THE EFFECT OF ROENTGEN RADIATION ON SPINAL GANGLIA
OF ALBINO RATS

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From the extensive and complete review of the literature on the effects of roentgen rays on the central nervous system by Lyman, Kupalov and Scholz (1), it seems well established that the spinal ganglion is remarkably radio-resistant. Heineke (2) in 1903 obtained different results in guinea-pigs which died as a result of roentgen irradiation. He found chromophilic changes in cerebral ganglion cells. Morowoka and Mott (3), in 1922, noticed large vacuoles in the irradiated cells of brains of cats and rabbits. While studying the general effect of roentgen radiation in rats, we observed more changes in the cells of the spinal ganglion, and the findings are communicated herewith.

METHOD AND MATERIAL

Normal albino rats weighing about 300 to 400 gm. were used, 150 $r$ in 6.8 minutes being delivered to the posterior surface of the right lower extremity of each animal. The x-rays were generated with a Douglas x-ray tube at 100 kv., 6 ma., and a filtration of 2 mm. aluminum. The skin-target distance was 25 cm. Three groups of animals were employed for the present study. Group I consisted of 4 rats, each receiving a total of 1,050 $r$ in seven days; group II, of 3 rats, each receiving a total of 2,400 $r$ in sixteen days; group III, of 4 rats, each receiving a total of 4,950 $r$ in thirty-three days. Treatment was administered daily.

For comparison, the posterior surface of the head and neck of another 4 rats, covering an area of about 4 x 6 cm., was similarly irradiated with x-rays. Two of them received a total of 1,050 $r$ each in seven days and the other two 2,400 $r$ each in sixteen days.

On the second day following the completion of the course of irradiation, the animals were killed with ether inhalation. The upper spinal ganglia were removed for cytological study. For mitochondria the sections were stained with anilin acid fuchsin and methyl green after Regaud's formalin bichromate fixation and for the Golgi apparatus a modified Kopsch-Kolatschev osmic chromate method was used (5).

OBSERVATIONS

(1) Normal Cells of the Spinal Ganglion: Normal cells of the spinal ganglion vary in size but are practically round in shape. A large and round nucleus occupies almost the center of the cell. In the nucleus one or more nucleoli may be found (Figs. 1 and 2).
Mitochondria in the form of granules, rods, and filaments are evenly distributed in the cytoplasm (Fig. 2). In the same preparation, the Nissl substance stained by the methyl green forms irregular masses. The Golgi apparatus appears as an incomplete network consisting of coarse strands, droplets, and granules located frequently in the vicinity of the nucleus (Fig. 1).

(2) The Non-irradiated Spinal Ganglion in the Irradiated Animal: The changes in the cells of the spinal ganglion represent the indirect effect of roent-
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Fig. 3 shows the Golgi apparatus. Note the presence of a network in the vicinity of the nucleus in cell A. $\times 445$. Fig. 4 shows the mitochondria. Note the very deeply stained cytoplasm in cell A and the reduced amount of mitochondria in the faintly stained cell B. $\times 1000$.

gen radiation and will be described more in detail according to the various dosages of the x-rays.

(a) In rats whose right lower legs were irradiated with a total of 1,050 $r$, some of the cells of the spinal ganglion retained their normal shape and appearance and some became irregular and deeply stained (Figs. 3 and 4).
Figs. 5 to 8. Changes of Mitochondria, Golgi Apparatus and Nucleus in the Spinal Ganglion Cells of Rats Whose Right Lower Extremities Were Irradiated with a Total Dosage of 2,400 r. × c. 500

Fig. 5. Mitochondria. Note the large vacuoles in cell A, and the granular and poorly stained mitochondria in cell B.

Fig. 6. Mitochondria. Note the lipoid droplets and the shrinkage in some of the cells.

Fig. 7. Mitochondria. Note the faintly stained mitochondria in some cells (A) and the deeply stained cell B.

Fig. 8. Golgi elements. Note absence in large part of cell A.

The nuclei, which are centrally situated, are small and irregular or elongated in the majority of the cells, especially in the deeply stained ones. In these cells the nuclei appear dark in the mitochondrial preparation (Fig. 4) and colorless in the preparation for the Golgi apparatus (Fig. 3). The nucleolus is large in size, round in shape, and centrally located.

The mitochondria in some cells are mostly granular, faintly stained, and slightly decreased in amount (Fig. 4B). In the deeply stained cells they are well stained and greatly increased in amount. In the very deeply stained cell the mitochondria become invisible and undifferentiated in the very deeply stained cytoplasm (Fig. 4A).

The Nissl substance stains faintly and is in the form of granules, more or less evenly distributed throughout the cell body in many of the spinal ganglion cells.

The Golgi apparatus in some cells shows a structure quite different from normal. It appears as segments, droplets, and granules spreading throughout the whole cell (Fig. 3). In cells of medium and small size, the network may be found in the vicinity of the nucleus (Fig. 3A). In the deeply, but not too deeply, stained cells the Golgi apparatus may be seen as a close network consisting of granules, droplets, and strands with osmium-stained fine dust. In
the very deeply stained cells no Golgi structure can be made out, but a black mass with some clear vacuoles and canals is visible.

(b) Cells of the spinal ganglion of rats whose right lower legs were irradiated with a total of 2,400 r do not differ significantly in general appearance from those in animals irradiated with a total of 1,050 r. The principal difference lies in the shrinkage of more cells of the former than of the latter. That this shrinkage cannot be attributed to unsuitable fixation or considered to be an artefact is shown by the fact that no shrinkage was observed in the cells of the normal spinal ganglion which were treated by the same method.

In addition, some cells contain large vacuoles (Fig. 5A), such as those described by Morowoka and Mott (3) in the irradiated nerve cells of brains of cats and rabbits.

The mitochondria in the majority of cells are granular, poorly stained, and well scattered throughout the cell (Figs. 5B and 7A), similar in appearance to the previous group. In some cells the granules become swollen and stain deeply in the form of droplets (Fig. 6). In a small number of cells normal mitochondria may be seen; and in the deeply stained cells the mitochondria cannot be made out (Fig. 7B).
The Nissl substance in many cells is not visible but a homogeneous ground substance stained by methyl green is seen in its place.

The Golgi apparatus in some cells is made up of segments and droplets smaller in diameter than normal. In most of the cells, the Golgi apparatus, which is accompanied by osmic acid-stained dust, is situated at one end of the cell while the nucleus is at the other (Fig. 8A). When examined in serial sections, the Golgi elements, though concentrated in a certain part of the cell, are on the whole less in amount, for they are absent in a large part of the cell (Fig. 8A). In some cells the Golgi apparatus is also less in amount when it appears as evenly distributed droplets. In the deeply stained cells the droplets and dust fuse together to form a black cytoplasmic mass.

c) Cells of the spinal ganglia of rats whose right lower legs were irradiated with a total of 4,950 r, show further changes. Many cells change in shape and become irregular in outline. Their nuclei are elongated, and indistinct in outline (Figs. 9 and 10), and in some of them the nucleoli are comparatively large (Fig. 9).

The mitochondria in general are granular in shape and greatly reduced in amount (Fig. 11), although a large amount of the substance may still be found in some cells (Figs. 9 and 11). In between the mitochondria varying quantities of lipoid droplets are to be found (Figs. 9 and 11C). In none of the deeply stained cells can mitochondria be made out.

The Nissl substance is not visible in any cell.

The Golgi apparatus is further reduced in amount (Fig. 12), and appears as droplets only. In the deeply stained cells the droplets are small in size and fused together with the osmic acid-stained dust.

3) In the directly irradiated spinal ganglion the changes in the form of the cells, nuclei, mitochondria, and Golgi apparatus were not strikingly different from those in the cells of the non-irradiated spinal ganglia in irradiated animals.

**DISCUSSION**

Our findings show that under careful cytological observation, with special staining technics, cells of spinal ganglia of rats subjected to direct roentgen irradiation reveal definite changes indicating cellular impairment. Furthermore, definite and similar changes were also found in the spinal ganglia when other parts of the body were irradiated. The extent of the changes varies with the amount of radiation delivered to the animals. The changes are as follows: (1) The outline of the ganglion cells may be normal or irregular or show marked shrinkage. (2) Their nuclei may be small, irregular, elongated or indistinct in outline. (3) The mitochondria are either reduced or increased in quantity. In general, they are granular and poorly stained. (4) The Nissl substance becomes invisible with increasing dosages of x-rays. (5) The Golgi apparatus varies from a network to segments or droplets, and from the normal distribution to a localized mass. At the same time the amount of the Golgi apparatus is reduced. The reduction of the mitochondria-Golgi complex and nuclear substance, the granular appearance of the mitochondria, and the segment or droplet formation of the Golgi apparatus, all indicate impairment of
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Cell function. With a small dosage of x-rays many ganglion cells will show evidence of hyperactivity as indicated by the increase of the mitochondria. Thus, as a result of roentgen irradiation, the cells of the spinal ganglia may show either hyperactivity, which indicates an attempt at repair; or hypoactivity, which represents a failure of the repair process.

Droplet formation in mitochondria of the spinal ganglion cells presents a picture similar to that described for fowls with beriberi (4). The appearance of the mitochondria-Golgi complex in many cells simulates that of the spinal ganglion cells of rats and guinea-pigs suffering from vitamin B, C, and D deficiency and starvation (6). From this close analogy, it may be inferred that the effect of irradiation upon the ganglion cells is brought about principally by a nutritional insufficiency, which may be explained as follows. In the first place, when a part of the body is irradiated by x-rays, the irradiated tissues are either damaged or destroyed and the disintegration products may circulate throughout the whole body and impair the function of the normal tissues and organs in other parts of the body, resulting in disturbed nutritional activity.

In the irradiation of any part of the body, such as the posterior part of the neck, blood vessels, which are very sensitive to radiation, are easily damaged and subsequently degenerate, causing thrombosis and obliteration of the vessels, resulting in a decreased blood supply to all the cells including the spinal ganglia. The effect upon all the cells including the spinal ganglia is, therefore, impaired function due to nutritional insufficiency. This may also explain the similarity of the changes observed in both directly irradiated spinal ganglia and non-irradiated spinal ganglia of irradiated animals. The changes of the irradiated spinal ganglion cells may, of course, be due in part to the toxic effects from the damaged or destroyed irradiated tissue, as is the case in the other group of animals.

The response of the spinal ganglion to roentgen radiation is different from that of the bone marrow. There is a definite difference between the irradiated and the unirradiated bone marrow in the same animal (7), and the changes in the former are more marked than in the latter. On the other hand, there is little difference between the changes in the irradiated and the unirradiated spinal ganglion. If the changes in the irradiated spinal ganglion cells resulted directly from radiation, one would expect more than those observed in the unirradiated spinal ganglion, as is in the case of the bone marrow. Thus the radioresistance of spinal ganglion cells, frequently mentioned in the literature, may indicate absence of changes in direct response to radiation, but presence of changes secondary to radiation.

The present experiment may explain the underlying cause of radiation sickness in patients receiving x-ray or radium therapy. The principal symptoms of radiation sickness are nausea and vomiting, which may result from irradiation of any part of the human body. As stated above, when a part of the human body is subjected to roentgen irradiation the tissues under treatment are either damaged or destroyed and the products of the destroyed cellular structures may affect the tissues of the unirradiated parts of the body. Some experiments (8) have shown that the sympathetic system is more easily affected than other parts of the nervous system. Since the gastro-intestinal tract
is supplied by both the sympathetic and parasympathetic nervous systems, when the cells of the sympathetic system are disturbed, the parasympathetic nervous system will act more energetically on the gastro-intestinal tract and thus cause the distressing symptoms. The acceptance of this hypothetical explanation, however, depends upon further studies.

**Summary and Conclusions**

In a parallel cytological study of the direct and indirect effects of roentgen radiation on the spinal ganglia of rats, with special staining technics, it was found that in both instances the extent of the cellular changes varies in direct proportion to the amount of radiation delivered to the animals. Briefly, the changes observed are as follows: The cell outlines vary from normal to irregularity and shrinkage, and the nuclei become small, irregular, elongated, and indistinct. The mitochondria vary from a reduced to an increased amount and are granular and poorly stained. The Nissl substance becomes invisible. The Golgi apparatus is reduced in amount, changes from a network to segments or droplets, and becomes a localized mass instead of being evenly distributed. All these changes indicate a functional disturbance of the cellular activity of the spinal ganglia in response to roentgen irradiation.

**Literature**