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Effects of thyroxine on L-cysteine desulfuration in mouse liver.

Maria Wrobel* Toshihiko Ubuka†

Wen-Bin Yao‡ Tadashi Abe**

*Jagiellonian University,

†Kawasaki University of Medical Welfare,

‡Beijing Tong-Ren Hospital,

**Okayama University,

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Maria Wrobel, Toshihiko Ubuka, Wen-Bin Yao, and Tadashi Abe

Abstract

The effect of exogenous thyroxine (T4) administration on the activity of rhodanese, cystathionase, and 3-mercaptopyruvate sulfurtransferase (MPST) in the mitochondrial and cytosolic fractions of mouse liver was investigated. Three groups of mice were treated for 6 consecutive days with subcutaneous injections of T4 (50 micrograms, 100 micrograms, and 250 micrograms per 100 g of body wt, respectively). The other 3 groups were given 100 micrograms of T4 per 100 g of body wt for 1, 2, or 3 days. The dose of 100 micrograms T4 per 100 g of body wt given for 6 days exerted the strongest effect on the activity of all of the investigated enzymes. In comparison to the control, rhodanese activity diminished in the mitochondrial fraction by 40% ($P < 0.05$), cystathionase activity diminished in the cytosolic fraction by 15% ($P < 0.05$), and MPST activity in the mitochondrial fraction was reduced by 34% ($P < 0.05$), whereas cytosolic MPST activity was unaltered. Simultaneously, in the liver homogenate, elevated levels of ATP and sulfate were observed after 6 days of T4 administration. Thus, the present results seem to suggest that in the mouse liver, after 6 days of administration of 100 micrograms T4 per 100 g of body wt, the desulfuration metabolism of L-cysteine is diminished, which is probably accompanied by an increase in oxidative L-cysteine metabolism. The dose of 100 micrograms per 100 g of body wt administered for a shorter period, and the use of a lower dosage (50 micrograms T4 per 100 g of body wt) for 6 days had a stimulatory effect upon MPST activity level, and an increased level of sulfane sulfur was observed.

KEYWORDS: thyroxine, rhodanese, 3-mercaptopyruvate, sulfurtransferase, cystathionase, sulfane sulful

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Effects of Thyroxine on L-Cysteine Desulfuration in Mouse Liver

Maria WRÓBEL[ab]*, Toshihiko UBUKA[ac], Wen-Bin YAO[ad] and Tadashi ABE[a]

[a]Department of Biochemistry, Okayama University Medical School, Okayama 700-8558, Japan, [b]Institute of Medical Biochemistry, Jagiellonian University, Kopernika 7, 31-034 Kraków, Poland, [c]Department of Clinical Nutrition, Kawasaki University of Medical Welfare, Kurashiki, Okayama 701-0193, Japan and [d]Department of Surgery, The Beijing Tong-Ren Hospital, Capital Institute of Medicine, Beijing 100730, The People's Republic of China

The effect of exogenous thyroxine (T_4) administration on the activity of rhodanese, cystathionase, and 3-mercaptopyruvate sulfurtransferase (MPST) in the mitochondrial and cytosolic fractions of mouse liver was investigated. Three groups of mice were treated for 6 consecutive days with subcutaneous injections of T_4 (50 μg, 100 μg, and 250 μg per 100 g of body wt, respectively). The other 3 groups were given 100 μg of T_4 per 100 g of body wt for 1, 2, or 3 days. The dose of 100 μg T_4 per 100 g of body wt given for 6 days exerted the strongest effect on the activity of all of the investigated enzymes. In comparison to the control, rhodanese activity diminished in the mitochondrial fraction by 40% ($P < 0.05$), cystathionase activity diminished in the cytosolic fraction by 15% ($P < 0.05$), and MPST activity in the mitochondrial fraction was reduced by 34% ($P < 0.05$), whereas cytosolic MPST activity was unaltered. Simultaneously, in the liver homogenate, elevated levels of ATP and sulfate were observed after 6 days of T_4 administration. Thus, the present results seem to suggest that in the mouse liver, after 6 days of administration of 100 μg T_4 per 100 g of body wt, the desulfuration metabolism of L-cysteine is diminished, which is probably accompanied by an increase in oxidative L-cysteine metabolism. The dose of 100 μg per 100 g of body wt administered for a shorter period, and the use of a lower dosage (50 μg T_4 per 100 g of body wt) for 6 days had a stimulatory effect upon MPST activity level, and an increased level of sulfane sulfur was observed.

Key words: thyroxine, rhodanese, 3-mercaptopyruvate sulfurtransferase, cystathionase, sulfane sulfur

The desulfuration pathway of L-cysteine metabolism may be an important source of metabolically active sulfane sulfur, *i.e.*, divalent sulfur atoms bonded only to other sulfur atoms (1). Such sulfane sulfur atoms occur in various compounds in biological systems (2). A number of distinct enzymes present in mammalian tissues, the sulfurtransferases, have been shown to catalyze reactions that either use or produce sulfane sulfur. The enzyme thiosulfate sulfurtransferase (rhodanese, EC 2.8.1.1) carries a sulfane sulfur atom from a variety of sulfur donors, *e.g.*, thiosulfate, cystine trisulfide (thiocystine), and persulfides, to various acceptors, for example, to cyanide for its detoxification (2) and to proteins for Fe-S cluster formation (5) or to apoenzymes for the regulation of their activity. Some enzymes in biological systems are known to be activated or inactivated by reduced sulfur via a mechanism that involves the incorporation of a sulfur atom (6, 7) (Fig. 1). 3-Mercaptopyruvate sulfurtransferase (MPST, EC 2.8.1.2) and cystathionine γ-lyase (cystathionase, EC 4.4.1.1) are known to be involved in forming sulfane sulfur (2). L-Cysteine is converted by transamination to 3-mercaptopyruvate; the substrate of MPST which catalyzes the transfer of sulfur atoms from 3-mercaptopyruvate to one of several acceptors including cyanide, thiols, sulfite, and sulfinates (2, 3). Cystathionase catalyzes the β-elimination reaction of cystine resulting in the generation of endogenous reduced sulfur, in addition to its main role of cystathionine catabolism that constitutes the final step in the pathway of cysteine synthesis from dietary methionine (4).

The treatment of rats with thyroxine (T_4) has been found to inhibit cystathionase; this mechanism of inhibition was assumed to be the result of the direct action on pyridoxal 5'-phosphate, the coenzyme of this enzyme (8).

* To whom correspondence should be addressed.

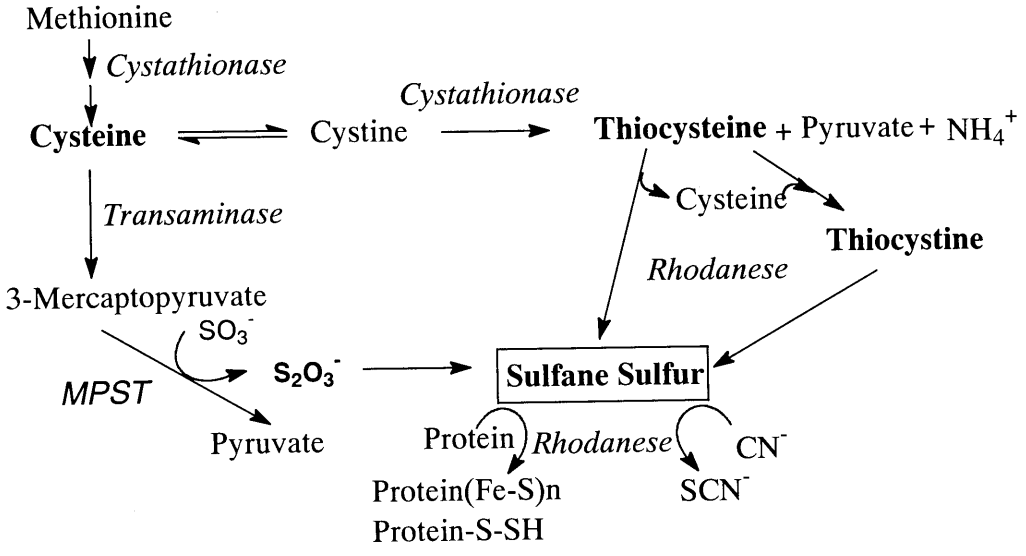

Fig. 1 The desulfuration pathways of L-cysteine metabolism.

In that study, it was also reported that electron microscopic analysis revealed no structural abnormalities in the mitochondria (8). On the other hand, a positive correlation between thyroid gland function and the activity of mitochondrial rhodanese and MPST was observed in an analysis of their levels in the frog liver, as measured during an entire year (9, 10). In frogs, as in mammals, MPST is present both in mitochondria and in the cytosol of liver cells; maximal activity of this enzyme in the mitochondria was detected in the Spring, the period of maximal thyroid activity (10). Rhodanese, a mitochondrial enzyme in the liver of mammals, was detected in both the mitochondria and the cytosol of frog liver cells (11). It was found that rhodanese activity in the mitochondria of frog liver cells is related to thyroid function. In the Spring, it reaches its maximum level and then rapidly decreases, parallel to thyroid function. In the Fall, another increase in its activity, corresponding to an increase in thyroid function, can be observed (9). Thus, it has been suggested that in the mitochondria of frog liver cells, the activity in the Spring of MPST, as well as the rhodanese activity in the Spring and the Fall, are evidently under the influence of thyroid activity.

The purpose of this study is to see as a pilot survey of whether or not the activities of MPST, rhodanese, and cystathionase are influenced by exogenously administered T_4. In order to clarify the effects of T_4 administration on the pathways of cysteine metabolism, we also examined levels of final metabolites such as sulfate (the final product of the oxidative pathway of L-cysteine metabolism), sulfane sulfur (the product of L-cysteine metabolism by way of desulfuration and transsulfuration), and ATP.

Materials and Methods

Animals and chemicals. Female Swiss mice weighing approximately 20 g were placed into groups of 8 animals per cage. They were fed a laboratory diet MF purchased from Oriental Yeast Co. (Tokyo, Japan) and water *ad libitum* for at least 1 week before the experiment. Sodium L-thyroxine pentahydrate was purchased from Sigma Chemical Company (St. Louis, MO, USA). Ammonium 3-mercaptopyruvate was synthesized according to Kun (1957). Sodium sulfite and N-ethylmaleimide were obtained from Wako Pure Chemical Ind. (Osaka, Japan). Lactate dehydrogenase (EC 1.1.1.27) from pig heart, glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and dithiothreitol were purchased from Böhringer-Mannheim GmbH (Mannheim, Germany). NADH, NADP and hexokinase (EC 2.7.1.1) from yeast were obtained from Oriental Yeast Co. Sodium thiosulfate was supplied by E. Merck (Darmstadt, Germany), and potassium cyanide by Katayama Chem. Ind. (Osaka, Japan). L-Homoserine, α-ketobutyrate and 2-mercaptoethanol were obtained from Wako, and pyridoxal 5'-phosphate

from Kyowahakko Co. (Tokyo, Japan).

Experimental procedure. The mice were maintained on the laboratory diet and water *ad libitum*. In experiment A, T_4 solution in 0.9% sodium chloride, neutralized with hydrogen chloride solution just before use, was injected subcutaneously at a dose of 50 μg T_4 per 100 g of body wt in group 1, 100 μg T_4 per 100 g of body wt in group 2, and 250 μg T_4 per 100 g of body wt in group 3, once a day for 6 days. In experiment B, 3 groups of mice received a daily dose of 100 μg T_4 per 100 g of body wt for 1, 2, or 3 days before they were sacrificed. Control mice were injected with the same volume of 0.9% sodium chloride solution. At 24 h after the last injection, the mice were killed, and the liver was taken and washed with cold 0.9% sodium chloride solution, and homogenized in 5 volumes of 0.25 M sucrose solution buffered with 10 mM Tris-chloride (pH 7.4), using a Potter-Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged at 600 xg for 5 min, and the obtained sediment was rehomogenized with 4 ml of the medium for 1 g of tissue and centrifuged as above. The pooled supernatants were further centrifuged for 15 min at 12,000 xg, yielding a final supernatant (cytosolic fraction). The pellets, crude mitochondrial fractions, were suspended in 4 ml of 0.05% Triton X-100 in 0.9% sodium chloride solution for 1 g of tissue (mitochondrial fraction). These fractions were kept frozen at -20 °C until used.

Enzyme assay. The activity of MPST was determined by measuring the amount of pyruvate formed during 15 min of incubation at 37 °C in accordance with the method of Valentine and Frankelfeld (12). The activity of rhodanese was assayed by Sörbo's method (13) measuring the amount of SCN^- formed during 5 min of incubation at 20 °C. The activity of cystathionase was determined by Matsuo and Greenberg's method (14) using L-homoserine as a substrate and the amount of 2-ketobutyrate formed during 30 min of incubation at 37 °C was measured. The activities of these enzymes were expressed in μmol of the product formed per 1 min per 1 g of fresh tissue.

ATP, sulfate and sulfane sulfur determination. ATP was determined by the UV-method with hexokinase and glucose-6-phosphate dehydrogenase (15). Sulfate was determined by ion chromatography as described by Ubuka *et al.* (16). Sulfane sulfur was determined by the method of Wood (17), based on cold cyanolysis and colorimetric detection of ferric thiocyanate complex ion. Statistical comparisons between groups were performed by means of Student's *t*-test.

Results

Mitochondrial enzymes. In mammals, rhodanese is known to be localized in the mitochondria (11). The activity value determined in the cytosolic fraction constituted about 6–7% of that in the mitochondrial fraction. This may be related to the leakage of the enzyme from the mitochondria during tissue homogenization and centrifugation. MPST shows bimodal distribution (18), namely, it is found both in mitochondrial and cytosolic compartments of the liver cell. Table 1 shows the activity values of MPST and rhodanese in the mitochondrial fraction of mouse liver obtained after subcutaneous T_4 administration at a daily dose of 50 μg, 100 μg, and 250 μg per 100 g of body wt, for 6 consecutive days (experiment A). T_4 administration resulted in the depletion of rhodanese activity but a dose-response relation was not observed. The lowest value, 60% of the control level, was that observed in the mitochondrial fraction of mouse liver after administration of 100 μg T_4 per 100 g body wt. The administration of T_4 at a dose of 50 μg and 100 μg per mouse caused a decrease in the activity of MPST to about 89% and 66%, respectively, of the control value. At a dose of 250 μg T_4, the level of MPST activity was almost the same as that in the control mice.

Fig. 2 presents results obtained after a single, 2, and 3 subcutaneous injections of 100 μg T_4 per 100 g body wt (experiment B). T_4 given at this dose inhibited rhodanese activity by 26% after a single injection, and by 12% or 16% after 2 or 3 days of administration. The changes in MPST activity were different depending on the doses.

Table 1 Mitochondrial enzyme activities of mouse liver homogenate after 6 days of thyroxine (T_4) administration

T_4 Dose (μg/100 g body wt)	Rhodanese (μmoles of product per 1 g of fresh liver/min)	MPST (μmoles of product per 1 g of fresh liver/min)
0 (n = 10)	102 ± 5	182 ± 21
50 (n = 16)	95 ± 5	163 ± 9
100 (n = 16)	62* ± 9	120* ± 11
250 (n = 16)	85* ± 4	188 ± 23

Enzyme activities were determined as described under Materials and Methods. MPST, 3-mercaptopyruvate sulfurtransferase; n, number of animals. *$P < 0.05$ compared to the control (dose 0).

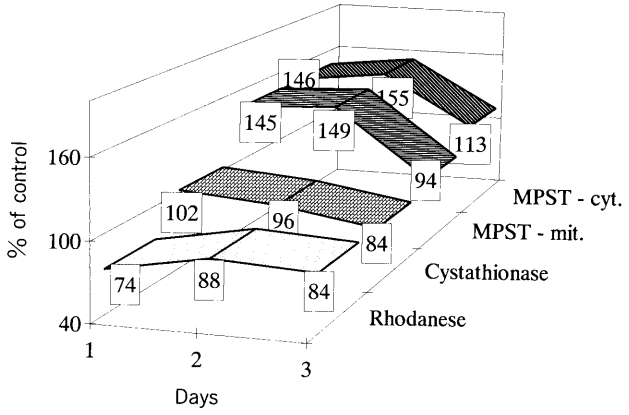

Fig. 2 Rhodanese, cystathionase and 3-mercaptopyruvate sulfurtransferase (MPST) activities in the mitochondrial (mit.) and cytosolic (cyt.) fractions of mouse liver homogenate after the dose of 100 μg T_4/100 g body wt daily, during 3 days.
The average control values of rhodanese and MPST activity determined in the mitochondrial fraction were: 99 ± 5 μmoles of SCN^- and 182 ± 12 μmoles of pyruvate per 1 g of fresh liver/min, respectively. The average control values of cystathionase and MPST activity determined in the cytosolic fraction were: 1.96 ± 0.09 μmoles of 2-ketobutyrate and 67 ± 4 μmoles of pyruvate per 1 g of fresh liver/min, respectively.

After a single or 2 injections of T_4, the level of MPST activity increased respectively by 45% and 49%. The third injection resulted in a decrease in MPST activity that approximated the range of the control value.

Cytosolic enzymes. Cystathionase is localized in the cytosolic compartment of the liver cell (18); the activity value found in the mitochondrial fraction constituted not more than 5% of that of the cytosolic fraction. Table 2 presents the activity values of cystathionase and MPST obtained in the cytosolic fraction of the mouse liver after the subcutaneous administration of T_4 for 6 days (experiment A). In comparison to the controls, the dose 50 μg per 100 g body wt did not cause any change in the cystathionase activity level. Doses of 100 μg and 250 μg T_4 per 100 g body wt resulted in a depletion of the activity level by 15% and 27%, respectively. In the case of MPST, the dose of 50 μg T_4 per 100 g body wt resulted in a significant increase (about 12%) in enzyme activity, when compared to the effects of this dose on the control animals. For the other 2 doses, the determined values were in the range of the control levels. Fig. 2 presents the results obtained for the cytosolic fraction of mouse liver after a single, 2, or 3 subcutaneous injections of 100 μg T_4 per 100 g of body wt (experiment B). In comparison with the activity in the controls, the activity

Table 2 Cytosolic enzyme activities of mouse liver homogenate after 6 days of thyroxine (T_4) administration

T_4 Dose (μg/100 g body wt)	MPST (μmoles of product per 1 g of fresh liver/min)	Cystathionase (μmoles of product per 1 g of fresh liver/min)
0 (n = 10)	86 ± 6	1.48 ± 0.07
50 (n = 16)	97* ± 7	1.43 ± 0.10
100 (n = 16)	87 ± 9	1.26* ± 0.17
250 (n = 16)	91 ± 11	1.08* ± 0.10

Enzyme activities were determined as described under Materials and Methods. MPST, 3-mercaptopyruvate sulfurtransferase; n, number of animals. *$P < 0.05$ compared to the control (dose 0).

of cystathionase in the test group decreased by 16% only after 3 days of thyroxine T_4 administration. The level of MPST activity increased after 1 and 2 doses by 46% and 55%, respectively, but the level determined after the third injection was only 13% higher than that of the controls.

Cyanide-reactive sulfane sulfur. The level of cyanide-reactive sulfane sulfur after T_4 treatment was investigated in the mitochondrial fraction of the mouse liver. In comparison with control levels, the levels of test animals were elevated by about 10% after a dose of 50 μg T_4 per 100 g body wt was given to the mice for 6 days. Higher doses, 100 μg and 250 μg T_4 per 100 g body wt, diminished the level of sulfane sulfur in the investigated fraction by 21% and 22%, respectively (Fig. 3).

The level of sulfate. The level of sulfate in whole liver homogenate of thyroxine-treated mice was 18 % higher than control levels after a dose of 50 μg T_4 per 100 g body wt was administered for 6 days. These levels increased 28% or 24%, respectively, in the case of the animals that received a dose of 100 μg and 250 μg T_4 per 100 g body wt (Fig. 4).

The level of ATP. The administration of 50 μg, 100 μg, and 250 μg T_4 per 100 g body wt to mice for 6 consecutive days caused the elevations of ATP levels by 3%, 13%, and 28% respectively, in the whole liver homogenate (Fig. 4).

Discussion

In the early work of Chatagner and Jolles-Bergeret (19) studied the effect of T_4 on pyridoxal phosphate-dependent enzymes, they indicated a decreased cystathionase activity in normal animals, but only when adminis-

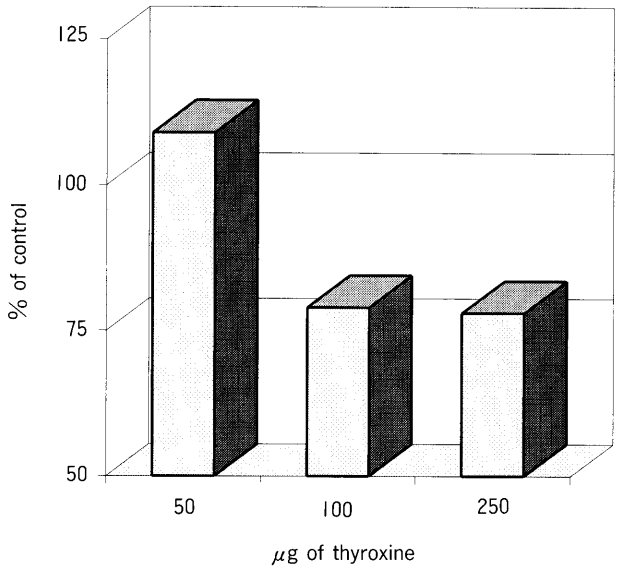

Fig. 3 Sulfane sulfur level after L-thyroxine administration for 6 successive days.
Sulfane sulfur level was determined in the mitochondrial fraction of liver homogenate and its average control value was 399 ± 55 nmol/g of fresh liver.

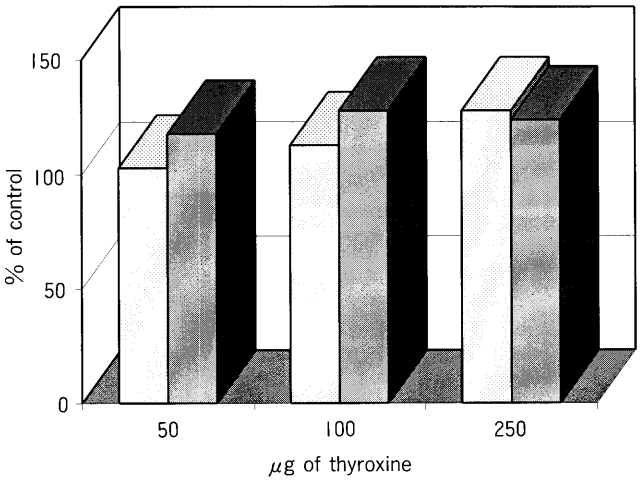

Fig. 4 ATP and sulfate levels after L-thyroxine administration for 6 successive days.
ATP and sulfate levels were determined in the whole liver homogenate. Their average control values were 3,170 ± 100 and 43.8 ± 7.1 nmol/g of fresh liver, respectively.
□, ATP; ▨, sulfate

tered doses were higher than physiological ones. In the present experiments, 3 doses of T_4 were used (50 μg, 100 μg, and 250 μg per 100 g of body wt) and it was noticed that even at the 50 μg dose, T_4 was effective in changing all the investigated enzymatic activities, as well as sulfane sulfur, ATP, and sulfate levels. It should be kept in mind that we used high doses of T_4 in experiments with normal rats, for we observed no variations in enzyme activities by subcutaneous injection of 25 μg T_4 per 100 g of body wt.

MPST activity increased in both fractions of liver homogenate following a single or 2 doses of 100 μg T_4 per 100 g of body wt. (Fig. 2). This enzyme activity also increased in the cytosolic fraction (Table 2). The level of sulfane sulfur compounds in the mitochondrial fraction (Fig. 3) also increased when a lower dose (50 μg T_4 per 100 g of body wt) was administered to mice for 6 days. These findings, when taken together, suggest that lower T_4 doses stimulate cysteine desulfuration through transamination to 3-mercaptopyruvate and the MPST reaction, and lead to the formation of sulfane sulfur (Fig. 1).

After 6 days of T_4 administration at a dose of 100 μg per 100 g of body wt, the activity of rhodanese in the mitochondrial fraction decreased by 40% ($P < 0.05$) and that of MPST decreased by 34% ($P < 0.05$), in comparison to the control levels (Table 1). In the cytosolic fraction, the activity of MPST was unaltered and the cystathionase activity showed a 15% lower ($P < 0.05$) level than that of the controls (Table 2). These results may suggest that a dose of 100 μg T_4 per 100 g of body wt, when administered for 6 days, results in a decrease of cystathionase activity in the cytosolic fraction of liver cells, which may lead to a drop in cysteine levels and in the levels of compounds which contain sulfane sulfur. The decrease of cystathionase activity level may affect a drop in MPST and rhodanese activities (Fig. 1), and such changes may result in a decreased level of sulfane sulfur observed in the mitochondrial fraction of liver homogenate after 6 days of T_4 administration at the dose of 100 μg T_4 per 100 g of body wt (Fig. 3). The transport of endogenous sulfur from the cytosol to the mitochondria is present *in vivo*, but its mechanism remains unclear (18). Simultaneous increase of sulfate, the final product of the oxidative pathway of L-cysteine metabolism, and ATP levels observed in liver homogenate after 6 days of T_4 administration (100 μg T_4 per 100 g of body wt) (Fig. 4), when taken together, indicates an increase of oxidative metabolism in the tissues and oxidative cysteine metabo-

lism.

Cystathionase is considerably inhibited by T_4 administration to the rat (8). It was also found that in the liver of vitamin B_6-deficient rats most cystathionase exists as an inactive apoenzyme, although the concentrations of the immunoreactive enzyme protein were virtually the same for controls and vitamin B_6-deficient livers (20). It is possible that the inhibition by T_4 is due to a Schiff-base formation between the amino group of the hormone and the formyl group of the coenzyme (8). It seems that the observed drop of cystathionase activity after 6 days of T_4 administration at a dose of 100 μg and 250 μg per 100 g of body wt is dose-dependent (Table 2); thus the decrease in cystathionase activity may be a direct effect of T_4 on the enzyme. The changes in MPST and rhodanese activities do not show any dependency in relation to the T_4 dose administered.

Thus, it can be supposed that the desulfuration and transsulfuration pathways of L-cysteine metabolism in the T_4-treated mouse liver are diminished when the dose of T_4 given to the mouse increases, which is probably accompanied by an increase in the oxidative L-cysteine metabolism. A shorter duration of administration of T_4 at a dose of 100 μg per 100 g of body wt as used in experiment B, and the use of a lower dosage (*i.e.*, 50 μg T_4 per 100 g of body wt) for 6 days in experiment A both had a stimulatory effect upon the MPST activity level. Such an effect was not observed in association with either rhodanese or cystathionase. The mechanism of this difference is unclear at present, but it seems to be related to the stimulating effect of T_4 administration on DNA-dependent RNA polymerase activity (21). Further studies are needed in order to elucidate the mechanism of T_4 action on cysteine metabolism.

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