

MORPHOLOGICAL STUDIES OF LAMINAR LESIONS

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(For Dr. John Lawrence, Donner Laboratory and Donner Pavilion, Berkeley, California, as one of a series of essays for use at the Meeting of AEC Biomedical Advisory Committee to be held in Berkeley, March 10 and 11, 1967.)

GPO PRICE \$ _____
CFSTI PRICE(S) \$ _____
Hard copy (HC) _____
Microfiche (MF) _____
ff 653 July 65

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FACILITY FORM 602	N 68-25623	
	(ACCESSION NUMBER)	(THRU)
	16	1
	(PAGES)	(CODE)
	TMX-59783	04
	(NASA CR OR TMX OR AD NUMBER)	(CATEGORY)

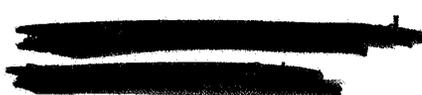
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As Jerzy Rose once related, the first effort to produce a laminar lesion had behind it a neurophysiological objective. If one or another of the six laminae of the cerebral cortex could be destroyed, leaving the others intact, then it might be possible to assess the contributions made by the various laminae to cortical electrical activity, as recorded from the cerebral surface. So the first experiment was done to see if a lamina could be selectively destroyed. This pioneer study, reported 10 years ago (1957) by Malis, Loevinger, Kruger and Rose,¹⁶ proved that the approach was feasible. In two cats in which a 5×5 mm area of the cerebrum was exposed to 10-MeV proton radiation they demonstrated that a longitudinal zone of the cortex 0.8 mm deep to the cerebral surface could be severely damaged without materially injuring the intervening tissue. In stained sections the damaged zone was found to have a sharp lower border and an uneven upper border, and its width was 40 to 100 μ , depending on dosage. Since tissue shrinks about 35% during processing, the actual width of the band would be correspondingly greater. To this "Bragg-peak zone," or "band," they gave the misnomer, "laminar" lesion, a term we shall probably have to live with. In actuality the zone was pseudolaminar, as would be expected from bombardment with a monoenergetic beam. Nonetheless, the lesions were precisely enough situated to serve



as a wholly satisfactory model for the neurophysiological purposes intended. Some results along this line are available.¹⁵

Rose and his associates,²³ in following up that first study in cats, decided that the initial step should be a careful histological study. They used rabbits and concentrated on silver methods that specifically impregnate nerve cells and their processes. As in the cats, the "Bragg-peak band" of damage in the rabbits was peculiar in that only nerve cells were destroyed, leaving intact the rest of the tissue elements, which included glia and fiber processes. From this study came the remarkable observation that new axons grew into the cell-depleted band. That nerve fibers in the brain can "regenerate" had long been known, but here in the laminar preparation was a model in which the phenomenon was open to study. When a peripheral nerve is sectioned, as many as 40 new axons sprout from a single nerve fiber in the proximal stump, and sprouting of much the same magnitude seemed also to be occurring in the laminar preparation. The comparison is, however, not entirely apt, as no proof was provided that the particle radiation amputated a sizable number of the fibers in their course vertically through the band. Be that as it may, the persisting tissue matrix in the band served as a latticework on which the new axons could climb. There was sometimes an orderliness in the regrowth in the band in that a semblance of the previous fiber-architecture was achieved, but usually not.

A subsequent study, by Kruger and Clemente,¹⁵ confirmed all this, and showed that new axons could grow out not only in the cat and rabbit,

but also in the rat and monkey. Further, in the rat Estable-Puig et al.⁵ observed that axonal growth occurred not only in the cerebral cortex but also in the cerebellar cortex. Such a heavy growth of axons occurred at both sites that after a few months the axons could easily be seen in stained sections with the unaided eye. Entrance of the new axons into the band was evident on about the 18th day postexposure. Many grew upward into the band from a long distance away, i.e., from a region of the white matter near the corpus callosum. Moreover, it was shown that the new axons in the band were being surrounded by a sheath of myelin, but the mechanism by which this occurred was obscure. Oligodendroglia appear to be requisite to myelin formation, in the sense that the myelin layers encircling axons are an extension of the oligodendroglial cytoplasm. One reason for mentioning all this is to indicate that in vivo models are needed if one is to get at the heart of the problem of how, for example, remyelination is achieved under some conditions of CNS injury,^{3,13} but not under other conditions, such as multiple sclerosis. The laminar preparation is admirably suited as such a model.

Speaking of possible applications of the laminar model to clinical problems in man, we wonder whether, with the use of the model, nerve fibers could be induced to grow more effectively up and down the spinal cord in paraplegics. It is a wild idea, but could not exposure of the cord to particle radiation in the acute stage of injury create a lattice-work on which new axons could more effectively grow?

Studies on laminar preparations were undertaken in the early days without any idea as to where they might lead. We used to come at times from Washington to work with Dr. Tobias and his associates. I would put

brain material into all kinds of fixatives, then, on coming home, stain sections in various ways. On one of these trips Igor Klatzo, of the NIH, came along and brought Rossman's fluid, which contains a number of fixing ingredients. He put some of the brain blocks in this fluid. On staining the sections by the PAS method with and without pretreatment with α -amylase he found that the astroglia in the irradiated area (but not in the control areas) contained particles of glycogen.¹² Glycogen is normally present in the brain, but is at a level (45-70 mg per 100 gm of tissue in the rat) usually not detectable with use of the PAS stain. Only on considerable increase does the glycogen become evident in sections, and then in the form of granules, which are stained red by PAS. It was appropriate that the glycogen increase should occur in the astrocyte, for carbohydrate metabolism in this cell is probably very intense. Through its cytoplasmic processes wrapped around vessels, this cell also has transport functions, carrying, for example, glucose from the blood stream and discharging it into the tissue, for use by such elements as nerve cells.

Klatzo, and shortly thereafter Miquel et al.,^{19,21} observed that the glucose coming from the blood stream into the irradiated part of the brain was not being used properly and was being stored as glycogen. This observation was a breakthrough, in that a dependable means was now available for the study of CNS energy metabolism. Essentially the glycogen accumulation represented a "biochemical lesion," the intensity of which reached a peak in 48 hours, then declined over the next week or two, when normality was reached.

The glycogen increase raised all kinds of questions as to its genesis. A favored view was that the radiation directly damaged the tissue such that inhibition of aerobic metabolism occurred, with the result that glucose in the tissue was converted to glycogen - in other words that the accumulation was the outcome of a profound reduction in oxydative metabolism. This view found support in an observation made by Snezhko,²⁵ in an experiment in rabbits in which determinations were made of free O₂ content in cerebral tissue following X-irradiation of the head at 1-3 kR. O₂ tension in the tissue fell transiently in some of the animals. However, in all the animals the tension soon increased drastically (by 70-100%). Subsequently - for at least 7 hours - the O₂ concentration fluctuated in a wave-like manner, and remained above the 70% increase level. No parallel was found with hemodynamic shifts, and thus it was concluded that the rise in O₂ concentration in the brain was indicative of lowered oxidative processes. There seems no better explanation of acutely developing glycogen increase.

The problem of O₂ tension in the brain following irradiation is, however, not that simple, as shown by Aleksandrovskaya¹ in a histology study in rats. The radiation conditions she used were, however, so different from those in the preceding experiment that comparison is not valid. The rats were totally irradiated in fractional doses of 50 R weekly for a maximum of 250 R, and they were sacrificed at various periods of time up to one month after the last exposure. Pathological changes favoring a hypoxic effect were laminar lesions in the cerebral cortex, destruction of nerve cells, and multiplication of oligodendroglia. According to the author: "On taking into account the high oxygen

requirement of an organism exposed to the action of penetrating radiation it can be assumed that the usual amount of oxygen supplied to the brain is found to be inadequate and a deficit develops in the oxygen supply of the brain, or to put it differently, a secondary anoxia develops." Lack of visible changes in vessels was considered to support this view. While lack of visible vascular change does not necessarily mean that it did not exist, and while vascular change may have occurred in this experiment and been pathogenic, nonetheless the viewpoint is refreshing, and certainly has some substance.

As if the O_2 factor were not enough to cope with in evaluating tissue damage, there is still another factor to be reckoned with, and that is brain edema. Here, a hypoxic state of the tissue is inevitably a complication.

To evaluate the significance of an edematous process in radiation injury a model was needed. None could be found that had more advantages than the laminar preparation, in which, as stated, only the upper part of the cortex is damaged. In studies on the rat and cat carried out with the use of this model, it was found²⁰ that very quickly after 48-MeV α -particle irradiation given in a large dose (24 krad) edema fluid began to permeate the white matter beneath the band, until in a day or two all the white matter throughout the cerebrum was flooded by the fluid. But the interesting point was that glycogen accumulated in astroglia throughout all the cerebral cortex, even in that farthest removed from the site of radiation injury (Miquel, unpublished). Then it was found that an equally far-flung glycogen disturbance occurred after a very small stab wound had been made in the cortex (Miquel and Ibrahim, unpublished).

This raised questions which have not as yet been answered. Could some reflexly-induced edematous process not visible under the light microscope be held accountable? Increased glycogen content in the cortex must necessarily go hand-in-hand with a reduction in brain function. Indeed, Krivánek¹⁴ found a correlation between duration of glycogen accumulation (following application of strychnine to the cortex, which should have induced an edematous state) and duration of abnormal conditioned reflexes. In this connection, Miquel and Haymaker¹⁹ observed in the totally irradiated brain (of the rat) that the largest accumulation of glycogen was in a structure heavily concerned in emotive functioning, i.e., the hippocampus. The future requires that more consideration be given to these wider implications of laminar and other radiation-induced lesions. A more sensitive means of glycogen detection is needed, as the glycogen response about which we have been talking is not detectable as doses below 1 krad. Perhaps further studies for the detection of glycogen biochemically, by a method already established for laminar preparations,²⁶ will prove profitable.

This gives something of the story of the investigations that were opened up in our own laboratory by the chance observation that particle irradiation resulted in glycogen accumulation.

This discussion is not supposed to exceed a certain length, and since something needs to be said about the nerve cell, little can be said about other matters. α -particle radiation in a dose of 12 krad (surface dose) invariably results in 2 or 3 days in the appearance of

mitotic figures at the sites of neuroglia. However, some say that neuroglia do not divide mitotically! At about 6 krad (surface dose) vascular permeability disturbances occurring in the laminar preparation can persist for as long as 13 days (as shown with the use of fluorescein-labelled albumin [FLA],^{8,11,12}) and prominent vascular changes, as viewed electron microscopically, can last some 22 days.¹⁷ Thus, vascular alterations during such time periods can be a source of parenchymal injury. Vessels in irradiated tissue can be functionally defective even though morphologically they appear perfectly normal, or practically so.^{4,24} In this connection, any claim that ionizing radiation primarily destroys nerve cells - say after a latency of some days or weeks - should be considered unfounded unless backed up by vascular permeability studies. The oligodendrocyte has such a refined digestion system that mesenchymal cells may not be called into the laminar preparations to clean up the debris.¹⁸ Lastly, if one is looking for a sensitive system for the detection of CNS radiation damage, the paper by Neumayr and Thurnher²² should not be neglected.

In regard to nerve cells, some people might think that the brain is composed mostly of these cells. But only about 1/10 the cells are nerve cells. It thus takes an average of 10 cells, such as vascular endothelial cells and astrocytes, to keep a nerve cell going. The nerve cell is thought to be particularly radioresistant because it is not subject to divisional processes which make other cells radiosensitive; the nerve cell uses practically all its energy in communication. The defenses set up for the preservation of the integrity of the nerve cells suffice when

the entire head is irradiated, as under these conditions the nerve cell can go uninjured following exposure to 1000's of rad, yet oligodendroglia not far away will be destroyed by 200 rad^{2,10} (primarily? secondarily?). However, despite all the defenses available, the nerve cell is the most vulnerable element when the radiation field is very small, such as in the laminar preparation. What is meant by "most vulnerable" in this context is that while vascular cells and glial cells react actively to the radiation injury and persist, the nerve cell dies.

There seems to be a discrepancy in the dosage needed to wipe out a narrow band of nerve cells. In one experiment, Janssen et al.¹¹ found that a 1.5 krad surface dose of 48-MeV α -particles (7.5 krad, peak dose) was sufficient to destroy a very narrow band of nerve cells - say a band 25 μ thick - in 7 months. Particle flux in this experiment was $1 \times 10^8 \alpha/\text{cm}^2\text{-sec}$, and dose rate 10 krad/min. On the other hand, Zeman et al.,²⁸ in bombarding the brains of mice with 22.5-MeV deuterons through an aperture 25 μ in diameter, found no discernible damage in 6-1/2 months after exposure to 225 krad ($10.7 \times 10^9 \text{ d}/\text{cm}^2\text{-sec}$; 8 krad/sec), though nerve-cell destruction did occur in 24 days when the dose was doubled. The need for massive dosage to destroy nerve cells in a 25 μ track was subsequently confirmed.²⁷ How is one to account for such wide differences in results? People say it must be a matter of geometry, that the greater volume of brain irradiated in the Janssen et al. experiment can account for the difference.

More concrete information is needed. Although much is known in regard to dose-tissue volume factors in pathogenesis when larger areas of

the brain are irradiated, much still remains to be learned of the factors involved in damage incurred in tiny brain areas. What we are hoping to do in collaboration with Dr. Tobias and his group in Berkeley is to bombard many 25 μ fields in the same brain with protons or α -particles in an effort to settle this problem. Tolerance doses would be established in relation to spacing of the particle tracks. This would be, as it were, a first approximation to a study of the effects of heavy primary primaries once the presently planned accelerator becomes available.

In this connection, there is the problem of the RBE of particle radiation. For most endpoints the RBE has been shown to be close to 1. But in regard to the brain it seems likely that the RBE of particle radiation is higher than 1, at least for certain particle energies. In the monkey, 6 krad proton radiation (55 MeV) given at 2 krad/min caused far greater damage in the same period (unpublished) than 30 krad γ rays (1.2-1.4 MeV) given at 1 kR/min.⁹ The comparison seems to be valid. Also it has been found that 138-MeV protons had less of an effect on the brain than 55-MeV or 32-MeV protons (unpublished). As to the effect of particles of different nuclear charge on the brain, we once ran an experiment with Dr. Tobias in which laminar lesions were made both with protons and α -particles of equal energy per nucleon (12 MeV), and given in the same dose and at the same dose rate. According to the published results, the width of the band was approximately the same in both series.¹¹ But there remains the hankering suspicion that the experiment was not well enough designed to provide a definitive answer. Repetition is therefore

in order. If we are to be ready to do the crucial studies when accelerated larger nuclei become available, and thus to get some idea of the RBE of galactic cosmic rays so far as the brain is concerned, then considerably more work is needed with particles now available.

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