The appearance of a focus of EA, which is a generator of pathologically enhanced excitation [4], and of primary generalized EA in the cerebral cortex is thus accompanied by activation of endogenous phospholipase hydrolysis in the cortex.

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HYPERTHYROIDISM AND THE TRANSMEMBRANE POTENTIAL OF RAT LIVER MITOCHONDRIA

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KEY WORDS: hyperthyroidism; mitochondria; transmembrane potential; calcium.

There is as yet no general agreement regarding the effect of the thyroid hormone level *in vivo* on the energy-transforming functions of the mitochondria. It was shown previously that the content of esterified phosphate per unit quantity of oxygen consumed is reduced in mitochondria isolated from the tissues of hyperthyroid animals [8]. Other workers did not observe this uncoupling effect of an excess of thyroid hormones [7]. Bronk [6], moreover, observed acceleration of oxygen consumption by isolated mitochondria in medium with thyroxine without any decrease in the efficiency of oxidative phosphorylation, ruling out the possibility of any direct uncoupling of oxidation and phosphorylation by thyroid hormones. Recently it was shown in the same laboratory that the transmembrane potential (TMP) of rat liver mitochondria rose after a single injection of thyroxine into animals, and on this basis it was postulated that a thyroxine-induced increase in the efficiency of oxidative phosphorylation takes place in the mitochondria [11], a concept directly opposite to that of the uncoupling action of thyroid hormone. In the case of mitochondria whose physiological state is determined by several cytoplasmic factors [4] it is evidently difficult to interpret an unambigu-

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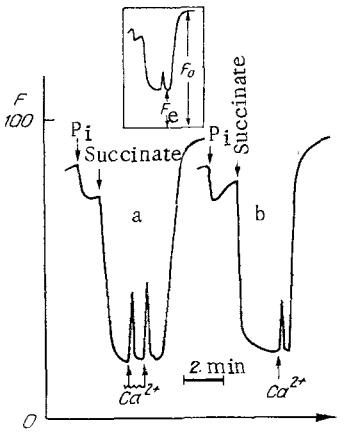

Fig. 1

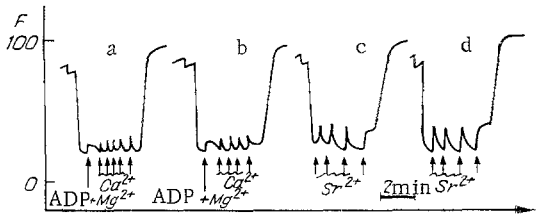

Fig. 2

Fig. 1. Effect of Ca^{++} on mitochondrial TMP. Fluorescence Dis-C_3-(5) probe in mitochondrial suspension from normal (a) and hyperthyroid (b) rats. Here and in Figs. 2 and 3: incubation medium contained 0.1 M KCl, 0.1 M sucrose, 5 mM Tris-buffer, pH 7.4 (20°C). Concentration of mitochondria 1 mg/ml as protein. Times of addition of inorganic phosphate (1 mM) and succinate (5 mM) to the suspension are indicated by an arrow. Total quantity of added Ca^{++} shown on figure. Ordinate: F) intensity of fluorescence (in relative units); abscissa, time (in min).

Fig. 2. Effect of conditions of incubation on mitochondrial TMP of normal (a, c) and hyperthyroid (b, d) rats. Mg^{++} 1 mM and ADP 300 μM. Ca^{++} 0.5 μM (a) or 0.3 μM (b); Sr^{++} 0.5 μM.

ous role for any modifier of mitochondrial functions, including thyroid hormones, on the basis of the recording of only one parameter *in vitro*. It would seem more appropriate when investigating so-called latent injuries to record the function under conditions of extremal or functional exposure (raised temperature, hypoxia, and so on). In particular, one such factor to which the mitochondria may be exposed is calcium loading.

It was accordingly decided to study the effect of hyperthyroidism on mitochondrial TMP under normal conditions and during calcium loading.

EXPERIMENTAL METHOD

Male Wistar albino rats weighing 200-220 g were used. Hyperthyroidism was induced by intraperitoneal injection of L-thyroxine in a dose of 100-120 μg/100 g body weight daily for 6-7 days. Control animals received an injection of the same volume of 0.05 N KOH solution. Mitochondria were isolated from the liver by the usual method in 0.25 M sucrose, 10 mM Tris-HCl, and 1 mM EDTA, followed by rinsing and keeping of the isolated mitochondria in medium without the complexone. The level of mitochondrial TMP was judged from the decrease in fluorescence of a Dis-C_3-5-(5) probe (3,3'-dipropylthiodicarbocyanine) during energization with succinate [3]. The protein concentration in the samples was determined by the biuret reaction.

EXPERIMENTAL RESULTS

Typical patterns of fluorescence of the probe in a suspension of liver mitochondria from normal and hyperthyroid rats are illustrated in Fig. 1. Energization of the organelles with succinate, leading to establishment of the maximal value of TMP, reduced the intensity of fluorescence of the dye. Addition of Ca^{++} to the suspension was accompanied by a brief decrease of TMP, the initial level of which was restored when all Ca^{++} was absorbed from the medium as shown by parallel experiments using a calcium electrode. After a certain time, the length of which was inversely proportional to the quantity of absorbed Ca^{++}, a small decrease of TMP to a level corresponding to a state of uncoupled mitochondrial respiration began. The relative level of the mitochondrial TMP was judged from the intensity of quenching of fluorescence — the ratio $(F_o - F_e)/F_o$ (Fig. 1). This ratio in preparations from hyperthyroid animals was found to be higher than the corresponding value for mitochondria

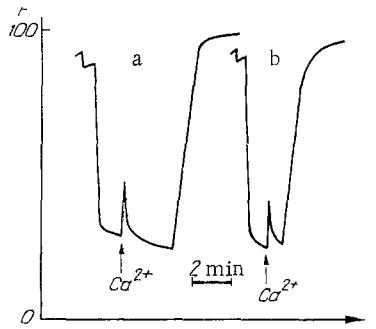

Fig. 3. "Retention time" of Ca^{++} by mitochondria of normal (a) and hyperthyroid (b) rats. Ca^{++} 25 nmoles/mg protein.

of euthyroid rats (111.9 ± 1.7% compared with 100% in the control; $P < 0.01$). These results point to an increase in mitochondrial TMP in hyperthyroid rats.

It was also found that the mitochondria of hyperthyroid rats, unlike those of normal animals, have increased sensitivity to Ca^{++} (Fig. 1). In preparations from hyperthyroid rats the fall in mitochondrial TMP took place in the presence of smaller Ca^{++} loads than normally. This phenomenon is evidently analogous to that of the decrease in the calcium capacity of the liver mitochondria observed by A. I. Gagel'gans in rats with experimental thyrotoxicosis, when the dose of calcium required for the development of high-amplitude swelling of the mitochondria was reduced [1].

The resistance of the mitochdonria of normal and hyperthyroid animals to the harmful action of Ca^{++} was increased on the addition of Mg^{++} and ADP to the medium. This was most probably due to the well-known ability of these substances to stabilize mitochondrial membranes [12]. It is interesting to note that the capacity of the mitochondria for Sr^{++} was about equal in normal and hyperthyroid animals, 4-6 times greater than their calcium capacity (Fig. 2).

The period of maintenance of the initial TMP level was later reduced in mitochondrial preparations from hyperthyroid rats after Ca^{++} loading compared with normal animals (Fig. 3).

In another series of experiments attempts were made to reproduce the experiments of Shears and Bronk [11], who observed an increase in TMP of liver mitochondria isolated 24 h after injection of thyroxine into rats (800 μg/100 g body weight). However, no difference was found in the TMP level: TMP in hyperthyroidism was 101.4 ± 0.61% of the control. The dose of thyroxine used by the authors cited [11], incidentally, was too large to allow conclusions on the physiological role of thyroid hormones in regulation of the energy-surviving functions of the cell to be drawn from the results. Moreover the time of action of the injected hormone (24 h) was too short to induce hyperthyroidism.

The increase in the mitochondrial TMP hyperthyroidism is connected with activation of electron-transporting units of the respiratory chain, which has been demonstrated for this particular state of the thyroid gland [5, 7]. As regards the decrease in resistance of the membrane system to Ca^{++}, this phenomenon may be based on activation of mitochondria phospholipase. The writers showed previously that activation of this kind takes place in liver mitochondria of hyperthyroid rabbits [2].

These results, and also those of recent experiments by Pfeiffer et al. [9, 10], showing that accumulation of free fatty acids, release of Ca^{++} from the mitochondria, and the reduction of TMP of these structures take place parallel to each other, suggest that differences observed in the trend of changes in TMP when limited calcium loading was present in preparations of the two groups studied were due to acceleration of hydrolysis of mitochondrial lipids in hyperthyroidism. This suggestion is supported not only by calcium loading experiments, but also indirectly by the approximately equal capacity of mitochondria of the two groups of animals for Sr^{++}. A difference between Sr^{++} and Ca^{++} was discovered in respect of activation of mitochondrial phospholipase [10].

In hyperthyroidism the mitochondrial TMP is thus higher than normally and, at the same time, the resistance of these organelles to the harmful action of Ca^{++} is reduced.

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BLOOD SERUM PROTEINS AS POSSIBLE INDICATORS OF BURN TOXINS

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KEY WORDS: burns; serum proteins; burn toxin.

The state of the blood proteins in thermal burns has often been investigated [5, 7, 8]. Changes in the relative concentrations of individual serum proteins [12], qualitative characteristics of protein fractions detectable by electro- and immunoelectrophoresis [1, 6, 11], the level of the so-called acute phase proteins [10], and other parameters have been regarded as prognostic signs in burns. On the whole, however, data on the pathogenetic significance and specificity of disturbances of the blood protein system in burns are highly contradictory. In previous studies of burned skin as the most likely source of autoimmunization and autointoxication the formation of a specific burn toxin was demonstrated and its important role in the pathogenesis of the initial period of burn toxemia was established [3, 4].

This paper describes the results of a comprehensive analysis of serum proteins corresponding in certain physicochemical characteristics to the high-molecular-weight toxin of burned skin.

EXPERIMENTAL METHOD

A thermal burn of the skin was inflicted on female Wistar rats weighing 100-120 g (anesthetized with 1 ml of 0.3% pentobarbital solution, flame burn from cotton soaked in alcohol, area of burn 15-20% of body surface, exposure 30 sec). Blood was taken from the animals' heart 48 h after burning under ether anesthesia and, after clot formation, the serum was separated by centrifugation. Serum was obtained under similar conditions from normal, unburned rats.

Fractionation of the serum proteins by two-stage precipitation with ammonium sulfate (fractions corresponding to 60-75 and 63-70% saturation) and gel-filtration on Sephadex G-200 was carried out as described previously [4]. All operations corresponded exactly to the isolation of toxic fractions from a saline extract of burned skin.

The gel permeation coefficient (K_{av}) was calculated by the equation:

$$K_{av} = \frac{V_e - V_0}{V_t - V_0},$$

where V_e is the elution volume of the corresponding fraction; V_0 the outer bed volume, deter-

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