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Studies on the Effects of Phthalate Esters on the Biological System: (Part 3). The *In vitro* Metabolism of Dibutyl Phthalate in the Small Intestines of Rats

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Considerable attention has been focused on the environmental contamination caused by phthalate esters (PAE) used extensively as plasticizers in recent years, in terms of toxicological considerations. So, many studies on their toxicities and metabolism have been carried out. (Singh et al.1972; Albro and Moore 1974; Ohta et al.1974; Schulz et al.1975; Gray et al.1977).

The previous studies in this series (Yamaguchi et al.1976; Kaneshima et al.1976) indicated that orally administered di-n-butyl phthalate (DBP) to mice or rats was readily absorbed from the gastrointestinal tract and metabolized rapidly in the small intestine, liver and kidneys. Additionally, it was also found in these studies that monobutyl phthalate (MBP) and its glucuronide were excreted in rat bile as the main metabolites after the oral administration of DBP. Recently, Rowland et al. (1977) described the important role of intestinal hydrolysis in the metabolism of PAE.

On the other hand, studies on the toxic effects of phthalate monoesters have recently increased (Yagi et al.1977; Carter et al. 1977; Takahashi 1977).

Therefore, it is of interest to ascertain the main metabolites and the rate of metabolism of PAE in the small intestine from the viewpoint of toxicology. The present paper describes the in vitro metabolism of DBP by the small intestine homogenates of rats.

Materials and Methods

Materials

DBP and phthalic acid (PA) were obtained from Tokyo Kasei Co. and MBP was prepared by the method of Albro et al. (1973). Other chemicals were of reagent grade.

In Vitro Metabolism

Male Wistar albino rats weighing about 300 g were sacrificed after fasting for 48 hr. Then the small intestines were removed immediately and homogenized in 4 volumes of an ice-cold 0.2 M potassium phosphate buffer (pH 7.0) using a potter glass homogenizer.

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After centrifugation at 2000 r.p.m. for 10 min, 5 ml of the supernatants were transferred to 5 test tubes respectively, containing 1 mg of DBP dissolved in 0.2 ml of 50 % ethanol. The mixture was incubated at 37° under air for various periods of time. At the end of incubation, the mixture was acidified to pH 3 with 4 N hydrochloric acid and extracted three times with 10 ml of ethyl acetate. The combined extracts were dried with sodium sulfate, concentrated at 40° under reduced pressure, and the residue was dissolved in 0.1 ml of ethyl acetate. For the preparation of trimethylsilyl (TMS) derivatives, the residue was combined with 0.2 ml of bis(trimethylsilyl) acetamide and allowed to stand at room temperature for 30 min. Then, the reaction mixture was evaporated under a N_2 gas stream and redissolved in 0.5 ml of ethyl acetate for gas chromatographic analysis.

Gas Chromatography (GLC)

A Shimadzu gas chromatograph GC-6A equipped with a flame ionization detector was used for analysis. The column used was a 3 mm i.d. glass, 2 m in length, packed with 2 % OV-1 on a 80-100 mesh chromosorb W treated with AW-DMCS. The operating parameters of the GLC were : column and injector temperatures of 190° and 220°, respectively, with a carrier gas (nitrogen) flow rate of 30 ml/min. The concentration of DBP and the TMS derivatives of MBP and PA were caliculated from each standard calibration curve.

Results and Discussion

From the results of the previous experiment (Kaneshima et al.

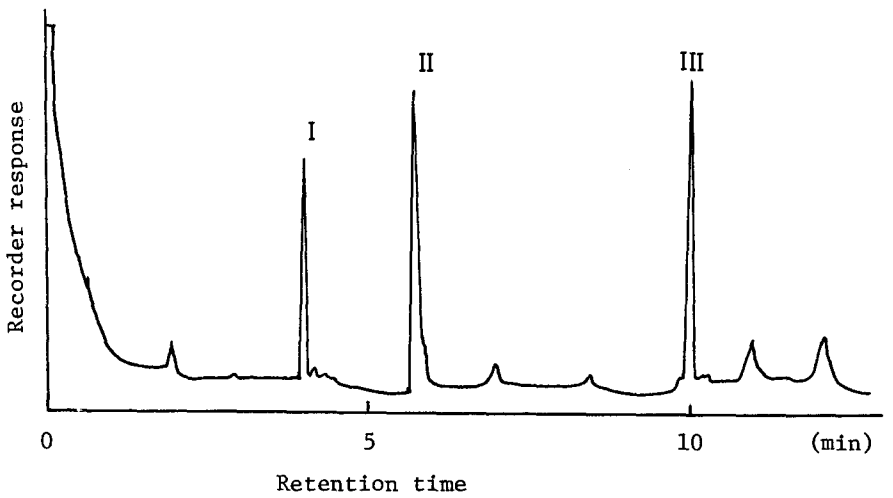

Fig.1 Gas chromatogram of DBP and its TMS-metabolites in ethyl acetate extract

I ; PA-TMS II ; MBP-TMS III ; DBP

1978), the main metabolites of DBP in the rat small intestine would be expected to be MBP and PA. However, the gaschromatographic separation of both compounds on 2 % OV-1 as a free form was not sufficient for quantitation, so their TMS derivatives were prepared for GLC determination.

As shown in Fig.1, DBP,and the TMS derivatives of MBP or PA included in the ethylacetate extract from rat intestinal homogenate were successfully separated on 2 % OV-1 without any disturbance of the other biological substances.

The in vitro metabolism of DBP in the rat small intestine homogenate at 37° is shown in Fig.2. As early as 30 min after incubation more than 90 % of the DBP initially added was degraded, while about 90 % of the MBP and a small amount of PA were simultaneously formed, indicating that there was a correlation between the rate of the degradation of DBP and that of the formation of MBP.

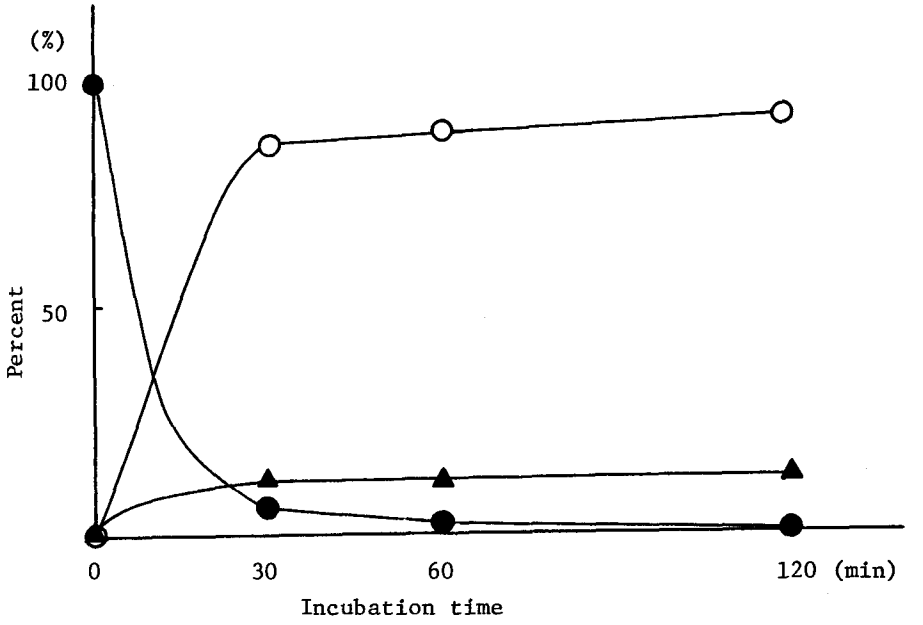

Fig.2 In vitro metabolism of DBP by the small intestine homogenates of rats

●—● ;DBP ○—○;MBP ▲—▲;PA

As reported previously, MBP and its glucuronide were found to be excreted as the main metabolites in the rat bile of orally administered DBP. Thus, these results may indicate that DBP orally given to rats would be absorbed mostly from the small intestine as MBP, and then excreted into the bile after passing through the liver.

In the in vitro studies with rat intestinal preparations, similar results were obtained by Lake et al. (1977) who reported that with six phthalate diesters examined the formation of the corresponding monoester accounted for more than 90 % of the total metabolite formed.

On the basis of these results they concluded that any toxic effects of orally ingested PAE would be governed essentially by the properties of the corresponding monoesters.

On the other hand, the toxic effects of phthalate monoesters have recently been clarified by other workers.

For example, when administered to rats, mono-(2-ethylhexyl) phthalate (MEHP) produced liver enlargement accompanied by mitochondrial changes (Lake et al.1975) and MBP also produced testicular damage (Carter et al.1977). In addition, the teratogenic effects of MEHP in mice were indicated by Yagi et al. (1977).

From the results obtained and the findings mentioned above, it could be concluded that the small intestine plays an important role in the metabolic fate of PAE, and thus the toxicities of the corresponding monoesters formed in the small intestine must be pursued further to evaluate the safety of PAE.

Acknowledgment

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