Biochemical Pharmacology, Vol. 27, pp. 2683–2688

006-2952/78/1201-2683$02.00/0

EFFECTS OF ADMINISTRATION OF DI-(2-ETHYLHEXYL)PHTHALATE ON RAT LIVER MITOCHONDRIA

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(*Received* 8 *December* 1977; *accepted* 11 *April* 1978)

Abstract—Rats were maintained on a diet containing di-(2-ethylhexyl)phthalate for 2 weeks. The hepatic contents of CoA, carnitine and their acyl-derivatives markedly increased. The activities of carnitine acetyltransferase and carnitine palmitoyltransferase in the hepatic mitochondria also increased. The β-oxidation of fatty acid in mitochondria was studied, particularly with respect to the transport mechanism of acyl-CoA in mitochondria. The rate of β-oxidation of palmitoylcarnitine by mitochondria was not affected by di-(2-ethylhexyl)phthalate administration. However, the palmitoyl-CoA transport *in vivo* appeared to be controlled by the intracellular concentration of carnitine. The transport of acetylcarnitine was the rate-limiting step in the conversion of acetyl units into ketone bodies or citrate in mitochondria.

The effects of di-(2-ethylhexyl)phthalate (DEHP), a widely used plasticizer, are very similar to those of a hypolipidemic drug, *p*-chlorophenoxyisobutyrate (CPIB). They are hypolipidemic action [1], hepatomegaly [1], peroxisomal proliferation [1], inhibition of gluconeogenesis [2], and enhancement of hepatic fatty acid synthesis [2]. Recently, it was reported that a system for palmitoyl-CoA oxidation is located in the peroxisomes of rat liver and that its activity is increased by the administration of various hypolipidemic reagents [3, 4]. The palmitoyl-CoA oxidizing system in peroxisomes is also induced by DEHP treatment [5]. The increase in activity by hypolipidemic reagents and DEHP appears to be responsible for lowering the plasma triglyceride levels.

CPIB treatment increases the activities of carnitine acyltransferases [6, 7] and the levels of CoA and its thioesters in liver [8]. The present study demonstrates that the administration of DEHP increases the activity of mitochondrial carnitine acetyltransferase (EC 2.3.1.7) and carnitine palmitoyltransferase (EC 2.3.1.23) and the levels of CoA, carnitine, and their acyl-derivatives in rat liver. Fatty acid oxidations in mitochondria and peroxisomes are also described.

MATERIALS AND METHODS

Animals and tissue preparations. Male Wistar rats weighing 200–250 g were used. Rats were fed a powder diet (Oriental Yeast Co., Tokyo) containing DEHP at a level of 0, 1, 2 or 4% (w/w) *ad lib.* The rats were anesthetized with diethylether. The livers were removed and frozen immediately by pressing them between two aluminum blocks precooled in liquid nitrogen for determinations of CoA, carnitine, and their acyl-derivatives. Unfrozen livers were homogenized in 4 vol. of 0.25 M sucrose containing 1 mM EDTA in a Potter–Elvehjem homogenizer with a tight-fitting Teflon pestle. The homogenate was fractionated according to the method of de Duve *et al.* [9]. The mitochondrial fraction was subjected to sucrose density gradient centrifugation for the separation of mitochondria from peroxisomes.

Assays of metabolites. CoA and its thioesters were assayed as described previously [8]. Carnitine, acetylcarnitine, and long-chain acylcarnitine were assayed spectrophotometrically [10]. When total CoA and total carnitine in tissues were determined, the samples were hydrolyzed under alkaline conditions Acetoacetate and 3-hydroxybutyrate were determined by the method of Williamson *et al.* [11]. Citrate was determined by the enzymatic method [12]. Protein was assayed by the method of Lowry *et al.* [13].

Assays of enzymes. The activities of carnitine acyltransferases were determined by measuring the rate of increase in absorbance at 232 nm in the direction of acetyl-CoA formation [14]. Each homogenate and each subcellular fraction were diluted with an equal volume of 10 mM potassium phosphate, pH 7.0, containing 1% Triton X-100 and then kept in ice for 10–30 min before use. The reaction mixture contained 100 mM Tris–Cl, pH 8.0, 0.25 mM EDTA, 0.2 mM CoA, 0.5 mM dithiothreitol and 1 mM acetylcarnitine or palmitoylcarnitine. The temperature was 25°.

Glutamate dehydrogenase (EC 1.4.1.3) [15], citrate synthase (EC 4.1.3.7) [16], 2-oxoglutarate dehydrogenase complex [17], succinate dehydrogenase (EC 1.3.99.1) [18], malate dehydrogenase (EC 1.1.1.37) [19], 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) [20], α-glycerophosphate dehydrogenase (EC 1.1.99.5) [21], urate oxidase (EC 1.7.3.3) [22] and lactate dehydrogenase (EC 1.1.1.27) [23] were assayed according to published procedures.

Reagents. The DEHP used was of analytical grade from Kanto Chemical Co., Tokyo. *l*-Carnitine, acetylcarnitine and palmitoylcarnitine were gifts from Otsuka Pharmaceutical Co., Ltd., Osaka.

CoA was purchased from Kyowa Hakko Kogyo Co., Tokyo. Dithiothreitol, ATP, ADP, NAD^+, NADH, citrate synthase, malate dehydrogenase, phosphotransacetylase (EC 2.3.1.8), 3-hydroxybutyrate dehydrogenase, citrate lyase (EC 4.1.3.6) and lactate dehydrogenase were from Boehringer Corp., Ltd. (Tokyo). Fatty acid-free bovine serum albumin was from Sigma Chemical Co., St. Louis, MO. [U-^{14}C]Palmitic acid (928 mCi/m-mole) was from the Radiochemical Centre, Amersham, England.

Preparations. Acetyl-CoA was synthesized from CoA and acetic anhydride by the method of Simon and Shemin [24]. An acetate-free preparation of acetyl-CoA was obtained by precipitation as the lithium salt. [U-^{14}C]Palmitoyl-CoA was prepared according to the method of Kornberg and Pricer [25], using the reaction mixture described by Bar-Tana *et al.* [26]. When [U-^{14}C]palmitoylcarnitine was prepared, carnitine (5 mM) and washed mitochondria (frozen mitochondria were washed twice with 0.4 M KCl; 0.5 mg protein/ml of reaction mixture) were added to the system for the preparation of [U-^{14}C]palmitoyl-CoA. The reaction volume was 2 ml. The reaction was stopped by the addition of an equal volume of 1 N $HClO_4$. The precipitated protein was washed three times with 4 ml of 0.1 N $HClO_4$ and suspended in 2 ml water. The suspension was extracted three times, using 2 ml of petroleum ether each time. The aqueous phase was neutralized by the addition of 1 M K_2CO_3 and extracted twice with 2 ml n-butyl alcohol. The alcohol layer was dried at 70° by bubbling with N_2. The residue was dissolved in a small amount of chloroform and chromatographed on a silicic acid thin-layer with chloroform–methanol–acetic acid–H_2O (50:25:7:3). The palmitoylcarnitine fraction was eluted with methanol and dried. The residue was dissolved in water. The yield was approximately 20 per cent, based on radioactivity. The preparation contained some phospholipid. The 2-oxoglutarate dehydrogenase complex was purified from pigeon breast muscle [17]. The residue of the muscle extract was used for the preparation of carnitine acetyltransferase. The crystalline preparation was obtained by the method of Chase *et al.* [14] without acetone fractionation and Sephadex G-100 gel filtration.

Measurement of β-oxidation. The basic assay medium consisted of 130 mM KCl, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.2, 0.1 mM EDTA. 1 mM P_i, 5 mM malonate, 1 mM dithiothreitol and 0.15% fatty acid-free bovine serum albumin. The following components were added when necessary: 1 mM carnitine, 0.1 mM CoA and 0.2 mM NAD^+. When measuring β-oxidation in the liver homogenate, 50 μM [U-^{14}C]palmitate (specific radioactivity, 1050 cpm/nmole), 5 mM ATP and 10 mM $MgCl_2$ were added. When particle fractions were used, 1 mM ADP and 30 μM [U-^{14}C]palmitoyl-CoA (268 cpm/nmole) or 30 μM [U-^{14}C]palmitoylcarnitine (765 cmp/nmole) were added. The final volume was 0.2 ml. The mixture was incubated at 25° for 5 min and the reaction was stopped by the addition of 0.2 ml of ice-cold 0.6 N $HClO_4$. After centrifugation, 0.2 ml of the supernatant fraction was used to measure the radioactivity. In typical experiments, 20–30 per cent of the total radioactivity was recovered in the supernatant fraction, depending on the amount of homogenate or particles added. The reaction proceeded linearly with time up to about 50 per cent conversion.

Formation of ketone bodies from acetyl-CoA by mitochondria. Formation of ketone bodies from acetyl-CoA was measured according to the method of Lee and Fritz [27].

RESULTS

Hepatic contents of CoA, carnitine, and their acyl-derivatives. Table 1 shows that the levels of these metabolites increased in the livers of rats which received a diet containing 2% DEHP for 2 weeks. CoA and carnitine increased 5- to 6-fold, their acetyl-derivatives about 4-fold, and long-chain acyl-derivatives 1.5-fold. The contents of these compounds in brain, kidney, heart and skeletal muscle (gastrocnemius muscle) were not changed by DEHP treatment (data not shown).

CoA and its derivatives were localized mainly in the mitochondrial and supernatant fractions. In contrast, carnitines were found mostly in the supernatant fraction (data not shown). Their distribution patterns were not affected by DEHP treatment. Although peroxisomes proliferated markedly in the liver after DEHP treatment, as reported by Reddy *et al.* [1], CoA and carnitines were not detected in this fraction.

Carnitine acyltransferases. Table 2 summarizes the effect of DEHP treatment on the enzyme activities and their subcellular distributions. Activities of carnitine acetyltransferase and carnitine palmitoyltransferase were increased 68-fold and 6.7-fold, respectively, after DEHP feeding. The glutamate dehydrogenase activity was not changed. The carnitine acetyltransferase activity of rat liver is very low compared to that of livers from other species or of other tissues [28, 29]. The activity almost reached the high level of activity found in other tissues after DEHP treatment. The activities of the enzyme in brain, kidney, heart and skeletal muscle were not changed by DEHP treatment (data not shown).

The recoveries of the enzymes and proteins ranged from 92 to 110 per cent after fractionation. The values and patterns of distribution of the marker enzymes corresponded to those observed by others [9, 30, 31]. Most of the activities of these carnitine acyltransferases were found in the mitochondrial

Table 1. Effects of DEHP on contents of CoA, carnitine and their acyl-derivatives in liver*

	Control	DEHP
CoA	0.070 ± 0.016	0.442 ± 0.036
Acetyl-CoA	0.040 ± 0.011	0.150 ± 0.019
Long-chain acyl-CoA	0.085 ± 0.002	0.127 ± 0.015
Carnitine	0.222 ± 0.035	1.146 ± 0.141
Acetylcarnitine	0.090 ± 0.021	0.374 ± 0.084
Long-chain acyl-carnitine	0.017 ± 0.005	0.029 ± 0.005

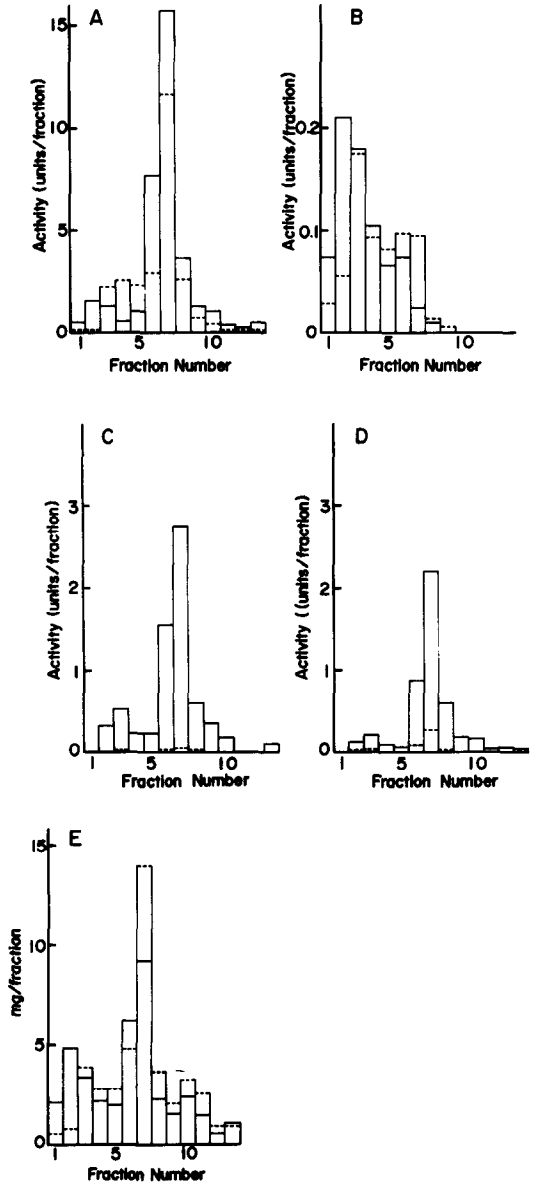
*Rats (N = 4) received the 2% DEHP diet for 2 weeks. Values are expressed as μmoles/g of wet weight ± S.D.

Table 2. Subcellular distribution of carnitine acyltransferase, glutamate dehydrogenase and proteins*

	Treatment†	Activity of Homogenate	Percentage of recovery				
			N	M	P	S	Total
Carnitine acetyltransferase	C	0.53 ± 0.12	15.1	60.4	3.8	17.0	96.3
	D	35.90 ± 0.42	26.2	58.5	2.8	9.2	96.7
Carnitine palmitoyltransferase	C	3.03 ± 0.39	28.7	82.0	1.0	0.0	111.7
	D	20.20 ± 0.86	8.5	72.8	1.9	25.6	108.8
Glutamate dehydrogenase	C	200 ± 15	30.5	56.5	3.5	1.6	92.1
	D	220 ± 20	26.2	63.0	2.0	2.2	93.4
Protein	C	225 ± 8	16.4	31.1	15.8	47.2	110.5
	D	255 ± 14	14.7	38.0	8.9	31.2	92.8

* Rats (N = 4) received the 2% DEHP diet for 2 weeks. Absolute activity values ± S. D. of the homogenate are expressed as μmoles substrate metabolized/g of liver (wet wt)/min; protein is expressed as mg/g of liver. Percentages of recovery were averaged. N is the nuclear fraction; M, the mitochondrial fraction (heavy + light); P, the microsomal fraction; and S, the final supernatant fraction.

† C, control; D, DEHP.



fraction. The distribution of the activities in the heavy and light mitochondrial fractions was examined. The heavy mitochondrial fraction was described as having a higher specific activity of carnitine acetyltransferase and a lower specific activity of carnitine palmitoyltransferase than the light mitochondria [30, 31]. In the present experiment, we found no difference between the enzyme activities of the two mitochondrial fractions on the basis of the glutamate dehydrogenase activity in both groups. The results on protein distribution between two mitochondrial fractions differed from those of some previous experiments [30, 31], but corresponded to those of de Duve *et al.* [9].

To clarify the localization of the carnitine acyltransferases which were induced by DEHP administration, the fluffy layer and the light mitochondrial fraction, which contain most of the peroxisomes, were pooled and subjected to sucrose density gradient centrifugation. As shown in Fig. 1, both of the carnitine acyltransferases were located in the mitochondria, and the increase in activities induced by DEHP treatment is due mostly to the mitochondrial enzymes.

Dose and duration of DEHP administration. Figure 2 summarizes the effects of dose and duration of DEHP treatment. The contents of CoAs and carnitines increased during the first 2 weeks, and the levels were slightly elevated from week 2 to week 4. The activities of carnitine acetyltransferase and carnitine palmitoyltransferase increased progressively up to week 4. The change in the DEHP dose did not affect the results. All of the rats fed the 4% DEHP diet died during a period of 3–4 weeks after the feeding was started.

Rate of β-oxidation. Table 3 summarizes the rates

Fig. 1. Pattern of sucrose denisty gradient centrifugation of rat liver mitochondrial fraction. The light mitochondrial fraction plus the fluffy layer of the heavy mitochondrial fraction from 1 g liver were used in both the control and the DEHP group. A, glutamate dehydrogenase; B, urate oxidase; C, carnitine acetyltransferase; D, carnitine palmitoyltransferase; and E, protein. Control (———), DEHP (———).

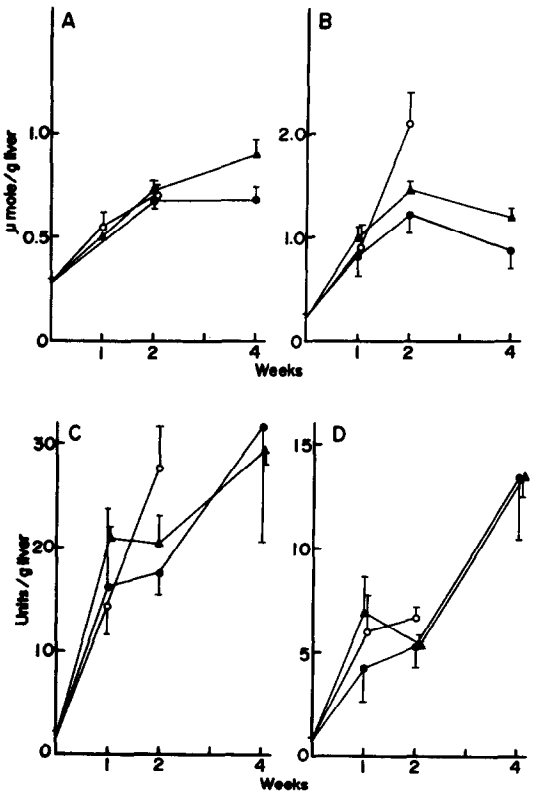


Fig. 2. Effects of DEHP treatment on CoA, carnitine and carnitine acyltransferases. A, total-CoA; B, total carnitine; C, carnitine acetyltransferase; and D, carnitine palmitoyltransferase. Key: 1% (●), 2% (▲) and 4% (○).

of β-oxidation by various enzyme preparations under various conditions. The KCN-sensitive fatty acid oxidation is catalyzed by the mitochondrial enzyme system, whereas the KCN-insensitive one is catalyzed by the peroxisomal system. In this table, therefore, the ratios are expressed as the rate of KCN-sensitive β-oxidation to the activity of the mitochondrial enzyme, glutamate dehydrogenase, and the rate of KCN-insensitive β-oxidation to the activity of the peroxisomal enzyme, urate oxidase. The rate of β-oxidation by homogenate was dependent on carnitine. The higher rate in the DEHP group without the addition of carnitine may be due to the endogenous carnitine. The endogenous carnitine concentrations in the reaction mixture were calculated to be 7 μM in the control and 30 μM in the DEHP respectively. The rates of oxidation of palmitoyl-CoA by the particle fractions was lower than that of palmitate by the homogenates. In a separate experiment, it was found that both mitochondria and peroxisomes oxidized palmitate at the same rate as that of the palmitoyl-CoA oxidation. The rates were 50–65 nmoles/min/g of liver and 10–20 nmoles/min/g of liver respectively. The rate of fatty acid oxidation by mitochondria was not affected by DEHP treatment. The rate of peroxisomes, however, was several times higher. The KCN-sensitive activity of the peroxisomal fraction seems to be due to the contamination by mitochondria, because the ratios of the activity to that of GDH were almost equal in both the mitochondrial and the peroxisomal fractions. For an unknown reason, the contamination of the peroxisomal fraction by mitochondria was more marked in the control than in DEHP group (see

Table 3. Rates of β-oxidation of [U-^{14}C]palmitate and [U-^{14}C]palmitoyl-CoA*

	Conditions				Control			DEHP		
	Carnitine	KCN	CoA	NAD$^+$	nmoles min/g liver	nmoles GDH	nmoles UOX	nmoles min/g liver	nmoles GDH	nmoles UOX
Homogenate	−	−	−	−	25.8			175.2		
	+	−	−	−	237.7			354.3		
	+	−	+	+	219.1	1.06		363.6	1.41	
	+	+	+	+	6.6		1.98	52.6		13.84
Mitochondrial fraction	−	−	−	−	4.7 (69.4)‡			5.1 (44.4)‡		
	+	−	−	−	67.7 (74.5)			57.1 (57.3)		
	+	−	+	+	50.1	0.24		55.1	0.19	
	+	+	+	+	2.1 (0.5)		0.64	13.3 (1.6)		3.5
Mitochondria†	−	−	−	−	4.8 (31.0)			13.1 (50.4)		
	+	−	−	−	57.0 (47.2)			67.6 (60.5)		
	+	−	+	+	57.4	0.28		63.1	0.25	
	+	+	+	+	1.4 (1.6)		0.80	8.1 (5.5)		5.4
Peroxisomes†	−	−	−	−	4.4 (0.0)			0.9 (1.2)		
	+	−	−	−	7.3 (0.0)			2.2 (2.2)		
	+	−	+	+	9.2	0.22		7.6 (2.0)	0.25	
	+	+	+	+	1.2 (0.0)		0.61	6,2 (0.7)		2.7

* Rats (N = 4) received the 2% DEHP diet for 2 weeks. Homogenates were pooled and fractionated. Experimental procedures were as described in Materials and Methods. The values are expressed as nmoles [U-^{14}C]palmitate or [U-^{14}C]-palmitoyl-CoA oxidized/min/g of liver. The rates of KCN-sensitive β-oxidation were divided by units of glutamate dehydrogenase (nmoles/GDH) and those of KCN-insensitive ones by units or urate oxidase (nmoles/UOX).

† These fractions were obtained by the sucrose density gradient centrifugation.

‡ Values in parentheses are nmoles [U-^{14}C]palmitoylcarnitine oxidized/min/g of liver.

Table 4. Rates of ketone body formation in mitochondria*

Substrate	Control		DEHP	
	Ketone body (nmoles/min/g liver)	Citrate	Ketone body (nmoles/min/g liver)	Citrate
Acetyl-CoA (1 mM)	37.4 ± 2.3	7.0 ± 0.6	54.3 ± 10.6	21.0 ± 5.3
Acetyl-CoA (1 mM) + carnitine (1 mM)	37.6 ± 11.8	23.3 ± 7.3	93.9 ± 9.3	19.8 ± 7.3
Acetylcarnitine (1 mM)	41.2 ± 3.6	24.5 ± 0.7	264 ± 33.7	24.0 ± 3.6

* Rats (N = 3) received the 2% DEHP diet for 2 weeks. Experimental procedures were as described in Materials and Methods. The recovery of mitochondria in the fractionation procedures was estimated by the measurement of glutamate dehydrogenase activity. The values are expressed as nmoles of ketone bodies (acetoacetate plus 3-hydroxybutyrate) or citrate/min/g of liver ± S.D. The amount of acetyl-CoA hydrolyzed was about 10 per cent of the total acetyl-CoA during the incubation. The rates of ketogenesis by the disrupted mitochondria were 2.8 μmoles/min/g of liver in the control and 3.9 μmoles/min/g of liver in the DEHP respectively.

Fig. 1). The rate of β-oxidation of [U-^{14}C]palmitoylcarnitine was not dependent on carnitine and was the same as that of [U-^{14}C]palmitoyl-CoA in the presence of 1 mM carnitine.

Rate of ketone body formation from acetyl-CoA. The rate of acetyl-CoA metabolism in mitochondria was determined by measuring the production of ketone bodies (acetoacetate plus 3-hydroxybutrate) and citrate. Table 4 shows no difference in the rate of ketone body formation from acetyl-CoA, acetyl-CoA plus carnitine, and acetylcarnitine in the control. The rate from acetyl-CoA by mitochondria of the DEHP group was 1.5 times higher than that of the control, and the rate was increased 1.7-fold by the addition of carnitine. The ketone body formation from acetylcarnitine was much higher than that from acetyl-CoA plus carnitine. The rates of production of citrate were lower than those of ketone bodies and did not vary among the different experimental conditions except that the rate from acetyl-CoA in the control was lower. In this experiment, the rate of formation of acetylcarnitine was determined. Acetylcarnitine was formed from acetyl-CoA plus carnitine in both groups, but the rate of the formation was much higher in the DEHP group.

DISCUSSION

The hepatic contents of CoA, carnitine and their derivatives were markedly increased after the administration of DEHP to rats. Hormonal and nutritional alterations affect the CoA levels [see Ref. 32]. As far as we know, however, a content as high as that found in this experiment has not been reported. The CoA-synthesizing activity from phosphopantetheine plus ATP and the CoA-hydrolyzing activity of the liver homogenate were not altered by DEHP administration (Y. Shindo, unpublished data). The addition of pantethine in the diets at a level of 0.1 per cent (w/w) did not increase the hepatic contents of CoA in either the control or the DEHP group.

The mechanism of increase in the carnitine content is unknown. The carnitine contents in the liver were increased 2- to 3-fold by starvation [33, 34]. The food intake and weight gain of the rats were depressed markedly by increasing the DEHP content in the diet [2], but the carnitine contents were nearly the same in all the DEHP groups.

Carnitine palmitoyltransferase is located exclusively in mitochondria [31]. There are outer and inner pools of carnitine acyltransferasese in the inner mitochondrial membrane [31], which is impermeable to CoA and fatty acyl-CoA derivatives [35]. The CPIB treatment increased the enzyme activities of the inner pool [6]. Carnitine palmitoyltransferase activity has been reported to increase during fasting [36]. The increases in activity by treatment with CPIB or DEHP may not be due to a shortage of food intake, as discussed above. The activities of the following mitochondrial enzymes were unchanged by DEHP treatment: citrate synthase, 2-oxoglutarate dehydrogenase complex, succinate dehydrogenase, malate dehydrogenase, 3-hydroxybutyrate dehydrogenase, and α-glycerophosphate dehydrogenase (data not shown).

Formation of the acylcarnitines has been proposed as the rate-limiting step in long-chain fatty acid oxidation [37], but Bremer and Norum [38] reported that the rate of palmitoylcarnitine formation exceeded that of palmitoylcarnitine oxidation by several times.

Mitochondria oxidize palmitate as well as palmitoyl-CoA. The rates of oxidation of each substrate were nearly the same. Palmitate oxidation was enhanced by the addition of carnitine as described by Rossi *et al.* [39]. Oxidation of palmitoyl-CoA in mitochondria was dependent on carnitine, and its rate in the presence of 1 mM carnitine reached that of palmitoylcarnitine oxidation. Table 3 suggests that the capacity of mitochondrial β-oxidation of palmitoyl-CoA in the presence of a saturation level of carnitine is not influenced by an increase in the activity of carnitine palmitoyltransferase or by the CoA content in mitochondria.

The increase in fatty acid oxidation and ketogenesis in livers of rats under various hormonal and nutritional conditions was ascribed to an elevation of carnitine content [34, 40]. The addition of carnitine to the medium perfusing rat liver or to the hepatocyte suspension medium accelerated ketogenesis [34, 41]. The reaction mechanism of carnitine palmitoyltransferase is complicated, but the K_m for carnitine

is 2.5×10^{-4} M [42] to 4.5×10^{-4} M [43]. The carnitine content was 0.222 μmole/g of liver in the control and 1.146 μmoles/g of liver in the DEHP group respectively (Table 1). It is, therefore, possible that long-chain fatty acid oxidation and ketogenesis in the liver of the DEHP group are enhanced by the elevation of carnitine content.

The rates of production of ketone bodies and citrate from acetyl-derivatives in the control were not altered by changing the substrates used, whereas those of the DEHP group were varied by the use of different substrates (Table 4). The maximal rate of ketone body formation from acetyl-CoA by the disrupted mitochondria was much higher and not altered after DEHP treatment; therefore, the rate of production of ketone bodies and citrate may be controlled by the rate of transport of acetyl-CoA or acetylcarnitine. The transport system, carnitine acetyltransferase, was enhanced by DEHP treatment. The acetylcarnitine formation from acetyl-CoA plus carnitine was higher in the DEHP group. The transport of the acetyl group seems to be intimately related to the production of acetylcarnitine. Rates of metabolism of acetylcarnitine in the DEHP group, however, were higher. This may be due to the fact that the intramitochondrial acetyl-CoA formation is increased by the elevated activity of the inner pool of the enzyme.

The peroxisomal proliferation is one of the characteristic features of the effects of hypolipidemic drugs and DEHP [1, 44]. It was found that rat liver peroxisomes oxidize palmitoyl-CoA and it was proposed that the hypolipidemic action of various agents is related to the increase in this activity [3–5]. However, in the present experiment, the rate of the peroxisomal β-oxidation was only about one-tenth of that of the mitochondrial rate (see Table 3). This may be due to the presence of a high concentration of albumin in the assay mixture because the peroxisomal β-oxidation is markedly inhibited by albumin (Y. Shindo, unpublished data). To clarify the physiological significance of the peroxisomal β-oxidation system, further studies should be carried out.

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