them cease to function. In other words, the level of the blood supply to the red muscle fibers during active work and at rest varies over a much wider range than the level of the blood supply to the white fibers with a glycolytic type of metabolism.

By contrast with the hind-limb muscles mentioned above, the number of functioning capillaries in the soleus muscle was not reduced after hypokinesia for 20 days (Table 2). Moreover, the study of the microcirculation of the soleus muscle of rats kept under conditions of hypokinesia showed signs of congestive stasis, reflected in the large number of dilated capillaries and small veins, congested with blood (Fig. 1). The congestion of the veins and capillaries of the soleus muscle was evidently connected with the fact that the function of the "muscular pump" is disturbed during hypokinesia and, as a result, the blood flow along the thin-walled intramuscular veins, incapable of contracting by themselves, is slowed. Slowing of the blood flow is invariably accompanied by the development of tissue hypoxia, and this most probably is the direct cause of the development of dystrophic changes in the muscles.

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INDUCTION BY THYROXINE OF STRUCTURAL CHANGES IN SARCOPLASMIC RETICULUM MEMBRANES OF RABBIT SKELETAL MUSCLES

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KEY WORDS: sarcoplasmic reticulum; rabbit skeletal muscles; thyroxine; structural changes in membranes.

Previously, the writers reported a fall in the level of functional parameters of fragments of the sarcoplasmic reticulum (FSR) of skeletal muscles of rabbits with thyrotoxicosis. In particular, a decrease in the efficiency of accumulation of Ca^{++} ions from the medium by FSR preparations was observed against the background of a decrease in Ca-dependent ATPase activity. The effects observed may be caused by various factors: a change in lipid composition occurring during thyrotoxicosis; disturbance of the stoichiometry of FSR proteins responsible for Ca^{++} transport and utilization; a change in the character of protein–lipid interactions in the membrane complex. In fact, if the energy barrier to passive outflow of Ca^{++} ions from the vesicles is lowered because of structural changes in the protein–lipid complex of FSR membranes, this must cause both

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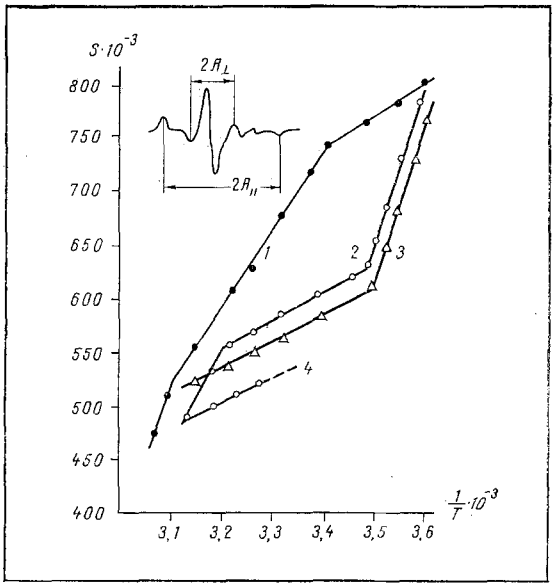

Fig. 1. Parameter of orderliness of fatty-acid chains of lipids as a function of temperature. 1) In membranes isolated from normal rabbits; 2) in membranes obtained from rabbits with thyrotoxicosis; 3) in liposomes from FSR of normal rabbits; 4) course of the curve during reversible fall of temperature.

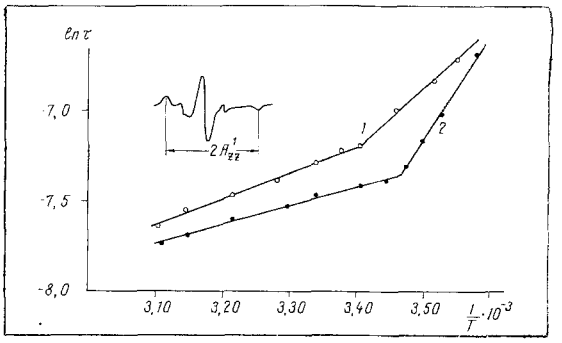

Fig. 2. Correlation time of rotation of spin label bound with protein thiol groups of FSR membranes as a function of temperature. 1) Preparations isolated from normal rabbits; 2) preparations isolated from rabbits with thyrotoxicosis.

a decrease in the rate of Ca^{++} uptake by FSR preparations and a decrease in the efficiency of the calcium pump.

Thyroid hormones, as among the more important factors regulating protein and lipid metabolism [8], are evidently capable of inducing the changes mentioned above in the structure of FSR in thyrotoxicosis.

EXPERIMENTAL METHOD

Male rabbits weighing 2.8-3 kg were used. Thyrotoxicosis was induced by intraperitoneal injection of thyroxine by the scheme described previously. The sarcoplasmic reticulum was isolated from the white muscles of the hind limbs and spinal region by the method described in [8]. The kinetics of FSR lipid peroxidation induced by Fe^{++} ions was recorded by a chemiluminescence method [1]. The temperature dependence of the

TABLE 1. Dependence of Chemiluminescence Parameters on Thyroid Status (M ± m)

Experimental conditions	Latent period, min	Intensity of luminescence, relative units
Normal	12,40±0,25	52,0±1,8
Thyrotoxicosis	4,00±0,29	76,2±0,8

Legend. Composition of incubation medium 0.115 M KCl; 0.003 M KH_2PO_4; 0.01 M Tris-HCl, pH 7.4. Reactions of lipid peroxidation initiated by the addition of Fe^{++} to the medium in a final concentration of 10^{-3} M.

rate of outflow of Ca^{++} from FSR was determined by the method described in [4].

Membrane lipids were tagged by the spin-labeled analog of palmitic acid, in which the nitroxyl fragment is located in position 6, relative to the COOH group. Before introducing FSR into the tube containing the probe, dissolved in ethanol, the alcohol was removed by evaporation. The final concentration of probe in the sample was 10^{-4} M. Thiol groups of FSR were modified with a spin label consisting of maleimide analog. The label was incubated with FSR in the ratio of 5:1 by molecular weight for 15 h. Unreacted label was removed by centrifugation twice. Electronic paramagnetic resonance (EPR) spectra were recorded on the Varian-E4 spectrometer under the following conditions: microwave power 10 mW, amplitude of modulation 1.6 G. The parameters observed are indicated on the EPR spectra of the palmitic acid probe illustrated in Fig. 1. By the use of these parameters the value of S [3], characterizing the degree of orderliness of the fatty-acid chains of the lipids in the bilayer, was calculated. The parameter of orderliness S was calculated by the equation:

$$S = \frac{A_{\parallel} - A_{\perp}}{2A_{\perp} \mp A_{\parallel}} \times 1{,}725.$$

The coefficient 1.725 allows for the electron-spin characteristics of the probe used.

For spin labels bound covalently with protein the correlation time τ was calculated by the equation [9]:

$$\tau = 5{,}1 \cdot 10^{-10}\left(1 - \frac{A_{22}}{68{,}65}\right)^{-1{,}36}$$

where A_{22} is the distance (in G) between the extrema of the spectrum; A_{22} = 68.25 for the case of slowest rotation of the radical.

Lipids were extracted from the FSR preparations by Folch's method. The protein concentration in the samples was determined by the biuret reaction.

EXPERIMENTAL RESULTS

Graphs showing the parameter of orderliness S as a function of temperature in FSR obtained from normal rabbits and from rabbits with thyrotoxicosis, plotted between Arrhenius' coordinates, are illustrated in Fig. 1. The discontinuity on the temperature curves of the thyrotoxic preparations compared with normal was shifted by 4°C toward the low temperature region. FSR of rabbits with thyrotoxicosis also had a discontinuity at 42°C, which is normally absent. If the temperature of the specimens, after reaching 42°C, was lowered to a certain limit, points obtained for preparations from normal rabbits again coincided with their initial values. Conversely, the analogous manipulation with preparations from rabbits with thyrotoxicosis revealed that graphs plotted for the newly obtained points did not coincide with the corresponding graphs obtained during a rise in temperature (Fig. 1). Protein–lipid interactions in FSR membranes in thyrotoxicosis are evidently weakened, and for that reason lower temperatures, insufficient to cause irreversible changes in normal preparations, were necessary to produce "disorder." The temperature transition observed at 42°C was probably due to denaturation of proteins, for it was not found in lysosomes prepared from FSR lipids of rabbits with thyrotoxicosis. Protein–lipid interactions in membranes of FSR from normal rabbits are evidently stronger and play the role of stabilizers, protecting the proteins against thermal denaturation.

A graph showing the correlation time τ of a spin label covalently bound with protein as a function of

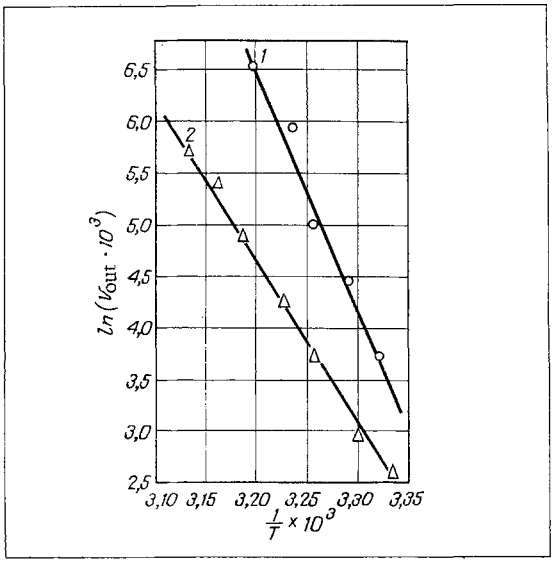

Fig. 3. Rate of outflow of Ca^{++} from vesicles as a function of temperature. 1) Preparations isolated from normal rabbits; 2) preparations isolated from rabbits with thyrotoxicosis, V_{out}) Velocity of outflow of Ca^{++} (in moles/min/mg protein).

temperature for membranes taken from normal rabbits and from rabbits with thyrotoxicosis is illustrated in Fig. 2. The discontinuity on the temperature curve in specimens from thyrotoxic preparations is shifted by 4°C toward low temperatures. This indicates an increase in mobility of the label in preparations obtained from rabbits with thyrotoxicosis, and is probably due to a decrease in viscosity of the microenvironment of the spin label.

It can thus be concluded from the results that both the protein and the lipid components of the membranes are substantially disturbed in thyrotoxicosis.

Additional information was obtained by the study of chemiluminescence of FSR of normal rabbits and of rabbits with thyrotoxicosis. As Table 1 shows, shortening of the period from addition of Fe^{++} to the beginning of development of a slow flash (latent period) took place in preparations obtained from rabbits with thyrotoxicosis. The intensity of luminescence increased under these circumstances. This was evidently connected with the more rapid formation of lipid hydroperoxides. Most probably, this effect was due to changes taking place in molecules of the oxidation substrate (lipids) in thyrotoxicosis. However, the possibility cannot be ruled out that FSR proteins make some contribution to the picture observed. Depending on the situation, thiol groups of proteins are known to be able to play the role of pro-oxidants or antioxidants [1, 2].

Further evidence in support of structural changes in FSR membranes in thyrotoxicosis is given by data obtained when studying the temperature dependence of the velocity of passive Ca^{++} outflow from FSR vesicles. The results of the appropriate experiments are illustrated in Fig. 3 as Arrhenius' graphs. A change in the angle of slope on the graph signifies that the activation energy of Ca^{++} outflow was lower than normal. This probably indicates the appearance of "facilitated" channels of passive transport for Ca^{++}, compared with the normal in the membranes in thyrotoxicosis.

In experimental thyrotoxicosis, marked changes thus take place in the structure of the membranes of the sarcoplasmic reticulum. The data presented in this paper suggest that these changes are largely connected with structural transformation both in the lipid bilayer and in the protein region.

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CHANGES IN THE PORTAL HEMODYNAMICS IN BURN SHOCK

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KEY WORDS: burn shock; hepatic circulation; blood volume and tone of portal vessels.

The anatomical features distinguishing the portal vascular system suggest that it plays the role of a pathological blood depot in stress states and, in particular, in severe burn trauma [1, 2]. However, no reference could be found in the literature to measurements of the blood volume in the portal vessels after burns.

This paper gives the results of measurement of the hepatic blood flow, the blood volume in the portal vessels, their tone, and the portocaval resistance in 15 dogs and eight rabbits in a state of burn shock.

EXPERIMENTAL METHOD

Burns of the IIIB degree affecting 20% of the body surface were inflicted by means of special heating elements. The animals were anesthetized with thiopental (50 mg/kg body weight). The total hepatic blood flow of the dogs was measured with the aid of colloidal ^{198}Au by the method of Restrepo et al. [7], the portal blood volume was measured by a slightly modified Bradley's method [4], and the cardiac output and circulating blood volume (CBV) were determined with the aid of the dye T-1824 [5]. To measure the pressure and to take blood samples from the aorta and hepatic veins, catheters were introduced through the femoral vessels into the right atrium, the hepatic vein, and the abdominal aorta under the control of a televisor of an image converter (Fig. 1). The catheter was introduced into the portal vein through one branch of the splenic vein, for which purpose the medial pole of the spleen was exteriorized through an incision in the abdominal wall. After introduction of the catheter the abdominal wound was closed in layers without drainage. Pressure curves were recorded by "Mingograph-81" electromanometers. The results are given in Table 1.

In rabbits the CBV, blood volume in the portal vessels, in the thorax, and in the region of the burns was determined by a method of regional radiometry based on the distribution of ^{51}Cr-labeled erythrocytes. Radiometry was carried out with three collimated sensors of the K-302 apparatus. The first sensor recorded the count from the head and chest, the second from the abdominal vessels, the third from the buttocks and lower limbs (the region of the burns). The sensitivity of the sensors was corrected for a two-dimensional phantom. Labeled erythrocytes were injected into the rabbit's auricular vein. Blood samples for determination of CBV were taken from the right atrium through the catheter introduced via the jugular vein. The first sample of labeled erythrocytes was injected before trauma, and CBV was then determined and the count obtained from each of the three collimated regions of the animal's body. These data were used to calculate regional blood volumes in the vessles of the thorax, abdomen, and region of the burns (Table 2).

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