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USE OF THE PROTON MAGNETIC RELAXATION METHOD TO STUDY EXPERIMENTAL BURNS

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KEY WORDS: proton magnetic relaxation; spin-lattice relaxation time; burns.

Various physical methods of investigation are nowadays widely used to study many problems in experimental and clinical medicine. One such method is proton magnetic relaxation (PMR), by means of which information can be obtained on proton mobility in biological and chemical objects by measuring the proton spin–lattice relaxation (T_1) and spin–spin relaxation (T_2) times [7]. In the study of biological cells and tissues the relaxation time characterizes mainly the mobility of protons of water molecules [12, 14]: the higher the water content, the longer the relaxation time. Since the beginning of the 1970s the PMR method has been used to investigate some pathological processes which are accompanied by changes in the water content in tissues and organs [6, 8, 10, 13].

In burns regulation of water metabolism in the body is disturbed, edema of the affected tissues arises, and the content of water in organs not directly affected by the burn is changed [1, 2, 11]. Hence the interest in the study of experimental burn pathology by the PMR method. Preliminary investigations showed an increase in T_1 in certain animal organs after burns [5]. In the present investigation the kinetics of changes in the proton spin–lattice relaxation time of the tissues of animals with experimental thermal trauma was studied parallel with the morphological changes in the affected tissues and internal organs.

EXPERIMENTAL METHODS

A burn of the IIIB degree, affecting 2 and 6% of the body surface, was inflicted under ether anesthesia on 160 SHK mice (weighing 16-20 g) by means of a metal rod heated to a tem-

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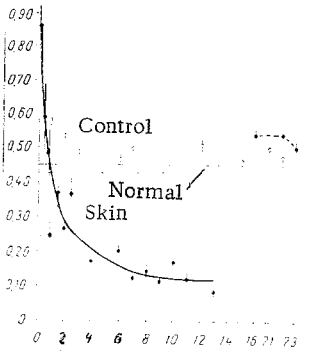

Fig. 1

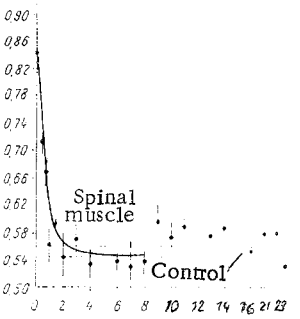

Fig. 2

Fig. 1. Spin–lattice relaxation time of skin (wound) in experimental burns. Here and in Figs. 2 and 3, abscissa, time after burning (in days); ordinate, T_1 (in sec).

Fig. 2. Spin–lattice relaxation time of muscle (wound) in experimental burns.

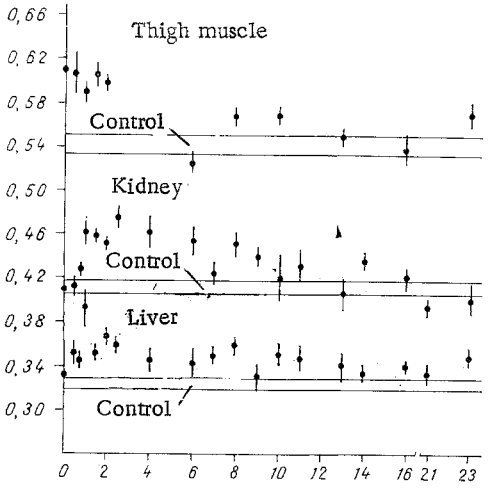

Fig. 3. Spin–lattice relaxation time of organs not affected by burning in experimental burns.

perature of 68-72°C (exposure 10 sec), on a previously epilated area of skin on the animal's back. In the course of the experiment the animals were allowed to drink *ad lib.*

The time course of changes in T_1 in the burned skin, muscle tissue in the region of the burn, and in tissues and organs unaffected by the burn — liver, kidneys, spleen, heart, brain, blood, lungs, and thigh muscle, was studied (using tissue samples weighing 50-100 mg).

A Minispec p-20 pulse spectrometer (from Brucker, West Germany) with a working frequency of 20 MHz was used to measure T_1. The method of measuring and calculating T_1 was described previously [8]. To obtain each experimental point, tissue samples were taken from 5-10 animals, and mean values of T_1 (M) and standard deviations (m) are given on the graphs. Epilated unburned animals (80 mice) served as the control, and a group of intact animals (40 mice) as the normal group.

The data were subjected to statistical analysis by Student's and Fisher's tests. The water content in the burned tissues was determined in parallel experiments by drying the samples to constant weight at 80-85°C. Standard methods [3] were used for histological control of the burned tissues, liver, kidneys, and thigh muscle [3].

EXPERIMENTAL RESULTS

In IIIB degree burns affecting 2 and 6% of the body surface a sharp increase in T_1 of the burned skin and muscle in the region of the wound was observed during the first few hours after burning (Figs. 1 and 2). This corresponded to marked edema and swelling of the the subcutaneous tissue and surface layers of the muscle in the region of the wound. The increase in T_1 was evidently caused by edema of the affected tissues. Tissue drying experiments showed that 15 min after burning the water content in the burned skin was up by 25% and in muscle by 20%. During formation and drying of the scab the values of T_1 and the water content of the tissues fell. After separation of the scab and healing of the wound (14th-16th day) the relaxation time of the newly formed skin was a little higher than in the control (Fig. 1). By this time the intensity of tissue edema in the floor of the wound and in the surrounding subcutaneous and muscle tissue was considerably reduced, and in some cases areas of perivascular inflammation and edema still persisted in the granulation tissue until the 21st-23rd days.

In burns affecting 2% of the body surface changes in T_1 in organs unaffected by burning were not statistically significant.

In burns affecting 6% of the body surface T_1 was increased in liver, kidney, and thigh muscle tissues (Fig. 3). High values of T_1 in the liver and kidney tissues were observed for a long time after burning. The functions of these vitally important organs are known to be seriously disturbed for a long time in burns. The increase in T_1 was probably due to changes in the water content of the tissues caused by increased capillary permeability. However, there are no data in the literature directly on the water content in these tissues in deep burns with small area. It has been shown for the liver and kidneys of intact animals by soaking in hypotonic sodium chloride solution that an increase in the water content by 2-3% leads to an increase of 10-20% in T_1 [14].

The results of histological investigation of the liver, kidneys, and thigh muscle tissue agree on the whole with those obtained by the PMR method. In the thigh muscle, from the time of trauma until the 4th-5th day swelling of some muscle fibers and edema of the intermuscular spaces were observed, but later they disappeared. Swelling of the cytoplasm of some hepatocytes, congestion, and moderate edema of Desse's pericapillary spaces were found until the 15th-17th day. Moderate swelling of the capillary endothelium of the glomeruli, congestion of the capillaries, interstitial edema of the medulla, and swelling of the epithelium, of the convoluted tubules were observed in the kidneys until the 8th-10th day.

The increase in T_1 in the organs reflects the action of burns on the whole of the animal's body. It is accepted that general disturbances arise in most cases in deep burns of the skin affecting more than 10% of the body surface. The results of the present investigation show marked biophysical changes in the organs of animals with deep burns of the skin affecting an area of 6%.

The PMR method thus is highly sensitive to changes in the water content of the tissues and can detect an increase in tissue hydration by 2-3%; moreover, the results are highly reproducible (scatter of the maxima 3-5% per sample). The technical simplicity of the PMR method (5-10 min per sample) enables it to be widely used in mass investigations. The results of the present study indicate that it is a valid method for investigating the state of water in experimental burn pathology and for the study of clinical biopsy materal. Development of the NMR-introscopy method [5, 9], which is based on the PMR method, provides a no contact method (by measuring biophysical shifts) of studying changes in the tissues in burns. The PMR method is a valuable modern method with which to study the mechanisms of burn trauma.

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CHANGES IN THE NORADRENALIN CONCENTRATION IN THE PORTAL VEIN AND AURICLES OF RATS IN THE COURSE OF STRESS

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KEY WORDS: noradrenalin; portal vein; auricle; emotional-pain stress.

Strong and prolonged excitation of the adrenergic system arising immediately after severe emotional-pain stress (EPS) leads regularly to a considerable fall in the noradrenalin (NA) content in the myocardium, adrenals, hypothalamus, and other organs due to the slower rate of resynthesis than of breakdown of catecholamines [3].

Increased liberation of NA into the blood is accompanied by damage to the myocardium [4]. The degree of this damage very probably varies in different parts of the cardiovascular system, depending on the intensity of the adrenergic effect therein.

The object of this investigation was to compare the degree of lowering of the NA content in different parts of the circulatory system in EPS, and to use this criterion to assess the intensity of the adrenergic effect. For this purpose the dynamics of the NA content in the myocardium of the auricles and ventricles and in the smooth muscle of a resistive vessel was compared. As the most adequate model of a resistive vessel the portal vein was used, for it has powerful muscles and intrinsic spontaneous activity, and so it closely resembles the resistive vessels in its properties [5, 6]. This vessel also has sufficient mass to allow quantitative determination of NA.

EXPERIMENTAL METHODS

Experiments were carried out on male Wistar rats weighing 180-200 g. Two groups of rats were investigated: the control (group 1) and animals exposed to EPS (group 2). EPS was reproduced in the form of an "anxiety neurosis" by Desiderato's method [7] for 6 h. The index of effectiveness of exposure to the harmful stressor was the formation of ulcerative lesions of the gastric mucosa in the rats. The NA concentration was determined in the portal vein and auricles 2 h and 1, 2, 4, and 8 days after the end of EPS and in the control. Measurements were made individually for each animal. The rats were decapitated and the test organs removed. NA was determined by the trihydroxyindole method [2]. Specific fluorescence was measured on the MPF-4 fluorescence spectrophotometer (Hitachi, Japan), with an excitation wavelength of 390 nm and was recorded at 485 nm. The results were subjected to statistical analysis by Student's test [1].

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