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Degeneration and Regeneration of Myelinated Fibers in the Cerebral and Cerebellar Cortex Following Damage from Ionizing Particle Radiation*

By

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With 19 Figures in the Text

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Although rather numerous studies have been carried out on the effects on the cerebral and cerebellar cortex of relatively wide beams of monoenergetic accelerated particles in the medium energy range (10 to 20 Mev/nucleon), only one has been concerned with axonal degeneration and regeneration (ROSE et al. 1960, 1961), and none with the behavior of the myelin under these conditions. To help fill this gap is the purpose of this paper. In the studies with which this report is concerned, damage was incurred on the basis that as particle velocity decreases in passing through tissue the rate of energy transfer and the absorbed dose increase, so that at the ionization peak ("Bragg peak") in the region of termination of the particle beam the damage is most intense (BAKER et al.; JANSSEN et al.; MALIS et al. 1958, 1960, 1962; TOBIAS).

The initial work in this field was carried out on the cerebral cortex of 2 cats (MALIS et al. 1957). Protons having an energy of 10 Mev were used and the radiation dose at the peak of the Bragg curve was approximately 5000 rad. The beam diameter was not indicated, but was relatively wide. At 6 months after irradiation virtually all the nerve-cell bodies in a band ("Bragg-peak band") in a region 100 μ in width and 800 μ deep[1] to the brain surface had disappeared, leaving relatively intact the pre-existing tissue framework, in which proliferated glia (type unspecified) were encountered. In accordance with the monoenergetic nature of the particles, the lower border of the band of nerve-cell loss was straight and sharp, and throughout its length was equidistant from the cyclotron aperture. The

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[1] All the widths and depths referred to in this paper were determined on stained sections.

upper border of the band was, by contrast, somewhat uneven because of the irregular loss of nerve cells.

In subsequent studies carried out by the same group of investigators, 20-Mev deuterons, 10-Mev protons and 40-Mev alpha particles were used, but preference was given to the deuteron beam because its range in the brain (about 2.5 mm) was twice that of the other beams. The cyclotron aperture was 3 to 4.5 mm in diameter. Cats and rabbits were utilized. At deuteron surface doses of 3000 to 9000 rad (peak doses, 15000 to 45000 rad) the pathological changes were of much the same nature as observed in the initial experiment described in the foregoing. The width of the "band" varied with the radiation dose, e.g., from approximately 60 to 100 μ at surface doses of 3000 to 5000 rad (peak doses, 15000 to 24000 rad), respectively. Under these conditions destruction of nerve cells was virtually limited to the "band," while with greater "band" widths, e.g., 180 μ at 9000 rad surface dose (37000 rad peak dose), nerve cells in the region just above the band or even at higher levels also underwent destruction. Calculations indicated that narrow to wide bands were produced by doses of 4×10^9 to 12×10^9 deuterons/cm^2, respectively (MALIS et al. 1958, 1960, 1962).

Detailed studies on axis-cylinder destruction and axonal regeneration in these brains were carried out by ROSE et al. (1960), whose approach was that of describing axonal regrowth after exposure to different radiation doses over various time periods, and not that of following the sequence of regrowth in point of time at any given dosage. Surface doses utilized in this study were approximately 3000 to 9000 rad (peak doses, 15000 to 45000 rad).

As to degenerative changes following irradiation, ROSE et al. (1960) reported that nerve fibers were more radiovulnerable than nerve-cell bodies. This was brought out in sections of brain exposed (a) to a surface dose of 4000 rad (peak dose, 20000 rad), in which, at 21 days following irradiation, the band was considerably rarefied as far as nerve fibers were concerned (Schultze's silver method was employed), but contained numerous nerve-cell bodies (their Figs. 30 and 31), and (b) to a surface dose of 10000 rad (peak dose, 40000 rad), in which, after a 30-day period, virtually all nerve fibers in the entire irradiated area of the cortex had disappeared, while most of the nerve cells above the band were intact (their Figs. 9, 10 and 11). A further point made was that apical dendrites traversing the band were considerably less radioresponsive than axis cylinders in the self-same location. This was evident, for instance, at the 132-day stage in a brain exposed to a surface dose of 12000 rad (peak dose, 20000 rad) (their Fig. 33).

Axonal growth occurred after a certain time lapse (exact time not specified), as was evident from the accumulation of delicate fibers within the band. Such fibers appeared much earlier when the radiation dose was relatively low than when nearly maximal for tissue destruction. With the passage of time, the nerve sprouts in the band increased greatly in number, ramified profusely, and formed rather dense networks which ran horizontally in the band; in some preparations the fibers extended more or less vertically across the band into the superjacent, less damaged, cortex. The morphological end-point of new growth was considered to have been reached by at least 7 weeks. At this time period the pattern of axonal growth within the band varied with the dose: at moderate radiation levels (surface dose, 6000 rad; peak dose, 28000 rad), restitution of the neuro-

fibrillary pattern was achieved, while at doses nearly maximal for tissue destruction (surface dose, 9000 rad; peak dose, 39000 rad) the newly grown fibers were much denser and were patternless.

These various papers deal only with cerebral cortex. No detailed study on the behavior of myelinated fibers after irradiation of the cerebellar cortex is known to us.

Materials and Methods

A total of 54 rats of the Long-Evans strain, 3 weeks of age and weighing approximately 60 gm, were exposed to alpha particles from the 60-inch cyclotron in Berkeley. The cyclotron aperture was 14.3 mm in diameter, allowing irradiation of most of the dorsal surface of the cerebrum and cerebellum. The energy of the alpha particles was 12 Mev per nucleon. The brain-surface dose was 6000 rad, and in the region of maximal penetration of the particles, i.e., in the region corresponding to the Bragg peak, the dose was approximately 30000 rad. During exposure, the average dose rate was 1000 rad/min to the brain surface, or approximately 10^8 particles/cm^2-sec. (Upon recalculation, the figure, 4×10^9 particles/cm^2-sec, given in previous publications [JANSSEN et al.; KLATZO et al. 1961, 1962; WOLFE et al.] was found incorrect; in those publications the value should have been 10^8 particles/cm^2-sec.) Further irradiation details are given elsewhere (JANSSEN et al.).

After exposure, the animals were sacrificed by decapitation at periods ranging from 4 hours to 7 months. The brains were fixed in chloral hydrate (10%), pyridine (70%), or alcohol-ammonia for silver nitrate impregnation *en bloc* (CAJAL), in formol bromide for silver carbonate impregnation, and in formalin (10%) for myelin staining (SPIELMEYER and WOELKE), silver impregnation of nerve fibers (GROSS-BIELSCHOWSKY, SOTELO-CAJAL and SCHULTZE), and polarizing microscopy. Blocks of brain prepared by the Cajal silver nitrate method, mostly according to formula 6, were embedded in paraffin or celloidin and occasionally toned with gold chloride after sectioning. Some brains fixed in Bouin fluid or in formalin (10%) were embedded in paraffin for staining by cresyl violet or by the hematoxylin-Van Gieson and Klüver-Barrera methods. Sudan IV was used as the fat stain.

Pathological Changes in Brain Components Other Than in Nerve Fibers

An initial change following exposure to the 6000 rad surface dose (30000 rad peak dose) consisted in damage of occasional nerve cells in much of the irradiated cerebral cortex, but particularly in the region of maximal penetration of the particles, which was approximately 1 mm deep to the brain surface. This was at 6 hours after irradiation. The same was true for the cerebellar granular layer, though, here, many more granule cells were altered, i.e., pyknotic. By 42 hours numerous nerve-cell bodies in the most intensely irradiated region, both in the cerebrum and cerebellum, showed evidence of necrosis, making evident a "band" (the "Bragg-peak" band), the lower border of which was straight and sharp while the upper border was indistinct because of irregular disappearance of nerve cells. The band of nerve-cell loss was consistently wider in the cerebral than in the cerebellar cortex (an average of 110:41 μ). Despite cellular devastation within the band, the apical dendrites of many immediately subjacent nerve cells that traversed the band appeared relatively unaltered (JANSSEN et al.). Pathological changes at the 16-day stage, as revealed in a hematoxylin-Van Gieson preparation, are indicated in Fig. 1.

Neuroglia were even more radiovulnerable than nerve cells. In hematoxylin-Van Gieson preparations, necrosis of occasional neuroglia was already evident both in the cerebrum and cerebellum at 6 hours after irradiation. In Cajal preparations, on the other hand, the earliest change observed was at 48 hours, and consisted in an increase in the intensity of metallic impregnation. By 72 hours, astrocytic hypertrophy was noted, but after the passage of a few more hours numerous astrocytes in the band had undergone disintegration or had vanished. Astrocytes just beneath the lower border of the band became hypertrophic, with their superior processes oriented to, and extending well up into, the band. By the 6th day, astrocytes throughout much of the irradiated area had undergone disintegration, and by the 16th day, few remained. Owing to capriciousness of impregnation, the relative radiovulnerability of oligodendrocytes at early stages was not established.

Activation of microglia, occurring in the more intensely irradiated cortex, was first observed at the 48-hour period. In time, the band, from which nerve cells and neuroglia had largely disappeared, was occupied by myriad thorny microglia-like cells together with some oligodendroglia, as in Fig. 14 (JANSSEN et al.; KLATZO et al. 1961, 1962).

A further significant alteration in astrocytes was the appearance, within them, of PAS-positive glycogen granules. At 6 hours after irradiation the granules were noted in occasional astroglia at various levels of the irradiated cortex. By 24 hours they were abundant and were concentrated in the region both above and below the band. Neither at this time period nor subsequently were granules found within the band. The peak of glycogen accumulation was at 48 hours. At subsequent time intervals, glycogen granules had been taken up by microglia. By the 36th day, PAS-positive granules were still found in various cells, but they contained no glycogen (KLATZO et al. 1961, 1962; MIQUEL et al.; WOLFE et al.).

Considering, now, the effects of the irradiation on vessels and on vascular permeability, vascular dilatation (as brought out by the Pickworth-Lepehne technique) was evident in the irradiated region at the 48-hour period and was most pronounced in the band. Within 3 or

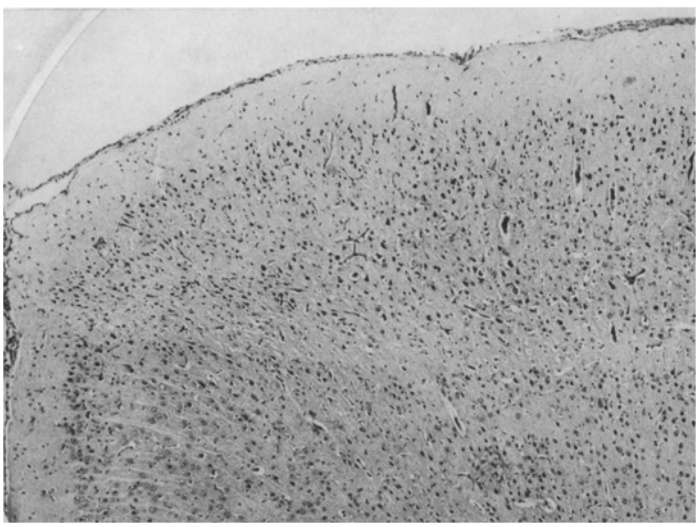

Fig. 1. Cerebral cortex. 16 days after irradiation. The "Bragg-peak band", from which most of the nerve cells have vanished, is clearly evident. In the irradiated area, tissue stainability has been reduced, some nerve-cell rarefaction has occurred, and vessels are dilated. ×50. Hematoxylin-van Gieson stain. (AFIP Acc. 961739, Neg. 61-2224)

4 days, many vascular filling defects were noted, especially in vessels within the band. By the 18th day the band was poor in capillaries, and in the rest of the irradiated tissue the capillary bed appeared disorganized and its target vessels were irregularly and strikingly dilated. Virtually simultaneous with the vascular dilatation, a disturbance of vascular permeability became evident. This was brought out through the use of fluorescein-labeled serum albumin (FLA), which was given intravenously some 24 hours prior to sacrifice. At 48 hours, numerous vessels in the irradiated cortex were surrounded by FLA droplets and, by 72 hours, the FLA, much of it in activated microglia, was widespread in the irradiated cortex, so that the entire irradiated cortex, and much of the subjacent white matter, was grossly fluorescent. "Barrier" penetration by the FLA, as observed microscopically, lasted 36 days (JANSSEN et al.; KLATZO et al. 1961).

Comment. Damage incurred by the irradiated cortex was due to a combination of factors. Degree of damage and the latent period required before damage became evident at different tissue levels coincided with the energy given off along the slopes and at the peak of the Bragg curve (JANSSEN et al.). The chief pathogenic factor was the radiation energy *per se* acting on tissue elements. This was considered the sole pathogenic factor in parenchymal cell damage

up to the 48th hour. Other factors, operative from 48 hours onward, consisted in circulatory disturbance and flooding of the tissue by plasma components as a result of breakdown in the blood-brain-barrier mechanism. As a consequence of these pathogenic influences, much of the irradiated tissue was gradually destroyed—so much so that after a lapse of some months tissue atrophy in the irradiated region was extreme. As the pathological process in the irradiated region underwent resolution, the capillary network gradually became re-established. As brought out in the foregoing, by the 16th day after irradiation the Bragg-peak band had the following characteristics: nerve cells, astrocytes, and, probably, oligodendrocytes, had disappeared, leaving intact a delicate neurogliopil; the apical dendrites of subjacent nerve cells and hypertrophied processes of subjacent astrocytes extended up into the band, reactive microglia in great numbers and a few oligodendroglia occupied the band, vessel walls were still being penetrated by FLA, and the capillary tree had become re-established.

Degeneration and Regeneration of Nerve Fibers

The sequence of change occurring in axis cylinders and in myelin of the *cerebral cortex* following exposure of the brain to 6000 rad surface dose (30000 rad peak dose) of alpha particles was as follows:

4, 8 and 24 Hours Following Irradiation (3 Animals). At these time intervals no brain changes were observed.

42 and 48 Hours (3 Animals). Sections prepared by myelin staining methods showed slight tissue pallor only in the region of the band in the interhemispheric cortex. In silver preparations a full complement of normal-appearing axis cylinders was observed.

96 Hours (3 Animals). Practically all the irradiated part of the cerebral cortex in all 3 animals showed advanced somewhat patchy reduction in myelin staining. Myelin fragments were particularly numerous in the heavily myelinated bundle of radial fibres in the interhemispheric cortex (Fig. 2). On the whole, little orientation of the myelin damage to the vascular tree was observed.

As brought about by silver impregnation, axis cylinders showed no change except in silver-nitrate block-impregnation preparations (CAJAL), in which some axis cylinders traversing the band were hyperargentophilic.

8, 9, 10, 11 and 12 Days (21 Animals). Myelin loss in the irradiated area had become more uniform and the band was more clearly established. That myelin was virtually non-existent in the band was verified by polarizing microscopy (Figs. 3 and 4).

Silver impregnation was carried out only on brains at the 8- and 9-day stages. Axis cylinders in the band and sometimes above the band exhibited hyperargentophilia. Only in an occasional brain was there a definite reduction in the number of axis cylinders, especially within the band.

16 Days (8 Animals). In all the brains the band was prominent, and its lower border sharp. In general, myelin within the band had vanished (Fig. 5), as was confirmed by the lack of birefringence by polarizing microscopy (Fig. 6). Above the band in occasional brains fragmented myelin unattended by reactive cells was found, although in considerably less measure than at the 4-day stage (Fig. 2).

In silver preparations it was evident that the axis cylinders were far less affected than the myelin. Only within the band was there conspicuous axis-cylinder rarefaction. However, from about the 16th day onward, horizontally-oriented fine axons, judged to be newly formed, were noted in the band.

24 and 30 Days (6 Animals). Spielmeyer preparations revealed generalized and uniform myelin loss in the irradiated region, except in 2 animals (AFIP Accs. 990620 and 990628) in which radial fibers extending into the interhemispheric cortex were heavily myelinated (Fig. 7). Myelin around these fibers took on a deeper hue than normally. In all 6 brains, Cajal preparations revealed a vast number of delicate axons within the band (Fig. 8). They were most abundant in the cortex adjacent to or bordering the interhemispheric fissure. Moreover, these new axis cylinders had become myelinated (Figs. 9 and 10) and in some brains had formed dense networks. The number of newly-formed regenerated fibers varied: They were most abundant in regions normally heavily myelinated, such as the interhemispheric cortex, and

somewhat less so in areas in which myelinated fibers are normally fine. From immediately beneath the band, delicate myelinated fibers could be seen bending into the band, in which they could be followed a short distance until lost in the maze of regenerated fibers. Such

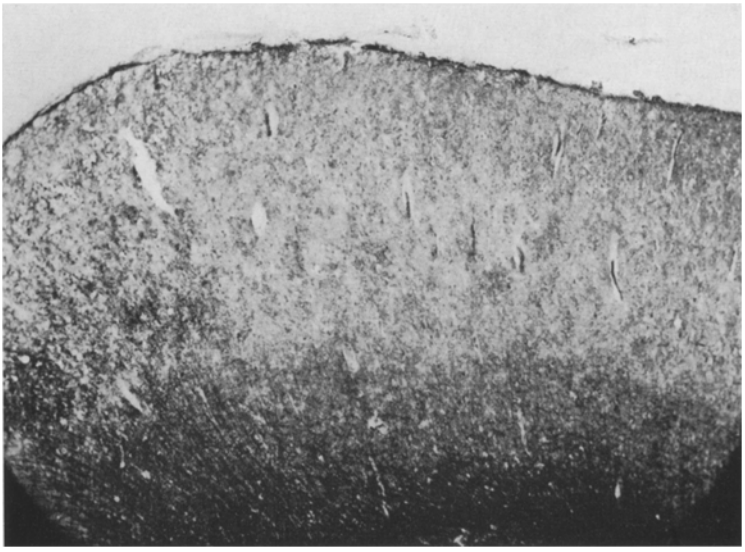

Fig. 2. Cerebral cortex. 96 hours after irradiation. Patchy but striking myelin loss is to be noted through much of the irradiated cortex, marked off inferiorly by a rather wavy line. There is little orientation of the myelin loss to the vascular tree. Numerous myelin fragments persist to the left, along the course of the radial fibers extending into the interhemispheric cortex. × 50. Spielmeyer myelin stain. (AFIP Acc. 990658; Neg. 61-5836)

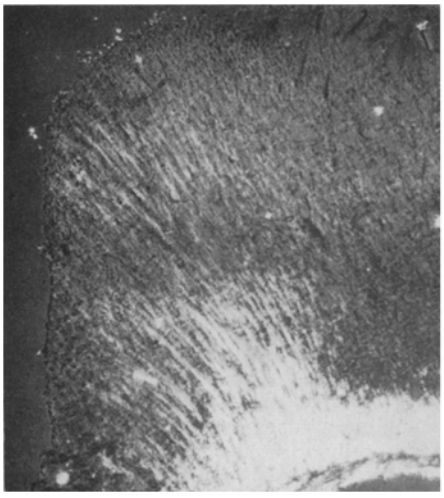

Fig. 3

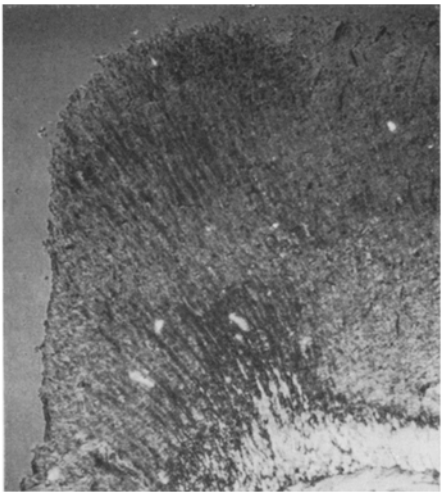

Fig. 4

Figs. 3 and 4. Cerebral cortex. 9 days after irradiation. Fig. 3. A myelin-free band is to be seen midway down the photograph, at the level of maximal ionization ("Bragg-peak band"). Fig. 4. From the same section, in which the specimen was rotated 90°, illustrating the absence of birefringement material in the "Bragg-peak band". × 30. Frozen section. Polarized-light photomicrographs. (AFIP Acc. 951521)

entering fibers were, by far, most numerous in the region of the radial bundle extending into the interhemispheric cortex. Elsewhere beneath the band, fewer fibers were seen to turn into the band. No fibers could be traced downward into the band from the superjacent irradiated tissue.

The newly formed fibers were of varying diameter, displayed a differing degree of argentophilia, and interweaved with one another. Polarized-light examination confirmed the presence of newly formed myelin (Figs. 11 to 13). When viewed under high magnification, birefringence

Fig. 5

Fig. 6

Figs. 5 and 6. Cerebral cortex. 16 days after irradiation. Fig. 5. Loss of myelin is to be noted in the striae of Baillarger (the vertical strip to the left) and in the radial fibers particularly in the region of peak ionization, where the border between irradiated and non-irradiated tissue is fairly sharp. × 50. Frozen section. Spielmeyer myelin stain. Fig. 6. Lateral region of visual cortex. Birefringency has disappeared from the irradiated region, marked off from the non-irradiated tissue by a sharp line. × 30. Frozen section. Polarized-light photomicrograph. (AFIP Accs. 990621 and 951523; Neg. for Fig. 5, 61-4838)

around larger axons was clearly evident (Fig. 13).

Another finding, confirming the observations in 24-day preparations (Fig. 7) as well as those at later stages, was the presence of many fully myelinated radial fibers in the interhemispheric cortex. Moreover, silver-impregnated sections revealed, within the band, many activated argentophilic cells which were oriented parallel to the persisting axis cylinders in the band. Most of these cells were microglia-like cells, but some exhibited dark cytoplasm with few processes and a clear nucleus characteristic of that of oligodendrocytes (Fig. 14).

64 Days (7 Animals). At this time period, newly myelinated fibers in the band, particularly rich in the interhemispheric cortex, had become more dense. In some of the brains the newly formed myelinated fibers predominated in the upper part of

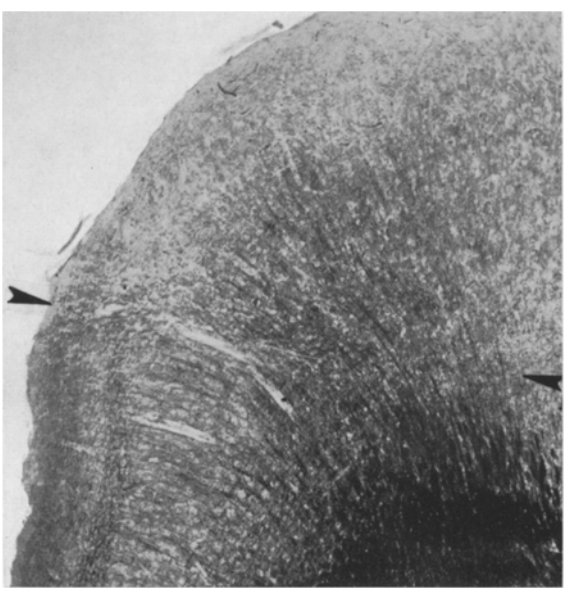

Fig. 7. Cerebral cortex. 24 days after irradiation. The extensive myelin loss illustrated in Fig. 5 is no longer apparent. Most radial fibers now have a myelin sheath, which, presumably, they have regained. The approximate lower border of the irradiated area is indicated by arrows. × 50. Frozen section. Spielmeyer myelin stain. (AFIP Acc. 990620; Neg. 61-4837)

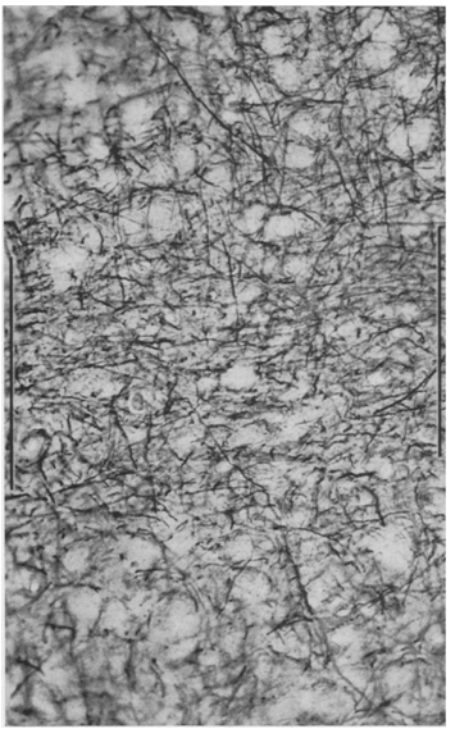

Fig. 8

Fig. 9

Figs. 8 and 9. Cerebral cortex. 30 days after irradiation. Fig. 8. Newly grown axons run horizontally in the "Bragg-peak band", the extent of which is indicated by the lines. × 300. Cajal silver-nitrate impregnation. Fig. 9. 24 days after irradiation. Delicate myelinated fibers are to be seen in the "band" (identified by the lines). × 115. Frozen section. Spielmeyer myelin stain. (AFIP Accs. 951526 and 990620; Negs. 61-4833 and 61-4836)

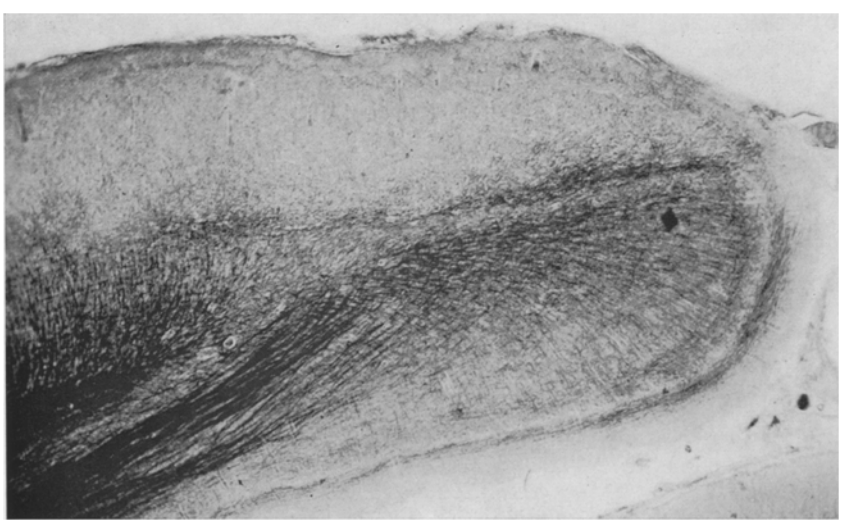

Fig. 10. Cerebral cortex. 30 days after irradiation. A bundle of newly formed myelinated fibers stretches across the cortex, in the upper part of the "Bragg-peak band". Numbers of newly formed fibers vary in the different regions. Complete demyelination is to be noted in the region above the band. × 80. Frozen section. Spielmeyer myelin stain. (AFIP Acc. 951527; Neg. 613212)

the band and even at higher levels, where they were intermixed with persisting nerve cells.

4 and 7 Months (3 Animals). Dense collections of newly formed myelinated fibers within the band were readily demonstrable. At 4 months the fibers were of multinodal distribution within the band, and individual fibers issuing from beneath the band could readily be seen to enter into the formation of the nerve-fiber plexus in the nodal regions (Figs. 15 and 16). In Klüver-Barrera preparations the myelin tubes were, as at previous stages, of differing diameter. As previously, the band was densest in regions in which the fibers were richest in myelin; this difference was, however, not as striking as at earlier time intervals. Myelinated fibers were

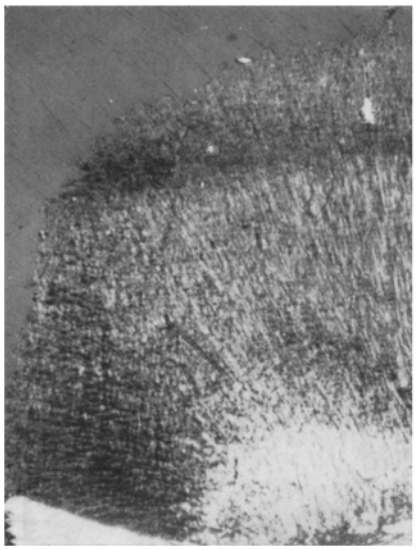

Fig. 11

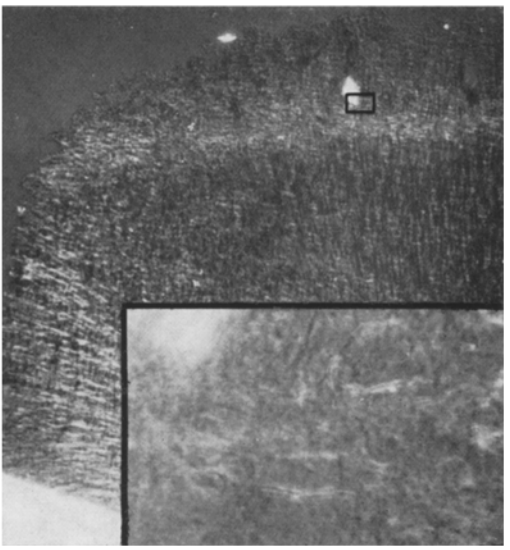

Fig. 12

Fig. 13

Figs. 11—13. Cerebral cortex. 30 days after irradiation. Under polarized light no evidence of myelin is to be seen (Fig. 11), but, as revealed by birefringence, myelinated fibers do become evident on 90° rotation of the specimen (Figs. 12 and 13). Fig. 13 is an enlargement of a field indicated in Fig. 12. Frozen sections. Polarized light photomicrographs. (AFIP Acc. 990635)

also present in the irradiated cortex above the band, but they were few and their architecture was distorted by general tissue shrinkage. By 7 months the neostria was fairly uniform (Fig. 17).

Changes highly similar to those occurring in the cerebral cortex were also visible in the cerebellum. Demyelination had also occurred in the irradiated crests of the cerebellar folia and was advanced by the 11th day (Fig. 18). At the 7-month stage, fibers within the irradiated part of the folia were well myelinated, and a sizable group of regenerated fibers had extended horizontally from the white-matter core through the granular layer and into the deeper part of the molecular layer. The neostria thus gave this region a "Lorain cross" appearance, instead of the normal Y, T or inverted L pattern (Fig. 19).

Discussion

The Degenerative Process. Decrease in myelin stainability in the irradiated region of these brains was well advanced at a time at which nerve cells and their axis cylinders were relatively intact, i. e. at 96 hours following irradiation. By this time period, astrocytes in much of the irradiated area had suffered heavily, circulatory disturbances were advanced, and flooding of all irradiated tissue by plasmatic fluid was in progress. The degree of myelin loss and the sharpness of

the lower border of demyelination, at a level corresponding with that of maximal ionization, strongly suggest a direct effect of radiation on the myelin. This is in the sense that radiation may initiate a chain of autoxidation of lipids, with formation of lipoperoxides, further oxidation, and polymerization. Whether myelin destruction in so short a time (it was advanced by 72 hours) could be dependent on oligodendroglial damage is unknown, but would appear doubtful because of the ubiquity of the myelin loss. An edemation process as a significant factor in the demyelination was ruled by the fact that myelin loss was far less pronounced in the frankly edematous region below the irradiated area, as revealed by fluorescence microscopy.

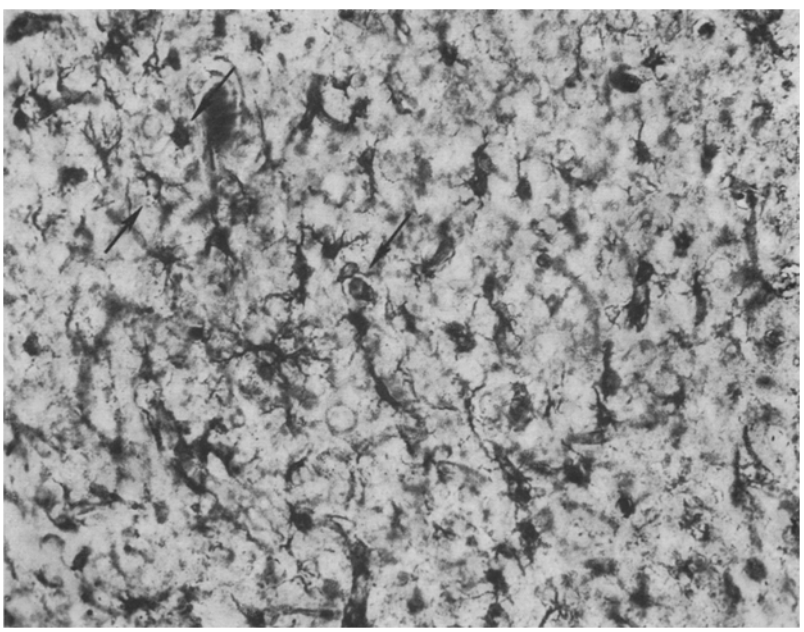

Fig. 14. Cerebral cortex at level of "Bragg-peak band". 30 days after irradiation. Most of the cells have characteristics of microglia. Three arrows point to 4 oligodendroglia. × 300. Frozen section. Hortega silver carbonate impregnation. (AFIP Acc. 990635; Neg. 61-2203)

The Regenerative Process. The Bragg-peak band in which nerve sprouts were found in our animals from approximately the 16th day onwards was characterized at that time period by a rather loose framework composed of naked axis cylinders, abundant reactive microglia-like cells and sparse oligodendroglia, and a rarefied capillary bed which was undergoing restitution. Extending up into the band from subjacent non-irradiated regions were robust processes of astroglia and the apical dendrites of nerve cells. This was the matrix, then, that was receptive to axonal regrowth. Much the same conditions were evident in the band as described by ROSE et al. (1960).

One of the aspects of the regenerative process in the brains of our animals was the remyelination of axis cylinders that had been rendered "naked" by the irradiation. As has been pointed out, many pre-existing axis cylinders of radial fibers traversing the band were devoid of myelin at earlier postradiation stages (Fig. 5),

while at subsequent stages the fibers in the self-same region were myelinated (Fig. 7). This was true not only for the cerebrum but also for the cerebellum (Figs. 18 and 19). The capacity for remyelination of axis cylinders deprived of their myelin

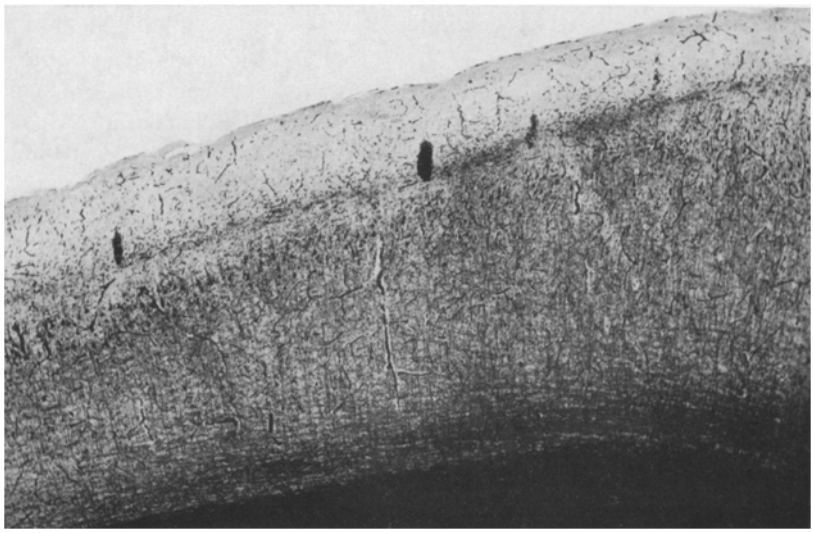

Fig. 15

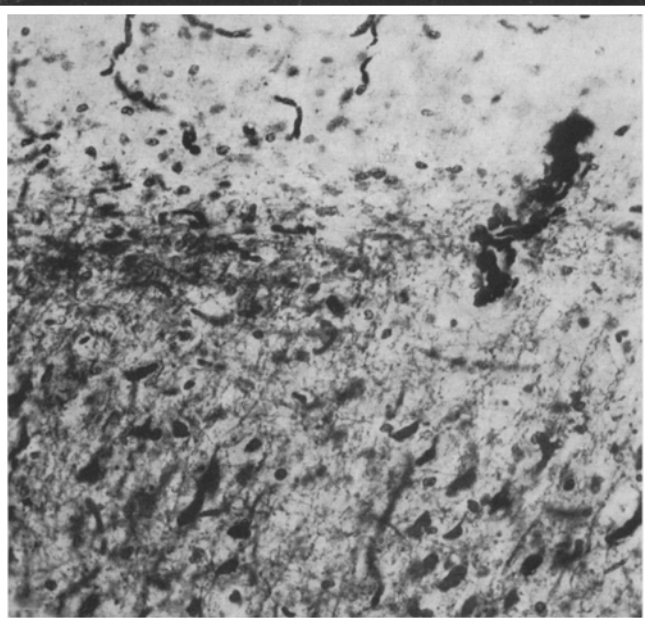

Fig. 16

Figs. 15 and 16. Cerebral cortex. 4 months after irradiation. Fig. 15. Visual cortex. Prominent multinodal myelinated fiber bundles extend across the cortex in the upper part of the "Bragg-peak band". Groups of myelinated fibers from lower laminae appear to be entering these nodal points. × 50. A field in Fig. 15 is illustrated in higher magnification in Fig. 16. Here, individual fibers can be seen extending into the nodal fiber aggregates in the band. × 305 Celloidin section. Woelke myelin stain. (AFIP Acc. 951 529; Negs. 61-4661 and 61-4660)

has, in the past, been considered unlikely in the central nervous system, but recent studies by BUNGE et al. have proved its occurrence in the long spinal

tracts in the cat. These investigators produced plaques of demyelination subpially in the spinal cord by repeatedly withdrawing and re-injecting cerebrospinal fluid into the spinal subarachnoid space. The axis cylinders were completely demyelinated by 6 days. Subsequently, occasional myelin sheaths were first seen at 19 days, and by 64 days all axis cylinders were at least thinly myelinated.

The other aspect of the regenerative process was the development of a robust bundle of newly-formed fibers within the Bragg-peak band. Apical dendrites coursing upward into the band both in our preparations and in those of ROSE et al. have been considered capable of giving off sprouts, as has been observed in hippocampal pyramidal cells in senile dogs (LAFORA), but such sprouting has not been observed following section of pyramidal-cell dendrites in the cerebral cortex of the cat (CAJAL). In our preparations, virtually all nerve sprouts within the band

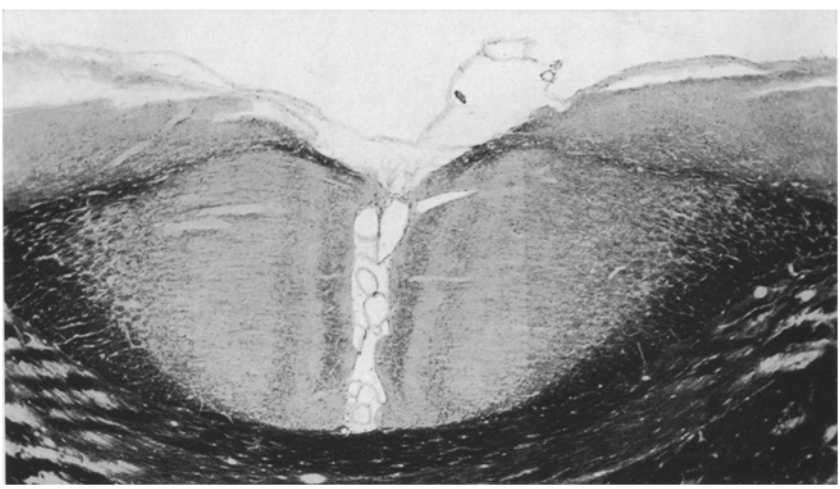

Fig. 17. Region of interhemispheric cerebral cortex. 7 months after irradiation. The irradiated part of the cortex is severely atrophic. A dense, myelinated bundle continuous with the subcortical white matter occupies the "Bragg-peak band". × 50. Paraffin section. Klüver-Barrera stain. (AFIP Acc. 943707; Neg. 61-4666)

issued from the subjacent tissue, namely from cortical afferent fibers. As brought out by various stains and by polarizing microscopy, myelinated fibers accumulated in the band from about the 16th postradiation day onward and formed a conspicous myelinated "neostria". The myelinated nature of the "neostria" was confirmed by electron microscopy, at the 9-month period after radiation exposure (ESTABLE-PUIG et al.). The fibers reconstituted the previous nerve-fiber architecture much less precisely than observed by ROSE et al. (1960) in brains exposed to the higher doses of deuterons. Up through the 4-month stage, the neostria tended to have a multinodal character (Fig. 15). It seemed that fibers entering the band accumulated in nodal regions of the band, then grew horizontally within the confines of the band until, at the 7-month stage (Fig. 17), the band was uniformly populated. Thus, it seemed that fiber growth in the brains of our animals continued well beyond the 7-week stage, the time at which the morphological endpoint of new growth was considered by ROSE et al. (1960) to have been reached in their animals.

One significant feature in the band into which nerve fibers grew was the presence of vast numbers of microglia-like cells. In a consideration of the problem

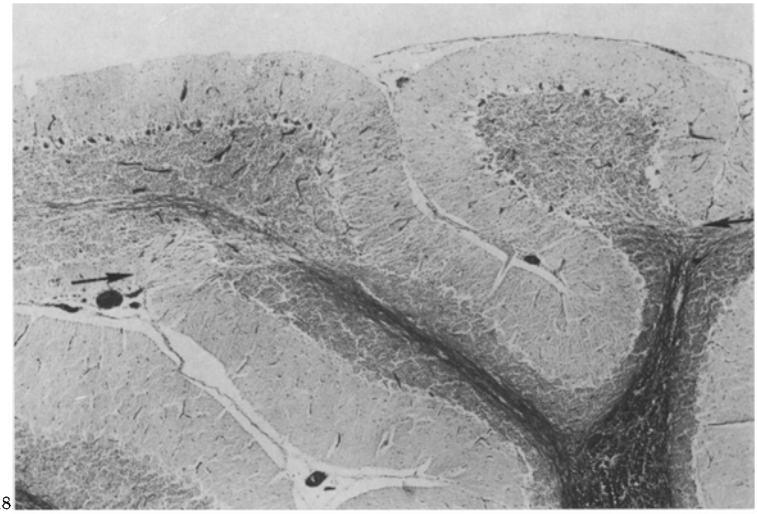

Fig. 18

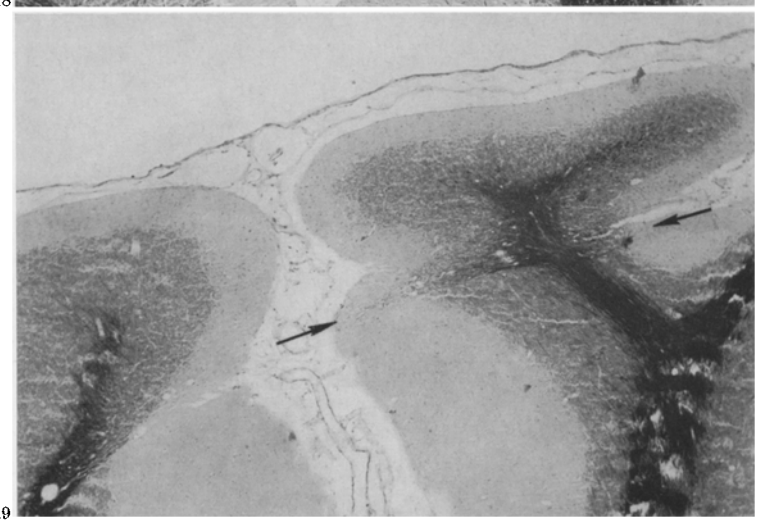

Fig. 19

Figs. 18 and 19. Cerebellum. Fig. 18. 11 days after irradiation. The area irradiated is marked off inferiorly by a sharp border (indicated by arrows). Striking loss of myelin is to be noted in the lowermost irradiated region, and above this level relatively few myelinated fibers are to be seen. In the folium to the left, Purkinje cells in the lower part of the irradiated area have vanished, while many of those remaining are hyperchromatic. × 5. Paraffin section. Fig. 19. 7 months after irradiation. The irradiated part of the folia is severely atrophic. Fibers in this region have become remyelinated, and a broad sheaf of myelinated neofibers in the region of the "Bragg-peak band" extends from the white-matter core laterally through the granular and into the molecular layer (*arrows*). A configuration simulating a Lorain cross has thus been created. × 50. Paraffin section. Klüver-Barrera stain. (AFIP Accs. 961735 and 943707; Negs. 61-6458 and 61-4669)

of growth of new myelinated fibers, SPATZ emphasized the importance of glial cell increase as a substrate necessary to nerve-fiber growth. Astrocytes appeared not to be implicated because they were absent from the band.

As to the mechanism of myelin formation on newly formed axons, we have little to offer. Oligodendroglia appeared not to be materially concerned because they were so few (Fig. 14).

In drawing on a voluminous literature, ROSE et al. (1960) have given the pros and cons as to whether the axonal sprouting represents a regenerative process following severance of axons—i.e., was provoked as a reaction to the injury that had been inflicted—or whether the new growth was an expression of an innate capacity possessed by the central nervous system for continual axonal growth as a reconstitutive process when conditions favorable to the reconstitution exist. Mainly on the basis of restoration of nerve-fiber pattern in the damaged laminae at the lower deuteron dosage they utilized, ROSE et al. (1960) favored the latter alternative. We have nothing to add in this connection. In our preparations the richness of the regenerative process impressed us, as did also its constancy. There must have been some unusual circumstance to account for this, for under other experimental conditions the regrowth of fibers is usually not as luxuriant or as constant. Obviously the setting was unusually conductive to normal growth in the band, i.e., rarefied tissue in which to grow, no disruption of tissue continuity and no obstructive influence of a connective-tissue scar. To what extent the cellular population in the band is of importance to the nerve-fiber growth still remains to be determined. Many axis cylinders were damaged or destroyed in areas of irradiated cortex above the band. The lack of fiber regrowth in this region may be attributed to a setting less conducive than the band to the new growth.

Summary

This study was concerned, on the one hand, with the effects of 48-Mev alpha-particle radiation, at a 6000 rad surface dose and a 30000 rad peak dose, on nerve fibers of the cerebral and cerebellar cortex and, on the other hand, with subsequent nerve-fiber regeneration.

Striking demyelination in the irradiated part of the cortex was already evident at the 96-hour period, but was most prominent in the region of maximal energy release, i.e., in the "Bragg-peak band". In time, the demyelination became still more profound. The myelin destruction was considered to be due chiefly to a direct effect of ionizing events on the myelin. Axis cylinders were much less radiovulnerable than the myelin.

At about the 16th day after irradiation, a regenerative process was underway. Evidence was obtained that pre-existing axis cylinders that had lost their myelin became remyelinated. Myelinated nerve sprouts issuing chiefly from afferent fibers beneath the band began populating the band at this time period and grew progressively within it. The end-stage of fiber regrowth was considered to be between the 4th and 7th month after irradiation.

The new growth was considered an expression of an innate capacity possessed by the central nervous system for continual axonal regrowth.

Only reactive microglia-like cells were present in abundance in the region of axonal regrowth. Whether oligodendroglia had any influence on the remyelination cannot be stated.

Similar changes were found to occur in cerebellar cortex under the same experimental conditions.

Résumé

Cette étude décrit les effets délétères des particules alpha avec des doses de 6.000 r avec une énergie de 48 Mev sur les fibres nerveuses et sur les gaines myéliniques de l'écorce cérébrale et cerebelleuse, aussi bien que la régénération consécutive des fibres nerveuses.

Une demyélinisation frappante est manifeste 96 heures après l'irradiation, spécialement au niveau de la bande du »pic de Bragg« correspondant à la zone dans laquelle la libération de l'énergie est à son maximum. Avec le temps la demyélinisation s'accroit. La destruction de la myéline semble due principalement à l'atteinte cellulaire directe.

Les axones semblaient beaucoup moins vulnérables à l'irradiation que la myéline.

Quelques 16 jours après l'irradiation un processus de régénération apparaissait.

Nos recherches parlent en faveur d'une rémyélinisation des cylindres-axes préexistants. En même temps des fibres néoformées myélinisées, qui prenaient leur origine des fibres afferents en dessous de la »bande« sous-mentionnée, commençaient à peupler la bande et augmentaient progressivement. La régénération finissait entre le 4ème et le 7ème mois après l'irradiation. Dans la région de la régénération il n'y avait que de cellules semblables à la microglie réactive. On ne pouvait pas préciser si l'oligodendroglie prenait une influence sur la néoformation ou rémyélinsation.

Le cortex cerebelleux présentait de changements semblables sous les mêmes conditions experimentales.

References

Baker, C. P., H. J. Curtis, and W. Zeman: The design and calibration of a deuteron microbeam for biological studies. Radiat. Res. **15**, 489—495 (1961).

Bunge, M. B., R. P. Bunge, and V. H. Ris: Ultrastructural study of remyelination in an experimental lesion in an adult cat spinal cord. J. biophys. biochem. Cytol. **10**, 67—94 (1961).

Cajal, Ramón y, S.: Degeneration and regeneration of the nervous system. (Transl. and edited by R. M. May) Vol. 2, p. 639. Oxford: Oxford Univ. Press 1928.

Estable-Puig, J. F., W. Bauer, J. Blumberg, W. Haymaker, and C. Tobias: Study of regenerated cortical nervous tissue after alpha particle irradiation. In: Proc. 5th Internat. Congr. Electron Microscopy vol. 2 Biology p. VV-4. New York: Academic Press 1962.

Janssen, P., I. Klatzo, J. Miquel, T. Brustad, A. Behar, W. Haymaker, J. Lyman, J. Henry, and C. Tobias: Pathological changes in the brain from exposure to alpha particles from a 60-inch cyclotron. In: Response of the Nervous System to Ionizing Radiation (T. J. Haley and R. S. Snider eds.) p. 383—409. New York: Academic Press 1962.

Klatzo, I., J. Miquel, W. Haymaker, C. Tobias, and L. S. Wolfe: Observations on appearance of histochemically-demonstrable glycogen in the rat brain as effect of alpha-particle irradiation. In: Effects of Ionizing Radiation on the Nervous System p. 309—358. Vienna: IAEA 1962.

— — C. Tobias, and W. Haymaker: Effects of alpha particle radiation on the rat brain, including vascular permeability and glycogen studies. J. Neuropath. exp. Neurol. **20**, 459—483 (1961).

Lafora, G. R.: Neoformaciones dendriticas en las neuronas y alteraciones de la neuroglia en el perro senil. Trab. Lab. invest. biol. **12**, 39—53 (1914).

MALIS, L. I., C. P. BAKER, L. KRUGER, and J. E. ROSE: Effects of heavy, ionizing, monoenergetic particles on the cerebral cortex. I. Production of laminar lesions and dosimetric considerations. J. comp. Neurol. **115**, 219—242 (1960).

— L. KRUGER, and J. E. ROSE: The use of deuterons in production of laminar lesions in the cerebral cortex of the rabbit. Trans. Amer. neurol. Ass. **83**, 78—80 (1958).

— R. LOEVINGER L. KRUGER, and J. E. ROSE: Production of laminar lesions in the cerebral cortex by heavy, ionizing particles. Science **126**, 302—303 (1957).

— J. E. ROSE, L. KRUGER, and C. P. BAKER: Production of laminar lesions in the cerebral cortex by deuteron irradiation. In: Response of the Nervous System to Ionizing Radiation (T. J. HALEY and R. S. SNIDER eds.) p. 359—368. New York: Academic Press 1962.

MIQUEL, J., and W. HAYMAKER: Astroglial reactions to ionizing radiation. In: Sympos. Internac. Invest. Neurológicas, Buenos Aires, Oct. 17—18, 1963.

ROSE, J. E., L. I. MALIS, and C. P. BAKER: Neural growth in the cerebral cortex after lesions produced by monoenergetic deuterons. In: International Symposium on Sensory Communication, 1961 (W. A. ROSENBLUTH, ed.), p. 279—301. New York: Wiley 1961.

— — L. KRUGER, and C. P. BAKER: Effects of heavy, ionizing, monoenergetic particles on the cerebral cortex. II. Histological appearance of laminar lesions and growth of nerve fibers after laminar destructions. J. comp. Neurol. **115**, 243—296 (1960).

SPATZ, H.: Morphologische Grundlagen der Restitution im Zentralnervensystem. Dtsch. Z. Nervenheilk. **115**, 197—231 (1931).

TOBIAS, C. A.: The use of accelerated heavy particles for production of radiolesions and stimulation of the central nervous system. In: Response of the Nervous System to Ionizing Radiation (T. J. HALEY and R. S. SNIDER, eds.) p. 325—343. New York: Academic Press 1962.

WOLFE, L. S., I. KLATZO, J. MIQUEL, C. TOBIAS, and W. HAYMAKER: Effect of alpha-particle irradiation on brain glycogen in the rat. J. Neurochem. **9**, 213—218 (1962).

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