Carbaryl Metabolism by the Selected Tissues of the Dog via the in Vitro Explant-Maintenance Technique

Recently we introduced the in vitro tissue-maintenance technique which faithfully reproduced the metabolites of carbaryl (1-naphthyl N-methylcarbamate) found in urine samples of similarly dosed animals. This study was undertaken to demonstrate the contributory metabolic role of kidney, liver, and lung of the dog toward carbaryl using this in vitro technique. Hepatic tissues of the dog incubated with low dosages of radioactivity labeled carbaryl actively performed demethylation, hydrolysis, hydroxylation, and oxidation, followed by sulfate conjugations. The kidney and lung of the dog made one and three of the five liver-generated metabolites, respectively. The metabolic profiles from these selected organs clearly show that the metabolic products found in vivo represent a composite of metabolites formed from at least the liver, lung, and the kidney.

The explant-maintenance technique (Sullivan et al., 1972a) using liver tissue of animals and man successfully reflected the in vivo metabolism of carbaryl (1-naphthyl *N*-methylcarbamate) in animals and man. Subsequently, the in vitro technique was extended to nonhepatic tissues of man (Chin et al., 1974, 1979) and of the rat (Chin et al., 1978). This in vitro technique clearly demonstrated the organ specificity of the metabolic products of carbaryl.

The purpose of this report on the metabolism of carbaryl by kidney, liver, and lung of dog using the in vitro technique is to demonstrate the contributory metabolic role of individual organs toward carbaryl. The designation of major metabolites of carbaryl is based on chromatographic behavior and fluorescence characteristics rather than actual isolation and structural verification of the metabolites.

EXPERIMENTAL SECTION

Two male beagle dogs were used for this study. Dog no. 76916, weighing 6 kg, was sacrificed 30 min after anesthetization with ether and dog no. 81620, weighing 11.5 kg was sacrificed 30 min after ip dosing with 33 mg/kg (BW) sodium pentobarbital (PB). Kidney, liver, and lung from these dogs were removed and processed in accordance with the explant-maintenance technique of Sullivan et al. (1972b) using 1-naphthyl-^{14}C *N*-methylcarbamate ([*naphthyl*-^{14}C]carbaryl) of specific activity 30 μCi/mg and *N*-methyl-^{14}C-carbaryl ([*methyl*-^{14}C]carbaryl) of specific activity 11.3 μCi/mg. These chemicals were provided by Union Carbide Corporation Technical Center, South Charleston, WV. The carbogen used was the commercially available 95% oxygen/5% carbon dioxide mixture. Trowell T8 medium (1959) and penicillin–streptomycin mixture were purchased from Microbiological Associates, Bethesda, MD. At the end of the incubation period, the culture medium was removed and subjected to diethylaminoethyl (DEAE)–cellulose column chromatography (Sullivan et al., 1972a,b) to determine the metabolic profile.

RESULTS

The metabolic profile analyses of naphthyl-^{14}C and methyl-^{14}C carbaryl for kidney, liver, and lung of the dog anesthetized with ether are summarized in Table I. Qualitatively, peaks A and B are considered to be neutral to the DEAE–cellulose anion-exchange system and no conjugates have been found in this area. Peak C is an unknown weakly acidic metabolite. Peak D was determined to be a group of metabolites, the major component of which was the 5,6-dihydro-5,6-dihydroxycarbaryl glucuronide (Sullivan et al., 1972b). Peak F has been shown to be glucuronides of ring hydroxylated carbaryls. Peak G was determined to be a naphthyl glucuronide in rat (Knaak et al., 1965) but the same peak in this study was labeled as G*, because even though it had chromatographic elution volumes characteristic of naphthyl glucuronide, it did not fluoresce as this compound should. The exact identification of peak G* was not made. Peaks I and J are sulfate conjugates of ring hydroxylated carbaryls and 1-naphthol, respectively (Knaak et al., 1965; Knaak and Sullivan, 1967).

As shown in Table I, all tissues produced neutral fractions with naphthyl-^{14}C. Significant amount of peak D (Sullivan et al., 1972b) was made by liver and not by kidney or lung of the ether-anesthetized dog. Both liver and lung appeared to ring hydroxylate carbaryl, followed by sulfate conjugation as evidenced by the formation of peak I. A small quantity of hydroxycarbaryl glucuronide (peak F) was made only in the liver. Significant quantities of peak G* were made in the liver as well as in the lung. This unique peak was also reported as the unknown dog urinary metabolite (Knaak and Sullivan, 1967) as described earlier. Naphthyl sulfate (peak J) was the major anionic metabolite of kidney and lung by a minor metabolite of liver.

Quantitative data were obtained from the metabolic profile analyses of carbaryl for kidney, liver, and lung of the dog anesthetized with PB. These data are not shown in Table I because the results were similar quantitatively to the metabolites generated from the three organs of the ether-treated dog with two major exceptions. These two major exceptions were found only in the liver-generated metabolites from naphthyl-^{14}C studies: (1) Peak D made by the liver of the PB-treated dog was ninefold less than that of the ether-treated dog. (2) On the other hand, peak J made by the liver of the PB-treated dog was sixfold more than that of the ether-treated dog.

DISCUSSION

The designation of major metabolites of carbaryl in this study was based on the chromatographic behavior and fluorescence characteristics rather than the actual isolation and verification of the metabolites. Based upon the anionic metabolic profiles of the in vitro metabolism of carbaryl by kidney, liver, and lung of the dog anesthetized with ether, all the tissues studied played a significant role in the metabolic processes. The major site of carbaryl metabolism appears to be in the liver of the dog. Hepatic tissues of the dog by the in vitro tissue-maintenance technique reproduced in vivo metabolites qualitatively and semiquantitatively (Knaak and Sullivan, 1967). It is evident that the kidney and lung tissues of dogs are capable of hydrolyzing carbaryl and conjugating the hydrolysis product with the formation of a sulfate. However, the kidney and lung of the dog made one and three of the five

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Table I. Comparison of in Vitro Metabolites of Carbaryl by Kidney, Liver, and Lung of the Dog[a]

	tentative identification by chromatographic position									
	A	B	C	D	F	G*	H	I	J	
tissue	neutrals to DEAE		UK[b]	dihydrodihydroxycarbaryl glucuronide, major aglycon	hydroxycarbaryl glucuronide	UK[b]	UK[b]	hydroxycarbaryl sulfate	naphthyl sulfate	pooled fractions[c]
					naphthyl-^{14}C-carbaryl					
kidney	44	0	0	0	0	0	0	0	51	5
liver	35	0	Tr	26	6	19[d]	0	4	3	7
lung	46	0	0	0	0	16[d]	0	4	27	7
in vivo[e]	⟵ 34 ⟶		0	16	11	14[d]	8	7	0	10
					methyl-^{14}C-carbaryl					
kidney	80	1	⟵ 18 ⟶		0	0	0	0	0	1
liver	69	0	⟵ 20 ⟶		5	0	0	1	1	4
lung	63	1	⟵ 27 ⟶		0	0	0	2	0	7
in vivo[e]	⟵ 46 ⟶		⟵ 31 ⟶		5	3[d]	3	4	0	8

[a] Calculated as percent of ^{14}C found in the medium. [b] UK = unknown. [c] Combined fractions from areas of chromatogram not containing a distinguishable peak. [d] Chromatographs as naphthyl glucuronide but does not fluoresce as this compound should. Identity unknown. [e] Data established by Knaak and Sullivan (1967).

liver-generated metabolites, respectively. Therefore, both kidney and lung failed to produce the complete in vivo carbaryl metabolic profile in the dog.

The most dramatic effect occurred only in quantities of peaks D and J in carbaryl metabolism by the liver of the PB-treated dog. Quantities of peak D decreased ninefold while peak J increased sixfold when compared to these peaks generated from the liver of the ether-treated dog. Because of this significant shift in quantities of peaks D and J, the present in vitro technique did not quantitatively reproduce the in vivo carbaryl metabolites of the untreated dog. Based upon this limited study, PB should not be used as an anesthetic prior to the sacrifice of the dog for in vitro metabolic studies.

The major conjugated metabolite of carbaryl in the human is naphthyl glucuronide (Sullivan et al., 1972a). However, the dog failed to produce naphthyl glucuronide both by in vivo (Knaak and Sullivan, 1967) and in vitro techniques. Therefore, the dog is definitely dissimilar to man in metabolizing carbaryl.

These comparative metabolic profiles from selected tissues clearly show that the metabolic products found in vivo represent a composite of metabolites formed from more than one organ. This view is supported by Gillette (1967). Investigation of the metabolic activity of individual organs for a given compound can be important in the interpretation of toxicological effects as they relate to a target organ.

LITERATURE CITED

Chin, B. H., Eldridge, J. M., Sullivan, L. J., *Clin. Toxicol.* 7, 37 (1974).
Chin, B. H., Eldridge, J. M., Anderson, J. H., Jr., Sullivan, L. J., *J. Agric. Food Chem.*, in press 1979.
Chin, B. H., Sullivan, L. J., Eldridge, J. M., Tallant, M. J., *Clin. Toxicol.* **14**(5) (1979).
Gillette, J. R., *Fed. Proc.*, **26**, *Fed. Am. Soc. Exp. Biol.* 1040 (1967).
Knaak, J. B., Sullivan, L. J., *J. Agric. Food Chem.* **15**, 1125 (1967).
Knaak, J. B., Tallant, M. J., Bartley, W. J., Sullivan, L. J., *J. Agric. Food Chem.* **13**, 537 (1965).
Sullivan, L. J., Chin, B. H., Carpenter, C. P., *Toxicol. Appl. Pharmacol.* **22**, 161 (1972a).
Sullivan, L. J., Eldridge, J. M., Knaak, J. B., Tallant, M. J., *J. Agric. Food Chem.* **20**, 980 (1972b).
Trowell, O. A., *Exp. Cell Res.* **16**, 118 (1959).

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