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A Comparison of *in Vivo* and *in Vitro* (Tissue Explant) Techniques: Metabolic Profile of Ethylbenzene in the Rat and the Dog

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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 this in vitro technique is also operative when hydrocarbon product - ethylbenzene (EB) - is used as the test chemical in rat and dog. To meet this requirement metabolic profiles of test chemical in urine of the rats and dogs exposed to vapor of test chemical were generated and these profiles were compared with in vitro liver-generated metabolic profiles which resulted from the incubation of test chemical with the rat and dog livers. The liver was the organ of choice for this investigation because many metabolites formed in liver predominated in the urine (PARKE 1968, CHIN et al. 1979).

MATERIALS AND METHODS

Rat Inhalation. Three Harlan-Wistar rats (100-120 g) were exposed for 6 h to a EB vapor at approximate concentration of 1 mg/L. Detailed chamber design and methodologies are described in CHIN et al. (1980) 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 air-tight fit. A 4-L plastic bag attached to one outlet was used to minimize the pressure changes produced in the box during respiration.

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 own breeding colony were used. Mature 4.5-year-old female beagle dog was anesthetized deeply with 10-15 mL methyoxyflurane

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and exsanguinated. The liver was then removed and immediately subjected to 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 carbogen 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 EB with specific activity of 0.1 mCi/mg was diluted with non-radioactive EB to give 16 to 30 x 10^6 cpm (counts per min)/uL of solution. For rat in vitro studies, a dose of 5 and 50 uL of ^{14}C-EB per 2-L stainless steel chamber was used. For dog in vitro study, a dose of 10 uL of ^{14}C-EB was used. Under these conditions, the uptake rate of radioactivity by various tissues in 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:

Seven 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 EB 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

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

Dog liver made most of the major in vivo metabolites except for metabolites A-1 and F. Dog liver made metabolite E not produced by dog in vivo study.

Major in vivo metabolites found in rat were B representing 51 to 57% and C representing 33 to 37% of the radioactivity applied to the column. Minor metabolite D represented 6.5 to 7.5% of the dose applied to the column. Metabolic profiles of EB in dog urine

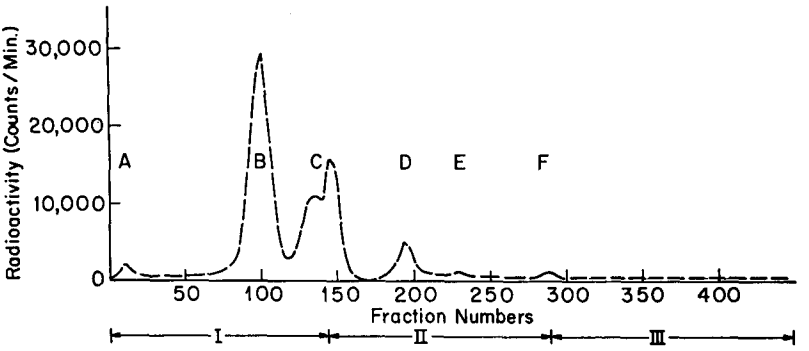

Figure 1. DEAE-Sephadex Chromatography of ^{14}C-Ethylbenzene Metabolites in Rat Urine

was very similar to that of rat except that quantity of metabolite C was twice that of metabolite B.

Rat in vitro studies with two different dosages produced two major in vivo metabolites B and C but were not able to generate in vivo metabolite D. Rat in vitro study with the dosage of 5 uL EB/2-L chamber, amount of metabolites A and B were 19 and 70% respectively of the radioactivity recovered from column. At 10x higher concentration, quantity of metabolites A and B were 47 and 45% respectively of the radioactivity recovered from the column.

DISCUSSION

A major portion (80-90%) of the absorbed EB was excreted in the urine of the rat when rats were exposed to the average chamber concentration of 1 mg EB/L (CHIN et al. 1980). Based on the profile analysis of this rat urine on DEAE-Sephadex, a total of 6 unknown EB metabolites were found. Two of the 6 metabolites accounted for 90% of the radioactivity in the 24-h urine. These two major metabolites must have similar polarity because further analysis using silica gel column chromatography did not resolve these components. 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).

Dog EB metabolites were very similar qualitatively to the rat, but the ratio of metabolites B to C in the rat were reversed in the dog. This species difference in the metabolism of EB is easily

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

Animal	Technique Used	uL EB per animal or *in vitro* Chamber	Metabolites						
			A	A-1	B	C	D	E	F
Rat	Inhalation[b]	20	2.7	0	57.2	33.3	6.5	0	0.3
		20	3.6	0	51.1	36.8	7.5	0.1	0.9
	In Vitro	5	19.3	0	70.0	10.7	0	0	0
		50	46.8	0	45.1	8.1	0	0	0
Dog	Inhalation[c]	50	1.2	4.7	25.0	51.8	10.4	0	5.9
	In Vitro	10	12.7	0	7.3	69.8	6.6	3.6	0

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

[b] 6-h exposure

[c] 3.5-h exposure

explained by examining the metabolic profiles of EB by the livers from each species. In vitro metabolite B produced by the rat liver was 10-fold higher than that of dog liver. On the other hand, the metabolic C produced by the rat liver was 6-fold lower than that of the dog liver. Consequently, concentration of metabolite B found in rat urine was higher than in the dog urine, and metabolite C in rat urine was lower than in the dog urine.

Based on the chromatographic profile analysis of in vitro derived metabolites of EB in rat and dog, the in vitro results semiquantitatively reproduced in vivo urinary metabolism of EB 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 without resorting to the direct dosing of human subject. The metabolic informations of man in vitro can facilitate selection of animals with a similar metabolic pattern to man for further in depth toxicity studies.

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