Studies on the Hydrolysis \textit{in vitro} of Phthalate Esters by Hepatic and Intestinal Mucosal Preparations from Various Species

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Esters of phthalic acid are extensively used as industrial solvents and plasticizers in the manufacture of a wide variety of plastics, including food-packaging materials. When administered orally to the rat, the metabolites in urine of a number of phthalate diesters appear as derivatives of the corresponding monoesters (Albro et al. 1973; Albro & Moore, 1974). Further, the most commonly used phthalate ester plasticizer, namely di-(2-ethylhexyl) phthalate (Autian, 1973), is metabolized \textit{in vitro} by a number of rat tissues to mono-(2-ethylhexyl) phthalate and 2-ethylhexanol (Albro & Thomas, 1973; Carter et al. 1974). In view of the widespread use of these compounds, we have studied the metabolism \textit{in vitro} of a series of phthalate esters by both hepatic and small intestinal mucosal preparations obtained from the rat, ferret and the olive baboon.

\(^{14}\text{C}\)-labelled dimethyl, diethyl, di-n-butyl, di-n-octyl, di-(2-ethylhexyl) and di-cyclohexyl phthalates were prepared from [carbonyl\(^{14}\text{C}\)]phthalic anhydride by the method of Albro et al. (1973). Whole liver homogenates (0.25g/ml) were prepared in 0.25\text{M}-sucrose and post-mitochondrial supernatant fractions obtained by centrifugation at 10000 \text{g}_{av.} for 20min. Intestinal-mucosal cells were scraped from the first 30–40cm of small intestine and homogenized in 0.25\text{M}-sucrose. The rates of hydrolysis of the

![Figure 1. Hydrolysis of di-n-butyl (●) and di-(2-ethylhexyl) (○) phthalates by rat hepatic (a) and small-intestinal-mucosal (b) preparations](image-url)
Phthalate diesters were studied by the method of Albro & Thomas (1973) for cholate-dispersed substrates. Portions of the incubated extracts were analysed by t.l.c. to permit the determination of the relative amounts of monoester and phthalic acid formed.

Both rat hepatic post-mitochondrial supernatant and intestinal-mucosal preparations hydrolysed di-n-butyl phthalate at a faster rate than di-(2-ethylhexyl) phthalate (Fig. 1). Generally, with both tissue preparations from all three species examined, the rates of hydrolysis of dimethyl, diethyl and di-n-butyl phthalates were much faster than those of di-n-octyl, di-(2-ethylhexyl) and dicyclohexyl phthalates. In the hepatic studies a considerable species variation in the rate of hydrolysis of the phthalate esters was observed. The rate of hydrolysis of dimethyl phthalate by baboon and rat liver preparations was 14 and 2.7 times respectively the rate of metabolism by ferret hepatic preparations. With all the six phthalate diesters, liver preparations from the baboon were always the most active, whereas ferret liver preparations were the least active.

In the intestinal-mucosal studies the preparations from the baboon and rat were again more active than the preparations from the ferret. For example, the rates of hydrolysis of dimethyl phthalate by baboon, rat and ferret intestinal-mucosal preparations were 6.7, 1.1 and 0.05 (µmol of product formed/h per mg of intestinal-mucosal-cell protein) respectively. The examination of both hepatic and intestinal-mucosal-tissue incubated extracts from the three animal species revealed that with all six phthalate diester substrates examined the formation of the corresponding monoester accounted for more than 90% of the total metabolite formed.

These results show that both hepatic and intestinal-mucosal preparations from all the three species examined were qualitatively similar with respect to the metabolism in vitro of phthalate diesters to the corresponding monoester derivatives. Thus orally administered phthalate diesters would probably be absorbed from the small intestine of the rat, ferret and baboon as the monoester derivatives. Further, these results show a similarity in metabolism between a rodent, a non-rodent and a primate species. It would clearly be desirable to study the metabolism of phthalate diesters by human intestinal-mucosal preparations to assess the present data in terms of the exposure of man to phthalates.

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Paracetamol Injury to Rat Liver Slices, and its Subsequent Prevention by some Anti-Oxidants

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Paracetamol (4-acetamidophenol) is a safe and widely used analgesic, but an overdose can cause acute necrosis of liver and kidney parenchymal cells (Davidson & Eastham, 1966; Mitchell et al., 1973; Chenery et al., 1976). Animals pretreated with phenobarbitone to induce synthesis of microsomal cytochrome P-450 are very sensitive to paracetamol, and in such animals, liver injury is found within a few hours of dosage. It was decided to investigate this cell injury in vitro (McLean & Day, 1975).

Rat liver slices were incubated in Ringer solution containing up to 8mm-paracetamol for 2h, and then moved to fresh Ringer solution without paracetamol. In a Ringer solution containing 15mm-Hepes [2-(N-2-hydroxyethylpiperazin-N'-yl)ethanesulphonic acid] buffer, and 5mg of albumin/ml, control liver slices generally maintained potassium content of over 240µmol/g dry wt., and leaked between 1 and 10% of their isocitrate dehydrogenase over the next 4h.