CYTOPLASMIC INCLUSIONS RESEMBLING NUCLEOLI IN SYMPATHETIC NEURONS OF ADULT RATS

MARY A. GRILLO

From the Departments of Physiology and Rehabilitation Medicine, New York University Medical Center, New York, New York 10016

ABSTRACT

A distinctive cytoplasmic inclusion consisting of a convoluted network of electron-opaque strands embedded in a less dense matrix was identified in the neurons, but not in the supporting cells, of rat sympathetic ganglia. This ball-like structure, designated "nematosome," measures $\sim$ 0.9 μ and lacks a limiting membrane. Its strands (diameter = 400–600 A) appear to be made of an entanglement of tightly packed filaments and particles $\sim$ 25–50 A thick. Cytochemical studies carried out with the light microscope suggest the presence of nonhistone proteins and some RNA. Usually only one such structure is present in a cell, and it appears to occur in most ganglion cells. Although they can be seen anywhere in the cell body, nematosomes are typically located in the perinuclear cytoplasm, where they are often associated with smooth-surfaced and coated vesicles. In fine structure and stainability, they bear a resemblance to the fibrous component of the nucleolus. Subsynaptic formations, which are a special feature of some of the synapses in sympathetic ganglia, appear similar to the threadlike elements in the nematosomes. The possibility that these three structures—nucleolus, nematosome, and subsynaptic formation—may be interrelated in origin and function is discussed.

INTRODUCTION

About 70 years ago both Rohde (41, 42) and Holmgren (18) observed incusions in the cytoplasm of nerve cells which they identified as nucleoli on the basis of staining properties. Since that time similar structures have been described by others. They were reported to occur in a variety of neurons in the central (6, 27, 45) and peripheral (15, 18, 27, 42, 47, 55) nervous system of vertebrates, and were also found in the ganglion cells of a mollusc (41). Although direct morphological evidence for a migration of nucleoli and nucleolar material into the cytoplasm was not always found, it was commonly agreed that the cytoplasmic nucleoli were of nuclear origin. Speculations on the fate and function of these bodies seemed to hinge on the question of whether they are restricted entirely to neurons or occur also in the neighboring intercellular spaces and nonneuronal cells. Observations bearing on these alternatives led to the proposal of three hypotheses: (*a*) the bodies are transformed into the nuclei of neuroglial cells (41); (*b*) they serve some unknown secretory or excretory function (27, 47); (*c*) they give rise to the Nissl substance. Of these hypotheses, the last was the most widely accepted and was supported by studies carried out not only on the adult nervous system (18, 41, 42, 55), but also on developing neurons during the period of Nissl substance differentiation (6, 15, 42).

Electron microscopic studies have thus far failed

to confirm the existence of cytoplasmic nucleoli in nerve cells. Possible exceptions to this statement are the recent studies by Shimizu and Ishii (48) on the normal rat hypothalamus and by Gambetti, Gonatas, and Flexner (10) on the entorhinal cortex of puromycin-treated mice in which cytoplasmic bodies described as "nucleoluslike" have been reported. These bodies consist of small (~0.8 μ in diameter), rounded, compact masses of an amorphous granular or fibrillar material which is unbounded by a limiting membrane. But, as both groups of investigators have pointed out, such aggregates look less like an entire nucleolus than a modified nucleolonema. They reportedly resemble the granular component of the nucleolus in particular (10). The chemical nature of the inclusions was not investigated, and no evidence was found to demonstrate a nuclear or nucleolar origin.

This paper presents a detailed description of the structure and distribution of still another cytoplasmic component of the nerve cell, as seen in the sympathetic ganglion cells of the adult rat, which has certain properties in common with the nucleolus. Although the identification of this inclusion as a nucleolus or a nucleolar component cannot be made with certainty at this time, its appearance and staining reactions in the light microscope indicate that it corresponds to at least some of the structures described as cytoplasmic nucleoli in the literature of light microscopy. A possible clue to its functional significance is to be found, perhaps, in its structural resemblance to certain postsynaptic specializations characteristic of sympathetic neurons. For the sake of brevity and convenience, inclusions of this kind will be designated throughout this paper by the descriptive term "nematosome," which means simply "threadlike body."

MATERIALS AND METHODS

Preparation for Electron Microscopy

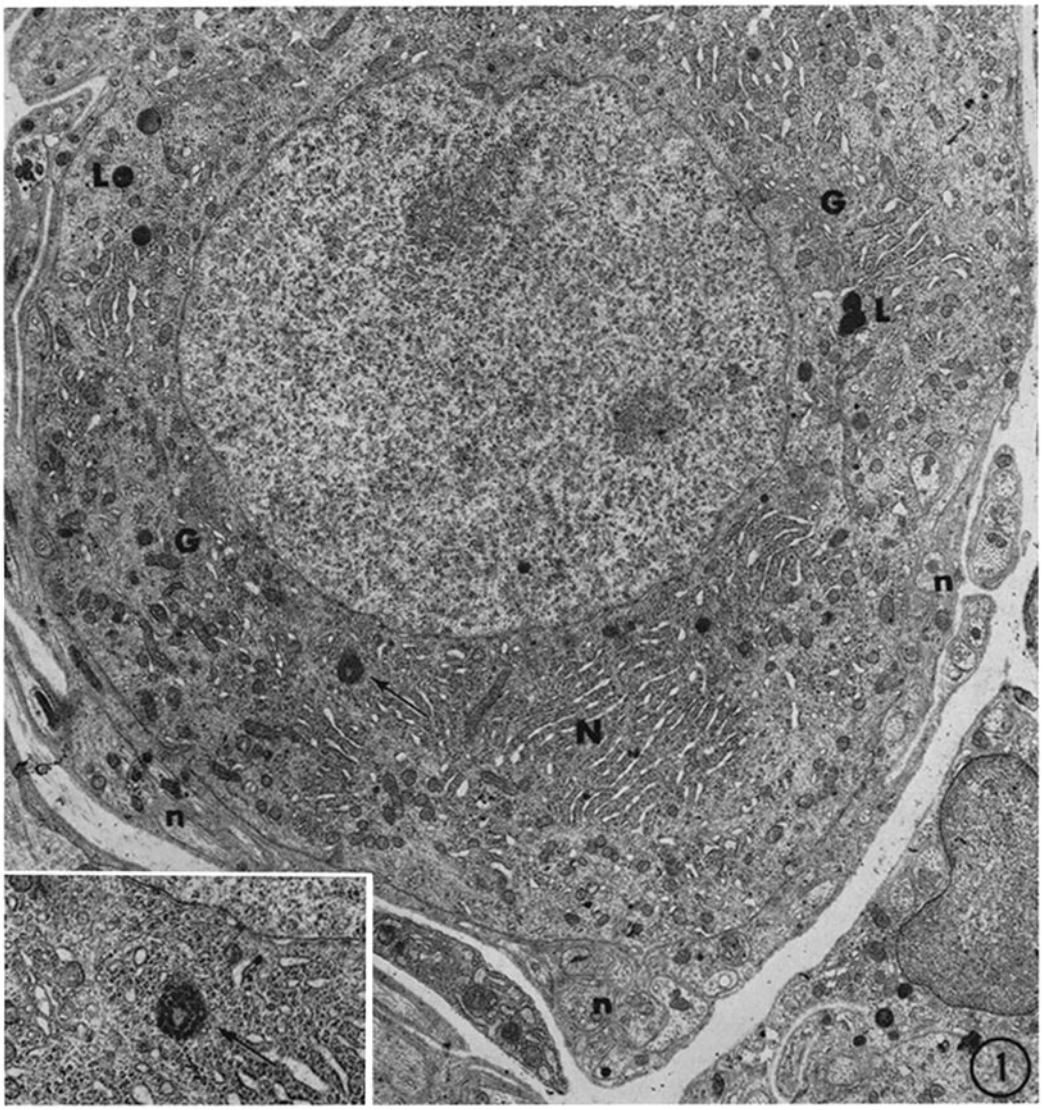
Specimens of ganglia and other tissues were obtained from 13 male and female rats of mixed strains, weighing 90–660 g and ranging in age from 7 wk to more than 18 months. Four rats were normal, nine experimental. In each experimental animal the preganglionic fibers innervating the right superior cervical ganglion were severed at some interval varying from several hr to several months before fixation. Since no significant differences were noted between the nematosomes of denervated ganglia and those of control ganglia taken from the contralateral side and from normal rats, the observations made on the various types of preparation have been pooled in the results. The sympathetic ganglia were obtained routinely from the cervical region; in some instances, the stellate and thoracic ganglia were also studied. In addition, a number of other tissues were explored for the presence of nematosomes; these were obtained from normal rats.

The tissues of nonfasted rats and rats fasted up to 72 hr were fixed by perfusion through the ascending or abdominal aorta, according to a method previously described (36). All but three animals were respired artificially. Of these three, two were rapidly chilled in an ice water bath to a rectal temperature of ~15°C min before fixation (D. E. Wolfe, personal communication); the third was maintained at normal body temperature, and the perfusion of its tissues was initiated within 20 sec after opening the chest. The perfusion of nine animals was carried out with solutions of 1–1.3% OsO_4 buffered to pH 7.2–7.4 with Veronal-acetate or a balanced salt solution (26, 44). The four remaining rats were perfused with 1.25–2% glutaraldehyde in Veronal-acetate or phosphate buffer at pH 7.2–7.4. Most of the glutaraldehyde-fixed specimens were postfixed by immersion in OsO_4 and processed routinely. A few were unexposed to OsO_4 and were treated instead with an ethanolic solution of phosphotungstic acid in the final step of the dehydration process (3, 4). All tissues were embedded in either Epon or Araldite and were sectioned with a glass or diamond knife on a Porter-Blum MT-2 microtome (Ivan Sorvall Inc., Norwalk, Conn.). Thin sections, mounted on naked or carbon-coated copper grids, were stained with uranyl acetate alone or doubly stained with uranyl acetate followed by lead citrate (58), and examined in a Philips EM 200 or 300. Some sections of tissue treated en bloc with ethanolic-phosphotungstic acid were examined with no further staining. Thicker sections (0.2–2 μ) were also cut from the same blocks, stained with toluidine blue, and examined in the light microscope.

Cytochemical Procedures

For study with the light microscope, the sympathetic ganglia of other rats were fixed *in situ* by intravascular perfusion with chilled 10% cacodylate-buffered formalin (pH 7.4) containing 7.5% sucrose or with 1.25% glutaraldehyde in Millonig's phosphate buffer (pH 7.4) at room temperature. The total fixation time was 2–3 hr. Following a 2–20 hr wash in multiple changes of buffer, the tissues were dehydrated in a graded series of methanol, embedded in methacrylate (38), sectioned serially at a thickness of 0.5–2 μ, and firmly affixed to glass slides by prolonged moderate heating. The plastic was removed with xylene and the preparations were stained by the fol-

lowing methods: (*a*) For proteins in general, fast green FCF at pH 2.0 (46) or mercuric bromphenol blue (37). (*b*) For basic proteins, fast green FCF at pH 8.1 (1) following hydrolysis in saturated aqueous picric acid at 60°C for 6 hr (2). (*c*) For RNA, azure B (8) or toluidine blue 0 at pH 4.0–4.3. To test the specificity of the basophilia, some slides were treated with 10% perchloric acid at 4°C for 5 or 10 hr;



Unless otherwise stated, all electron micrographs represent specimens fixed in OsO_4 and doubly stained with aqueous uranyl acetate and lead citrate.

FIGURE 1 Nematosome (arrow) in the perinuclear region of a sympathetic ganglion cell. The cytoplasm is crowded with organelles, including large masses of Nissl substance (*N*) and Golgi membranes (*G*). Because of its size and density, the nematosome can be easily mistaken for a pigment granule (*L*) at this magnification. Two clumps of chromatin are visible in an otherwise evenly dispersed nucleoplasm. The cell is completely enveloped by Schwann cell cytoplasm in which a multitude of small neurites (*n*) is embedded. *Inset*: An enlargement of the nematosome showing its pale core and dense periphery in more detail. Fig. 1, × 6200; *inset*, × 12,000.

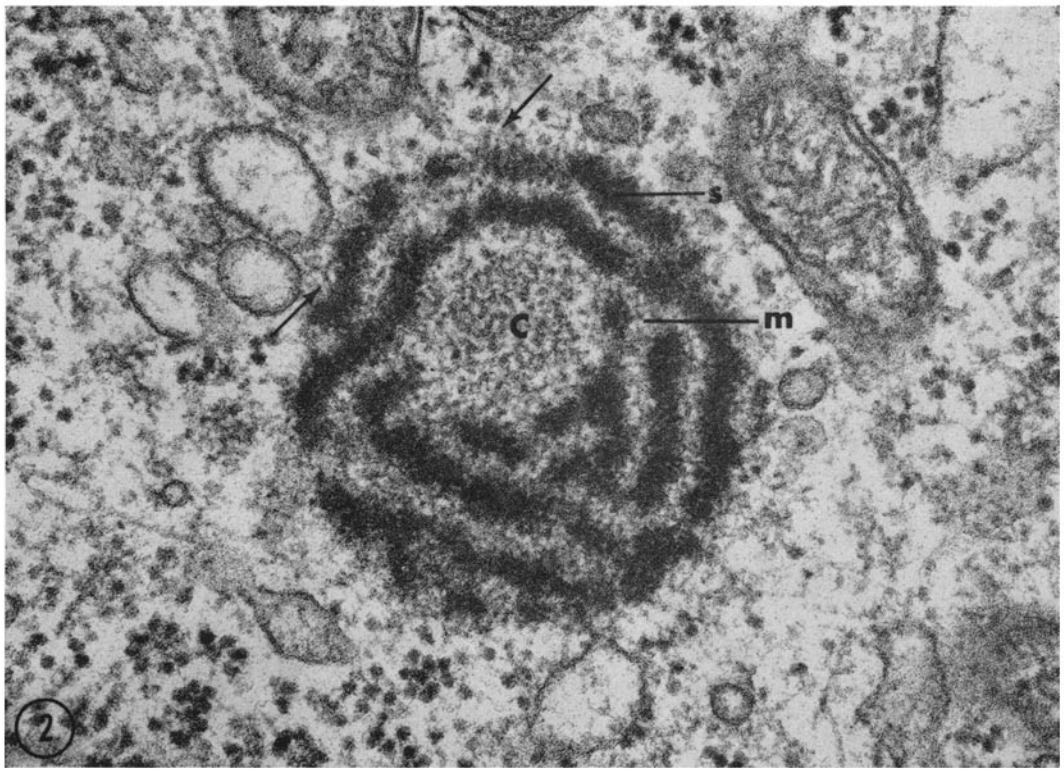


FIGURE 2 This figure depicts a typical nematosome with its component strands (*s*) and matrix (*m*). Two distinct zones can be recognized: an inner core (*c*) made of matrix material only, and an outer cortex containing, in addition, branched and unbranched strands of different lengths. Where parallel, the strands have a center-to-center spacing of ~900–1000 A. The matrix appears to be made of a meshwork of filaments and particles, whereas the strands show little definable substructure, except in a few areas (arrows) where filaments can be seen. × 92,000.

others were digested with RNase[1] (0.2 mg/ml in distilled water at pH 6.5) for 3, 8, or 20 hr at 45°C followed by 5% trichloroacetic acid at 4°C for 10 min. As controls for the RNase method, parallel slides were incubated in the absence of enzyme. (*d*) For DNA, the Feulgen reaction. Control slides were stained with the Schiff reagent without acid hydrolysis. (*e*) For polysaccharides, the periodic acid-Schiff (PAS) method. Some staining with Haidenhain's iron hematoxylin and with the Fontana-Masson silver technique was also carried out.

The staining procedure adopted was, in general, to first locate and photograph cells containing identifiable nematosomes in sections stained with fast green at acid pH. The sections were then destained by prolonged immersion in distilled water (pH 5–7) and 50% ethanol, restained by some other method, and rephotographed. By such a multiple staining procedure, the reactions attributable to the nematosome could be distinguished from those due to other components of the cell cytoplasm. For the determination of RNA, the sections previously stained with a basic dye were again destained, extracted with RNase or acid, and restained with azure B or toluidine blue. Simultaneous staining with more than one dye was avoided except in some of the determinations for RNA. The results were read by the combined use of photography and microscopy.

[1] Obtained from Worthington Biochemical, Freehold, New Jersey.

RESULTS

General Morphology

In survey micrographs of sympathetic neurons the nematosome is visible as a small electron-

opaque body embedded in the perikaryal cytoplasm (Fig. 1). Although the nematosome is seemingly identical to lipid droplets, pigment granules, myelin figures, and various other inclusions at low magnifications, it appears distinctly different from all of these on further enlargement. The nematosome has no limiting membrane. It is typically circular or oval in profile, but may be polygonal (Fig. 12). It measures on the average ~0.9 μ (range 0.3–1.4 μ). Each nematosome is a composite of two distinct parts: a conglomeration of coarse strands ~400–600 A thick and of indefinite length, and an intervening matrix of variable but lower density (Figs. 1–3, 5–14, and 16). The strands, in turn, are composed of a finely textured substance which appears to consist of an entanglement of tightly packed filaments and particles ~25–50 A thick (Fig. 3). Whether any of the particles represent granules rather than transverse sections of filaments could not be determined. The filaments are best defined in grazing sections and at the edges of the strands where they project at close but irregular intervals into the adjacent matrix. They are seemingly short, and only rarely can any of them be traced beyond a few hundred Ångstroms (Fig. 13). Images of filaments which vaguely suggest a helix can be seen (Fig. 3, arrow).

Although organized more loosely, the elements comprising the matrix are morphologically indistinguishable from those which form the strands (Figs. 2, 3, and 13). Indeed, it seems likely, though unproven, that they may simply represent unraveled extensions of the latter.

The density of the strands seems to vary somewhat with the type of fixative and the staining method used. In general, it is greater in tissue fixed with osmium tetroxide alone (Figs. 5–9) than in tissue fixed with glutaraldehyde followed by osmium tetroxide (Figs. 10–12). Maximal density was obtained with osmium tetroxide fixation in the presence of excess calcium (Figs. 2, 5, 8, 9, and 14); minimal density with 2% glutaraldehyde (Fig. 10). The strands are well stained by aqueous uranyl acetate (Fig. 8) but show no special affinity for lead. In specimens block-stained with ethanolic-phosphotungstic acid after fixation in glutaraldehyde alone, the strands appear consistently dense (Figs. 12 and 16), unlike certain other organelles which stain unreliably in the thickness of the block.

The threadlike component of the nematosome is represented in sections by a variety of profiles. In general, the number of profiles is inversely proportional to their length. Branching and bent forms are common (Figs. 2 and 14), but spirals, loops, and rings of various dimensions up to a measured length of 5.33 μ are also encountered (Figs. 5, 6, 9, and 10). The occurrence of periodic inflections along the length of some profiles suggests that the strands may be twisted in a ropelike or helical configuration (Fig. 3). Cross-sections of the strands are seen as circular or polygonal profiles (Figs. 8 and 11).

From the many anastomoses and branchings observed in single sections, it may be inferred that the strands form a three-dimensional network. Their organization within this network does not appear to be entirely at random, however, for as shown by Fig. 10, and to a lesser degree by other micrographs (Figs. 5 and 12), the strands may be disposed in parallel rows which show few interruptions or branchings in the plane of section and which are spaced a uniform distance apart from one another. Furthermore, examples are numerous in which the strands appear to be limited to the outer portion of the nematosome and surround more or less circumferentially an inner core made up of matrix material only (Figs. 1–3, 5, 10, and 13); this architectural arrangement is reminiscent of that found in certain nucleoli wherein the nucleolonema and the pars amorpha bear a similar relationship to each other.

Intracellular Location and Associations with Other Organelles

Nematosomes were found to occur in the perikarya but not in the processes of sympathetic neurons. They are located typically in the vicinity of the nucleus and the perinuclear Golgi apparatus (Figs. 1, 5, 8, and 9), but they can occur anywhere in the cell body. However close to the nucleus (Fig. 5) or surface (Figs. 13 and 16) of the cell they may be, they are always separated from the membranes of each by a width of cytoplasm measuring 0.1 μ or more. Nematosomes are sometimes seen at the base of a cell process, i.e. near the mouth of a funnel-shaped region of the cell from which the process emerges (Fig. 13). They have not been identified, however, in any of the more distal extensions of these processes, whether these extensions lie within or outside of the ganglia proper. (The extraganglionic tissues examined include the internal and external carotid nerves,

the cervical and thoracic sympathetic trunks, and the nerves innervating the heart and mesenteric arteries.)

Situated as they are in a cytoplasm crowded with organelles, nematosomes inevitably appear in close association with one or another of its components. None of these associations is constant, however, and at present there is little basis for judging which, if any, may be significant. In general, the associations found reflect the local composition of the cell. In the Golgi region, for example, the nematosomes often lie near numerous small vesicles of the smooth-surfaced and coated varieties, either of which may have a small dense inclusion (Figs. 5, 9, and 14); in some instances, filaments from the outermost strand appear to extend and "attach" to their surfaces. More peripherally, associations with free ribosomes predominate (Figs. 10 and 11), and in these instances attachments are made with this organelle. All told, virtually every type of cytoplasmic inclusion has, at one time or another, been seen to contact a nematosome; in none of these cases could the possibility of optical overlay be excluded.

That the association with the smooth-surfaced vesicles may be more than fortuitous is suggested by the fact that vesicles occur not only around the periphery of the nematosome but also in its interior where they are surrounded on all visible sides by profiles of strands (Fig. 7). A similar internalization of ribosomes and other organelles has not been observed. In other instances (Fig. 14), one may find, just outside of the nematosome proper, several small dense clumps of nematosome-like material which appear attached to the walls of the adjacent vesicles, suggesting (among other possibilities) that the vesicles may function in transporting bits of this material either to or from other regions of the cell.

Frequency and Distribution

Examples of nematosomes were encountered in all 13 of the animals studied. Their presence in the thoracic as well as cervical sympathetic ganglia and in normal as well as surgically denervated ganglia was established. Although no attempt was made to investigate systematically possible differences in the morphology, frequency, or distribution of these bodies in the various preparations used, no differences were noted on first inspection.

The number of nematosomes found in any one section through a cell was not more than one. Although the incidence of these bodies is low in thin sections, they occur with about the same frequency as the centriole/basal body complex of these cells. In the superior cervical ganglion, for example, the percentage of perikaryal profiles containing these bodies was found to be about 1.3%. This figure is somewhat less than the calculated value of 2.2% which would be expected if all of the ganglion cells were equipped with one of these organelles—assuming a section thickness of 600 A, a diameter of 40 μ for the cell body and 0.9 μ for the nematosome, and a random distribution of the nematosome within the perikaryon. It is reasonable to conclude from these data that a nematosome probably is present in a majority of the cells.

Attempts to demonstrate nematosomes in other neurons of the central (spinal cord and cerebral and cerebellar cortex) and peripheral (nodose, spinal, and sphenopalatine ganglia) nervous system were unsuccessful. However, granulo-filamentous inclusions of a type previously described by others in certain vertebrate neurons (5, 10, 23, 35, 43, 44, 48) and thought by some (10, 44, 48) to resemble nuclear or nucleolar material *were* found in some of these locations, namely in the granule cells of the cerebellum and in the dorsal root ganglion cells. With regard to the occurrence of nematosomes in other cell types within and outside of the nervous system, cells of ectodermal (Schwann and glial cells, perineurial cells, and chromaffin cells of the adrenal medulla), mesodermal (fibroblasts, macrophages, and mast cells, cardiac and smooth muscle fibers) and entodermal (intestinal lining cells) origins were examined; here, too, none was found. At least one centriole/basal body complex or one cilium was encountered in each of the cell types surveyed.

Other Structures Morphologically Related to Nematosomes

Structures which are not clearly identifiable as nematosomes but which nevertheless resemble them in certain respects were encountered occasionally within the processes of sympathetic neurons. The first of these structures, as illustrated in Fig. 18, consists of a collection of dense masses, each about 500 A in diameter and roughly spherical or rodlike in shape, spaced a variable distance apart. At the resolutions obtained, the fine structure of the dense masses appears similar to that

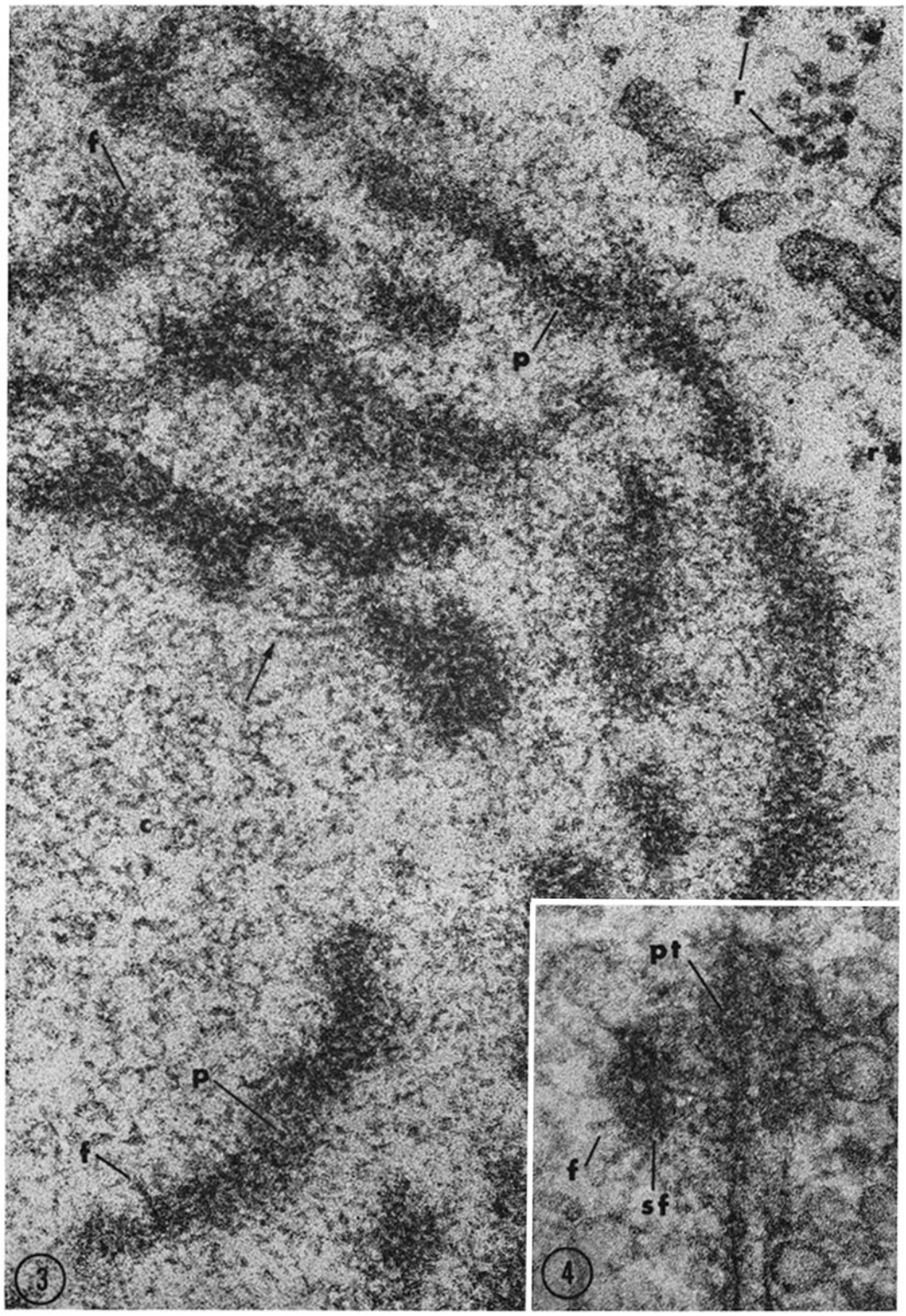


FIGURE 3 A high magnification micrograph of a portion of a nematosome having its core (*c*) at the left and its cytoplasmic surface at the right. The strands appear to be composed of an entanglement of tightly packed filaments (*f*) and particles (*p*) ~25–50 A thick. A loose array of similar elements occurs in the matrix. Note the corkscrew-like appearance of the filament (arrow) and the inflections in the strand above it; both configurations suggest a helix. Coated vesicle (*cv*); its filaments resemble those found in the nematosome. Ribosomes (*r*). × 192,000.

FIGURE 4 Portion of an axosomatic synapse, taken from a sympathetic ganglion, with the presynaptic process (right) and the postsynaptic component (left). Note the similarity between the subsynaptic formation (*sf*) and the strands of the nematosome (Fig. 3). Filaments (*f*); paramembranous postsynaptic density (*pt*). × 192,000.

of the threadlike component of the nematosome. As in the threads, numerous filaments, ~50 A in diameter, radiate out from the surfaces of the dense masses into the adjacent matrix. The aggregate as a whole is surrounded by a cytoplasm containing rosettes of ribosomes and swarms of agranular vesicles of various sizes and shapes. Somewhat similar aggregates have been described in the rat and cat spinal cord by Gray (12), who identified them as the dense projections of a presynaptic membrane seen in full-face view. That the structures here described are not of this kind is suggested by the fact that vesicles are virtually absent from the interspaces between the masses; the probability of this happening at a synapse is not very great, as can be seen from sections cut normal or slightly oblique to the synaptic membranes. Furthermore, in none of three consecutive sections through one of these structures was there any evidence for the presence of such vesicles or another cell process. It is tentatively suggested that the aggregates may represent a modified form of the nematosome.

Similarities in fine structure and staining properties also appear to exist between the strands of the nematosome and certain subsynaptic bodies and bands (Figs. 4, 15, and 17), which are a special though apparently not a unique (11, 25, 28, 32, 33) feature of a number of the synapses in sympathetic ganglia (52). In sections which are near normal to the synaptic membranes, the subsynaptic bodies appear as a row of one to eight profiles which underlies some portion of the postsynaptic thickening at a distance of 200–400 A; they are ~250–300 A wide (range, 125–600 A) and up to 3000 A in length. Their contour is irregular, and in favorable sections filaments, ~50 A in diameter, can be seen to project from their surfaces (Fig. 4). Their staining reactions appear similar to those of nematosomes, not only with the conventional methods used in electron microscopy, but also with the ethanolic-phosphotungstic acid stain recently devised by Bloom and Agahajanian (3, 4) (Fig. 17). Relatively frequently, agranular vesicles of various sizes are closely associated with their surfaces (Fig. 15).

Nematosomes also resemble structures in the nucleus, such as: (*a*) the dense fibrillar, as opposed to granular, component of the nucleolus which, too, is composed of 50 A-thick filaments arranged in a system of branching and anastomosing strands (Figs. 19 and 20), and (*b*) the accessory body of Cajal which consists of a spherical aggregate of globular and rodlike profiles randomly disposed in a background of paler material; their constituent filaments and particles are within the size and density range of those seen in nematosomes (Figs. 19 and 21).

Cytochemical Studies

The identification of nematosomes with the light microscope proved difficult because their size and number are such as to make their study from adjacent thick and thin sections unfeasible and, in my hands, impossible. Nevertheless, by use of the characteristics revealed by electron microscopy as criteria for their identification with the light microscope, it was possible to demonstrate a population of cytoplasmic inclusions which correspond in size, shape, and location to nematosomes, and whose frequency of occurrence and distribution within the various cell types found within the superior cervical ganglion are compatible with this interpretation. This demonstration was achieved through the use of 0.5–2 μ thick sections of methacrylate-embedded formaldehyde- or glutaraldehyde-fixed tissues stained with either fast green at pH 2 or mercuric bromphenol blue. With these stains, the inclusions appear as small spherical or ovoid masses of intensely stained material immersed in a faintly colored cytoplasm (Fig. 22 *A*). Other fixatives and embedding media were less satisfactory, and increasing the section thickness obscured the differential staining. With respect to the application of all of the other stains used, presumed nematosomes were first identified in sections stained with fast green; this was followed by an appropriate sequence of destaining and restaining by some other method (s).

The results obtained on nematosomes with the cytochemical tests and other staining procedures are summarized in Table I and are compared with those obtained on nucleoli in the same tissue sections. The intensely positive reactions obtained with fast green at acid pH and mercuric-bromphenol blue indicate a high concentration of protein. Histones could not be demonstrated by the fast green method at pH 8.1. Positive PAS and Feulgen reactions were both faint and infrequent.[2]

[2] The PAS and Feulgen reactions could not be determined in the glutaraldehyde-fixed specimens because of a nonspecific background coloration obtained with the Schiff reagent even in the absence of acid hydrolysis.

With azure B and toluidine blue 0, the structures display a faint to moderate basophilia (purple color) which is sensitive to RNase digestion[3] and acid hydrolysis (Figs. 22 *B–D*); within the limitations of the methods employed, it thus appears that some RNA is present. Ammoniacal silver stains the structures a golden brown, iron haematoxylin a brown-black.

In serial 1 μ sections stained with fast green at pH 2, the cells usually exhibit only one such body, although up to four were occasionally observed. Indications are that most ganglion cells, if not all, contain at least one of these inclusions.

DISCUSSION

The findings indicate that the nematosome is a definable organelle, morphologically distinct from other components of the cell cytoplasm (except, perhaps, for the glomerular body, as noted later). The absence of a limiting membrane distinguishes it from lysosomes, autophagic vacuoles, fibrillary inclusions (50), and other membrane-limited bodies. Even when compared with the non membrane-bounded inclusions of cells, the nematosome is found to be quite distinct. For example, the multilaminated bodies (9, 17, 29, 30, 40, 49), fibrous banded structures (54), and filamentous corpuscles (7, 31) differ from the nematosome in almost every detail, ranging from the dimensions of their fibrous elements to their gross morphology. Also different are the granulofilamentous inclusions described by some investigators as nuclear- or nucleolar-like in appearance and found in a variety of vertebrate neurons (10, 23, 35, 43, 44, 48, and L. Kruger and E. B. Masurovsky, personal communications), either *in situ* or in tissue culture (5); like the other inclusions already mentioned, these too lack the ropelike component characteristic of the nematosome, and it is not

[3] RNase did not work on tissues fixed in glutaraldehyde.

FIGURES 5–12 Associations, stainability, and configuration of the nematosome in various preparations. Figs. 5–9. Specimens fixed in OsO_4. Figs. 10–12. Specimens fixed in glutaraldehyde.

FIGURE 5 Core-containing nematosome in a paranuclear position. Arrows indicate nuclear pores. × 25,000.

FIGURE 6 Cluster of mitochondria near a nematosome. Note the Fig 8 configuration of the strand at *s*. × 40,800.

FIGURE 7 Two smooth-surfaced vesicles (*v*) among the strands of a nematosome. × 63,000.

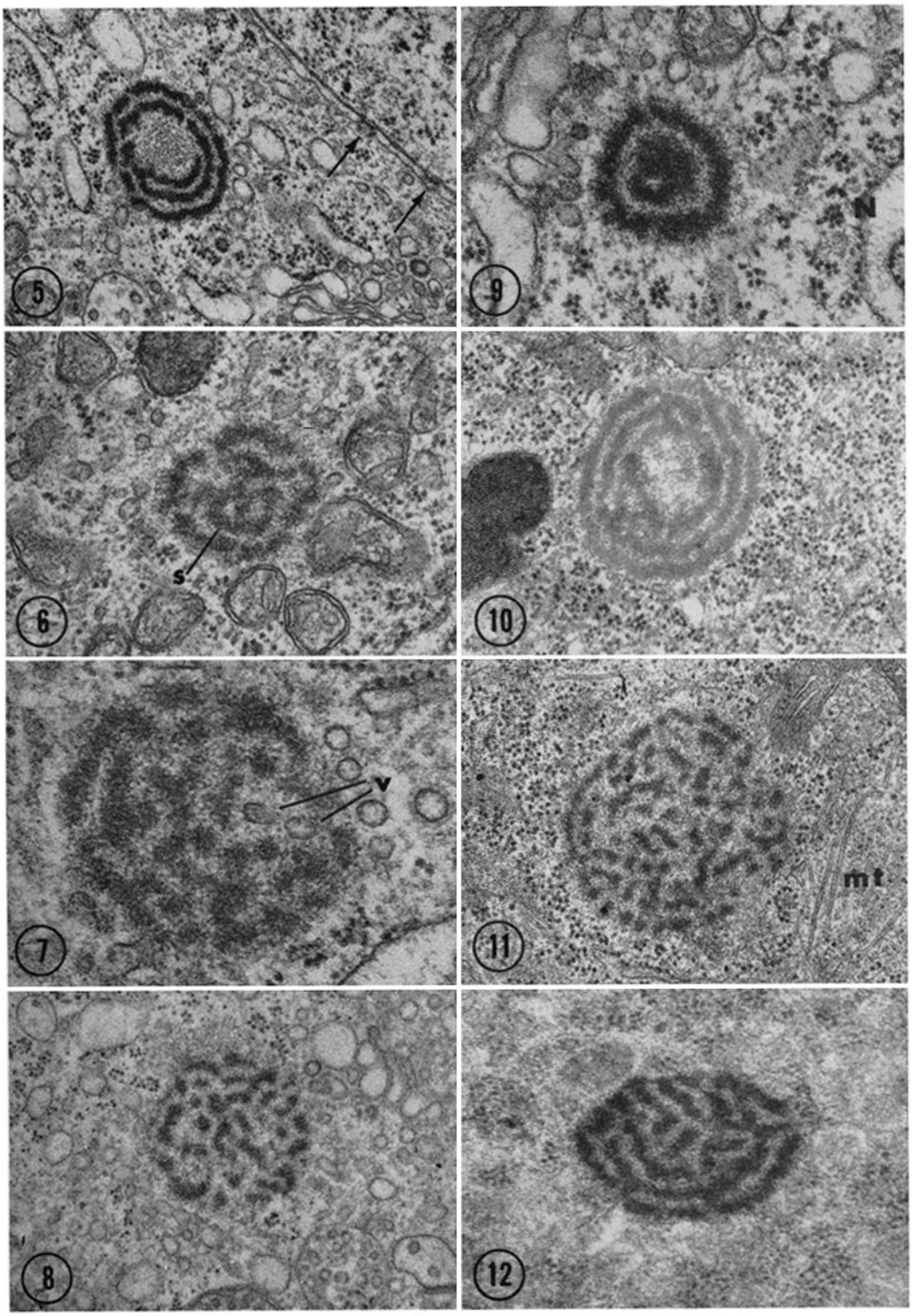
FIGURE 8 Illustration of the dense appearance of the nematosome after staining with uranyl acetate alone. × 25,000.

FIGURE 9 Nematosome interposed between a stack of perinuclear Golgi membranes on the left and a Nissl body (*N*) on the right. Occupying the center of this small diameter profile, presumably taken from the edge of a nematosome, is a knot of electron-opaque material; the latter, in turn, is surrounded by a single strand in the form of a ring. × 50,000.

FIGURE 10 The strands of this nematosome are oriented parallel to the plane of section and show few interruptions. Particularly well demonstrated is the fact that the nematosome contains no identifiable ribosomes, which in this case are noticeably denser than the strands. Fixed in 2% glutaraldehyde, postfixed in OsO_4. × 44,200.

FIGURE 11 Here the strands are mostly short and give the nematosome a granular rather than a threadlike appearance. Microtubules (*mt*). Fixed in 1.25% glutaraldehyde, postfixed in OsO_4. × 35,000.

FIGURE 12 Specimen fixed in glutaraldehyde and block-stained with ethanolic-phosphotungstic acid, with no exposure to osmium tetroxide or other stains. The nematosome is well stained by this method. × 36,500.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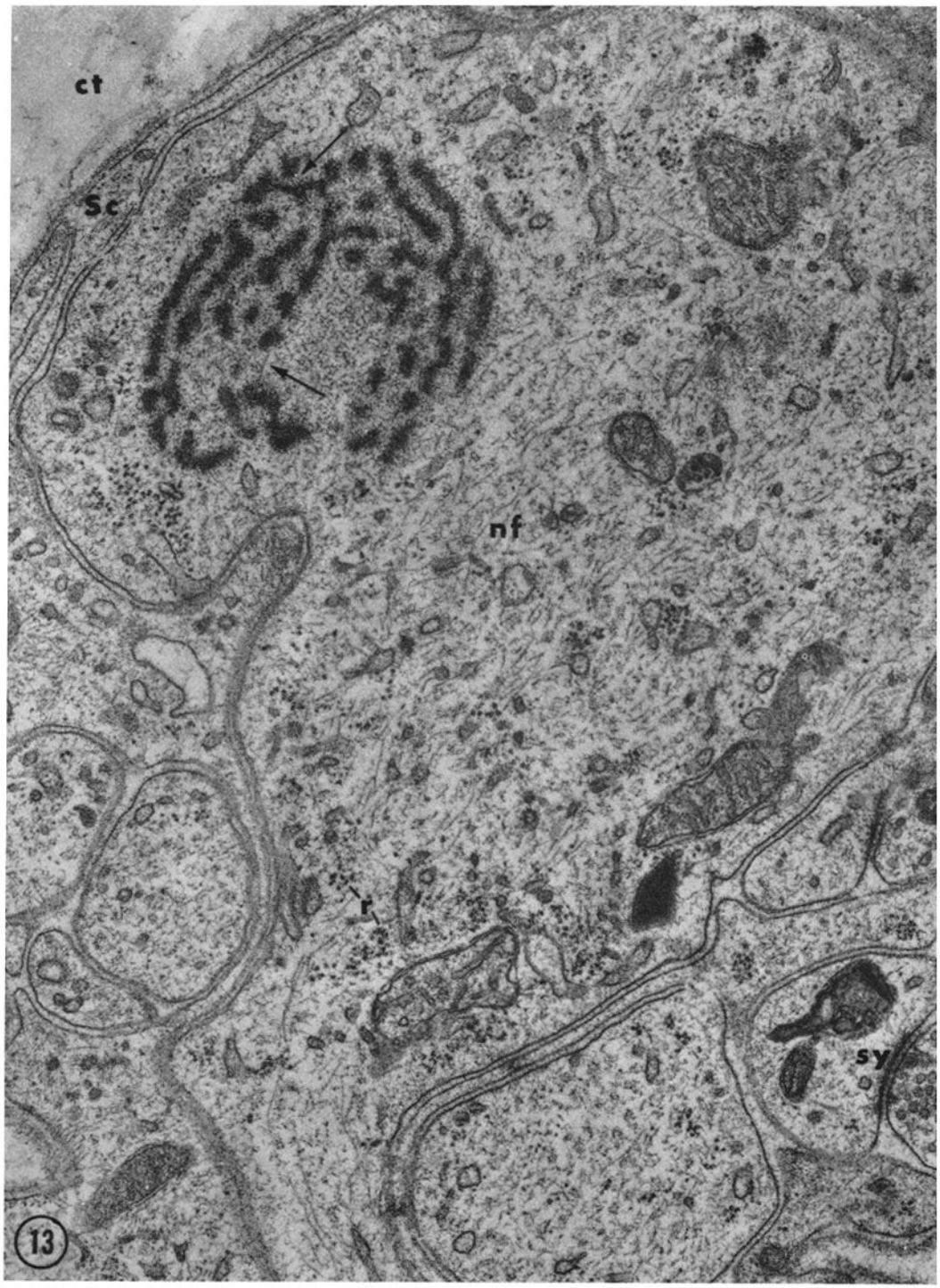


FIGURE 13 Core-containing nematosome located in a bulge near the origin of a cell process. Numerous filaments can be seen radiating out from the surfaces of the strands; the arrows indicate some filaments which appear to extend across an interspace, bridging two strands together. Microtubules show poorly in this type of specimen which has been fixed with OsO_4 in McEwen's balanced salt solution. Connective tissue (*ct*), Schwann cell cytoplasm (*Sc*), ribosomes (*r*), neurofilaments (*nf*), axodendritic synapse (*sy*). × 47,600.

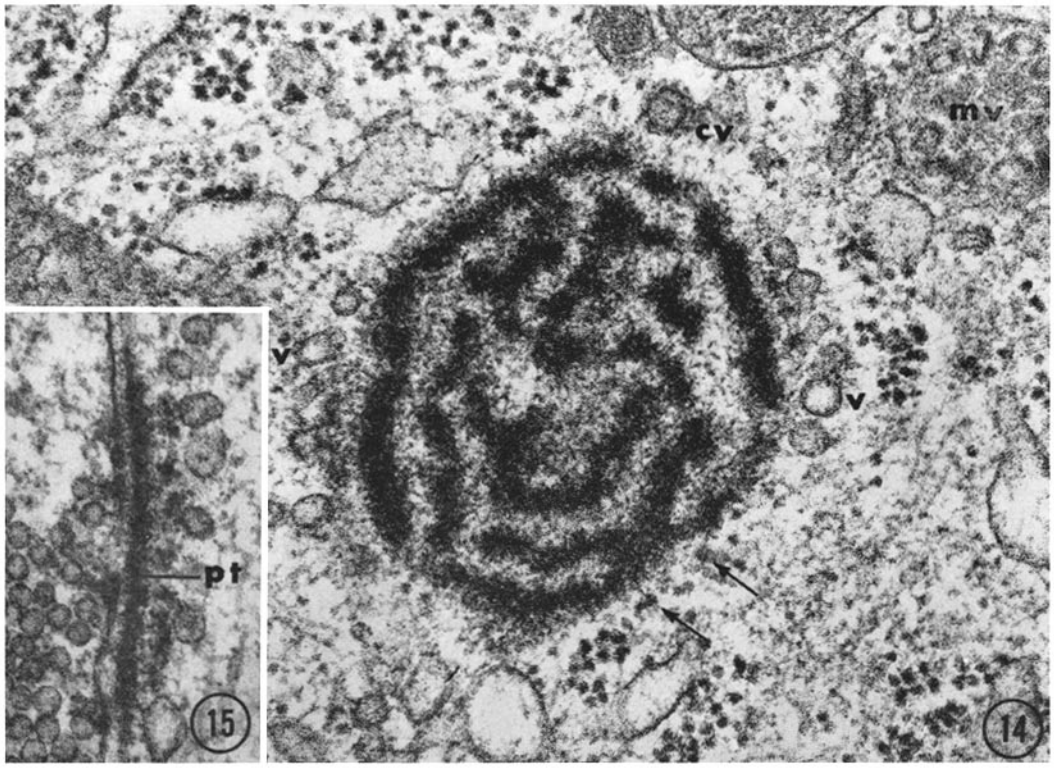


FIGURE 14 Situated between the main body of the nematosome and clusters of vesicles (*v*) are several small clumps of dense material similar to that found in the strands. Filamentous connections between these various structures can be seen. Arrows indicate ribosomes which appear to be attached to nematosome filaments, probably due to overlap. Multivesicular body (*mv*); coated vesicle (*cv*). × 75,000.

FIGURE 15 Portion of a ganglionic synapse with the presynaptic process on the left and the postsynaptic component on the right. Aligned in a row lying parallel to the postsynaptic thickening (*pt*) are several clumps of dense material comprising a subsynaptic formation. Note the similarity between the nematosome-associated clumps in Fig. 14 and the subsynaptic formation. × 75,000.

clear, from the published reports, that their subunits are the same.

In 1965 Takahashi and Hama (51) noted the presence of a peculiar structure composed of a pile of fine filaments (their term), 150–200 A thick, in the nerve cell bodies of the ciliary ganglion cells of the chick; however, their brief description and published micrographs are insufficient for ascertaining the possible identification of this peculiar structure with nematosomes.

On the other hand, there is little doubt that the nematosomes bear a striking resemblance to the inclusions called "glomerular bodies" by Toro and Rohlich (57) in the syncytial trophoblast of the mouse and rat placenta. Apart from being substantially more numerous, the placental inclusions are somewhat larger than nematosomes, and they usually have a "granular" rather than ropelike appearance. The matrix of the nematosomes is delicately structured from filaments and particles, and it often forms a prominent core near the center of the inclusion; the matrix of the glomerular bodies is reported to be optically empty and uniformly distributed. To what extent these differences can be explained by factors such as sampling, fixation, states of functional and metabolic activity, etc., is at the moment unclear. Certainly, the similarities between the two structures are impressive, and there is even the intriguing possibility that the "isolated threads" observed in the placental cells have a counterpart in certain configurations encountered in neurons.

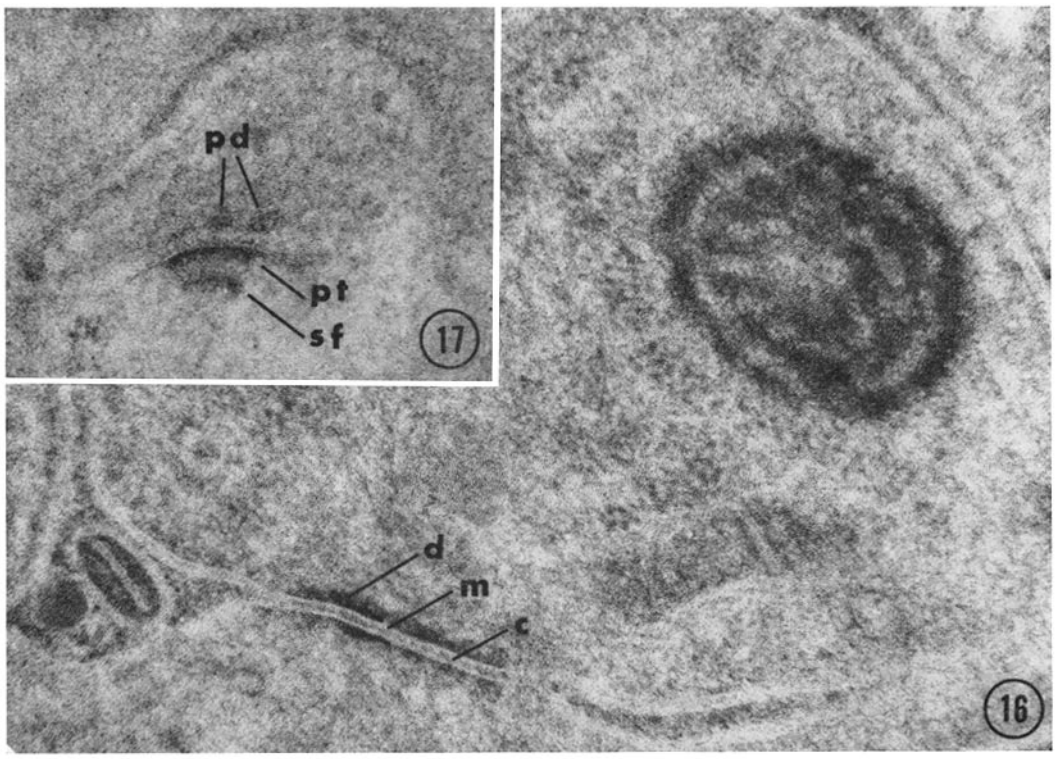


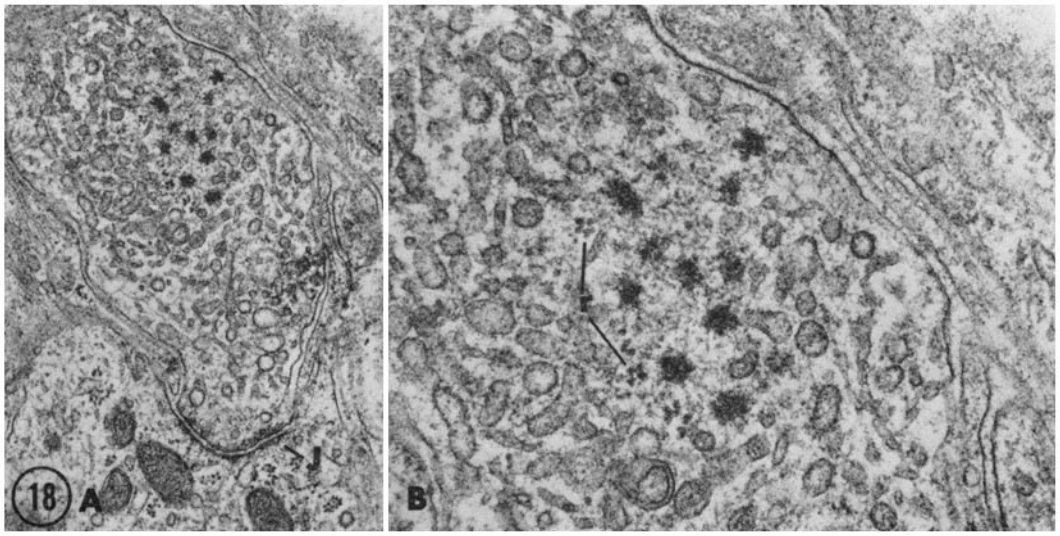
FIGURE 16 Demonstration of the dense appearance and filamentous structure of a nematosome fixed in glutaraldehyde alone and block-stained with ethanolic-phosphotungstic acid. Reliably and equally well stained by this method are the membrane thickenings of specialized junctions such as the one shown at the lower left. Paramembranous density (*d*); stainable cleft material (*c*); junctional membrane, seen as a white line (*m*). × 77,000.

FIGURE 17 Specimen fixed and stained as in Fig. 16. Ganglionic synapse with the presynaptic process above and the postsynaptic component below. Note that the presynaptic densities (*pd*), postsynaptic thickening (*pt*), and subsynaptic formation (*sf*) are all stained and also are comparable in density to the strands of the nematosome shown in Fig. 16. × 77,000.

Recently, an unequivocal example of a nematosome has been seen in a neuron of the cerebral hemisphere of the adult toad (J. Rosenbluth, personal communication).

Possible Relationship to Nuclear Components

The fine structural appearance of the nematosome immediately suggests that the nematosome may be related to the nucleolus and other components of the cell nucleus. It resembles nucleoli in shape, general configuration, and staining affinities, but differs from them in size and complexity. Nematosomes have no ribosomes; their fine structure is similar to that of only the fibrous component of the nucleolus. In this respect they are more akin to the prenucleolar bodies or cleavage nucleoli found in early amphibian embryogenesis (16, 22) and to the fibrous pseudonucleoli of the anucleolate mutant of *Xenopus* (16, 21). This interpretation is consistent with the observation that the nematosomes display a weak to moderate basophilia, but stain intensely for proteins (16, 20, 24). In size and structure they also bear a resemblance to the accessory bodies of Cajal. These accessory bodies, however, are granular rather than threadlike in form, and they have a less structured matrix. They are believed by some investigators to be of nucleolar origin (13, 14, 56), and they reportedly contain large



FIGURES 18 *A* and *B* Nerve cell process containing an unidentified aggregate of dense masses which may represent a special form of the nematosome. The masses have a mottled appearance, an irregular outline, and radiating filaments which occupy much of the space between them. Note that the individual masses and the aggregate they form are not surrounded by membranes. Unlike the matrix between the masses, the adjacent cytoplasm contains clusters of ribosomes (*r*) and numerous agranular vesicles of various sizes and shapes. At *J* the process forms a broad desmosome-like junction with the perikaryon of a nerve cell. Fig. *A*, × 21,000; Fig. *B*, × 49,500.

amounts of protein but no nucleic acid (14, 34). All three of these structures—nematosomes, nucleoli, and accessory bodies—are argyrophilic and they react similarly to iron hematoxylin and bromphenol blue. This confirms the observations of light microscopists (42, 55) who, using similar stains, found that neurons contain within their cytoplasm a structure which has the appearance of being a nucleolus. Whether or not the nematosomes are in fact nucleoli or derivatives of nucleoli in origin and function has yet to be determined.

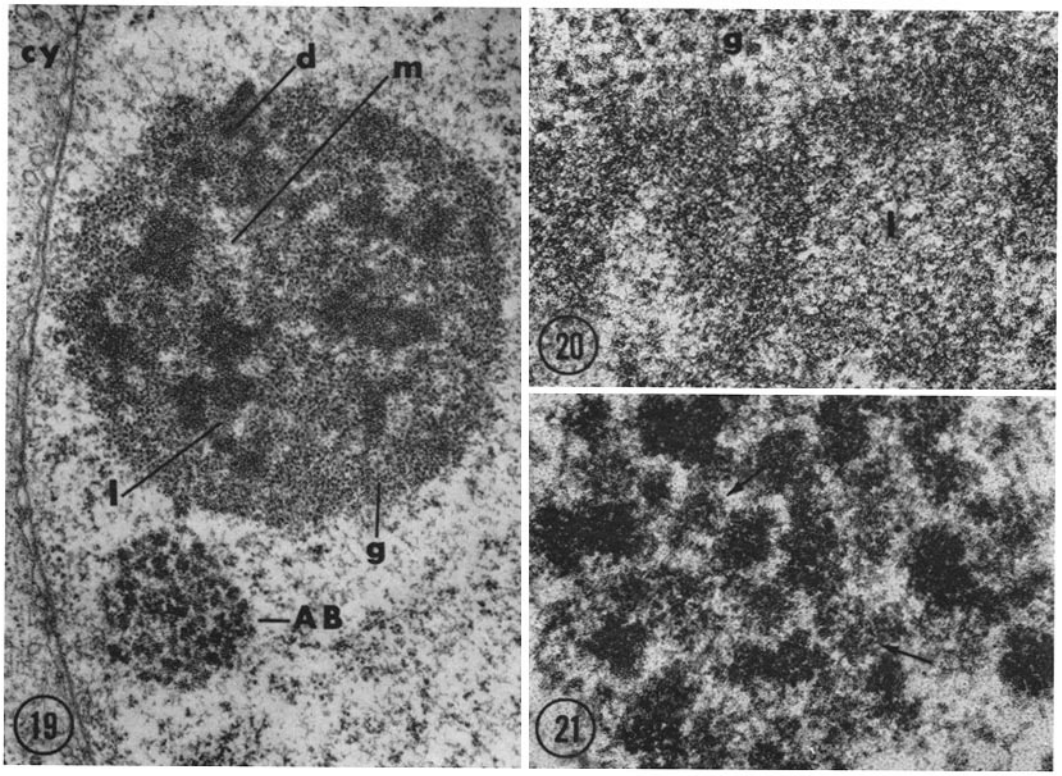
Possible Relationship to Cytoplasmic Components

In the results, note was made of morphological and staining similarities between the nematosome and the subsynaptic formations. It should be emphasized that both structures are a component of sympathetic neurons; and, indeed, it would appear, from the data, that both of them must occur in the same cells. The nature of the material comprising the subsynaptic formations is unknown. Milhaud and Pappas (28) have related the occurrence of similar structures in the habenula and interpeduncular nuclei to the high monoaminoxidase content of these areas. It may be inferred, from their stainability with ethanolic-phosphotungstic acid as demonstrated in this study, that they contain some protein (3, 4). As would be expected if nucleic acids were present

TABLE I

Staining Characteristics of Nematosomes and Nucleoli

Stain	Nematosome	Nucleolus
Mercuric bromphenol blue	+	+
Fast green pH 2.0	+	+
pH 8.1	−	−
Toluidine blue 0, pH 4.3	+	+
Azure B, pH 4.0	+	+
PAS	±	+
Feulgen	±	±
Iron hematoxylin (Haidenhain)	+	+
Silver method of Fontana-Masson	+	+



FIGURES 19–21 Nuclear inclusions which resemble the nematosome.

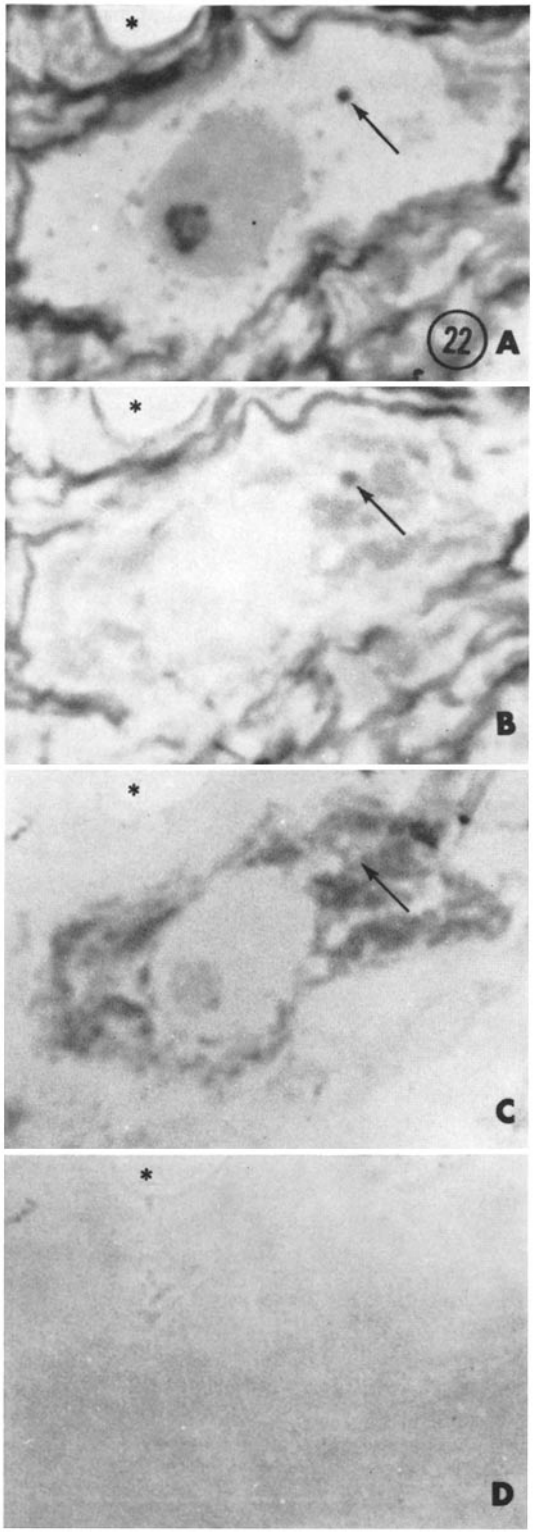
FIGURE 19 Nerve cell nucleolus and accessory body of Cajal (*AB*). In the nucleolus four distinct zones can be recognized: a granular component (*g*), a dense fibrous component (*d*), a less dense fibrous component (*l*), and a very pale matrix-like substance (*m*). The accessory body, on the other hand, is composed of dense and less dense globular masses embedded in a pale background. Cytoplasm (*cy*). × 21,300.

FIGURE 20 Dense fibrous component of a nucleolus at high magnification, with adjacent light fibrous (chromatin⁻) (*l*) and granular (*g*) components. Note the similarity in shape, texture, and substructure between the dense fibrous component of the nucleolus and the strands of the nematosome shown in Fig. 3. × 100,000.

FIGURE 21 Accessory body at higher magnification. The less dense masses are grazing sections through the denser ones. Although rodlike or globular in form rather than elongated, they are similar in both density and texture to the strands of the nematosome illustrated in Fig. 2. Filaments and particles ~50 A thick can be seen in some areas (arrows). × 100,000.

(19), they strongly bind uranyl acetate. This evidence is suggestive rather than conclusive (4, 19, 39), however, and further studies are needed to elucidate their chemistry. On the basis of the report by Taxi (52) that the subsynaptic formations in amphibian sympathetic neurons rapidly fragment and disappear following degeneration of the presynaptic processes, it was considered of interest to determine whether this phenomenon in the rat could be correlated with some visible change in the nematosomes. The unexpected finding that the subsynaptic formations in mammals persist apparently indefinitely without change precluded the study (unpublished observations). No differences were detected between the nematosomes of denervated ganglia and those of the controls.

To what extent these morphological similarities between the nucleolus and the nematosome and between the nematosome and the synaptic special-



izations imply a chemical similarity, a common origin, and a functional interrelationship is not at all clear at the moment. The observations described are consistent with the possibility that the nematosome may be an intermediary in the transfer of nuclear-derived material to the synapse where presumably it serves some special function, related perhaps to specificity or to the control of synaptic transmission or intercellular adhesion by some such mechanism as providing receptors, enzymes, or other substances which affect the operation of the synapse. On the other hand, in a survey of 95 nerve cells taken from frog sympathetic ganglia, no nematosomes were found;[4] yet, as is well known from the work of Taxi (52, 53), subsynaptic specializations are present in this type of cell. These findings emphasize the need for additional data concerning the life history and distribution of nematosomes. Information on their occurrence in other cell types and in other neurons whose synapses do and do not have a subsynaptic formation, and a knowledge of their response to specific changes in protein and nucleic acid metabolism, would help to clarify their significance.

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[4] In a similar number of rat ganglion cells one would expect to find one or two nematosomes.

FIGURE 22 Photomicrographs of a sympathetic neuron fixed in glutaraldehyde, embedded in methacrylate, and sectioned at ~1 μ. *A–D*, Identical specimen stained sequentially with fast green and azure B. The nematosome in each figure (except *D*) is indicated by an arrow, the capillary lumen by an asterisk *A*. Stained with fast green FCF at pH 2.0. The nematosome appears a deep green. *B*. Partially destained followed by staining with azure B at pH 4.0. The exact position of the nematosome in relation to the masses of Nissl substance can be seen. The nematosome is stained green, the Nissl substance deep blue to purple. Photographed with a red filter. *C*. Completely destained and restained with azure B alone at pH 4.0 Both the nematosome and the nucleolus stain metachromatically. *D*. Again destained, treated with 10% perchloric acid at 4°C to remove RNA, and restained with azure B at pH 4.0. No color was visible to the eye. The nematosome could not be identified except under phase optics. × 2200.

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