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Short Communications

Effect of Plasticizers on the Polyprene Distribution in the Liver *

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Our most common plastic material, polyvinyl chloride (PVC), contains 40 % phthalate esters in the soft form. Di(2-ethylhexyl)phthalate (DEHP) is used most commonly. Because of the migration of the substance to the environment, a sizeable contamination occurs. Experimental studies and also observations on humans suggested that phthalate esters may cause a number of chronic toxic effects.[1] It is claimed that these substances have carcinogenic, mutagenic and teratogenic effects, and also cause testicular atrophy and pituitary hypertrophy. DEHP has been shown to induce peroxisomes and mitochondria and to influence a number of metabolic processes.[2] Lipid metabolism and turnover is one of the main targets of phthalate esters, and it appears to be of importance to analyze to which extent polyisoprenoid content and distribution is influenced by these substances.

The experiments in this investigation were performed on rats which recieved a basal diet (consisting of 24 % protein, 49 % carbohydrates, and 5.4 % total fat) mixed with 2, 0.2 and 0.02 % DEHP. After various time periods, the livers were removed and subcellular fractions were prepared.[3] The pattern and amount of dolichol was determined by high performance liquid chromatography[4] – which was also used for dolichyl-P determination after dephosphorylation with an acid phosphatase.[5]

Dolichol is synthesized in the microsomes and an extensive transport occurs to various intracellular and plasma membranes. The concentration in the microsomes is relatively low, but high amounts are present in the lysosomes (Table 1). After 5 weeks of treatment of rats with 2 % DEHP the microsomal dolichol remained at an unchanged level, but in the lysosomes the amount had trebled. Dolichyl-P is present both in microsomes and lysosomes; in the former location it participates in the glycoprotein synthesis as an essential intermediate in the establishment of *N*-glycosidically linked oligosaccharide chains. After a few weeks of phthalate treatment, the microsomal dolichyl-P content, contrary to the lysosomal one, had decreased considerably.

The total dolichol fraction consists of a number of polyprenes with various numbers of isoprene residues. At present, no explanation is available for the functional importance of the presence of a polyisoprenoid family in the same membrane. Contrary to many other drug treatments, phthalate administration introduces a change in the dolichol distribution pattern (Table 2). Gradually, changing with time, dolichols with 17 and 18 isoprene residues are decreasing while those with 19 and 20 residues are increasing. This new pattern resembles the one found in normal human tissues.

Mitochondria have a very low concentration of dolichol, and the combined mitochondrial-lysosomal fraction has practically all its dolichol associated with lysosomes. During prolonged treatment with 2 % DEHP, the maximal dolichol concentration is attained in this fraction after about 40 weeks which is four times as much as in the control (Fig. 1). A significant increase of dolichol occurs with age even in the control fractions prepared from

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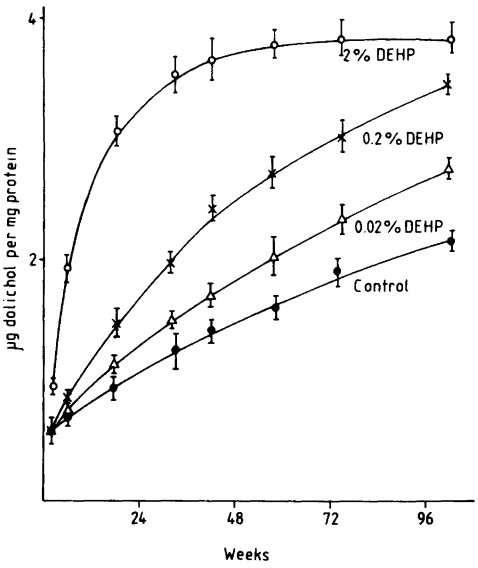

Fig. 1. The effect of prolonged DEHP-treatment on the lysosomal dolichol content. Rats received a diet containing 2, 0.2 and 0.02 % DEHP, and after various intervals during the 102 week period the mitochondrial-lysosomal fractions from liver were analyzed for dolichol content. The values represent 8 experiments, the vertical bars S.E.M.

Table 1. Distribution of dolichol and dolichyl-P in liver fractions after treatment of rats with 2 % DEHP during 5 weeks. Rats weighing 180 g at the start of the experiment received a diet containing 2 % DEHP over a five week period. Subcellular fractions were prepared after this time and were used for extraction of lipids. Dolichol was isolated and quantitated by high performace liquid chromatography.[4] The values are given as mean values ± S.E.M. (n=6). µg per mg protein.

	Dolichol		Dolichyl-P	
	Control	Treated	Control	Treated
Homogenate	0.192±0.018	0.289±0.020	0.039±0.003	0.035±0.003
Mitochondria	0.072±0.007	0.100±0.015	0.003±0.0004	0.003±0.0002
Lysosomes	3.73 ±0.40	9.51 ±0.84	0.070±0.006	0.064±0.0070
Microsomes	0.215±0.0018	0.238±0.024	0.062±0.0062	0.037±0.004

Table 2. Distribution of individual dolichols in liver mitochondrial – lysosomal fraction after DEHP treatment. Rats recieved 2 % DEHP in the diet for various lengths of time. The individual dolichols isolated from the mitochondrial – lysosomal fraction were quantitated by high performance liquid chromatography. Values are means ± S.E.M. (n=7).

DEHP treatment Weeks	Composition, % of total				
	D17	D18	D19	D20	D21
0	12±1.2	38±1.6	34±1.9	11±0.9	5±0.4
33	8±1.0	34±2.2	39±1.3	13±1.1	6±0.5
42	5±0.2	30±1.4	41±2.1	17±2.2	7±0.5
57	4±0.1	28±1.7	42±1.8	19±2.8	7±0.8

Table 3. Glycosyl transferase activities in liver microsomes. Rats received 2 % DEHP in the diet for 6 weeks; microsomes were isolated and incubated with nucleotide activated sugars as described earlier. After incubation at 30 °C for 5 min, the lipids were extracted and the radioactivity was determined in the chloroform fraction. The radioactive product was identified as dolichyl-P sugar by thin layer chromatography. The radioactivity in the protein pellet was measured by scintillation counting. The values represent the means of 5 experiments ± S.E.M. Cpm/mg protein.

	Dol-P monosaccharide		Protein	
	Control	Treated	Control	Treated
UDP-GlcNAc	1559± 78	826± 74	589±34	308±20
GDP-Mannose	2385±291	1486±142	606±55	420±22
UDP-Glucose	1735±169	1462±109	451±32	445±28

untreated rats. When decreasing the dose ten times, to 0.2 % the effect is less pronounced but still continuously increasing until 102 weeks of treatment have passed. Even the 100 times smaller dose, 0.02 % has a significant effect and elevates the dolichol content of the lysosomes. This latter dose is very interesting from the toxicological point of view in human since the amount administered to dialysis patients approximates this level.

The three dolichyl-P dependent glycosyl transferase activities were also followed since protein glycosylation is important for normal function. A sizeable decrease of sugar transfer to dolichyl-P is observed, particularly in the case of GlcNAc and mannose (Table 3). As could be expected, glycosylation of the protein is also influenced. The significant reduction of the glucosamine and mannose incorporation demonstrates that the oligosaccharide transfer to endogenous protein acceptor is effected.

Dolichyl-P has a well established function as intermediate in glycoprotein synthesis but its impaired function induced by plasticizers may have deleterious consequences on the biosynthesis of necessary membrane components. It has been established in a number of previous investigations[6] that dolichyl-P is the rate limiting component of the glycosyl transferase system and it is reasonable to suppose that this is also the case during phthalate ester treatment. A deficient protein glycosylation may be one of the underlying reasons for various pathological functions. Recent investigations suggest that dolichol may play an important role in membrane structure.[7] In model membranes these polyprenes destabilize lipid structures by inducing hexagonal H_{II} phases; they also increase phospholipid fatty acid fluidity. The increased amount of dolichol in lysosomal membranes may change their permeability for various molecules. In this way, partial release of the lysosomal content can lead to various types of intracellular damage.

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1. Thomas, J.A. and Northup, S.J. *J. Toxicol. Environ. Health 9* (1982) 141.
2. Ganning, A.E., Brunk, U. and Dallner, G. *Hepatology 4* (1984) 541.
3. Autuori, F., Brunk, U., Peterson, E. and Dallner, G. *J. Cell Sci. 57* (1982) 1.
4. Eggens, I., Chojnacki, T., Kenne, L. and Dallner, G. *Biochim. Biophys. Acta 751* (1983) 355.
5. Carsson, D.D. and Lennarz, W.J. *J. Biol. Chem. 256* (1981) 4679.
6. Potter, J.E.R. and Kandutsch, A.A. *Biochim. Biophys. Res. Commun. 106* (1982) 691.
7. Valtersson, C., Duÿn, G., Verkley, A.J., Chojnacki, T., Kruijff, B. and Dallner, G. *J. Biol. Chem. In press.*

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