ORIGIN, DEVELOPMENT, AND NATURE OF INTRANUCLEAR RODLETS AND ASSOCIATED BODIES IN CHICKEN SYMPATHETIC NEURONS

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ABSTRACT

Correlative data are presented here on the developmental history, dynamics, histochemistry, and fine structure of intranuclear rodlets in chicken sympathetic neurons from in vivo material and long-term organized tissue cultures. The rodlets consist of bundles of $\sim 70 \pm 10$ A proteinaceous filaments closely associated with ~ 0.4–$0.8\ \mu$ spheroidal, granulofibrillar (gf) bodies of a related nature. These bodies are already present in the developing embryo a week or more in advance of the rodlets. In early formative stages rodlets consist of small clusters of aligned filaments contiguous with the gf-bodies. As neuronal differentiation progresses these filaments increase in number and become organized into well-ordered polyhedral arrays. Time-lapse cinemicrography reveals transient changes in rodlet contour associated with intrinsic factors, changes in form and position of the nucleolus with respect to the rodlet, and activity of the gf-bodies. With the electron microscope filaments may be seen extending between the nucleolus, gf-bodies, and rodlets; nucleoli display circumscribed regions with fine structural features and staining reactions reminiscent of those of gf-bodies. We suggest that the latter may be derivatives of the nucleolus and that the two may act together in the assemblage and functional dynamics of the rodlet. The egress of rodlet filaments into the cytoplasm raises the possibility that these might represent a source of the cell's filamentous constituents.

INTRODUCTION

Intranuclear rodlets have been observed by light microscopy in a variety of cells constituting the nervous system of diverse species since the mid-1890's (1–3). In 1899 Holmgren (4) described rodlets in certain avian (and other vertebrate) neurons, placing special emphasis on those in sympathetic neurons of the chicken. Ramon y Cajal (5, 6) demonstrated such structures in various neurons of the mammalian central nervous system with his elegant silver staining techniques and referred to them as "the rodlets of Roncoroni" after the Italian investigator who reported them in neurons of several mammalian species, including the human (2). It is by this appellation that they are sometimes designated today. Fig. 1 illustrates some of these early light microscopic observations.

Siegesmund et al. (7) provided the first definitive ultrastructural description of intranuclear rodlets in a correlative light and electron microscopic study of some of the same mammalian neurons originally reported by Cajal (6) to contain them. They noted that the rodlets consisted of oriented

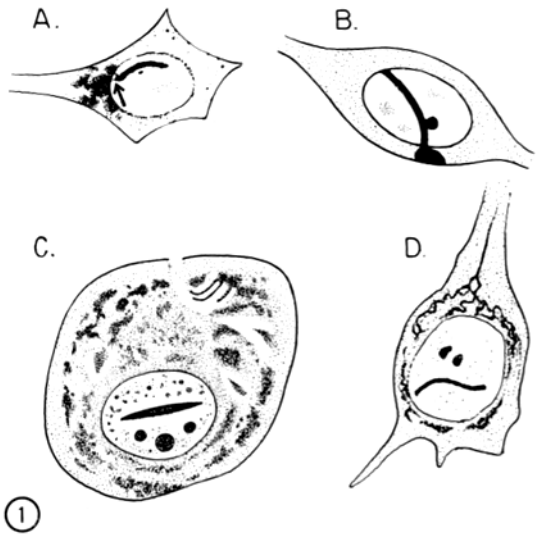


FIGURE 1 Illustration of intranuclear "rodlets" in neurons of diverse species. (*A*) Redrawn from Mann (1). Rodlet in the nucleus of an occipital lobe neuron of the rabbit arcing over to the vicinity of the nuclear membrane where fine strands (arrow) appear to extend from it to the nuclear membrane. Similarly stained material may be seen in the adjacent perinuclear cytoplasm (Mann's toluidine blue stain). (*B*) Redrawn from Roncoroni (2). Prominent, broadly arcing presumed rodlet in a neuron of the mammalian cerebral cortex. Notice the similarly stained area in the immediate juxtanuclear cytoplasm. The nucleolus appears to be directly in contact with the rodlet (Roncoroni's methylene blue-borate stain). (*C*) Redrawn from Holmgren (4). Tangentially sectioned intranuclear rodlet appears spindle-shaped in a chicken sympathetic ganglion neuron; the nucleolus and two smaller spheroidal bodies are situated nearby (Heidenhain's iron hematoxylin stain). (*D*) Redrawn from Cajal (5). Sinuous intranuclear rodlet in a rabbit pyramidal neuron. Note the similarly stained network of material in the cytoplasm of this cell (Cajal's reduced silver stain).

fibrils or filaments ~50–70 A in diameter, which appeared to be distinctly different from the chromatin in the surrounding nucleoplasm. Very similar filamentous formations have subsequently been described in electron microscopic studies of a variety of cells comprising the vertebrate and invertebrate nervous systems (8–21). Neither the origin, composition, nor significance of the formations was ascertained in the foregoing investigations.

Our correlative light and electron microscopic studies of these intranuclear rodlets and associated bodies have been carried out principally with highly organized, developing cultures of chicken sympathetic ganglia. These culture preparations enable us to observe directly, and photograph sequentially, such vital phenomena in the *living* neurons as the movement and interrelationships of the rodlets with other nuclear structures. Sympathetic ganglia also were excised from embryonic and adult birds, fixed and handled in an analogous manner with the culture preparations, and assiduously compared as to the details of the structural and histochemical properties of the rodlets and associated bodies. The present study reports the findings of investigations embodying this coordinated in vivo and in vitro analytical approach designed to elucidate the origin, development, chemical nature, and possible functional significance of these enigmatic structures.

MATERIALS AND METHODS

Sympathetic chain ganglia from chick embryos ~4–17 days *in ovo* were explanted onto collagen-coated cover slips (22) and were carried as organized, developing cultures in Maximow double cover slip assemblies for periods exceeding 160 days. Details of culture preparation, maintenance, and organotypic development in vitro appear elsewhere (15). These cultures were observed at regular, almost daily intervals in the *living* state by bright field light microscopy and were sequentially photographed at appropriate stages with Kodak High Contrast Copy or Constrast Process Ortho film. Time-lapse cinemicrographic studies of the *living* neurons in culture were conducted with a similar system of bright field optics utilizing a Zeiss inverted microscope, a Sage Series 100 Cinephotomicrographic apparatus (Sage Instruments Inc., White Plains, N. Y.), and Kodak Shellburst Linograph SP 430 film.

Light microscopic (LM) histochemical determinations were carried out on specimens fixed with 10% neutral formalin, Carnoy's solution (23), or 3% redistilled (24) Fisher Biological Grade glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4); electron microscopic (EM) histochemistry was done on material fixed with the latter aldehyde solution. For LM histochemistry, standard staining procedures for proteins and nucleic acids, together with appropriate nuclease, protease, and/or acid hydrolysis controls (23, 25), were employed on whole mount culture preparations as well as 7-μ paraffin sections of sympathetic ganglia from in vivo and in vitro sources; the various stains used and their reactions are indicated under Observations. EM histochemistry was carried out on ~50-μ slices cut from glutaraldehyde-fixed

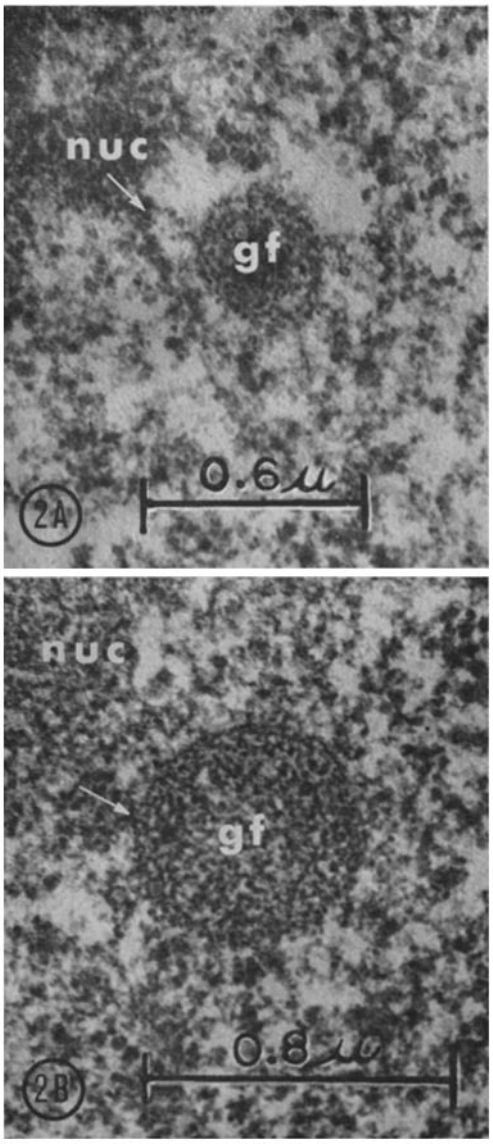


FIGURE 2 Electron micrographs of granulofibrillar bodies (*gf*) in embryonic chick sympathetic ganglion neurons. (Glutaraldehyde-OsO_4 fixation). Fig. 2 *A* ~0.4 μ *gf*-body in a 6-days *in ovo* neuronal nucleus. Fibrous elements (arrow) appear to extend between the coiled cortical region of this body and the outer margin of the nucleolus (*nuc*). × 48,000. Fig. 2 *B* ~ 0.7 μ *gf*-body in a 13-days *in ovo* neuronal nucleus. This more voluminous body, composed chiefly of intermeshed and concentrically arranged fibrillar elements, appears more typical of later developmental material than Fig. 2 *A*. Note that this body is contiguous (if not continuous) with the nucleolus (*nuc*) at the arrow. × 50,000.

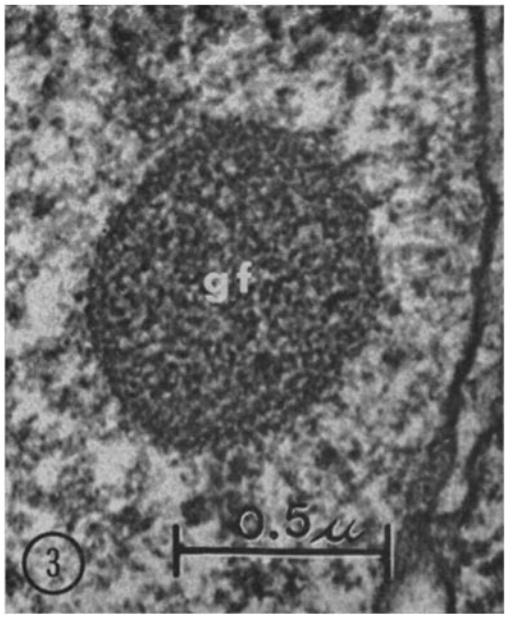


FIGURE 3 Electron micrograph of a ~0.9 μ granulofibrillar body (*gf*) in the nucleus of a highly differentiated sympathetic neuron 90 days in vitro. Note the remarkably intercalated, labyrinthian arrangement of elements comprising this body, and its close proximity to the nuclear envelope. (Glutaraldehyde-OsO_4 fixation). × 58,000.

tissues subjected to digestion with DNase (Sigma Deoxyribonuclease I, ribonuclease-free, DN-EP [Sigma Chemical Co., St. Louis, Mo.]), RNase (Sigma Ribonuclease A, protease-free, Type I-A), or trypsin (Difco Trypsin 1:250 [Difco Laboratories, Inc., Detroit, Mich.]) solutions for appropriate durations (23, 25), then postfixed with 2% OsO_4, embedded in Epon 812, sectioned, and stained as below for routine ultrastructural study.

In addition, LM and EM radioautography was conducted on cultures >50 days in vitro containing developing rodlets that were incubated for 24 and 72 hr with thymidine-methyl-^3H (specific activity 19.2 Ci/mmole, purity assayed over 98%) or uridine-5-^3H (specific activity 29.2 Ci/mmole, purity assayed over 99%), obtained from the New England Nuclear Corp., Boston, Mass., at final concentrations in the culture medium of 10 and 30 μCi. LM and EM radioautograms were prepared from these glutaraldehyde-fixed, Epon-embedded cultures with Ilford L-4 nuclear emulsion (Ilford, Ltd., Ilford, Essex, England) according to the procedures described in (26), and developed with Kodak Microdol-X for 5 min at 18°C after appropriate periods of exposure at 4°C.

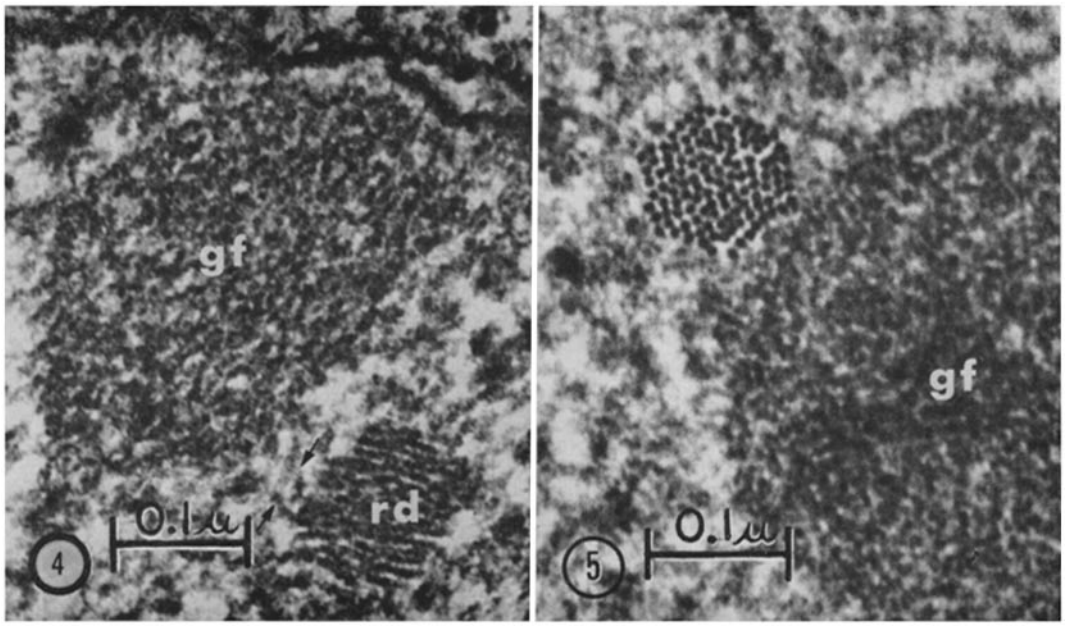
Fine structural studies were conducted with sym-

pathetic ganglion cultures fixed at 22 key intervals from 2–158 days in vitro with either 2% OsO_4 in Veronal-acetate buffer, containing 0.05% $CaCl_2$, pH 7.4 (27), or 1.5–3% redistilled (24) Fisher Biological Grade glutaraldehyde in 0.1 M cacodylate buffer containing 0.05% $CaCl_2$, pH 7.4, for 1 hr at 4°C, then postfixed with the aforementioned 2% OsO_4 solution. Dehydration was accomplished with an ascending series of ethanol solutions and two changes of propylene oxide, followed by embedment in Epon 812 (27). After polymerization and suitable mounting (27), thin sections were cut from these preparations with a diamond knife on a Porter-Blum MT-2 ultramicrotome (Ivan Sorvall Inc., Norwalk, Conn.) and stained with 50% ethanolic uranyl acetate for 10 min, followed by Reynolds' lead citrate (28) for 8 min. These sections were examined in an RCA EMU-3G and/or a Philips 200 electron microscope; in the latter instrument, stereo pairs were taken of rodlets and associated structures at tilt angles of 6° (total included angle of 12°). Embryonic (4, 6, 9, 12, 13, 14, and 15 days *in ovo*) and adult sympathetic ganglia were prepared for electron microscopy in essentially the same manner as the cultures. Since the rodlet appears in neurons of both male and female animals (7), rooster sympathetic ganglia were selected for these studies to avoid any possible confusion arising from sex chromatin formations (Barr bodies) in histochemical and/or ultrastructural studies concerned with rodlet-associated bodies. Data from over 16 dozen cultures and in vivo ganglion preparations are presented in this paper.

OBSERVATIONS

Early Development of Intranuclear Rodlets and Associated Bodies in Embryonic, Developing Neurons

In neuronal nuclei of freshly excised sympathetic ganglia from 4–9 day embryos filamentous rodlets were not discerned either in the living cells or in fixed and stained preparations at the light and electron microscopic levels. Small (~0.4 μ) spheroidal, granulofibrillar (gf) bodies composed chiefly of coiled and intermeshed fibrillar elements may sometimes be seen, however, in various locations within such neuronal nuclei, especially in the immediate environs of the nucleolus (Fig. 2 *A*) At 12–15 days *in ovo* similar, more voluminou



FIGURES 4 and 5 Electron micrographs of early rodlet formation in relation to granulofibrillar bodies (*gf*) in chick sympathetic neurons. (Glutaraldehyde - OsO_4 fixation.) Fig. 4 shows a sparse, primordial rodlet (*rd*) in a 7-days in vitro neuronal nucleus closely juxtaposed to, or continuous with, (arrows) an adjacent granulofibrillar body (*gf*) that is in close proximity to the inner nuclear envelope. × 180,000. Fig. 5 shows a cross-section through a rodlet contiguous with a granulofibrillar body (*gf*) in an 18-days in vitro neuronal nucleus exhibiting regular polyhedral arrangement of component filaments. × 190,000.

bodies may be found that already display many of the fine structural features seen at later stages of development in vitro, as well as in the adult animal (Fig. 2 *B*). They also frequently appear close to, or contiguous with, the nucleolus. Numerous observations indicate that these bodies are not membrane-bounded; rather, they seem to maintain their form by a remarkably intercalated, labyrinthine arrangement of fibrillar elements intermingled with various granules. The fibrillar components making up their cortical regions not infrequently appear to be wound around the bodies in a manner reminiscent of a ball of twine (Figs. 3, 8, and 13 *B*). These bodies have been observed to precede the appearance of the filamentous rodlets at the *electron microscopic* level by some 1–3 wk in vitro, and at the *light microscopic* level by about 4–5 wk of development in organized culture, depending upon the age of the embryo from which the sympathetic ganglia were explanted.

In early formative stages the primordial rodlets consist of sparse clusters of aligned filaments in close proximity to, or continuity with, the granulofibrillar bodies (Fig. 4). By the second to third week in culture the organization of these ~70 ± 10 A filaments into well-ordered, polyhedral arrays is clearly in evidence, as is their continuing close spatial relationship with the spheroidal gf-bodies (Fig. 5). Contributing to the ordered arrangement of the filaments in such a formation are side- or cross-bridges located at various intervals along their length (Fig. 6), which set the filaments ~50 ± 10 A apart (center-to-center spacing ~130 ± 10 A). Each filament making up such an array appears to consist of fine strands of subunits entwined into cable-like or helical conformations (Fig. 7), the pitch and period of which have

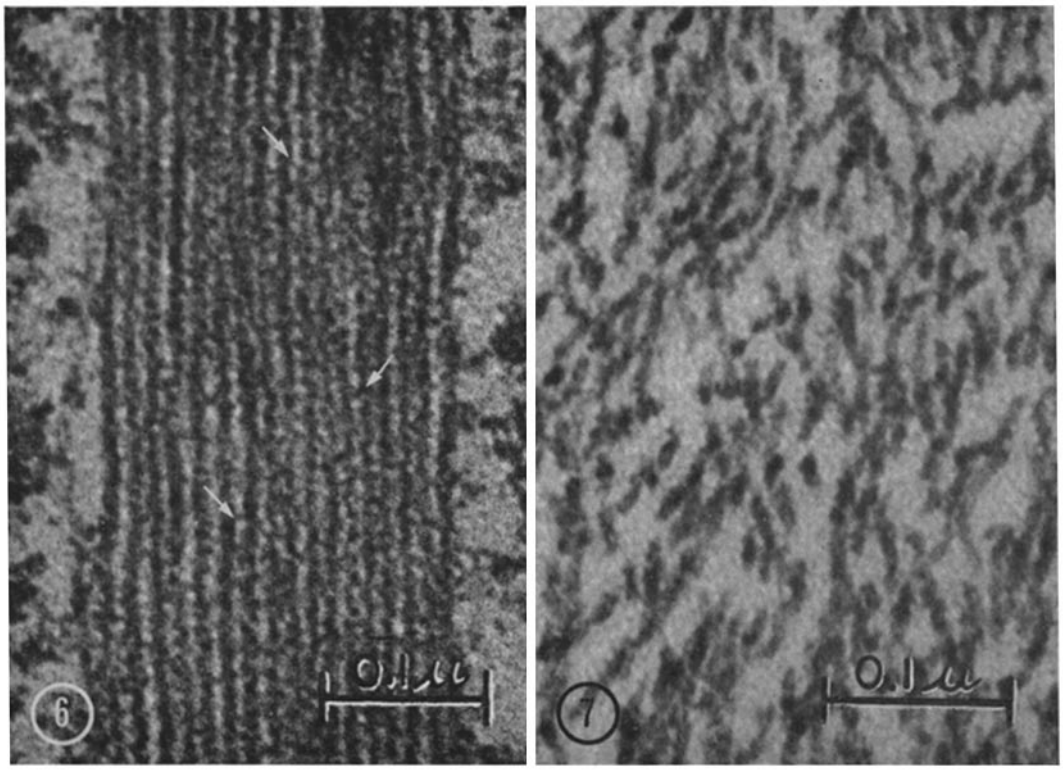


FIGURE 6 Electron micrograph of a longitudinal section through a glutaraldehyde-OsO_4-fixed rodlet array in a 21-days in vitro neuronal nucleus in which numerous side- or cross-bridges (e.g., at the arrows) may be seen that set the filaments ~50 ± 10 A apart (center-to-center spacing ~130 ± 10 A). × 220,000.

FIGURE 7 Electron micrograph of a glancing section through an opened-out rodlet array in OsO_4-fixed material 24 days in vitro showing the cable-like or helical arrangement of filament subunits. × 250,000.

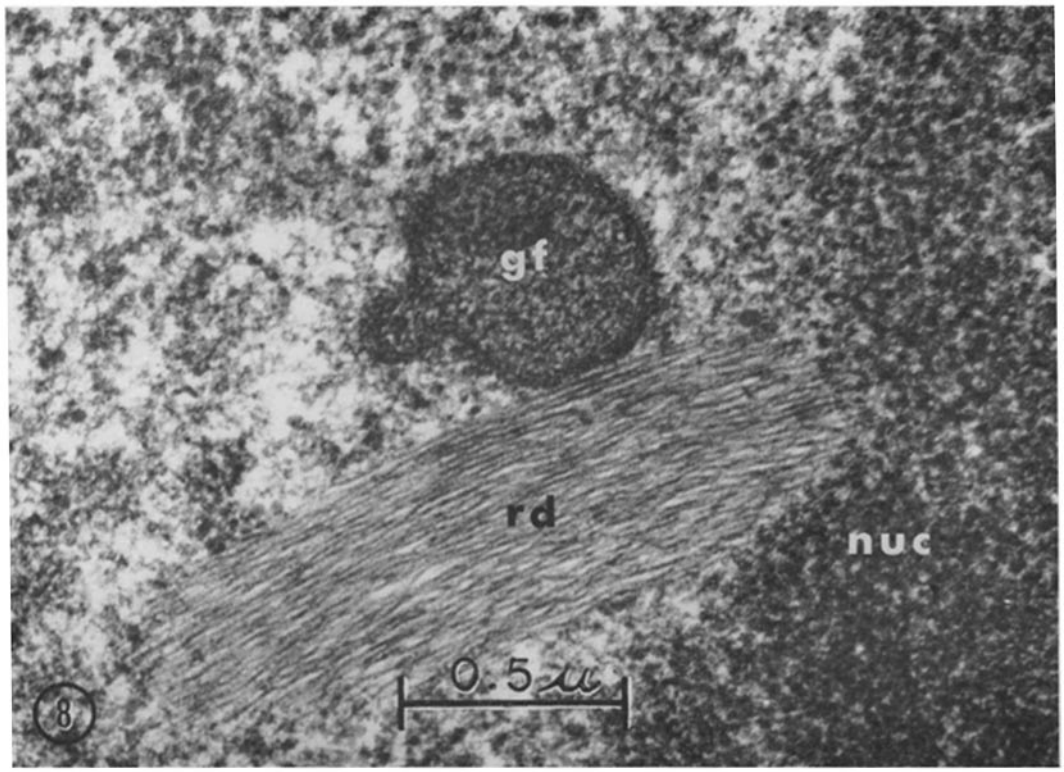


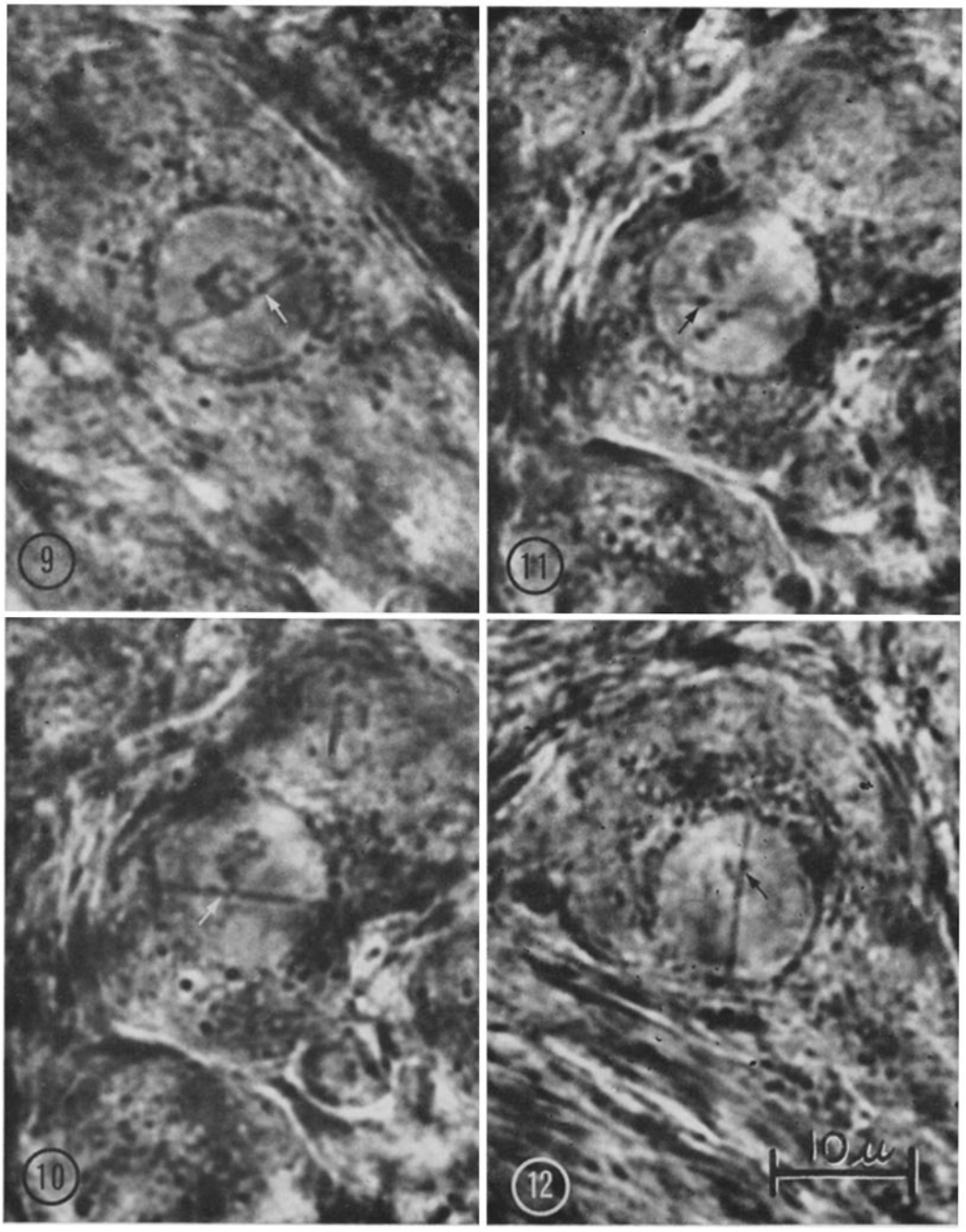
FIGURE 8 Electron micrograph of the nucleus in an OsO_4-fixed neuron 28 days in vitro showing a portion of the rodlet (*rd*) in contact with the margin of the nucleolus (*nuc*). A granulofibrillar body (*gf*) appears atop the rodlet in close juxtaposition with the nucleolus. × 60,000.

been noted to vary depending upon fixation, and perhaps physiological, conditions.

In addition to the frequently observed close apposition and/or continuity between the enlarging rodlets and granulofibrillar bodies encountered during the first month of growth and development of the neurons in culture, it was also noted that the rodlets not infrequently appeared in close juxtaposition or contact with the periphery of the nucleolus (Fig. 8). The nature of this relationship, and its possible connection with the granulofibrillar body-rodlet system, will be considered in greater detail below.

Later Development and Dynamics of the Rodlets, Granulofibrillar Bodies, and Nucleolus in Cultured Neurons

Rodlet enlargement in both girth and length continues from 30–160 days in vitro, enabling them to be seen clearly now in the *living* neurons as sharply demarcated, cable-like formations traversing various regions of the nucleoplasm. The curvilinear nature of these rodlets is readily perceived by focusing up and down through the nucleus of the *living* neurons; one can then trace their course through the nucleoplasm along arcing paths, which not infrequently bring them in close proximity to, or direct contact with, the nucleolus (Fig. 9). Rodlets are often seen crossing the nucleus from pole to pole, as well as running for some distance subjacent to the nuclear membrane. The contour and optical density of the rodlet may vary when it is viewed by bright field optics in the *living* state, indicating dynamic changes in material distribution or movement along it (Fig. 10). Contributing to some of the changes noted above may be the presence of punctate spheroidal bodies that are considerably smaller and denser in appearance than the nucleolus, and which are frequently seen in close juxtaposition or contact with the rodlet, both in the vicinity of the nucleolus and at some distance from it along the rodlet (Figs. 11, 12).



FIGURES 9–12 Photomicrographs of *living* chick sympathetic neurons 63 days in vitro. × 1,900. Fig. 9 shows a curvilinear rodlet in close proximity to, or direct contact with, the nucleolus displaying a small, dense, spheroidal body on the rodlet proximal to the nucleolus (arrow). Fig. 10 shows a rodlet crossing near the nuclear equator displaying an irregular contour with an inflection and small elevated ridge of material near the nucleolus (arrow). Fig. 11 illustrates a prominent, dense, spheroidal body (arrow) on a thin rodlet near, but not in contact with, the nucleolus. Fig. 12 reveals a rodlet displaying irregular contour and density with a dense, pleomorphic spheroidal body complex at the arrow.

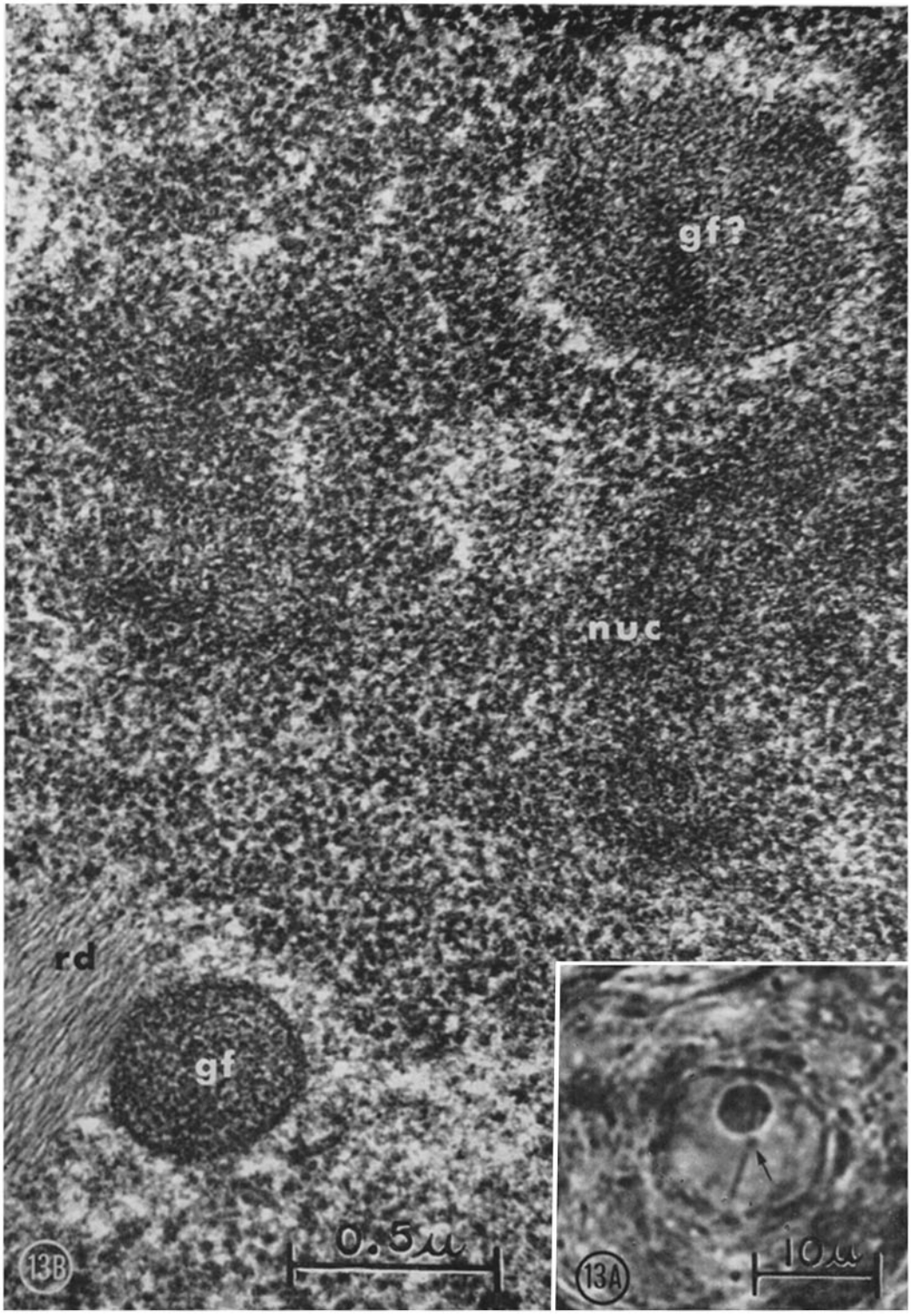


FIGURE 13 Chick sympathetic neurons 86 days in vitro. In the photomicrograph of a *living* neuron (Fig. 13 *A*) a dense spheroidal body (arrow) appears to be situated on a rodlet in close juxtaposition with the nucleolus. × 1,900. In an electron micrograph of a section through such a nucleus (Fig. 13 *B*) a granulofibrillar body (*gf*) is seen on, or connected to, a filamentous rodlet (*rd*) that is in contact with the nucleolus (*nuc*). Note the circumscribed, largely fibrillar region possibly representing a forming granulofibrillar body (*gf?*) within the nucleolar complex. (OsO_4 fixation.) × 62,000.

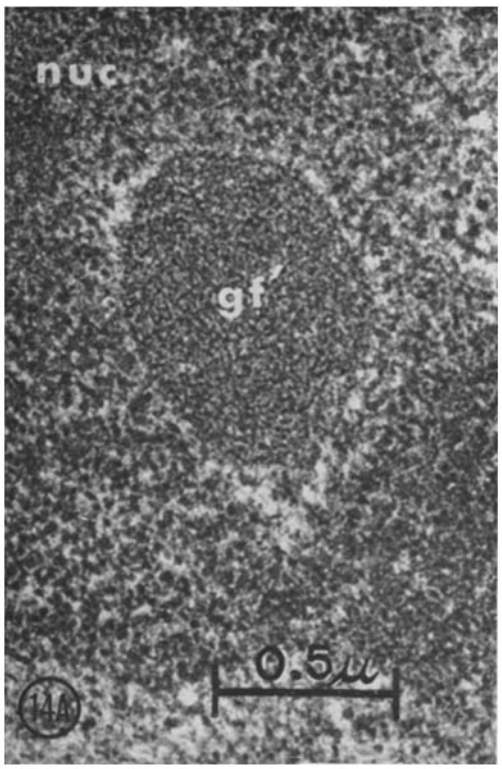
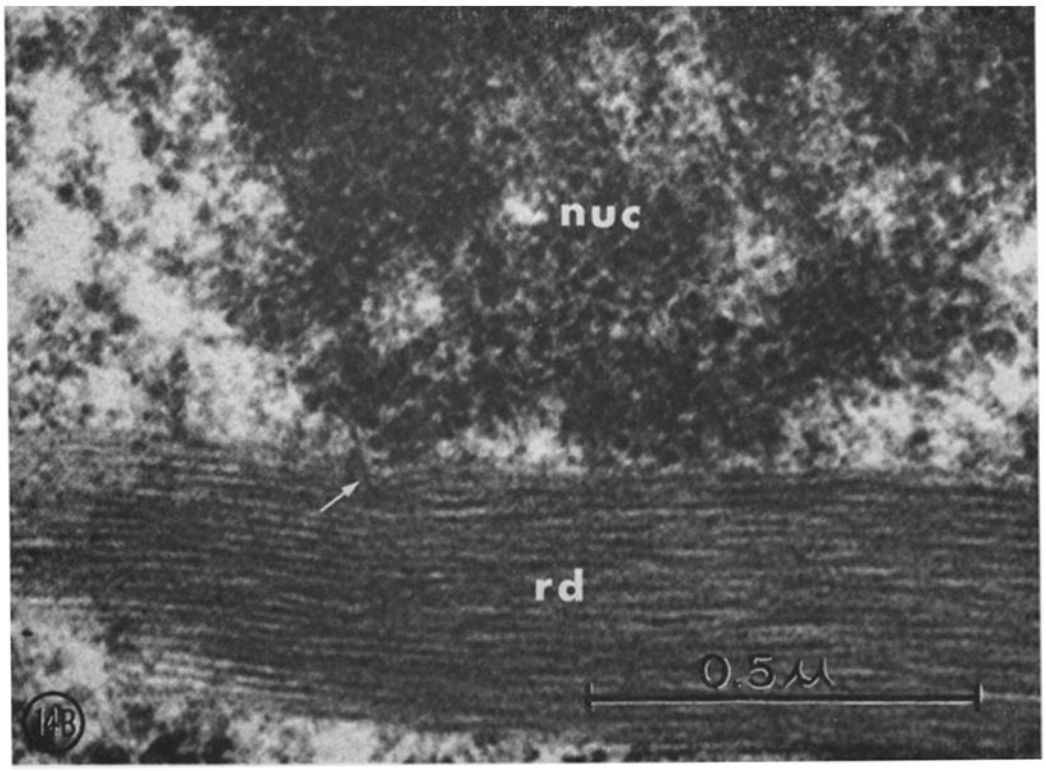
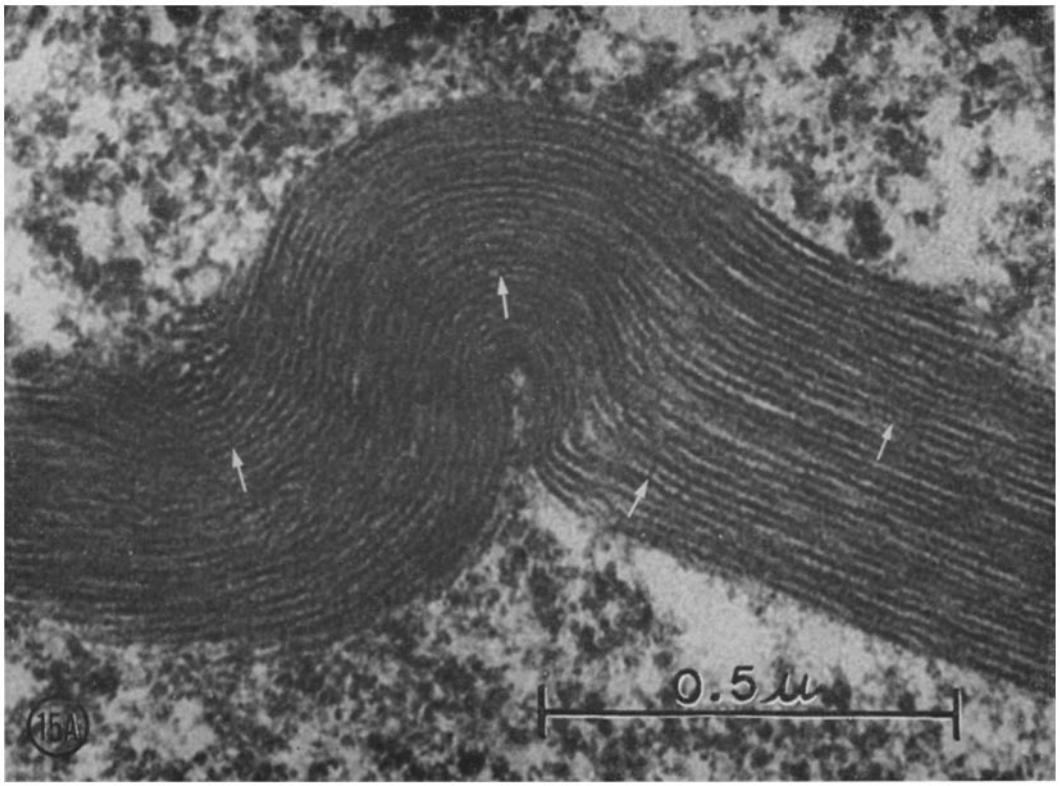


FIGURE 14 *A* Electron micrograph of an intermeshed, largely fibrillar region within an OsO_4-fixed neuronal nucleolus (*nuc*) displaying component ultrastructure and heavy-metal staining very reminiscent of a granulo-fibrillar body (*gf'*). (65 days in vitro.) × 50,000.

FIGURE 14 *B* Electron micrograph depicting the nucleolus (*nuc*) in contact with the rodlet (*rd*) in an avian sympathetic neuron. Note the filament (arrow) that appears to extend between the two structures and bends alongside the neighboring rodlet filaments. (Glutaraldehyde-OsO_4 fixation.) (125 days in vitro.) × 102,000.





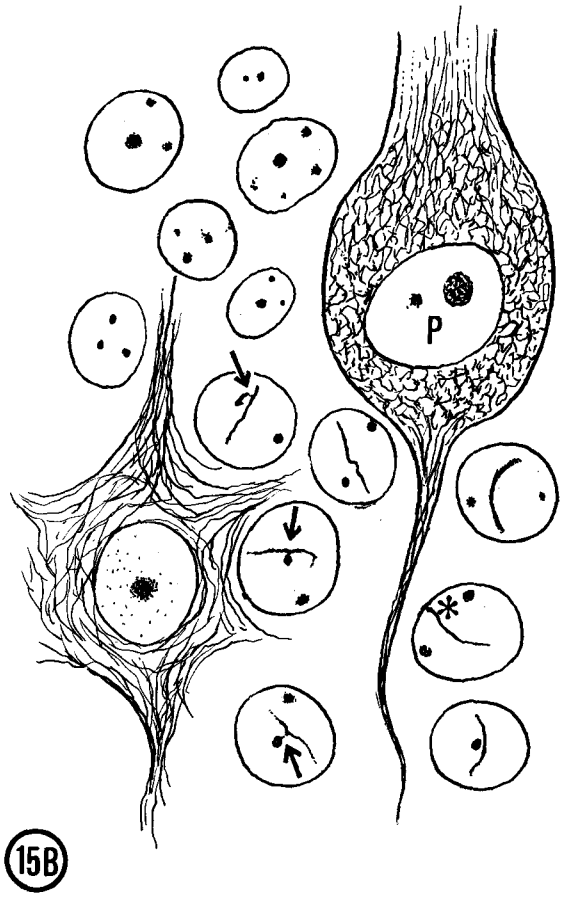


FIGURE 15 *A* Electron micrograph of a rodlet in a glutaraldehyde-OsO_4-fixed neuron 110 days in vitro displaying a sharp bend or inflection. Notice that the rodlet bends as an organized unit, possibly being held together by the numerous side- or cross-bridges between filaments situated at various positions within the array (e.g., arrows). × 110,000.

FIGURE 15 *B* Rabbit cerebellar granule cell nuclei displaying a variety of arcing and flexural rodlet configurations. Note in this silver-stained preparation that the neurofibrillar network of the Purkinje cell (*P*), and other neurons, stains under the same conditions as the rodlets. Observe the approximately spheroidal bodies, the larger of which may be nucleoli, in close proximity to and/or connection with some of the rodlets (arrows). One rodlet appears to be in contact with the nuclear membrane (*). (Redrawn from Cajal [5]).

This kind of body, and the various positions it may assume near or on the rodlet, is seen especially clearly in silver-stained preparations of these cultures. These small spheroidal structures are identifiable with the granulofibrillar bodies of the same general size and shape seen in the electron microscope at different locations along the rodlet, including such juxtanucleolar positions as indicated in Fig. 13 *A* and *B*. In this connection it is also to be noted that certain circumscribed regions within the nucleolus (especially near its periphery) display ultrastructural features and staining reactions very similar to those of the gf-bodies (Fig. 14 *A*).

Time-lapse cinemicrographic studies of *living* sympathetic neurons in culture confirm and extend the above observations concerning relationships between the rodlets, small spheroidal bodies, and the nucleolus by directing attention to dynamic activities that are not readily apparent in either routine visual observations or sequential still photographs of these structures. Such films disclose, for example, a flexural, somewhat undulatory movement of the rodlet, particularly in portions of it near the nuclear membrane, which appears to be distinguishable from oscillations of the nucleus as a whole and not unlike the propagation of movement along a bundle of flexible cables (see Discussion). The rodlet is also observed to change its orientation with respect to the viewer at various intervals, due to internal nuclear activity and/or nuclear rotation. The nucleolus is not infrequently seen in close proximity to, or direct contact with, the rodlet and may maintain this relationship for varying lengths of time. It has been observed to move as if attached to the rodlet during nuclear rotation, then move away from it following such an excursion. The nucleolus also undergoes translational and conformational changes in relation to the rodlet, which sometimes give the distinct impression of distributing some of the nucleolar substance along the rodlet. This complex and intimate association of the nucleolus with the rodlet is confirmed at the electron microscopic level where filaments of the same type as those comprising the rodlet may be traced between the two structures (Fig. 14 *B*). It is not known at this time whether such nucleolar-rodlet interrelationships follow any cyclical patterns, or involve specific regions of the nucleolar complex (see Discussion).

Transient, localized changes in contour (and optical density) are not infrequently observed along the rodlets, signifying movement and/or distribution of material in those regions related to spheroidal body activity (Fig. 12). Such spheroidal body complexes have been noted to change shape and seemingly diminish in size en route along the rodlet. In contradistinction to such spheroidal body activity, sharp, localized inflections are seen along the rodlet, which may be quite conspicuous in regions near the nucleolus (Fig. 10). When such an inflection is viewed in the electron microscope (Fig. 15 *A*), it becomes clear that the rodlet bends as an integral unit, possibly being held together by the filament side- or cross-bridges that appear in various positions within the array. This, and other electron microscopic observations, corroborate the photomicrographic findings in the *living* neurons of a wavelike displacement that appears at times along these closely coupled formations. What may represent this same kind of flexural activity was depicted by Cajal ([5] in silver-stained prepa-

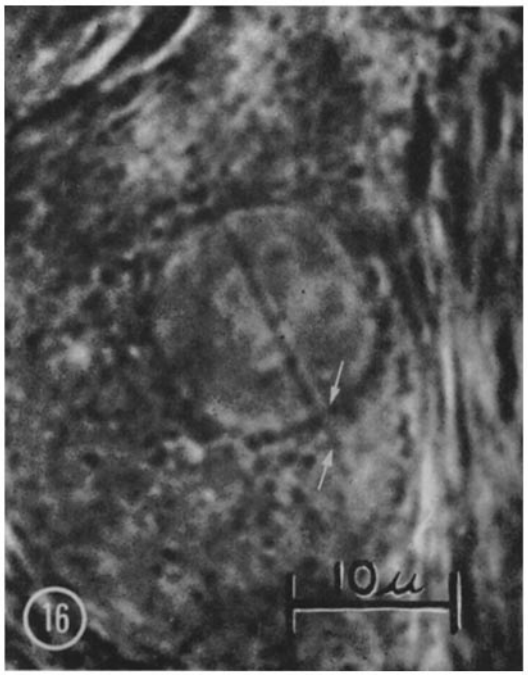


FIGURE 16 Photomicrograph of a *living* chick sympathetic neuron 125 days in vitro displaying an irregularly contoured rodlet that is especially noticeable in the region where it appears to have penetrated through the nuclear membrane (arrows), and may be transferring some filaments into the perinuclear cytoplasm. × 2,200.

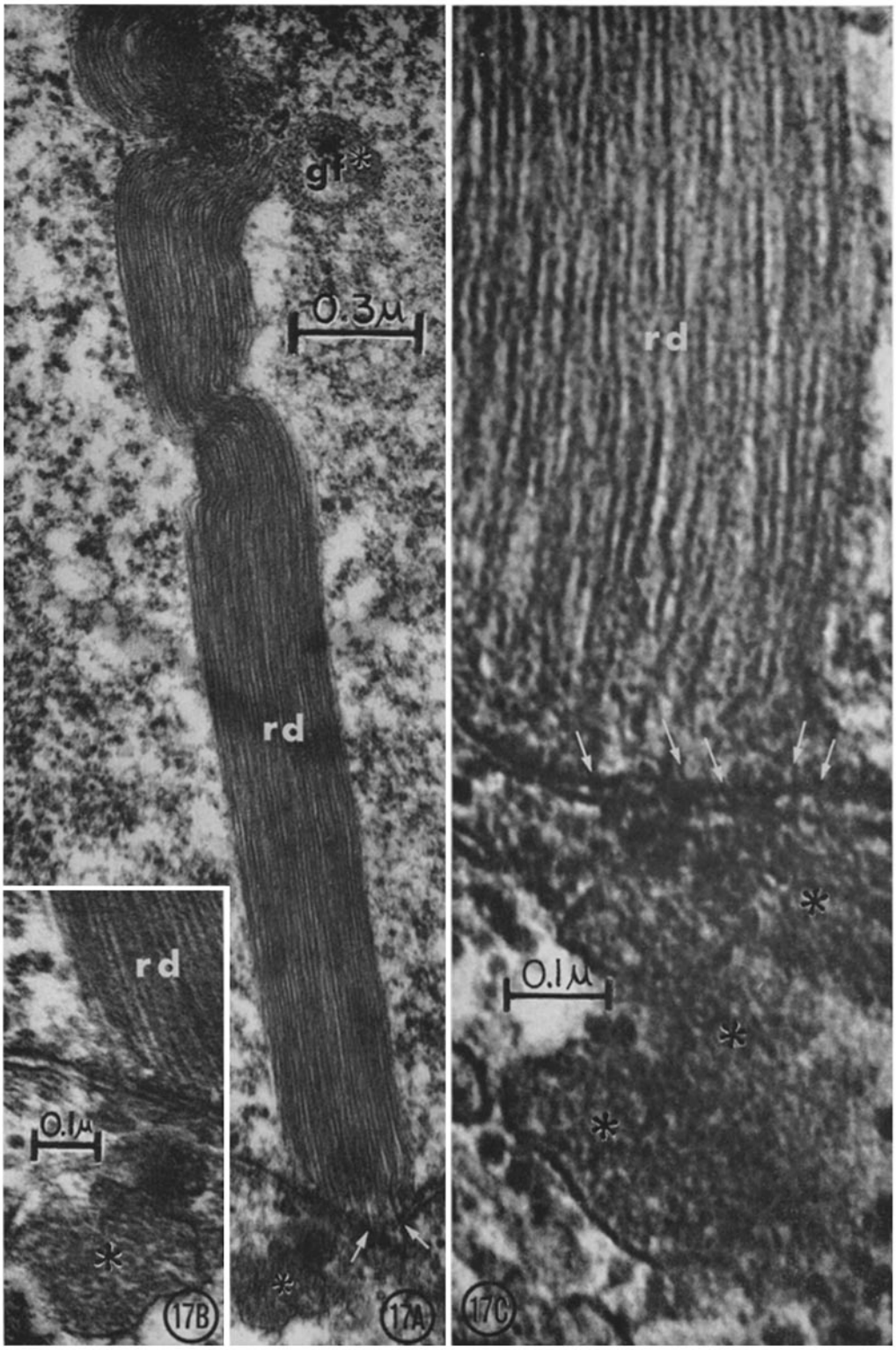


FIGURE 17 Electron micrographs of sections through rodlets where filaments are being extruded into the cytoplasm at the point where the rodlet indents the nuclear envelope. (Glutaraldehyde-OsO_4 fixation.) In Fig. 17 *A* a series of inflections distort the rodlet (*rd*) in the region where a portion of a granulofibrillar body (*gf**) is attached. Rodlet filaments (arrows) project into the perinuclear cytoplasm; an irregular mass of extruded filaments (*) appears near the point(s) of their egress from the nucleus. × 70,000. At a higher magnification (Fig. 17 *B*) of the region where the rodlet (*rd*) indents the nuclear envelope, individual (~70 ± 10 A) filaments are discernible in the cytoplasmic agglomerate of extruded rodlet material (*) shown in Fig. 17 *A*. × 100,000. At a still higher magnification (Fig. 17 *C*) of another region of rodlet indentation of the nuclear envelope, several filaments (arrows) extend across the nuclear envelope and become intermingled with other extruded, somewhat crimpled rodlet filament material (*). × 165,000.

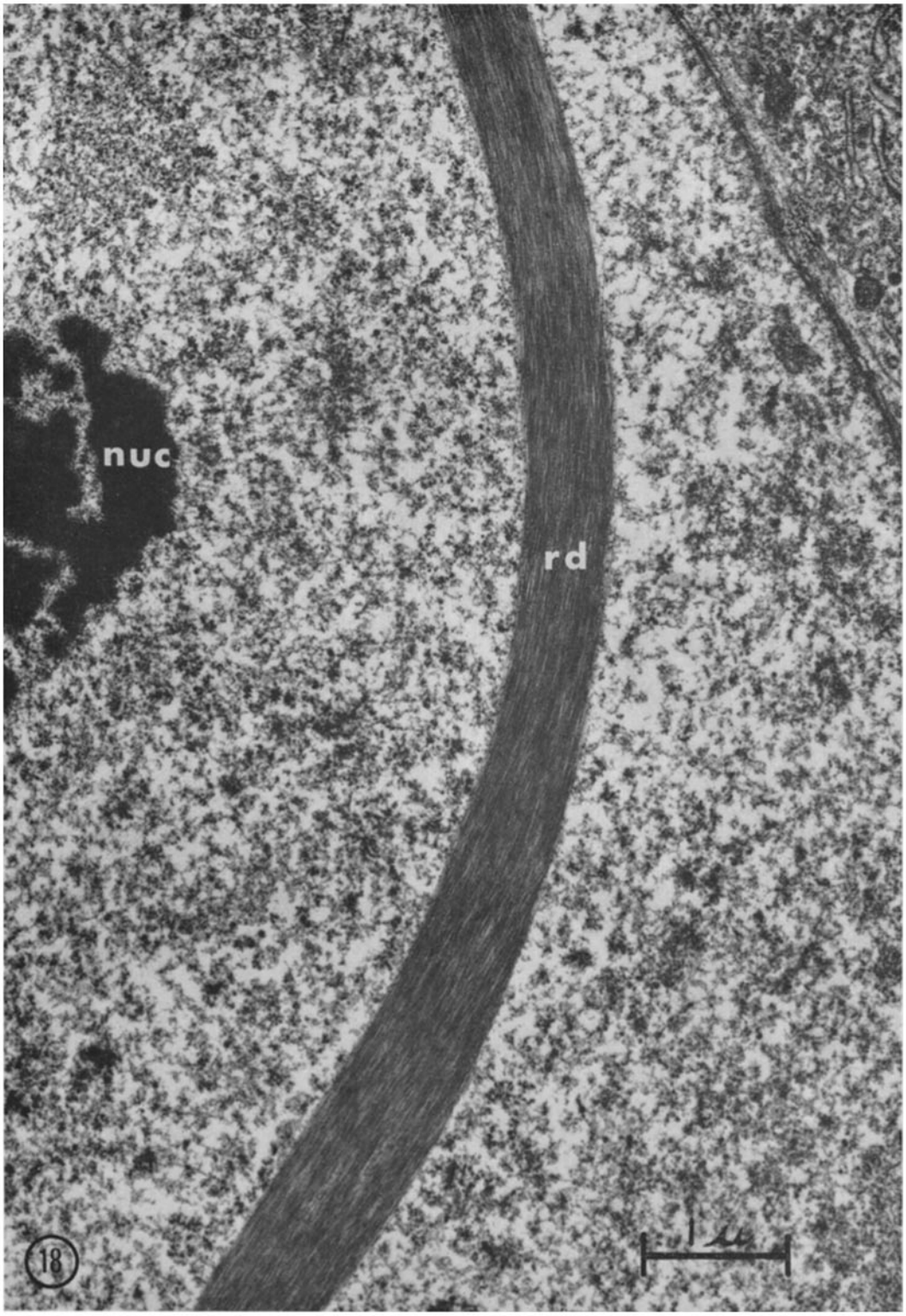


FIGURE 18 Electron micrograph of a portion of a nucleus in an adult rooster sympathetic neuron containing a broadly arcing filamentous rodlet (*rd*). Part of the nucleolus (*nuc*) appears to the left. (Glutaraldehyde-OsO_4 fixation.) × 22,000.

rations showing the nuclei of cerebellar granule cells in the rabbit) as various serpentine or irregularly wavy configurations of rodlets, together with the close juxtaposition and/or connection of approximately spheroidal structures, some of which appear large enough to be nucleoli (Fig. 15 *B*). As can be seen in Figs. 16 and 17 it is possible that some rodlet inflections may be manifestations of the process by which rodlet filaments are sometimes propelled through the nuclear envelope into the cytoplasm. In the photomicrograph of a *living* neuron in culture (Fig. 16) the rodlet displays an irregular contour most notably near the region where it extends through the nuclear membrane into the cytoplasm. At the electron microscopic level (Fig. 17 *A*, *B*) such rodlet regions display a series of inflections, some of which may be due to resistance offered by the nuclear envelope to the force that causes the filaments to indent the envelope, and some of them to penetrate through it into the cytoplasm. The clearly discernible indentation of the nuclear envelope by the rodlet, concomitant with the passage of some of its filaments into the perinuclear cytoplasm, has been convincingly observed on several occasions at the electron microscopic level in the plane of section approximately parallel to the long axis of the rodlet and perpendicular to the nuclear envelope at the point(s) of penetration; this is especially evident in stereo pairs of such regions. An irregular mass of extruded rodlet filaments not infrequently appears in the perinuclear cytoplasm immediately adjacent to these point(s) of egress from the nucleus (Fig. 17 *B* and *C*). The possible disposition of these transposed filaments, as well as the broader implications of the filament translocation phenomenon, will be discussed below.

Relationships Between the Rodlets, Granulofibrillar Bodies, and Nucleolus in Adult Avian Neurons

The findings presented above, based upon observations of *living* and fixed embryonic and later

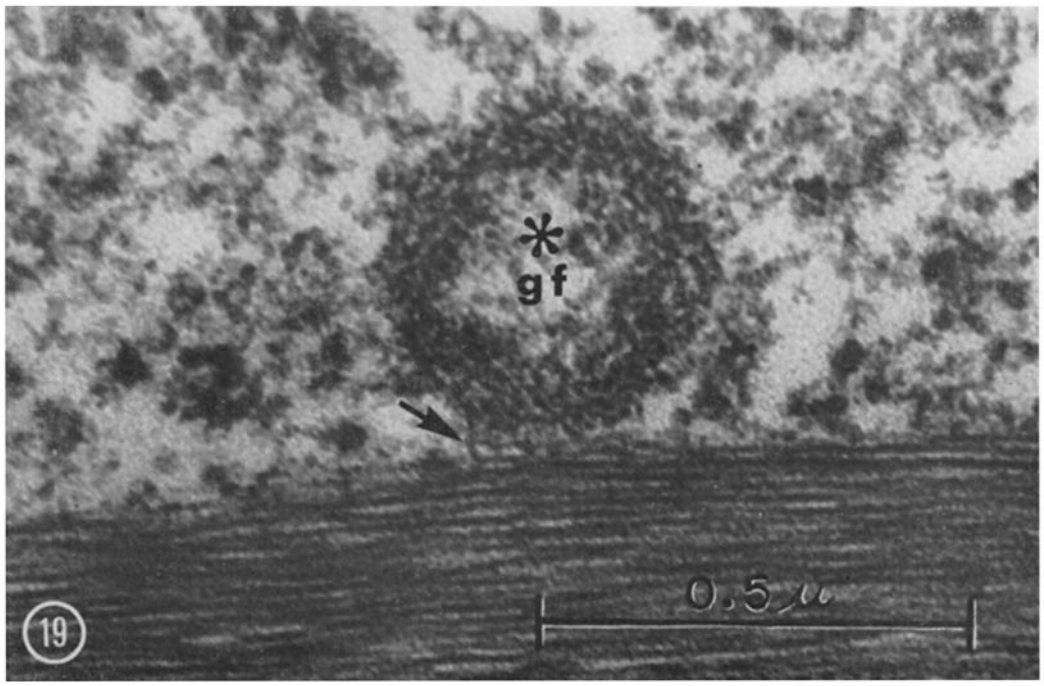


FIGURE 19 Electron micrograph of a glutaraldehyde-OsO_4-fixed neuronal nucleus from an adult bird in which filament material of the type comprising the rodlet extends between a portion of a granulofibrillar body (*gf**) and the rodlet, then appears to bend as if for alignment along the other rodlet filaments (arrow). Notice the side- or cross-bridges that are also present between filaments in this in vivo material. × 115,000.

developmental material, are recapitulated on a somewhat larger and more diversified scale in similar neurons obtained from the adult animal. The rodlets found in vivo within these somewhat larger neurons, though generally thicker and longer than those seen in vitro, vary perceptibly in their dimensions from cell to cell. As in the culture preparations, rodlets display notable inflections that sometimes give them the appearance of the letter "Z." They may be seen in close proximity to, or direct contact with, the nucleolus–at times appearing to be suspended from it, and may traverse the nucleoplasm along broadly arcing paths such as depicted in Fig. 18. Granulofibrillar bodies displaying essentially the same ultrastructural features as those seen in vitro are similarly found in intimate contact with the rodlet (Fig. 19). In addition, various configurations of filament looping, twisting, and intercalation are seen, which may represent either stages of filament alignment along the rodlet, or their possible detachment and folding back from the array. Factors bearing on the positioning and alignment of rodlet filaments, as well as their possible retraction from these formations, are considered below.

Chemical Nature of the Rodlet and Granulofibrillar Bodies

Results of histochemical studies of in vivo and in vitro material indicate that the rodlets are constituted largely (if not exclusively) of protein, and the spheroidal gf-bodies of similar protein and what appears at times, based upon variable stainability with basic dyes and occasional labeling with uridine-^{3}H, to be some associated RNA. Alkaline-fast green (pH 8.1) does not stain the rodlet, whereas acid-fast green (pH 2.8), eosin Y, mercury-bromophenol blue, and methyl blue (1) react definitively with this formation. A variety of silver impregnation procedures, such as the Cajal (6) and Bodian (29) techniques, also bring out this structure (and the gf-body) quite vividly. By contrast, the rodlets are Feulgen negative and do not stain with buffered thionine (pH 3.8 and 4.6), methyl green-pyronine, or gallocyanine-chromalum (pH 0.88 and 1.73), indicating that neither DNA nor RNA is present in these ordered formations (at least in amounts detectable by these procedures).

These findings are substantiated by the appropriate enzyme extraction (trypsin, DNase, and RNase) and acid extraction (hydrochloric and/or perchloric acid) controls (23), as well as by the radioautographic findings of no definitive labeling of the rodlets by either thymidine-methyl-^{3}H or uridine-5-^{3}H. These observations appear to be in keeping with the recent cytochemical and radioautographic findings of Lane (30) on similar appearing intranuclear filamentous formations in actinomycin D-treated oocytes of *Triturus viridescens*. Among the amino acids constituting the rodlet filament (and gf-body) protein in avian sympathetic neurons are tyrosine, arginine, and tryptophan (Kim, Masurovsky, Benitez, and Murray, submitted for publication). Electron microscopic histochemical and radioautographic studies generally confirm the aforementioned light microscopic findings and are now directed to characterizing further the nature of the macromolecules comprising these structures.

DISCUSSION

To all appearances the filamentous rodlets described in this report are analogous with some, if not most, of the intranuclear rodlets observed by the classical histologists around the turn of the century, and more recently by electron microscopists studying a variety of neural (and non-neural) tissues in diverse species, including man. None of these investigators has shed any definitive light on either the origin or the nature of these intriguing formations. Through the use of organized nerve tissue cultures we have been able to follow the development and dynamic activities of the rodlets and associated bodies in chicken sympathetic ganglion neurons from early embryonic stages through a high degree of maturation in vitro. By combining these sequential observations in *living* material with cinemicrography it has been possible to gain insights difficult to obtain otherwise about the essential nature of these structures. Moreover, the tissue culture findings provide an important continuum with, and in many ways serve to illuminate, those from in vivo preparations; they also appear to be in keeping with observations made by other investigators at both the light and electron microscopic levels (4, 5, 7, 16–21, 32, 45, and 46). It may therefore be appropriate to correlate our findings with these other data, and to advance hypotheses concerning the origin, nature, and possible functional significance of intranuclear rodlets and related filamentous formations.

As to the possible origin of the filaments comprising the rodlets we have evidence from time-lapse cinemicrographic studies of the *living* neurons, as well as light and electron microscopic observations of fixed sympathetic ganglion preparations, which suggests that the nucleolus and its presumed spheroidal gf-body derivatives are involved in the assemblage and functional dynamics of the rodlet. Other workers have considered this a likely possibility (20, 30–31), based principally upon the close proximity of the rodlet formations to the nucleolar complex or its environs, as well as the similarity in morphology, fine structure, and/or staining reactions between certain well-delineated, largely fibrillar regions within some neuronal nucleoli and the gf-bodies. The frequent observation of the intimate association (and comovement) of the rodlet with the nucleolus in time-lapse films, together with the electron microscopic observations of filamentous connections between the two, could be interpreted as indicating that the nucleolus plays a significant role in the assemblage and/or functional dynamics of the rodlets. Concerning the possible relationship of the rodlets to the nucleolus, Waddington (cited in 30) pointed out that intranuclear protein filaments were most commonly found near nucleoli in certain amphibian embryonic cells and further noted that they were not encountered in pregastrula cells in which a nucleolus had not yet been formed, or in adult tissues where nucleoli were poorly developed. Lane (30) also suggested that if the association of these filamentous formations with the nucleolus is meaningful, their presence in actinomycin D-treated amphibian oocytes, which contain hundreds of nucleoli, could be significant and may possibly represent a manifestation of actinomycin D alteration in DNA-RNA metabolism. This notion is intriguing and may signify that in normal cells, such as these sympathetic neurons, certain regions of the nucleolus in which nucleic acid metabolism is undergoing some kind of physiological change might contribute related protein components to the building up of these filamentous formations. It may also mean that in pathological tissues (13, 17, 20, 36) this process is perhaps abnormally altered or accelerated so as to provide a greater profusion of these elements than normally would occur (18, 33) Preliminary findings in actinomycin D-treated avian neurons in vitro of additional nuclear deposits of filament material indistinguishable morphologically from rodlet filaments lends support to this view.

Regarding the spheroidal gf-bodies seen in close relationship to the rodlets, we have observed such distinctive granulofibrillar complexes within, as well as directly connected to, the periphery of the nucleolus. The close apposition and/or connection of the nucleolus to the rodlet at times when the gf-bodies are in juxtaposition with both structures further suggests a nucleolar-related origin of at least some of these bodies. It is not clear whether their appearance elsewhere in the nucleoplasm means that there may exist other sites of origin for them, or that they have simply migrated to those locations from the nucleolar complex. Similar appearing bodies have been reported by electron microscopists in a variety of neural tissues and other cells (19, 20, 33–35, 45) with filamentous elements sometimes emanating from them; and they have been *broadly* classified as "nuclear" bodies (34). In *normal* nervous tissue (14, 17, 18, 20) such bodies display fine structural features that would place them in the Type I category of Bouteille et al. (33). Apparently no prior electron microscope studies have been carried out relating modulations in their size, shape, or component distribution to various developmental stages of neurons.

The present authors have shown that nuclear bodies of an apparently similar kind are present in chicken sympathetic neurons even at early embryonic stages, and that in the course of neuronal differentiation and growth some of these nuclear bodies display changes in size and shape commensurate with their participation in the elaboration and/or functional dynamics of the filamentous rodlets. At early developmental stages these macromolecular complexes appear as intricately folded and entwined spheroidal bodies which, at some later point, apparently begin to unfold and become aligned in orderly register, perhaps through the formation of different and/or more stable chemical bonds. These arrayed filaments may then act as orientation or alignment sites for other like macromolecules that are subsequently added to the assemblage, contributing to its growth in both girth and length until it spans the nucleus. Such phenomena should not be entirely unexpected (33–35); moreover, the stepwise assemblage of the rodlets beginning in perinatal life and continuing through various stages of neuronal differentiation strongly

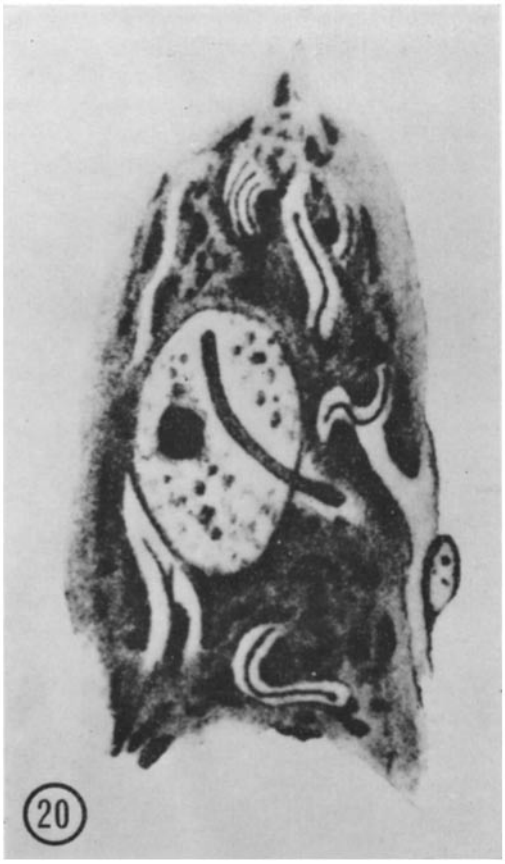


FIGURE 20 Photocopy of an illustration by Holmgren (4) of a rodlet straddling the nuclear membrane in a chicken sympathetic neuron. Heidenhain's iron hematoxylin stain. (Reproduced through the courtesy of Veb Gustav Fischer Verlag, Jena, of *Anatomischer Anzeiger*.)

suggests that some correlation may exist between the mode and rate of construction of these arrays and the developmental processes of the neuron. The observation that rodlet arrays in sympathetic neurons from the adult animal exhibit noteworthy variability in their dimensions (3, 4) and number of component filaments further suggests that the formation and turnover of these filaments may be related as well to the ongoing patterns of the neuron's basic physiological activities.

This may also be true for intranuclear "lattices" composed of very similarly appearing filaments that have been reported by several investigators (10, 13, 17–19, 36 and 45) in a variety of neurons from diverse species. That they not infrequently appear in the same neuronal nuclei in which rodlets are found (10, 13, 17, 19)[1] leads one to suspect that both types of formations may represent variations in the arrayal of similar (if not identical) macromolecules.

Whatever the geometrical form of these formations it is likely that the initial alignment, as well as the ultimate ordering of filaments within an array, are determined by those conformational states of the filament macromolecules that satisfy their intrinsic physicochemical behavior within the array. Thus, the various helical, extended, and/or other conformational states these molecules may be capable of assuming as a function of contractile or other activity, as well as the dimensions, angular orientation, conformations, and bonding characteristics of the side-bridges, would be important determinants of the spacing and angles between filaments within such formations. Local ionic, pH, and related microenvironmental factors would also influence the physicochemical processes involved in filament alignment, retraction, or contraction in these arrays. One manifestation of possible contractile processes within a rodlet array might be the "flexing" seen at various points along the rodlets, especially at loci near the nucleolus where dynamic interrelationships may either give rise to or accentuate such activity. It would seem reasonable to suppose that transient movement of this kind, initiated at a given point within these closely coupled formations, might well be propagated throughout the rest of the array, perhaps setting up a kind of reverberatory activity not unlike that observed in time-lapse films of rodlet activity in *living* neurons.

As alluded to earlier, some of these movements probably constitute manifestations of the process by which filaments may be extruded into the cytoplasm, that is, by a kind of wavelike activity propelling them through the nuclear membrane. Once the stimulus is provided to set such a process in motion, filaments are moved out into the perinuclear cytoplasm where they sometimes appear as loose agglomerates of multifarious dimensions. These findings would appear to be in keeping with some of the early cytological observations of these structures (1, 2), including

[1] Similar intranuclear rodlets and lattices have been seen in neurons of the rat cerebral cortex (by R. L. Schultz) and the cat olefactory bulb (by T. J. Willey, personal communications).

those of Holmgren (4) who depicted rodlets straddling the nuclear membrane in the same kind of avian sympathetic neurons we have worked with (Fig. 20). In further support of the transposition of nuclear filaments into the cytoplasm is the electron microscopic observation cited in references 16 and 20 of the extension of a similar appearing intranuclear fibrillar formation (rodlet) through the nuclear envelope into the cytoplasm of an experimental ependymo-blastoma cell in the mouse. Nuclear-cytoplasmic filament movement has also been suggested for other cells containing apparently similar nuclear formations (18, 46). The very nuclear setting for such events implies some association with genetic processes occurring during interphase, the nature of which remain to be determined.

The limited reports thus far of rodlet filaments bridging the nuclear envelope raises the question of whether this might represent a transient occurrence requiring extensive sampling to detect, or is in some way related to the stage of development of the rodlet and/or the type of cell in which it appears. A definitive answer cannot be provided at present, nor is it possible to say whether the transposed filaments follow the same distribution pattern (or postulated activities) (37–39) as similar appearing filaments in neurons. The reader, however, is referred to a recent discussion and review by Schmitt (40), which should be consulted particularly on matters pertaining to possible transport and related functions of filaments and other fibrous proteins in neurons. His remarks on the likely interplay of genetic factors with the ongoing pattern(s) of bioelectrical activity and other vital neuronal processes, and their possible facilitation by fibrous protein-mediated transfer of biochemical "information" from and to the cell center, might be considered germane to the nuclear-cytoplasmic filament system described in the present study. In thin sections of neural tissue, such as the kind we have worked with, it does not seem unreasonable to suggest that the transposed rodlet filaments might contribute in some measure to the medium diameter ($\sim 70 \pm 10$ A) complement of the general filament population in these cells. Indeed, there is no a priori reason to believe that *certain* "neurofilaments" might not originate in the nucleus, since the origin of such filaments as a whole remains a matter of conjecture (40–42). It awaits the careful isolation and physicochemical characterization (41) of these rodlet filaments to determine whether they in fact fall within the presently defined norms for such fibrous proteins. What applies for the neuron and its filaments of nuclear origin may perhaps also be the case with glia and intranuclear glial filaments (18, 20, 43), as well as certain nuclear filaments in ependymal (16, 20) and other cells (17, 44, 46).

It is clear from the above discussion and speculations that much work remains to be done with the most advanced experimental and analytical techniques in order to ascertain exactly what these *particular* filaments are capable of doing in neurons (and other cells). With the judicious utilization of suitable in vivo and in vitro preparations it should be possible to provide answers, at both the organized structural and the molecular levels, to many of the tantalizing questions remaining about the fundamental nature of these filaments, and the complex, exquisitely precise, and quite possibly important system of their assemblage and hierarchical ordering in the nucleus, and timely transfer into the cytoplasm.

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Note Added in Proof: L. Boquist (1969. *J. Cell Biol.* **43:** 377) has recently reported apparently similar intranuclear rodlets, and closely associated spheroidal bodies reminiscent of gf-bodies, in islet β-cells of the murine pancreas.

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