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#### TIME COURSE OF MORPHOLOGICAL CHANGES IN THE SPINAL CORD AFTER EXPOSURE TO NONIONIZING MICROWAVE RADIATION

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According to data in the literature nonionizing microwave radiation (NIMR) causes extensive neurological disorders and disturbs many functions of the body, including visceral functions [2-6, 8, 9]. Nevertheless, the pathogenesis of these disturbances and, more especially, of structural changes in the nervous system itself under the influence of NIMR has been inadequately studied. Only isolated investigations have been carried out in this direction so far as the brain is concerned [1, 7, 10]. No work devoted specially to the study of spinal cord structure under the influence of NIMR has been undertaken.

The object of this investigation was to study the character and time course of development of morphological changes arising in different segments of the spinal cord under the influence of NIMR.

#### EXPERIMENTAL METHOD

Experiments were carried out on cats and dogs (50 animals altogether), which were exposed to NIMR (wavelength 12.6 cm, intensity 400-500 mW/cm<sup>2</sup>) for 1 h (cats) or 4 h (dogs). A number of intact animals constituted the control group. During irradiation the animals were kept in special cages, located in a screened anechoic chamber. The "Luch-58" treatment apparatus, operating on a continuous schedule, was used as the generator.

The spinal cord was studied 1, 10, 20, and 30 days after the end of exposure to NIMR, and for this purpose the animals were anesthetized with ether, decapitated in groups, the spinal cord was removed and pieces of tissue from it, of the necessary size, were fixed in 12% neutral formalin solution and alcohol. Paraffin sections or frozen sections were stained with hematoxylin and eosin and by Van Gieson's, Nissl's, Zimmermann's, and Cajal's methods,

Segments of the spinal cord were identified by their roots, and also by the shape of the gray and white matter, depicted on photographs of unstained sections. An atlas of the spinal cord [11], in which the author distinguishes 10 regions in each segment and conventionally calls them laminae, was used for this purpose. In each lamina neurons of various types were present and formed the nuclei of the spinal cord, with specific functions.

#### EXPERIMENTAL RESULTS

Changes in structure of the spinal cord tissue were found by the first day after the end of irradiation in animals exposed to NIMR. The changes increased to the 10th, 20th, and 30th days. The character of the changes was identical, but their intensity differed in neurons located in different regions (laminae) of the spinal cord. The changes were similar in corresponding regions of the cord in different individuals of the same species (cats) and also in animals of the other species (dogs), so that a general description is in order.

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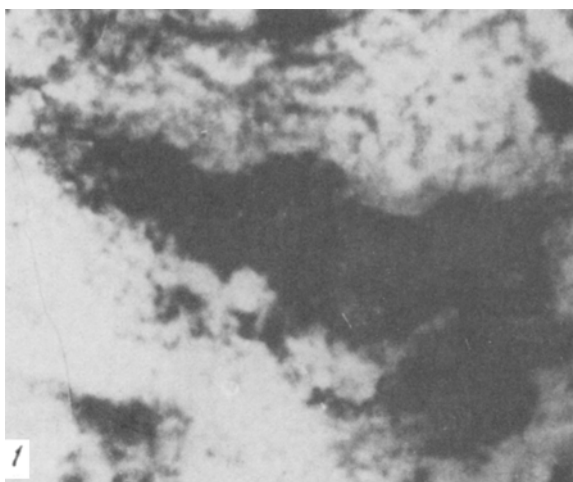


Fig. 1

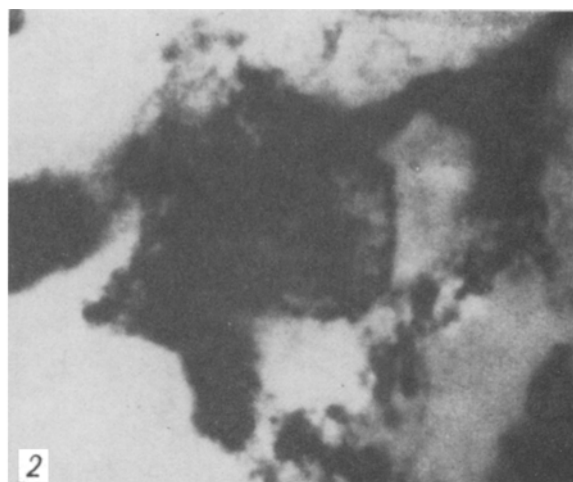


Fig. 2

Fig. 1. Tigrolysis and vacuole formation in cytoplasm of neuron (lamina VII) of the sacral portion of the cat spinal cord after end of exposure to NIMR. Nissl's stain, 630  $\times$ .

Fig. 2. Large vacuoles in cytoplasm with partial change of nucleus in neuron (lamina VIII) in lumbar region of cat spinal cord 1 day after exposure to NIMR. Nissl's stain, 630  $\times$ .

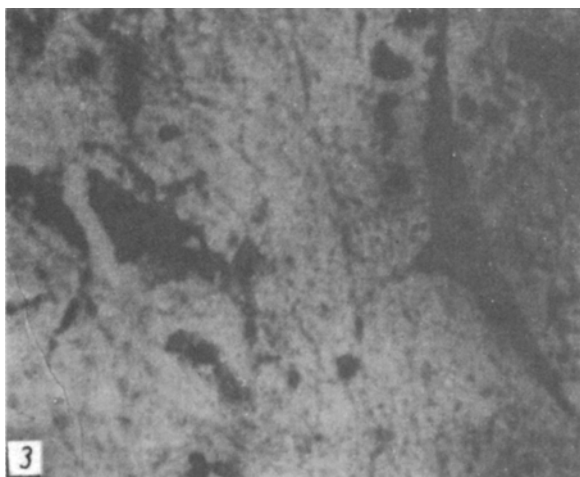


Fig. 3

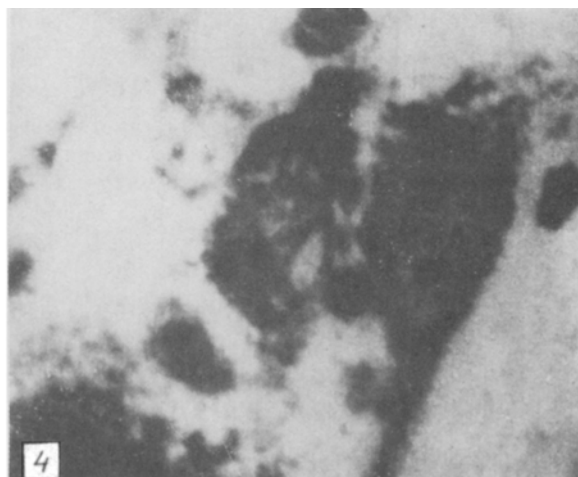


Fig. 4

Fig. 3. Vacuolation of nucleus of one neuron in intermediate zone (lamina VII) of lumbar region of cat spinal cord 1 day after exposure to NIMR. Nissl's stain, 900  $\times$ .

Fig. 4. Death and incrustation of cell (lamina VII) in cervical region of dog spinal cord 10 days after exposure to NIMR. Nissl's stain, 400  $\times$ .

Among cells forming the lateral group of the anterior horn of the spinal cord, besides some neurons with almost normal appearance there were others with increased hyperchromatism. The nuclei of these cells were a little enlarged, with uneven contours, and displaced somewhat toward the periphery of the neuron body. Their nucleoli were considerably reduced in size and often located eccentrically. An uneven distribution with partial dispersion of the basophilic substance was observed much less frequently in motoneurons.

In cells of the anterolateral sympathetic nucleus, located in the lateral horn of the spinal cord, marked liquefaction of the basophilic substance was observed, with the formation of separate islets in different regions of the cytoplasm. The neurons were much reduced in size, more elongated in shape, and their borders were blurred and ill-defined. Their nuclei

also were reduced in size, oblong in shape, and contained a small, intensely stained, eccentrically located nucleolus, resembling a small dot. Many neurons contained a palely stained nucleus.

Neurons of the posterior horns showed less marked changes, compared with those of the lateral horns. Many neurons were palely stained.

Vascular changes in the spinal cord tissue and also activation of neuroglial cells may also be included among these general features of structural changes in the spinal cord neurons of the irradiated animals. However, besides general histopathological changes in the spinal cord of the irradiated animals, some special features were found in each segment of the cord. These were concerned mainly with the severity of the structural changes and the number of affected neurons (Figs. 1-3).

Morphological investigation of the spinal cord of animals irradiated under these conditions thus shows that NIMR has a considerable effect on neuron structure. This state of the neurons is characteristic morphological evidence of developing degeneration in the cells and, according to Nissl's classification, it corresponds to severe disease of the nerve cells or to a state of "fluidification" (liquefaction) according to Spielmeyer (Marinesko, 1964).

In the final stages of the investigation (10th, 20th, and 30th days) after exposure of the animals to NIMR it was found that this "disease" of the neurons progressed. On the 10th day many neurons (over 50%) were already in a state of complete destruction (Fig. 4). Cell "ghosts" were frequently found, alongside which atrophied neurons could be seen. Their bodies were considerably reduced in size, their nuclei were elongated in shape, and in their staining properties they differed only a little from the cytoplasm. The nucleoli were contracted and displaced to the periphery of the nucleus; they had the appearance of a small dot, which was stained a darker color. The total number of neurons in the plane of the section was appreciably reduced. The response of the gliocytes, manifested as marked hyperplasia and hypertrophy on the 1st day after irradiation, later became considerably depressed. Neuroglial cells were less frequently found and were reduced in size.

Considerable changes also were found in the synaptic apparatus of the neurons under these circumstances. Swollen synaptic plaques on the bodies of many neurons could be seen even on the first days after irradiation. By the 10th day partial destruction of the synapses and their separation from preterminals were found. Later the number of pathologically changed synapses increased. However, the synaptic apparatus remained intact longer on the bodies of large motoneurons.

Analysis of these morphological changes in the CNS of animals exposed to NIMR and of the general state of the animals for 1 month after irradiation gives good grounds for concluding that neurological disorders and disturbances of many functions observed by clinicians in persons exposed to the action of such radiation are based on pathogenetic changes in the structure of nerve cells.

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# HISTOCHEMICAL STUDY OF THE PILOTROPIC ACTION OF 1-(CHLOROMETHYL)- SILATRANE ON GUINEA PIG SKIN

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Much interest has recently been shown by research workers in a new class of organosilicon compounds — the silatranes; this is due, in particular, to the broad spectrum of their biological activity.

It has been shown that silatranes stimulate growth of the hair in laboratory animals. Microscopic and macroscopic investigations of the pilotropic action of 1-substituted silatranes with the general formula  $\text{XSi}(\text{OCH}_2\text{CH}_2)_3\text{N}$  have shown that the most active of them is 1-(chloromethyl)silatrane ( $\text{X} = \text{ClCH}_2$ ) [1, 3, 5].

To shed light on some aspects of the mechanism of the pilotropic action of this silatrane a histochemical study of the skin of experimental animals was undertaken after its application: The effect of 1-(chloromethyl)silatrane was studied on the principal components of connective tissue, namely glycogen, mucopolysaccharides, and nucleic acids.

## EXPERIMENTAL METHOD

The hair was removed from an area of skin of guinea pigs by means of 2% Epilin ointment. For the next 3 months, 5% 1-(chloromethyl)silatrane ointment was rubbed into the epilated areas of skin. At the end of the experiment the animals were killed and the skin subjected to histochemical analysis. The material was processed by the usual methods. The distribution of polysaccharides was revealed by the PAS reaction (with amylase control), mucopolysaccharides by alcian blue at different pH values (with hyaluronidase control), and RNA by Einarson's method (with ribonuclease control). These substances were determined quantitatively microspectrophotometrically [3, 4].

## EXPERIMENTAL RESULTS

Under the influence of 1-(chloromethyl)silatrane the content and concentration of glycogen was increased both in the basement membrane and in the outer epithelial sheath of the hair follicle (Table 1).

It can be postulated that this compound has a beneficial effect on a certain stage in carbohydrate metabolism, acting as a stimulator intensifying this process. An increase in the glycogen content can also be explained on the grounds that the structure of the synthesized glycogen becomes "cross-linked," evidently on account of silicon "bridge" atoms.

Of all the mucopolysaccharides in the guinea pig skin, heparin was chosen for demonstration (Table 2).

It will be clear from Table 2 that the heparin content in the skin of the experimental animals was sharply reduced. There are several possible reasons for this: 1) Heparin is neu-

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