HISTOCHEMICAL CHANGES IN L-GLYCEROL-3-PHOSPHATE: MENADIONE-TETRAZOLIUM OXIDOREDUCTASE ACTIVITY IN THE LIVER OF ALBINO RATS WITH EXPERIMENTAL THYROTOXICOSIS

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Activity of L-glycerol-3-phosphate: menadione-tetrazolium oxidoreductase was studied histochemically in the liver of albino rats with experimental thyrotoxicosis. Induction of this enzyme in the liver cells was detected when the level of thyroid hormones in the animals was excessively high. If 6-methyl-2-thiouracil was injected together with aminophylline, complete blocking of the induction of this enzyme took place.

Biochemical investigations conducted on the liver mitochondria, heart muscle, and kidneys of albino rats with experimental thyrotoxicosis induced by administration of T_3, T_4, and their analogs have demonstrated that the activity of mitochondrial L-glycerol-3-phosphate: (acceptor) – oxidoreductase (1.1.99.5) is dependent on the level of thyroid hormones in the body [7, 10, 12]. If an excess of thyroid hormone is given to albino rats, the activity of this enzyme was considerably increased in the liver cells. Combined administration of thyroid hormones and substances blocking protein synthesis at different structural levels (actinomycin D, puromycin, cyclohexylimide, etc.) does not increase the activity of this enzyme. These results show that thyroid hormones reinduce the synthesis of this enzyme [11, 17-19, 21]. Thyrostatic substances, especially derivatives of 2-thiouracil (2-TU), widely used in the clinical treatment of thyrotoxicosis, partially block induction of the synthesis of mitochondrial L-glycerol-3-phosphate: menadione-tetrazolium oxidoreductase in hepatocytes. Partial blocking of induction of the synthesis of this enzyme is observed only if T_4 is given along with the blocking agent, and not other analogs of L-thyronine possessing the peripheral action of thyroid hormones [6, 7, 9, 10, 12, 16, 17].

No histochemical studies of induction of enzymes by thyroid hormones in the liver of experimental animals could be found in the literature. The object of the present investigation was to use a histochemical method to study the activity of L-glycerol-3-phosphate: menadione-tetrazolium oxidoreductase in the liver of albino rats with experimental thyrotoxicosis and during its treatment with 6-methyl-2-thiouracil (6-MTU) and aminophylline.

EXPERIMENTAL METHOD

Sexually mature male albino rats weighing 200-250 g were subdivided into 8 groups with 6 animals in each group. The experimental conditions are shown in Table 1. T_4 was injected subcutaneously as the sodium salt of DL-thyroxine, T_3 as L-triiodothyronine, and 6-MTU was given with the food throughout the day. Aminophylline was injected intramuscularly in a dose based on body weight. The experiment lasted 25 days. The animals were decapitated, and pieces of liver taken from the rats of each group were

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TABLE 1. Experimental Conditions

Group of animals	Preparation			
	T_4	T_3	6-MTU	aminophylline
	μg/100 g body wt.		mg/100 g body wt.	
1 Control	—	—	—	—
2 (T_4)	30	—	—	—
3 (T_3)	—	4	—	—
4 (T_4+6-MTU)	30	—	20 after 6th day	—
5 (T_3+6-MTU)	—	4	20 after 6th day	—
6 (6-MTU+T_4)	30 after 6th day	—	20	—
7 (T_4—6-MTU + aminophylline)	30	—	20 after 6th day	30 after 6th day
8 (6-MTU)	—	—	20	—

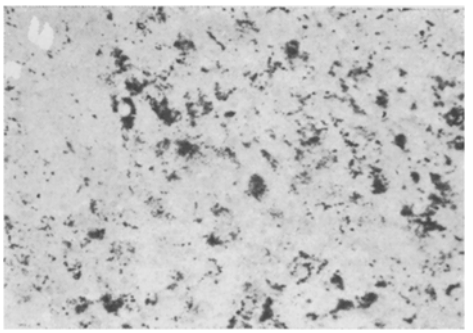

Fig. 1. Histochemical reaction for GPMTO in liver of control albino rat. Magnification: objective 20 ×, Homal 5 ×.

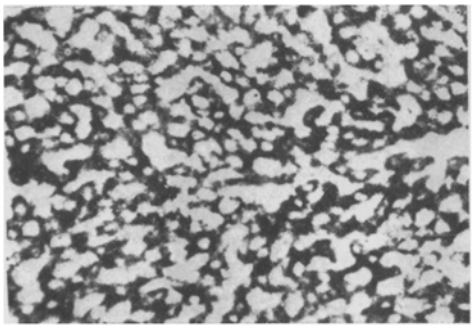

Fig. 2. Histochemical reaction for GPMTO in liver of albino rat receiving T_3. Magnification: objective 20 ×, Homal 5 ×.

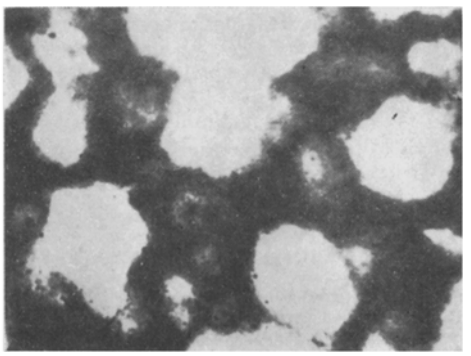

Fig. 3. Histochemical reaction for GPMTO in liver of albino rat receiving T_4. Magnification: objective 60 × (oil immersion), ocular Homal 5 ×.

mounted on a freezing microtome en bloc and frozen with "dry ice." Serial sections were cut to a thickness of 8 μ in the cryostat at −16°C with a very cold knife and affixed to dry slides.

Mitochondrial L-glycerol-3-phosphate: menadione-tetrazolium oxidoreductase (GPMTO) was detected by the histochemical method of Wattenberg and Leong [20] and Hess and Pearse [5], modified in the writers' laboratory. Nitro-BT and MTT-tetrazolium bromide were used as hydrogen acceptors. The incubation time was 15 min. Frozen survey sections were fixed with Gendre's solution at −60°, washed in water and 70% ethanol, and stained with gallocyanin−chrome alum by the method of De Boer and Sarnaker [15].

EXPERIMENTAL RESULTS

The results of investigation of the GPMTO activity in the liver of albino rats showed a low intensity of the histochemical reaction, based on the quantity of mono- or diformazan deposited as an index of the positive histochemical reaction in the hepatocytes, was weak (±) in the animals of Groups 1, 7, and 8, strong (5+) in group 2, and less strong in groups 3 and 4 (4+), group 5 (3+), and group 6 (3±1).

In the hepatocytes of the control animals, GPMTO activity was detected histochemically as only slight deposits of mono- or diformazan (Fig. 1).

After administration of thyroid hormones to the animals (groups 2 and 3), a strongly positive histochemical reaction of uniform intensity was found in the peripheral and central zones of the hepatic lobules and also in the individual hepatocytes. Mono- or diformazan was located in the cell mitochondria as granules of identical shape and size (Figs. 2 and 3).

GPMTO activity in the hepatocytes was somewhat reduced in albino rats receiving 6-MTU against the background of exogenous thyrotoxicosis (groups 4 and 5), but it was much higher than in the control group. Similar results were obtained by preliminary administration of 6-MTU and subsequent saturation of the animals with thyroid hormones (group 6).

Complete blocking of the induction of GPMTO synthesis was observed by the combined administration of 6-MTU and aminophylline to the hyperthyroid albino rats (group 7). Administration of 6-MTU to the control rats had no effect on mitochondrial GPMTO activity (group 8).

Mitochondrial L-glycerol-3-phosphate: (acceptor)-oxidoreductase is one of the enzymes particpating in active H^+ transport from the hyaloplasm into the mitochondria by means of the L-glycerol-3-phosphate cycle, and facilitating the subsequent transfer of H^+ to the respiratory chain at the coenzyme level [2-4, 13]. The increase in activity of the mitochondrial enzyme by the action of thyroid hormones accelerates the transfer of protons to the respiratory electron transport chainand the accumulation of energy through coenzyme Q, leading to a disturbance of D-glucose metabolism in the hepatocyte [8].

Theophylline and its analogs are inhibitors of the adenylate-cyclase system [1, 14]. The present experiment showed that combined administration of 6-MTU and aminophylline, in the presence of exogenous thyrotoxicosis, leads to complete blocking of the induction of GPMTO, one of the enzymes of the α-glycerol-3-phosphate shunt, so that one of the pathways of proton transfer from hyaloplasm to mitochondria is restored to normal.

Administration of aminophylline together with the thyrostatic agents may perhaps be used in the clinical treatment of thyrotoxicosis to restore normal metabolism in the liver.

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