Electron Microscopic Observations on Spinal Ganglion Cells of *Rana pipiens* after Injection of Malononitrile*

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PLATES 30 TO 33

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ABSTRACT

Spinal ganglionic cells of *Rana pipiens* were studied with light and electron microscopes in normal animals and in animals which had received graded dosages of malononitrile intraperitoneally. After treatment no increase in the intensity of staining was noted in the Nissl substance when spinal ganglion cells were examined with the light microscope. The electron micrographs demonstrated the following in malononitrile-treated animals:

1. The cisternae of the endoplasmic reticulum composing the Nissl bodies appeared to fragment and lose their parallel orientation.

2. The microvesicular components of the Golgi complex appeared to increase in number, and the increase was apparently due to fragmentation of the membrane system of the Golgi complex.

3. The mitochondria enlarged and became pleomorphic, but displayed no alterations of internal structure.

The morphological changes may be interpreted as reflections of biochemical alterations.

In 1948 Hyden and Hartelius (14) reported that malononitrile administration was followed by an increase in Nissl substance in spinal ganglion and anterior horn cells of rabbits. Although other investigators (4, 15, 17, and 24) have not confirmed the findings of the above authors, the original report was so convincing that it appeared desirable to investigate the problem with the aid of the electron microscope and to study the alterations in the ultrastructure of neurons after malononitrile administration. It was hoped that such studies would also yield further information on the correlation of function and fine structure of the cells.

Material and Methods

In this study, the grass frog (*Rana pipiens*) was used. As control material, spinal ganglia were removed from ten normal animals; half of these ganglia were fixed in Bouin's fluid and half in 1 per cent OsO_4 buffered at pH 7.6–7.8. Approximately forty animals were divided into four experimental groups. These were injected intraperitoneally, respectively with dosages of 6, 10, 20, and 40 mg./kg. body weight of malononitrile[1] solution. Four hours after the 6 and 10 mg./kg. dosages and 1 hour after the 20 and 40 mg./kg. dosages, spinal ganglia were removed, and half the ganglia from each animal were fixed in Bouin's fluid and half in buffered osmium tetroxide, as were the control specimens. Bouin's fixed tissues were embedded in paraffin, sectioned at 2 μ, and stained with a modified chrome alum hematoxylin stain. The osmium tetroxide–fixed tissues were embedded in methacrylate, sectioned with a Servall Porter-Blum microtome, and examined with a Philips electron microscope (model EM-100 A).

OBSERVATIONS

The perikaryon of the dorsal root ganglion cell of *R. pipiens* displays the same general structural features as extensively reported for the rat neuron by Palay and Palade (23). Figs. 1 and 2 are electron micrographs depicting some of these features. The Nissl body (*nb*, Fig. 1) is shown to be com-

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[1] The malononitrile was obtained from the Nutritional Biochemical Corporation, Cleveland, Ohio.

posed of elongated profiles of granular reticulum (20) arranged approximately parallel. The Golgi complex (*gc*, Fig. 2) is composed of a series of smooth, paired membranes lying close together in a parallel pattern. Adjacent to this membrane complex are microvesicles which vary in diameter. A possible variant of the Golgi complex is seen at *c* in Fig. 1. The cytoplasm contains small oval and elongated mitochondria (*m*, Figs. 1 and 2), displaying the characteristic internal structure described by other investigators (1 and 19). At *a* in Fig. 2 is a type of cytoplasmic body which is found in many of our preparations; it typically displays a dense homogeneous core surrounded by an area of lower density which sometimes contains small granules or vesicles. The composition and significance of this structure are as yet unknown.

A portion of a satellite cell may be seen at *sc* in Fig. 1, in the cytoplasm of which are elongated profiles of a smooth membrane system which is probably the lamellar component of the Golgi complex (*s*, Fig. 1). In addition to the lamellar system, a small vesicular component is observed at *v*, which is similar to the vesicles that De Robertis and Bennett (9 and 10) reported to occur in Schwann and satellite cells of sympathetic ganglia of *R. pipiens* and *R. catesbiana* and in synapses of frog and earthworm. These small vesicles are also similar to those seen by Palade (18 and 21) in the cytoplasm of the endothelial cells of blood capillaries.

In our experiments, intraperitoneal injection of malononitrile was followed by a characteristic alteration in the behavior and color of the frogs. Approximately 10 minutes after injection the animals became moderately sluggish and changed color from the normal green to a dull brown. These changes persisted for 4 hours, which was the maximum time before sacrifice.

Light microscopy of preparations of spinal ganglion cells from animals treated with the 6, 10, 20, and 40 mg./kg. dosage of malononitrile revealed no detectable increase in intensity of staining of the Nissl substance. Commonly observed variations in the Nissl pattern were the same in the experimental neurons as in the controls.

The observations by electron microscopy were as follows:

Four hours after the injection of a 6 mg./kg. dosage of malononitrile, the reticulum of the Nissl substance appeared to lose its orientation and to fragment into numerous circular and oval elements of varying diameters (*er*, Fig. 3). These fragments of the reticulum were studded with small granules and were reminiscent of the "microsomal" vesicles found in tissue homogenates by Palade and Siekevitz (22). Similar changes were observed after the injection of the 10 (*er*, Fig. 4), 20 or 40 mg./kg. dosage (*er*, Fig. 6).

The microvesicular elements (*mv*) of the Golgi complex of the neurons appeared to increase in size after 6 mg./kg. and in both size and number after larger dosages (Figs. 4 and 6). Dilatations within the lamellar portion of the Golgi complex were commonly observed. These dilatations were slight to moderate after the 6, 10, or 20 mg./kg. dosage, but were extreme after the 40 mg./kg. dosage (*d*, Fig. 6).

For the most part, the mitochondria retained their normal membranous structures, but increased in size and in irregularity of shape; both alterations were progressively more marked with increase of dosage of malononitrile (*m*, Fig. 7).

The cytoplasm of the satellite cells in animals which received malononitrile contained many small, oval or irregularly shaped vesicles as well as a number of large vacuoles, which seemed to be related to the Golgi complex of these cells.

DISCUSSION

After treatment with malononitrile, alterations in the ultrastructure of the spinal ganglion cells of the frog were fairly well defined, especially in terms of the disorganization of the structure of the Nissl bodies, the vacuolation of the Golgi complex, and the enlargement and pleomorphism of the mitochondria.

It is impossible to decide whether the observed changes were due to malononitrile's acting directly upon the cytoplasmic components of the neurons, or indirectly *via* interference in the general metabolism of the animal, or both directly and indirectly.

Nissl Substance.—Our staining procedures did not show an increase in basophilia of the neurons after malononitrile treatment, nor a discernible change in the commonly observed variety of patterns of the Nissl bodies, in light microscopy. The alterations observed in fine structure did not include a great increase in Nissl substance, though the amount present can only be estimated from the two dimensional electron micrographs. Our results are, therefore, in disagreement with the observation of Hyden and Hartelius (14), that malononitrile administration to rabbits is followed by an increase in Nissl substance in the neurons.

However, in noting this *disagreement*, it must be mentioned that the results compared were obtained with different methods and materials. Basophilic staining is a fairly reliable criterion, but is, of course, different from the ultraviolet absorption criterion employed by Hyden and Hartelius. It is possible that the nucleic acid may have increased after malononitrile administration, as reported by Hyden and Hartelius, but that it may have occurred in such a form that it was not demonstrable in either the stained preparations or electron micrographs. With regard to differences in materials, Mendelson *et al.* (17) has previously pointed out from their spectrophotometric analysis that the malononitrile used by Hyden and Hartelius was not the same as that used by American investigators, that the Swedish preparation contained a different substance, and that this substance appeared in a solution of the American product only after aging of the solution. The solution of malononitrile used in our study was freshly prepared from the American product. If differences exist between the activities of fresh and old solutions, it would be important to compare the fine structure of the perikaryon after the injection of aged solutions. It would be further advisable to follow the same course of study using the Swedish preparation.

Whether the fragmentation of the reticulum of the Nissl bodies impaired or enhanced their function is not ascertainable from our findings. However, our data suggest possible agreement with the views of Commoner (4), who speculated that malononitrile induces a disorganization of the molecular orientation in Nissl substance, which, he claimed, reached a high degree in normal neurons. As a consequence of this disorganization, according to Commoner, the ultraviolet absorption coefficient of Nissl substance is spuriously increased after malononitrile. We do not imply that the Nissl granules (fine particulate component), as seen with the electron microscope, are nucleic acid molecules, but it does seem reasonable to consider them to be highly oriented multimolecular aggregates.

Golgi Complex.—After treatment, there was an increase in both size and number of Golgi microvesicles in the perikarya of the spinal ganglia, as well as dilatation of the paired membranes of the Golgi complex. The increase in number of the microvesicular elements is possibly the result of repeated constrictions of the terminals of the paired systems, and perhaps also over the expanse of the paired membrane component. It may be supposed that this is the mechanism of formation of the microvesicles in untreated ganglion cells, and that the procedure is increased in rate after malononitrile administration.

Except for the extreme dilatation after high dosage levels, it is possible that the vacuolation of the Golgi system after malononitrile treatment is not an abnormal situation, though it rarely appears in normal neurons. Golgi vacuoles do commonly appear in other cells (1–3, 7, 8, and 11) and seem to represent an accompaniment of physiological activity and cyclic functioning.

After treatment with malononitrile, the satellite cells were observed to be packed with much greater than normal numbers of small oval vesicles. Consistent with the stimulation of the Golgi system in the neurons would be the interpretation that the Golgi system in the satellite cells is also stimulated to produce more vesicles. This apparent increase in the vesicular component may also be viewed as a result of enhanced submicroscopic pinocytosis (21).

Mitochondria.—Malononitrile treatment also was followed by an increase in size and by varying degrees of pleomorphism in mitochondria. This is apparently a functional change, and not an osmotic swelling. From light and electron microscopic studies, a number of investigators have reported morphological changes of mitochondria after treatment with mitotic inhibitors (16), cytochrome inhibitors (5, 6, 12, and 25), or cortisone (13). It is possible, therefore, that changes in size and shape of mitochondria found in neurons of malononitrile-treated frogs may reflect induced biochemical alterations.

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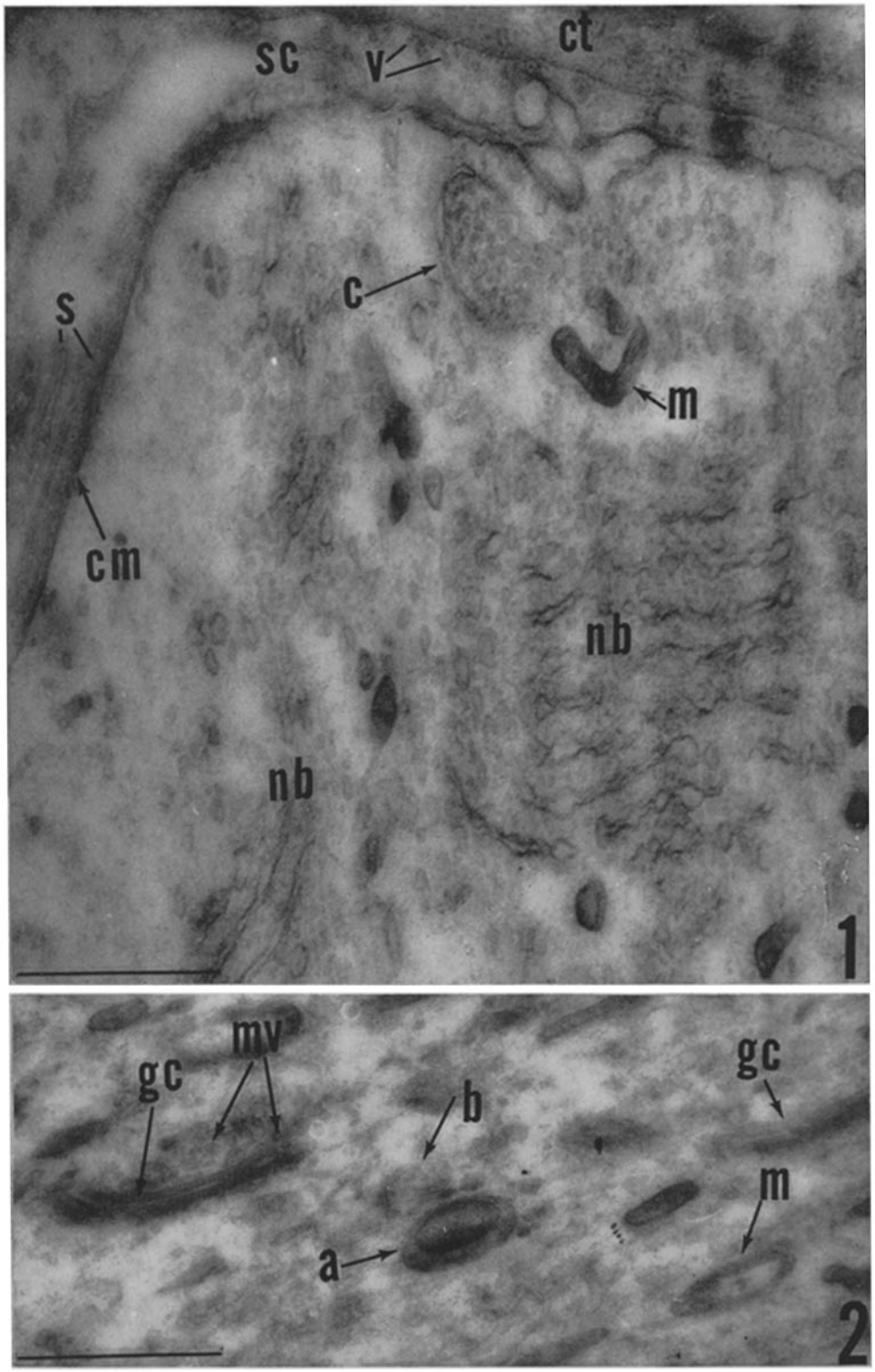
Explanation of Plates

The line in the lower left corner of each figure represents one micron.

Plate 30

Figs. 1 and 2. A small field of the cytoplasm of normal spinal ganglion cells, showing the orientation of the cisternae of the granular reticulum of the Nissl body (*nb*, Fig. 1). Profiles of the Golgi complex at *gc*, microvesicles, *mv* (Fig. 2), mitochondria (*m*), and a body (*b*) consisting of a thin membrane (*b*) enclosing smaller vesicles may be observed. A possible variant of the Golgi complex is seen at *c* (Fig. 1). The cytoplasm also contains a body at *a* (Fig. 2), the identity of which is unknown. The cell membrane is labelled *cm*.

A portion of a satellite cell is labelled *sc* (Fig. 1). A vesicular component at *v* and the lamellar components of the Golgi complex (*s*) are evident in the cytoplasm of this cell. Note fibrils of connective tissue at *ct* (Fig. 1). ×30,000.

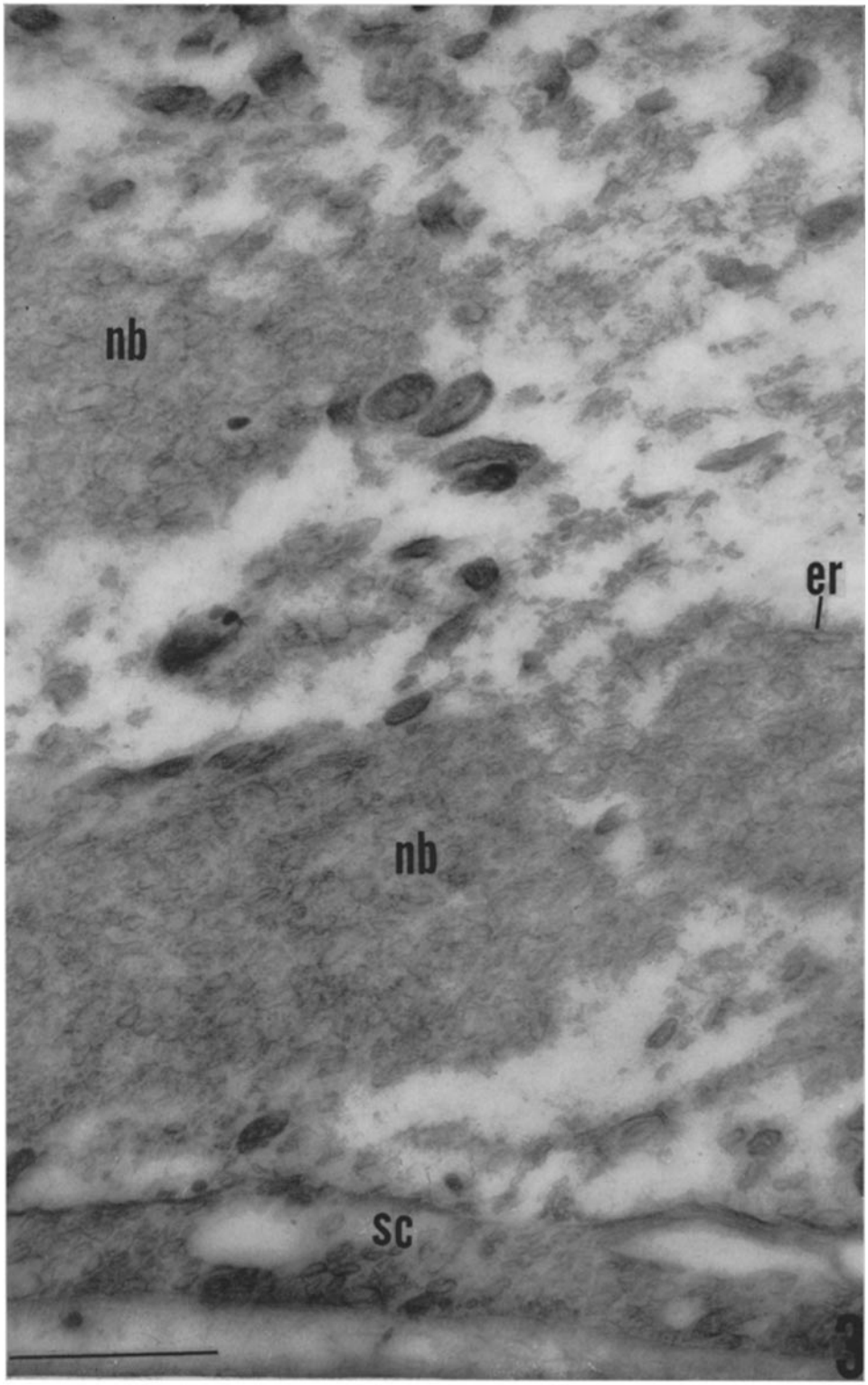


(Anderson and van Breemen: Spinal ganglion cells of *Rana pipiens*)

PLATE 31

FIG. 3. Section showing the organization of the Nissl bodies (*nb*) after 6 mg./kg. dosage of malononitrile. Note the many vesicular elements of the granular reticulum of the Nissl bodies and the remnants of the original orientation at *er*.

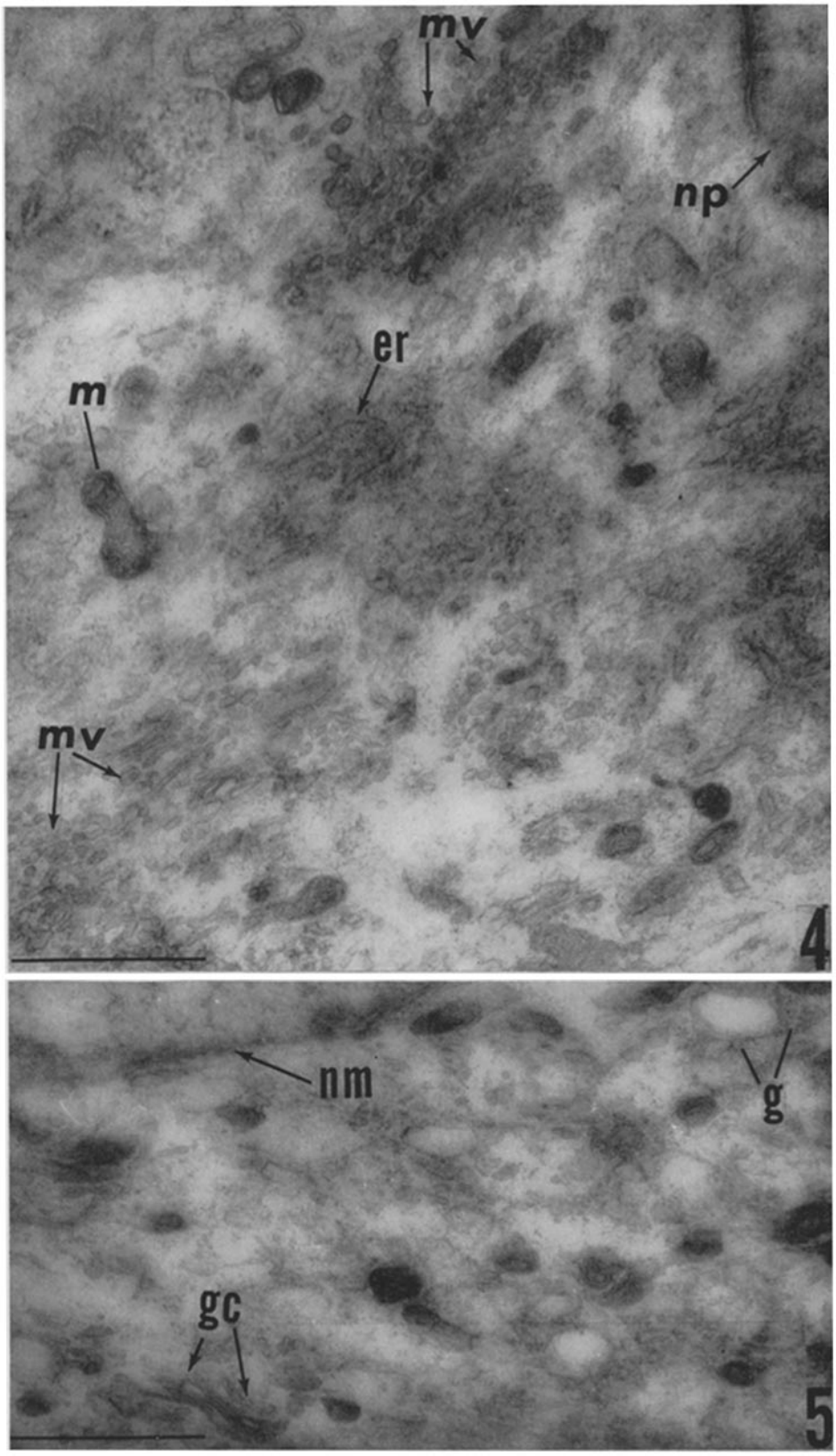
At the bottom of the figure the satellite cell cytoplasm (*sc*) is seen packed with many oval and elongated vesicles as well as three large vacuoles. ×30,000.



(Anderson and van Breemen: Spinal ganglion cells of *Rana pipiens*)

PLATE 32

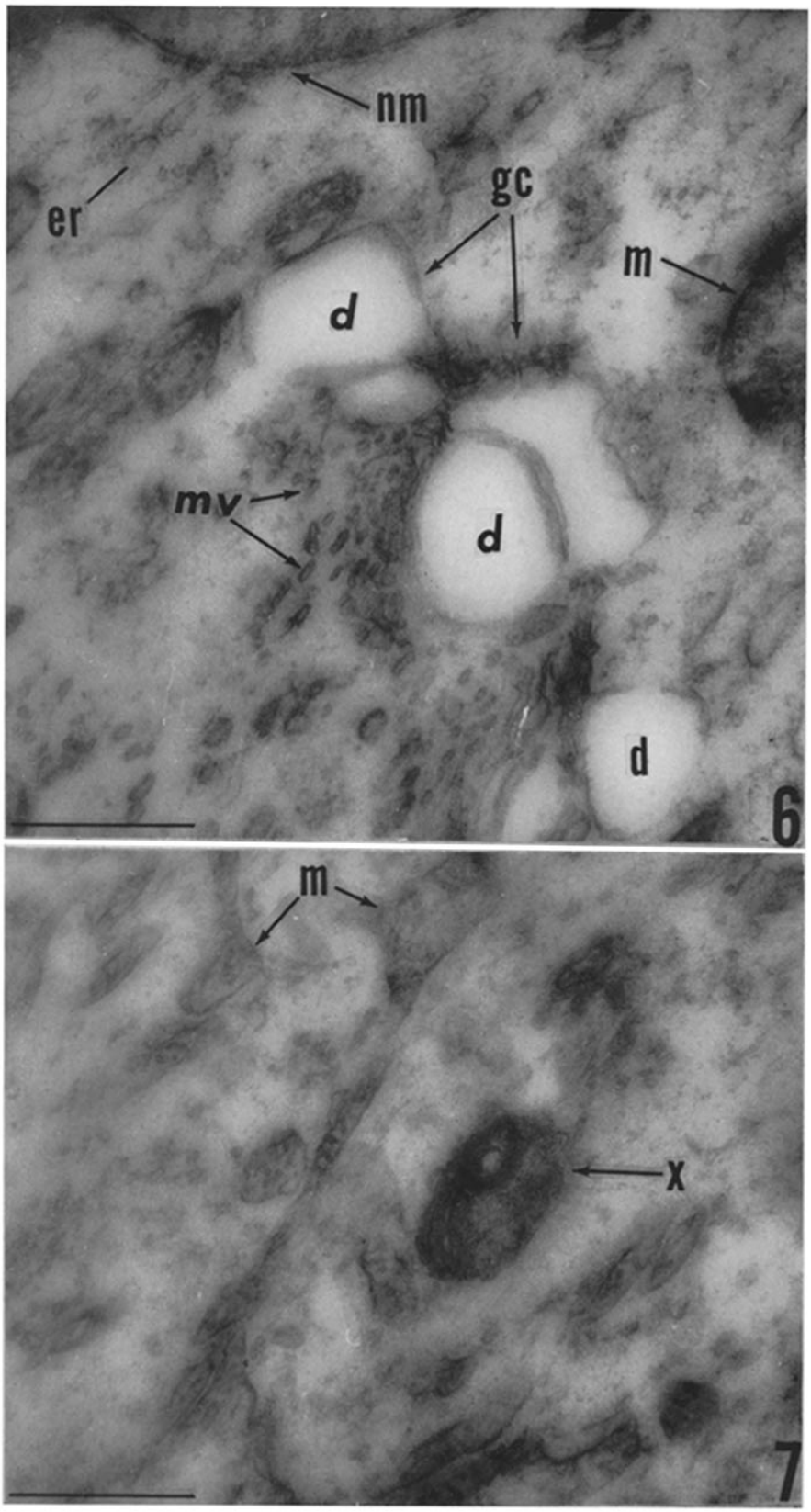
FIGS. 4 and 5. Sections of a portion of the cytoplasm after a 10 mg./kg. dosage, showing profiles of mitochondria (*m*, Fig. 4), the Golgi complex (*gc*, Fig. 5), and microvesicles (*mv*, Fig. 4). The nuclear membrane is shown at *nm* (Fig. 5), and what appears to be a nuclear pore at *np*. (Fig. 4). Note the profiles of the endoplasmic reticulum (*er*, Fig. 4) and the dense granules surrounding a larger vesicular element *g* (Fig. 5). ×30,000.



(Anderson and van Breemen: Spinal ganglion cells of *Rana pipiens*)

PLATE 33

FIGS. 6 and 7. Small fields showing the disorganization of cytoplasmic components after a 40 mg./kg. dosage. Profiles of the Golgi complex at *gc*, with conspicuous dilatations (*d*) within the lamellar component and many microvesicles (*mv*). The nuclear membrane is seen at *nm* (Fig. 6), and profiles of mitochondria at *m* (Figs. 6 and 7). Note the irregular shape of the mitochondrion at the right of the arrow labelled *m* in Fig. 7. At *x* (Fig. 7) is seen a body which appears to be either a degenerating mitochondrion or remnants of a dense body like the one abelled *a* in Fig. 2. ×30,000.



(Anderson and van Breemen: Spinal ganglion cells of *Rana pipiens*)