not identical, in agreement with what was observed at the crystallization stage. Determination of the temperature optimum and thermolability showed that activity of the "pathological" enzyme was reduced at temperatures of above 20°C. Under normal conditions at this temperature activity increased, to reach a maximum at 40-55°C. In atherosclerosis the enzyme was more thermolabile: Inactivation began at 60°C; in enzyme from unchanged tissue it was observed at 70°C (Fig. 2).

Investigation of dependence of FDA activity on pH showed that in atherosclerosis the pH optimum was shifted a little toward the acid side: 8.2 under normal conditions, 8.0 for the "pathological" enzyme (Fig. 3).

FDA from muscle tissue in atherosclerosis had lower substrate affinity: The value of the Michaelis constant for fructose-1-6-diphosphate increased from 3.4×10^{-6} to 4.6×10^{-6} M.

This paper thus describes the first isolation of FDA in crystalline form from the skeletal muscles of patients with atherosclerosis. The enzyme is characterized by high catalytic activity and also by changes in certain physicochemical parameters.

LITERATURE CITED

1. M. F. Gulyi, B. D. Dvornikova, A. M. Kolomiichenko, et al., in: Problems in Muscle Biochemistry [in Russian], Kiev (1954), p. 244.
2. L. T. Litvinenko, M. F. Gulyi, F. N. Gil'miyarova, et al., Ukr. Biokhim. Zh., No. 2, 214 (1972).
3. A. Dzugaj, W. Wnuk, and T. Baranowski, Acta Biochim. Pol., 21, 237 (1974).

ACTIVATION OF LIPID PEROXIDATION IN LIVER MITOCHONDRIA OF HYPERTHYROID RABBITS

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If thyroxine is added to medium containing liver mitochondria or homogenates it inhibits lipid peroxidation (LPO) in these systems [2, 5, 6, 9, 10]. Since the doses of the hormone used in these systems were close to physiological (10^{-6}-10^{-7} M) and since the antioxidant activity of the hormone was comparable with the ability of α-tocopherol to slow the rate of LPO processes [2, 10], it was suggested that thyroxine may have a specific function as a natural antioxidant [6]. It is not known, however, whether the fall in the thyroid hormone level leads to activation of LPO reactions or, conversely, to their inhibition at the expense of lipid metabolism. It has been shown that the concentration of α-tocopherol in the serum and liver rises in hyperthyroidism [12]. Meanwhile liver homogenates from hyperthyroid animals in the course of incubation accumulate products reacting with thiobarbituric acid with higher velocities than samples prepared from the liver of normal and hypothyroid animals [13]. Acceleration of LPO reactions in fragments of the sarcoplasmic reticulum in hyperthyroid rabbits also was observed by the present writers previously [3]. Direct addition of thyroxine or other derivatives of the thyroid gland, moreover, is not always accompanied by inhibition of LPO and, on the contrary, it may actually activate LPO, as has been shown in the case of isolated erythrocytes [11]. Relations between thyroid hormones and LPO are further complicated by the fact that deiodination of the hormone, considered to be essential for the realization of its biological activity, is increased when the diet is deficient in vitamin E [8], whereas elevation of the body's thyroid levels leads to an increase in the concentration of this vitamin in the serum and liver.

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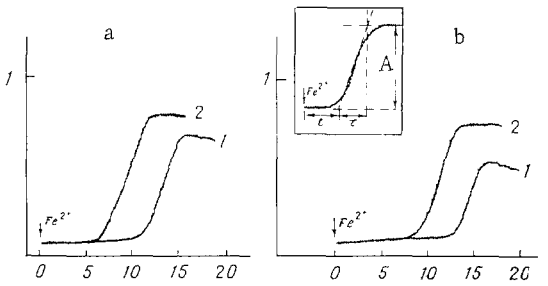

Fig. 1. Chemiluminescence of mitochondria (a) and lipids (b) as a function of thyroid hormone concentration in normal (1) and hyperthroid (2) rabbits. a) Incubation medium contained 105 mM KCl, 3.0 mM KH_2PO_4, pH 7.4. Fe^{++} (time of addition indicated by arrow) was added up to a final concentration of 1.0 mM. Protein concentration in measuring cuvette 1 mg/ml. Temperature 28-30°C; b) lipid concentration 1 mg/ml. Unlike incubation medium for mitochondria, KH_2PO_4 concentration in liposome suspension was 16 mM, Fe^{++} concentration 5×10^{-4} M. Scheme of analysis of chemiluminescence shown inside frame: 1) latent period of onset of "slow flash," τ) rise time of "slow flash" with maximal velocity, A) amplitude of "slow flash." Abscissa, time (in min); ordinate, intensity of chemiluminescence (in relative units).

In the investigation described below the velocity of LPO reactions initiated by Fe^{++} in liver mitochondria of rabbits with hyperthyroidism was studied.

EXPERIMENTAL METHOD

Rabbits weighing 2.0-3.0 kg were used. Hyperthyroidism was induced by intraperitoneal injection of L-thyroxine in a dose of 100-150 µg/kg body weight daily for 16-21 days. Mitochondria were isolated from a 12% liver homogenate in medium containing 0.25 M sucrose, 10 mM Tris buffer, and 1 mM EDTA (pH 7.4). The supernatant obtained after centrifugation (650g, 10 min) was sedimented (10,000g, 20 min) and rehomogenized in washing medium containing 0.25 M sucrose and 10 mM Tris buffer (pH 7.4) and again sedimented. Lipids were isolated from the mitochondria by the method of Bligh and Dyer [4]. Chemiluminescence of the mitochondria and liposomes, reflecting the velocity of LPO reactions, was recorded on a standard apparatus [1]. To exclude drift ot the parameters of the apparatus and to standardize the conditions, in each series of experiments chemiluminescence of the mitochondria and lipids of normal and hyperthyroid animals was measured. The preparations mentioned were isolated from two animals (control and experimental) practically synchronously and under identical conditions after sacrifice. The series of chemiluminescence tests include at least four pairs of curves of luminescence of mitochondria and the same number for lipids. The protein concentration was determined by the biuret reaction.

EXPERIMENTAL RESULTS

Typical chemiluminescence curves of mitochondria from normal and hyperthyroid animals are illustrated in Fig. 1a. Compared with preparations from normal animals the mitochondria of hyperthyroid rabbits had a shorter latent period of onset of the "slow flash" and a higher amplitude of that parameter, evidence of the higher velocity of the LPO reaction of the liver mitochondria in hyperthyroidism. It should be noted that in liproprotein structures and, in particular, in mitochondria peroxidation reactions depend not only on the physicochemical properties of the unsaturated fatty acids composing the lipids, but also on proteins, which may affect the rate of oxidation of Fe^{++} (the LPO initiator in the system used), thereby introducing modifications into the course of the process we are discussing. However, it was found that in the case of luminescence of lipids isolated from mitochondria, the differences observed in the organelles between normal and pathological were still preserved (Fig. 1b; Table 1).

TABLE 1. Effect of Thyroid State on Latent Period and Amplitude of "Slow Flash" in Mitochondria and Liposomes

Parameter	Mitochondria (n = 7)		Liposomes (n = 4)	
	normal	hyperthyroidism	normal	hyperthyroidism
Latent period	100%	85,5±5,2	100%	75,2±4,6
Amplitude of "slow flash"	100%	141,2±8,1	100%	136,5±13,4

Legend. Here and in Table 2, values obtained in preparations of mitochondria and lipids of normal animals taken as 100%: experimental conditions indicated in caption to Fig. 1.

TABLE 2. Dependence of Time of Development of "Slow Flash" in Mitochondria and Liposomes on Body Level of Thyroid Hormones

Object	Value of τ	
	normal	hyperthyroidism
Mitochondria	100%	115,1±13,5
Liposomes	100%	69,9±1,7

It can thus be concluded that the ability of mitochondrial lipids to take part in LPO reactions is greater in hyperthyroidism than in the normal state. This may be due to an increase in the index of unsaturated fatty acids, which is observed in rat liver mitochondria after administration of thyroid hormones [7]. Meanwhile it can be tentatively suggested that intensification of chemiluminescence of mitochondria in hyperthyroidism was not caused by a fall in their antioxidant content. In the latter case the rise time of the "slow flash" (Fig. 1, parameter τ) is usually shortened. In the present experiments there was a contrary increase in τ, which can be explained by a rise in the antioxidant level (Table 2). This is in agreement with data on an increase in the α-tocopherol content in the liver in hyperthyroidism [12]. The volume of τ in the liposomes of hyperthyroid rats was much lower than in the control (Table 2), possibly due to loss of antioxidant factor during isolation of the lipids.

An increase in the thyroid hormone concentration thus intensifies LPO in liver mitochondria, although the mechanism of this prooxidant action of thyroxine (and also, evidently, of its metabolic products) is not yet clear. Whatever the explanation, the observed effects of hyperthyroidism were not the result of the direct action of thyroxine on mitochondria, as Gukasov and Fedorov [2] showed in a model system (mitochondria + thyroxine), for in the present experiments the addition of different concentrations of thyroxine to isolated mitochondria from the liver of normal animals (3.25×10^{-8}-1.6×10^{-7} mole/mg protein) caused virtually no change in the kinetics of luminescence (not shown in Fig. 1). Consequently, in hyperthyroidism changes take place in the liver mitochondria which facilitate processes of peroxidation of membrane lipids.

LITERATURE CITED

1. Yu. A. Vladimirov and A. I. Archakov, Peroxidation of Lipids in Biological Membranes [in Russian], Moscow (1972).
2. V. M. Gukasov and V. K. Fedorov, in: Role of Changes in Membrane Structure in Cell Pathology [in Russian], Moscow (1977), p. 8.
3. A. I. Marzoev, A. G. Maksima, O. A. Azizova, et al., Byull. Eksp. Biol. Med., No. 4, 410 (1980).
4. E. G. Bligh and W. J. Dyer, Can. J. Biochem. Physiol., 37, 911 (1959).
5. J. Bunyan, J. Green, E. E. Edwin, et al., Biochim. Biophys. Acta, 47, 401 (1961).
6. W. D. Cash, M. Garby, H. E. Carlson, et al., J. Biol. Chem., 241, 1745 (1966).
7. I. Y.-D. Chen and F. L. Hoch, Arch. Biochem., 181, 470 (1977).
8. V. A. Golton and S. H. Ingbar, Endocrinology, 77, 169 (1965).

9. F. E. Hunter, A. Scott, J. Weinstein, et al., J. Biol. Chem., 239, 622 (1964).
10. F. E. Hunter, J. M. Gebicki, B. E. Hoffstein, et al., J. Biol. Chem., 238, 828 (1963).
11. S. K. Kumar, R. Walls, and P. Hochstein, Arch. Biochem., 180, 514 (1977).
12. M. Neradilova, F. Hruba, V. Novakova, et al., Čas. Lek. Česk., 117, 264 (1978).
13. O. M. Saka and H. A. Impren, in: Advances in Thyroid Research, Oxford (1961), p. 520.

INCREASE IN BREAKDOWN POTENTIAL OF LIPOSOMES FORMED FROM LIVER MITOCHONDRIAL LIPIDS OF HYPOTHYROID RABBITS

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It was shown previously that the breakdown potential is increased in liposomes and bilayer membranes formed from liver mitochondrial lipids of hyperthyroid rabbits [4]. The value of the breakdown potential, reflecting the electrical stability of model membranes, is determined by the spectrum of phospholipids composing them and the physicochemical characteristics of the fatty acid chains of these compounds. Thyroid hormones take part in the regulation of lipid metabolism, and under these circumstances properties of the lipids that are important for the value of the breakdown potential are changed [5, 6-9]. It was natural to suggest that if the electrical stability of the lipid membranes is increased in hyperthyroidism, a fall in the value of the breakdown potential ought to be observed in hypothyroidism, as it it is in nearly all other cases in which the biological action of thyroid hormones has been studied. The investigation described below was devoted to an experimental verification of this hypothesis.

EXPERIMENTAL METHOD

Rabbits weighing 2.0-3.0 kg were used. Hypothyroidism was induced by thyroidectomy under pentobarbital anesthesia. Mitochondria were isolated from the liver by the method previously described [1]. Lipids were extracted from the mitochondria by the method of Bligh and Dyer [6]. Liposomes were obtained by dispersion of the isolated lipids (0.2 mg/ml) in a 10 mM sucrose solution (pH 7.0). The value of the breakdown potential of the liposomes and the "aggregation parameter," reflecting the negative charge on their surface, were measured by the method in [3]. Puchkova, working in the writers' laboratory, has shown that water-soluble lipid peroxidation products formed during autooxidation or as a result of UV-irradiation can lower the breakdown potential of liposomes. Accordingly, in a series of experiments the antioxidant β-ionol was added to all the isolation media of mitochondria and liposomes in a final concentration of 0.1 μM. However, no difference could be found in the electrical parameters between lipids isolated in media with or without the antioxidant.

EXPERIMENTAL RESULTS

Typical curves showing the change in scattering of light by the liposomes on addition of potassium acetate are given in Fig. 1. The minimum of the curves corresponds to the transition from compression of the liposomes in hypertonic medium (a decrease in light transmittance) to swelling of the liposomes, taking place as a result of electrical breakdown of the membranes and entry of electrolyte inside the liposomes [3]. The concentration of potassium acetate which induced breakdown of these structures was higher in liposomes formed from liver mitochondrial lipids of hypothyroid animals than in the corresponding preparations from normal animals. This increase signifies an increase in the breakdown potential of the liposomes, calculated by the method in [3] (Table 1). It should be noted that a similar value of the breakdown potential of liposomes was observed in hyperthyroid animals also [4]. Changes in

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