Effects of Beta Rays on Central Nervous Tissues.* (17323)

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The present report is made specifically to define the gross morphological changes evoked in the neurons and glial cells of the nervous system by high doses of ionizing radiations. The use of local irradiation by means of a radon applicator was made in order to study a sharp gradient of tissue reaction in the cerebral cortex. Similar technic has been described by Levin[1] in studies of other tissues and by Peyton[2] in an investigation on the effects of radium on the central nervous system. By comparing the various concentric zones of cellular alteration in a lesion the center of which showed complete destruction of cells, the pattern of progressive cytologic alteration has been made clear. In this series of experiments, the high gradient was caused both by the short trajectory of the bombarding electrons in the tissue and by the exponential operation of the law of the inverse square. For the present study, a solution of the difficult question of dosage from radon applicators will be deferred until, in a later report, the effects of dosage will be more fully discussed. Nor will attention be paid in this paper to the possible effects of the very small fraction of ionization caused by the hard gamma radiation which is delivered by the radon applicators. We have no data which would cause one to believe that the action of one type of ionizing radiation is essentially different than of another. The effects of the gamma rays in the present experiments is thought to make the gradient of exposure somewhat less steep by reason of their great tissue penetrating properties. They are, of course, also subject to the law of the inverse square and, forming a small fraction of the total ionization, seem not to complicate matters.

Cats were used in the experiments. Trephining of the calvarium over the parietal region of the cerebral cortex was performed under barbiturate anaesthesia and the dura mater carefully slit and raised. The administration of the irradiation was by means of a small glass bubble (ca 2 mm in diameter) containing emanation of radium. The heads of the cats were held in a rigid holder and the radon applicator was fastened to a 3-directional microdrive and thus directed to the surface of the cortex. The duration of the exposures varied from 5 to 32 minutes, the calculated activity of the applicators from 30 to 78 millicuries. The dura was then returned to place (in the longer experiments) and the skin carefully sutured over. Killing was by overdose of sodium pentobarbital. Where imprints of the lesions were made, the cortex was dissected out fresh and after the imprints, the material of the lesion was fixed by immersion in 10% formalin. In several of the experiments, the animal was first perfused with formalin and the lesions subsequently dissected out. Though maps of the sulci and the positions of the sites of irradiation were kept, some difficulty was had in locating those lesions which were not strongly enough irradiated to cause a visible pink spot.

Observations. Fig. 1 shows the edge of a typical lesion in the cerebral cortex 5 hours after exposure. To the right is the area, just visible in the photograph, of complete destruction. Towards the left the affected area borders the normal cortex. The alteration towards basophily of the neurons and of the glia is visible. The meninges and the surface of the brain frequently are much altered at the point of contact with the applicator. In all of the marked lesions, as in Fig. 1, there was considerable breakdown of the layered structure of the cerebral cortex. The disturbance of the tissue seldom extended into the neighboring white matter. The neuronal changes in the area bordering that of complete tissue

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1 Levin, PROC. SOC. EXP. BIOL. AND MED., 1924, **21**, 462.

2 Peyton, W., *Am. J. Cancer*, 1934, **20**, 558.

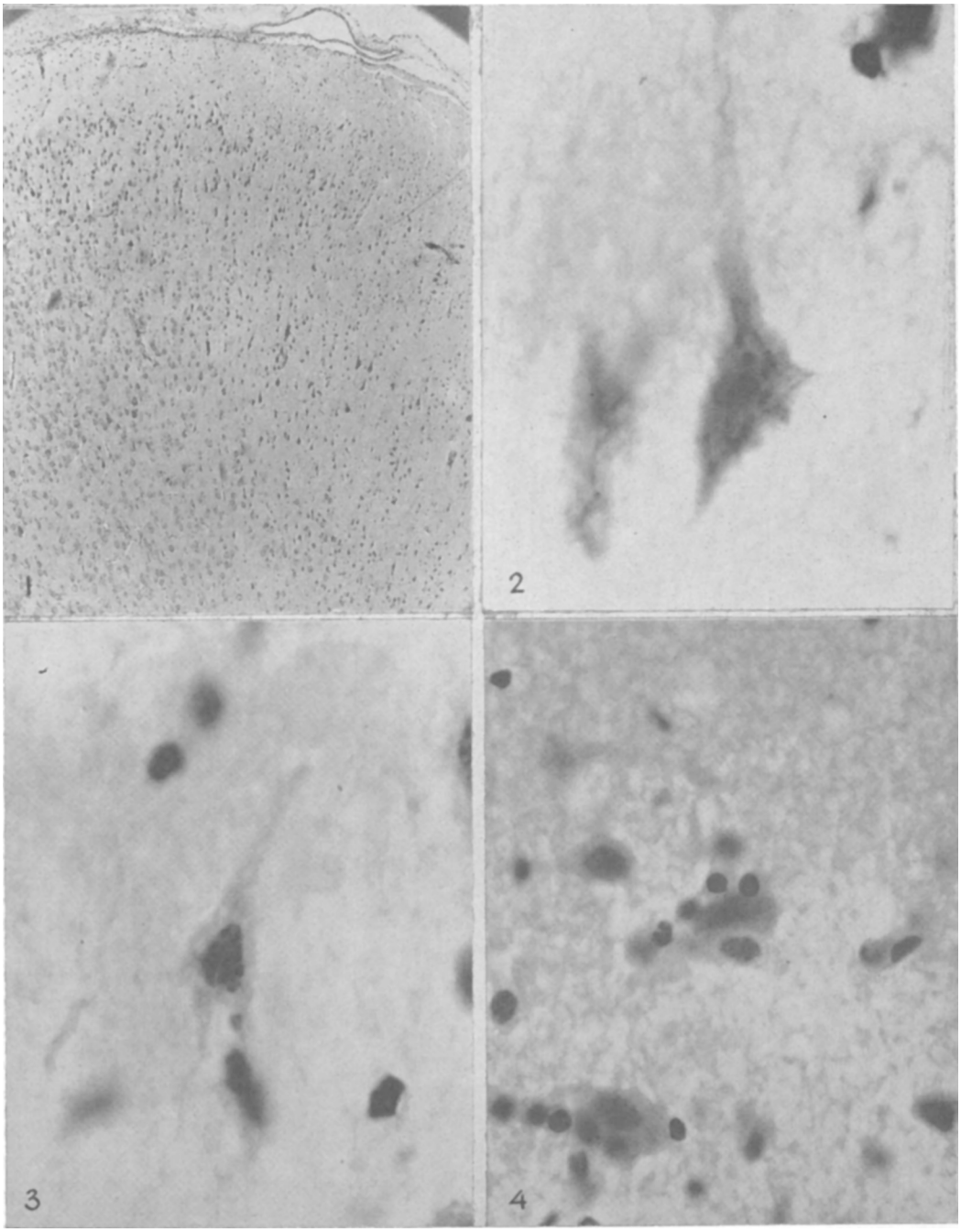

FIG. 1. Edge of small lesion in cerebral cortex 5 hours after irradiation. To the right is the area of heavy destruction, to the left that of unaffected tissue. Cresyl violet stain.

FIG. 2. Hyperchromic nerve cell of fifth cell layer of cerebral cortex. Cresyl violet stain.

FIG. 3. "Polka-dot" degeneration of cell from fifth cell layer of cerebral cortex. Cresyl violet stain.

FIG. 4. Plasma cell like changes in satellite oligodendroglia in fifth cell layer of cerebral cortex. Hematoxylin and eosin stain.

destruction are severe and segregate into two general patterns. First is the hyperchromic reaction which leads to cells similar to those described by Miller[3] and Hartmann[4] in cerebral cortex of cats and other animals. This is characterized by a simultaneous increase in basophilia of all elements of the cell and a shrunken, ragged appearance. The second type of change observed leads to fragmentation of the nuclear chromatin and a progressive loss of nuclear membrane and cytoplasmic structure. In the area of heaviest irradiation all of the neurons of the cerebral cortex are affected. Toward the edge of the lesions it is seen the changes continue for a greater distance from the source in the large pyramidal cells of Layer 5 than in the granule cells of Layer 4. Inspection of the deeper layers closer to the center of the lesion shows that the large pyramidal cells, which seem affected by a lower dosage, also show a greater resistance to the rays in that they persist, as hyperchromic cells, in areas quite devoid of other neuron types. Further towards the center of the lesion, they, too, suffer dissolution.

The hyperchromic cells are conspicuous in Fig. 1. In Fig. 2 is shown a large pyramidal cell from Layer 5 with a long apical dendrite. All of the dendrites are deeply stained, but the cork-screw appearance mentioned by Miller and Hartmann[3,4] is not conspicuous. The margin of the cytoplasm is heavily vacuolated, giving the cell body a scalloped effect. The Nissl bodies are visible and discrete and of normal appearance except for the intense affinity for basic stains. The cytoplasmic matrix (the unstainable substance of Nissl), which is usually refractory to staining by most dyes, is heavily colored by the basic stain and in many of the more pycnotic cells completely occludes the finer structure of the cell. In the example seen in Fig. 2, the nucleus contains a characteristically hypertrophied nucleolus. In many instances, the nucleus has the appearance of being filled with nucleolar material. The nuclear membrane is usually heavy and covered with small densely staining plaques of chromatin. The axons are not stained.

More common than the hyperchromic alterations in the areas of heavy irradiation are those cells showing fragmentation of the nucleus and dissolution of the cytoplasm as their main features. In these cells, both the Nissl bodies and the rest of the cytoplasm have very little affinity for the stain and the nuclear changes may be followed with considerable ease. There is an increase in the amount of chromatin in the nucleus and it is seen to assume a pattern quite unlike that seen in neurons under other conditions. Heavy rounded accumulations of chromatin are formed giving the nucleus a "polka dot" appearance. In the cells least altered, as in Fig. 3, this nuclear material is of considerable bulk and a thin nuclear membrane may be made out. Other cells, apparently more affected, show loss of size of the granules and a thinning of the nuclear membrane. The nucleolus disappears. The cytoplasm of the more affected cells as well as the cell membranes are visualized only with difficulty or not at all, and near the center of the lesion remains of the neurons consist merely of an accumulation of small heavily staining granules.

The small lesions produced by these experiments are especially useful in studying the glial responses to irradiation. Across the steep gradient from normal tissue to the necrotic center of the lesion (a distance of only a few millimeters) the changes brought about by a full spectrum of adequate dosage may be observed. The fate of the astrocytes, as traced in these lesions is that of slight nuclear swelling followed by dissolution. No cytoplasm is visible in any of the stages. The nuclear material becomes finely stippled in the terminal stages, reminiscent of the dotted chromatin pattern of some of the neurons. The position in the lesion where the astrocyte may last be recognized indicates that it is more radiosensitive than either the neurons or the other glial cells.

The oligodendroglia and the microglia, whose naked nuclei may be quite well distinguished in the normal appearing periphery of the lesion show convergent changes as

[3] Miller, R., *Am. J. Anat.*, 1949, **84**, 201.

[4] Hartmann, F. P., unpublished manuscript.

they are followed through the zones of higher exposure. These consist of a marked hypertrophy of the nuclear membranes and the accumulation of visible cytoplasm. There is no sign of either cell undergoing destruction in the outer zones and the phenomenon of convergence to a common cell type is well marked. In the region of intense destruction of the neurons, these altered cells of the mesoglia show a tendency to clump around the remains of the nerve cells but signs of phagocytic activity were not made out with certainty (Fig. 4). Instead, the cytoplasm showed an absence of the foamy and detritus ladened appearance of true macrophages. Examination of these altered glial cells in air-dried imprints of the lesions stained with Wright-Giemsa shows that the cells resemble, in some particulars, the brain plasma cell. The nuclei tend to be eccentric, the chromatin particles very coarse. There is a basophilic cytoplasm which resembles in its staining and texture that of the plasma cell. A perinuclear halo is, however, infrequently seen.

The changes undergone by these mesoglial cells (cligodendroglia and microglia) are similar to those of other cells of supportive tissue in inflammatory reactions. Thus the nucleolar chromatin increases in amount, the nuclear membrane hypertrophies, and both the volume and the nucleic acid content of the cytoplasm is altered upward. As is the rule in other supporting tissues, the rounding up of the cells occurs as a correlate of these processes.

In the older lesions, infiltration of hematogenous cells, first polymorphonuclear leucocytes and later lymphocytes occurs. The former species degenerate, the lymphocytes differentiate into intermediate polyblasts as described by Good.[5]

Discussion. A limiting factor in the interpretation of results such as presented here is the impossibility of ruling out the indirect effect of the irradiation on the cell components of nervous tissue by disturbance of the vascular system. Work is underway to circumvent the complication by irradiation of cells in tissue culture. Prior to such improved data, however, the present findings are not without considerable interest.

The ability of such small radioactive sources to make restricted lesions in the nervous system has obvious uses. Not only does the small size of the lesion furnish a gradient from normal to destroyed tissue along which changes may be traced with especially good control, but bloodless destruction of certain tracts and nuclei whose vascular system is related to surface vessels may be more practical than hitherto. Details necessary for exploitation of this method in surgery must be worked out in long term experiments of this sort.

The chromophilic "sclerotic" nerve cell, so conspicuous in these preparations, has been previously studied in this laboratory (Miller,[3] Hartmann[4]) and found to be more than a fixation artifact. Production of this cell change by such means as ischemia (Tureen[6]), inanition, or chronic methylene blue poisoning (Näätänen[7]) has not been successfully duplicated in this laboratory.

Both the changes in the neurons and in the glia show the protein synthetic mechanisms of the cells to be susceptible to alteration by ionizing irradiation. The as yet undeciphered link between the genic chromatin in the nucleus, the cytoplasmic (and nucleolar) ribose nucleic acid, and physiological states associated with inflammation is apparently a target in the experiments described here. Not only in the chromophilic neuron, but particularly in the glia, is this obvious. The mesoglial elements give rise to plasma-cell like cells rather than to macrophages as would be expected in lesions of similar magnitude formed through other means. While these cells show some tendency to cluster around the neurons, phagocytic activity, as judged by the appearance of the cytoplasm, could not be postulated. As recent work on the relations of macrophages and plasma cells in experimental brain lesions has shown that

[5] Good, R. A., *J. Neuropath. and Exp. Neurol.*, in press.

[6] Tureen, L. L., *Arch. Neurol. and Phychiat.*, 1936, **35**, 789.

[7] Näätänen, E., *Acta path. et microbiol. Scand.*, 1945, **22**, 603.

the functions of secretion of antibodies and of scavenging are sharply differentiated,[5] the most reasonable interpretation is that these destructive ionizing radiations have produced aberrant secretory cells, possibly analogous to the neoplastic cell in multiple myeloma.

The phenomenon of convergence of the oligodendroglia and of the microglia seen in these preparations is further evidence of the thesis supported by the senior author[8,9] that the microglia and the oligondendroglia are functional states of the same cell type and that both contribute to the formation of inflammatory cells in encephalitic disease. The clarity with which this process can be seen in the transition zones of the beta ray lesions makes the demonstration of similar evolution of the two species of mesoglia more obvious than any other preparation. That it is not simply a particular response to this highly unusual stimulation is substantiated by the parallelism of the changes in herpetic virus disease.

Summary. 1. The application of small, high-energy, applicators of radium emanation to the cerebral cortex of cats produces restricted lesions in which progressive changes of the cells of that tissue may be followed from normal appearing tissue to that showing complete necrosis over a distance of several millimeters.

2. Nerve cells so affected show two types of destruction. One involves lysis of the cytoplasmic structures and degeneration of the nucleus into a small aggregation of basophilic granules. The other consists of chromophilic alteration of the cells. The pyramidal cells of layers 2, 3, and 5 seem especially susceptible to this change. The cells become shrunken, extremely heavy staining, and show hypertrophy of the nucleolus.

3. The astrocytes are the most susceptible of the various cells of the cerebral cortex. They undergo lysis in the middle zones of the lesions.

4. The mesoglial cells, oligodendroglia and microglia, are highly resistant. They show progressive changes, from the edge to the center of the lesions, which by convergence, make the two types indistinguishable. The product of these changes is a plasma-cell like structure which shows no phagocytic activity.

8 Campbell, B., *Anat. Rec.*, 1947, **97** (Suppl.), 7.

9 Campbell, B., *J. Neuropath. and Exp. Neurol.*, 1949, **8**, 347.

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Lysozyme Content of Granulation Tissue.* (17324)

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The highest lysozyme concentrations in the mammalian body occur in tears and in the mucosa of the antrum, pylorus, and duodenum.[1] The origin of these high local concentrations presumably is epithelium, *i.e.*, the tear glands and as yet undetermined cell types in the gastrointestinal mucosa. In contrast, the lysozyme titer in mesodermal tissue is low; for example, serum averages 1 unit/cc,[1] and human leucocytes (from the buffy coat of normal blood) contain only 1.8 units per 5,000,000 cells.[2] However, human cartilage averages about 40 units/g, although this value is without doubt too low because of the difficulty in extracting this tissue. Normal human skin (including a considerable quantity of fibrous tissue) was found to have less than 1 unit/g.

The finding of high lysozyme titres in gran-

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1 Meyer, K., Prudden, J. F., Lehman, W. L., and Steinberg, A., *Am. J. Med.*, 1948, **5**, 482.

2 Meyer, K., in Modern Trends in Ophthalmology, 2:71, Paul B. Hoeber, New York, 1947.