A Novel Route of Excretion of 2, 4, 3′, 4′-Tetrachlorobiphenyl in Rats

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Polychlorinated biphenyls (PCBs), one of the environmental pollutants, in parallel with other organic chlorocompounds (BHC, DDT, dieldrin and others) have been accumulated in human tissues through food chains, because of their high degree of biochemical stability and lipid solubility, and found to be hardly eliminated from human tissues (BIROS et al. 1970). However, in our previous study on the elimination of PCBs preparation, Kanechlor-400 (KC-400), with about 48 % chlorine content, it was found that even the components with high chlorine content (more than tetrachloro-derivatives) were eliminated very gradually from mice tissues (YOSHIMURA and ÔSHIMA 1971). WILLIAMS et al. (1965) found that dieldrin injected intravenously into the bile duct cannulated rats was excreted in part as unchanged in the feces, suggesting the excretion of this organic chlorocompound from the gut wall. These results imply that a part of PCBs may be excreted also as unchanged in the gastrointestinal tract.

This assumption was supported from the follwing findings. (I) In rats, both 2,4,3′,4′-tetrachlorobiphenyl (2,4,3′,4′-TCB) and 3,4,3′,4′-tetrachlorobiphenyl (3,4,3′,4′-TCB), which were found to be abundant components of KC-400 (SAEKI et al. 1971), were metabolized to monohydroxy-derivatives [5- and 3-hydroxy-2,4,3′,4′-TCB (YOSHIMURA et al. 1973, YAMAMOTO and YOSHIMURA 1973) and 5- or 2-hydroxy-3,4,3′,4′-TCB (YOSHIMURA and YAMAMOTO 1973, YOSHIMURA and YAMAMOTO 1974), respectively], and excreted into the feces through biliary system, but the bile did not contain the unchanged compound in both cases (YOSHIMURA et al. 1974). Nevertheless, rats which were orally administered with either 2,4,3′,4′-TCB or 3,4,3′,4′-TCB, excreted a small amount of the unchanged compound together with above metabolites in the feces for long period of time (YOSHIMURA et al. 1973). It was hardly believable that a part of the orally administered compound, which was unabsorbed from the gastrointestinal tract, continued to excrete for more than 10 days after the administra-

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tion. (II) In the case of mice which was administered intraperitoneally with 2,4,3',4'-TCB, both unchanged 2,4,3',4'-TCB and its metabolites were excreted into the feces (YOSHIMURA et al. 1974). Recently it was also reported that some parts of pure mono-, di-, tetra- and hexachlorobiphenyl isomers injected into rats were excreted as unchanged in the feces (HUTZINGER et al. 1972). Purpose of the present study is to present the first evidence that 2,4,3',4'-TCB in the body of the rat is excreted gradually through the small intestinal wall. The toxicological importance of this excretion route for neutral, lipid-soluble and biochemically stable compounds will be also discussed.

Materials and Method

2,4,3',4'-TCB, which was prepared according to the method of SAEKI et al. (1971), was dissolved in a mixture of Tween 80 - saline (1:3) to make 1 % (W/V) suspension. This suspebsion (0.1 ml), which contained 1.0 mg of 2,4,3',4'-TCB, was intravenously injected into the adult male rat of Wistar King strain anesthetized with ether. For the studies on determination of unchanged compound in the feces and in the content of gastrointestinal tract, 3 rats weighing 180-196 g and 12 rats weighing 152-210 g were used, respectively. In the latter experiment the bile duct of animals was ligated with a silk thread. Each animal was housed in an individual metabolic cage giving free access to food and water, and the urine and feces were separately collected for 4 days after the injection. The contents of stomach, small intestine, caecum, large intestine and rectum were also taken at 1, 3, 6, 18 and 24 hr after the intravenous injection of 2,4,3',4'-TCB.

The feces, after dried in a desicator (P_2O_5) and powdered with mortar, were extracted with $CHCl_3$ by Soxhlet extractor for 14 hr. The content of gastrointestinal tract was mixed with anhydrous Na_2SO_4 (ca. 10 g) for drying and shaked three times with 20 ml of n-hexane. This extract was diluted with n-hexane to adjust to 100 ml and submitted to gas liquid chromatography (GLC). The instrument used for this GLC was a Shimadzu GC-3AE gas chromatograph equipped with electron capture detector. The column (4 mm x 2.5 m) packing was 1.5 % SE-30 on Chromosorb W (60-80 mesh) and the column temperature was maintained at 200°. Nitrogen was used as a carrier gas with the flow rate of 60 ml/min (1.5 Kg/cm^2).

Result

Excretion rate of unchanged 2,4,3',4'-TCB in the rat feces

Excretion rate of unchanged compound in the rat feces was determined by GLC during a period of 4 days after intravenous injection of 2,4,3',4'-TCB at a single dose of 1.0 mg/body. The results are summarized in Table I.

TABLE I
Fecal excretion of unchanged 2,4,3',4'-TCB in rats after I.V. injection (1.0 mg/body)

(% of dose)				
0 - 24	24 - 48	48 - 72	72 - 96 hr	Total
0.8±0.18	0.5±0.03	0.5±0.07	0.7±0.31	2.5±0.47

Values are means ± S.D. of 3 rats

As can be seen in Table I, unchanged compound excreted during 4 days accounted for about 2.5 % of the dose, and as average about 0.6 % of the dose were excreted everyday for 4 days in rat feces. This result suggests that the gastrointestinal tract may be an important site of excretion of 2,4,3',4'-TCB in rats, because it has been established already that unchanged 2,4,3',4'-TCB is not excreted into the bile (YOSHIMURA et al. 1974).

Excretion of unchanged 2,4,3',4'-TCB in the gastrointestinal tract of rats

In order to eliminate the possibility of biliary excretion of 2,4,3',4'-TCB, bile duct of rats was ligated with a silk thread. These rats were injected intravenously with 2,4,3',4'-TCB and were killed at 1, 3, 6, 18 and 24 hr after the injection. The contents of stomach, small intestine, caecum, large intestine and rectum were taken out separately and extracted with n-hexane and ethylacetate according to the procedure shown in Fig. 1.

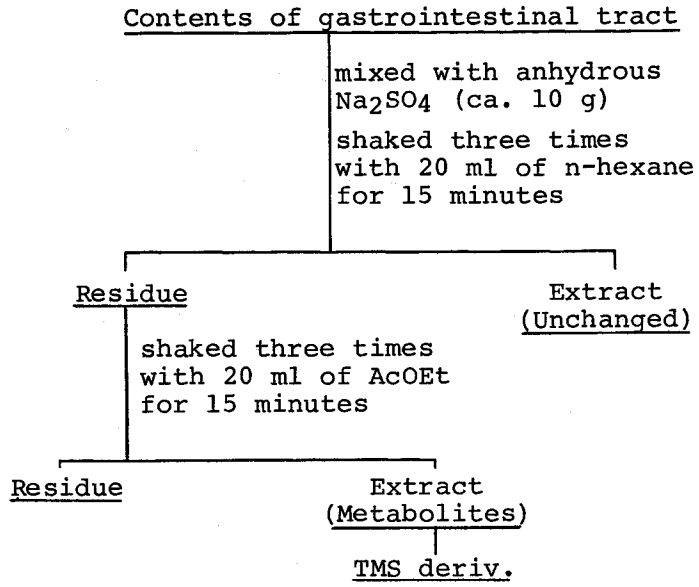

Fig. 1. Extraction procedure of contents of gastrointestinal tract

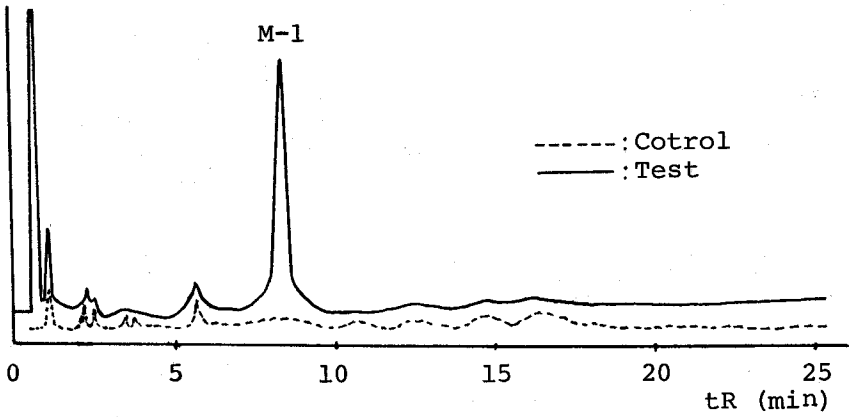

Fig. 2. Gas chromarogram of trimethylsilylated extract of small intestinal content in rat administered with 2,4,3',4'-TCB

A typical gas chromatogram of the n-hexane extract of the small intestinal content obtained 1 hr after the administration is shown in Fig. 2. As can be seen in Fig. 2, one peak having retention time of 8.5 min. (M-1) was shown to be same as that of unchanded compound. No metabolites were detected in any samples from these bile duct ligated rats.

Cumulative excretion rate of unchanged 2,4,-3',4'-TCB in each gastrointestinal content was illustrated in Fig. 3. It was evident from Fig. 3 that at 1 hr after the injection, unchanged compound (about 0.1 % of the dose) appeared already in the small intestinal content but not in the contents of any other parts of intestine. During a period of 3 and 6 hr after the injection the unchanged 2,4,3',4'-TCB excreted in the small intestine accounted for about 0.2 % and 0.3 % of the dose, respectively, and a trace amount of 2,4,-3',4'-TCB was detected also in the contents of caecum and rectum during both periods of time. These results suggest that the rat small intestinal wall is a major site of excretion of unchanged 2,4,3',4'-TCB. About 0.6 % of the dose was detected as the unchanged compound in the content of gastrointestinal tract during a period of 24 hr after the injection of 2,4,3',4'-TCB.

Discussion

In general, PCB components of high chlorine content are hardly eliminated from the mammalian body, because they are not metabolized easily and therefore difficult to be excreted into either urine or feces. However, in our previous study on the elimination of KC-400 in mice (YOSHIMURA and ÔSHIMA 1971), it was found that even these components which seemed not to be metabolized disappeared little by little from the tissues. The present investigation was undertaken to solve these problems and explored that as average about 0.6 % of the dose were excreted everyday into the small intestine of rats after the intravenous injection of 2,4,3',4'-TCB. It was also found that no metabolites were excreted from any parts of the gastrointestinal wall. These results strongly suggested that from the toxicological point of view this excretion route through the small intestinal wall is very important for the neutral, highly lipid-soluble and metabolically stable compounds such as PCBs. Because, this finding may be useful for considering promotion of excretion of PCB components from human tissues.

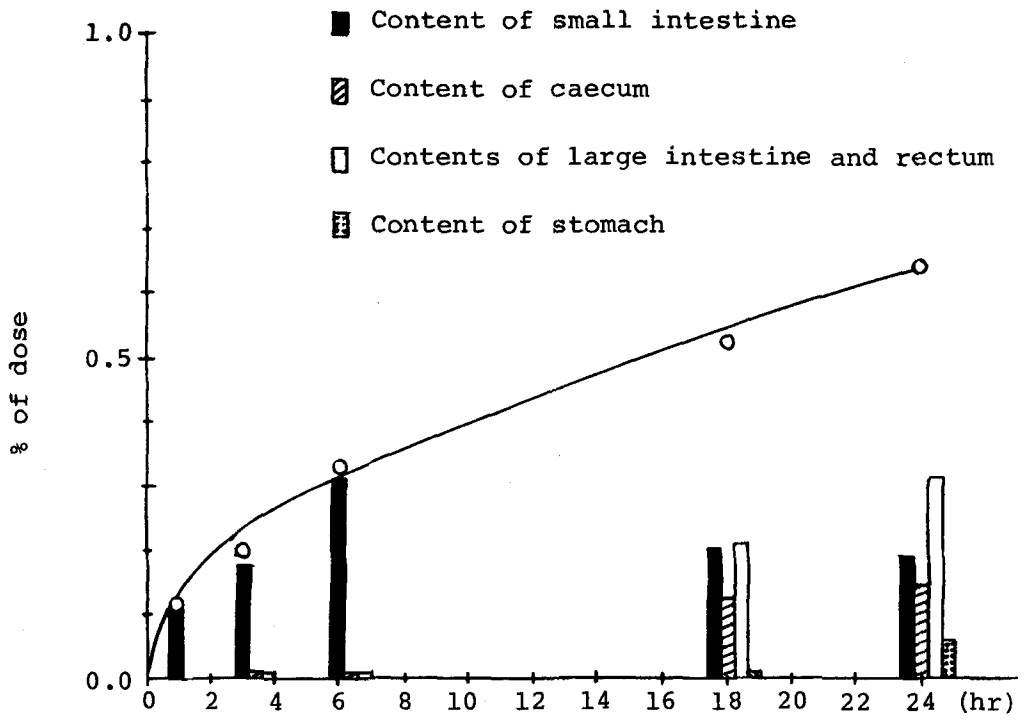

Fig. 3. Cumulative excretion of unchanged 2,4,3′,4′-TCB into gastrointestinal tract

Concerning with the studies on excretion of foreign compounds into the gastrointestinal tract, SHORE et al. (1957) already established that the basic compounds with pKa 5.0 or higher (aminopyrine and others) were excreted into the stomach. For acidic (diphenylhydantoin) or neutral compounds (dieldrin and digoxin), suggestions were provided that these compounds were excreted into the small intestine (NOACH et al. 1958, WILLIAMS et al. 1965, HARRISON, JR. et al. 1966). However, as far as we know, no definite conclusion has been obtained before the present report for the excretion of neutral compounds from the small intestinal wall. Considering the present results, it is very likely that the site of excretion of PCB isomers which were reported by HUTZINGER et al. (1972) would be also the small intestinal wall. The mechanism of this excretion is unclear at present and must be elucidated in near future.

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