The most characteristic components of the glycoproteins were hexoses, hexosamines, and sialic acids. Hexosamines could not be found in the Pacinian corpuscles, whereas hexoses and neuraminic acid were present in both water-soluble and salt-soluble proteins. The high content of neuraminic acid will be noted. The functions of the glycoproteins are varied. One of them maintains the constancy of the microenvironment surrounding the cell. Neuraminic acid, in the form of acyl derivatives, so-called sialic acids, is an essential component of the neutral glycoproteins. Neuraminic acid participates in the formation of collagen and is also present in large quantities in albumins and globulins. Hexuronic (glucuronic and iduronic) acids are specific components of the proteoglycans. They are also present in both groups of proteins. The most important function of the proteoglycans is considered to be regulation of ionic equilibrium and of movement of water in the tissues. In this respect the data showing the increased content of potassium ions in the fluid of Pacinian corpuscles are particularly interesting. This is probably due to the presence of glycosaminoglycans, active anions capable of binding small cations, in the intercellular medium of the external capsule of the receptors.

The characteristics of the soluble proteins of Pacinian corpuscles described above thus indicate that they can perform metabolic functions and provide the specific microenvironment for the tissue mechanoreceptor.

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CHANGES IN SPATIAL ORGANIZATION IN SARCOPLASMIC RETICULUM MEMBRANES IN RABBITS WITH EXPERIMENTAL THYROTOXICOSIS

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Membrane structures of some biological objects, namely mitochondria, erythrocytes, and the sarcoplasmic reticulum (SR), are a unique target for thyroid hormones [5, 7, 12, 14]. Structural changes in SR membranes in rabbits with hyperthyroidism caused by prolonged administration of thyroxine to the animals have been described [4]. In particular, labilization of protein–lipid bonds and a decrease in the orderliness and viscosity of the lipid bilayer were noted.

In the present investigation the spatial organization of proteins and lipids in SR membranes was studied depending on the thyroid state of the animal by means of the fluorescent probe pyrene.

EXPERIMENTAL METHOD

Fragments of SR were isolated by the method in [10] from skeletal muscle proteins of rabbits weighing from 2 to 3 kg. Thyrotoxicosis was simulated by intraperitoneal injection of L-thyroxine in 0.01 N KOH according to the scheme described in [5]. Control animals received the corresponding volume of 0.01 N KOH solution. Fluorescence of the proteins was measured on a spectrofluorometer used in [3]. The efficiency of energy transfer from tryptophan residues of protein to pyrene molecules was judged by the degree of extinction of the

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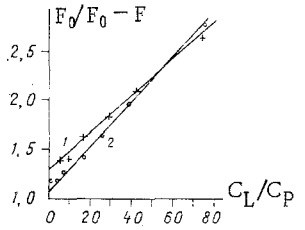

Fig. 1

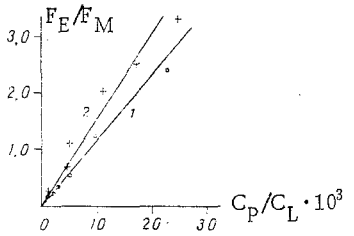

Fig. 2

Fig. 1. Efficiency of quenching of fluorescence of SR membrane proteins as a function of pyrene concentration. F_o, F) Intensities of fluorescence of SR proteins without and after addition of pyrene respectively. C_L, C_P) Molar concentrations of membrane lipids and pyrene. 1) Control, 2) hyperthyroidism. Protein concentration 0.1 mg/ml.

Fig. 2. Efficiency of eximerization of pyrene molecules in SR membranes as a function of probe concentration. F_E, F_M) Intensities of fluorescence of pyrene at 470 nm (eximer form) and 400 nm (monomer form) respectively. C_P, C_L) Molar concentrations of pyrene and lipids. Remainder of legend as to Fig. 1.

protein fluorescence by the molecules of the probe. The viscosity of the membrane lipids was determined from the rate of eximerization of pyrene, a function of the velocity of diffusion of the probe molecules in the lipid phase [1]. The protein concentration was determined by the microbiuret reaction. The content of membrane lipids was calculated from the weight of proteins, using the protein/lipid (w/w) ratio of 2:1, obtained previosly for SR membranes [11]. Lipids were extracted by the method of Folch et al. [8]. Electrophoresis of proteins was carried out in polyacrylamide gel as described in [9].

The results of extinction of protein fluorescence and fluorescence of pyrene were analyzed by computer by the method of least squares. Points were interpolated by the first degree polynomial $y = kx + b$, where $k = \tan \alpha$. Student's t-test was used for the statistical analysis of the results.

EXPERIMENTAL RESULTS

The previous investigation showed that on addition of pyrene to SR membranes, fluorescence of proteins is quenched as a result of the transfer of energy to the pyrene. The maximal degree of quenching occurred in SR membranes isolated from the muscles of normal rabbits, about 77% of the original intensity of fluorescence [2]. To determine this value, reciprocal coordinates were used, plotting the value $F_o/F_o - F$ against $1/C$, where F_o and F represent the intensity of protein fluorescence in the absence and presence of pyrene respectively, and C the concentration of pyrene. In that case, as $C \to \infty$ a segment equal to $F_o/F_o - F_\infty$ is intercepted on the vertical axis; the value $F_o - F_\infty/F_o$ is the maximal degree of quenching. In the present experiments it was 0.79 ± 0.02 in the group of control animals, in agreement with data obtained in [2]. In thyrotoxicosis, however, the value of $F_o - F_\infty/F_o$ was 0.96 ± 0.02, and it differed from the control (Fig. 1).

As has been shown by the method of electron paramagnetic resonance (EPR), the viscosity of lipids in the SR membranes was reduced in thyrotoxicosis [4]. We also observed a tendency for the viscosity of the lipid bilayer to fall in preparations obtained from animals with thyrotoxicosis (Fig. 2), although the difference between the control and experiment was not statistically significant (1.37 ± 0.04 and 1.51 ± 0.05, respectively).

The observed increase in the efficiency of energy transfer in SR preparations from animals with thyrotoxicosis (Fig. 1) could have several causes. The efficiency of this process may increase as a result of a decrease in the fraction of large proteins (for example, Ca-ATPase) in the general mass of membrane proteins. In addition, the maximal degree of quenching depends on the protein–lipid ratio. The very important role of thyroid hormones in the regu-

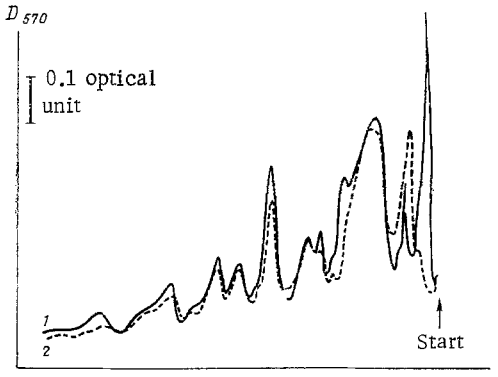

Fig. 3. Densitograms of samples of polyacrylamide gels each containing 100 µg SR membrane protein from normal animals (1) and from rabbits with thyrotoxicosis (2). Ordinate) optical density at 570 nm.

lation of synthesis of proteins and lipids is well known [6], and these two possibilities therefore deserved special study. However, as electrophoresis of proteins in polyacrylamide gel showed, no significant changes took place in either the composition or the relative proportions of the protein fractions of the SR membranes in thryrotoxicosis. No difference likewise was observed between the control and experiment in the protein–lipid ratio: In thyrotoxicosis it remained at its previous level (2:1), as was shown by quantitative analysis of phospholipids extracted from SR preparations.

In thyrotoxicosis there is thus a significant increase in the transfer of energy from proteins to the fluorescent probe (pyrene). Under these circumstances no significant changes are observed either in the protein composition or in the protein–lipid ratio. In that case, the cause of the changes in energy transfer could only have been a change in the spatial organization of the membrane protein–lipid complex. According to data in the literature [1, 2], an increase in the maximal degree of quenching of proteins by pyrene must be due to deeper embedding of proteins (especially large proteins of ATPase type) into the lipid bilayer. In some normal preparations Ca-ATPase projects above the surface of the lipids by 4 nm — this is clear from results obtained with the electron microscope [13] and from the results of a study of the efficiency of energy transfer [2]. In thyrotoxicosis, Ca-ATPase is embedded for a distance of 1 nm and projects above the surface by not more than 3 nm, as shown by results obtained during a study of the efficiency of energy transfer.

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