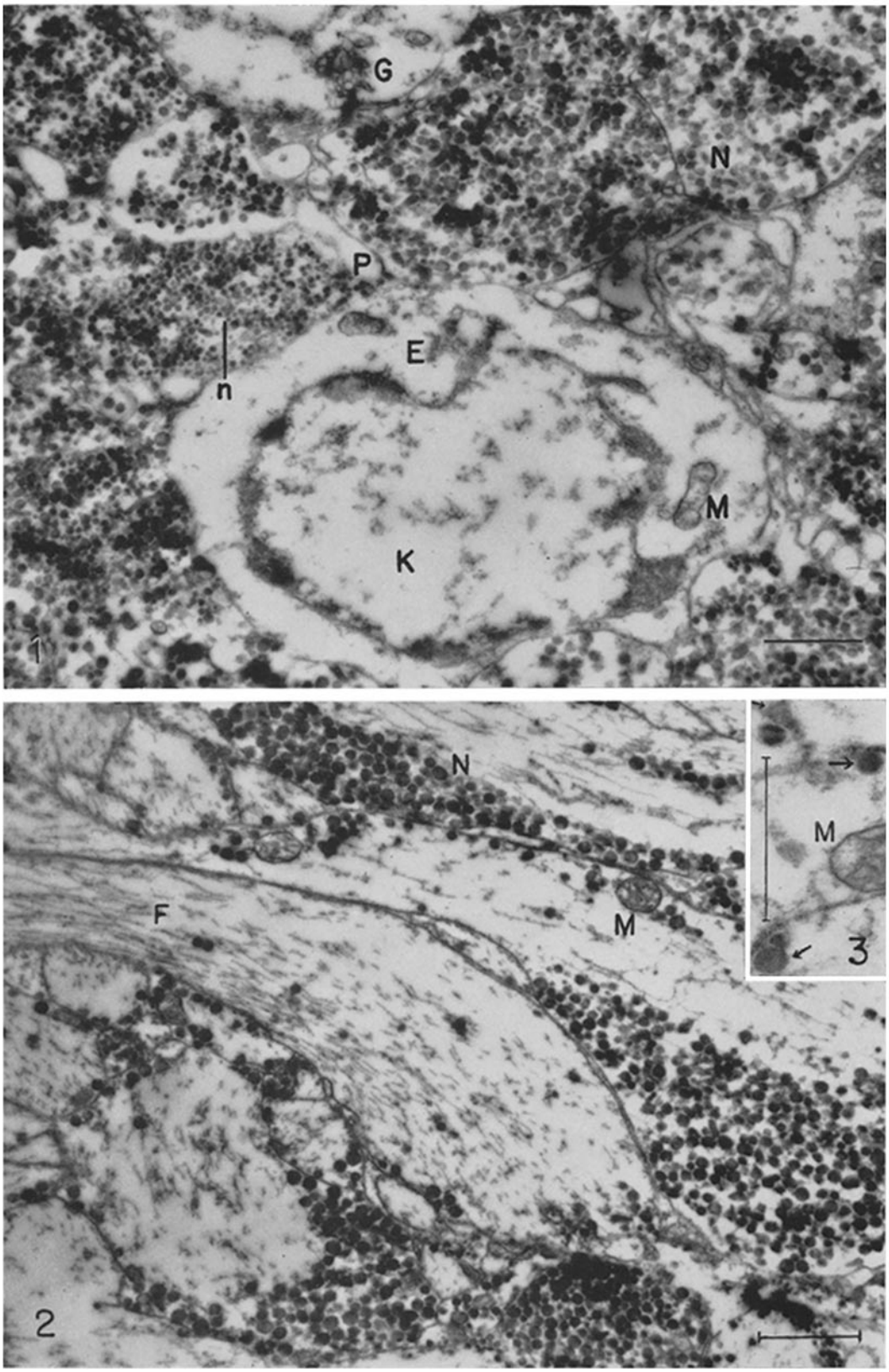
Fig. 1. Cross-section of several dilated axon terminals filled with neurosecretory granules, and two endogenous or glial cells in the sinus gland of *Gecarcinus lateralis*.

N, axon terminal with neurosecretory granules, 0.15—0.2 μ; *n*, axon terminal with granules, 0.05—0.1 μ; *M*, mitochondrion; *K*, nucleus of glial cell; *G*, Golgi region of glial cell; *E*, endoplasmic reticulum of glial cell; *P*, process of glial cell. × 15,600.

Fig. 2. Cross and longitudinal sections of dilated axons in the sinus gland. *F*, neurofibrils. × 15,600.

Fig. 3. Several neurosecretory granules at higher magnification, showing the limiting membrane (arrow). *M* mitochondrion. × 25,000.

Magnification marks for Figs. 1 to 3 = 1 μ.



(Hodge and Chapman: Fine structure of sinus gland of land crab)