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Thiamine and Thiamine Diphosphate in Liver and Brain from Rats Treated with CCl_4

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The amount of thiamine and thiamine diphosphate in liver and brain of rats and their distribution in liver cytoplasm fractions has been studied.

The supernatant (including the microsomal fraction) contains nearly twice as much thiamine diphosphate as the mitochondria, while thiamine is present almost exclusively in the supernatant.

In rat livers with fatty infiltration, obtained by injections of CCl_4, the amount of thiamine diphosphate is slightly reduced. The decrease happens mainly in the mitochondria. An increased decomposition of thiamine diphosphate is found in fatty infiltrated liver homogenates as compared with normal ones.

No significant difference is found in thiamine diphosphate concentration between brains from normal and CCl_4-treated rats. The amount of thiamine is very low in either case.

Fatty infiltration of the liver is easily obtained in rats by injections of CCl_4 or white phosphorus, or by feeding them with a diet deficient in choline. Fatty liver may be obtained also by giving rats alcohol for a long time. In rats suffering from fatty liver caused by CCl_4 or white phosphorus, or by feeding on a choline deficient diet the liver mitochondria are morphologically changed in the same way as when treated with hypotonic medium [1]. They also contain less than normal amounts of thiamine diphosphate [2], cytochrome c, pyridine nucleotides, and adenylnucleotides [3-5].

In the present communication the content of thiamine diphosphate and thiamine in liver and brain are analysed and evidence is put forward for an increased decomposition of thiamine diphosphate * in fatty liver produced by CCl_4 treatment as compared with normal conditions.

* Abbreviations used: TDP thiamine diphosphate, T thiamine, P_i inorganic phosphate.

EXPERIMENTAL

Male rats from the same litter and weighing about 200 g were used for the experiments. In each experiment two rats were used parallelly, one with fatty infiltration obtained by CCl_4 treatment and the other a control rat. Fatty infiltration was obtained by daily intraperitoneal injection of CCl_4 (0.2 ml of a 20 % solution in olive oil).

The rats were killed by decapitation, the livers immediately removed and chilled on ice, weighed, minced with a pair of scissors in cold isotonic sucrose, washed twice, and homogenized in a Potter-Elvehjem homogenizer in five volumes of cold 0.25 M sucrose. The homogenisations of the tissues from all animals were performed as uniform as possible, *i.e.* the same homogenizer was used, the pistel was moved with the same rapidity, and the time was always the same. From the homogenate samples were taken for incubation with TD^{32}P or T and $^{32}P_i$ in order to study the decomposition and synthesis of TDP as described elsewhere [6]. From the remaining part of the homogenate mitochondria and supernatant (soluble and microsomal fraction being taken together) were prepared.

Free thiamine was determined as thiochrome according to Hjarde [7], TDP manometrically as described by Westenbrink and Steyn-Parvé [8], and with the thiochrome method as the difference between total and free thiamine. TDP isolated by paper chromatography (*iso*butyric acid—versene—NH_3 [9] followed by propanol — HAc—H_2O [10]) was used as standard in the manometric method. Generally the values for TDP obtained with the thiochrome method were slightly lower than with the manometric method. In the tables the values for TDP obtained with the latter method are given. For determination of protein the Biuret method of Cleland and Slater [11] was used.

RESULTS

Table 1 gives figures about the content and distribution of TDP and T in the liver cells from normal and fatty infiltrated rat livers. The figures are mean values from six experiments, all determinations having been performed in duplicate. To avoid leakage of T and TDP from mitochondria to supernatant during the preparation of the cell fractions the tissues were homogenized very cautiously in a Potter-Elvehjem homogenizer. Our figures for the TDP concentration in mitochondria and supernatant are therefore very low as compared with those for liver given by Goethart [12] and Dianzani and Dianzani Mor [2]. In our experiments about 50 % of the TDP was recovered in the fraction removed by low speed centrifugation, and which contains mainly intact liver cells, blood corpuscles, and nuclei.

When, however, nuclei from rat liver were isolated according to Logan, Ficq, and Errera [13], the content of TDP in this purified fraction was found to be only 0.03 μg TDP per gram of normal liver.

Thus our figures in Table 1 for TDP and T in mitochondria and supernatant (soluble and microsome fraction) only intend to supply an idea about the relative distribution of the two compounds in the cell fractions, and to show the ratio between them. The latter agrees with that obtained by Goethart and Dianzani and Dianzani Mor, *i.e.*, in normal liver the TDP content of mitochondria is about half that of the supernatant. In fatty infiltered liver the mitochondrial TDP is reduced.

Compared with TDP the amount of T in the liver cells is low (Table 1), and is found mainly in the supernatant. In fatty infiltrated liver T is reduced, but since also TDP is reduced, the ratio between T and TDP is nevertheless the same as in normal liver.

Table 1. Content and distribution of thiamine and thiamine diphosphate in normal and fatty infiltered rat liver (T and TDP are mean values from 6 expts., total lipids from 4).

		TDP		T		$\frac{T}{TDP}$	Total lipids mg/g wet weight
		μg/g liver	μg/10 mg prot.	μg/g liver	μg/10 mg prot.		
Normal liver	Homogenate	8.96 ±0.55	0.38 ±0.03	0.59 ±0.01	0.026 ±0.001	$\frac{1}{16}$	41
	Supernatant	3.08 ±0.23	0.23 ±0.02	0.51 ±0.04	0.049 ±0.02	$\frac{1}{6.2}$	
	Mito-chondria	1.64 ±0.18	0.76 ±0.05	0.03 ±0.007	0.012 ±0.001	$\frac{1}{63}$	
Fatty liver	Homogenate	7.17 ±0.34	0.36 ±0.02	0.36 ±0.01	0.019 ±0.002	$\frac{1}{20}$	77
	Supernatant	2.43 ±0.31	0.27 ±0.02	0.38 ±0.03	0.044 ±0.004	$\frac{1}{6.8}$	
	Mito-chondria	0.98 ±0.16	0.48 ±0.06	0.03 ±0.005	0.012 ±0.002	$\frac{1}{42}$	

Table 2 gives figures for the amount of TDP and T in the brain from normal and CCl_4-treated animals. With regard to TDP no obvious differences are found. The amount of T in normal brain is only 1/20 of that in liver, and is still lower in CCl_4-treated animals.

The decomposition of TDP in homogenates from normal and fatty infiltrated rat livers has been investigated by means of radioactive TDP added to the homogenates. The amount of TDP decomposed after 60 min at 37°C is signi-

Table 2. Thiamine and thiamine diphosphate in brain from normal and CCl_4 treated rats (mean value from 6 expts.).

	TDP		T		$\frac{T}{TDP}$
	μg/g liver	μg/10 mg protein	μg/g liver	μg/10 mg protein	
Normal rat	3.68 ± 0.36	0.23 ± 0.04	0.03	0.0025	$\frac{1}{77}$
CCl_4 treated rat	3.61 ± 0.22	0.27 ± 0.02	<0.03	<0.0025	$< \frac{1}{77}$

Table 3. Synthesis and decomposition of thiamine diphosphate in homogenates from normal and fatty infiltered rat livers. Homogenate from 1/5 of a gram of liver has been used. (The values represent the mean of 6 expts.)

	Synthesis (per cent of added ^{32}P)	Decomposition (per cent of added $TD^{32}P$)
Normal liver	2.06 ± 0.25	5.92 ± 0.55
Fatty liver	2.25 ± 0.30	17.14 ± 1.61

ficantly higher in fatty infiltrated liver homogenates than in normal ones (Table 3). The same table shows that the ability to synthesize TDP from added T is uninfluenced by CCl_4 treatment.

DISCUSSION

The figures for TDP in rat liver homogenates (Table 1) are rather low as compared with those given by Goethart [12] and by Dianzani and Dianzani Mor [2], but are more in agreement with those obtained by Siliprandi and Siliprandi [14] and by Ochoa [15]. Possibly the differences can be ascribed to different rat strains.

The total amount of TDP in fatty infiltrated rat livers, produced by injections of CCl_4, is only slightly less than in normal livers (Dianzani and Dianzani Mor [2] and Table 1 in the present paper). The decrease is mainly localized to the mitochondria. Christie and Judah [16], in a study of the mechanism of action of CCl_4, advanced the opinion that the primary locus of action were the membranes of the liver mitochondria. This action is, according to Dianzani and Dianzani Mor, comparable with the displacement of TDP from swollen mitochondria as a consequence of hypotonic treatment.

However, no corresponding increase of the TDP concentration in the supernatant occurs (Table 1). Our results even show a slight decrease also in the supernatant from fatty liver (calculated as μg TDP/g liver).

Table 3 shows that in liver homogenates from CCl_4-treated rats, as compared with normal ones, the decomposition of TDP is increased. This increase of the phosphatase acting on TDP may explain the decrease of TDP in the supernatant and in the mitochondria. In this connection it may be of interest to point to an increased mitochondrial ATP-ase activity observed recently by Recknagel and Anthony [17] in CCl_4-treated rat liver.

On the other hand, an increased decomposition of TDP to thiamine, should result in an increased thiamine level. This is, however, not the case (Table 1). This can be explained by the assumption that tissues are capable of retaining only a limited amount of free thiamine. Thus when the amount is enhanced by increased dephosphorylation of TDP, the excess of thiamine is eliminated by excretion or decomposition.

As CCl_4 is thought to be a hepatotoxin only, no differences in TDP should occur in tissues other than liver. The only tissue, apart from liver, studied by us is brain, where CCl_4 is found to be without effect on the TDP level.

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REFERENCES

1. Dianzani, M. U. *Giorn. biochim.* **2** (1953) 180.
2. Dianzani, M. U. and Dianzani Mor, M. A. *Biochim. et Biophys. Acta* **24** (1957) 564.
3. Dianzani, M. U. and Viti, I. *Biochem. J.* **59** (1955) 141.
4. Dianzani, M. U. *Biochim. et Biophys. Acta* **17** (1955) 391.
5. Dianzani, M. U. *Biochem. J.* **65** (1957) 116.
6. Kiessling, K.-H. *Biochim. et Biophys. Acta.* **39** (1960) 412.
7. Hjarde, W. *Nogle undersøgelser over B_1-vitaminet in vitro og in vivo.* Diss. Copenhagen 1950.
8. Westenbrink, H. G. K. and Steyn-Parvé, E. P. *Intern. Rev. Vitamin Research 21* (1950) 4.
9. Kiessling, K.-H. *Acta Chem. Scand.* **10** (1956) 1356.
10. Siliprandi, D. and Siliprandi, N. *Biochim. et Biophys. Acta* **14** (1954) 52.
11. Cleland, K. W. and Slater, E. C. *Biochem. J.* **53** (1953) 547.
12. Goethart, G. *Biochim. et Biophys. Acta* **4** (1952) 479.
13. Logan, R., Ficq, A. and Errera, M. *Biochim. et Biophys. Acta* **31** (1959) 402.
14. Siliprandi, D. and Siliprandi, N. *Nature* **168** (1951) 422.
15. Ochoa, S. and Peters, R. A. *Biochem. J.* **32** (1938) 1501.
16. Christie, G. S. and Judah, J. D. *Proc. Roy. Soc. London,* **B 142** (1954) 241.
17. Recknagel, R. O. and Anthony, D. D. *J. Biol. Chem.* **234** (1959) 1052.

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