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A Comparison of *in Vivo* and *in Vitro* (Tissue Explant) Techniques: Metabolic Profile of Ethylcyclohexane in Rat and 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, 1979b).

The object of the study reported herein was to evaluate whether the in vitro technique is also operative when the hydrocarbon product - ethylcyclohexane (ECH) - is used as the test chemical in rat and dog. To meet this requirement, metabolic profiles of the test chemical in the urine of rats and dogs exposed to the vapor of the test chemical were generated and these profiles were compared with in vitro metabolic profiles generated by liver. The latter resulted from the incubation of the test chemical with the livers of either rats or dogs. 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. 1974, 1979b).

MATERIALS AND METHODS

^{14}C-ring labeled ECH, having a specific activity of approximately 5 mCi/mmole, was obtained from New England Nuclear, Inc. Unlabeled ECH was obtained from Phillips Petroleum Co.

Rat Inhalation. Three Harlan-Wistar rats (100-120 g) were exposed for 6 h to ECH 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.

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.

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In Vitro Studies. Livers from Harlan-Wistar rats (100-120 g) from our own 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 ECH with specific activity of 0.1 mCi/mg was diluted with non-radioactive ECH to give 16 to 30 x 10^6 cpm /uL of solution. For all in vitro studies, a dose of 10 uL of ^{14}C-ECH 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:

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 for use to separate ECH 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-ECH in 24-h urine specimens following a 6-h inhalation period of ECH vapor is shown in Figure 1. The quantitative results obtained from both the in vivo and in vitro metabolites of ECH by rat and dog are given in Table 1.

Major in vivo metabolites found in rat were fractions from 70 to 130 (metabolite B) which represent 91 to 92% of the total radioactivity recovered from the column. Minor metabolites which

represent 1 to 20% of the dose on the column were metabolites A, C, D, E, and F. Rat liver produced in vivo metabolites A, B, C, and D but was not able to generate minor in vivo metabolites E and F.

Major in vivo metabolites found in dog were fractions 70 to 130 (metabolite B) which represent 77% of the total radioactivity recovered from the column. A neutral component (metabolite A) and metabolite C consist of 12.6 and 6% of the radioactivity recovered from the column respectively. Minor metabolites, amounting to 2%, were metabolite F and a new metabolite G (fractions 280 to 320). Metabolites D and E were not found.

Dog liver made all of the major in vivo dog metabolites except for metabolite G. In addition to the in vivo metabolites, dog liver also made metabolites D and E.

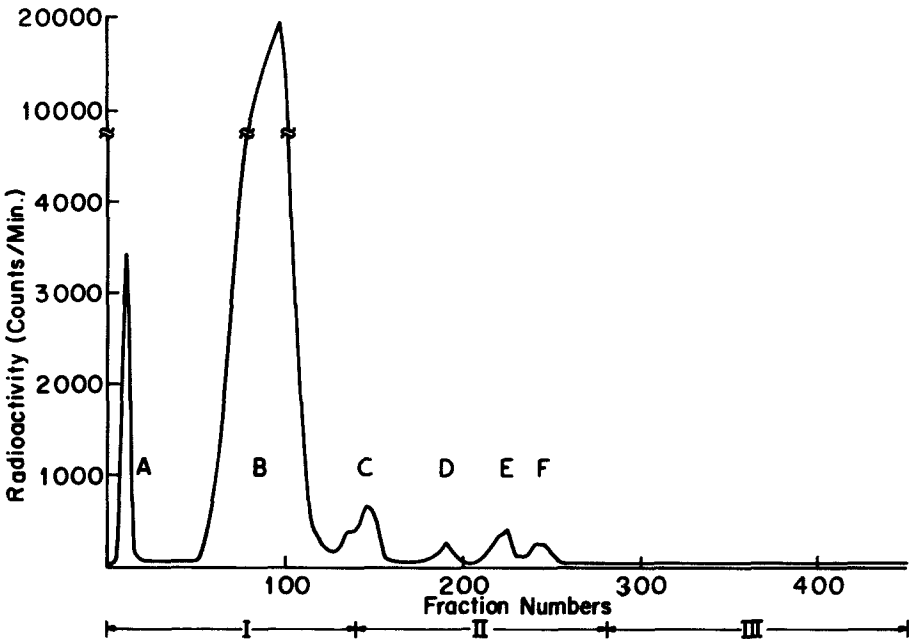

Figure 1. DEAE-Sephadex Chromatography of ^{14}C-Ethylcyclohexane Metabolites in Rat Urine

DISCUSSION

A major portion (65%) of the absorbed ECH was excreted in the urine of the rat when rats were exposed to the average chamber concentration of 1 mg ECH/L (CHIN et al. 1980b). Based on the profile analysis of the rat urine on DEAE-Sephadex, a total of 6 unknown ECH metabolites were found. One of the 6 metabolites

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

Animal	Technique Used	uL ECH per animal or *in vitro* Chamber	Metabolites							
			A	A-1	B	C	D	E	F	G
Rat	Inhalation[b]	20	2.0	0	91.4	2.3	1.4	1.9	1.0	0
		25	2.4	0	92.2	2.1	0.7	1.4	1.1	0
	In Vitro	10	8.7	0	75.7	9.8	5.8	0	0	0
Dog	Inhalation[c]	50	12.6	0	77.0	6.2	0	0	2.0	2.2
	In Vitro	10	22.9	2.2	57.6	4.9	8.6	1.7	2.1	0

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

[b] 6-h exposure

[c] 3.5-h exposure

accounted for 90% of the radioactivity in the 24-h urine. A possible major ECH metabolite in rats is the glucuronic acid conjugate of side chain hydroxylated ECH. Chromatographic profiles of ECH metabolites from dog were very similar to those of rats qualitatively and were slightly different quantitatively. Metabolite A in rats and dogs amounts to 2% and 13% of the urinary metabolites, while metabolite C accounts for 2% and 6% of the urinary metabolites respectively.

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

This in vitro study confirmed earlier studies (SULLIVAN et al. 1972, CHIN et al. 1979a, 1980a) 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 from man obtained by the in vitro technique can facilitate selection of animals with a metabolic pattern similar to that of man to be used for further in-depth toxicity studies.

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