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Phthalate Esters in Normal and Pathological Human Kidneys

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During the course of studies on the lipid composition of human kidneys (DRUILHET et al. 1975, OVERTURF et al. 1976), we observed the presence of unusual esters in various purified lipid preparations. We have identified these compounds as dibutyl phthalate (DBP) and di(2-ethylhexyl)phthalate (DEHP).

Phthalate esters, one of the most common groups of plasticizers utilized by the polymer industry, are added to synthetic plastic resins to impart flexibility. They are widely used in the formulation of plastic housewares, food wrappings and closures, and medical tubing and intravenous transfusion bags. According to PEAKALL (1975) and others*, approximately 440 million pounds of DEHP and 30 million pounds of DBP are released into the environment annually.

Within the last decade phthalate esters have been recognized as environmental pollutants (MAYER et al. 1972, MAYER & SAUNDERS 1973, CORCORAN 1973). Several studies have also shown that DEHP could be recovered from plasma, liver, spleen, lung, abdominal fat, and gastrointestinal tissue of patients who received blood transfusions (JAEGER & RUBIN 1970a, 1972, 1973, HILLMAN et al. 1975, RUBIN & NAIR 1973). Others have found DBP and DEHP in human adipose tissue obtained from victims of accidental death (MES et al. 1974, MES & CAMPBELL 1976). However, the incidence of phthalate contamination of human tissue as a result of environmental exposure has not been well documented.

We chose to investigate further the occurrence of DEHP and DBP in human kidney because of the enormous quantities of phthalate esters being produced each year and the probable ubiquitous distribution of these compounds in the ecosystem. Particular impetus was provided by the dearth of information on the levels of DBP and DEHP in human tissue, and the recent report of DBP and DEHP contamination in the Gulf of Mexico and Mississippi delta atmospheric, marine, and shore environments (GIAM et al. 1978).

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METHODS

Human kidneys were obtained at autopsy from seventeen individuals of the Houston Gulf Coast area who died suddenly from either trauma, myocardial infarction or ruptured ascending aorta. One donor was hospitalized at the time of death. None had a history of occupational or medical exposure to phthalate esters. All kidneys were removed within four hours of death; superficial fat and connective tissue were removed immediately. Fifteen kidneys were histologically normal and two were nephrosclerotic. Seven kidneys were split bilaterally and the cortical and medullary tissue were separated prior to extraction; the others were homogenized intact.

The tissue samples were extracted with chloroform-methanol (FOLCH et al. 1957), freed of non-lipid soluble substances by passage through a Sephadex G-25 column (WUTHIER 1966), and separated into neutral and phospholipid fractions using a silicic acid column (MICELI & FERRELL 1972). Five neutral lipid and four phospholipid subclasses were separated and purified to homogeneity using thin-layer chromatography and solvent systems previously described (DRUILHET et al. 1975). The lipid subclasses were methylated with methanolic-HCl and purified (OVERTURF & DRYER 1969). Preliminary GLC was performed using glass columns 1.82 m X 6 mm OD packed with 100-200 mesh Chromosorb WAW and coated with either 15% DEGS or 15% EgSS-X. The esters were analyzed isothermally at 170C with a carrier nitrogen flow of 25 cm^3/min, and a hydrogen flame detector. When lipid subclasses were found to contain unidentified peaks, they were analyzed with a Finnigan 3200 gas chromatograph-mass spectrometer interfaced with a Finnigan 6000 Computer System. The compounds were separated on a glass column 1.32 m X 2 mm ID packed with 3% OV-17 on Gas Chrom Q. The injector temperature was held at 250C and the column temperature was programmed from 150C to 290C at a rate of 4C/min. The mass spectrometer was operated at 70eV in the electron impact mode. Quantities of DEHP and DBP were determined relative to dihexyl phthalate internal standard.

We are aware that tissue samples can easily be contaminated in the laboratory with phthalates arising from the use of contaminated solvents (MAXWELL et al. 1973), solvent extraction of plastic ware (PASCAL & ACKMAN 1974) and rubber closures (DICKSON et al. 1974), and by the use of phase-separating filter paper and phthalate-contaminated silica gel (MAXWELL et al. 1973, ROLL et al. 1974). Thus, only glass containers and closures were used, and solvent, reagent and silica gel contamination was excluded by the analysis of control extractions and purifications of phthalate-free lipids. Further consideration of possible laboratory contamination was obviated by the demonstration that simultaneous extraction of various kidneys revealed high levels of phthalates in some and negligible amounts of phthalates in others.

RESULTS AND DISCUSSION

We initially found measurable amounts of DEHP and DBP in triglyceride fractions of donors 1 and 2 (Table 1). Insufficient quantities of 1,3-diglycerides, monoglycerides and cholesteryl esters were isolated for mass spectrometric analyses. Subsequent to these findings we screened fifteen donor kidneys and found phthalates in the cortex and medulla of donors 3 and 4. Amounts varied with different tissue samples and no anatomical distribution was apparent. Values for DBP ranged from 0.6 - 144 μg/mg. Phthalate esters were not detected in the phosphatidylcholines, phosphatidylethanolamines, lysophosphatides, or sphingomyelins isolated from these kidneys.

These data clearly indicate that some human renal cortical and medullary tissues contain quantities of DEHP and DBP which were associated with neutral lipid fractions exclusively. Only two of fifteen normal kidneys contained measurable levels of phthalates, while phthalates were present in both nephrosclerotic kidneys. The significance of this observation is unknown but several animal studies demonstrated associations between phthalates and organ size, pathology, and lipid metabolism (CARPENTER et al. 1953, HARRIS et al. 1956, NEERGAARD et al. 1975, STEIN et al. 1974, BELL & NAZIR 1976, BELL et al. 1976, 1978, ALBRO et al. 1973).

The source of phthalate esters in tissue of patients who received blood transfusions was explained by the observation that DEHP readily elutes into plasma lipids during storage in plastic transfusion packs (MARCEL & NOEL 1970, JAEGER & RUBIN 1970a, 1970b). Recent studies also showed that higher phthalate levels in human neonatal tissues were associated with larger amounts of transfused blood products, extensive use of plastic catheters, and early death (HILLMAN et al. 1975). According to medical records, no intravenous infusions were administered to the kidney donors of this study.

In vivo rat studies showed that DEHP accumulated in the heart and the epididymal fat pad, and that the physiological effect of DEHP was dependent on dietary fat levels (STEIN et al. 1974). Dietary fat and DEHP acted synergistically in increasing total lipid content of the liver. Later studies showed that the addition of DEHP to stock diet of rats resulted in a 50% decrease of control values of ^{14}C-acetate into total lipid of liver and kidney tissue slices (BELL et al. 1976, 1978). Other organs such as heart, testes, and aorta were unaffected. In rats fed 0.5% DEHP for 10 days, incorporation of ^{14}C-acetate into total lipid of kidney was similar to the control value, but incorporation into the triglyceride and sterol ester plus hydrocarbon fractions was decreased 38 per cent.

Little is known regarding the catabolism of either DEHP or

TABLE 1

Phthalate Esters in Neutral Lipid Classes of Human Kidney Tissue (μg/mg lipid).

Donor: (No.), Age & Sex	Cause of Death	Kidney Pathology	Tissue	DBP				DEHP			
				TG	1,3-DG	MG	CE	TG	1,3-DG	MG	CE
(1) 54 Male	Myocardial Infarction	Nephro-sclerosis	Cortex	33.6				64.4			
			Medulla	4.4				20.3			
(2) 15 Male	Cerebral Infarction	None	Cortex	52.0				102.0			
			Medulla	55.5				58.0			
(3) 56 Female	Ruptured Aorta	Nephro-sclerosis	Cortex	3.6	22.0	3.8	15.7	2.5	10.5	0.4	0.7
			Medulla	15.0	ND	143.7	ND	1.6	ND	5.6	ND
(4) *	Trauma	None	Cortex	2.8	3.6	1.3	1.9	2.2	0.1	0.1	0.1
			Medulla	0.6	ND	4.9	2.3	3.6	ND	1.6	0.1

Blank areas indicate that these lipid classes were not assayed by mass-spectrometry. Phthalate esters were not detected in the tissue of thirteen additional normal human kidneys. DBP, dibutyl phthalate; DEHP, di(2-ethylhexyl)phthalate; TG, triglycerides; 1,3-DG, 1,3-diglycerides; MG, monoglycerides; CE, cholesteryl esters; ND, not detected.

* Unknown.

DBP. Detailed studies of the metabolism of DEHP have been performed using rats fed [^{14}C] DEHP (ALBRO et al. 1973). Neither glucuronide, nor sulfate, nor glycine conjugates were found. Indeed, > 98% of the radioactivity in the urine was excreted without conjugation and it was concluded that rats do not modify the phthalic moiety of DEHP in producing urinary metabolites. According to ALBRO et al. (1973), it appears that DEHP was first hydrolyzed to MEHP [mono(2-ethylhexyl) phthalate], which then undergoes ω-oxidation and ω-1 oxidation, probably in the liver. The alcohol intermediates may then be oxidized to the level of ketone after ω-1 oxidation, or acid after ω-oxidation. The acid metabolite may then undergo one round of β-oxidation to yield another metabolite. The metabolites found in the urine thus suggest that mono(2-ethylhexyl) phthalate is metabolized like a fatty acid in the rat. We found no DEHP or DBP metabolite in any of the tissues analyzed suggesting that they were either not in an actively cycling pool, or that human kidney tissue cannot metabolize them effectively.

Early acute toxicity studies led to the conclusion that phthalates possess a low order of toxicity; however, recent chronic experiments indicate that DEHP has a cumulative toxicity when administered to mice over an 11 to 25 week course (LAWRENCE et al. 1975). The chronic toxicity of DEHP was 28 times greater than its acute toxicity. Other studies have revealed subtle toxicity effects of phthalate esters including fetal resorption and birth defects in experimental animals (SINGH et al. 1972) and toxicity to cells in culture (DeHAAN 1971, DILLINGHAM & AUTIAN 1973, JONES et al. 1975). LAKE et al. (1975) reported that oral administration of DEHP leads to swelling of mitochondria with shortening of the cristae, and a remarkable increase in liver weight in rats. Succinic dehydrogenase and adenosine triphosphatase activity in rats measured 21 days after three intraperitoneal injections of DEHP was decreased in heart, lung, and kidney while it remained unaltered in brain (SRIVASTAVA et al. 1977). Recent studies suggest that DBP interacts with mitochondrial membrane causing a change in the permeability to ions that results in uncoupling of oxidative phosphorylation (INOUYE et al. 1978), and secondarily in the inhibition of electron transport at higher levels of DBP (INOUYE et al. 1978, OHYAMA 1976).

The presence of DBP and DEHP in human kidneys obtained from individuals without a recent history of medical or occupational exposure to phthalates and especially the occurrence of these compounds in nephrosclerotic tissue in apparent association with neutral lipids emphasizes the potential for change in lipid metabolic processes and a possible relationship between phthalate esters and disease states. This observation and recent biochemical, toxicological, and ecological studies also emphasize the need for additional studies regarding the effects of chronic environmental phthalate exposure in biological systems.

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