lipids in the membrane, the unique composition of lipids with receptor functions, and so on. The importance of each of the above parameters for effectiveness of fusion calls for further investigation.

In conclusion, it should be emphasized that despite the lack of clarity in the causes of the differences found, increased uptake of liposomes from homologous lipids by nonphagocytic cells compared with liposomes of egg lecithin and selective uptake of "homologous" liposomes in preference to incorporation of vesicles from a natural mixture of lipids of other cells can be used to make the selective application of therapeutic substances to target tissues more effective.

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# THYROID HORMONES AND ELECTRICAL STABILITY OF RAT LIVER MITOCHONDRIAL MEMBRANES


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Administration of thyroid hormones to animals is accompanied by their rapid binding with liver mitochondria [10], activation of endogenous phospholipase of liver mitochondria [4], stimulation of the process of $Ca^{++}/2H^{+}$ exchange through the mitochondrial membrane [3], and acceleration of lipid peroxidation (LPO) reactions in these organelles [6]. In electron micrographs of the liver of animals with hyperthyroidism the mitochondria appear more swollen than normally [14]. Resistance of the barrier systems of mitochondria isolated from animals with different thyroid states to $Ca^{++}$ is reduced in the order hypothyroidism > normal > thyrotoxicosis [1, 2, 5]. These data can be interpreted as evidence of a connection between membrane stability and hormonal state. The general stability of a membrane is characterized by its electrical stability, determination of which in the case of artificial phospholipid membranes has found widespread application [6, 7].

The aim of this investigation was to determine the electrical stability of mitochondrial membranes in animals in different thyroid states.

## EXPERIMENTAL METHOD

Male Wistar rats were used. Thyroidectomy as a model of hypothyroidism was performed on animals weighing 100-120 g. By the beginning of the experiment (2 months after the operation) the animals weighed 140-


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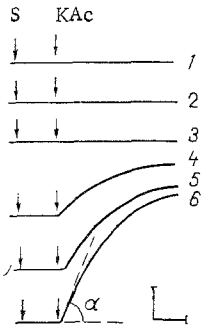


Fig. 1. Dependence of light transmission of mitochondrial suspension on potassium acetate concentration. Incubation medium (in mM): KCl 125, Tris-HCl 5, EGTA 0.1, succinate 10; pH 7.4 (20°C). Final KAc concentration: 1) 0.8 mM, 2) 1.6 mM, 3) 3.2 mM, 4) 6.25 mM, 5) 9.4 mM, 6) 12.5 mM. Arrows indicate addition of succinate (S) and KAc. Concentration of mitochondria (as protein) 1 mg/ml. Ordinate, light transmission 680 nm. Calibration: 0.1% (680 nm); 1 min.

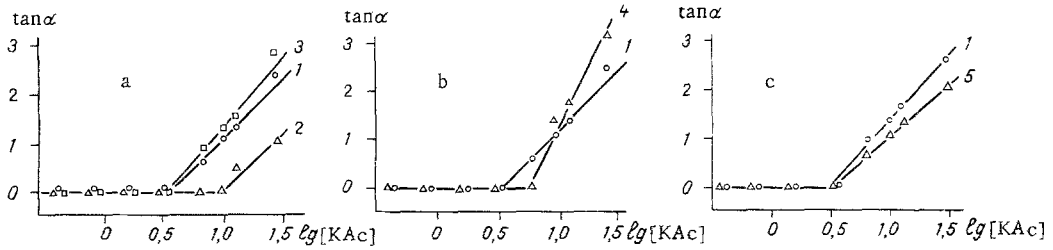


Fig. 2. Effect of thyroid state on swelling of rat liver mitochondria induced by electrical failure. Incubation medium as in Fig. 1. Curves represent typical results for three series obtained on preparations of normal (1), hyperthyroid (2), hypothyroid (3), and thyrotoxic (4) rats and rats receiving thyroxine 1 h before sacrifice (5). Each preparation consisted of mitochondria from two or three rats of the corresponding group. Abscissa, logarithm of molar concentration of KAc; ordinate, tan $\alpha$.

170 g, whereas control rats at this age weighed 200-230 g. Hyperthyroidism or thyrotoxicosis was induced by daily administration of L-thyroxine to the animals in a dose of 100 $\mu$g/100 g body weight for 9 days or 4 mg/100 g body weight for 6 days respectively. Mitochondria were isolated from the liver by the method in [9] in medium of the following composition (in mM): sucrose 250, Tris-buffer 10, EDTA 1; pH 7.4. The mitochondria were washed and kept in a similar medium, but without EDTA. The relative value of the mitochondrial transmembrane potential ($\Delta p$ or $\Delta\bar{\mu}H^+$) was judged from the degree of quenching of fluorescence of the carbocyanin probe Dis-$C_3$-(5) in a suspension of energized mitochondria [12]. The electrical stability of the mitochondrial membranes was assessed from the quantity of potassium acetate (KAc) which, under standard conditions, must be added to medium with mitochondria to induce "electrical failure" in the membranes (for details, see [6] and [7]). Under the conditions of this investigation the experiment was carried out as follows. Addition of KAc to energized mitochondria was accompanied by diffusion of electrically neutral acetic acid in the mitochondrial matrix [8]. Dissociation of its molecules in the matrix led to acidification of the latter with $H^+$ which, in turn, caused a decrease in the pH gradient on the mitochondrial membrane. Since the proton-motive force $\Delta p$ is made up of electrical ($\Delta E$) and osmotic (chemical) ($\Delta pH$) components,

$$\Delta P = \Delta\bar{\mu}H^+/F = \Delta E - 59\Delta pH,$$

TABLE 1. Effect of Thyroid State on Intensity of Fluorescence (in relative units) of Dis-$C_3$-(5) Probe in Mitochondrial Suspension (M ± m)

| Parameter | Control ($n=4$) | Thyrotoxicosis ($n=4$) |
|---|---|---|
| F | 166±6 | 194±3 |
| ΔF | 45±5 | 32±1 |
| ΔF/F | 27,5±2,9 | 16,3±1,7 |

Legend. F) Intensity of fluorescence of probe in mitochondrial suspension (125 mM KCl, 18 mM Tris-buffer, pH 7.4); F) differences between value of F and intensity of fluorescence of probe after addition of succinate (10 mM) to the medium. Concentration of probe in medium 15 μM. Concentration of mitochondria as protein 1 mg/ml. Number of animals shown in parentheses. Each experiment was done on a combined preparation of mitochondria from two rats; number of repetitions in each experiment 6-8.

lowering of the pH gradient (under natural conditions this is directed from the external medium into the matrix) on account of addition of acetic acid will lead to a corresponding increase in the electrical component ΔE [8]. This increase, proportional to the quantity of added KAc, continues until the electrical potential difference across the mitochondrial membrane reaches a certain critical level, after which "electrical failure" arises. As a result of failure membrane permeability increases for $K^+$ present in the medium, and its entry into the mitochondrial matrix leads to swelling of the organelles. The beginning of mitochondrial swelling and, consequently, of the electrical failure responsible for it, was monitored by recording light transmission by the mitochondrial suspension at 680 nm in a nephelometer. As an illustration of the above, light transmission curves of mitochondrial suspensions with different KAc concentrations are shown in Fig. 1. Clearly the change in light transmission of the mitochondria began with KAc in a concentration of 6.25 mM. A further increase in the KAc concentration in the medium caused swelling to take place more rapidly (an increase in tan $\alpha$).

The protein concentration was determined by the biuret reaction.

## EXPERIMENTAL RESULTS

Graphs showing light transmission by suspensions of mitochondria from rats with different thyroid states are given in Fig. 2. It follows from these results, which are typical for the corresponding series, that for failure to develop the largest quantity of KAc is required in the case of mitochondria from hyperthyroid animals (Fig. 2a). Rather less KAc than in hyperthyroidism, but more than in the case of normal animals or hypothyroidism, was needed to induce electrical failure in preparations from thyrotoxic rats (Fig. 2b). Between mitochondrial preparations of normal and hypothyroid animals, judging from the light transmission curves, no differences were observed in electrical stability of the membrane. To this must be added that, among the mitochondrial populations studied, those from animals with thyrotoxicosis swelled most rapidly.

It was shown previously [16] that highly specific receptors for thyroid hormones exist in mitochondria of several tissues. This can evidently explain the phenomenon of rapid binding of thyroxine by liver mitochondria (minutes after injection into the animal) followed by active metabolizing of the hormone [9]. This situation enables in vivo assessment of the rapid action of thyroid hormones, unconnected with processes of cytoribosomal synthesis, on electrical stability of the mitochondrial membranes. To study this problem, 1 h before isolation of the organelles, the animals were given an intraperitoneal injection of thyroxine (300 μg/100 g body weight). As regards the electrical stability of their mitochondria, these rats were shown not to differ from control animals (Fig. 2c), receiving the appropriate quantity of solvent (0.05 N KOH) 1 h before isolation of the mitochondria. However, injection of thyroxine was accompanied by a significant decrease in the rate of swelling of the mitochondria compared with the control.

It is interesting to compare the results of this investigation with previous observations made on artificial membranes formed from phospholipids of mitochondria of animals with experimental thyroid disease [6, 7]. In particular, it was shown that in hyperthyroidism electrical stability of liposomes of flat membranes is higher than normal [7]. This correlates well with the increased electrical stability of the mitochondria of hyperthyroid rats observed in the present investigation. What is more surprising is that in hypothyroidism also, characterized by more stable mitochondrial phospholipids than normal [6], the mitochondria on the whole were not more stable than those of normal animals (Fig. 2a).

It is difficult to determine correlation between electrical stability and transmembrane potential at a given moment, although it may be pointed out that in hyperthyroidism the value of $\Delta p$ of the liver mitochondria is increased [5, 15].

In hypothyroidism, on the other hand, a decrease in the transmembrane potential of rat liver mitochondria was observed [1]. Recording fluorescent responses of the Dis-$C_3$-(5) probe in mitochondrial suspensions from normal and thyrotoxic rats showed a considerable fall in the parameter $\Delta F/F$ in preparations from thyrotoxic animals (Table 1).

Considering that the value of $\Delta F/F$ is proportional to $\Delta p$ [12], a decrease in the transmembrane potential of mitochondria from thyrotoxic rats can be postulated, in agreement with the well-known ability of toxic doses of thyroid hormones to inhibit oxidative phosphorylation.

Thyroid hormones have a many sided action on metabolism of membrane (including mitochondrial) phospholipids. Parameters such as the spectrum of mitochondrial phospholipids, and their fatty-acid composition [11], which is important for electrical stability of model lipid membranes [13], also are determined by the hormonal level. This conclusion is confirmed indirectly by the presence of correlation between increased electrical stability of mitochondrial lipids [7] and the increased electrical stability of mitochondrial membranes of hyperthyroid rats demonstrated in the present investigation. The absence of such correlation for hypothyroidism, in which the mitochondria as a whole are not more stable than in euthyroidism, whereas electrical stability of the mitochondrial phospholipids of hypothyroid animals was higher than the normal level [6], indicates only that electrical stability of native membranes may be determined by factors other than lipids (for example, by protein–lipid interactions).

The concrete cause of the increased electrical stability of the mitochondria of hyperthyroid animals is difficult to distinguish, but it can be considered that it is not connected with the direct action of excessive quantities of hormone on the mitochondria. This can be judged by the absence of any evident effect of thyroxine, injected 1 h before sacrifice of the animal, on the magnitude of this mitochondrial parameter (Fig. 2c).

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