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THE SOURCES AND ACTIVITIES OF TWO CHROMATOPHOROTROPIC HORMONES IN CRABS OF THE GENUS SESARMA. II. HISTOLOGY OF INCRETORY ELEMENTS [1]

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In a previous paper dealing with physiological analyses concerning the activities and possible sources of chromatophorotropic hormones in three Japanese species of *Sesarma* (Enami, 1951), I have shown the sites of origin of two kinds of hormones, namely S and N hormones, on a gross anatomical scale. On the one hand, S hormone was concluded to be produced in both the sinus gland and all the principal ganglia, its occurrence in the latter being independent of the secretory activity of the former. This suggested a strong possibility of the universal existence in ganglionic tissues of a certain incretory mechanism essentially similar to that involved in the sinus gland.

On the other hand, N hormone was demonstrated to be much restricted in its distribution, only a few of the central nervous organs, *i.e.*, the brain and the medulla terminalis in the adults, the brain, the medulla terminalis, the commissural ganglion, and the thoracic ganglion in the juvenile forms, apparently producing the hormone. This suggested the presence in the said ganglia of specific incretory elements not as widely distributed as the presumed sources of the S hormone.

A histological study was carried out in order to determine the ultimate sources of the two hormones as well as the cytological details of their elaboration. The present work also includes a closer investigation of the sinus gland, whose secretory activity has hitherto been studied only superficially, in spite of the generally admitted significance of the gland as the predominant incretory organ in crustaceans.

Materials and Methods

Observations were mainly based on histological sections of tissues of the adults of *Sesarma dehaani,* weighing 25–30 g. and showing no sign of ecdysis (*cf.* Enami, 1951). For the purpose of the present study standard sections were prepared from the whole soft eyestalk tissues carefully freed from the exoskeleton, the isolated sinus gland, and the body ganglia such as the brain, the commissural ganglion, and the thoracic ganglion dissected out separately. The tissues were fixed in Heiden-

[1] This work was carried out at the Mitsui Institute of Marine Biology, Shimoda, Japan, with the support of a grant-in-aid from the Ministry of Education of Japan in 1948.

hain's Susa for 4 to 8 hours, and paraffin sections 8–10 μ thick were stained with Mallory's triple stain.

For specific purposes, especially for a detailed examination of the sinus gland, various other techniques including both vital and supravital staining were employed, references to which will be made in the following accounts.

Information obtained in the adults of *Sesarma dehaani* was compared with that in the adults of two other species of the same genus, *viz., S. intermedia* and *S. haematocheir,* with corresponding results. Accordingly, the following account applies to all these species.

Observations

Histological signs of an incretory activity in the adults of *Sesarma* were observed in the following tissues and cells:

(1) Sinus gland
(2) Neurilemma of all ganglionic tissues examined
(3) Specific nerve-cells located within certain ganglia. Among these, three types can be distinguished as to their secretory behavior; they are here tentatively designated as α, β, and γ neurosecretory cells, respectively.

Thus, altogether five kinds of incretory elements are dealt with, their topography being diagrammatically illustrated in Figure 1.

1. The sinus gland and its secretory behavior

The sinus gland of *Sesarma* is well developed and, in fresh or even in formalin-fixed eyestalks, is macroscopically visible owing to its well-known characteristic opacity and slightly bluish hue (Brown and Cunningham, 1939, etc.). It is located at the dorsal aspect of the junction between the medulla interna and the medulla terminalis (Fig. 1, SG). Its outline roughly conforms to a triangle, one of whose apices is tapering off in a proximal direction. When viewed through transmitted light, the gland appears brownish yellow in marked contrast to the almost colorless nervous tissues nearby. The approximate volume of the gland was measured in animals of different sizes by means of a simple calculation from the area of optical section and the thickness of the isolated gland sandwiched between the slide and cover glass of a Thoma-Zeiss hemacytometer (Fig. 2).

The proximal tapering indicates the innervation by the sinus-gland nerve which is traceable along the frontal surface of the neuropile of the medulla terminalis up to a disc-like cluster of giant nerve-cells located at the proximal ventral-lateral corner of the medulla (Fig. 1, A). The nerve leaves the nerve-cell group at the center of the latter's internal concavity, and within a short distance from the point of its departure sends off a slender branch proximally, which enters into the pedunculus lobi optici (Fig. 1). Concerning the innervation of the gland in *Cambarus,* Welsh (1941) has described, besides the unquestionable innervation by the sinus-gland nerve originating in the medulla terminalis, a distribution to the surface of the gland of a branch of the oculomotor nerve II, a fact which demonstrates a dual innervation of the gland. However, as far as the present study is concerned, the innervation of the sinus gland of *Sesarma* takes place by the sinus-gland nerve exclusively, no other routes having been found, although some of the finer branches

of the oculomotor nerve II, in supravital staining with methylene blue, appear to be in superficial contact with the glandular tissue.

The glandular tissue is, just as described in the sinus glands of various other crustaceans (Hanström, 1933, 1937; Sjögren, 1934; Ståhl, 1938; Carstam, 1941; Pyle, 1943; etc.), composed of a folded continuous lamella of syncytium, including a variable amount of large and small acidophile droplets. Several branches of the dorsal radial blood-sinus are enclosed, a continuous structureless membrane of about 0.3 μ thickness separating the glandular lamella from the blood-sinus (Fig. 3, L.M.). This limiting membrane was made visible in deep blue color by means of Mallory's triple staining after fixation with 5% sulfosalicylic acid. It is noteworthy that,

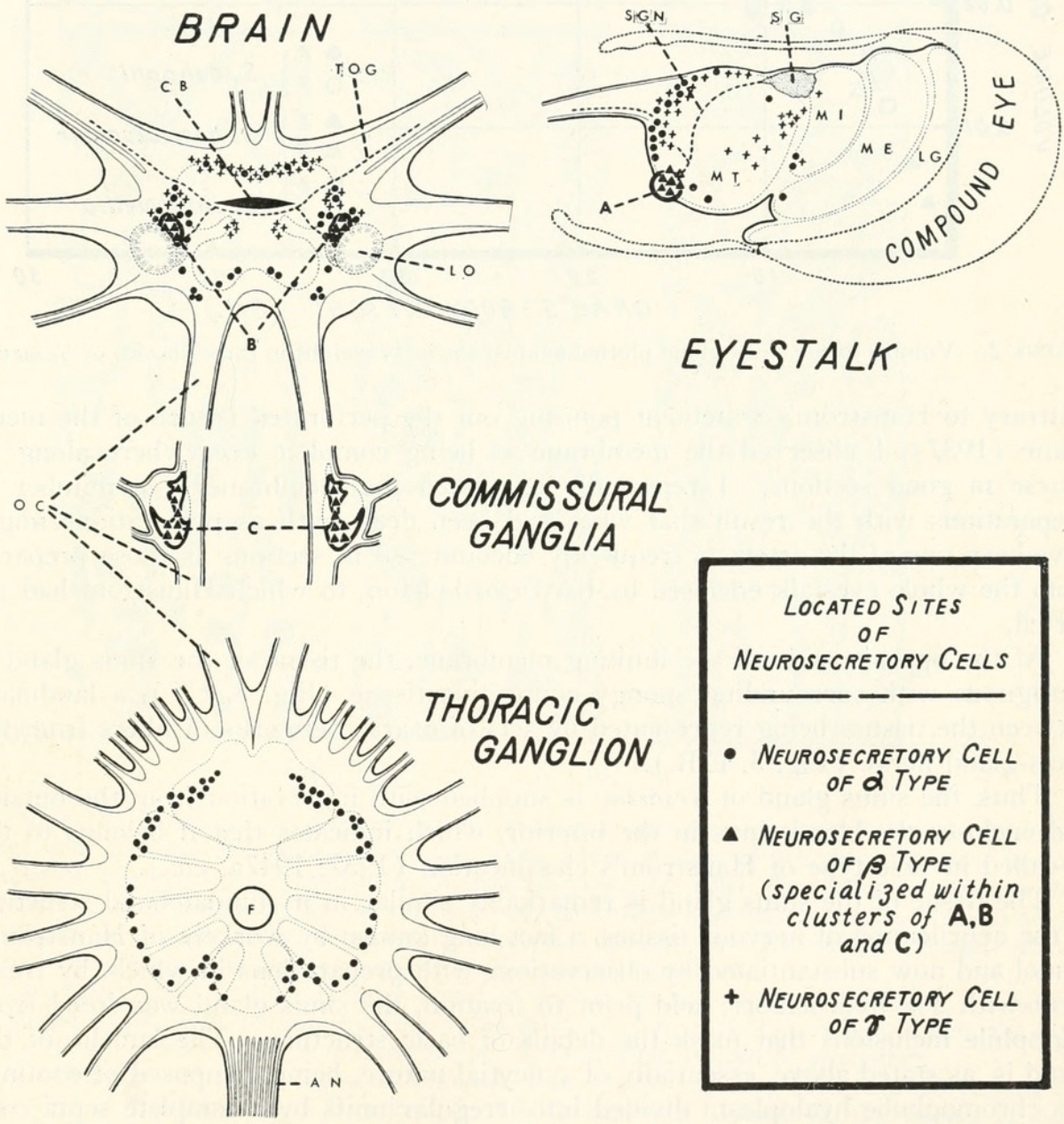

FIGURE 1. Diagram of the central nervous organs and the eyestalk tissues of *Sesarma* showing distribution of incretory elements. AN, abdominal nerve; CB, central body of the brain; F, foramen; LG, lamina ganglionaris; LO, lobus olfactorius; ME, medulla externa; MI, medulla interna; MT, medulla terminalis; OC, oesophageal commissure; SG, sinus gland; SGN, sinus-gland nerve; TOG, tractus olfactorio-globularis.

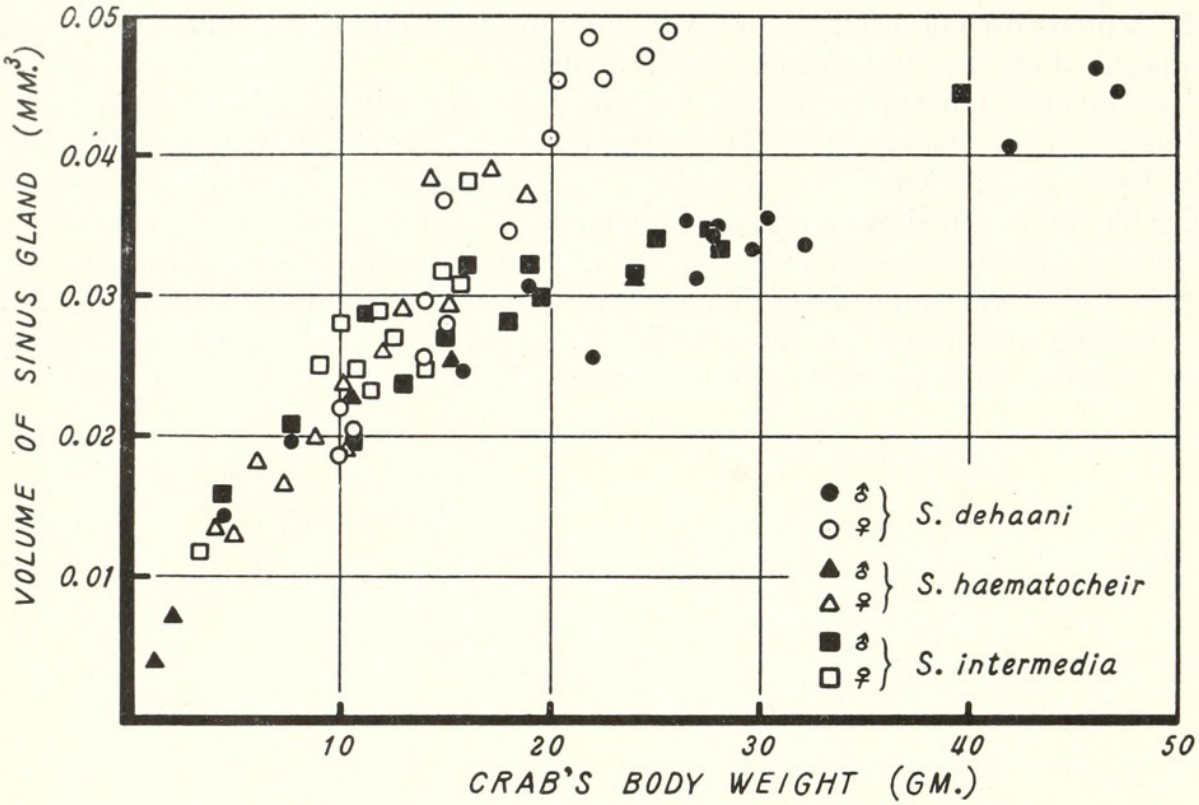

FIGURE 2. Volume of the sinus gland plotted against the body weight in three species of *Sesarma*.

contrary to Hanström's statement pointing out the perforated nature of the membrane (1937), I observed the membrane as being complete everywhere along its course in good sections. I repeatedly examined the membrane in a number of preparations with the result that what had been dealt with as perforations might have been one of the artefacts frequently encountered in sections as those prepared from the whole eyestalk enclosed by hard exoskeleton, to which Hanström had resorted.

At the opposite side of the limiting membrane, the tissue of the sinus gland is contiguous with surrounding spongy connective tissue (Fig. 3, CT), a landmark between the tissues being represented by sets of coarse networks of fibers from the sinus-gland nerve (Fig. 3, E.B.).

Thus, the sinus gland of *Sesarma* is supplied with innervation from the outside and encloses the blood-sinus in the interior, which indicates that it belongs to the so-called inverse type of Hanström's classification (1937, 1947a, etc.).

The tissue of the sinus gland is remarkably similar in its fundamental structure to the neurilemma of nervous tissues, a fact long known by workers of Hanström's school and now substantiated by observations with preparations in which, by treatment with 5% hydrochloric acid prior to fixation, the sinus gland was freed from acidophile inclusions that mask the details of basic structure. The lamella of the gland is, as stated above, essentially of syncytial nature, being composed of continuous chromophobe hyaloplasm divided into irregular units by incomplete septa consisting of coalesced and half-coalesced granules (Fig. 3, S). Within each of the units are a variable number of nuclei of different appearance. Such characteristics are in complete accord with those observed in the neurilemma tissue (Fig. 4). Surrounding the blood-sinus, units of the sinus-gland syncytium are cylindrical, standing vertically against the limiting membrane (Fig. 3).

A remarkable difference between the sinus gland and the neurilemma exists in regard to the amount and morphological variability of the acidophile inclusions, the sinus gland predominating in these respects. As to configuration, stainability, plasticity, microscopic composition, etc., three types of inclusions within the sinus gland are distinguishable which are here tentatively dealt with as colloids A, B, and C, respectively.

Colloid A (Fig. 3, middle figure, CA). Ovoid or ellipsoid masses ranging from 4 μ to 8 μ in length, stained most intensely with the acid fuchsin component of Mallory's triple stain. It is found either in isolation or in small aggregations, its

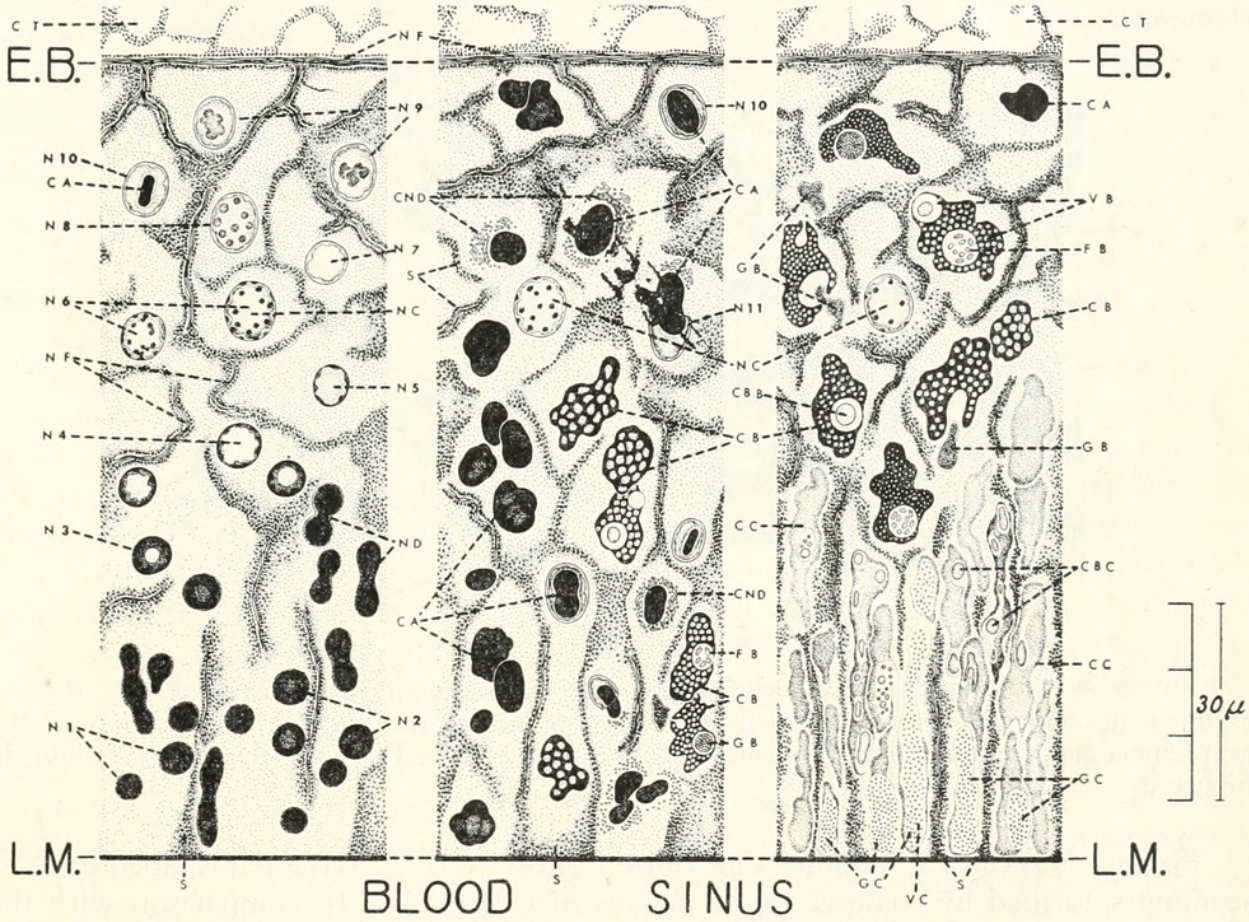

FIGURE 3. Histology of the sinus gland illustrated semi-diagrammatically on the basis of studies with sections and supravital mounts. The whole process of secretory behavior is, for the sake of convenience, arranged in three separate views, of which the left one represents the process of transformation of the nucleus, and both the middle and the right ones are indicating the process of transformation of secretory precursor derived from the nuclear change. E.B., external boundary of the glandular tissue; L.M., limiting membrane.

Left figure: N 1–N 10, successive states of nuclear change; NC, nucleus most commonly seen in the gland; ND, nucleus showing direct division; CA, colloid A formed within the nucleus; NF, nerve-fibers; CT, connective tissue; S, incomplete septum of syncytium.

Middle figure: N 11, liberation of colloid A from nuclear capsule; CND, nuclear capsule in the process of disintegration; CB, colloid B; FB, fragmentary inclusion in the vacuole of colloid B; GB, intra-vacuolar granules of colloid B; other notations are the same as those in the left figure.

Right figure: VB, vacuole in colloid B; CBB, central body in the vacuole of colloid B; CC, colloid C; CBC, central body in the vacuole of colloid C; GC, granules in the vacuole of colloid C; VC, vacuole in colloid C; other notations are the same as those in the left figure.

occurrence being inconsistent at different spots of the same glandular lamella. In the supravital state the colloid shows a marked affinity to alizarine S, the resulting coloration being associated with pronounced refractility, and it displays considerable plasticity, which suggests a high viscosity of its composite material. In both fixed and *in vitro* preparations, its staining is homogeneous, no structural differentiation being present. Characteristic staining reactions of the inclusions are further observed in sections impregnated with silver according to the techniques of Bielschowsky and of Hortega, in which the colloid reduces silver almost selectively. In sections treated with $N/4$ sodium hydroxide and stained with Janus green B, the colloid appears deep greenish blue in contrast to the unstained surrounding tissue elements.

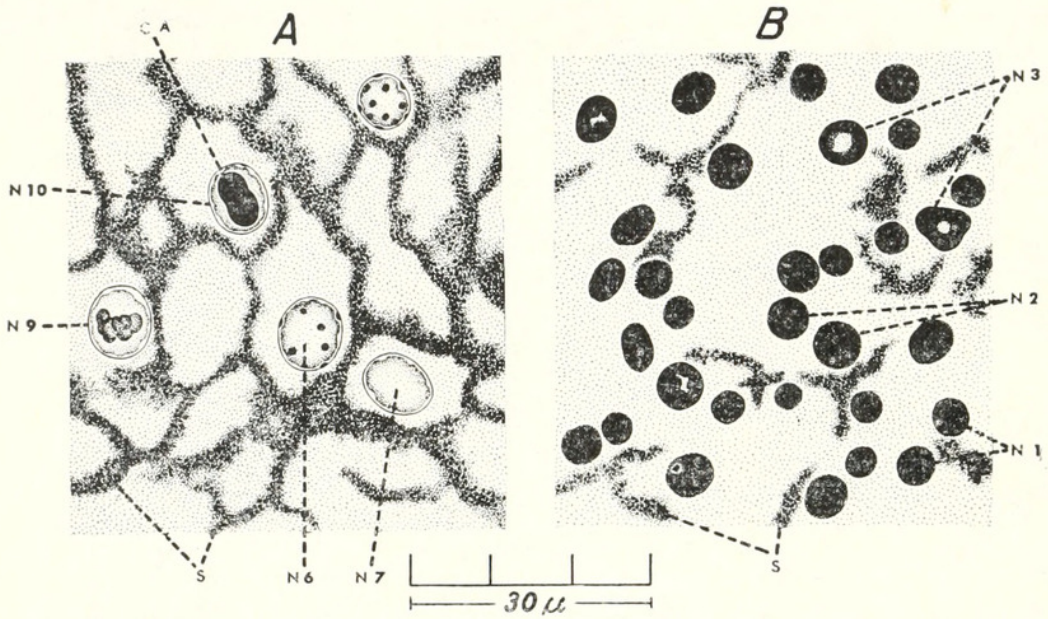

FIGURE 4. Histology of the neurilemma semi-diagrammatically illustrated. A—Portion of the neurilemma showing formation of colloid A within the nucleus. B—Portion of the neurilemma including a crowd of small basophile nuclei. Notations are the same as those in Figure 3.

Colloid B (Fig. 3, middle and right figures, CB). Irregular-shaped masses seemingly formed by coalescence of masses of colloid A. In comparison with the latter, it shows weak stainability with both acid fuchsin in Susa-Mallory preparations and alizarine S applied *in vitro*. Together with the lowering of the affinity to these dyes, a marked decrease in the power of silver reduction together with the stainability with Janus green B is characteristically observed with this kind of glandular inclusion. In contrast to the apparent homogeneity of colloid A, colloid B is differentiated into a feebly acidophile matrix substance which appears less viscous than colloid A, and almost chromophobe contents of various sizes and amounts. Vacuoles occur in the colloid (Fig. 3, right figure, VB), which remain achromatic in Susa-Mallory preparations but are selectively stained with trypan blue, anilin blue, Congo red, eosin and tropeolin *in vitro*. They may or may not include formed elements, whose appearance ranges from single large chromophobe bodies (Fig. 3, right figure, CBB) to several irregular achromatic fragments (Fig. 3, middle and right figures, FB) and a number of granules deeply stained with anilin blue in Susa-Mallory sections (Fig. 3, middle and right figures, GB). Such vacuolar

inclusions are unstainable *in vitro,* but are clearly demonstrated when their surroundings are stained with the dyes mentioned above. Irrespective of their size, no sign of Brownian movement is observed *in vitro,* which is suggestive of considerable viscosity of their surroundings within the vacuoles. It was not infrequent to observe in both sections and supravital mounts of the sinus gland that the said anilin-blue granules together with their surrounding viscous substance were on the way of freeing themselves from colloid B into the hyaloplasm of the glandular tissue (Fig. 3, right figure).

Colloid C (Fig. 3, right figure, CC). Almost formless masses which, by coalescence, grow to larger forms conforming to the contours of the divisions of the glandular tissue. In Susa-Mallory preparations, this kind of inclusion is stained fairly well and homogeneously with Orange G, no morphological differentiation of composition being obvious. In whole mounts of the sinus gland *in vitro,* a wide affinity of the colloid to basic dyes such as Dahlia, Cresylecht violet, Gentian violet, methylene blue, and neutral red was disclosed, and an evident sign of its low viscosity was its tendency to assume a rounded appearance. In contrast to both colloid A and colloid B, this colloid rejects alizarine S *in vitro,* and in sections it reacts negatively to silver impregnation and also to staining with Janus green B following treatment with NaOH. Moreover, it is characterized by active vacuole formation (Fig. 3, right figure, VC). In vacuoles of various sizes one finds a number of granules of uniform size (Fig. 3, right figure, GC), several irregular fragments, or one or several central bodies (Fig. 3, right figure, CBC), all of which remain unstainable in both fixed and supravital conditions. *In vitro,* such vacuolar inclusions exhibit more or less pronounced Brownian movement, the smaller their size, the more active the visible motion. This fact indicates a certain fluid nature of the vacuolar content surrounding the inclusions. The development of such vacuoles in this colloid is very pronounced, and there are figures indicating that a given mass of the colloid may be almost wholly occupied by an enlarged vacuole.

Possible transformation of secretory materials within the sinus gland. The features described above are characteristic of the three representative forms of colloid inclusions of the sinus gland, but there are other forms derived from the respective colloids, which lie either between colloids A and B or between colloids B and C. This fact indicates a possible continuity of all the colloidal inclusions, *i.e.,* the possibility of a transformation of colloid A to colloid C by way of colloid B. In relation to such a possibility, the following observation appears to be of interest.

Upon treatment with 10% pyridine, a freshly isolated sinus gland loses most of its characteristic opaque whiteness in less than 30 minutes. Formalin-fixed and Mallory-stained sections of such material reveal that the vanishing of whiteness is due to the almost complete disappearance of colloid C. Nevertheless, both colloid A and colloid B are known to be resistant to the treatment. Of these remaining inclusions, colloid A as a whole retains a remarkable affinity to acid fuchsin, while colloid B reacts to the dye in a manner different from that shown by the one not treated with pyridine. The fragmentary inclusions within colloid B take the dye almost selectively, whereas the homogeneous ground substance around them is but feebly stained. That is, the affinity to acid fuchsin is reversed in the two kinds of components of colloid B as a result of the treatment with dilute pyridine.

After all, there are indications that the initially highly viscous and homogeneous

mass (colloid A) differentiates into a less viscous substance as the result of progressive disintegration into finer particles, which further changes into a more fluid substance as the result of a separation of viscous substance by means of vacuole formation. The fluid substance thus produced (colloid C) may then change into intravacuolar fluid and granules, both of which appear to be the ultimate products of transformation of colloid A, taking place within the tissue of the sinus gland.

Derivation of secretory material from nucleus. Inasmuch as the acidophile inclusions of the sinus gland are considered as secretory material (*cf.* Hanström, 1937, etc.), the preceding account of the possible fate of colloid A throws some light on the process of formation of the active material. Another problem, so far left untouched, concerns the origin of the glandular inclusions.

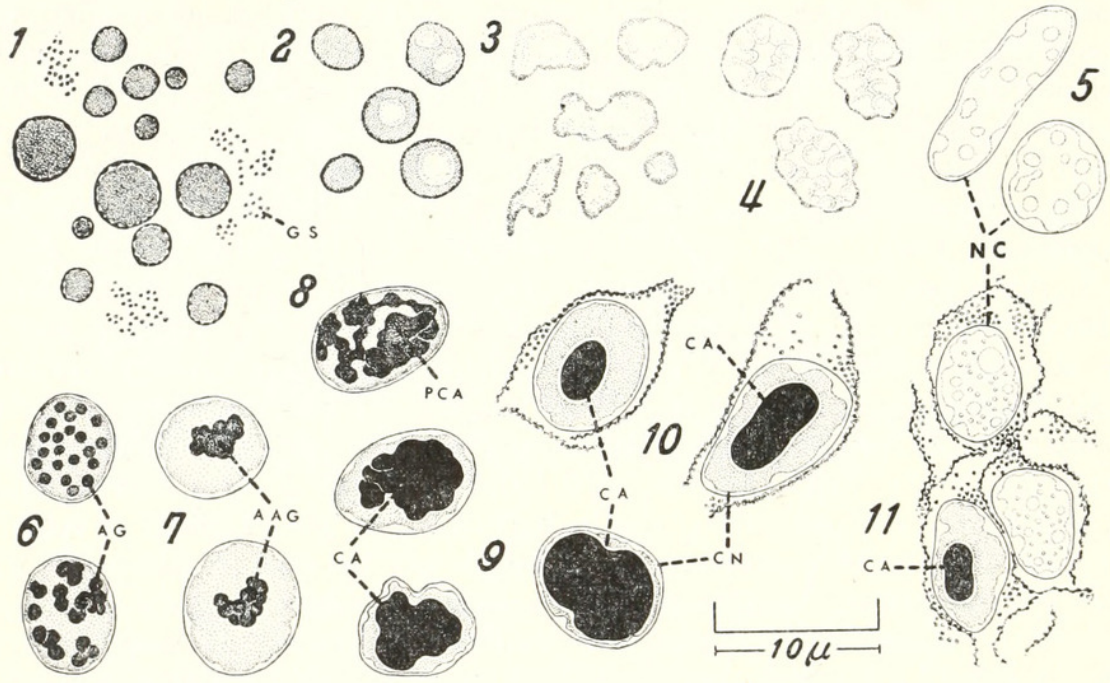

FIGURE 5. Variety of nuclear figures in both the sinus gland and the neurilemma. Susa, Mallory. NC, common appearance of nucleus; GS, granules of septum of syncytium; AG, globules stained with Orange G; AAG, aggregated Orange G-globules; PCA, precursor of colloid A; CA, colloid A; CN, capsule of nucleus surrounding colloid A.

It was not infrequent to observe in both sections and supravital mounts of the sinus gland that colloid A in its isolated form was enclosed by an ovoid or ellipsoid capsule of considerable thickness which, although being refractory to any dyes, is visible on account of its high refractility (Fig. 3, left and middle figures, N 10; Fig. 5, *10*). Some figures showed the release of the colloid masses from their respective capsules through rupture of the latter (Fig. 3, middle figure, N 11; Fig. 7, *2–5*). In areas of the glandular lamella where such figures were found in close aggregation, freed masses of the colloid were seen in coalescence with each other to form larger masses which were associated with empty capsules (Fig. 7, *1*). The capsule, after releasing the colloid mass, appeared to disintegrate, being at first decomposed into irregular pieces and eventually reduced to finer granules (Fig. 3, middle figure, CND; Fig. 7, *6–9*).

In the state of full integrity, the encapsulated body conforms in size and form

to the nucleus, a fact which led me to an inquiry regarding the role of the nucleus of the sinus gland. In the routine hematoxylin-eosin preparations, the nuclei of the gland most commonly met with are of vesicular appearance, containing varying amounts of basophile granules which may be taken for granular chromatin, but no acidophile elements corresponding to nucleoli. In sections treated after Feulgen's method modified by Rafalko (1946),[2] it was found that the chromatin or, more precisely, the basophile elements are arranged at the periphery of the nucleus, the latter's interior being occupied by chromophobe caryoplasm (Fig. 3, NC or N 6; Fig. 6, *7*). Besides this appearance there were other features of the nucleus, indicating its derivation from a smaller structureless basophile body.

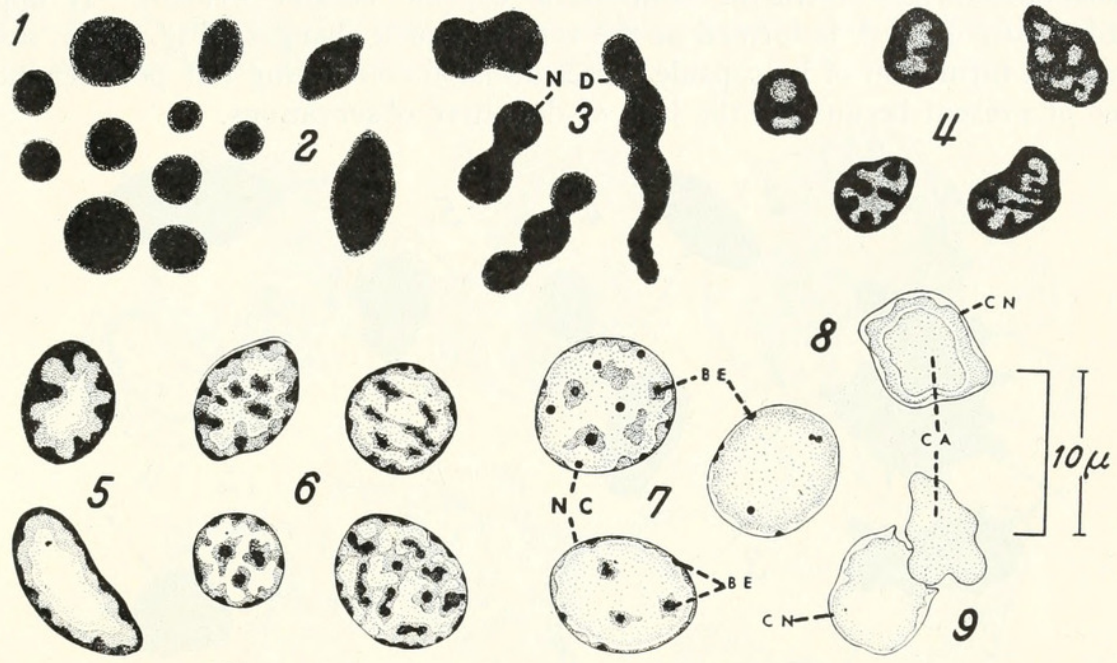

FIGURE 6. Figures of nuclei in both the sinus gland and the neurilemma. Helly, Feulgen (Rafalko's modification). ND, amitotic figures of nuclear division; NC, common appearances of nuclei. BE, basophile elements at the periphery of nucleus; CA, colloid A; CN, capsule of nucleus.

What is designated to be the original form of the nucleus is a minute sphere, ovoid or ellipsoid, of dimensions ranging from 3 μ to 6 μ, which stains with hematoxylin, safranin and muci-carmine and also exhibits a marked uniform reaction with Feulgen's stain except for a thin peripheral area (Fig. 3, left figure, N 1; Fig. 6, *1* and *2*). In Susa-Mallory preparations, such a body takes anilin blue uniformly, but the peripheral area predominates in this stainability (Fig. 5, *1*). Nuclei of such structureless appearance were at times found in dense aggregation in certain restricted areas of the sinus gland, where they were observed in association with groups of melanin granules, and occasionally in the process of direct division (Fig. 3, left figure, ND; Fig. 6, *3*).

Such a state of the nucleus appears to change to a state in which the chromophobe portion is differentiated in the center of the basophile mass (Fig. 3, left figure, N 2 and N 3; Fig. 5, *2*; Fig. 6, *4*), and there were figures showing further successive

[2] Use of this technique was aided by the kind information received from Dr. K. W. Cleland, Sydney University, Australia.

changes, in which the nucleus becomes enlarged by the expansion of the central chromophobe mass which is likely to take place at the expense of the basophile portion restricted to the periphery (Fig. 3, left figure, N 4 and N 5; Fig. 5, *3* and *4*; Fig. 6, *5* and *6*). These changes seem to result in the common vesicular state of the nucleus already referred to.

Figures were observed indicating the formation of strongly acidophile droplets within the chromophobe substance of the nucleus, which stained with the Orange G component of Mallory's stain and in this respect resembled the ordinary nucleoli (Fig. 3, left figure, N 8; Fig. 5, *6*). In some of the nuclei, such droplets showed a marked tendency to coalesce (Fig. 3, left figure, N 9; Fig. 5, *7*), and along with this phenomenon the refractile achromatic capsule became evident. It appears probable that colloid A is formed as the result of such changes (Fig. 5, *8*), accompanying the formation of its capsule, but a decision concerning this point cannot be reached at present because of the lack of definitive observations.

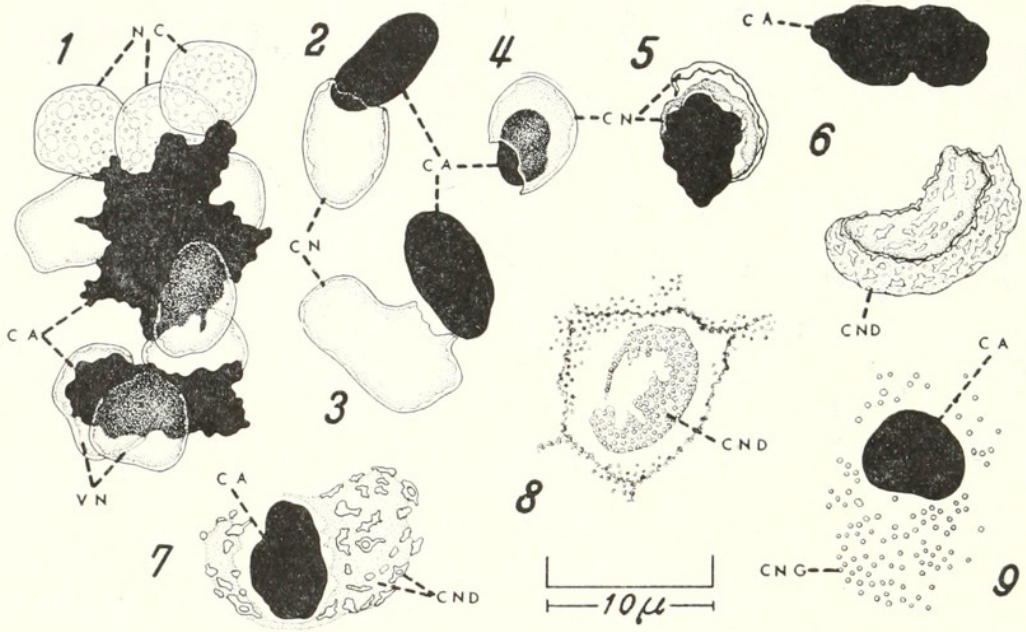

FIGURE 7. Figures showing liberation of colloid A from the nucleus and the later fate of nuclear capsule in the sinus gland and the neurilemma. Formalin, *N*/4 NaOH, Janus green B. NC, common forms of nuclei; CA, colloid A; VN, vacant nuclei; CN, capsule of nucleus; CND, disintegrating capsule of nucleus; CNG, granules resulted from disintegration of nuclear capsule.

2. Secretory activity of the neurilemma

Just as in the sinus gland, the nucleus plays a significant role in the production of acidophile inclusions in the neurilemma. The neurilemma of all ganglionic tissues was observed to produce a kind of colloid which was morphologically quite similar to colloid A of the sinus gland (Fig. 4, *A*, CA). In Susa-Mallory preparations or more clearly in sulfosalicylic acid-Mallory preparations of each of the principal ganglia, the colloid in question was found in varying amounts in different portions of the neurilemma, whose brilliant red coloration stood out impressively from the light blue tinge of the background. It was further observed that what was known about the morphological change of the nucleus within the sinus gland in relation to the process of the formation of colloid A was also the case regarding the nucleus of the neurilemma (Fig. 4). Such facts indicate that the same secretory mechanism is

operating in the two kinds of tissue in question, which, as stated above, are remarkably similar in their fundamental structure.

However, the neurilemma was observed to be inferior to the sinus gland with respect to the nuclear activity, and, moreover, present investigation failed to ascertain the transformation of colloid A into other forms, because of the apparent absence of both colloid B and colloid C or their homologues within the neurilemma tissue.

3. The neurosecretory cells

The present study includes the search for possible incretory elements in the nervous tissues outside the neurilemma, which, as already stated, resulted in the finding of three kinds of neurosecretory cells.

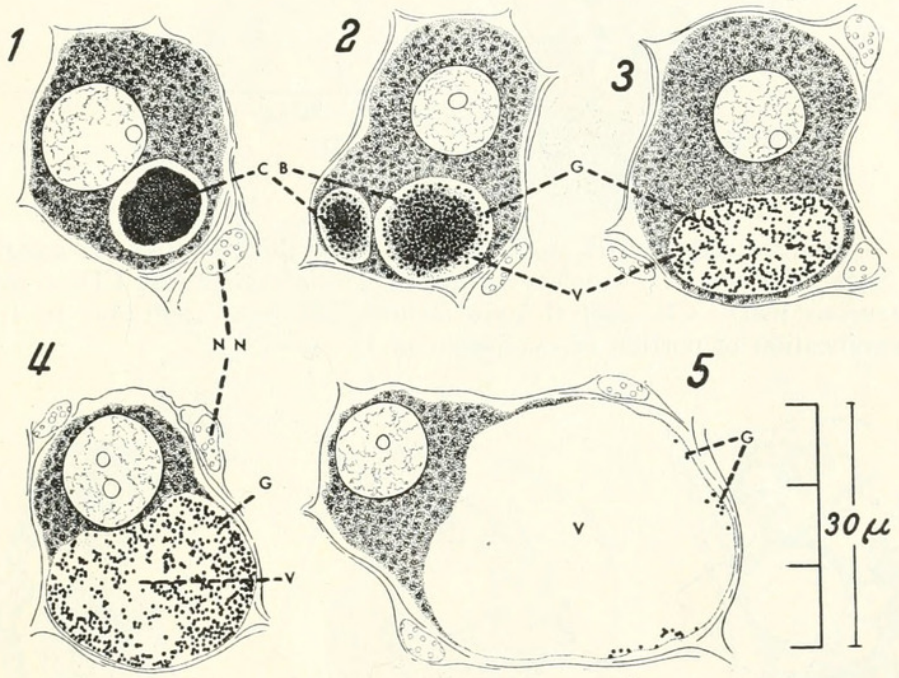

FIGURE 8. α neurosecretory cells from the brain and the medulla terminalis. Susa, Mallory. CB, central body formed in vacuole; G, granules derived from the central body; V, vacuole; NN, nucleus of the neurilemma.

α *neurosecretory cell* (Fig. 8). Secretory behavior was observed in some unipolar nerve-cells of the common type widely distributed in the brain, the medulla terminalis, the medulla interna and the medulla externa as well as the thoracic ganglion (Fig. 1). Excepting those found in the thoracic ganglion which were represented by giant cells attaining about 60 μ in length and including a large vesicular nucleus of about 20 μ diameter with one or two nucleoli of about 4 μ, the cells in other ganglionic tissues are generally of moderate size, measuring about 30 μ in length and including a nucleus of about 12 μ in diameter in which one or two nucleoli of about 2 μ are present. Irrespective of the difference in cellular dimensions, all of the cells are rich in cytoplasm, in which significant vacuole formation takes place. In Susa-Mallory preparations of the said ganglia, formation of a large fuchsinophile body within each vacuole (Fig. 8, *1*), splitting of the body into a number of fuchsinophile granules (Fig. 8, *2*), and dispersion of isolated granules within the

largely expanded vacuole (Fig. 8, *3* and *4*) were seen. Sometimes, expanded vacuoles were observed to be almost free of formed inclusions except for a few fuchsinophile granules (Fig. 8, *5*). With the formation of such vacuoles, the cytoplasm of the cells exhibited a variable affinity to acid fuchsin; the cytoplasm rejected the dye in the presence of many fuchsinophile granules within the vacuole, but was

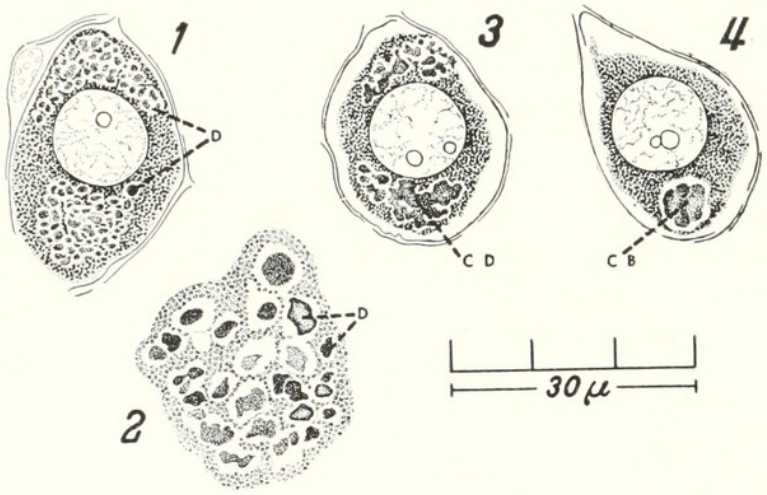

FIGURE 9. β neurosecretory cells from the brain and the commissural ganglion (*cf.* Fig. 1, B and C). Susa, Mallory. D, droplets appearing within cytoplasm; CD, droplets coalesced to form homogeneous mass; CB, central body formed. Scale is applicable to 1, 3, and 4. 2 is a further magnification of portion of cytoplasm in 1.

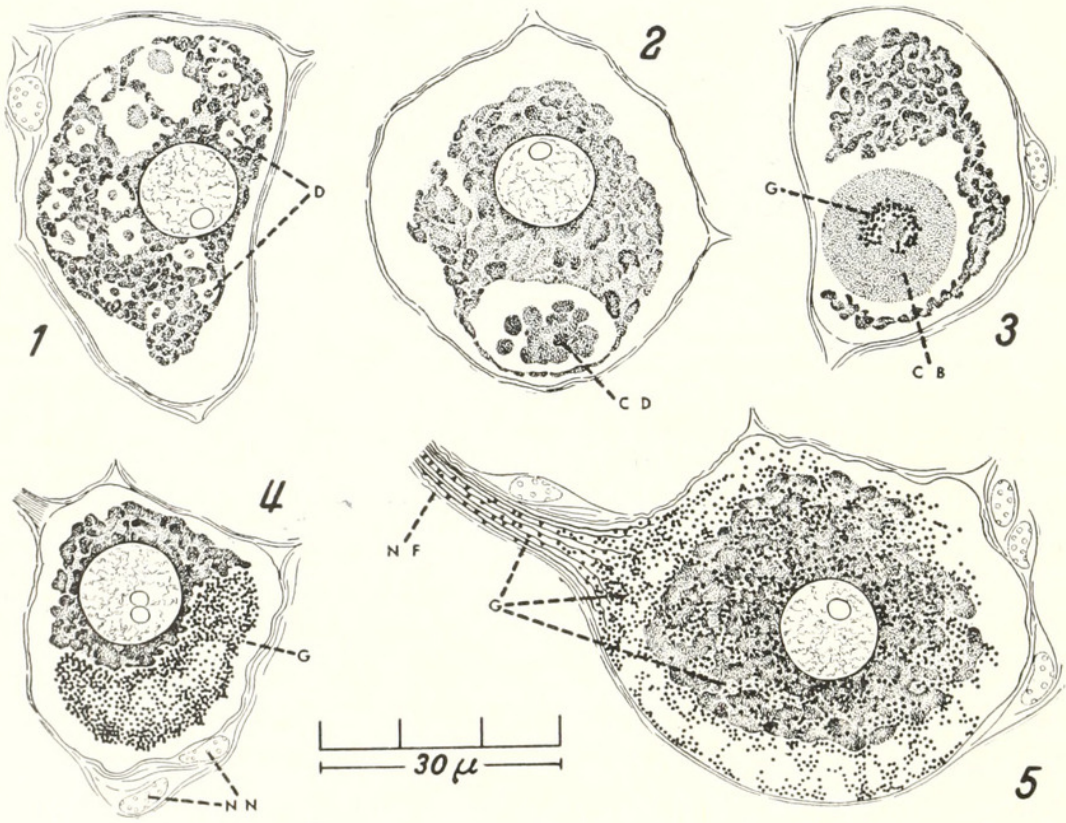

FIGURE 10. Giant β neurosecretory cells from the medulla terminalis (*cf.* Fig. 1, A). Susa, Mallory. NF, nerve-fiber innervating the sinus gland; NN, nuclei of the neurilemma; G, secretory granules; other notations are the same as those in Figure 9.

stainable in higher or lower degrees when the vacuole was deprived of most of its granular inclusions. Pictures were observed indicating a possible absorption of the intra-vacuolar granules by the cytoplasm, a fact which might explain the fuchsinophilic character of the cytoplasm during the disappearance of the granules.

β neurosecretory cell (Figs. 9, 10, and 11). The second type of neurosecretory cells differs from the preceding one in its mode of secretory activity and in the predominant affinity of the secretory material to the anilin blue component of Mallory's stain and also in the mode of secretory behavior. Cells showing this type of secretion were detected in the brain, the medulla terminalis and the commissural

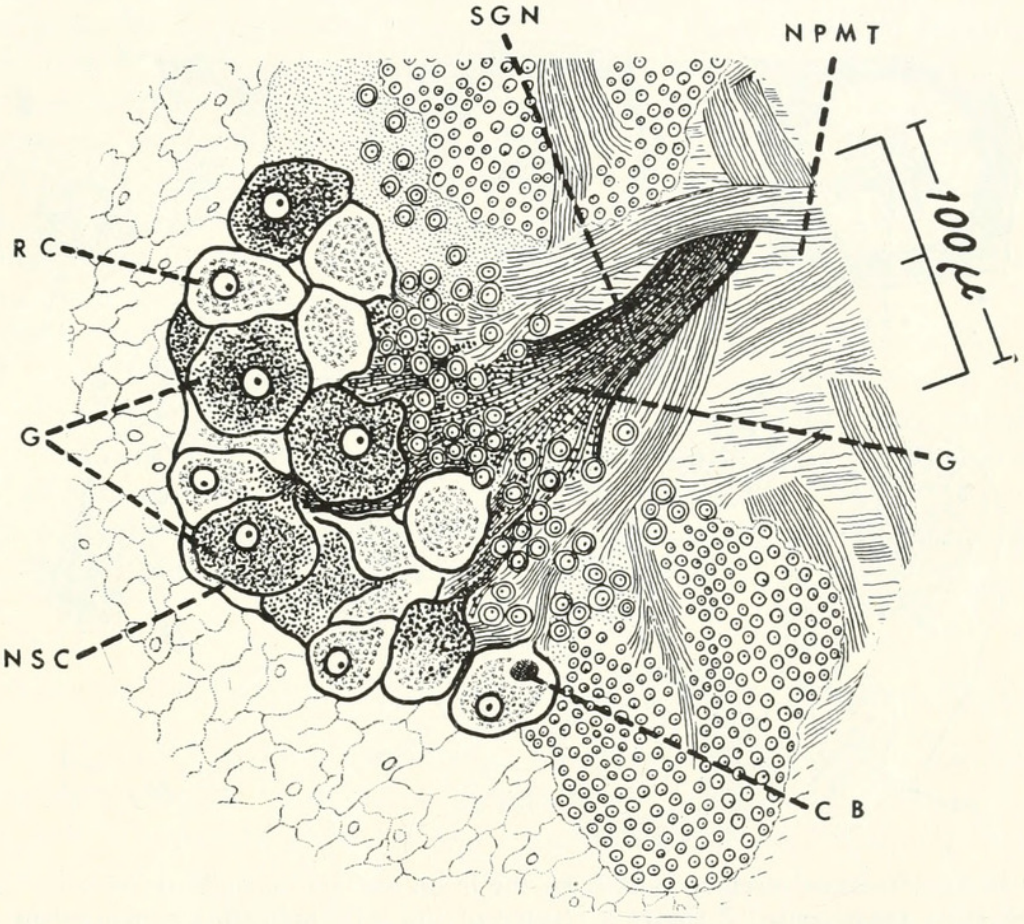

FIGURE 11. Section of the cluster of giant β neurosecretory cells of the medulla terminalis (*cf.* Fig. 1, A). Susa, Mallory, sagittal to axis of the eyestalk. SGN, sinus-gland nerve; NPMT, neuropile of the medulla terminalis; G, secretory granules; RC, resting cell; NSC, cell in secretory activity; CB, central body formed in the cell.

ganglion, but not in other ganglia. In the brain, such cells were located exclusively in a group of nerve-cells situated just anterior to the lobus olfactorius (Fig. 1, B). They, together with those found in the commissural ganglion (Fig. 1, C), are of moderate size, measuring ca. 30 μ in length and including a vesicular nucleus of ca. 10 μ in diameter (Fig. 9). Their cytoplasm is fairly homogeneous and of compact appearance, showing but slight contraction upon fixation. The cells belonging to the medulla terminalis are represented by a number of giant unipolar cells which constitute the conspicuous cluster at the ventral surface of the proximal portion of the neuropile of the medulla, dispatching nerve-fibers innervating the sinus gland

(Fig. 1, A; Fig. 11). These cells are composed of loose cytoplasm which, upon fixation with Bouin, Allen-Bouin, formalin, Helly, sulfosalicylic acid, Susa, and Zenker, contracts more or less conspicuously. The average dimension of the cells, measured by the contour of the surrounding neurilemma capsule, was about 50 μ, and the nucleus attained about 15 μ in diameter (Fig. 10).

In the cytoplasm of the β-type neurosecretory cells occurred many droplets feebly stained with anilin blue (Fig. 9, *1* and *2*; Fig. 10, *1*). Also observed in the cytoplasm of some cells were one or two spherical bodies taking up anilin blue considerably, which seemed to result from coalescence of the said droplets (Fig. 9, *4*; Fig. 10, *3*; Fig. 11, CB). Each of these spherules was observed to split into fine

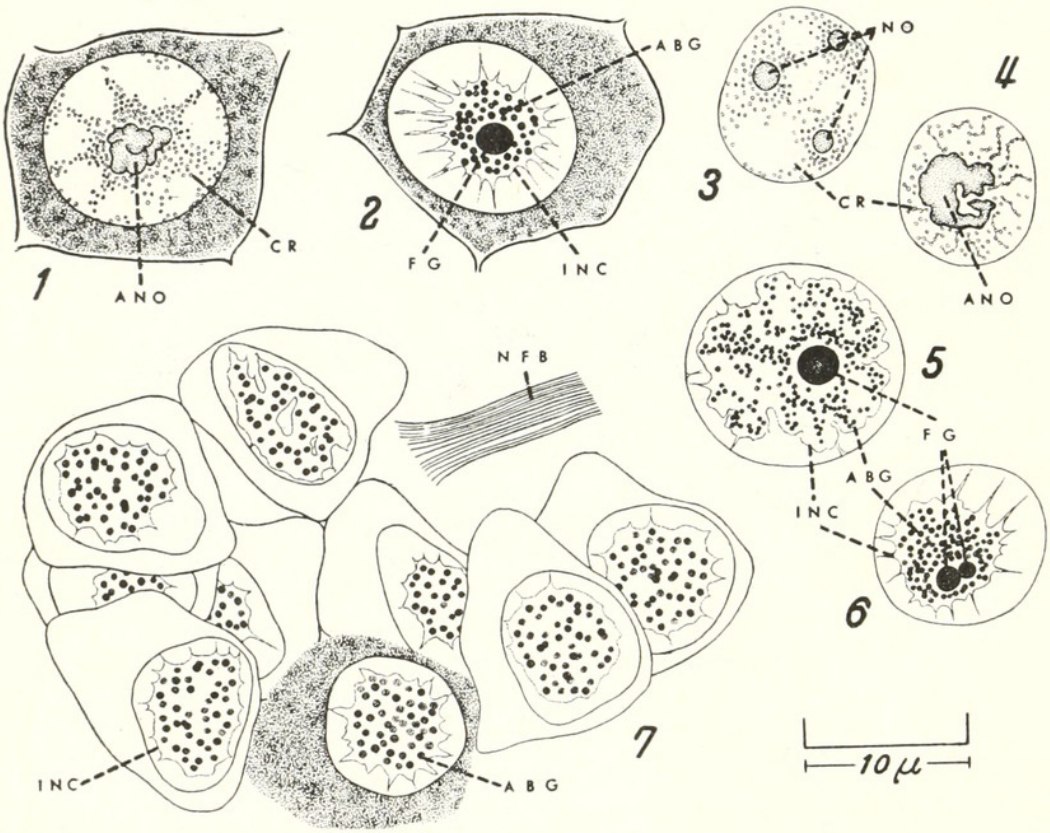

FIGURE 12. γ neurosecretory cells from the brain and the medulla terminalis. Susa, Mallory. 3–6 show nuclei only; 7 shows a cluster of the cells indicating synchronism of nuclear activity. ANO, nucleoli aggregated at the center of nucleus; CR, chromatin granules; FG, fuchsinophile globule; ABG, anilin-blue granules; INC, intra-nuclear colloid; NO, nucleoli; NFB, nerve-fiber bundle.

granules stained deeply with the dye (Fig. 10, *3* and *4*). The resulting granules were dispersed over the whole area of the cytoplasm, and it was of much interest to find in a few instances that they were present also along the path of axons arising from the cells with secretory activity (Fig. 10, *5*; Fig. 11, G). In one case, the granules were detected along the course of the sinus-gland nerve for approximately two-thirds of its whole length.

γ neurosecretory cell (Fig. 12). Unlike the preceding two types of neurosecretory cells, the third type is characterized by a kind of nuclear secretion. In some small unipolar nerve-cells measuring about 20 μ in length, as well as in some of the cells belonging to the so-called globulus type, measuring about 10 μ in length,

the nucleus underwent a series of changes unassociated with any visible change in the cytoplasm. There were pictures showing formation of a large intra-nuclear droplet uniformly stained with the Orange G component of Mallory's stain, within some of which were differentiated a fuchsinophile spherule and many anilin blue granules (Fig. 12, *2* and *5*). The fuchsinophile spherule was different from the nucleolus in the ordinary state of the cells in question in that it was far larger than the latter and was stained conspicuously with acid fuchsin in contrast to the latter's affinity to Orange G. Similarly the anilin blue granules were different from the granular chromatin in size and in stainability, the former far exceeding the latter in this respect. Other figures showed that both the fuchsinophile spherule and the anilin blue granules were reduced to smaller forms. That is, two or more globules developed from a single spherule, which, unlike their preceding form, took up Orange G considerably and resembled nucleoli, and a number of fine granules were derived from the anilin blue granules, which resembled the chromatin (Fig. 12, *6*). These figures are suggestive of a reduction of intra-nuclear inclusions to nucleoli and chromatin granules. As regards the process of formation of the intra-nuclear colloid, there were indications suggesting its origin from a central aggregation of both the nucleoli and the chromatin (Fig. 12, *1, 3,* and *4*).

Such being the case, it would appear plausible that the nucleus of this type of nerve-cell shows a kind of reversible change, and that the secretion of a diffusible substance takes place in the course of the change. The present study failed to obtain any sign of discharge of formed elements from the nucleus to the surrounding cytoplasm of the cell.

Nerve-cells showing such nuclear change were located in abundance in the brain as well as in the medulla terminalis, but were scarcely detected in other ganglia (Fig. 1).

Considerations on Possible Sources of Chromatophorotropic Hormones

On the basis of the histological findings so far described, possible sources of the two chromatophorotropic hormones of *Sesarma* may be analyzed.

Taking into account the results of the experimental study (Enami, 1951) which have demonstrated that one of the hormones, S hormone, is originating not only in the sinus gland but in all the principal ganglia in the adults, one may attach some significance to the remarkable identity of the fundamental structure as well as the mode of secretion of the sinus gland and the neurilemma universally associating with the nervous tissues. These structures are equivalent in that their secretions appear to originate as the result of a transformation of the nucleus, although the fate of the secretory material within the respective tissues appears to be different. The secretory precursor, or colloid A, in the sinus gland undergoes a sequence of complicated changes to be transformed into a semi-fluid form (colloid C) which is further transformed through vacuole formation into liquid and granules. Now, in connection with the problem of what form the final secretion may assume, the observed completeness of the limiting membrane is to be taken into consideration. It appears possible that the final secretion containing S hormone is of diffusible nature, to which the limiting membrane is permeable, and that no kind of formed element can take its way out through the membrane, in which no sign of microscopic

perforation was observable. In this respect, the vacuolar fluid of colloid C rather than the co-existing granules appears to be of significance.

Concerning the role of the vacuolar contents of colloid B, which are liberated as a whole from the latter, no concrete information could be obtained. They may somehow be responsible for the formation of certain secretory materials, or they may be nothing but by-products of the transformation of the colloid into less viscous colloid C, having no direct relation to the formation of secretory products.

At any rate, such complicated secretory behavior was not found in the neurilemma, which fact may be correlated with a presumably simpler mode of hormone production in this tissue.

Recent investigations by several authors have demonstrated that the crustacean sinus gland produces various kinds of hormones in addition to the generally admitted chromatophorotropic one, such as that acting on the motility of the heart, the hormone inhibiting molting, the diabetogenic principle, the one inhibiting ovarian growth, the hormone controlling retinal pigment migration and that controlling general motor activity. With the exception of the chromatophorotropic hormone, all of these principles seem to originate in the sinus gland exclusively (*cf.* Brown, 1944; Hanström, 1947a). While it remains to be seen whether such hormones are different substances or represent only one or a few substances, it should be taken into account that there is no concrete information concerning the capacity of the neurilemma for such manifold production of hormones. It may tentatively be proposed that the observed simplicity of the secretory behavior in this tissue appears to be correlated with a restricted productivity of hormonal substances.

With respect to the histological elements responsible for the production of another kind of chromatophorotropic hormone, N hormone, experimental results have indicated that such might be located in the adult crabs in the brain and the medulla terminalis. Such a restriction in the distribution of this hormone points to incretory elements specifically present within the said two nervous organs as the possible sources. Therefore, the neurilemma universally associating with the nervous tissues is to be excluded. A marked parallelism exists between the distribution of the neurosecretory cells of the γ type and the experimentally ascertained sites of origin of N hormone.

As for the other types of neurosecretory cells, there is little possibility of their participation in the production of N hormone. This might be deduced from the experimental results demonstrating the absence of the hormone in concentrated extracts of such ganglia as the thoracic ganglion, in which the cells of the α type are present in greatest abundance, and the commissural ganglion containing the β type cells. Absence of the hormone was also demonstrated in the extract of the isolated cluster of β neurosecretory cells of the medulla terminalis.

It is of much interest that, as demonstrated above, neurosecretion of the γ type is characterized by a kind of nuclear secretory activity, which has also been reported in certain neurosecretory cells as well as in certain hypophyseal cells in some vertebrates (Scharrer, 1934; Scharrer and Scharrer, 1945; Hanström, 1947b). Thus, the production of N hormone can be regarded as being in principle identical with that of S hormone, inasmuch as both involve nuclear activity, even though the mode of nuclear behavior differs. S hormone appears to take its origin at the

expense of the nucleus, whereas N hormone results from a reversible change of intra-nuclear elements, during which the nuclear integrity remains intact.

Thus, the chromatophorotropic hormones in *Sesarma* are elaborated by two kinds of nuclear secretion, an observation which contributes further to the recently increasing knowledge regarding the participation of the nucleus in secretory activities in both the vertebrates and the invertebrates. As Palay (1943) has supported the concept of nuclear secretion against the former negative view of Bowen (1929), present work also demonstrates it in certain secretory activities.

Concerning the neurosecretion of the α and β types, no information about their physiological significance is available at present. Of interest is the role played by the secretory material of the β neurosecretory cells, especially those supplying nerve-fibers to the sinus gland. As reported above, the anilin blue granules interpreted as the final product of secretory activity of the cells were observed to take their way along the path of the axon toward the sinus gland, a fact which is reminiscent of similar figures demonstrated by Scharrer and Scharrer (1944) and Palay (1945) in the preoptico-hypophyseal tract in some vertebrates and also in the intercerebralis-cardiacum-allatum pathway in some insects. It appears plausible that the neurosecretion in question may play a significant role in the control of the secretory activity of the sinus gland. However, the present study is short of data for such considerations, except for the following. In an experiment, the nerve-cell cluster in question was carefully isolated from the medulla terminalis and extracted to be injected into eyestalk-less specimens of juvenile *Sesarma haematocheir* and eyestalk-less *Paratya compressa,* with the result that a considerable effect of S hormone (pigment concentration in reddish chromatophores in both recipients; *cf.* Enami, 1951), but not of N hormone was produced. However, whether this result is attributable to a temporary promotion of secretory activity in the recipients' sinus gland brought about by the secretory product contained in the extract, or whether it is due to the direct effect of the donor's S hormone derived from the neurilemma around the nerve-cell cluster in question, is left undetermined.

Summary

1. In this part of my contemplated series of papers are reported some results concerning the histological investigation of the incretory elements responsible for the production of the two chromatophorotropic hormones in *Sesarma,* whose activities and distribution were experimentally dealt with in the preceding study.

2. On the basis of the present histological findings, and their comparison with the experimental data, it has been concluded that the sinus gland and the neurilemma are responsible for the secretion of S hormone, whereas N hormone has its origin in a type of neurosecretory cell (γ neurosecretory cell) occurring in abundance in both the brain and the medulla terminalis.

3. The secretory activity in all of these hormone sources is alike in that it shows nuclear secretion, but there are differences in the secretory behavior.

4. The close relationship between the sinus gland and the neurilemma was substantiated on the basis of their fundamentally similar histological structure and their secretory behavior. However, certain differences were observed as to the fate of the secretory material derived from the nucleus, a more complicated sequence of transformations taking place in the sinus gland than in the neurilemma. The sig-

nificance of these differences and the possible nature of the final secretory material of the sinus gland were discussed.

5. Besides the cells regarded as possible sources of N hormone, two kinds of neurosecretory cells (α and β neurosecretory cells) were described, which are located in areas of the nervous system more or less distant from the area of distribution of the γ cells. These show a cytoplasmic secretory activity with particulate elements as their ultimate products. The physiological significance of their respective secretions is still unknown.

6. The sinus gland is innervated by a cluster of giant β neurosecretory cells of the medulla terminalis. Figures show a distal flow of secretory granules from the cells along the course of the sinus-gland nerve, a fact which is indicative of an interesting type of chemical transmission resembling those reported to occur in the innervating systems of the vertebrate hypophysis and of the insect cardiacum-allatum complex.

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