HISTOCHEMICAL INVESTIGATION OF THE NUCLEI OF SUBCUTANEOUS CONNECTIVE TISSUE CELLS OF WHITE RATS EXPOSED TO THE ACTION OF ULTRASOUND

A. P. Speranskii and I. L. Martsveladze

Institute of Health Resorts and Physiotherapy and Department of Anatomy and Physiology, V. I. Lenin Pedagogic Institute, Moscow
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In view of the increasing application of ultrasound in the treatment of a number of diseases, we considered that it would be of interest to investigate its effect on connective tissue, because this tissue evidently reacts to such treatment.

EXPERIMENTAL METHOD

As ultrasound generator we used the Zonostat-515 apparatus with a piezo-quartz pick-up (not water-cooled), the active surface of the vibrator being 7 cm^2, and giving a continuous supply of ultrasound waves with a frequency of 800 cps. In view of the small size of the animal, we used a stable method of application (through a tube of water). As a rule, two applications were given (2 minutes each, on alternate days) to the inguinal region. Immediately after the second exposure of the animals to ultrasound, and subsequently after 3, 10, and 30 days, fine films of subcutaneous connective tissue were taken from the superficial layer of the inguinal and axillary (not exposed to ultrasound) regions. The connective tissue films (7 films from each area) were prepared by Maximow's method, fixed in Helly's mercuric chloride solution, and stained with iron-hematoxylin by Yasvoin's method.

EXPERIMENTAL RESULTS

Examination of preparations (50 fields of vision in each) from films taken immediately after the second exposure to ultrasound of relatively high intensity (1.17 W/cm^2) showed profound changes in the connective tissue structures: homogenization of the ground substance, a marked decrease in the number of cells, and the presence of only 2-3 "bare", irregularly shaped nuclei in a field of vision or their complete absence. In the case when ultrasound of comparatively low intensity (0.2 W/cm^2) was used, the changes were much slighter in degree: the number of cells was 10.1 (compared with 18 in the controls), and in half of them the nuclei were irregular in shape; the protoplasm was preserved on the average in only two cells. We also observed swollen collagen fibers, a few elastic fibers, and dilated capillaries.

The pattern of the changes in the axillary region was similar but less marked.

After the initial depression of the tissue, during the first few days of the after-effect processes of recovery of the connective-tissue structures were observed (an increase in the number of cells and normalization of the fibrous structures). When ultrasound with an intensity of 1.17 W/cm^2 was used, however, the process of recovery

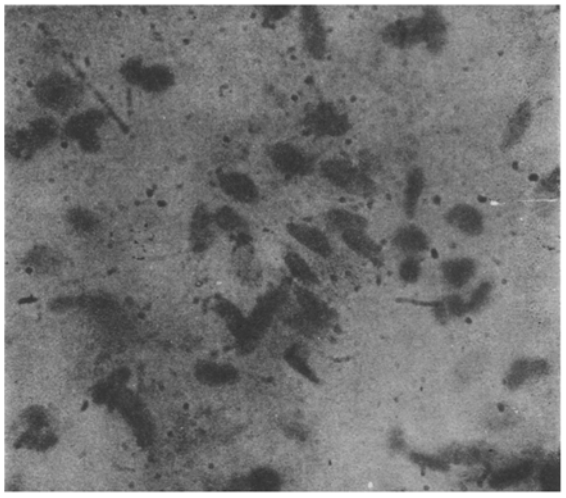

Fig. 1. Subcutaneous connective tissue of a white rat, not exposed to ultrasound. Stained with fuchsin. Magnification: ocular 15 ×, objective 40 ×.

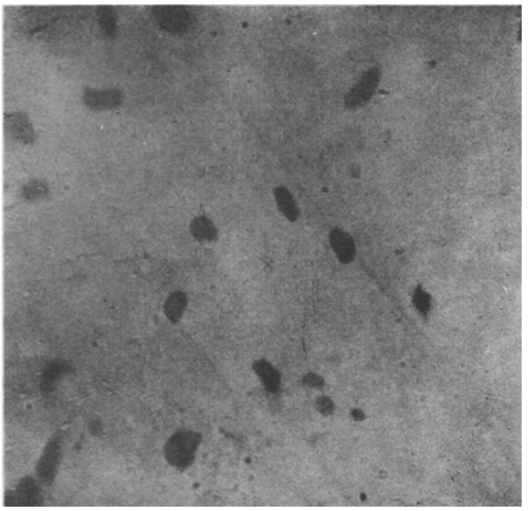

Fig. 2. Subcutaneous connective tissue of a white rat one month after the second exposure to ultrasound (intensity of irradiation 1.17 W/cm^2). Stained with fuchsin. Magnification: ocular 15 ×, objective 40 ×.

Fig. 3. Subcutaneous connective tissue of a white rat one month after the second exposure to ultrasound (intensity of irradiation 0.2 W/cm^2). Stained with fuchsin. Magnification: ocular 15 ×, objective 40 ×.

of the connective-tissue structures was not complete on either the 10th or the 30th day after exposure. After the action of ultrasound with an intensity of 0.2 W/cm^2 the phase of initial moderate depression of the tissue quickly gave way to a phase of restoration and renovation of the tissue. In preparations from films of tissue taken 10 days after exposure, evidence of stimulation of the vital activity of the tissue was seen. This was especially noticeable one month after exposure: an increase (by comparison with the controls) in the number of cells with more intensively stained protoplasm than the controls; an increase in the number of young cells and of mast cells; the predominance of elastic fibers among the fibrous structures, which is characteristic of younger tissue.

There are reports in the literature [1, 2, 3, 4] of the stimulating effect of small doses of ultrasound on various organs and tissues of the intact animal.

The histological evidence which we obtained of the stimulation of connective tissue by small doses of ultrasound suggests an increase of its functions and an intensification of its vital activity, which may be associated with an alteration in and a stimulation of the biochemical processes of metabolism within the tissues and cells. We accordingly considered it important to study the effect of ultrasound on certain processes of the metabolism of nucleic acids, and in particular of desoxyribonucleic acid (DNA).

We studied the DNA content of the subcutaneous connective tissue of white rats. The experimental conditions were the same as those described above. Experiments were carried out on 21 rats (adult females weighing 200-250 g), seven of which acted as controls. DNA was determined by Feulgen's method.

Relatively slightly stained cell nuclei are seen in Fig. 1 (control)*. When the changes in the connective tissue were studied one month after exposure to ultrasound in a dose of 1.17 W/cm^2, an increase in the intensity of staining of the cell nuclei was observed by comparison with the controls; this demonstrates intensification of the nucleic acid metabolism. It must be pointed out that when this dose was used only part of the nuclei was stained more brightly than in the control (Fig. 2). The intensity of staining of the nuclei was more marked one month after exposure to ultrasound of relatively low power (0.2 W/cm^2). In this case most nuclei were more intensively stained than in the control (Fig. 3).

The results obtained from our investigation of the influence of small doses of ultrasound on the DNA content of the connective-tissue cells thus also demonstrate that this effect is one of stimulation.

SUMMARY

The work involves histological and histochemical examinations.

Inguinal region of the animals was subjected to ultrasound action of relatively high (1.17 W/cm^2) and low (0.2 W/cm^2) intensity. Connective tissue membranes were prepared according to Maximov, and stained according to Yasvoin with iron hematoxylin. Since ultrasound may affect the metabolism of nucleic acid, the study of the dynamic changes in the amount of desoxyribonucleic acid, conducted for a period of one month after ultrasound treatment was made. The Felgen staining method was used to detect desoxyribonucleic acid.

Ultrasound of relatively low intensity caused connective tissue stimulation with changes in the nucleic acids metabolism (sum total rise of desoxyribonucleic acid content). Ultrasound dispensed in dose of 1.17 W/cm^2 caused prolonged depression of loose connective tissue function, and, apparently, some disturbances on the nucleic acid metabolism.

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* With the staining method used in the preparations illustrated, only the nuclei are stained.