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INVESTIGATIONS ON THE MECHANISM OF THE HYPOCHOLESTEROLEMIC ACTION OF DIETHYLHEXYL PHTHALATE IN RATS

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Abstract—The plasticizer di(2-ethylhexyl)phthalate (DEHP), used widely in the manufacture of food packages and medical plastic devices, depressed serum cholesterol (40%) and proliferated hepatic mitochondria (100%) when administered in the diet (2%, w/w) to the rat. Microsomes isolated from the livers of animals administered DEHP showed lowered specific activity of 3-hydroxy-3-methyl glutaryl coenzyme A reductase (50%). The incorporation of acetate but not of mevalonate into hepatic cholesterol was decreased (52%) in these animals. The release of bile acids was greatly enhanced (100%), and the activity of cholesterol 7α-hydroxylase, the regulatory enzyme in the pathway of bile acid formation, was stimulated (70%) on DEHP administration. Even though the capacity of mitochondria to oxidize the side chain of cholesterol was not enhanced in DEHP-fed animals, the larger population of mitochondria would ensure that the amount of cholesterol oxidized per gram of liver was significantly higher.

The common plasticizer di(2-ethylhexyl)phthalate (DEHP; also called dioctyl phthalate, DOP; see Fig. 1) is used widely in the manufacture of food packages and medical plastic devices in which the concentration of the chemical may be as high as 60% [1, 2]. Interest in the toxic and carcinogenic potential of the compound was aroused because of its presence ubiquitously in the environment, in stored blood, and in the tissues of patients. Extensive studies have revealed that DEHP is mildly toxic to experimental animals [2] and tends to induce hepatocellular tumors in rodents on chronic administration in the diet [3–5]. The plasticizer has also been claimed to be a tumor promoter [6] and suppressor [7]. However, studies on the disposition and metabolism of the compound in African Green monkey and humans failed to show any toxicity [8].

When administered to experimental animals, DEHP causes profound morphological and biological changes in the liver which accumulates large concentrations of the compound [2]. Mention in this regard may be made of hepatomegaly [4], induction of carnitine acetyltransferase [1, 9–12] and catalase [1, 13], inhibition of mitochondrial respiratory enzymes [9, 10, 14, 15] and catalase [9, 10], and proliferation of peroxisomes [9, 10, 16, 17] as well as of mitochondria [9, 10].

The pivotal role of peroxisome proliferation and induction of carnitine acyltransferase in the hypolipidemic action of clofibrate has been elucidated [18–20]. In agreement with this, DEHP which proliferates peroxisomes and induces carnitine acetyltransferase has been shown to cause decreases in serum triglycerides [1] and increases in peroxisomal β-oxidation [13] when fed to experimental animals.

The report from our laboratory that administration of clofibrate to the rat proliferates hepatic mitochondria [21, 22] has been confirmed [23–25] and extended to many species including humans. In the degradation of cholesterol to bile acids, the cleavage and oxidation of the side chain take place in mitochondria [26–28]. The hypothesis was developed [29] that the hypocholesterolemic action of the drug rested largely on the enhanced destruction of the lipid by mitochondria. Keeping this in view, we have investigated the effect of administration of DEHP to the rat on cholesterol metabolism. The results presented in this paper demonstrate that the plasticizer inhibits the synthesis and hastens the degradation of cholesterol.

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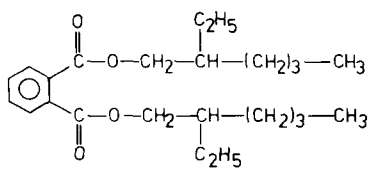

Fig. 1. Structure of diethylhexyl phthalate.

MATERIALS AND METHODS

Animals. Male albino rats (130–150 g) obtained from the Central Animal Facility of this Institute and maintained on a commercial (Hind Lever) diet were used in the studies reported here. The animals were kept in a room with artificial lighting from 9:00 a.m. to 5:00 p.m. daily. Since it is recognized that the presence of DEHP in food materials arises primarily by contamination from polyvinyl containers, the diet was transported and stored in jute bags. Animals

were fed with DEHP (2%, w/w) mixed in the diet powder for the time period indicated. For canulation of the bile duct, larger animals (180 g) were used. To minimize errors due to variation, the same stock of diet was fed in all the experiments, and an equal number of control and experimental animals were killed at a time.

Isolation of mitochondria. Animals were killed by cervical dislocation; livers were removed, blotted free of blood, weighed, and homogenized, and mitochondria were sedimented by differential centrifugation as described earlier [21]. The particles were washed once with 0.25 M sucrose and used with a minimum of delay.

Preparation of supernatant factor. Liver homogenate (10%, w/v) prepared in 100 mM potassium phosphate buffer, pH 7.4, was centrifuged at 12,000 *g* for 10 min and then at 100,000 *g* for 1 hr. The supernatant fraction thus obtained was kept in a waterbath at 95° for 15 min. The precipitated protein was removed by centrifugation, and the clear supernatant fraction was used in the assay of cholesterol oxidation.

Canulation of bile duct. The rats were anesthetized by injection (i.p.) of urethane (0.9 g/kg body wt). The peritoneum was opened, and an incision was made on the bile duct. A polyethylene tubing was introduced through the incision to collect the bile.

Estimation of cholesterol. Blood drawn by cardiac puncture was allowed to clot (1 hr). Cholesterol in the serum and liver was estimated after saponification and extraction into petrol, by the Lieberman–Burchard reaction [30]. The content of cholesterol in bile was estimated by the cholesterol oxidase method [31] which is specific for cholesterol [32].

Estimation of bile acids. The bile (400 μl) was extracted with chloroform–methanol (2:1) and treated with alkali as described by Paul and Ganguly [33]. The bile acids were estimated according to Mosbach *et al.* [34]. Standards (cholic acid and deoxycholic acid) were run simultaneously.

Precursor incorporation. The rate of cholesterol synthesis was followed by determining the incorporation of the radioactive precursor, [1-^{14}C]acetate (10 μCi/rat) or [2-^{14}C]mevalonate (0.5 μCi/rat). The precursor was injected intraperitoneally, and the animals were killed 30 min later. To determine the turnover of cholesterol, rats were injected with radiolabeled acetate and killed at the time intervals indicated. The liver was excised and saponified. The non-sponifiable lipids were extracted into petroleum ether and fractionated on a 5% (v/w) deactivated alumina column [35].

Enzyme assays. For the measurement of 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMGCoA) [mevalonate:NADP oxidoreductase (acylating CoA), EC 1.1.1.34], hepatic microsomes were isolated from rats killed at the peak of the diurnal cycle (midnight), and kept frozen at −15° overnight. The enzyme activity was assayed according to Shapiro *et al.* [36]. The reaction system contained NADPH-regenerating system and 400 μg of microsomal protein in a total volume of 150 μl. After the addition of 50 nmoles of DL-[3-^{14}C]HMGCoA (specific radioactivity 166 nCi/μmole), the reaction was allowed to proceed for 30 min at 37°. After stopping the reaction with 2 M HCl, the mevalonate formed was separated by thin-layer chromatography, and the radioactivity was determined.

The activity of cholesterol 7α-hydroxylase [cholesterol 7α-monooxygenase; cholesterol, NADP:oxygen oxidoreductase (7α-hydroxylating), EC 1.14.13.17] was assayed in liver microsomes isolated at 6:00 p.m. The reaction system contained 1 mg of microsomal protein and 554 nmoles of [4-^{14}C]cholesterol (specific radioactivity 213 pCi/nmole) in a volume of 2.5 ml. The reaction was started by the addition of NADPH-regenerating system and incubated for 1 hr at 37°. After stopping the reaction with ethanol, the cholesterol was extracted into petrol, separated by thin-layer chromatography, and the radioactivity in 7α-hydroxy-cholesterol was determined [37, 38].

The oxidation of cholesterol by liver mitochondria was assayed according to Lee and Whitehouse [28]. The reaction system contained 50 mg (protein) of mitochondria and 20 nmoles of [26-^{14}C]cholesterol (110,000 dpm) in a total reaction volume of 3 ml. The supernatant fraction (0.5 ml) obtained from liver homogenate (boiled) was added as indicated. Oxidation was allowed to proceed for 6 hr at 37° in a Warburg flask. The CO_2 liberated was trapped in 0.2 ml of 1 N NaOH in the central well.

Measurement of radioactivity. Samples of non-saponifiable lipids were dissolved in benzene (0.5 ml) and added to vials containing 10 ml of counting fluid consisting of 0.5% (w/v) 2,5-diphenyl oxazole (PPO) in toluene. Samples of bile (100 μl) were added to 10 ml of aqueous cocktail consisting of 0.5% (w/v) PPO and 0.005% (w/v) 1,4-bis-2-(5-phenyl oxazolyl) benzene (POPOP) in toluene–methanol (1:1, v/v). Radioactive CO_2 liberated from cholesterol by oxidation was trapped on a piece of filter paper soaked in 0.2 ml of 1 N NaOH. The paper was placed in the aqueous cocktail for counting. Radioactivity was measured in an LKB Rack-Beta II liquid scintillation counter. In every set of measurements, the extent of quenching was determined by the use of internal standards. Quench correction (16% in aqueous cocktail) was applied.

Protein was estimated by the biuret method [39], deoxycholate being used for solubilization. All centrifugations were made in a Sorvall-RC-5B refrigerated centrifuge or in a Kontron TGA-50 ultracentrifuge. Operations for the isolation of mitochondria were carried out at 4°. Solutions were prepared in water double-distilled in an all quartz apparatus and adjusted to the desired pH before use.

Chemicals. The sample of DEHP in these experiments was purchased from the Solvents & Chemicals Co., Bangalore, India. Cholesterol oxidase, cholesterol esterase and authentic samples of 7α-hydroxy-cholesterol were gifts from Dr. S. R. Panini, Department of Biological Chemistry, University of Cincinnati Medical Center, Cincinnati, OH, U.S.A. Radioactive cholesterol was purchased from the New England Nuclear Corp., Boston, MA, U.S.A., and acetate and mevalonate were from the Bhabha Atomic Research Centre, Bombay, India. All other biochemicals were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A.

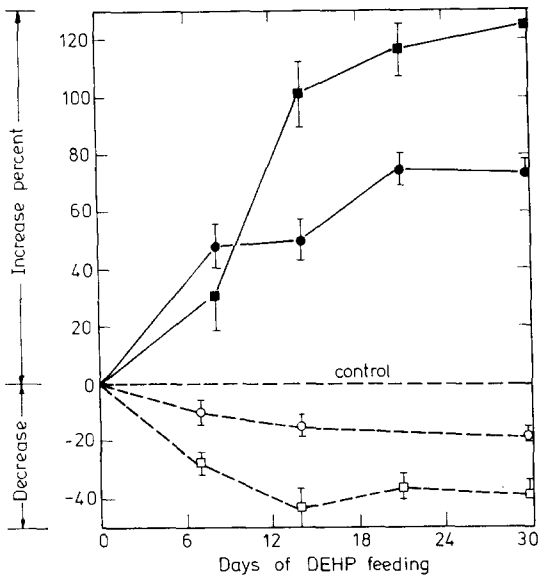

Fig. 2. Effect of administration of DEHP on body and liver weight, mitochondrial content and serum cholesterol of rats. Animals were fed with DEHP (2%, w/w) in the diet for the period indicated. The values represent the increase or decrease of body weight (○—○), serum cholesterol (□—□), liver weight per 100 g body weight (●—●) and liver mitochondria (■—■), taking the *corresponding control* as 100, and are the mean of four independent determinations. Some typical values of standard deviation are indicated. The values for zero time control were: body weight, 135 ± 21 g; liver weight/100 g body weight, 4.0 ± 0.5; serum cholesterol, 74 ± 1 mg/100 ml; and mitochondrial protein content, 36 ± 6 mg/g liver. Except in the case of body weight, the values for control animals did not vary by more than 10% at different time intervals. Therefore, those values are not indicated.

RESULTS

Hepatic mitochondria and serum cholesterol. The gain in body weight of rats administered DEHP was about 10–20% lower than that of control animals (Fig. 2). The difference in body weight, however, was not statistically significant. The slight growth-retarding effect of the chemical has been reported earlier [2]. The decrease in growth was not due to decreased food consumption. In these sets of experiments, control and DEHP-fed animals consumed, respectively, 16.6 ± 2.0 and 16.9 ± 3.3 g of the diet per day per animal.

The results in Fig. 2 also confirm the hepatomegalic effect of the compound observed earlier [4]. It may be noted that the increase in liver weight plotted in Fig. 2 is computed for constant body weight (100 g). The actual increase varied from 40 to 50%. Thus, the liver mass increased from 5.6 ± 0.4 to 8.9 ± 1.0 g in 14 days and from 6.8 ± 0.5 to 10.4 ± 0.3 g in 30 days of feeding with DEHP.

Serum cholesterol was depressed by about 40% in 2 weeks and remained at that level for the duration of the experiment. This may be compared with a 30% decrease reported under similar experimental conditions [16]. The protein recovered in the mitochondrial fraction of the liver also increased progressively with the administration of DEHP and almost doubled in 2 weeks (Fig. 2). Ganning *et al.* [9, 10] observed a 3-fold increase in the protein content of the mitochondrial fraction in 14 days under similar experimental conditions. It may be pointed out, however, that the protein recovered in the mitochondrial fraction by these authors (about 18 mg/g liver) was only about half of what is normally obtained from control animals. We have confirmed by counting the organelle population that the increased protein recovery reflected proliferation of mitochondria (data not given).

Synthesis of cholesterol. To gain insight into the mechanism of the hypocholesterolemic effect of the chemical, the activity of HMGCoA reductase, the rate-limiting enzyme in the biosynthetic pathway of cholesterol, was assayed in liver microsomes. It may be noted from the results presented in Table 1 that the specific activity of the enzyme was decreased by 50% in hepatic microsomes of DEHP-fed animals. However, the total enzyme activity in liver was only about 25% less than that of control animals. Apparently, the hepatomegalic effect of DEHP could partially compensate for the decrease in specific activity. Dietary intake of cholesterol is known to decrease

Table 1. Effect of administration of DEHP on the HMGCoA reductase activity of rat liver

	HMGCoA reductase (mevalonate formed/min)	
Treatment	Specific activity (pmoles/mg microsomal protein)	Total activity (nmoles/whole liver)
Control	705 ± 47	234 ± 12
DEHP-fed	353 ± 29*	171 ± 8*

Where indicated, animals were fed with DEHP (2%, w/w) in the diet for 4 weeks. Total activity (whole liver) was calculated from microsomal content (52 ± 1 mg and 56 ± 3 mg/g liver) and liver weight (6.4 ± 0.3 g and 8.6 ± 0.2 g for control and DEHP-fed animals respectively). Other details are given in Materials and Methods. The values are the mean ± S.D. of six independent determinations (animals).

* $P < 0.01$ (control vs DEHP-fed).

Table 2. Effect of administration of DEHP on cholesterol synthesis in rat liver

Precursor	Lipid fraction	Cholesterol synthesis			
		dpm/g liver		dpm/whole liver	
		Control	DEHP	Control	DEHP
Acetate	Non-saponifiable	4,859 ± 691 (100)	3,391 ± 511* (100)	31,693 ± 3,464 (100)	30,942 ± 3,056 (100)
	Cholesterol	3,560 ± 703 (73 ± 4)	1,705 ± 187* (51 ± 4)	23,196 ± 3,840 (73 ± 5)	15,673 ± 1,876* (51 ± 1)
	Cholesterol (dpm/mg cholesterol)	2,160 ± 297	1,135 ± 93*		
Mevalonate	Non-saponifiable	8,474 ± 1,464 (100)	8,440 ± 1,562 (100)	58,596 ± 6,156 (100)	84,157 ± 9,286 (100)
	Cholesterol	7,428 ± 1,132 (88 ± 1)	6,649 ± 1,085 (79 ± 3)	51,358 ± 5,601	66,381 ± 5,706
	Cholesterol (dpm/mg cholesterol)	4,556 ± 349	4,656 ± 402		

Rats were injected (i.p.) with [1-^{14}C]acetate (10 μCi/rat) or [2-^{14}]mevalonate (0.5 μCi/rat) as indicated and killed 30 min later. The plasticizer was administered (2%, w/w) in the diet for 30 days. The values are the mean ± S.D. of eight (acetate) and four (mevalonate) independent determinations. The values in parentheses represent percent incorporation.
* $P < 0.01$ (control vs DEHP).

HMGCoA reductase activity [40]. Since the diet used in these experiments was largely vegetable oil-based and low in cholesterol content (48 μg/10 g diet, the source of cholesterol being fish meal), the intake was not more than 80 μg of cholesterol per rat per day.

It may be pertinent to mention in this context that addition of DEHP (up to 400 μM) to the reaction system did not result in any inhibition of enzyme activity. Also, the enzyme has now been shown to be partially localized in peroxisomes [41]. Since the microsomes were sedimented from the post-mitochondrial supernatant fraction, our preparation would include peroxisomes also.

The pattern of incorporation of acetate and mevalonate into cholesterol depicted in Table 2 is consistent with the decrease in specific activity of HMGCoA reductase. In DEHP-administered animals the incorporation of acetate into the non-saponifiable lipid fraction of liver (per g) was significantly lower (53%) than that observed in control animals. Cholesterol accounted for almost three-fourths of the radioactivity of the non-saponifiable fraction in control animals, whereas in DEHP-fed animals it accounted for about half. In agreement with this, the specific radioactivity of cholesterol in the livers of DEHP-fed animals was only about half of that of control animals. The incorporation of radioactivity into hydrocarbons was not different in control and DEHP-fed animals. However, the incorporation of the precursor into ubiquinone was substantially higher in the animals administered the plasticizer (data not given).

Even when computed for whole liver, the incorporation of acetate into cholesterol was significantly lower (30%) in DEHP-administered animals. This agrees with the decrease of HMGCoA reductase activity by the same margin (Table 1) and confirms that the hepatomegalic effect of the compound does not completely compensate for the inhibition of enzyme activity. In these experiments, the weight of liver in control and DEHP-fed animals was 6.5 ± 0.2 and 9.2 ± 0.9 g respectively. It may be mentioned in this context that the specific radioactivity of serum cholesterol was also depressed by 48% in DEHP-administered animals.

In contrast to the results obtained with acetate, the incorporation of mevalonate into the non-saponifiable fraction or into cholesterol was not inhibited in DEHP-administered animals (Table 2). This is consistent with the identification of HMGCoA reductase as the site sensitive to DEHP.

Degradation of cholesterol. Since conversion to bile acids is the more important route of degradation of cholesterol in the liver [42], the effect of DEHP on the release and composition of bile was studied. The results presented in Table 3 give clear indication that the rates of bile flow and degradation of cholesterol were significantly higher in DEHP-administered animals. Expressed on a gram liver basis, the radioactivity associated with bile was significantly higher ($P < 0.01$) in DEHP-administered animals (748 ± 41) in comparison with control animals (252 ± 48 dpm/g liver). The volume of bile released in 1 hr/g liver which agrees with the values reported previously [33] also was significantly different ($P < 0.05$) in control and experimental animals.

It has been reported that hypocholesterolemic agents like nicotinic acid [43] and polyunsaturated fatty acids [44] increase the biliary excretion of cholesterol. Our data show that the plasticizer decreased the release of cholesterol in bile, apparently due to more rapid conversion to bile acids.

In the degradation of cholesterol to bile acids in the liver, the rate-limiting enzyme is believed to be cholesterol 7α-hydroxylase [38]. However, in steroidogenesis, the rate-limiting step is believed to be

Table 3. Effect of administration of DEHP on the conversion of cholesterol to bile acids in rat liver

	Bile analysis					
	Volume (μl/hr per g liver)	Total volume (μl/hr)	Radioactivity (dpm/100 μl)	Radioactivity (dpm/hr)	Cholesterol content (μg/100 μl)	Bile acid content (μg/100 μl)
Control	37 ± 8	242 ± 46	680 ± 78	1632 ± 278	18 ± 4	813 ± 18
DEHP	48 ± 4*	423 ± 35†	1558 ± 206†	6439 ± 356†	9 ± 1†	1106 ± 23†

Rats were injected with [4-^{14}C]cholesterol (1 μCi/rat) and killed after 1 hr. Where indicated, DEHP was fed (2%, w/w) for 30 days. The values are the mean ± S.D. of four independent determinations (animals). The total concentration of bile acids (cholate + deoxycholate + chenodeoxycholate) is given.
* $P < 0.05$.
† $P < 0.01$.

the cleavage of the side chain, which takes place in mitochondria [44]. The ability of hepatic mitochondria to oxidize [26-^{14}C]cholesterol to [$^{14}CO_2$] was first detected in 1953 and has since then been investigated in many laboratories [45]. We have assayed this activity in hepatic mitochondria isolated from DEHP-administered animals.

Mitochondria isolated from the livers of control and DEHP-fed animals showed no significant difference in cholesterol oxidase activity (Table 4). The large stimulation in the rate of oxidation on the addition of heat-denatured supernatant fraction is in agreement with previous reports [26–28, 45]. The nature of the cofactor present in the supernatant fraction is obscure, but has been suggested to be similar to that involved in 11β-hydroxylation of steroids in the adrenals [27, 46]. It may be noted that the supernatant fraction prepared from the livers of DEHP-fed animals stimulated the oxidation as effectively as did the fraction obtained from control animals (Table 4). This would indicate that the administration of the plasticizer produced no adverse effect on the cofactor.

In contrast, cholesterol 7α-hydroxylase activity of hepatic microsomes increased by almost 70% in DEHP-administered animals (12.0 ± 1.4 nmoles 7α-hydroxycholesterol formed/min/mg protein). The enzyme activity in control animals (7.2 ± 1.4 nmoles 7α-hydroxycholesterol formed/min/mg microsomal protein) agreed with the values reported by Sanghvi *et al.* [47]. It may be stated that administration of DEHP did not affect the cholesterol content of microsomes. The preparations used in these experiments contained 35 ± 3 and 36 ± 4 nmoles cholesterol/mg of microsomal protein, respectively, from control and DEHP-fed animals.

Turnover of cholesterol. The increase in the activity of cholesterol 7α-hydroxylase and of cholesterol oxidation and the enhanced secretion of bile revealed that the degradation of cholesterol was higher in the livers of DEHP-administered animals. We sought to confirm this by determining the turnover rate of cholesterol in livers of DEHP-fed animals. The method of measurement of the decay of the lipid involved incorporation of the precursor (acetate) and is based on the assumption that the incorporation of radioactivity due to synthesis is negligible under the conditions of the experiment. The disappearance of the lipid is governed by the equation $A_t = A_0 \cdot e^{-K_d \cdot t}$ in which A_0 represents the amount of lipid (radiolabel) at "zero" time and A_t at time "t", K_d being the rate constant of degradation. Taking logarithms, the equation may be represented as $\ln A_0/A_t = K_d \cdot t$. The determination of K_d involves

Table 4. Effect of administration of DEHP on the oxidation of cholesterol by rat liver mitochondria

	Release of $^{14}CO_2$ from cholesterol (dpm)	
Addition	Control	DEHP
None	10,016 ± 962	11,066 ± 1,075
Supernatant factor from control animals	17,558 ± 1,352*	16,008 ± 1,121*
Supernatant factor from DEHP-fed animals	18,799 ± 1,397*	17,093 ± 1,234*

Experimental details are given in Materials and Methods. The plasticizer was fed (2%, w/w) in the diet for 30 days. The values represent the total release of $^{14}CO_2$ from cholesterol under the conditions of the experiment and are the mean ± S.D. of three independent experiments (animals).
* $P < 0.05$ (mitochondria alone vs supernatant added).

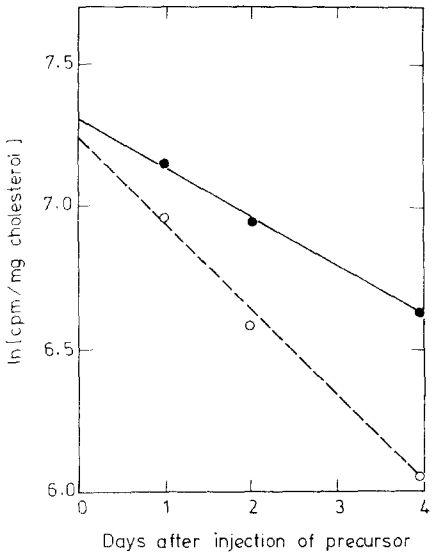

Fig. 3. Effect of administration of DEHP on the turnover of liver cholesterol in the rat. Animals injected with [2-^{14}C]acetate were killed at the time intervals indicated. The incorporation of the label/mg of cholesterol in liver was determined. The values given for hepatic cholesterol of control (●) and experimental (○) animals are the mean of three independent determinations. Experimental animals were fed with DEHP (2%, w/w) in the diet for 30 days. The regression line for each set is drawn. The correlation coefficient "r" is 0.94 ± 0.05 (control) and 0.98 ± 0.03 (DEHP-fed). The values of decay rate (K_d) are 0.168 ± 0.01 day^{-1} and 0.307 ± 0.04 day^{-1} in control and DEHP-fed animals respectively. Other details are given in Materials and Methods.

the plot of ln A_t against "t" when the slope of the line yields K_d. By definition, half-life ($T_{\frac{1}{2}}$) is given by the equation $t = 0.693/K_d$.

Animals were injected with [1-^{14}C]acetate, and at different time intervals (1, 2 and 4 days) the radioactivity associated with liver cholesterol was determined and plotted. The decay curves are presented in Fig. 3. An increase in the slope of the regression line for the decay of liver cholesterol in DEHP-fed animals is discernible. The half-life of cholesterol in the liver was decreased by almost 50% in DEHP-administered animals (2.3 ± 0.3 days). The half-life of liver cholesterol of control animals (4.2 ± 0.4 days) agrees with that reported in the literature. Thus, by the continuous isotope administration technique, Pihl *et al.* [48] obtained a value of 6 days for liver cholesterol in the rat. The turnover of rat hepatic cholesterol has been shown to range from 2.0 to 4.2 days depending on the type of diet fed [45, 49].

DISCUSSION

The results presented in this paper give convincing evidence that the hypocholesterolemic action of DEHP arises largely from the ability of the compound *in vivo* to stimulate the degradative removal of the sterol as bile acids. The stimulation of the activity of the rate-limiting hydroxylase enzyme and the proliferation of mitochondria in the liver contribute to this process. The contribution of the inhibition of HMGCoA reductase in determining the level of serum cholesterol may be partial in view of the hepatomegalic effect of DEHP which tends to compensate for this effect to a certain extent.

A comparison of the action of DEHP with that of other hypolipidemic compounds may not be out of place. It would appear that these compounds act through different mechanisms. Thus, D-thyroxine which is widely used as an anti-hypercholesterolemic drug does not directly influence cholesterol biogenesis but improves elimination primarily as neutral sterols and less significantly as bile acids [43, 50, 51]. There is no convincing evidence that poly-unsaturated lipids exert any inhibitory influence on cholesterol biosynthesis. Their hypocholesterolemic action appears to rest to a small extent on interference with absorption and enhanced withdrawal into tissues [50] but largely on increased biliary excretion of cholesterol and bile acids [52]. Bile acid sequestrants like cholestyramine interrupt enterohepatic circulation of bile and cause a several-fold increase in faecal excretion of cholesterol [43]. The antihypercholesterolemic drug clofibrate, many of whose effects on the liver are mimicked by DEHP, inhibits HMGCoA reductase and cholesterol synthesis from acetate but not from mevalonate [53, 54]. However, degradative removal of cholesterol in the form of bile as the major cause of the hypocholesterolemic action of the drug was high-lighted by Kritchevsky and associates [29]. The hepatomegalic and mitochondria-proliferating actions of the drug play a decisive role in this process. It may be pointed out that the cholesterol side chain oxidizing capacity of mitochondria did not increase with administration of either clofibrate or DEHP, the higher rate of bile excretion in both cases being accomplished by the increase in mitochondrial population. However, the large increase in the biliary excretion of free cholesterol in clofibrate-treated animals and patients [43, 55, 56] may be contrasted with a large decrease in the content of cholesterol in the bile of DEHP-administered animals. Since high concentrations of cholesterol in bile tend to produce gall stones, DEHP should be free of this harmful effect if used in therapy. There is no evidence that clofibrate administration increases microsomal 7α-hydroxylase activity [57].

It is interesting that in DEHP-administered animals, the synthesis of cholesterol was inhibited and the decay rate was enhanced, but the concentration of cholesterol per gram of liver did not suffer any significant decrease (1.7 ± 0.03 and 1.5 ± 0.04 mg cholesterol/g liver in control and 30-day DEHP-administered animals respectively). Administration of clofibrate also does not bring about any significant decrease in the content of cholesterol in liver [57]. This conforms to the view that the cholesterol content of liver is finely regulated [55]. Under conditions of decreased synthesis and enhanced degradation, homeostasis is achieved by enhanced mobilization of tissue cholesterol via the serum [43].

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