"Reticular" and "Areticular" Nissl Bodies in Sympathetic Neurons of a Lizard*,‡

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PLATES 46 TO 51

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ABSTRACT

Sympathetic ganglia of the horned lizard, *Phrynosoma cornutum*, were fixed in OsO_4 and imbedded in methacrylate. Thin sections were cut for electron microscopy. Some adjacent thick sections were cut for light microscopy and were stained in acidified, dilute thionine both before and after digestion by RNase.

In the light microscope two types of Nissl bodies are found, both removable by RNase: (1) a deep, diffuse, indistinctly bounded, metachromatic variety, and (2) a superficial, dense, sharply delimited, orthochromatic sort.

Electron microscopically, the former ("reticular" Nissl bodies) corresponds to the granulated endoplasmic reticular structure of Nissl material previously described by others, whereas the latter ("areticular" Nissl bodies) comprises compact masses of particles of varying internal density and devoid of elements of endoplasmic reticulum. The constituent particles of the areticular Nissl material are 4 to 8 × the diameter of single ribonucleoprotein granules of the reticular Nissl substance and seem, near zones of junction with the reticular type, to arise by clustering of such granules with subsequent partial dispersion of the substance of the granules into an added, less dense material.

It is suggested that the observed orthochromasia of the areticular Nissl substance is due to accumulation of a large amount of protein bound to RNA and, further, that these Nissl bodies may represent storage depots of RNA and protein.

INTRODUCTION

Investigations of the fine structure of normal vertebrate neurons, using modern methods of tissue preparation, have been largely confined to studies of the neurons of small mammals such as rats and mice (2, 9, 10, 17, 26, 31). Single accounts of some aspects of the fine structure of neurons of sympathetic (7) and spinal (1) ganglia of the frog and of the spinal cord of the lamprey (30) have, however, appeared. In these studies it has been consistently reported that Nissl bodies comprise more or less dense and ordered aggregates of endoplasmic reticulum with small, dense granules (100 to 300 A) externally studded upon and scattered among the reticular elements. The reticular elements may be vesicles, tubules, or cisternae. The presumably equivalent granulated endoplasmic reticula of murine hepatic cells (23) and of the pancreatic acinar cells of the guinea pig (24) have been shown by Palade and Siekevitz to be the sources of the microsomal fractions recoverable from homogenates of these cells by fractional centrifugation. They demonstrated, furthermore, that the ribonucleoprotein component of microsomes is identifiable with the dense granules, and the lipid component, with the membranes of the reticulum.

The emphasis which has been placed on the attachment of the dense granules to the elements of the endoplasmic reticulum has led to the inference

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(21, 22, 32) that the association has significance for the role in protein synthesis which RNA is widely believed to play not only in gland cells, but also in other cell types including neurons (4, 5, 13, 19, 26). The occurrence of Nissl bodies consisting of large accumulations of RNA-containing particles unassociated with endoplasmic reticular elements appears, therefore, to be germane to the question of that significance in neurons. This paper presents descriptions of the fine structure and staining properties of such "areticular" Nissl bodies and of the usual "reticular" type of Nissl bodies which occurs concomitantly in sympathetic neurons of the horned lizard, and discusses their possible significance.

Materials and Methods

The "stellate" ganglia of five specimens of *Phrynosoma cornutum* were fixed in 1 per cent or 2 per cent OsO_4 in veronal-acetate buffer (pH 7.5–7.6) for 1 to 2½ hours at 0°C. The osmolarity of the fixative for four of the animals was adjusted to 0.273 with sucrose. Three of the animals were anesthetized by intraperitoneal pentobarbital, and two by immersion in crushed ice. Tissues were rinsed 5 minutes in buffer, dehydrated in 10 minute changes of successive 10 per cent grades of methanol (beginning with 10 per cent), and imbedded in a prepolymerized (2 per cent luperco) 1:19 mixture of methyl and *n*-butyl methacrylates by completion of polymerization at 45–50°C.

Gold, silver, or gray sections were cut with glass knives on a Servall Porter-Blum microtome onto 10 to 50 per cent acetone, mounted on collodion-coated slit grids, and examined at 80 or 100 KV in a Phillips EM 100A electron microscope fitted with a 30 μ objective aperture. Micrographs were made on Kodak medium lantern slide plates at initial magnifications of 2,000 to 30,000 and exposures of 1 to 4 seconds and were photographically enlarged up to 5 ×.

A number of thick (~1 to 4 μ) sections were cut, some sequential with preceding thin sections, and mounted with Mayer's albumin on clean glass slides. After methacrylate removal in a mixture of di- and tetrachloroethanes, they were stained in 0.05 per cent solutions of thionine in either 0.01 M or 1 per cent acetic acid, following which they were rinsed in equivalent solutions of acetic acid, dehydrated in acetone, cleared in xylene, and mounted in HSR (harleco synthetic resin) (16). After examination and photomicrography, the thionine was washed out of some which were then subjected to digestion by ribonuclease[1] (27) and restained with thionine as before.

Sections of material previously prepared by perfusion with Zenker-formalin and stained by Masson's or by McGregor's azocarmine methods were used for general orientation. Sections of the same material were subjected to the periodic acid–Schiff procedure both with and without prior digestion by salivary amylase.

[1] Mann Research Laboratories, Inc., New York. Lot No. A5077.

OBSERVATIONS

With the exception of the Nissl bodies and of certain remarkable inclusions (33, 35) found in some of the ganglion cells, the fine structure of these perikarya is similar in principle to that previously reported for other vertebrate neurons (1, 2, 7, 9, 10, 17, 26, 30, 31).

As is true of sympathetic neurons in vertebrates generally, the Nissl bodies of the ganglionic perikarya of *P. cornutum* are localized peripherally (Figs. 1 and 3). The Nissl bodies viewed both light and electron microscopically in these cells comprise two types. One is superficially located, is relatively dense, and is sharply circumscribed (Figs. 1 to 3, *ANB*). The other is more deeply located, is relatively diffuse, and is indistinctly delimited (Figs. 1–3, *RNB*). Of these, the deep type corresponds essentially in its fine structure to the descriptions of Nissl bodies given by Palay and Palade (26) and others. It differs somewhat, however, in the sparseness of its elements, in the casual arrangement of the predominantly vesicular elements of its endoplasmic reticular component, in the almost random scattering of its smaller (~ 50 to 150 A), dense granular component, and in the relatively lesser frequency of attachment of its granules to the vesicles of its reticulum (Figs. 2 and 8).

The superficial type of Nissl body, on the other hand, is not immediately recognizable in the electron microscope as Nissl material (Figs. 1, 2, 6 to 8, *ANB*). It is virtually completely lacking in any elements identifiable as endoplasmic reticulum. It consists of frequently extensive, flattened, crescentic masses with relatively abrupt boundaries which either abut directly upon the inner surface of the cell membrane or are separated from it only by a thin layer of usually unspecialized cytoplasm.

The masses comprise accumulations of closely packed, dense particles[2] ranging from 200 A to 400

[2] To avoid lengthy circumlocutions in designation, the term "particle" will be applied exclusively to the unit structure of these masses within the text of this paper. The term "granule" will be reserved exclusively for the small, dense ribonucleoprotein granules first described by Palade, even though he originally called

A in diameter. The usual particle (Figs. 2, 7, 8) is irregularly polygonal in profile with a slightly blurred boundary. In relatively thick sections, or at low magnifications, the particles appear to be of relatively uniformly high density (Figs. 1, 2, 6). In thinner sections, however, the densities of the particles are seen to be markedly varied internally (Figs. 7 and 8). The most dense internal regions of the particles are indistinguishable with respect to size and density from the small dense granules of the deep Nissl bodies. Some of the particles, particularly those at the inner boundaries of the accumulations, appear in fact, to be tight clusters of a few such small dense granules (Figs. 7 and 8). The density of the remainder of the interiors of the particles grades irregularly down to approximately 2 to 3 times that of the cytoplasmic ground substance.

The density of the particles is apparently somewhat dependent on the duration of fixation. In material fixed 1½ hours (Fig. 7), the particles appear on the average to be almost twice as dense as those in material fixed 2½ hours (Fig. 8), using the density of the small dense granules of the deep Nissl bodies as a crude standard for comparison. It is noteworthy, however, that the densest regions (granules) within the particles seem to suffer no diminution in density after the longer period of fixation.

In one of the ganglia fixed for 2½ hours, generalized dilatation of the vesicles of the endoplasmic reticulum occurred (Fig. 8) due to unknown causes. It was noteworthy, however, that the constituent particles of the superficial Nissl bodies showed no evidence of enlargement or expansion in this specimen.

The demonstration that the superficial masses are indeed Nissl bodies is provided by comparison of light and electron microscopic observations as illustrated in Figs. 4 to 7, and by the effects of ribonuclease digestion. These figures are selected from a series of pairs of sequential thin and thick sections. The thick (~1 μ) one (Fig. 4) and the thin (gray to silver) one (Figs. 5 to 7) were separated from each other only by the thickness of one other gray to silver section. With the thin section in the electron microscope, a cell was selected which was distinctive in size, shape, location, and surroundings, and it was then micrographed, first at high magnification (Figs. 6 and 7) and then at low magnification (Fig. 5). The thionine-stained adjacent thick section was examined in the light microscope, and the same cell was identified by the same distinctive criteria and photographed (Fig. 4). Identification was unequivocal. A portion of the cytoplasm of the cell in the thick section was occupied by a basophilic mass (Fig. 4) which was clearly characteristic of a stained Nissl body. The corresponding area of the electron micrographs of the adjacent thin section (Figs. 5 to 7) showed the characteristic fine structure of a superficial Nissl body as described above. The thick section, after rehydration, digestion by ribonuclease, and restaining, was devoid of any basophilic masses within the neuronal cytoplasm, but retained basophilia of the chromatin in nuclei of connective tissue and satellite cells and metachromasia of mast cell granules.

The less densely basophilic, deep Nissl material in the same and an adjacent cell (Fig. 4) appeared faintly metachromatic in the thick section prior to ribonuclease digestion, but was not stainable after digestion. In still thicker (~3 to 4 μ) sections, however, after staining in thionine in 1 per cent acetic acid, the deep Nissl bodies were lightly to moderately but sharply metachromatic (red-violet) whereas the crescentic, superficial Nissl bodies and neuronal nucleoli were moderately to intensely orthochromatic (grayish blue) (Fig. 3). In similar sections stained in less acid thionine (0.01 M acetic acid) the superficial Nissl bodies and nucleoli were also found to be metachromatic when examined immediately after staining, dehydrating, clearing, and mounting (see above). During 24 to 48 hours of aging at room temperature in the mounting medium, however, the superficial Nissl bodies and nucleoli became virtually completely orthochromatic, whereas the deep Nissl bodies retained their metachromasia indefinitely.

Sections of Zenker-formalin–fixed material showed no intraneuronal material reactive to the periodic acid–Schiff procedure.

DISCUSSION

The original descriptions of Nissl bodies specified them to be basophilic masses within the cytoplasm of neurons. Later cytochemical investigations demonstrated that the basis for their basophilia resides in their content of ribonucleic acids. The elegant study by Palay and Palade (26) of the fine

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them particles. There is growing use of the terms "RNP granules," "Palade granules," and "granular reticulum," which makes the distinction selected the less ambiguous one.

structure of mammalian neurons has conditioned the expectation that Nissl bodies everywhere will be found to consist of more or less ordered and compact arrays of granulated endoplasmic reticulum similar to those composing the basophilic ergastoplasm of pancreatic acinar cells. Heretofore, this expectation has been borne out by studies subsequent to theirs. In the sympathetic ganglionic neurons of *P. cornutum*, however, there are two types of Nissl bodies which can be distinguished light microscopically on the basis of staining and cytochemical criteria. The fine structure of one of these (deep) coincides with previous descriptions of Nissl material. The fine structure of the other, however, has no precedent in the previously reported fine structure of RNA-rich cytoplasm either of neurons or of other cell types. Because the constituent particles of the latter type (superficial) include within them small dense granules closely similar to those of usual Nissl material but are unassociated with any endoplasmic reticular elements, the type will hereinafter be designated as "areticular" Nissl substance as opposed to the usual or "reticular" type.

The propriety of using the term "areticular" rests upon the correctness of the assertion that the constituent particles of the areticular Nissl substance contain no closed, membranous components (which in this case could only be vesicular). The fact that there was no expansion of the particles in a specimen in which the endoplasmic reticular vesicles were swollen has already been cited above. It is consistent with but does not prove the view that no vesicles are present within the particles. Additionally consistent is the size of the particles, which is on the average approximately half to two-thirds that of the smallest endoplasmic reticular or synaptic vesicles encountered in these ganglia. The less dense regions of the particles are still smaller, usually by at least a factor of two. Most convincing, however, is the fact that within virtually all of the particles the less dense regions are irregularly angular, or contain small densities (granules), or appear as tortuous interstices incompletely separating the granules. Rarely the profile of a particle is roughly circular with a partial rim of unevenly high density which is, however, incomplete. The particles do not contain smooth, linear profiles of even thickness which close upon themselves to enclose a circular or oval area of lower density.

The existence, as well as the morphological, staining, and cytochemical characteristics, of areticular Nissl material raises a number of additional questions the answers to which are less readily evident. They include the questions of: (*a*) the composition of the constituent particles other than their demonstrable content of RNA; (*b*) the origin of the constituent particles; and (*c*) the function of the areticular material, particularly as it may be distinct from that of the reticular material. The data at hand are quite insufficient to support firm answers to any of these questions, but do provide starting points for a number of speculative proposals which can subsequently be tested.

The intensity of the basophilia of the areticular Nissl bodies bespeaks a large content of RNA accessible for dye binding—significantly larger, in fact, than in the reticular Nissl material. The effects of ribonuclease digestion show beyond reasonable doubt that the basophilia of both forms of Nissl material is due entirely to RNA. Finer localization of the RNA within the elements of the two types is, however, another matter. Upon the basis of Palade's (21, 22) correlation of distribution of small dense granules with cytoplasmic basophilia and Palade and Siekevitz's (23, 24, 32) exquisite dissections of hepatic and pancreatic microsomes, it has (26) and continues to seem reasonably safe to extend their concepts to the morphologically similar reticular Nissl material and to localize the RNA in the small dense granular component thereof. A natural sequel is to extend the reasoning by morphological similarity to the small densities which appear as constituents in the structure of some of the particles of the areticular Nissl material. Close scrutiny of appropriately thin sections, however, reveals that even after correction is made for the compactness of the areticular Nissl material, there are still too few equivalents of the small dense granules to account for the remarkably intense basophilia of the material, which approaches that of nucleoli. If, as seems generally acceptable, the cytoplasmic ground substance is excluded as a major locus of RNA it becomes necessary to include some of the regions of lesser density in the particles of the areticular Nissl material as loci of RNA, presumably relatively dispersed. A direct approach to the answer to this problem has thus far been prevented by technical difficulties which have made it impossible to apply RNase digestion to one of a pair of adjacent thin sections prior to electron microscopic examination of both.

Palade (21, 22) has suggested that attachment or non-attachment of small dense granules to elements of the endoplasmic reticulum may reflect

qualitative differences in the small granules and/or differences in various regions of the reticulum. The characteristics of areticular Nissl material seem to accord more with the notion that the difference resides in the RNA-containing elements, particularly in view of the orthochromasia of this material in contrast to the metachromasia of the reticular Nissl material.

Unfortunately, the literature dealing with metachromasia (3, 8, 14, 15, 27, 29) is distinguished by lack of agreement with respect to: (*a*) the limits of the territory enclosed by the term, (*b*) the mechanisms of alteration of light absorption by metachromatically bound dyes, and (*c*) the extent to which specific configuration (other than occurrence of multiple anionic sites) of the dye-binding substrate (chromotrope) determines metachromasia by coupled interaction with the dye. It is generally, although not unanimously agreed, however, that nucleic acids, particularly RNA, are among the more effective chromotropes and that in acid media the presence of protein generally leads to suppression, or quenching, of metachromasia, presumably by competition for anionic sites. It is also generally agreed that metachromasia is due in part to the polymeric state of the chromotrope since its constituent monomers are not in themselves chromotropic.

It seems unlikely that any extensively depolymerized RNA sufficient to produce orthochromasia could survive the procedures of preparation which permit demonstration of the areticular Nissl bodies. Lacking other probable explanations for such orthochromasia it seems not unreasonable to suggest that it may be the result of quenching of metachromasia by the presence of larger proportions of protein bound to the RNA of the areticular Nissl bodies than are present in the ribonucleoprotein granules of the reticular Nissl substance. Precedent for this suggestion is found in the report of Himes (11) that chemical treatment presumed to deaminate proteins resulted in conversion of nucleolar orthochromasia to metachromasia in amphibian and mammalian cells. This possibility is consistent, furthermore, with the observation that longer fixation leads to partial lessening of the densities of the particles of areticular Nissl substance. It is well known (6, 28) that progressive protein extraction results from lengthening fixation times in OsO_4. It may well be, therefore, that the areticular Nissl particles are composites of more or less dispersed RNA and relatively large amounts of closely bound protein. Unfortunately, the histochemical methods for protein have been found thus far to be blocked by osmium fixation. The methods have not yet been applied to ganglia fixed otherwise.

There can be little hope that the origin of the areticular Nissl substance can be settled by electron microscopic examination of sections of fixed tissues. Certain morphological features do, however, shed some light on the matter. The characteristic particles have thus far been found nowhere else in the cytoplasm of these neurons than in the superficially located masses described above. These masses lie very like crusts (Figs. 2, 3, 8) upon the subjacent reticular Nissl material and frequently in close contact with it. The frequent occurrence of tight clusters of small dense granules at the inner boundaries of the areticular Nissl bodies suggests that the particles thereof arise by clustering of small groups of unattached granules of the reticular Nissl material. These clusters are supposed subsequently to become progressively transformed by accumulation of closely bound protein which leads to wider dispersion of the contained RNA and results in diminution of particle density and quenching of metachromasia. It is supposed, furthermore, that these marginal "crusts" may be deposited, beginning close to the cell membrane, by continued transfer of granules from the reticular Nissl bodies, which are crowded centrally as they add to the deep surface of the accumulating areticular Nissl material. The reticular Nissl material, in turn, is assumed to arise in part from the nucleus in these cells, since it is common to find a greatly modified face of the nucleus from which numerous streamers of granule-studded, evaginated outer nuclear membrane protrude fan-like into the adjacent cytoplasm.

The question of function of the areticular Nissl material is the most hazardous one of all to approach, partly because it is inextricably bound to current uncertainties about the functional roles of ribonucleic acids (4, 5, 12, 20, *et al.*) and of granulated endoplasmic reticulum (24, 25, 32, *et al.*). Ribonucleic acids are perhaps most commonly asserted to be intermediary templates linking genetic structures to the specific patterning of protein synthesis. They are also invoked, however, as labile reservoirs of energetic nucleotides directly linked to the energy-yielding metabolic processes, and, more recently, linked in turn to protein synthetic processes within the framework of the template theory. Similarly, the granulated endoplasmic reticula have been advanced as sites of

protein synthesis and accumulation, on the one hand, and as sites of protein degradation (36, 37), on the other. Regardless of the exact roles of RNA and granulated endoplasmic reticulum, however, it is agreed that there are close positive correlations among a cell's content of RNA, its content of small dense granules, and its activity in protein synthesis.

The accumulation of frequently large masses of areticular Nissl material at or close to the surfaces of these cells, their absence in regions of emergence of cell processes or of axosomatic synaptic contact, and the virtual lack within them of all but a rare mitochondrion or other cell organelle, temptingly invite the speculation that these masses are relatively inactive storage depots. If so, they are certainly storage depots of RNA and, if the interpretation of their orthochromasia is correct, also of large amounts of protein already synthesized, but perhaps not yet mobilized from its template RNA.

A different but attractive potential correlate to the similarity of nucleolar and areticular Nissl materials (as opposed to reticular Nissl material) is found in the work of McMaster-Kaye and Taylor (18). They observed that nucleolar RNA may be distinguished from chromosomal and cytoplasmic RNA's in *Drosophila* salivary gland by its sharply different rate of incorporation of P^{32} administered as inorganic phosphate.

Palade has pointed out (21, 22) that attachment of ribonucleoprotein granules to endoplasmic reticulum is associated with highly differentiated cell function, whereas numerous, randomly scattered, unattached ribonucleoprotein granules are characteristic of undifferentiated, proliferating cells. It seems that the apparently complex constituent particles of the areticular Nissl bodies of the sympathetic neurons of *P. cornutum* constitute a third, more specialized member of this family of RNA-containing cytoplasmic moieties.

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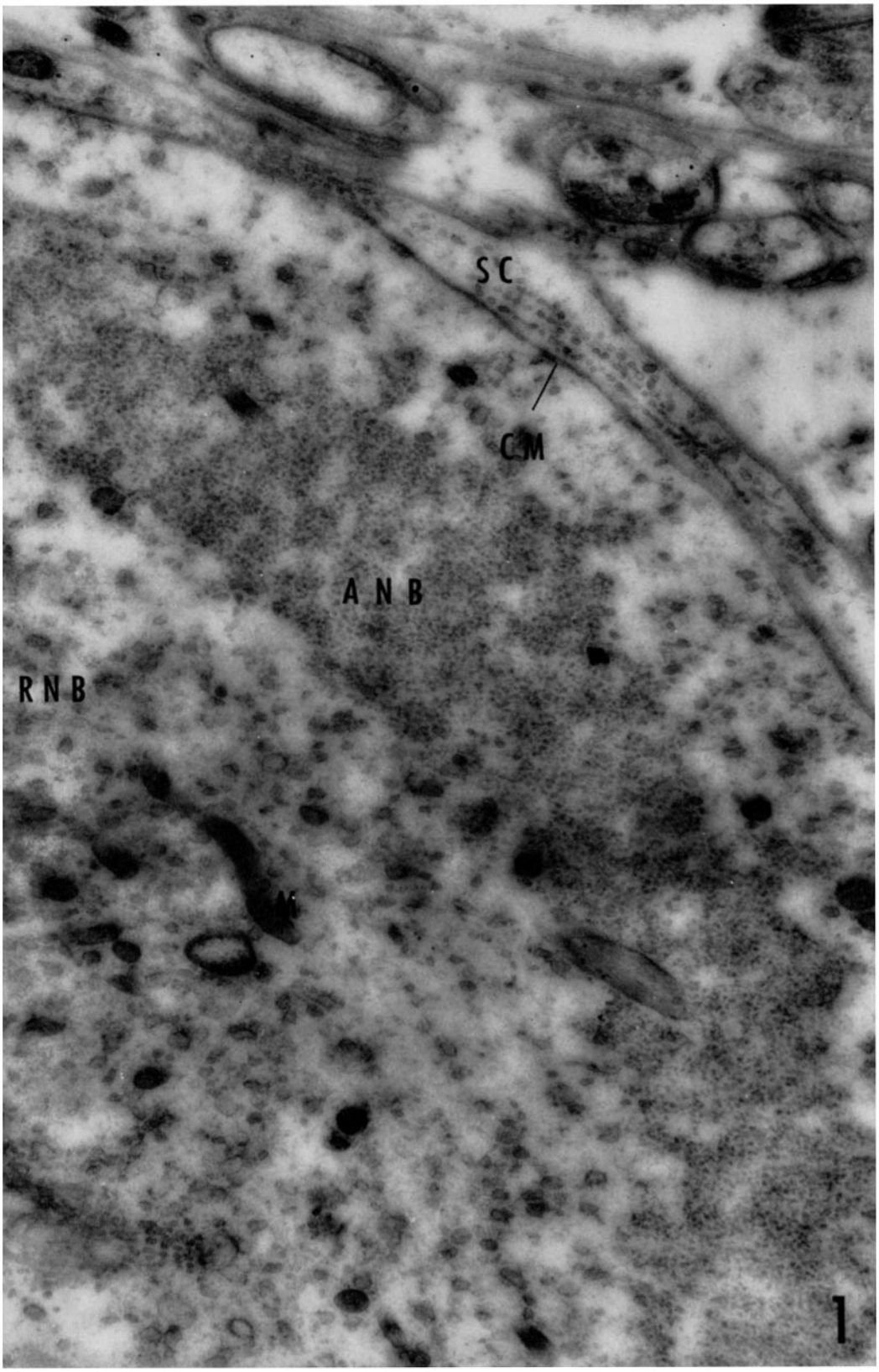
Explanation of Plates

All figures are of "stellate" ganglia of *Phrynosoma cornutum* which were fixed at 0°C. by immersion in veronal-acetate-buffered (pH 7.5–7.6), 2 per cent OsO_4 with sucrose added to 0.273 osmolarity. All tissues were methacrylate imbedded.

Plate 46

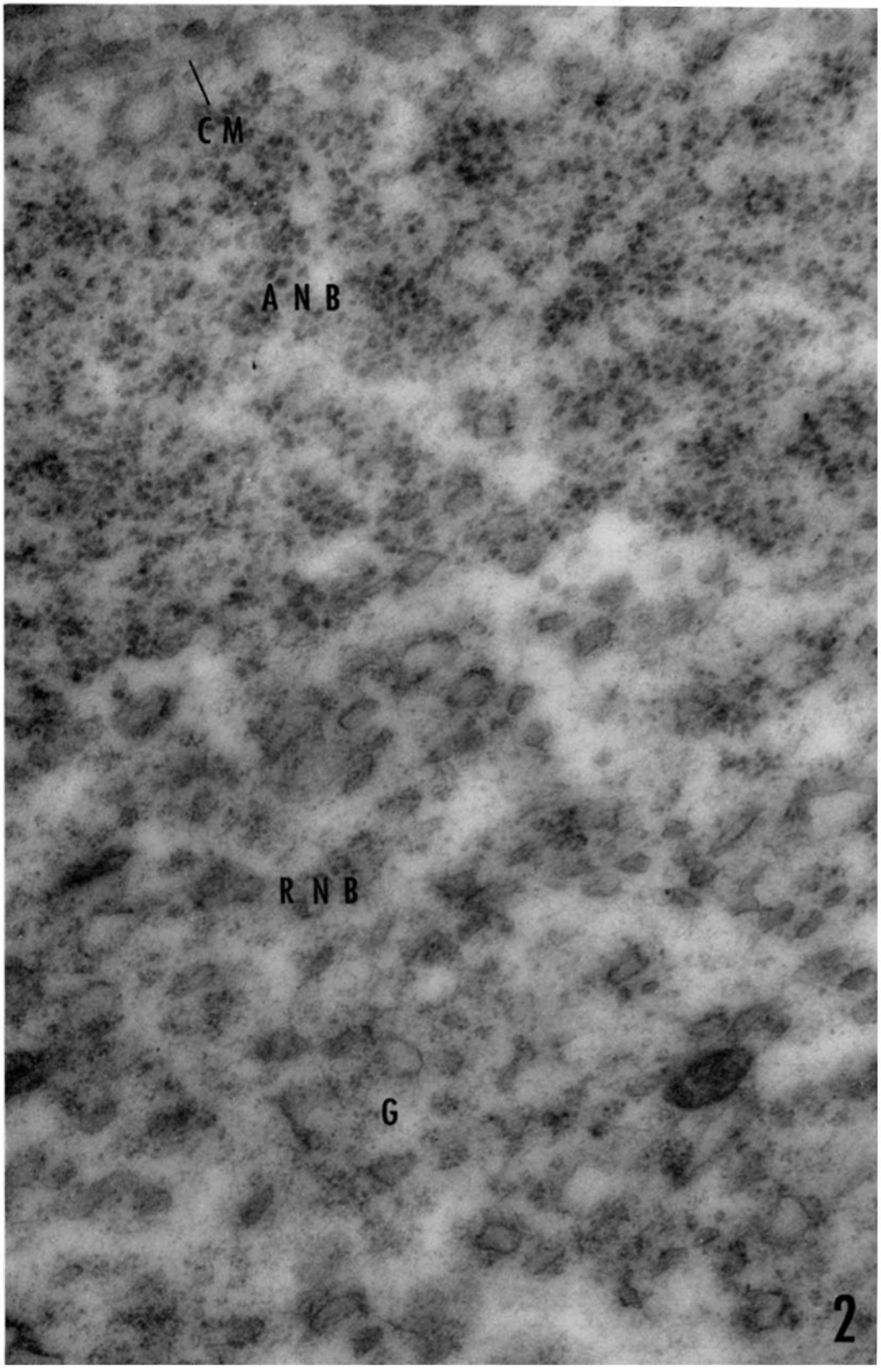
Fig. 1. Portion of perikaryon showing a moderately sized areticular Nissl body (*ANB*) crossing the field diagonally less than 1 μ beneath the cell membrane (*CM*). Elsewhere, the cytoplasm is occupied by scattered elements of reticular Nissl substance (*RNB*) and by the characteristically minute mitochondria (*M*). Covering the cell surface is a thin layer of satellite cell (*SC*) cytoplasm containing serried vesicles. Fixation 1½ hours. Gold section. × 34,000.



(Smith: "Reticular" and "areticular" Nissl bodies)

PLATE 47

FIG. 2. Portion of perikaryon similar to that in Fig. 1 showing an areticular Nissl body (*ANB*) just beneath the cell membrane (*CM*) and an immediately subjacent portion of a reticular Nissl body (*RNB*), in which can be seen the vesicular elements of endoplasmic reticulum to some of which are affixed a few, and between which are scattered many small dense granules (*G*) of ribonucleoprotein. Fixation 1½ hours. Gold section. × 55,000.

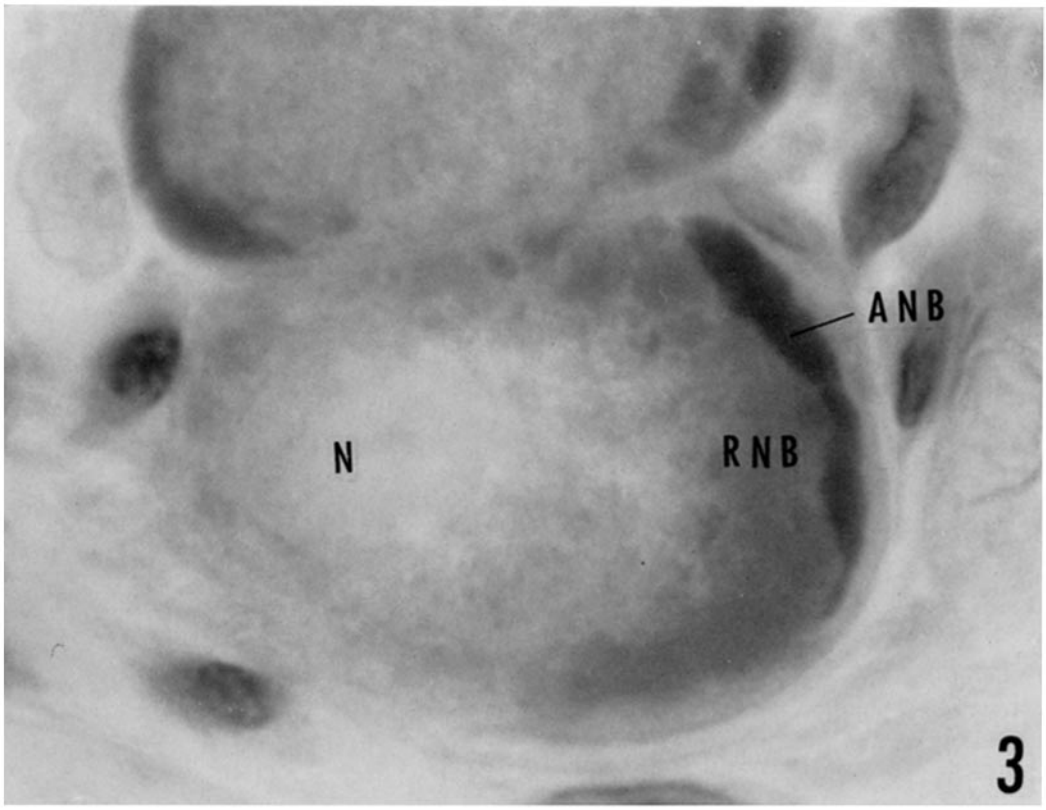


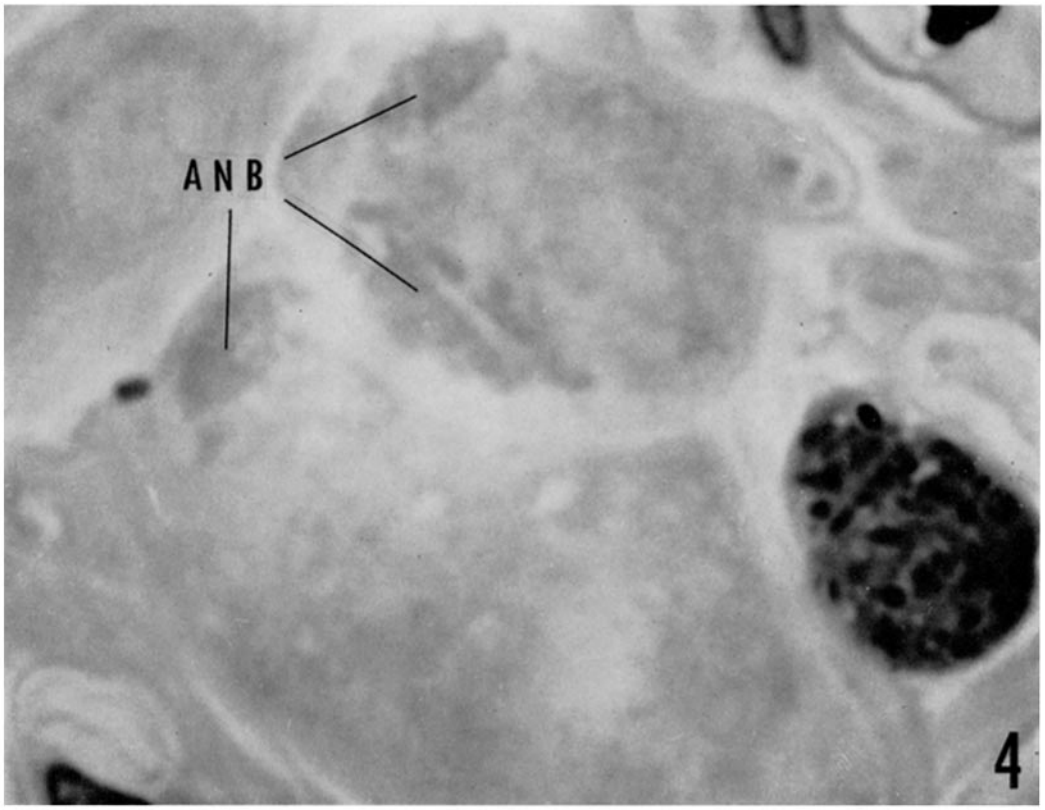
(Smith: "Reticular" and "areticular" Nissl bodies)

PLATE 48

FIG. 3. Section of one perikaryon and part of its neighbor cut at ~3 to 4 μ and affixed to a glass slide. Methacrylate removed in chlorinated ethanes. Stained in 0.05 per cent thionine in 1 per cent acetic acid, dehydrated in acetone, and mounted in HSR. In the centrally located perikaryon a dense, orthochromatic, areticular Nissl body (*ANB*) is seen as a dark crescent beneath the cell membrane at the right. Subjacent to it is a more extensive coalescent series of metachromatic, reticular Nissl bodies (*RNB*) which extend more than halfway around the periphery of the perikaryon. The nucleus (*N*) can be seen indistinctly at the left end of the cell. (The nucleolus is in an adjacent section.) Fixation 1½ hours. × 2,000.

FIG. 4. Section similar to that in Fig. 3, but cut at ~1 μ sequential to that in Fig. 5. The cell in the upper center has been cut almost tangentially through its end in which are located two orthochromatic areticular Nissl bodies (*ANB*), which appear paler than those in Fig. 3 because of the thinness of the section. The cell fits partly into a concavity in the upper surface of the larger cell at the bottom. To the right is a mast cell crowded with intensely metachromatic granules. Fixation 1½ hours. × 2,500.





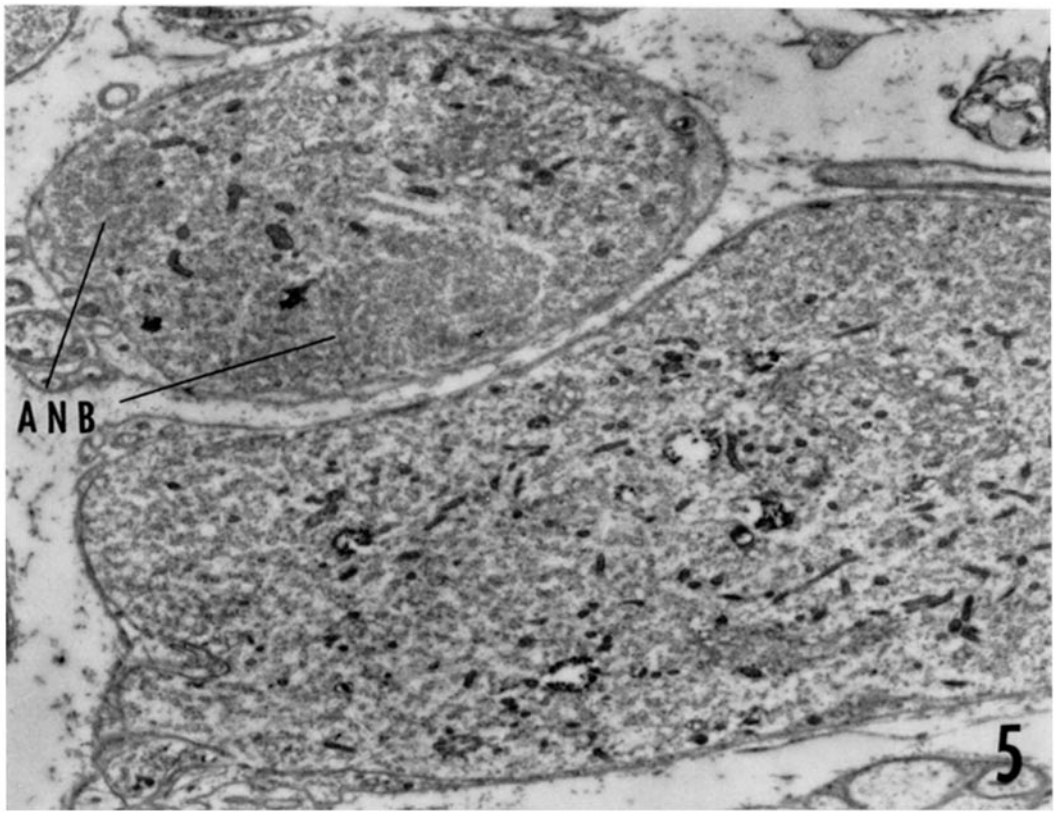
(Smith: "Reticular" and "areticular" Nissl bodies)

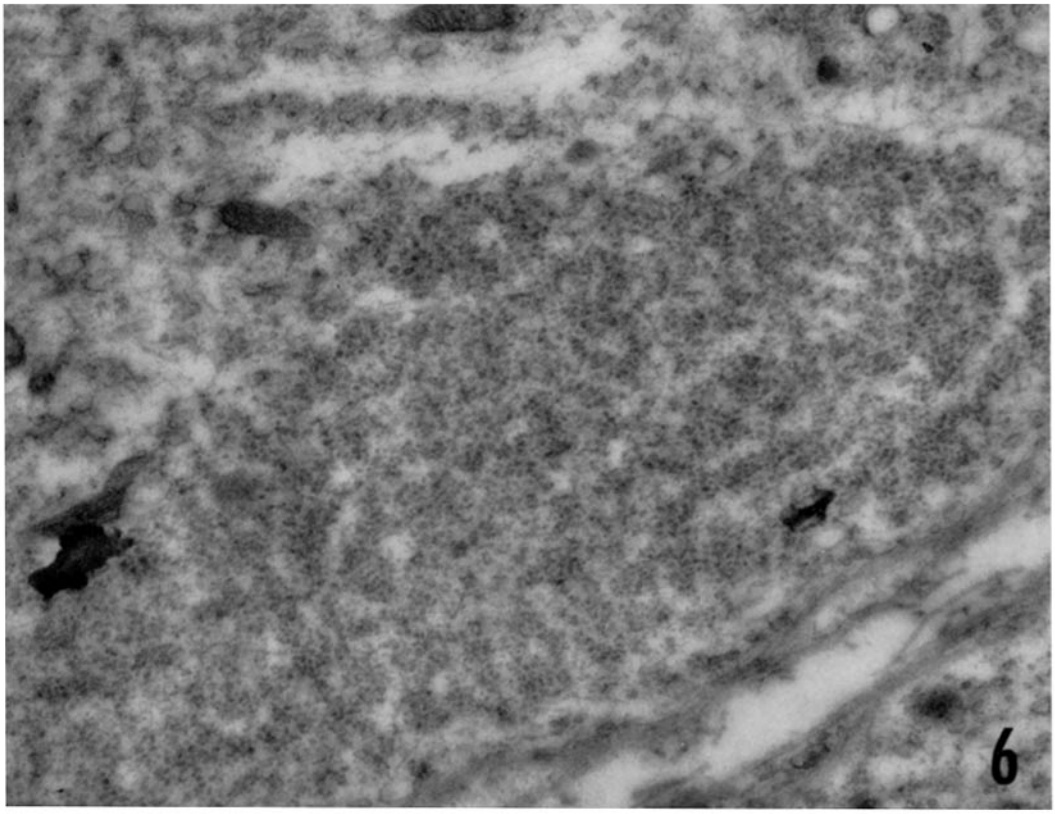
PLATE 49

FIGS. 5, 6, and 7 are all electron micrographs of a single area of the thin (gray to silver) section preceding the thick section shown in Fig. 4. The high magnification micrograph (Figs. 6 and 7) was taken first. Before the low magnification micrograph (Fig. 5) was taken, the specimen was inadvertently exposed to full beam intensity with the resulting noticeable damage. Fixation 1½ hours.

FIG. 5. The same cells pictured in Fig. 4 are seen in this micrograph, but the field of view is rotated approximately 30° counterclockwise, and there is evidence of some sectioning compression along the vertical axis of the picture. The same two areticular Nissl bodies (*ANB*) are labelled in the tangential section of the upper cell. In the upper right corner is the tip of the mast cell seen in Fig. 4. × 4,200.

FIG. 6. Slight enlargement of high magnification micrograph of upper cell of Figs. 4 and 5. The field is occupied by much of the lower of the two areticular Nissl bodies labelled in Fig. 5. Orientation as in Fig. 5. × 25,000.

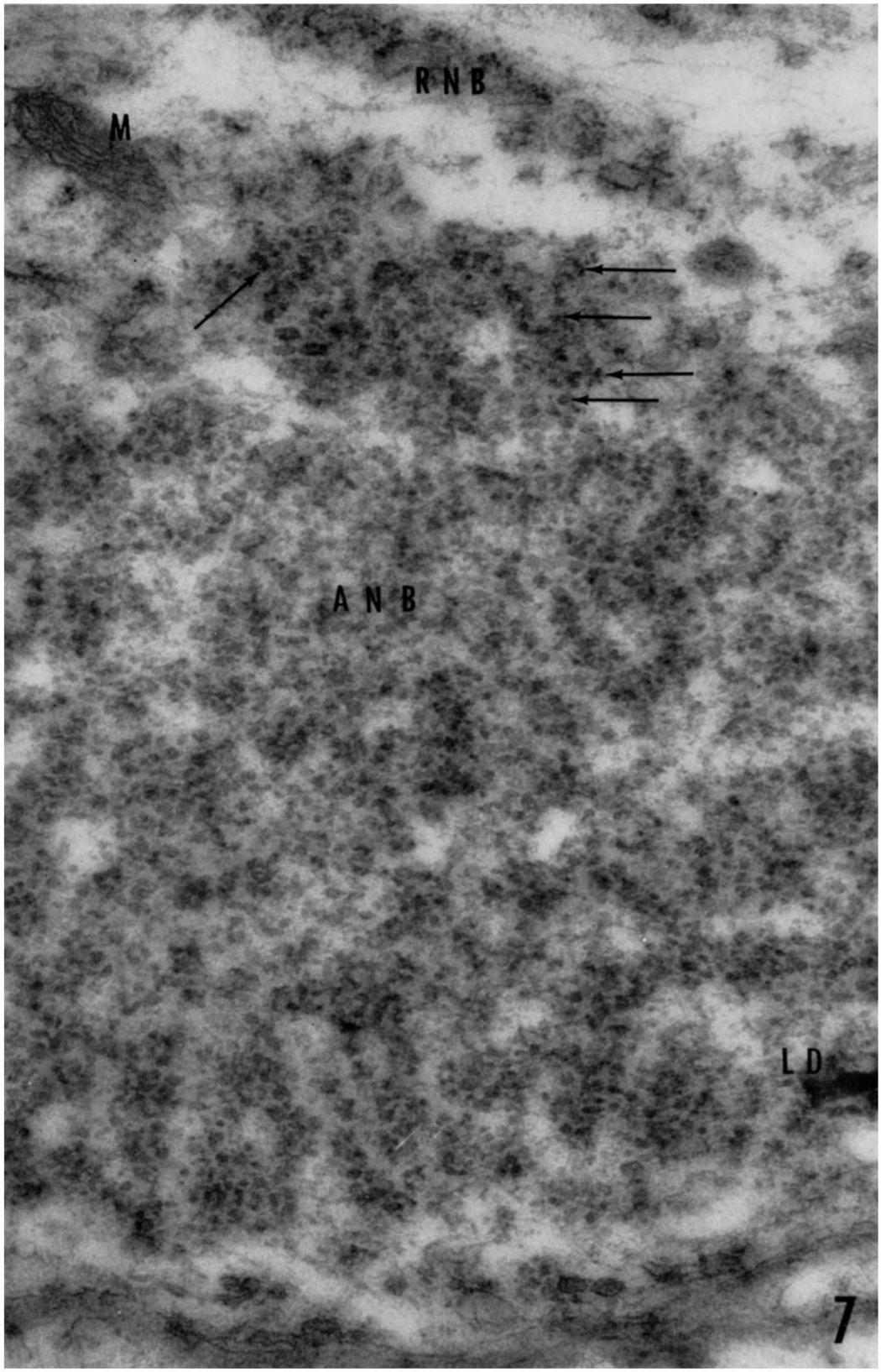




(Smith: "Reticular" and "areticular" Nissl bodies)

PLATE 50

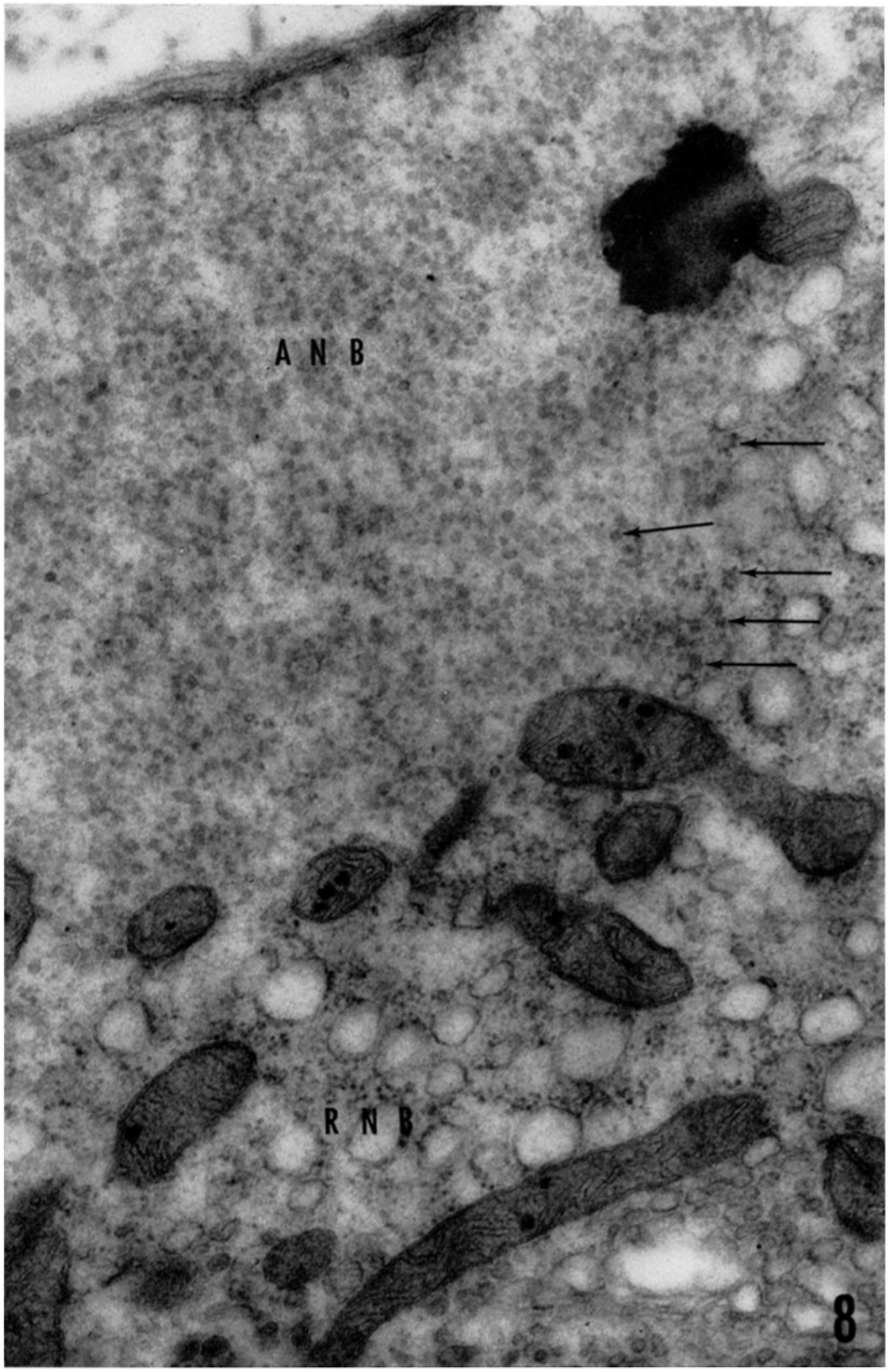
FIG. 7. Further enlargement of micrograph pictured in Fig. 6. Cell membrane cut quite obliquely is seen at bottom, overlaid by a very thin layer of satellite cell cytoplasm. At upper left is a mitochondrion (*M*), and at lower right, a portion of a partially extracted lipide droplet (*LD*). The lower four-fifths of the field is occupied by a portion of the areticular Nissl body (*ANB*) of Fig. 6. Above is the region of transition from a wispy strand of reticular Nissl material (*RNB*). Particularly in the transitional region, tight clusters (arrows) of small dense granules indistinguishable from those of the reticular Nissl material may be seen. Deeper within the mass the constituent particles display less well defined internal densities and a matrix of lower density which, at its lowest, is two to three times that of the cytoplasmic ground substance. × 70,000.



(Smith: "Reticular" and "areticular" Nissl bodies)

PLATE 51

FIG. 8. Area of perikaryon similar to that pictured in Fig. 7, but from tissue which had been fixed 2½ hours. In this particular specimen an unidentified factor resulted in rather marked dilatation of the vesicles of the endoplasmic reticulum. In all other respects, however, the cytoplasmic elements of this specimen are like those in the other ganglia fixed for the same length of time. Note particularly that there is no evidence of enlargement of the constituent particles of the areticular Nissl body (*ANB*), which renders unlikely the possibility that they may contain microvesicles. Note also the general lightening of the cytoplasmic structures, presumably due to leaching of protein. The small dense granules of the reticular Nissl bodies (*RNB*) consequently stand out in greater contrast, as do the mitochondrial membranes. Correspondingly, the particles of the areticular Nissl substance are not only on the average less dense, but also show greater internal contrast of densities. The process of clustering (arrows) of the small dense granules of the reticular Nissl material at its zone of junction with the areticular Nissl material is readily seen in several regions. Gray to silver section. × 70,000.



(Smith: "Reticular" and "areticular" Nissl bodies)