# DEMYELINIZATION INDUCED IN THE BRAINS OF MONKEYS BY MEANS OF FAST NEUTRONS

## PATHOGENESIS OF THE LESION AND COMPARISON WITH THE LESIONS OF MULTIPLE SCLEROSIS AND SCHILDER'S DISEASE

BY F. STEPHEN VOGEL, M.D.

AND

JOHN E. PICKERING

*Colonel, United States Air Force*

*(From the Department of Pathology of The New York Hospital-Cornell Medical Center, New York, and United States Air Force School of Aviation Medicine, Randolph Air Force Base, Texas*)*

PLATES 32 TO 35

(Received for publication, May 25, 1956)

Demyelinization can be induced in laboratory animals in various ways, notably by injecting emulsions of brain (1, 2), lipolytic enzymes (3), or toxic substances (4–6), and by inducing nutritional deficiencies (7). The experimental lesions differ from those seen in the two common forms of naturally occurring encephalomyelitis, namely multiple sclerosis and Schilder's disease, in the following particulars: they are acute and non-progressive rather than chronic and progressive; they are often characterized by degenerative changes in axons and neurons as well as by demyelinization, and they are generally accompanied by marked inflammation. In the work now to be reported, a demyelinization that was chronic and progressive and unaccompanied in its early stages by changes in other neuronal structures or by noteworthy inflammation was regularly found in the brains of monkeys sacrificed some months after exposure to fast neutron radiation.

### *Materials and Methods*

In tests of the biological effects of neutron radiation, seven monkeys were exposed over the ocular regions to ionizing rays from a Cockcroft-Walton accelerator (8). The animals were maintained on well balanced diets in individual cages. Detailed ophthalmological and

* This study was conducted in part at the Radiobiological Laboratory of The University of Texas and the United States Air Force, Austin. It was supported by funds provided under Contract A.F. 18(600)-165 with the United States Air Force School of Aviation Medicine, Randolph Air Force Base, Texas, and by a research grant from the National Institute of Neurological Disease and Blindness of the National Institutes of Health, Public Health Service.

limited neurological examinations were made at least once a month. One monkey died of enteritis 2 months after radiation and 6 others were sacrificed 12 to 20 months later, at times when mature cataracts had developed (9). Detailed gross and microscopic postmortem studies were made, the tissues of five normal monkeys serving as controls.

*Monkeys.*—Before radiation the 12 male *Macaca mulatta* monkeys weighed approximately 8 pounds each and were estimated to be 3 years of age. They had been maintained on well balanced diets for 3 months and were considered to be in excellent health.

*Radiation*[1].—The monkeys were placed within restraining boxes and arranged facing the target area of the Cockcroft-Walton accelerator so that the distance from the nose of each animal to the center of the target was exactly 9 cm. The target, a stainless steel sphere 4 cm. in diameter, contained zirinium sponge metal that absorbed the target gas tritium. A deuteron beam of approximately 90 micro amperes was generated and focused through a tungsten diaphragm to bombard the tritium. Each neutron that was produced gave rise to an alpha particle. These were collimated into an alpha counter and by this means the concentration of neutrons per square centimeter was determined. A total dose of 850 r.e.p. was administered without interruption to the head of each animal, over a period of 2 hours and 9 minutes. The energy of the neutrons was 14 mev. The gamma contamination was less than 3 per cent.

*Histological Techniques.*—Representative blocks of each brain were embedded in celloidin, sectioned, and stained as follows—hematoxylin and eosin, Masson's trichrome method, Weil's method for myelin, Nissl's cresyl violet method for neurons, Von Kossa's method for inorganic phosphate, and Turnbull's method for iron. Tissues prepared by frozen section were stained by Cajal's silver nitrate method for axis cylinders, Hortega's silver carbonate methods for astrocytes, oligodendroglia, and microglia, and by Sudan III for neutral fat.

## RESULTS

### *Gross Changes in the Brains of Monkeys Exposed to Neutrons*

The brain of the monkey that died of enteritis at the end of the 2nd month following radiation was normal on gross examination. By contrast, symmetrical atrophy of the frontal lobes was conspicuous in the brains of 6 animals sacrificed more than a year later. Furthermore the degree of atrophy was directly related to the survival time: it was minimal in the animals sacrificed 14 and 17 months after exposure, moderately advanced in the 2 monkeys examined after 20 months, and was marked in the 2 monkeys that survived 22 months. The frontal lobes of the latter 2 animals were approximately one-half normal size, the gyri being greatly narrowed and the sulci correspondingly widened (Fig. 1). The atrophy was particularly conspicuous on the orbital surfaces of the frontal lobes, and the olfactory bulbs and tracts were reduced in size. The cerebral tissues throughout the rostral portions of the brain were firmer than normal; there were no areas of malacia. The subarachnoid and subdural spaces were widened over the frontal lobes. The meninges were delicate and devoid of adhesions or areas of thickening. The white matter of the frontal lobes, as seen

[1] The radiation was given at Los Alamos Laboratory of The University of California, Los Alamos, under the direction of Dr. E. R. Graves.

in transverse sections, was regularly and markedly decreased in amount; no longer pearly white and glistening, it had become mottled and lusterless, and it contained a few granules of grumous, friable, calcific material. The cortical grey matter was uniform in thickness and normal in appearance. The frontal horns of the lateral ventricles were moderately dilated. The choroid plexuses were unaltered.

*Histological Changes in the Brains of Monkeys Exposed to Neutrons*

Detailed histological examinations were made of the cerebral tissues of the monkey that died of enteritis 2 months following radiation; these failed to disclose alterations in the frontal lobes or elsewhere. So too in the brains of the 5 non-radiated (control) animals. Focal and confluent areas of demyelinization were regularly found, however, in the frontal lobes of monkeys exposed to neutron radiation and sacrificed after more than 1 year. In the 2 animals killed 14 and 17 months after exposure the loss of myelin was patchy and confined to the frontal lobes (Fig. 2), but in the animals sacrificed a few months later, the frontal lobes and the rostral portions of the parietal lobes, were almost devoid of myelin (Fig. 3). In regions of partial demyelinization the individual myelin sheaths were varicose and fragmented and stained poorly with ferric-alum-hematoxylin (Fig. 4). In tissues stained by Cajal's method, there were dense plexuses of well preserved axis cylinders and many of these crossed large areas of demyelinization (Fig. 5). A few were beaded and fragmented. The white matter contained multitudes of large fibrillary astrocytes (Fig. 6). The cortical grey substance showed little or no astrocytic gliosis. In areas of the white matter that were devoid of myelin, the oligodendroglial cells were moderately decreased in number but, except for some loss and shortening and swelling of the neuroglial processes, were normal in appearance (Fig. 7). The oligodendroglia in the cortex were normal in distribution and had small rounded nuclei with fine chromatin material and delicate neuroglial processes. The microglia were increased in number throughout the frontal lobes (Fig. 8). Those in the outer margins of the cortex had small ovoid or elongated nuclei and fine, branching spines. Deeper in the cortical layers and in the white matter, there were many transitional forms of microglia and numerous phagocytes that were distended with sudanophilic lipide. The tissues were practically devoid of extravascular leukocytes and erythrocytes. Deposits of finely granular material were present in a few areas that showed demyelinization. These stained basophilic in the hematoxylin and eosin preparations and, in part, gave positive staining reactions for calcium, iron, and neutral fat. They dissolved partially during fixation in formalin and in these properties resembled calcium soaps. The nerve cells were notably well preserved and rarely appeared altered in tissues stained by Nissl's or Cajal's methods. A few cortical neurons showed ferrugination and

TABLE I

*Changes in the Brains of Monkeys Given 850 r.e.p. of 14 Mev. Neutron Radiation*

| Animal No. | Quantity of radiation | Changes in living animal | Survival time after radiation* | Gross changes in brain | Essential microscopic changes in brain |
|---|---|---|---|---|---|
| | *r.e.p.* | | *days* | | |
| 1 | 850 | Alopecia in frontal region of the scalp. No abnormal behavior or neurological signs. | 52 | Nil | Nil |
| 2 | 850 | Alopecia followed by partial regeneration of grey hair. Photophobia. No abnormal neurological signs. Cataracts. | 441 | Slight atrophy of frontal lobes. Meninges normal. | Focal and confluent areas of demyelinization in frontal lobes, olfactory bulbs, and tracts. Neurons and axons normal. |
| 3 | 850 | Alopecia with partial regeneration of hair. Induration of skin in the orbital regions. No abnormal neurological signs. Cataracts. | 498 | Similar to animal 2. | Similar to animal 2. (See Fig. 2). |
| 4 | 850 | Alopecia followed by partial regeneration of grey and brown hair. Marked induration of skin over upper face. No abnormal neurological signs. Cataracts. | 613 | Moderate degree of symmetrical atrophy of frontal lobes, olfactory bulbs, and tracts. Meninges normal. | Moderately extensive demyelinization of frontal lobes; rostral portions of parietal lobes, olfactory bulbs, and tracts. |
| 5 | 850 | Alopecia with partial regeneration of hair. Marked induration of skin of face with necrosis of upper lip. No abnormal neurological signs. Cataracts. | 615 | Similar to animal 4. | Similar to animal 4. |
| 6 | 850 | Alopecia followed by partial regeneration of hair. Wasting and rigidity of muscles of extremities. Marked induration of skin of face and necrosis of upper lip. Cataracts. | 656 | Marked symmetrical atrophy of frontal lobes, olfactory bulbs, and tracts. Meninges unaltered. (See Fig. 1). | Frontal lobes and rostral portions of parietal lobes almost devoid of myelin. Cortical grey matter intact. Most blood vessels normal; a few slightly thickened. (See Fig. 3). |
| 7 | 850 | Alopecia with partial regeneration of hair. Photophobia. Rigidity of muscles of extremities. Induration of skin of the face. Cataracts. | 660 | Similar to animal 6. | Similar to animal 6. |
| 8 | 0 | Nil | — | Nil | Nil |
| 9 | 0 | Nil | — | Nil | Nil |
| 10 | 0 | Nil | — | Nil | Nil |
| 11 | 0 | Nil | — | Nil | Nil |
| 12 | 0 | Nil | — | Nil | Nil |

* Animal 1 died of enteritis; all other animals were sacrificed.

calcification, the cell bodies being pyknotic and covered with basophilic material that stained positively for iron and calcium. The olfactory bulbs and tracts showed a partial loss of myelin; the ganglion cells were intact (Fig. 2) Almost without exception, the walls of the cerebral, meningeal, and choroidal blood vessels were normal. Only a few medium sized arteries and veins were minimally altered by hyalinization, sclerosis, fragmentation of the elastica or endothelial cell proliferation. The vessels were regularly patent and their lumina were rarely narrowed. The collagen of the meninges and choroid plexuses was not structurally altered.

Table I provides a summary of the findings as a whole.

## DISCUSSION

From the findings here given it is plain that fast neutrons can destroy myelin without inducing notable changes in other neuronal structures or in the cerebral blood vessels. The findings do not disclose whether the effects are to be attributed to the physical characteristics of neutrons as opposed to other forms of ionizing radiation, or to one or another of the conditions of exposure, notably the energy, the dose, or the dose rate. Many observations have shown that x-ray and gamma radiation produce areas of necrosis in the grey and white matter of the brain; but these lesions are regularly associated with marked vascular sclerosis, and they have generally been attributed to ischemia (10–12). A degeneration of the cerebral tissues has been noted, however, in the absence of conspicuous changes in the blood vessels, in animals exposed to 23 mev. x-rays from the betatron (13).

The fact that small, discrete areas of demyelinization were present in the frontal lobes of 2 monkeys sacrificed 14 and 17 months after radiation while extensive demyelinization was present in the frontal and parietal areas of the brains of 4 animals sacrificed a few months later, makes it seem probable that the demyelinization was progressive. The examination of lesions of various sizes and at different times after exposure, by means of special staining techniques, showed clearly that the initial focal demyelinization was regularly followed, in the absence of notable inflammation or changes in the cerebral blood vessels, by a degeneration of the axis cylinders and later by a proliferation of the astrocytes and microglia, and by minor changes in the number and appearance of the oligodendroglia. In these respects the lesion resembles closely those that typify disseminated encephalomyelitis in human beings (14).

## SUMMARY

Demyelinization was regularly conspicuous in the white matter of the rostral portions of the brains of 6 monkeys sacrificed 14 to 22 months after exposure of the ocular regions to 850 r.e.p. of 14 mev. neutron radiation and it was not present in the brain of a monkey 2 months after radiation under identical con-

ditions; or in those of 5 non-radiated animals serving as controls. In early lesions, the individual myelin sheaths were varicose and fragmented, while the neurons, axons, and glial cells remained normal in appearance. With the passage of time, the degeneration of myelin became more marked and in later stages was accompanied by a degeneration of the axis cylinders, a proliferation of astrocytes and microglia, and minor cytological changes in the oligodendroglia, the whole process occurring essentially without inflammation or notable changes in the cerebral or meningeal blood vessels. The findings show that neutron radiation has the property of destroying myelin in the living animal and inducing changes that are notably similar in their pathogenesis to those that characterize disseminated encephalomyelitis in human beings.

## BIBLIOGRAPHY

1. Rivers, T. M., Sprunt, D. H., and Berry, G. P., Observations on attempts to produce acute disseminated encephalomyelitis in monkeys, *J. Exp. Med.*, 1933, **58,** 39.
2. Kabat, E. A., Wolf, A., and Bazer, A. E., The rapid production of acute disseminated encephalomyelitis in *rhesus* monkeys by injection of heterologous and homologous brain tissue with adjuvants, *J. Exp. Med.*, 1947, **85,** 117.
3. Vogel, F. S., Demyelinization induced in living rabbits by means of a lipolytic enzyme preparation, *J. Exp. Med.*, 1951, **93,** 297.
4. Ferraro, A., Experimental toxic encephalomyelopathy: Diffuse sclerosis following subcutaneous injections of potassium cyanide, *Arch. Neurol. and Psychiat.*, 1933, **29,** 1364.
5. Frazer, A. C., Elkes, J. J., Sammons, H. G., Govan, A. D. T., and Cooke, W. T., Effects of *Cl. welchii* Type A Toxin on body tissues and fluids, *Lancet*, 1945, **1,** 457.
6. Hurst, E. W., Experimental demyelinization of the central nervous system, *Australian J. Exp. Biol. and Med. Sc.*, 1942, **20,** 297.
7. Wintrobe, M. M., Miller, M. H., Follis, R. H., Stein, H. J., Mushatt, C., and Humphreys, S., Sensory neuron degeneration in pigs. Protection afforded by calcium pantothenate and pyridoxine, *J. Nutrition*, 1942, **24,** 345.
8. Manley, J. H., Haworth, L. J., and Luebke, E. A., Developments in ion-accelerating tubes, *Rev. Sc. Instr.*, 1941, **12,** 587.
9. Brown, D. V. L., Pickering, J. E., Cogan, D. G., and Graves, E., Radiation studies on the monkey eye. Effects of fast neutrons on the lens, in preparation.
10. Lyman, R. S., Kupalov, P. S., and Scholz, W., Effects of roentgen rays on the central nervous system. Results of large doses on the brains of adult dogs. *Arch. Neurol. and Psychiat.*, 1933, **29,** 57.
11. Russell, D. S., Wilson, C. W., and Tansley, K., Experimental radionecrosis of the brain in rabbits, *J. Neurol., Neurosurg., and Psychiat.*, 1949, **12,** 187.
12. Clemente, C. D., and Holst, E. A., Pathological changes in neurons, neuroglia and blood-brain barrier induced by x-irradiation of heads of monkeys, *Arch. Neurol. and Psychiat.*, 1954, **71,** 66.

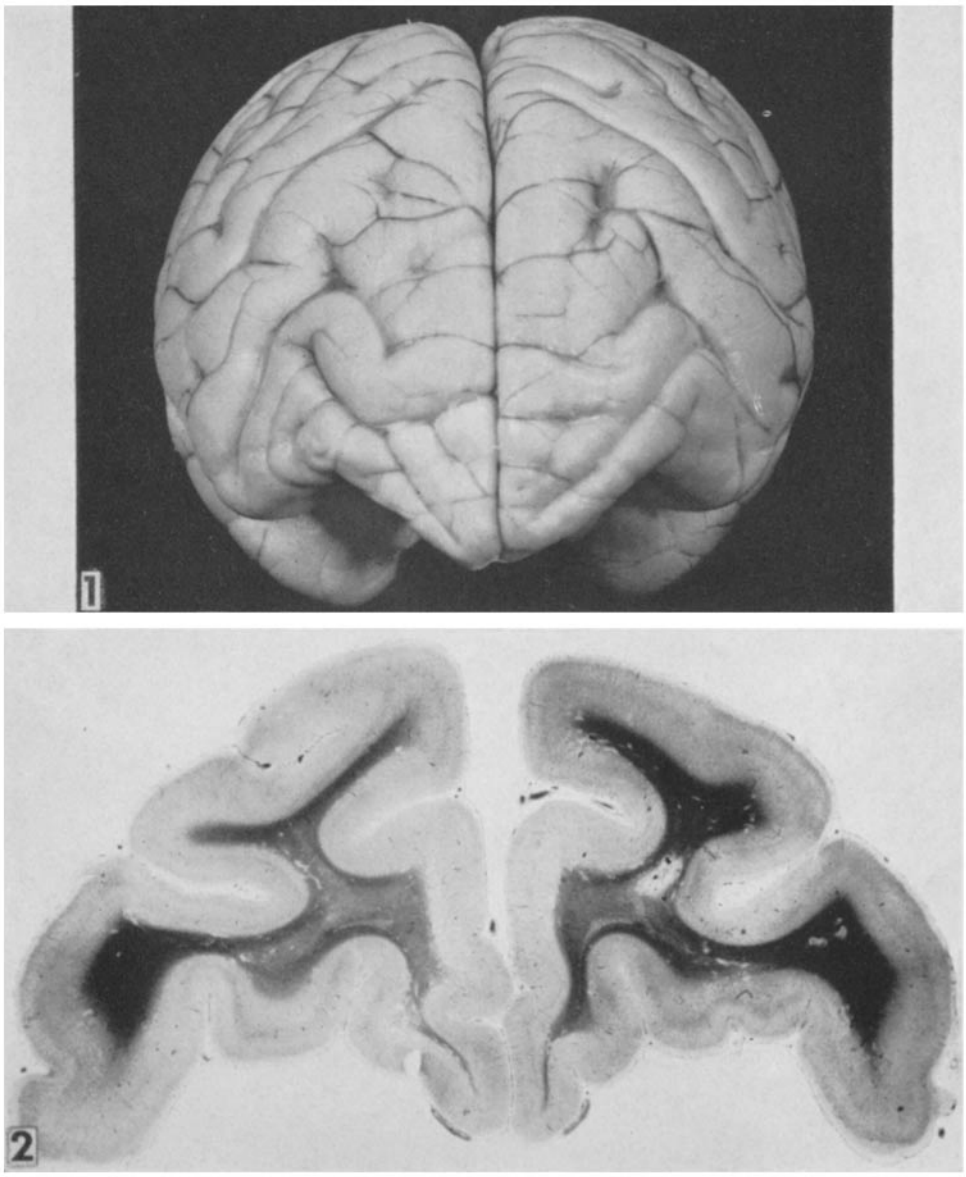
13. Arnold, A., Bailey, P., Harvey, R. A., Haas, L. L., and Laughlin, J. S., Changes in the central nervous system following irradiation with 23 mev. x-rays from betatron, *Radiology*, 1954, **62**, 37.
14. Globus, J. H., and Strauss, I., Progressive degenerative subcortical encephalopathy, *Arch. Neurol. and Psychiat.*, 1928, **20**, 1190.

## EXPLANATION OF PLATES

### PLATE 32

FIG. 1. The brain of a monkey that was sacrificed 1 year and 10 months after exposure of the frontal area to 850 r.e.p. of 14 mev. neutron radiation. The frontal lobes are approximately one-half normal size. The gyri are greatly narrowed and the sulci correspondingly widened. The leptomeninges are normal.

FIG. 2. Coronal section of the brain of a monkey 17 months after exposure to neutron radiation. The white matter in the medial portions of the frontal lobes stains less intensely than that in the lateral regions and conspicuously less than the white matter of the normal monkeys serving as controls. In addition, there are small focal areas in the white matter that show almost no staining for myelin; so too with the olfactory tracts. Weil's stain. × 2.8.

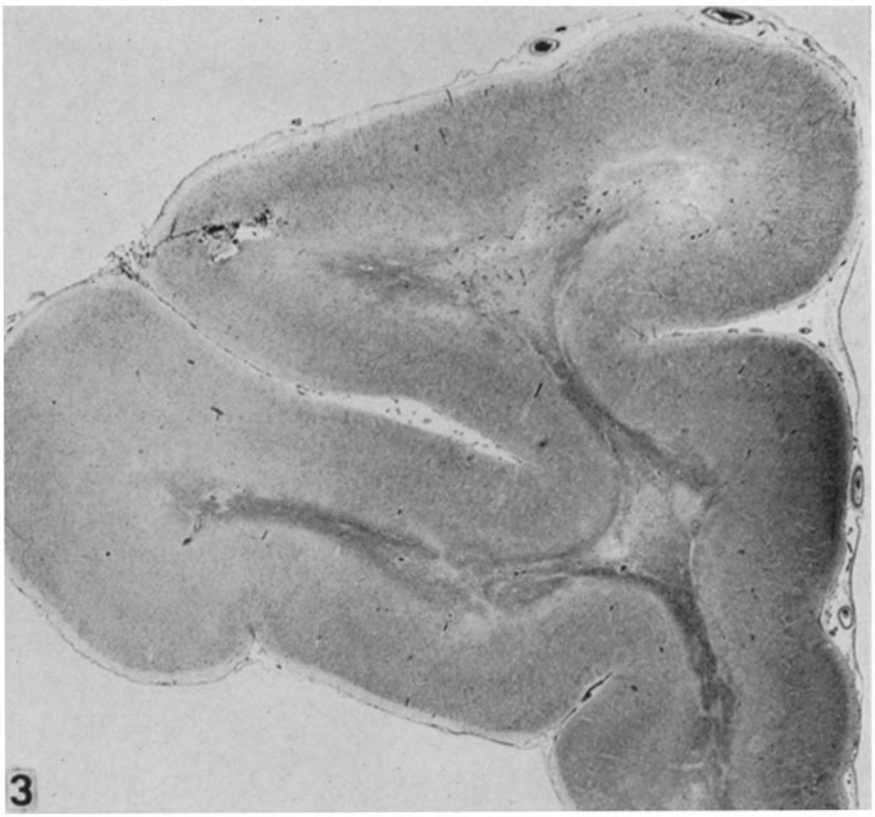


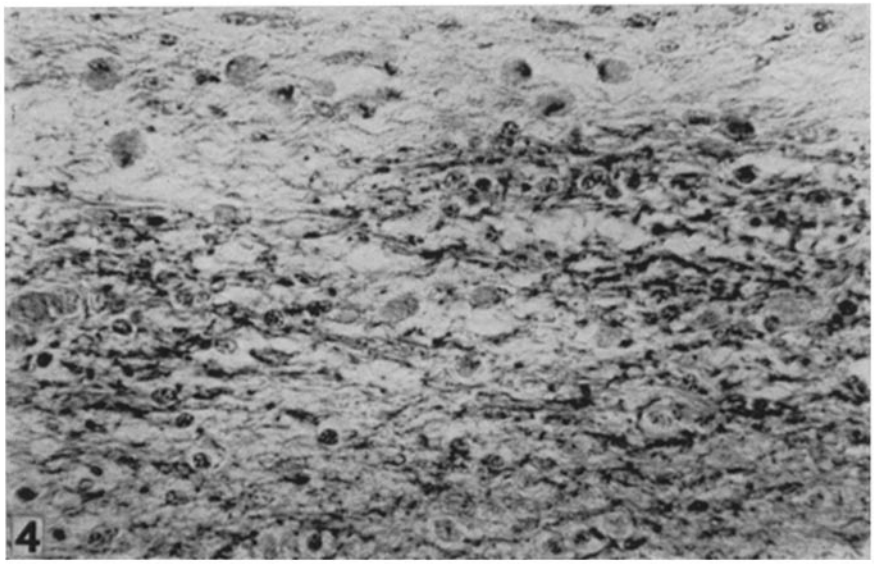

(Vogel and Pickering: Demyelinization induced with neutrons)

PLATE 33

FIG. 3. Coronal section of the left frontal lobe of a monkey 22 months after radiation. The white matter is almost devoid of myelin. It appears pale while the erythrocytes in the vessels of this section and the tissues of normal animals, processed at the same time, stained dark black. Weil's stain. × 8.

FIG. 4. Higher magnification of Fig. 3. The white matter in the upper portion of the illustration shows no staining for myelin. The individual myelin sheaths in the adjoining area are fragmented. The tissues contain phagocytic microglia and astrocytes but are not infiltrated by other inflammatory cells. Weil's stain. × 450.



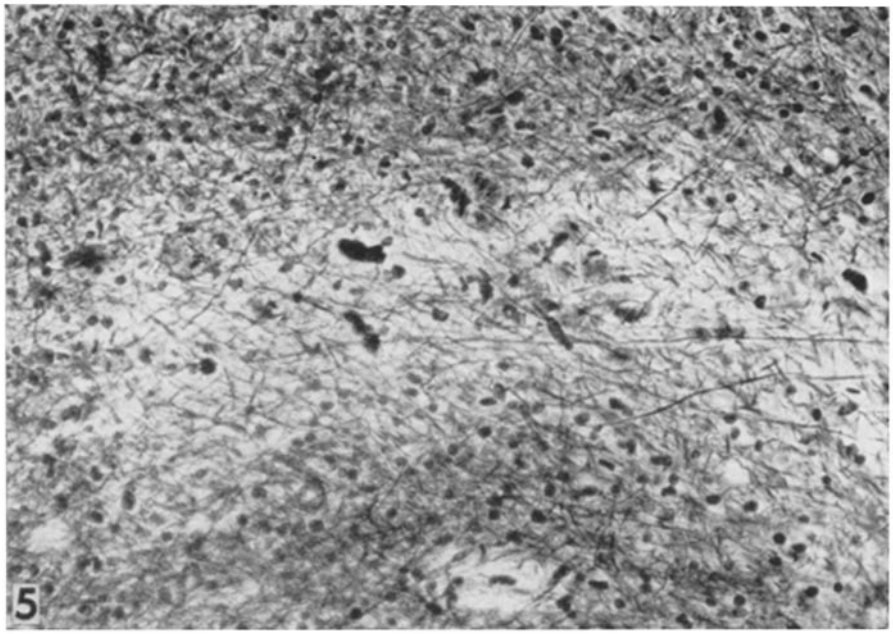



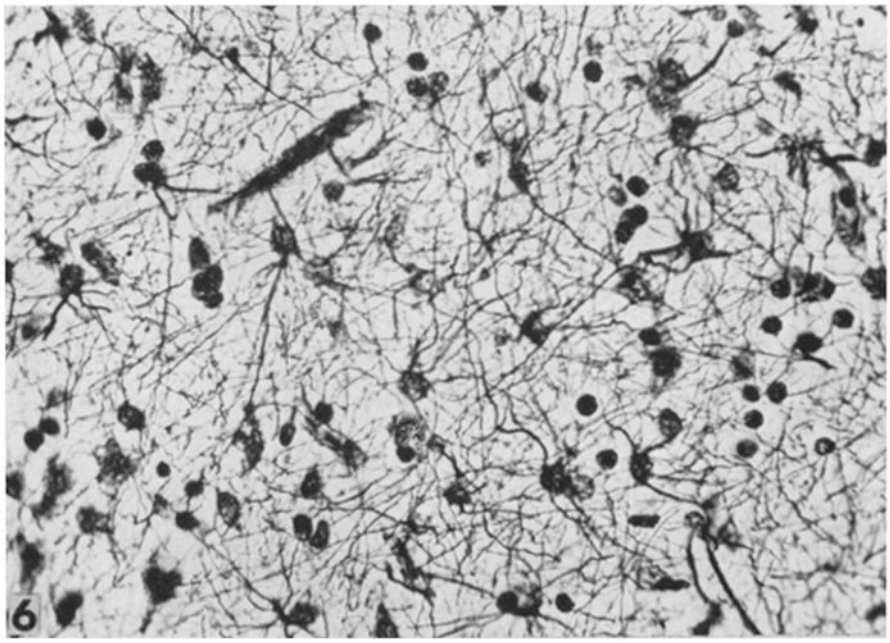

(Vogel and Pickering: Demyelinization induced with neutrons)

## PLATE 34

FIG. 5. An area of demyelinization in a monkey 17 months after exposure to neutron radiation, that is crossed by many well preserved axis cylinders. Cajal's silver nitrate preparation. × 240.

FIG. 6. Marked astrocytic gliosis in the white matter of the frontal lobes of a monkey that had been exposed to neutron radiation 22 months before being sacrificed. Hortega's silver carbonate preparation. × 450.



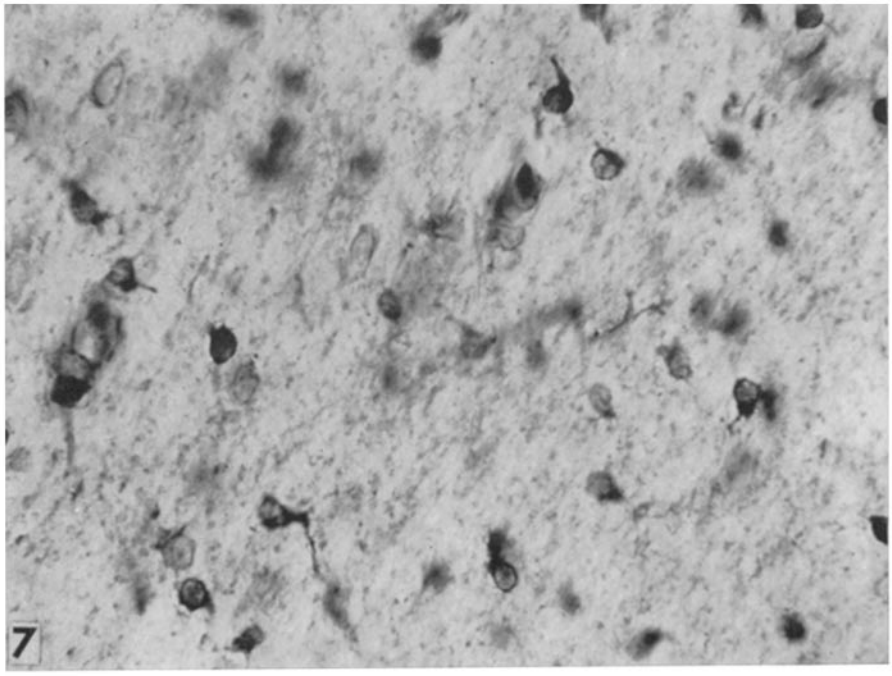



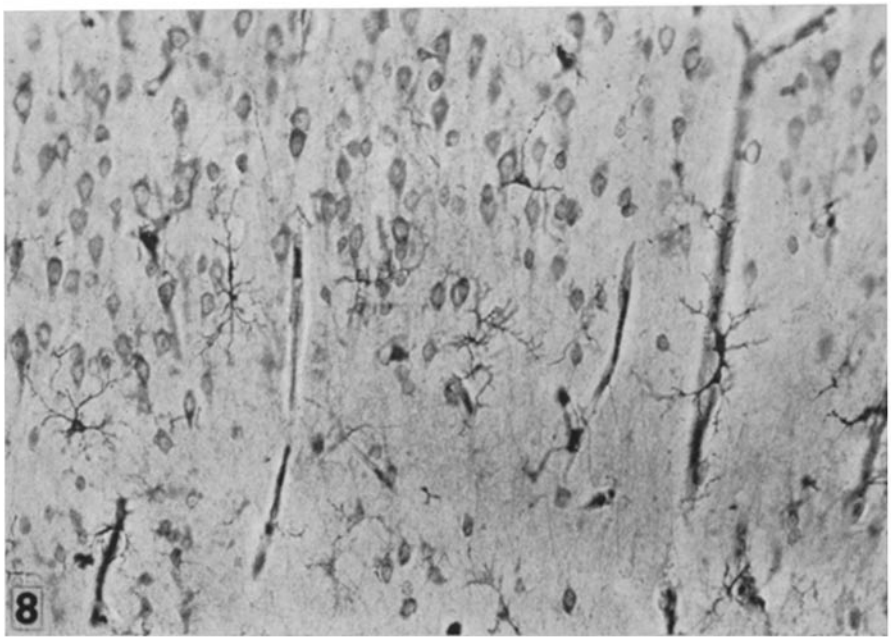

(Vogel and Pickering: Demyelinization induced with neutrons)

## PLATE 35

FIG. 7. Oligodendroglia cells in an area of total demyelinization in the white matter of a monkey 22 months after exposure to neutron radiation. In this preparation the nuclei of the oligodendroglia are not impregnated by silver and only the cytoplasm and neuroglial processes are stained. Many oligodendroglia are present. Their neuroglial processes are decreased in number and are shortened and thickened. Hortega's silver carbonate preparation. × 600.

FIG. 8. A greater than normal number of microglia in the cortex of a monkey 20 months after neutron radiation. Hortega's silver carbonate preparation. × 240.




(Vogel and Pickering: Demyelinization induced with neutrons)