METABOLISM OF L-THYROXINE BY MITOCHONDRIA OF VARIOUS RAT TISSUES IN THE PRESENCE OF PYRIDOXAL PHOSPHATE AND α -KETOGLUTARATE, DIPHOSPHOPYRIDINE NUCLEOTIDE OR FLAVIN MONONUCLEOTIDE

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The authors have been engaged in a series of *in vitro* studies on the deaminative degradation of L-thyroxine (T_4), and demonstrated the presence of the T_4 -transaminase employing rat kidney homogenate⁹⁾ and mitochondria¹¹⁾, to which were added pyridoxal phosphate and some amino group acceptors as the enzyme cofactors. On the other hand, a DPN-linked enzyme was reported by ALBRIGHT and his collaborators to metabolize T_4 ⁸⁾ and triiodothyronine (T_3)¹⁾. This enzyme was obtained in a soluble state from rat kidney mitochondria. Our recent work indicated that the DPN-linked enzyme is less active than the transaminase in deaminating T_4 , when kidney mitochondria are used as enzyme sources¹¹⁾.

L-amino acid oxidase was regarded by many workers as the main enzyme to deaminate thyroid hormones. As far as the authors are aware, however, this has never been confirmed in experiments with mammalian tissues, although such indications were given by NAKANO *et al.*²⁾, who have shown that L-amino acid oxidase derived from snake venom actively metabolizes T_4 and T_3 .

The present investigation was undertaken in order to obtain information concerning the distribution of these three enzymes among mitochondrial fractions of rat tissues. The results have shown that the transaminase and the DPN-linked enzyme are markedly active only in the kidney. Regarding L-amino acid oxidase, the attempt to detect the enzyme activity was not successful. However, during this study it was found that flavin mononucleotide (FMN), the coenzyme of L-amino acid oxidase, enhances the deiodination of T_4 by kidney or liver mitochondria; the distribution of this deiodinase being described below.

The results have been included in part in a paper read in the First Asia and Oceania Regional Congress of Endocrinology in Kyoto, Nov. 1, 1959.

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MATERIALS AND METHODS

Mitochondrial fractions, isolated from the kidney, liver, heart, femoral muscles and the whole brain of adult male Wistar rats, were used as enzyme sources. The isolation procedures, the method of preparation of radioactive T_4 and the analytical procedures of the reaction mixtures for the radioactive metabolites were essentially the same as described previously¹¹). The activity of the transaminase and DPN-linked enzyme was estimated on the basis of the conversion of T_4 to TA_4 , and that of the FMN-linked deiodinase on the basis of the liberation of inorganic iodide from T_4 . In these experiments, α -ketoglutarate was applied in a final concentration of 10^{-2} M and coenzymes, pyridoxal phosphate, DPN and FMN, in 10^{-4} M respectively. Pyridoxal phosphate was a gift from Dr. K. OOIKE, Wakamoto Drug Lab. The other three cofactors were obtained from commercial sources.

These experiments were carried out from September to November.

RESULTS

Table 1 is a summary of representative data obtained from one experiment, in which mitochondria derived from the same rat organs were employed. Table 1,

TABLE 1
Metabolites of T_4 Formed by Three Enzymes of Rat Tissue Mitochondria

Enzyme cofactors added	Tissues	Percent distribution of radioactivity among					TA_4/T_4
		T_4	TA_4	I ⁻	O	Residual	
A Pyridoxal phosphate, α -Ketoglutarate	Control*	74	5	10	2	9	0.07
	Kidney	36	28	12	17	8	0.78
	Liver	60	14	9	7	10	0.23
	Heart	59	11	10	8	12	0.19
	Skeletal muscle	65	12	11	4	8	0.18
	Brain	63	10	9	5	13	0.16
B DPN	Control*	71	6	7	3	13	0.08
	Kidney	53	14	8	11	14	0.26
	Liver	62	10	9	7	12	0.16
	Heart	62	12	7	10	9	0.19
	Skeletal muscle	66	11	6	4	13	0.17
	Brain	61	11	9	5	14	0.18
C FMN	Control*	61	6	12	6	15	—
	Kidney	25	9	20	24	22	—
	Liver	25	6	26	26	17	—
	Heart	49	8	14	12	17	—
	Skeletal muscle	47	11	12	16	14	—
	Brain	52	10	12	11	15	—

* T_4 was incubated only with respective cofactors, without addition of tissue mitochondria.

A and FIG. 1 show the distribution of T_4 -transaminase. The amount of TA_4 formed and T_4 consumed was the greatest in the case of kidney mitochondria. The ratio TA_4/T_4 , which was calculated in order to show roughly the degree of deamination and was listed in Table 1, was also the greatest in this case. Liver mitochondria were much less active, though in a few of the other experiments was an activity comparable to that of kidney observed. The other tissues exhibited only a little activity in all experiments.

FIG. 1. Formation of TA_4 from T_4 by mitochondrial T_4 -transaminase of various rat tissues. T_4 : L-thyroxine, TA_4 : tetraiodothyroacetic acid, I $^-$: inorganic iodide, O: origin. (Butanol: Acetic acid: H_2O).

TABLE 2

Tissues	Nitrogen content (mg)
Kidney	0.499
Liver	0.647
Heart	0.252
Skeletal muscle	0.166
Brain	0.664

FIG. 2. Formation of TA_4 from T_4 by mitochondrial DPN-linked deaminase of various rat tissues.

FIG. 3. Liberation of inorganic iodide from T_4 by mitochondrial FMN-linked deiodinase of various rat tissues.

These comparisons were made on the basis of equal amounts of fresh tissue, *i.e.*, 400 mg. of wet weight. Although the nitrogen content of mitochondrial fractions isolated from this amount of respective tissues differed considerably

(Table 2), the differences in transaminase activity of the tissues cannot be ascribed to the nitrogen content, because diluted kidney mitochondria show yet a considerable activity as described before¹¹).

The distribution of the DPN-linked enzyme is shown in Table 1, B and FIG. 2. The enzyme was fairly active in kidney mitochondria, but the amount of TA_4 produced and the ratio TA_4/T_4 were much less than those in the case of T_4 -transaminase described above. All tissues except for the kidney showed much less activity.

The results shown in Table 1, C and FIG. 3 indicate the following. FMN caused no marked accumulation of TA_4 in all cases of tissues. But, unexpectedly, FMN resulted in a significant deiodination of T_4 when kidney and liver mitochondria were employed as enzyme sources. The liberation of inorganic iodide and the diminution of T_4 were conspicuous. At the same time, a significant amount of radioactivity appeared at the filter paper origin, indicating that the radioactive iodide released from $I^{131}-T_4$ is bound by mitochondrial protein. In the case of a control, in which T_4 was incubated only with FMN, deiodination was not apparent. With mitochondria of the heart, skeletal muscle and brain, no marked deiodination took place.

DISCUSSION

The results obtained above show the activity of three different enzymes connected with respective cofactors, because neither mitochondria from various tissues nor each enzyme cofactor alone yielded any appreciable amount of TA_4 and iodide.

Two deaminative enzymes, the transaminase and DPN-linked enzyme, were especially active in kidney mitochondria, and much less active in mitochondria of all the other tissues, the liver, heart, skeletal muscle and brain. ALBRIGHT *et al.*¹⁾ reported a similar distribution of the DPN-linked enzyme extracted from mitochondria. The DPN-linked enzyme of the kidney was far less active than the kidney T_4 -transaminase in deaminating T_4 , as reported before¹¹).

Though the attempt to detect L-amino acid oxidase was unsuccessful, the experiment made clear that there is present in the kidney and liver a potent mitochondrial deiodinase which is linked with FMN. The liver enzyme appeared to be more active. Whether the L-amino acid oxidase acting upon T_4 is actually absent in these tissues or the enzyme activity is only undetectable on account of the rapid deiodination of both T_4 and TA_4 , is unknown under the present experimental conditions. The deiodinase was only slightly active in the cases of mitochondria of the heart, skeletal muscle and brain. FMN alone did not appear to cause any notable deiodination. These facts indicate that the deiodination is caused enzymatically.

The deiodination of thyroid hormones has been investigated by many workers both *in vivo* and *in vitro* employing tissue slices; earlier work in this field has been reviewed recently^{3,4,10}). However, up to the present time only a few reports concerning deiodinase of thyroid hormones have appeared and, moreover, there is no agreement in these reports. In the liver, the enzyme was found mainly within the mitochondrial fraction¹²). The enzyme activity in the skeletal muscle⁵) and brain⁷) was also studied and it was observed that the enzyme was chiefly located in the soluble cytoplasmic fraction⁶). All these experiments were carried out without adding any enzyme cofactors to the tissue preparations.

The FMN-linked mitochondrial deiodinase described here is interesting because it appears very potent and this type of enzyme has apparently never been demonstrated. More detailed investigation is now in progress and will be reported in the near future.

The fact demonstrated in the present study, that T_4 is mainly deaminated by kidney mitochondria and mainly deiodinated by liver mitochondria, is in exact accordance with the well-known fact that thyroid hormones are metabolized most actively in these two organs *in vivo*.

SUMMARY

Employing mitochondria of various rat tissues as enzyme sources, and pyridoxal phosphate, DPN and FMN as the coenzymes, a study was made of the distribution of three types of enzymes among the kidney, liver, heart, skeletal muscle and brain. These enzymes are transaminase, DPN-linked deaminase and FMN-linked deiodinase acting respectively upon thyroxine.

The former two deaminases were found to be the most active in the kidney, and of these two the transaminase was far more active than the DPN-linked enzyme.

The FMN-linked deiodinase, which appears to be a new type of enzyme of mitochondrial origin, was the most active in the liver.

The specific feature of such distribution is in accord with the fact that thyroid hormones are deaminated mainly in the kidney and deiodinated in the liver *in vivo*.

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