EARLY AND LONG-TERM EFFECTS OF DEUTERON IRRADIATION ON CELLS IN THE CEREBELLUM OF ADULT MICE

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INTRODUCTION

Reactions to ionizing radiation can range from mild and transient cellular disturbances to death of the organism. Experiments have shown that radiosensitivity varies markedly among different cells, tissues, and organs of the body, as well as among species, sex, and different age levels (5). Organs with high rates of cell renewal are highly vulnerable, whereas the brain and other organs with postmitotic or nondividing cells are considered to be more resistant (7, 24). Since recovery after brain irradiation cannot include the replacement of neurons, the loss of cells and the maintenance and possible repair of the remaining neurons represent two of the more important considerations in the interpretation of radiation damage to the brain.

Observations of neurons with the electron microscope shortly after heavy particle irradiation have indicated prompt cellular alterations, including contraction of the nucleus (22), deposition of intracellular glycogen (10), changes in the fine structure of lysosomes (13), and swelling of the endoplasmic reticulum and mitochondria (17). While it may be assumed that some of these early changes result in the ultimate loss of many neurons, the long-term effects of irradiation on the surviving neurons have not been reported. This investigation was undertaken as an examination of ultrastructural changes in individual cells within the cerebellum of normal and deuteron-irradiated mice at two postirradiation intervals.

MATERIALS AND METHODS

A total of eight C57BL/10 female mice were used in this study. At the time of irradiation the subjects were 100 days of age. Four animals were exposed to deuteron beams delivered by the 60 inch Brookhaven cyclotron at 10,000 rads and covering an area 9 × 5 mm over the parietal, visual, and cerebellar cortex. The desired beam profile was achieved by means of an aperture of 0.4 mm platinum foil clamped to the end of a beam pipe (1). For irradiation, animals were subjected to Nembutal anesthesia and placed in a stereotactic type head holder mounted in the vise of a milling machine which could be moved accurately in any direction. The beam was continuously monitored during exposure. Irradiation was performed at a dose rate of 1925 rads sec^{-1} for 5.2 sec through an anti-Bragg wheel. By this means, only the calvaria, dorsal meninges, and brain tissue extending to a depth of 2 mm from the skull are uniformly irradiated (1). An additional group of four animals served as sham-irradiated controls. Two animals from each group were sacrificed at 40 days and again at 480 days after irradiation for a comparison of early and long-term ultrastructural changes in the cells of the cerebellum. Experimental lesions produced in the brain by heavy particles are particularly advantageous for examination with light and electron microscopy since the tissue sections provide material in which cells within the lesion area can be compared with adjacent cells located within nonirradiated tissue in the same block (10, 17).

The procedure for fixing tissue included immersion of small pieces of excised cerebellum for 6–12 hr in 4% glutaraldehyde buffered to pH 7.2 with 0.2 M Sorenson's phosphate chilled to 4°C. After preliminary fixation, selected segments of cerebellum were trimmed into smaller pieces and postfixed for an additional 2 hr in chilled 1% osmium tetroxide in 0.2 M cacodylate buffer. After fixation, the tissues were rinsed in Sorenson's phosphate buffer, dehydrated through graded concentrations of cold ethyl alcohol, embedded in Maraglas (3), and cut with an LKB ultratome microtome. So that identifica-

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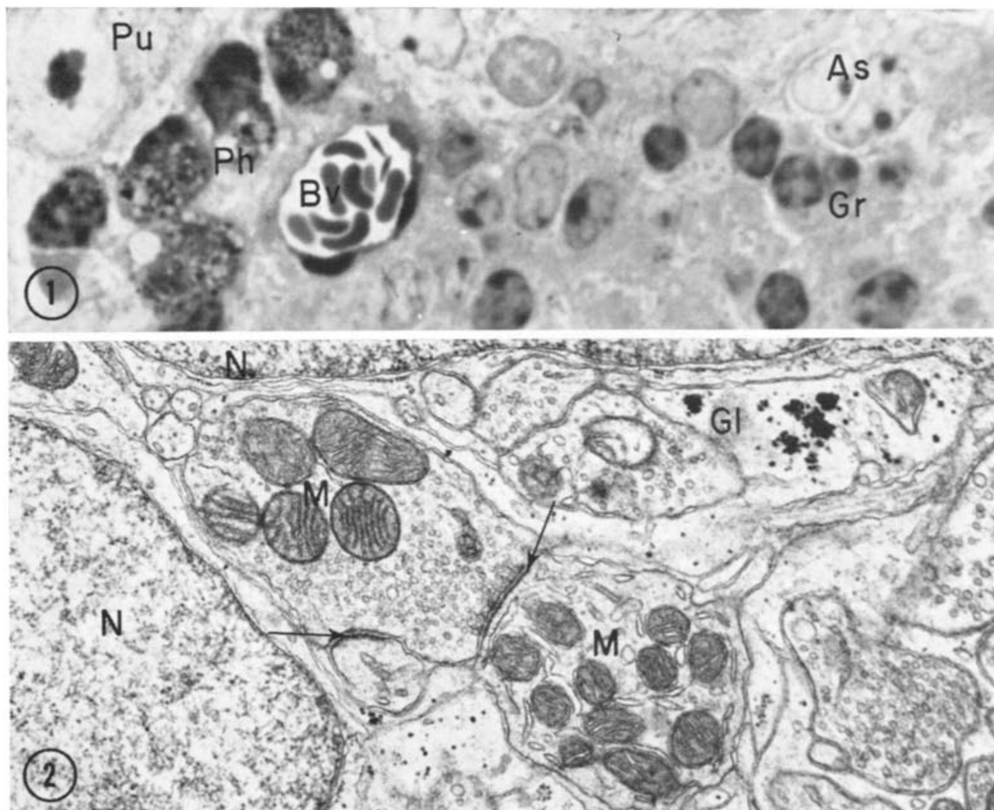

FIGURE 1 A light micrograph of cerebellum 480 days after irradiation showing several phagocytes (*Ph*) near a blood vessel (*Bv*). A Purkinje cell (*Pu*), astrocytes (*As*), and granule cells (*Gr*) are illustrated. × 1000.

FIGURE 2 An electron micrograph of a portion of cerebellum 480 days after irradiation showing normal-appearing nuclei (*N*), mitochondria (*M*), and synaptic structures (arrows). An astrocytic process contains a small deposit of ~260–300-A granules presumed to be glycogen (*Gl*). × 21,500.

tion of the irradiated region could be facilitated, sections ~1 μ thick were stained with Azure II (6) and examined with a light microscope. When an irradiated area was identified, further study was continued on adjacent thin sections which were cut at ~600 A and stained with lead citrate (21). All electron micrographs were obtained with a Siemens Elmiskop I.

RESULTS

One of the more prominent histological findings in the irradiated region of the cerebellum at both 40 and 480 days after irradiation was a marked accumulation of phagocytes ~12 μ in diameter and frequently localized adjacent to blood vessels (Fig. 1). The origin of these cells in injured brain has variously been attributed to vascular adventitia (pericytes), pia mater, glial cells, and circulating blood elements. However, after ionizing particle irradiation, the phagocytes are probably derived from the pericytes, and their appearance is dependent upon and correlated with a perivascular reaction (9). The presence of phagocytes in brain tissue is generally associated with mechanisms of repair. The role of "microglia" in repair of brain injury remains undetermined. Although the neurons in the irradiated zone may also be injured, some normal nuclei, mitochondria, dendrites, axons, and synaptic terminals can be identified in the lesion zone at the two intervals studied (Fig. 2).

The lesion zone can be readily identified in lead-stained preparations with the electron micro-

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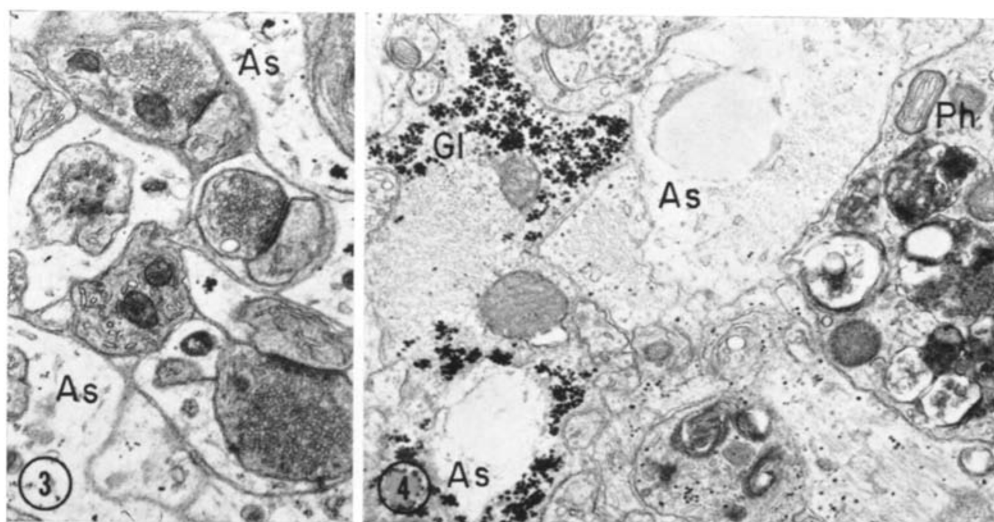

FIGURE 3 Synaptic structures surrounded by "clear" astrocytic processes (*As*) in cerebellum of non-irradiated (control) brain. × 15,000.

FIGURE 4 Cerebellum of mouse at 40 days after irradiation. The astrocytic processes (*As*) contain an abnormally large deposit of presumed glycogen granules (*Gl*) and many fibrils. A portion of a phagocyte (*Ph*) is identified. × 18,000.

scope by the presence of reactive astrocytes which contain increased numbers of cytoplasmic fibrils measuring ~100–120 A in diameter and abnormally large deposits of ~260–280-A granules presumed to be glycogen (Figs. 3 and 4). An increase in the amount of glycogen in astrocytes has been observed as early as 24 hr after exposure to alpha particle irradiation (10). There was no apparent difference in the number and appearance of reactive astrocytes at 40 and 480 days after irradiation.

The effects of irradiation on the fine structure of different types of neurons have been described by several investigators. Most studies are in agreement that the granule cells of the cerebellum are the most vulnerable neurons of the brain to irradiation. Observations with the electron microscope in the present study revealed a considerable loss of granule cells in the irradiated area of the cerebellum at 40 and 480 days after irradiation. However, many granule cells were still present at both postirradiation intervals in all four of the irradiated animals studied.

For determination of whether irradiation may produce some long-term subcellular changes discernible only at the ultrastructural level, a more detailed examination of the surviving cells was made at both postirradiation periods when the animals were 140 and 580 days of age, respectively. Surprisingly, many of the surviving granule cells, approximately one out of 20, contained unusual membranes. Some of these membranes appeared to be extracellular duplications of the plasma membrane (Fig. 5), while others appeared to be intracytoplasmic and in proximity to the cell nucleus (Figs. 6–8). The "extraplasma" membranes were seen in about equal numbers at both 40 and 480 days postirradiation; the "extraperinuclear" membranes were seen only at 480 days. No granule cells with similar membranes were observed in tissue sections obtained from the cerebellum of sham-operated control mice.

Structurally, the two main types of membranous figures differed in several ways. First, the extraplasma membranes were associated with granule cells only and appeared as bilaminar or four-layered structures (insert, Fig. 5). These membranes did not appear to be continuous with the plasma membrane at any point. The extraperinuclear membranes, on the other hand, were found in a variety of cell types of undetermined origin, and they contained 4–18 apposed dense lines. A second difference was the length of the extra-

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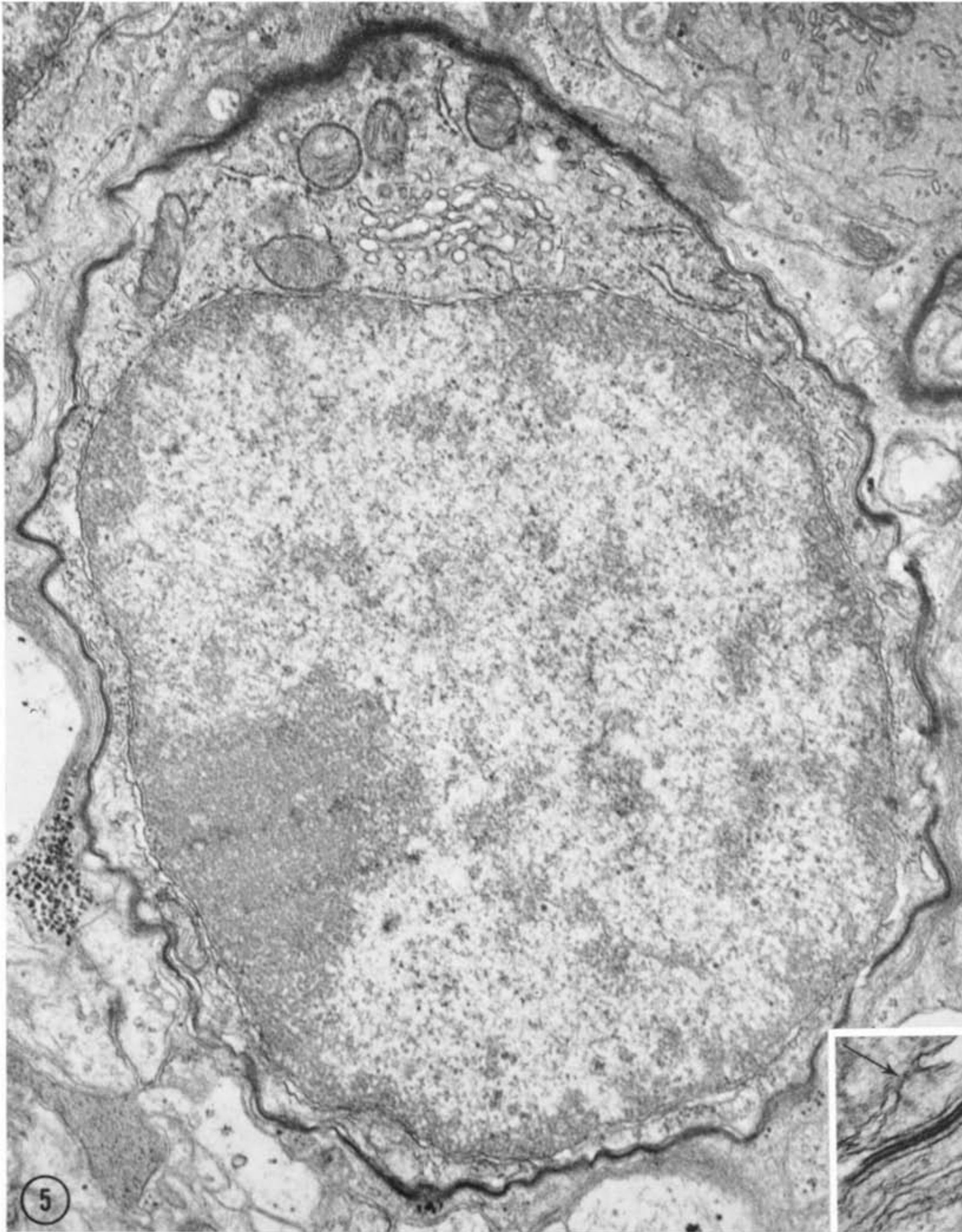

FIGURE 5 A granule cell surrounded by a dense membranous structure at 40 days' postirradiation. The insert shows a nuclear pore (arrow) and contrasting densities of the membranous lamellae surrounding the plasma membrane. × 21,500; insert, × 60,000.

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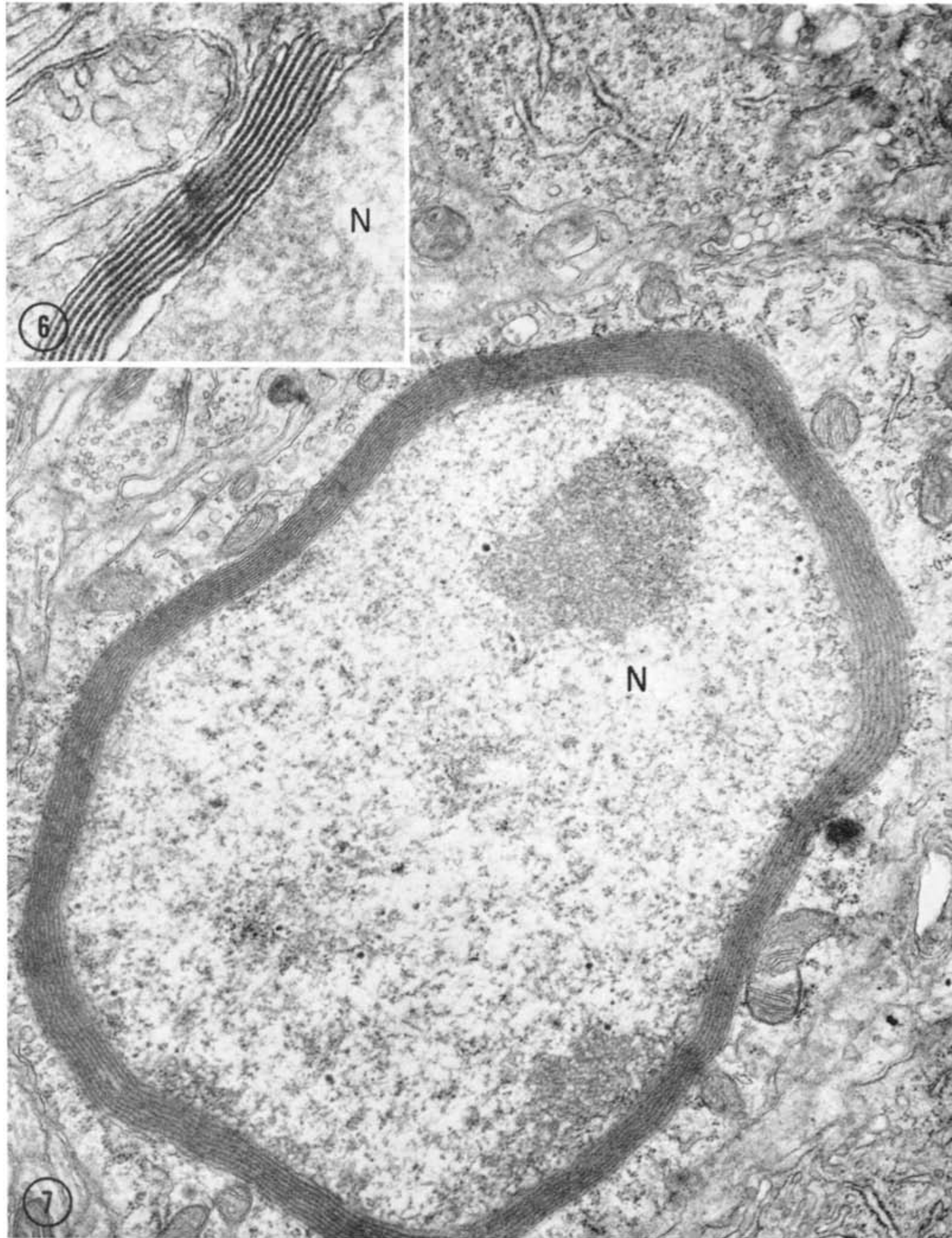

FIGURES 6 and 7 Cerebellar cells at 480 days after irradiation showing membranous figures adjacent to the nuclei (N). $\times$ 60,000 and $\times$ 14,500, respectively.

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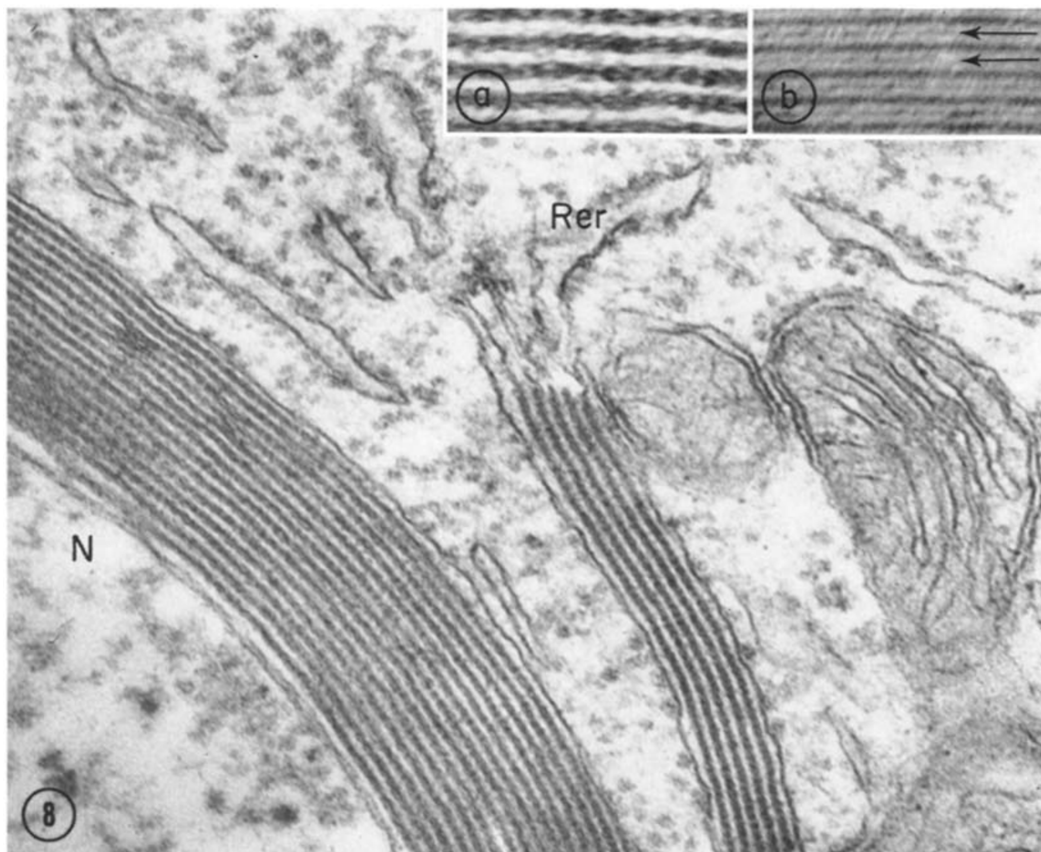

FIGURE 8 An irradiated cerebellar cell at 480 days showing one group of membranous figures adjacent to the nucleus (*N*) and another group between membranes of rough-surfaced endoplasmic reticulum (*Rer*). For comparison of their structural arrangements, the membranous lamellae (*a*) and myelin (*b*) with its characteristic intraperiod lines (arrows) are shown at high magnification. × 108,000; inserts, × 240,000.

membranous systems; the extraplasma membranes often completely surrounded the perikaryon, whereas the extraperinuclear membranes extended either part way around the nucleus (Fig. 6) or, less frequently, completely around the nucleus (Fig. 7). A third difference was in the appearance of the membranous figures. The extraperinuclear membranes were characterized by alternating periods of light and dark material distributed in a manner suggesting a double-stranded structure (insert, Fig. 8 *a*); the extraplasma membranes appeared thinner, more uniform in density, and of higher contrast in relation to the apposing membranes (insert, Fig. 5). It was also evident that the extraperinuclear membranes were contained within or associated with the nuclear envelope and the contiguous endoplasmic reticulum (Fig. 8). Neither variety of membrane revealed the intraperiod line characteristic of the myelin membrane system (compare inserts, Figs. 5, 8 *a* and *b*). The dense lines forming the extraperinuclear membranes and myelin were regularly spaced at ~160 A. However, the extraperinuclear membranes were slightly thicker (~100 A) than the dense lines of myelin (~60 A). The extraplasma membranes were ~50 A wide and were spaced at an interval of ~170 A.

COMMENT

Multilamellar membranous inclusions have been reported in a variety of irradiated and nonirradiated neurons. The extraplasma membranes

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associated with the granule cells observed in the present study at 40 and 480 days postirradiation may be comparable to the lamellae of myelin found to surround some cerebellar granule cells in toad (16). The lamellae associated with the nuclear envelope and the endoplasmic reticulum of irradiated cells seen at 480 days after exposure to 10,000 rads of ionizing energy are not unlike those reported previously by several authors who have described the fine structure of cytoplasmic lamellae in a variety of vertebrate and invertebrate neurons of both the peripheral and central nervous systems (4, 11, 12, 15). Membranous inclusions have been observed in cultures of rat dorsal ganglia (8) and in sympathetic neurons of adult frogs (13) after exposure to a single dose of X-rays. Whorls of similar membranes have been observed in astrocytic end feet in the cerebral cortex of rats after irradiation, and it is reported that they may represent duplications of the plasma membrane (10).

Regeneration of axis cylinders rendered "naked" by irradiation has also been reported (2), and laminar sprouts arising from Purkinje cell dendrites have been described in an ultrastructural study of the cerebellar cortex of rats after chronic intoxication with Dilantin (18). Laminar bodies associated with mitochondria were found in human sympathetic neurons, but it could not be determined whether these structures occurred during life or were artifacts of fixation (14). Myelin figures associated with mitochondria have been produced experimentally in the liver by a variety of conditions, and it has been proposed that the formation of these figures may be triggered by a modification in the lipoprotein complexes normally residing in the mitochondrial membranes (23).

In the present experiment, it seems likely that irradiation of the brain with the relatively high dose of 10,000 rads may have altered the neuronal DNA or messenger RNA required for priming protein and lipid synthesis in the cytoplasm. As a consequence, the membranous configurations observed in some irradiated granule cells may represent an unusual variety of lipid or lipoprotein resulting from nucleic acid damage. Alternatively, the resulting membranous configurations within the irradiated cortical cells may represent abnormal protein formed by free radicals released by lipid peroxidation (19). Both mitochondria and microsomes contain a great deal of polyunsaturated lipid and are very vulnerable to peroxidation effects with resulting metabolic derangements (20). It should also be noted that the changes observed in cells at 40 and 480 days after irradiation with a dose of 10,000 rads may represent responses in the lethal part of the response spectrum for the cells observed (24). Whatever the origin or composition of these unique multilamellar membranes, it remains to be determined to what extent these membranes are involved in the decline of metabolic functions or in the ultimate loss of individual cells which is associated with long-term radiation damage to the brain.

Although recovery after brain irradiation cannot include the replacement of neurons, the neurons of the nervous system must possess more "plasticity" than is usually believed, and cellular metabolism of various degrees, if not repair, is evidently possible depending upon dose level and a variety of experimental conditions.

SUMMMARY

This report describes morphologic studies on cerebellar neurons of adult C57BL/10 female mice which were exposed to deuteron beams delivered by the 60 inch Brookhaven cyclotron at a dose of 10,000 rads over the parietal, visual, and cerebellar cortex and extending to a depth of 2.0 mm from the skull. Observations with the light microscope indicated an accumulation of phagocytes containing cellular debris adjacent to blood vessels at 40 and 480 days after irradiation. Comparisons in the electron microscope of early and long-term ultrastructural changes revealed an accumulation of cytoplasmic fibrils and glycogen granules in reactive astrocytes and the formation of multilamellar membranes surrounding the perikarya of granule cells at 40 and 480 days after irradiation. Multilamellar intracytoplasmic membranes adjacent to the nuclei of some unidentified cells were observed only at the longer postirradiation interval.

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