INDUCTION OF PEROXISOMES AND MITOCHONDRIA BY DI(2-ETHYLHEXYL)PHTHALATE

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1. Introduction

Plasticizers have a broad use in different industrial processes and large amounts of these substances occur in soil, water, air and food [1]. A number of investigations performed to date indicate that various plasticizers possess toxic, teratogenic, mutagenic and carcinogenic properties [2]. Previous investigators found an increase in the β-oxidation of fatty acids in rat liver homogenate after treatment of rats with a plasticizer [3]. Since this oxidation was insensitive to KCN, the increase was attributed to an induction of the peroxisomal system. Here, we have analyzed the dietary effect of the most common plasticizer, di(2-ethylhexyl)phalate (DEHP) on rat liver and found significant induction of both peroxisomes and mitochondria. This unusual type of membrane and enzyme induction was not associated with damage of mitochondrial functions.

2. Materials and methods

Male rats (180 g body wt) were starved for 20 h before the experiments were performed. The experimental animals received a diet containing 2% DEHP (w/w) ad libitum. Mitochondria and microsomes were prepared as in [4,5]. Peroxisomes were prepared on a discontinuous sucrose gradient using a vertical rotor. Briefly, the 2800 × *g* supernatant in the presence of 1 mM glutaraldehyde is layered over a continuous 1.4–1.85 M sucrose gradient with a cushion of 2.1 M sucrose at the bottom of the tube and centrifuged in a VTi-50 rotor (Beckman) at 120 000 × *g* for 1 h. The peroxisomal fraction appearing at the lower sucrose interface was essentially free from mitochondria but was contaminated to an extent of 14% with microsomes. Palmitoyl-CoA oxidation was monitored by measuring the reduction of NAD in an Aminco-Chance spectrophotometer. The incubation medium contained 30 mM phosphate buffer (pH 7.5) 0.2 mM NAD, 0.1 mM CoA, 1 mM KCN, 6 mM dithiothreitol, 0.14 mM palmitoyl-CoA, 0.15 mg bovine serum albumin, 0.01% Triton-X-100 and 50–70 μg peroxisomal protein in a final volume of 1 ml. During the measurement the temperature was maintained at 37°C.

The other peroxisomal enzymes, as well as the mitochondrial and microsomal enzyme activities or amounts were determined as in [6–10]. Protein was measured according to [11].

3. Results and discussion

The male rats which received a diet containing 2% DEHP for 2 weeks showed an increase in liver weight of ~50% relative to body weight (fig.1A). The composition of the hepatocytes is obviously changed, since the protein content/g liver is increased by 30%. Electron microscopic investigations detected a clear proliferation of peroxisomes and mitochondria with an obvious increase in the number of these two organelles, and for this reason the livers were fractionated and the amount of particles determined. The protein content of the peroxisomal fraction is elevated ~4-times in the 14 day period, but the major portion of this increase was already apparent during the first week (fig.1B). The mitochondria were also induced in a similar fashion and the protein content of this fraction was increased 3–4-fold after 2 weeks. Unexpect-

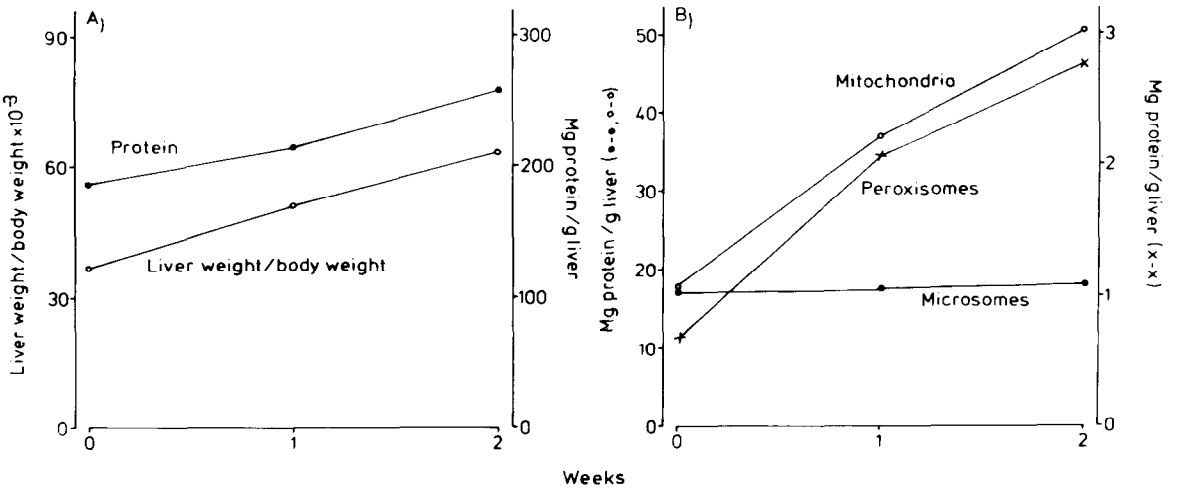

Fig.1. Effect of DEHP on rat liver and on subcellular fractions: (A) liver weight was measured before homogenisation and the amount of protein was determined in the homogenate; (B) the protein content was analyzed in the isolated final fractions of peroxisomes, mitochondria and total microsomes.

edly, the protein content of the microsomal fraction did not increase.

The most abundant enzymes of the peroxisomal luminal compartment, catalase and urate oxidase, show decreased specific activity of ~40% (table 1). The β-oxidation system of fatty acids, which is also present in the luminal compartment, is affected in a specific way by the plasticizer employed. Both substrates used in these experiments, octanoyl-CoA and palmitoyl-CoA, are metabolized to a very high extent and the specific activity of their oxidation increases 5–7-times. The various mitochondrial respiratory enzymes, among them cytochrome *c* oxidase activity are unchanged (table 2). The mitochondria display an undamaged structure and unaffected functional capacity, which is demonstrated by the high degree of respiratory control and unchanged P/O-ratio after 2 weeks treatment with the plasticizer. The activity of carnitine–acetyl transferase undergoes a dramatic change by showing a 50-fold increase in the 2 week period.

The isolated microsomal fraction, which has about the same protein content in the case of control and treated rats, possesses an elevated NADPH-cytochrome *c* reductase activity and the amount of cytochrome *P*-450 also increases >50% (table 3). The induction is not completely restricted to these 2 enzymes of the hydroxylation system, but also affects nucleoside diphosphatase. Other phosphatases are either unchanged or even decreased.

Table 1
Effects of DEHP on peroxisomal enzyme activities

	Specific activity	
	Control	DEHP-treated
Catalase[a]	29.3	17.6
Urate oxidase[b]	0.21	0.13
Octanoyl-CoA oxidation[c]	32.6	195.6
Palmitoyl-CoA oxidation[c]	22.2	111.0

[a] μmol H_2O_2 decomposed . min^{-1} . mg $protein^{-1}$;
[b] μmol urate oxidized . min^{-1} . mg $protein^{-1}$;
[c] μmol NAD reduced . min^{-1} . mg $protein^{-1}$

Table 2
Effect of DEHP on mitochondrial functions

	Specific activity	
	Control	DEHP-treated
Cytochrome *c* oxidase[a]	1.05	1.10
Respiratory control[b,c]	3.5	3.2
P/O-ratio[c,d]	2.3	2.2
Carnitine-acetyl transferase[e]	0.04	2.01

[a] μmol cytochrome *c* oxidized . min^{-1} . mg $protein^{-1}$;
[b] state 3/state 4;
[c] β-OH-butyrate as substrate;
[d] mol ADP/mol oxygen;
[e] μmol CoA produced . min^{-1} . mg $protein^{-1}$

Table 3
Effect of DEHP on microsomal enzymes

	Specific activity	
	Control	DEHP-treated
NADPH-cytochrome *c* reductase[a]	0.05	0.08
NADH-cytochrome *c* reductase[a]	0.8	0.7
Cytochrome *P*-450[b]	0.8	1.3
Glucose-6-phosphatase[c]	0.40	0.40
ATPase[c]	0.29	0.20
IDPase[c]	0.05	0.10

[a] μmol NADPH or NADH ox . min^{-1} . mg $protein^{-1}$;
[b] nmol/mg protein;
[c] μmol P_i . min^{-1} . min $protein^{-1}$

This investigation demonstrates that di(2-ethylhexyl)phthalate is an unusual and valuable inducer of the intracellular membranes in hepatocytes. In contrast to most other inducers, the endoplasmic reticulum is not much affected and only the hydroxylating system displays a limited increase. The size and type of induction in peroxisomes have similarities to that described for rats treated with hypolipidemic agents [12]. The number of peroxisomes increases 3–5-times in electron micrographs and the β-oxidation capacity of the particles also increases on protein basis. It is obvious that peroxisomes in the treated rats perform a large part of the fatty acid oxidation of the liver and to a certain extent a functional redistribution of the oxidation takes place in the intramembranous compartment. The induction of mitochondria is a unique event exhibited by DEHP, which is not seen in the case of most other inducers. One would suspect that the reason behind mitochondrial induction is a toxic effect, that is, dissociation of the respiratory chain or damage of the oxidative phosphorylating system may require a physical induction to keep up the level of ATP synthesis. However, this is not the case. The unchanged respiratory control and P/O-ratio give evidence for the intactness of the mitochondrial structure and function and indicate that the increase in the number of mitochondria has some specific function. The great increase in the carnitine–acetyl transferase activity suggests that the fatty acid-transport system is heavily involved in the process, but further investigations along this line will be necessary in the future. Thus, some of our most common environmental pollutants, the plasticizers, greatly affect the structure and the function of the liver cell and this may be one of the explanations for the effects described in toxicological studies.

Acknowledgements

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