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A Comparison of *in Vivo* and *in Vitro* (Tissue Explant) Techniques: Metabolic Profile of Methylethylbenzene Isomers in Rats and Dogs

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This laboratory developed an in vitro technique which semiquantitatively reproduced in vivo metabolic processes of the insecticide carbaryl in dog, guinea pig, rat and man (SULLIVAN et al. 1972, CHIN et al. 1974, 1979).

The object of this study was to evaluate whether the in vitro technique is also operative when the hydrocarbon product - methylethylbenzene isomers (MEB) - is used as the test chemical in rats and dogs. To meet this requirement, metabolic profiles of the test chemical in the urine of rats and dogs exposed to the vapors of the test chemical were generated and these profiles were compared with in vitro liver-generated metabolic profiles which resulted from the incubation of the test chemical with livers from rats and dogs. The liver was the organ of choice for this investigation because many metabolites formed in liver were found in the urine (PARKE 1968, CHIN et al. 1979).

MATERIALS AND METHODS

^{14}C-ring lableled MEB, having specific activity of approximately 5 mCi/mmole, was obtained from New England Nuclear, Inc. Unlabeled MEB was obtained from Pfaltz & Bauer, Inc. The sample of MEB was a mixture of ortho, meta, and para isomers at a ratio of approximately 1:2:1 respectively. The stock solutions of radioactive MEB isomers used were diluted with corresponding nonradioactive samples to give 2 to 3 x 10^6 cpm (counts per min)/uL of solution. The ratio of isomers for the sample of MEB was same between labeled and unlabeled material.

Rat Inhalation. Three Harlan-Wistar rats (100-120 g) were exposed for 6 h to MEB vapor at an approximate concentration of 1 mg/L. Detailed chamber design and methodologies are described in CHIN et al. (1980b) and CARPENTER et al. (1975).

Dog Inhalation. A restrainer attached to a 12-L Plexiglas® box was used for head exposure of a 6-year-old female beagle dog weighing 11 kg. The head of the beagle was confined in the box by means of a split Masonite® plate fitted to the neck size of the average adult beagle, and gasketed with rubber to insure an airtight fit. A 4-L plastic bag attached to one outlet was used to minimize the pressure changes produced in the box during respiration.

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A Komhyr Teflon® pump delivering 1 L/min was used to circulate the air and an Ascarite® trap used to collect the CO_2 built up during respiration. Oxygen was delivered into the system at approximately 70 mL/min to maintain an oxygen concentration of 20.9% in the system.

In Vitro Studies. Livers from Harlan-Wistar rats (100-120 g) from our breeding colony were used. A mature 4.5-year-old female beagle dog was anesthetized deeply with 10-15 mL methyoxyflurane and exsanguinated. The liver was then removed and immediately prepared for the in vitro metabolism studies.

Liver explants were prepared and transferred to a 60 x 15 mm petri dish containing 3 mL of TROWELL T8 (1959) medium according to the methods of SULLIVAN et al. (1972), then the dish was placed in a leak-tight 2-L stainless steel chamber. The entire unit was flushed with a 95% oxygen; 5% carbon dioxide mixture at the rate of 1.5 L/min for 10 min and the appropriate dose of test chemical was injected.

For all in vitro studies, a stock solution of ring ^{14}C-labeled MEB with specific activity of 0.1 mCi/mg was diluted with non-radioactive MEB to give 16 to 30 x 10^6 cpm (counts per min)/µL of solution. For all in vitro studies, a dose of 10 uL of ^{14}C-MEB per 2-L stainless steel chamber was used. Under these conditions, the uptake rate of radioactivity by various tissues in an in vitro chamber was 0.5 to 3% of the administered dose; which is sufficient for further column chromatographic analyses.

Analytical Procedures for Hydrocarbon Metabolites. Diethylaminoethyl-sephadex (DEAE-Sephadex) columns were used for the analyses of all the metabolites present in animal urines or growth medium with the column prepared as follows:

Fourteen g of DEAE-Sephadex were weighed into a beaker and 100 mL 0.01 N NH_4OH added. This slurry was placed on a steam bath for approximately 2 h and then poured into a 1.2 x 24 cm glass column. The column was then washed with approximately 700 mL 0.01 N NH_4OH followed by washing with 0.005 N ammonium acetate (pH 6.5) until the eluent was brought to pH 6.5. The column was then ready to be run for MEB metabolites. The elution gradients for these columns consisted of 0.005 to 0.05, 0.05 to 0.5 and 0.5 to 1 N ammonium acetate utilizing 300 mL of each concentration gradient. Four mL fractions were collected, and every fifth fraction was analyzed by liquid scintillation counting techniques.

RESULTS

A typical DEAE-Sephadex chromatogram of the in vivo rat metabolites of ^{14}C-MEB in 24-h urine specimens following a 6-h inhalation period of MEB vapor is shown in Figure 1. The quantitative results obtained from both the in vivo and in vitro metabolites of MEB by rat and dog are given in Table 1.

Major in vivo metabolites found in rat urine were B, C, and D representing 35, 29, and 31% of the radioactivity respectively. Minor metabolites were A, E, F, and G representing 0.8 to 2% of the dose applied to the column.

Rat liver produced all of the major in vivo MEB metabolites B, C, and D and minor in vivo metabolites E, F, and G. In addition to these metabolites, liver made significant amounts of metabolite A.

Major in vivo dog metabolites found were B, C and D representing 19.8, 27.9 and 48.5% respectively. Minor metabolites were A and F representing 1.0 and 2.2% of the ^{14}C recovered from the column respectively. Dog liver produced all of the major in vivo MEB metabolites B, C, and D. In addition to these metabolites, liver made significant amounts of metabolite A (neutral fractions). Minor in vivo MEB metabolites F and G found in dog liver amounts to 1.1 to 1.5% of ^{14}C recovered from the column.

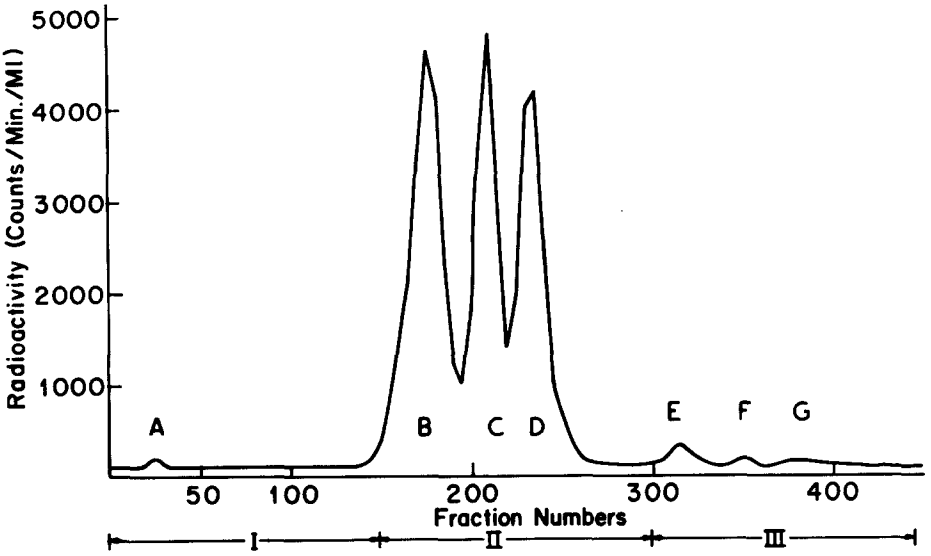

Figure 1. DEAE-Sephadex Chromatography of ^{14}C-methylethylbenzene Isomers Metabolites in Rat Urine

DISCUSSION

A major portion (72%) of the absorbed MEB was excreted in the urine of the rat when rats were exposed to the average chamber concentration of 1 mg MEB/L (CHIN et al. 1980b). Based on the profile analysis of the rat urine on DEAE-Sephadex, a total of 7 unknown MEB metabolites were found. Two of the 7 metabolites accounted for 65% of the radioactivity in the 24-h urine. The identities of these major metabolites B to D, which account for 95% of the urinary metabolites are unknown. They have anionic characteristics and polarity similar to the major ethylbenzene (EB) metabolites (CHIN et al. 1980a) based on DEAE-Sephadex and

TABLE 1. Metabolic Profiles of ^{14}C-Methylethylbenzene Isomers in Rat and Dog[a]

Animal	Technique Used	uL MEB per animal or in vitro Chamber	Metabolites A	B	C	D	E	F	G
Rat	Inhalation[b]	20	0.8	34.9	29.1	31.1	2.1	1.2	0.8
	In Vitro	10	15.0	28.6	35.0	18.1	1.2	1.1	1.0
Dog	Inhalation[c]	110	1.0	19.8	27.9	48.5	2.2	trace	trace
	In Vitro	10	14.3	40.0	31.5	11.6	0	1.1	1.5

[a] Metabolites are expressed as percent of total radioactivity recovered from column

[b] 6-h exposure

[c] 3.5-h exposure

silica gel column chromatographic profiles. Because it appears that aliphatic oxidation is readily accomplished (ELLIOTT et al. 1964) when there is a choice between aliphatic and aromatic hydrocarbon oxidation, the possible major EMB metabolites would be very similar to major EB metabolites. At least one of the major metabolite could be hippuric acid (glycine conjugate of a benzoic acid) because characteristic elution pattern of the major EB metabolite by silica gel chromatography was the same as that of the hippuric acid by silica gel chromatography (CHIN 1973).

Chromatographic profiles of EMB metabolites from dog were very similar to those of rats qualitatively and were slightly different quantitatively.

Based on the chromatographic profile analysis of *in vitro* derived metabolites of MEB in rat and dog, the *in vitro* results semiquantitatively reproduced the *in vivo* urinary metabolism of MEB in the corresponding animal species.

This *in vitro* study confirmed earlier studies (SULLIVAN et al. 1972) that the *in vitro* technique is species specific and therefore offers promise as a method to determine metabolism in man semiquantitatively without resorting to the direct dosing of a human subject. The metabolic information of man obtained by the *in vitro* techniquecan facilitate selection of animals with a metabolic pattern similar to man to be used for further in-depth toxicity studies.

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