THE FINE STRUCTURE OF SYMPATHETIC NEURONS IN X-IRRADIATED FROGS

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ABSTRACT

The effects of whole body x-irradiation on the fine structure of sympathetic neurons were studied in 15 unanesthetized adult frogs (*Rana pipiens*), as seen at intervals ranging from 1 hour to 2 weeks after single exposures to 1000 r and 2000 r. Using standard procedures, the lumbar sympathetic ganglia of experimental and 20 control animals were prepared for electron microscope examination. Radiation produced conspicuous but irregular and variable deterioration, swelling, and clearing of neuronal lysosomes. These changes may have been due to an increased permeability of lysosomal membranes, causing the entry of fluid into lysosomes and their swelling and deterioration, but a pronounced escape of lysosomal enzymes into the cytoplasm was questionable. Less frequent were the dilatation and the parallel layering or complete fusion and tight packing of the rough-edged endoplasmic reticulum. The number of vacuoles, probably derived from Golgi cisternae, was somewhat increased. These vacuoles were conjectured to serve the "sequestration" of damaged cytoplasmic areas. Abnormal amounts of presumptive glycogen granules occupied some axons of myelinated and unmyelinated fibers, especially of presynaptic nerve fibers. This was assumed to be due to a decreased breakdown of glycogen and probably caused the interruption of the transmission of nerve impulses in presynaptic fibers. The maximal incidence of these alterations seemingly occurred 8 days after exposure to 1000 r, and 1 hour after x-irradiation with 2000 r. Signs of recovery appeared 2 weeks after exposure to 2000 r.

INTRODUCTION

The few reports on the influence of ionizing radiation on the fine structure of nervous tissue have been based chiefly on studies of the central nervous system (16, 17, 20, 30, 41, 44, 52, 53). The peripheral nervous system has received even less attention. The fragmentation of the ergastoplasmic channel system in the axon and the degeneration of the Schwann cells have been observed in the sensory nerve fibers of frog skin after irradiation with Po^{210} particles (24). A detailed description has been given of the changes which occur in the fine structure of the neuronal and non-neuronal components of the spinal root ganglion of the rat after exposure to 185 Mev protons (2–5). The effect of radiation on the ultrastructure of autonomic nerves has never been studied. A search for radiation-induced submicroscopic alterations in sympathetic neurons seemed promising, because clinical (28, 46, 54), electrophysiological (6, 18, 19), and cytochemical (48) observations have suggested the occurrence of subtle changes in the structure of irradiated nerves.

MATERIALS AND METHODS

Fifteen adult frogs (*Rana pipiens*) of both sexes were kept in quarantine for 1 week, according to previously described precautions (37), to ensure that only healthy animals were used in this study. Total

body x-irradiation was administered to these animals by a Picker therapeutic x-ray machine run at 220 kvp and 20 ma, at a target distance of ±48 cm to the center of the body. Inherent filtration of the tube was the glass equivalent of 0.25 mm Cu and the oil equivalent of 1.0 mm Al. External filtration included 0.5 mm Cu and 1.0 mm Al. The half value layer was equivalent to 1.1 mm Cu. The dose delivered (as measured by a Victoreen dosimeter) was 34.5 RPM in air, to the center of the body. Unanesthetized frogs were put in individual plastic containers and placed on a rotating disc in such a manner that they were equidistant from the center of the beam. A single dose of 1000 r was delivered to 6 frogs, and another group of 9 frogs was exposed to a single dose of 2000 r. Pairs of frogs irradiated with 1000 r were sacrificed at 1 hour, 2 days, and 8 days, and those irradiated with 2000 r, at 1 hour, 2 days, 3 days (1 animal only), 8 days, and 2 weeks after exposure. After these periods, the animals were anesthetized by injections of 2 ml of a 10 per cent solution of urethane into their dorsal lymph sacs. Subsequently, the lumbar sympathetic trunks of both sides were exposed, but left *in situ*, immersed in an ice cold 1 per cent solution of osmium tetroxide buffered with 0.1 M phosphate at pH 7.8, and kept in the refrigerator for 15 minutes. Thereafter, individual sympathetic ganglia and rami were excised, placed in the same fixative, and refrigerated for 2 hours more. This material was dehydrated in graded alcohols and embedded in Epon (29). Ultrathin sections were cut from 70 blocks of this material, on the LKB and Huxley ultramicrotomes, using glass and diamond knives. The sections were placed on Formvar-coated or uncoated copper grids, stained with lead hydroxide or lead acetate, examined with a Siemens Elmiskop I microscope, and studied on 1500 electron micrographs. Previously reported studies (38, 39) of the fine structure of sympathetic neurons of 20 non-irradiated frogs, which had been fixed in 1 per cent osmium tetroxide buffered with Veronal-acetate or 0.1 M phosphate, and had been embedded in methacrylate or Epon, served as controls. Ultrathin sections from 98 blocks of this material were examined with the electron microscope and studied on 2000 electron micrographs.

OBSERVATIONS

The exposure to x-rays did not produce any obvious changes in the vigor or behavior of the 15 frogs before they were sacrificed for electron microscope examination. The most striking effect seen microscopically involved those structures in the sympathetic perikaryon which were previously referred to as "large inclusion bodies" (39). Although distinct from other well characterized subcellular structures, these particles varied morphologically to such an extent that their identification by constant anatomical criteria became difficult. Nevertheless, all these bodies possessed a single limiting membrane which surrounded a polymorphic content. Particles of like appearance, now known as lysosomes, were first observed in liver and later also in other cell types (31), and were shown to contain a number of hydrolytic enzymes (13, 14). The cytochemical identification of these structures was made primarily by cell fractionation studies (8, 13, 33). Using specific staining procedures, the activity of some of these enzymes, notably that of acid phosphatase, was demonstrated in these particles on ultrathin tissue sections with the electron microscope (15, 21, 32, 45). "Dense bodies" of similar morphology and biochemical composition also occur in the neurons of the central nervous system (34–36) as well as in sympathetic neurons of the frog (49). On the basis of this evidence, the "large inclusion bodies" (39) were tentatively interpreted as lysosomes and are referred to as such in this study, although some of these particles may have differed cytochemically from lysosomes.

Normally, lysosomes of round or oval shapes and surrounded by single membranes were scattered as single structures or in small groups throughout the sympathetic perikaryon (Fig. 1). Larger aggregates were extremely rare (39). These particles varied in texture and size, in that their interiors consisted of variously arranged, densely packed membranes and/or of well circumscribed homogeneous areas of varying electron opacity. Their sizes ranged from 0.22 by 0.4 μ to 0.67 by 0.88 μ. Irrespective of these differences, lysosomes in sympathetic perikarya of non-irradiated animals gave the impression of being compact bodies, although a few had small cracks or were slightly disrupted (Fig. 1).

Whole body x-irradiation produced a wide range of changes in the distribution, texture, and size of lysosomes. Those with relatively slight structural alterations retained their normal sizes (0.3 to 0.78 μ) and pattern of distribution, but others which were severely disrupted and enlarged to 1.4 μ along their greatest diameters tended to form conspicuous conglomerations (Figs. 2 and 3). The type and degree of lysosomal deterioration varied greatly (Figs. 2 and 3). Some lysosomes had one or several clefts, which also occurred normally, but their dense content was broken up abnormally into several irregular pieces (Fig. 4 *a*). Or, the dense material retained

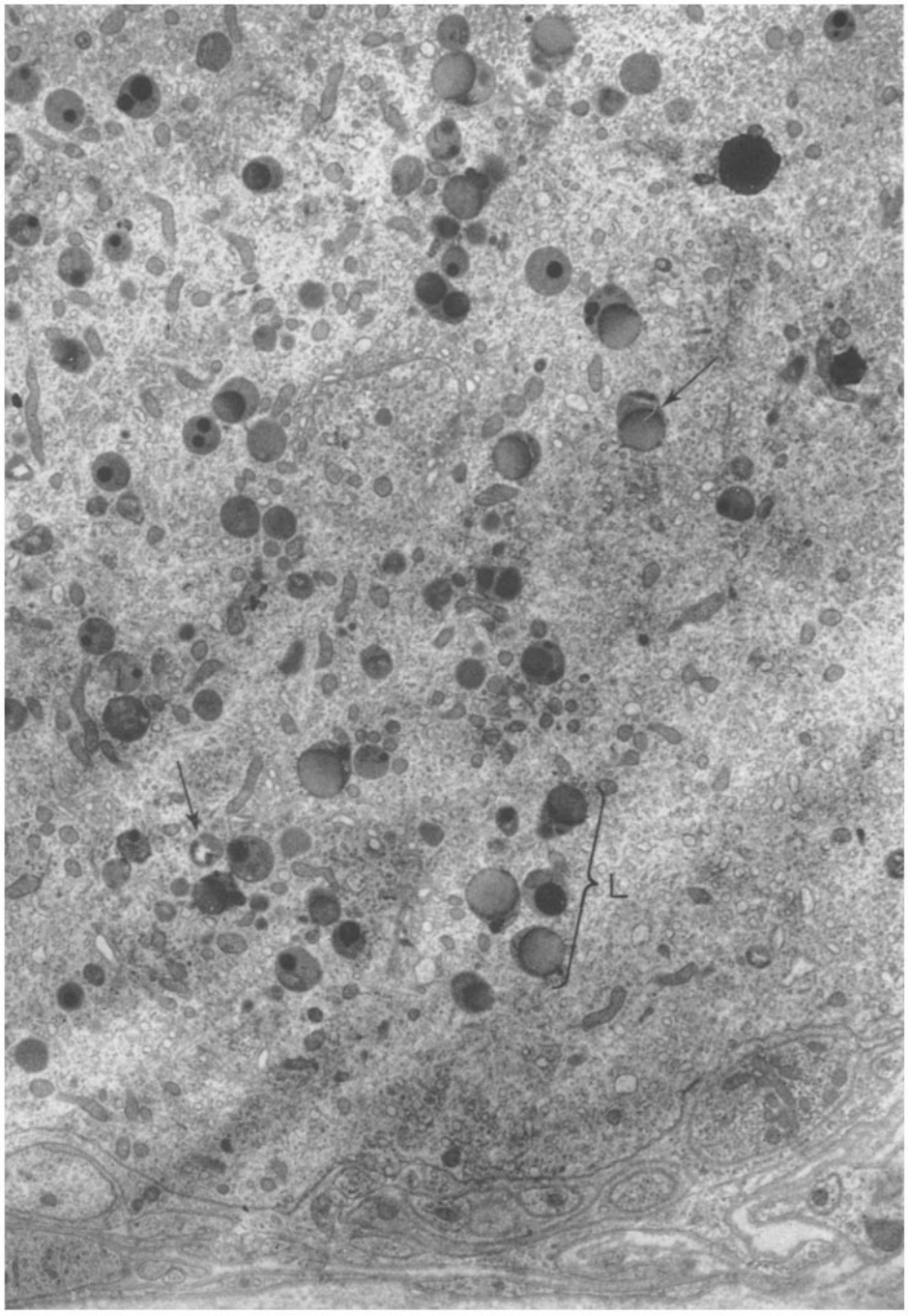


FIGURE 1 Sympathetic perikaryon of a normal adult frog. Note the round or oval shape and variable texture of lysosomes (*L*) and related structures which are scattered throughout the cytoplasm as single structures and in small groups; the largest of these particles measured 0.79 μ in diameter. A few of the structures (arrows) are slightly cracked or disrupted. × 10,000.

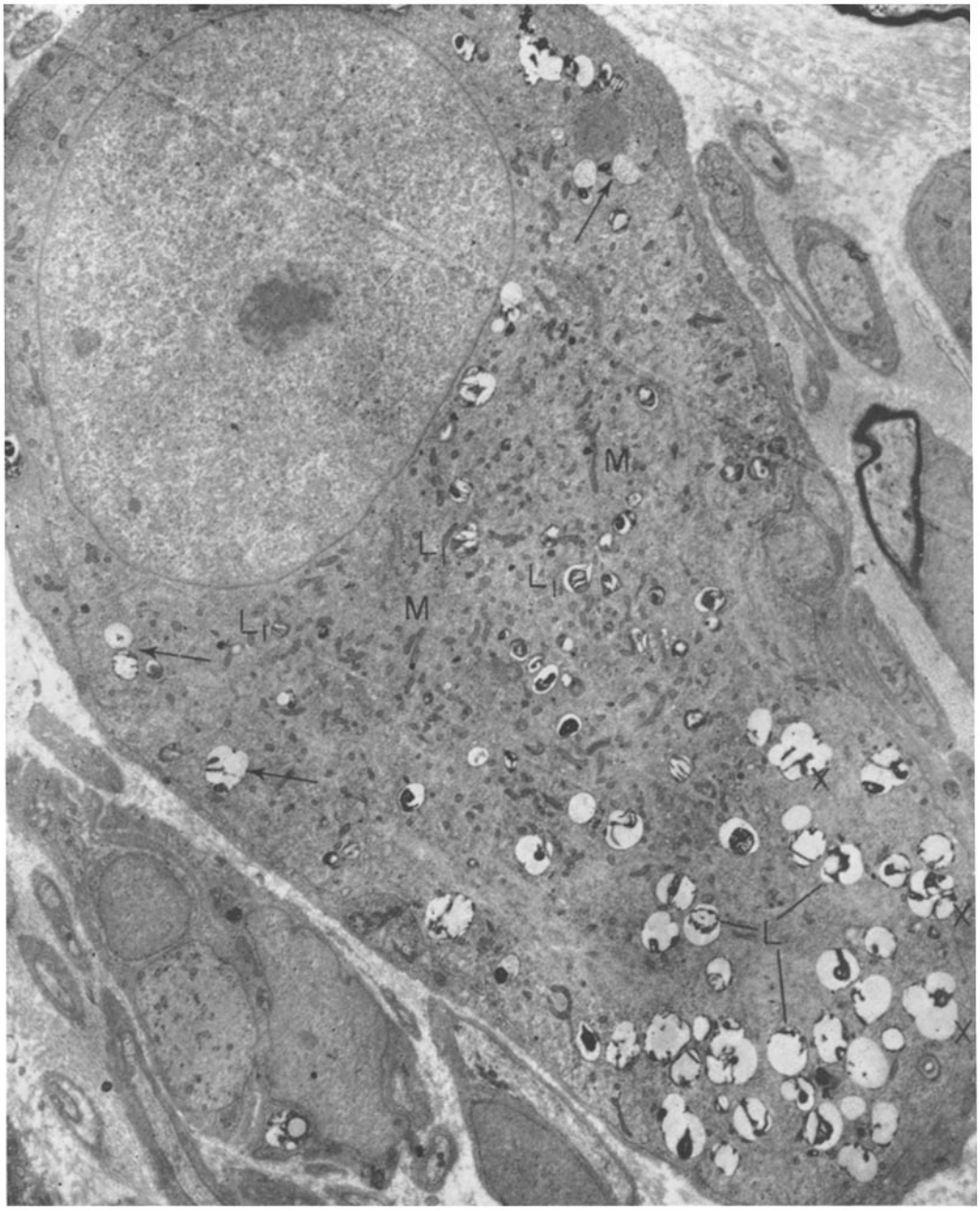


FIGURE 2 Sympathetic ganglion cell of an adult frog, 1 hour after x-irradiation with 1000 r. Note a cluster of variously enlarged lysosomes (L) at the bottom of the figure; some of these particles contain irregularly shaped debris, whereas others are almost entirely clear. Similar lysosomes also occur as single structures or in small groups in other areas of the perikaryon. Enlarged lysosomes with scalloped outlines (X) probably result from the conflux of several disintegrating lysosomes. Still other lysosomes (L_1) are less conspicuously disrupted and enlarged. The sizes of these lysosomes range from 0.3 by 0.3 μ to 0.5 by 1.5 μ (arrows). Note the normal appearance of mitochondria (M). × 6000.

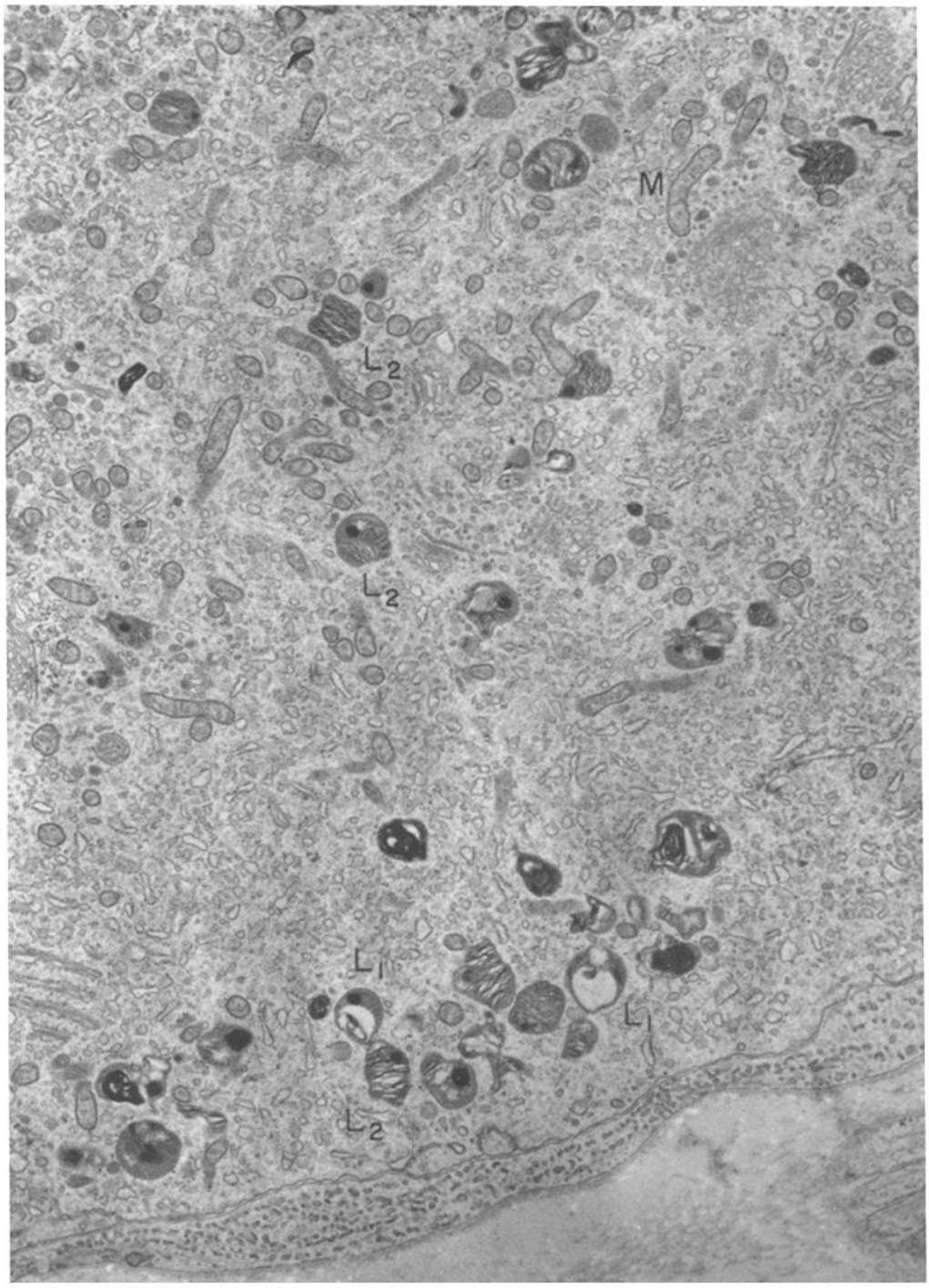


FIGURE 3 Sympathetic perikaryon of an adult frog, 1 hour after x-irradiation with 2000 r. Note the small aggregate of moderately enlarged lysosomes at the bottom of the figure: some of these organelles have a clear center capped by a crescent of normal looking dense material (L_1); in others (L_2) the disruption into loosely arranged lamellae is more pronounced. Lysosomes of type L_2 also occur singly in other areas of the perikaryon. The sizes of these lysosomes ranged from 0.5 by 0.43 μ to 0.5 by 0.68 μ. Mitochondria (M) appear normal. × 14,000.

its normal appearance, but the central lamellae were of abnormal, loose arrangement, and the entire corpuscle became enlarged (Fig. 4 *b*). Disruption and disintegration of central lamellae of certain lysosomes progressed, with concomitant enlargement of the entire particle, although the central electron-opaque material still looked normal (Fig. 4 *c*). More severely deteriorated lysosomes were enlarged, had only small remnants of lamellae near the periphery, and consisted otherwise of a moderately dense, finely granular material; the opaque core, though still sharply delineated, also showed signs of disruption as judged by its mottled appearance (Fig. 4 *d*). In extreme cases, the swelling and disintegration of the entire central material had simultaneously occurred. These particles formed large bags, some of which still contained a variable amount of debris, while others had completely clear centers (fig. 2). There was also evidence that several disintegrating lysosomes had merged to form large corpuscles, characterized by their scalloped outlines (Fig. 2). In still other lysosomes, radiation produced peculiar changes of the central dark material. The opaque center became elongated, with or without concomitant changes in size and texture of the lysosomes (Fig. 4 *e*). In extreme cases (Fig. 4 *f*), this elongated dark material extended in form of a rod beyond the organelle, and was surrounded by the evaginated lysosomal membrane. Bipolar extensions of the dark central material also occurred in certain lysosomes (Fig. 4 *g* and *h*). Finally, there were abnormally large lysosomes, measuring 1.05 by 1.44 μ, in

FIGURE 4 Various types of submicroscopic changes of lysosomes in frog sympathetic neurons following x-irradiation.

FIG. 4 *a*. Lysosomes, measuring 1.02 by 0.78 μ and 1.09 by 0.65 μ, showing small clefts and irregularly disrupted dense material. Two days after x-irradiation with 2000 r. × 32,000.

FIG. 4 *b*. Lysosome with moderately loose arrangement of its lamellae and normal appearance of the central dense material, but enlarged to a size of 1.4 by 1 μ. Two days after x-irradiation with 2000 r. × 32,000.

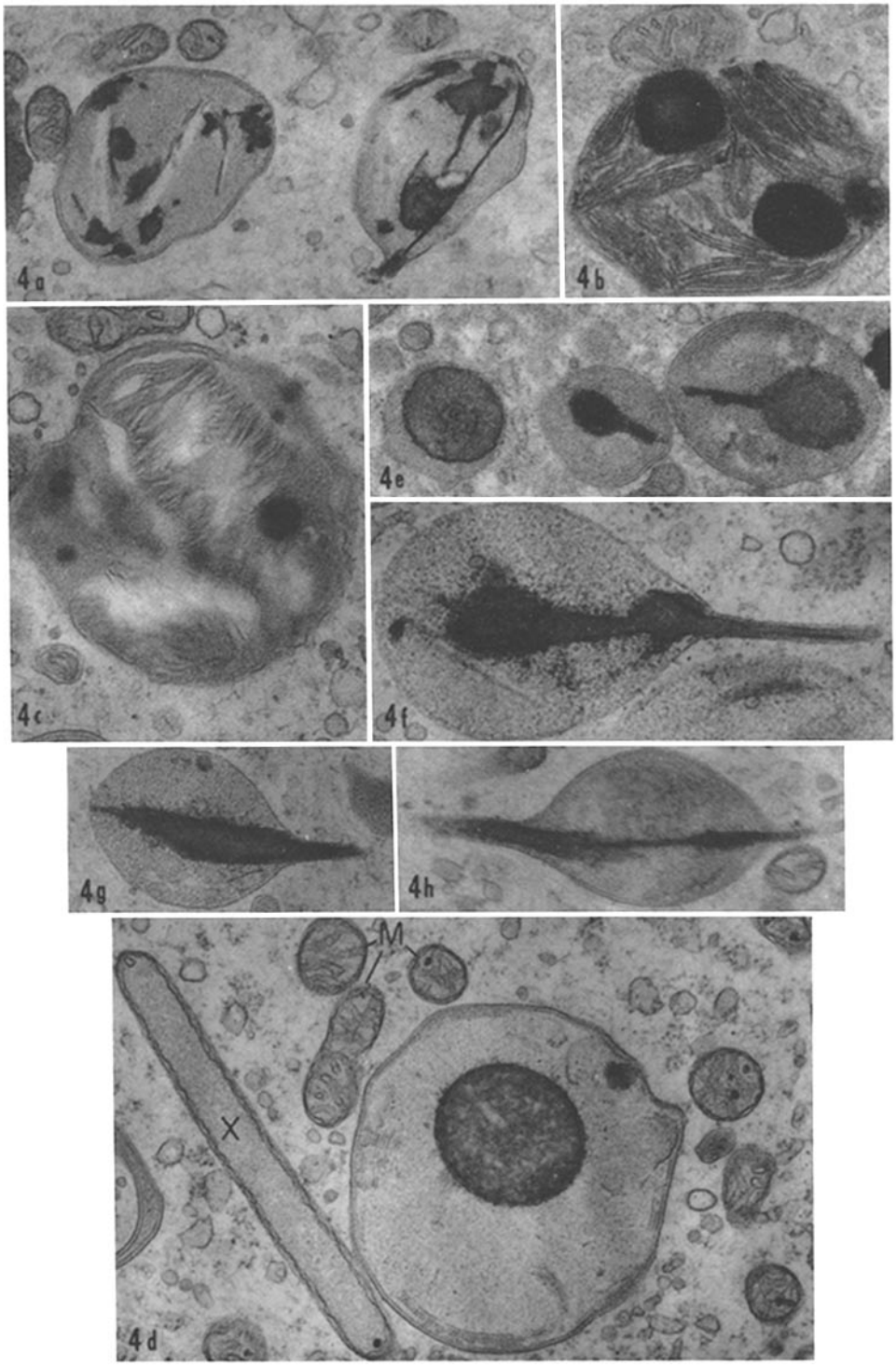
FIG. 4 *c*. Lysosome, measuring 1.15 by 1.13 μ, with loosely arranged and partially disrupted lamellae. Two days after x-irradiation with 2000 r. × 41,000.

FIG. 4 *d*. The lysosome in center measures 1.63 by 1.45 μ. Its mottled dense core is surrounded by a finely granular, moderately dense material, and remnants of lamellae can be discerned near the periphery. The long but unusually flat structure (*X*) may also represent a lysosome, since its finely granular inner texture resembles that of the adjacent lysosome. The interpretation that structure *X* is a mitochondrion which has lost its cristae is unlikely, because all other perikaryonal mitochondria (*M*) looked normal after irradiation, and a loss of cristae should have been associated with a greater width. One hour after x-irradiation with 2000 r. × 31,000.

FIG. 4 *e*. Two of these three lysosomes show various degrees of elongation of the central dark material. The normal looking lysosome on the right measures 0.61 by 0.56 μ, the middle one 0.67 by 0.61 μ, and the one on the left side 1.12 by 0.9 μ. Eight days after x-irradiation with 1000 r. × 26,000.

FIG. 4 *f*. Lysosome with a rodlike extension reaching beyond the organelle and simultaneously evaginating the peripheral lysosomal membrane. This lysosome measured 0.99 by 0. 71 μ, and the extension was 0.49 μ long and over 500 A thick. Two days after x-irradiation with 2000 r. × 48,000.

FIG. 4 *g*, *h*. Lysosomes with various degrees of bipolar elongation of their dark centers. The lysosome in Fig. 4 *g* measured nearly 0.9 by 0.68 μ, and its extension was 0.31 μ long. The lysosome in Fig. 4 *h* measured 1.09 by 0.68 μ, and the extensions were approximately 0.46 μ and 0.3 μ long. Two days after x-irradiation with 2000 r. × 32,000.


4a
4b
4c
4e
4f
4g
4h
M
X
4d

sympathetic perikarya of frogs which were studied 2 weeks after the administration of 2000 r. However, these particles did not look disrupted, as their central material was of nearly normal density and configuration. In fact, some gave the impression that they were in a state of fusion or division (Fig. 5).

The type and degree of the structural changes of the lysosomes did not clearly depend on the dose rate, or on the length of the interval between irradiation and electron microscope examination. Moreover, these morphological alterations differed in the neurons of different frogs which had been given the same dose of x-rays and were studied after the same interval; they also differed in different ganglia of the same frog, in adjacent neurons of one and the same ganglion, and even in different areas of the same neuron. In fact, some neurons appeared entirely normal, irrespective of dose rate or time interval.

It therefore became difficult to state accurately the time or dose required for the onset, full development, and repair of lysosomal changes. Nevertheless, one gained the impression that the maximal numerical incidence and severity of lysosomal alterations had taken place 8 days after irradiation with 1000 r, and 1 hour after x-irradiation with 2000 r. Signs of recovery had occurred 2 weeks after the administration of 2000 r (Table I). The available evidence was not sufficient to permit one to recognize the steps which led to this restitution (Fig. 6). Nevertheless, one may speculate that some enlarged and disrupted lysosomes regained their normal contents and density, whereupon they divided into smaller particles (Fig. 5, arrows at right).

Other subcellular components of sympathetic neurons of irradiated frogs also differed from the norm in several respects. These changes were far less conspicuous and occurred with even greater irregularity and infrequency than those observed in lysosomes.

Normally, the Nissl substance consisted of a diffusely dispersed rough-edged endoplasmic reticulum. However, in approximately 1 per cent of the micrographs of non-irradiated frogs, the cisternae of the endoplasmic reticulum were situated parallel to one another in small areas of the sympathetic perikaryon. Elongation and moderate dilatation of closely arranged parallel layers of the endoplasmic reticulum occurred more often after x-irradiation, in 10 per cent of a total of 169 electron micrographs, particularly 8 days after exposure to 1000 r. It remained dubious whether this arrangement could be attributed to the exposure to x-rays. In extreme cases, however, several adjacent rough-edged tubules came into actual contact to form tightly packed layers. This apposition was then so close that it resulted not only in the disappearance of the granules bordering

FIGURE 5 Sympathetic perikaryon of an adult frog, 2 weeks after x-irradiation with 2000 r. It contains lysosomes of various sizes and textures. Note at arrows lysosomes, measuring 1.05 by 1.44 μ, which contain a regularly shaped dark material; some of these structures (arrows at right) seemingly are in the process of fusion or division. × 9000.

FIGURE 6 Sympathetic perikaryon of an adult frog, 2 weeks after x-irradiation with 2000 r. Note the normal size, texture, and distribution of the lysosomes. × 9,000.

FIGURE 7 Sympathetic perikaryon of an adult frog, 8 days after x-irradiation with 1000 r. Note the tightly packed layers of adjacent cisternae of the rough-edged endoplasmic reticulum. At certain places (between arrows) the lumina of the cisternae are narrow and the number of membranes is uneven (five), indicating that fusion of adjacent membranes may have occurred. × 32,000.

FIGURE 8 Sympathetic perikaryon of an adult frog, 2 days after x-irradiation with 2000 r. Note the vacuole which is formed by smooth-surfaced cisternae (probably Golgi cisternae). The limiting membrane (M_1) surrounds more or less tightly packed cisternae which seemingly encapsulate a smaller, more regular-looking vacuole. The latter is bounded by an outer (M_2) and several inner concentric membranes, and contains a debris of vesicles and granules. × 29,000.

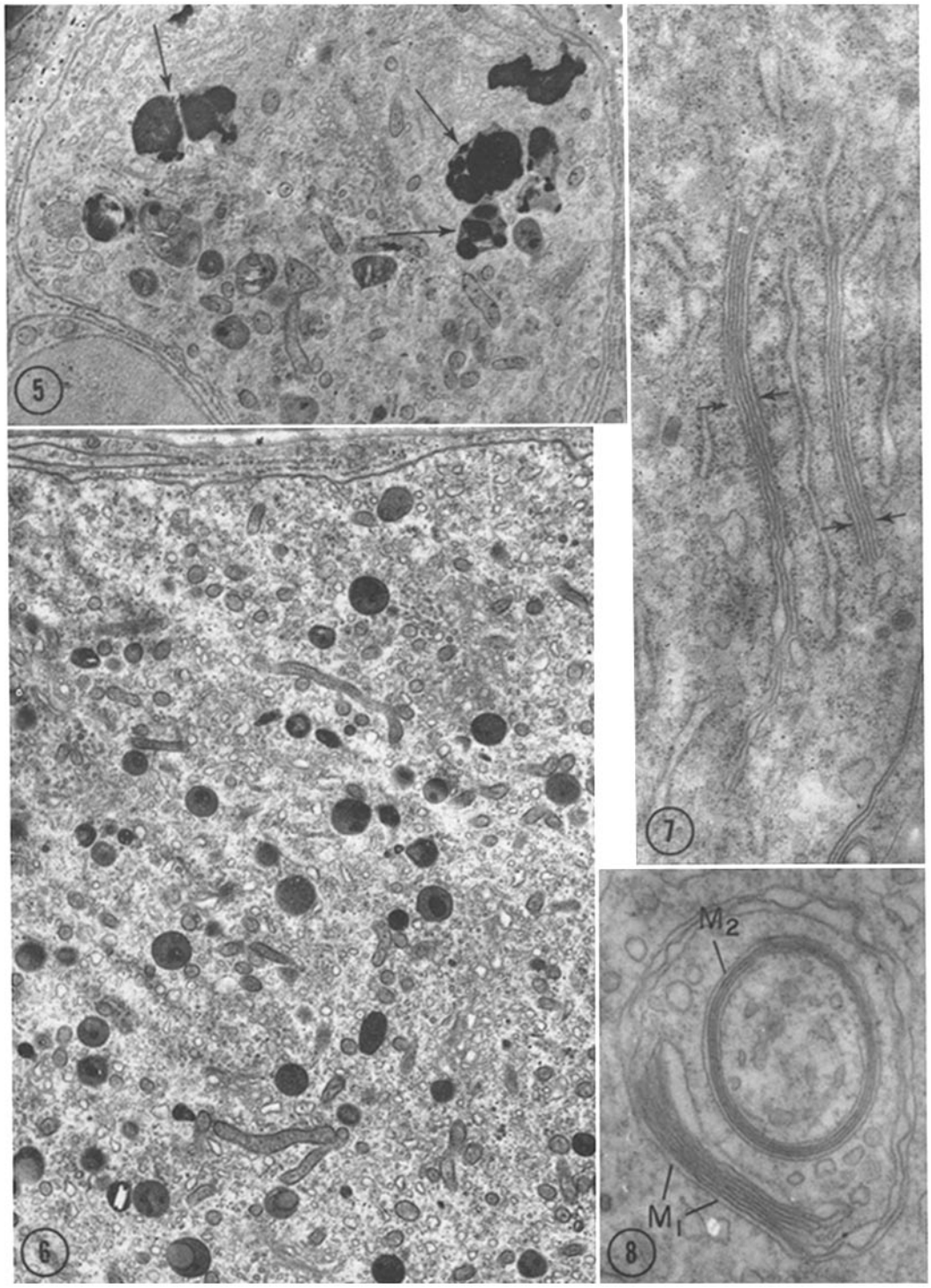

5
6
7
8
M2
M1

TABLE I

Effect of Total Body X-Irradiation on the Fine Structure of Lysosomes and Related Particles of the Lumbar Sympathetic Neurons in the Adult Frog (Rana pipiens)

Dose rate and interval	(1000 r) 1 hr.	2 days	8 days	(2000 r) 1 hr.	2 days	8 days	2 wks.
No. of electron micrographs*	227	109	169	93	130	76	75
Lysosomal changes (per cent)	13	30	35	41	31	19	14
Recovery (per cent)	—	—	—	—	—	—	21

* These figures represent the number of electron micrographs of different neurons which were taken from 17 to 13 blocks of 2 frogs after each interval. The electron micrographs which were taken of identical areas but at different magnifications were not included in this count.

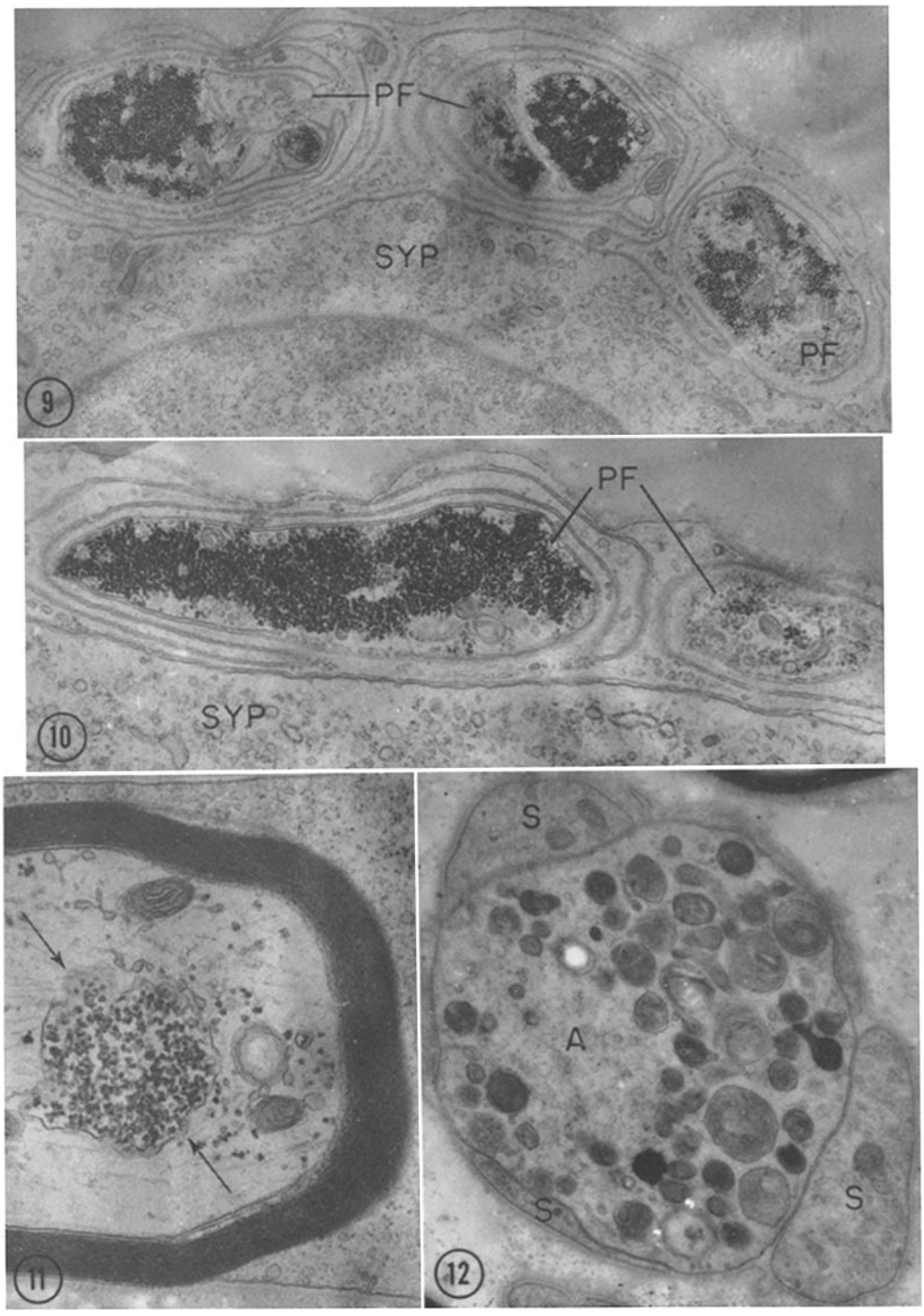
the tubular membranes, but evidently also in a fusion of some of these membranes, as judged by their uneven numbers (Fig. 7, between arrows). At the same time, the cisternae seemingly became flattened, giving such layers an appearance reminiscent of myelin figures. This arrangement was observed in 30 instances, and again occurred most frequently 8 days after the administration of 1000 r, being observed in 6 per cent of 169 electron micrographs. This merger of membranes of the rough-edged endoplasmic reticulum was not observed in the control material, and was, therefore, regarded as a radiation effect.

Vacuoles were rarely observed in normal sympathetic perikarya; their derivation from Golgi membranes was probable in only one instance and doubtful in two additional instances. After x-irradiation, the cisternae of the Golgi apparatus were occasionally arranged in tightly packed layers. This arrangement was associated with a flattening of the individual cisternae and the more or less complete merger of adjacent membranes. Vacuoles which may have been formed by modified Golgi membranes occurred as single structures and were seemingly in various stages of development; others had a more complex arrangement. As may be seen in Fig. 8, the presumptive Golgi cisternae were surrounded by a limiting membrane (M_1) and varied as to state of fusion and packing; they appeared to encapsulate a smaller, more regular looking vacuole bounded by an outer (M_2) and several inner membranes concentrically arranged around some debris. Such vacuoles were seen 13 times in the sympathetic perikarya of irradiated frogs; their incidence was highest 8 days after the administration of 1000 r (2 per cent of 169 electron micrographs) and 2 days after the administration of 2000 r (4 per cent of 130 electron micrographs). The rare incidence of these vacuoles rendered their interpre-

FIGURE 9 AND 10 Two electron micrographs showing the variable amount of dense granules (probably glycogen) in the presynaptic nerve fibers (*PF*) at the sympathetic perikaryon (*SYP*). The content of granules seen in the presynaptic nerve fibers at the extreme left in both figures indicates the range of the number of dense granules occurring in normal frogs. Note the abnormal amount of dense granules in the remaining nerve fibers, especially at the right in Fig. 10. Adult frog, 3 days after x-irradiation with 2000 r. Fig. 9, × 11,000; Fig. 10, × 19,000.

FIGURE 11 The axon of myelinated sympathetic nerve fiber contains an aggregate of dense granules (probably glycogen) surrounded by a limiting membrane (between arrows). Adult frog, 8 days after x-irradiation with 2000 r. × 21,000.

FIGURE 12 A tunicated sympathetic nerve fiber, characterized by individual Schwann cell expansions (*S*), of an adult frog, 1 hour after x-irradiation with 1000 r. Note the variety of abnormal particles in the axon (*A*); some may be derivatives of mitochondria, others of multivesicular bodies, and still others may represent lipid droplets. × 14,000.


PF
SYP
PF
9
PF
SYP
10
S
A
S
S
12
11

tation as radiation-induced structures doubtful. This possibility, however, could not be excluded, because such vacuoles were identified with certainty only once in the control material.

The fine structure of myelinated, unmyelinated, tunicated, and presynaptic nerve fibers differed occasionally from the norm, particularly as regards the axonic granules and mitochondria. Normally, the axons of tunicated and other nonmyelinated nerve fibers contained electron-opaque granules, measuring 80 to 340 A in diameter, which were usually scattered throughout the axoplasm, but occurred seldom as small aggregates (39). The axons of myelinated nerve fibers contained dense granules, measuring 150 to nearly 700 A, which were always diffusely distributed and, under normal conditions, never formed conspicuous aggregates (38).

After irradiation, abnormally large accumulations of dense granules, averaging 400 A in diameter, almost completely filled the axons of certain unmyelinated fibers, especially of presynaptic nerve fibers, while the content of such granules in neighboring axons was well within normal range (Figs. 9 and 10). The abnormal accumulation of such granules, which were tentatively regarded as glycogen, occurred in 25 different axons, and was considered to be another response to radiation. The highest incidence of this effect was observed 8 days after irradiation with 2000 r (10 per cent of 76 electron micrographs).

Abnormal aggregates of dense granules surrounded by a limiting membrane occurred only 5 times in the axons of myelinated nerve fibers, especially 8 days after exposure to 2000 r (Fig. 11). Because of this small incidence, further studies must decide whether or not this observation can be attributed to radiation.

The mitochondria in the axons of some myelinated and tunicated nerve fibers were enlarged, with a concomitant shortening or loss of their cristae, various degrees of deterioration, and arrangement in closely packed groups. Some of these particles may have derived from other subcellular structures such as multivesicular bodies or small lipid droplets (Fig. 12). These changes, which were seen 16 times, especially in the axons of myelinated nerve fibers, also may have been caused by radiation.

COMMENT

Previous investigations (16, 17, 20, 30, 41, 44, 52, 53) are difficult to compare with this study, because of the differences in dose rate of ionizing radiation, species, techniques, and degree of neuronal damage. The previous reports deal with the central and not the peripheral nervous system. The latter may have reacted differently to radiation on account of the connective tissue, which is absent in the brain and spinal cord.

The observations on radiation-induced alterations of the fine structure of the rat dorsal root ganglion (2–5) are more pertinent and are, therefore, interesting to compare with this study, although the work on the rat was carried out under considerably different experimental conditions. In the rat, 18 to 42 hours after radiation, there were holes in the nucleus of the spinal root ganglion cell, due to the disappearance of the granular component of the karyoplasm, dispersion of nucleolar material, and karyolysis. In contrast, the nuclei of sympathetic neurons looked normal in the frog. In the rat neurons the most striking changes occurred in the Nissl substance: the Nissl granules were displaced to the periphery after 42 hours and began to disintegrate after 2½ days, a process which reached its height after 13½ days, but regressed somewhat after 17½ days. This observation indicates that the Nissl substance in the rat also responded to radiation, though with more conspicuous and different anatomical changes as compared with the occasional alterations of the Nissl substance in the frog. In the rat, the mitochondria of the neuronal perikaryon became assembled around the nucleus, but rarely changed their normal appearance, even when the endoplasmic reticulum had undergone severe destruction. On the other hand, the mitochondria of satellite cells and macrophages were enlarged and lost their cristae. Similar observations were made on the rat cerebellum, in which the mitochondria of the granular cells retained their normal appearance, whereas the mitochondria of glial cells became swollen and lost their cristae (41). Likewise, the mitochondria of frog sympathetic perikarya retained their normal fine structure. The formation of dense lamellated bodies from mitochondria and microbodies, in addition to normally occurring lipofuscin granules, was ascribed to radiation in the rat neuronal perikaryon (5). This was not observed in the neurons of the frog. The initial

changes in the rat spinal nerve fiber (3) were similar to the alterations in the sympathetic nerve fiber of the frog, in that the axons of both animals contained more or less closely packed aggregates of degenerating mitochondria and other lamellated bodies. In the rat, these axonal changes were considered secondary to the alterations of the perikaryon. Similar changes observed in the frog axons did not support this interpretation, and were therefore thought of as radiation effects which ensued independently of perikaryonal alterations. The degeneration of the Schwann cells and myelin sheath which occurred in the rat, but not in the frog, was regarded as a direct radiation effect (3). An abnormal accumulation of electron-opaque granules (glycogen) or an alteration of the Golgi apparatus in irradiated spinal neurons was not recorded in the rat (2–5). However, the presence of glycogen granules was associated with mitochondrial changes in the astrocytes of irradiated rat cerebral cortex (30), and was also demonstrated histochemically in the cerebral cortex, in the white matter, and in cerebellar glial cells of the rat (26).

Occasional disintegration of the Golgi complex was seen in irradiated rat cerebellar neurons (41). However, none of the reports dealing with radiation-induced submicrosopic alterations of nervous tissue mention responses of the lysosomes such as occurred in the sympathetic neurons of the frog.

The observations made in this study raise several questions. It is not clear whether the ultrastructural changes are due to direct action of the x-rays on the neurons or are secondary to primary radiation damage to other tissues such as the vascular or hormonal systems. The uneven effects of x-irradiation are perplexing. Some neurons or parts of neurons may not have been struck at all by radiation, or, if they were, they may have been in a state of insensitivity to x-rays. It is also puzzling that the response of lysosomes was pronounced as compared with the slight effect on the rough-edged endoplasmic reticulum and on the Golgi cisternae. Most baffling is the difference of the reaction of perikaryonal mitochondria, which remained unaffected, from that of axonal mitochondria, which tended to degenerate, a finding which was corroborated by previous observations (2–5, 41). The lack of any visible change in perikaryonal mitochondria of neurons is the more interesting, in that ionizing radiation produced early changes in the non-neuronal components of peripheral (4) and central nervous tissue (30, 41) as well as in other cell types such as the intestinal epithelium (23). One is therefore immediately confronted with the question whether or not the various subcellular components of neurons are endowed with a different degree of resistance against ionizing radiation. The evidence also poses the problem whether the radiation-caused changes develop independently or are secondary to a primary lesion, perhaps to lysosomal disintegration. Although these questions cannot be answered on mere morphological grounds, some of the observations made in this study are tentatively interpreted in the light of existing information.

Evidence is accumulating that ionizing radiation causes initially an increase in the permeability of cellular and intracellular membranes (7, 9–11, 22, 27). In particular, the lipoprotein membranes of lysosomes can be damaged by exposure to various free radical generating systems such as ionizing radiation (^{60}Co) (48). Free radicals supposedly cause the opening of membranes and the release of typical lysosomal enzymes. Other cytochemists (55, 56) have demonstrated cathepsin-like enzyme activity in nerve cell cytoplasm after irradiation, and conclude that "ionizing radiation can either activate the precursors of such enzymes or liberate them from the lysosomes in which they were locked up." Subsequent nerve cell necrosis is regarded by these authors as an autolytic process due to the liberated enzymes acting freely in the cytoplasm. Further evidence of radiation-induced release of enzymes from lysosomes is furnished by the demonstration of a reversible conglomeration and diffuse distribution of acid phosphatase–containing particles in irradiated rat brain (25).

One can, therefore, conjecture that the structural changes in the lysosomes observed in this study were also initiated by an increased permeability of their membranes. This, in turn, may have caused the release of their contents, which manifested itself anatomically, first in various forms of disruption, and later in complete elimination of the dense material of their interiors. Simultaneously, cytoplasmic fluid had probably entered the lysosomes in increasing amount, causing a watery appearance, swelling, perhaps rupture of their membranes and merger with their adjacent fellows. Had this been the sequence

of events, it should be haveen accompanied by severe damage to the rest of the cytoplasm, because the escape of hydrolytic enzymes has been related to the phenomena of intracellular digestion, autolysis, and necrosis (12, 51). This did not occur. Even when the lysosomes showed severe deterioration, the rest of the neuronal perikaryon retained in most cases its normal appearance, and was altered only slightly in relatively few instances. It is, therefore, more reasonable to assume that radiation produced only a moderate increase in the permeability of the lysosomal membranes, just sufficient to permit the entrance of cytoplasmic fluid into the lysosomes, causing their swelling and perhaps the dissolving of their contents. This increased permeability was presumably inadequate for the escape of the larger molecules of hydrolytic enzymes into the cytoplasm. Yet, here and there, the release of a small amount of enzymes may have occurred, producing minute areas of focal cytoplasmic degradation (47) which became enclosed by the packing of Golgi cisternae. This process of "sequestration" (47) may have accounted for the presence of the vacuoles, which presumably were derived from modified Golgi membranes and were seen in somewhat increased numbers, in the sympathetic perikarya of irradiated frogs. This, however, is not necessarily the full explanation of the anatomical alterations of the lysosomes. The enlargement, deterioration, and swelling of lysosomes, and actual rupture of their membranes, associated with a conspicuous escape of acid phosphatase into the cytoplasm, nucleus, and urine, has been convincingly demonstrated in experimentally produced sucrose nephrosis in rats. Yet the fine structure of the endoplasmic reticulum and mitochondria remained unchanged in the kidney cells (50). The neurons described in the present paper may have responded in a similar manner. Therefore, a release of considerable amounts of enzymes from neuronal lysosomes without severe concomitant changes in other cytoplasmic organelles may have taken place in irradiated frogs, a possibility which must be tested by additional methods.

There is no evidence to suggest that the lysosomal changes were immediately related to the moderate alterations of the fine structure of the endoplasmic reticulum; radiation may have independently caused an increased permeability of its membranes, resulting in the passage of cytoplasmic fluid into its cisternae, which subsequently became slightly dilated and elongated. On the other hand, the parallel arrangement and particularly the fusion of adjacent membranes of rough-surfaced cisternae are difficult to account for.

The lack of any structural response of the perikaryonal mitochondria to radiation, not only in amphibian but to some degree also in mammalian neurons, remains an enigma. One may speculate that neuronal mitochondria in perikarya are endowed with a special protecting mechanism which the mitochondria of axons or of other cell types do not possess.

The granules which were observed in abnormal amount in some axons of unmyelinated fibers, especially of presynaptic nerve fibers, are tentatively interpreted as glycogen. The presence of glycogen in the form of a small crescent has been demonstrated histochemically, at the periphery of normal sympathetic perikarya (39). However, the exact location of this glycogen within the perikaryon and/or in the presynaptic nerve fibers and synaptic nerve endings around the perikaryon could not be seen with the light microscope. Experiments to ascertain with the electron microscope the exact location of this glycogen in frog sympathetic ganglia, which had been fixed first in glutaraldehyde, treated with human saliva, and subsequently postfixed in osmium tetroxide and stained with lead salts, were not successful (40). These granules therefore could be identified as glycogen only by the less reliable criterion of their deep staining quality with lead salts (42). Nevertheless, this interpretation is supported by similar observations (26, 30) in which the accumulation of glycogen was emphasized as an early and very sensitive indicator of radiation effect.

The appearance of an abnormal amount of these dense granules, especially in presynaptic nerve fibers, could have been due to an accelerated synthesis or to a decreased utilization of glycogen. Assuming that the latter is the cause of this phenomenon, one has to conjecture that the storage of these granules must interfere with the conduction of the nerve impulse. Available evidence indicates (6, 18, 19) that the bioelectric activity of both vertebrate and invertebrate nerve fibers is altered by radiation. Of particular interest is the block of neuromuscular transmission which has been observed in irradiated rat diaphragm

(1). The action potentials from the phrenic nerve remained unchanged, but this nerve no longer initiated an end-plate potential in the muscle. Neither the amplitude and time courses of miniature end-plate potentials nor the acetylcholine release was affected by the doses of radiation used. It was, therefore, postulated that this failure of impulse transmission was due to a block in the finest presynaptic branches of the motor nerve fibers in the muscle, but no explanation for this differential sensitivity of fine nerve branches was offered (1). The underlying reason for this phenomenon may have been a decreased breakdown of glycogen leading to an abnormal accumulation of glycogen granules in the presynaptic branches of the phrenic nerve similar to that found in the presynaptic sympathetic nerve fibers in the frog. This interpretation is the more likely, in that a decreased metabolism of glycogen is expected to interfere first with the nerve impulse transmission in the smallest nerve fibers, for the following reasons.

Glycogen breakdown is normally the major source of energy for pumping Na out of axons. Impulse transmission is blocked if the intracellular Na concentration becomes too high. The amount of Na which enters the axon with each impulse depends on the surface area of the nerve fiber. Therefore, with each impulse the intracellular Na concentration is raised more in small axons than in large axons, because of the high surface-to-volume ratio in a small fiber. It follows that the impulse conduction is blocked first in nerve fibers of smallest diameter, whenever the energy available to the Na pump is reduced (43). The unusual amount of electron-opaque granules, probably glycogen, in myelinated, large unmyelinated, and tunicated sympathetic nerve fibers cannot be commented upon further, and is recorded merely as an anatomical finding. The signs of morphological restitution of lysosomes and other cytoplasmic components are interesting in view of the reversible release of acid phosphatase following irradiation of rat neurons (25), but the mechanism of this recovery remains obscure.

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