UNION CARBIDE CORPORATION
CHEMICALS AND PLASTICS
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SOUTH CHARLESTON PLANT

October 8, 1973

Kenneth D. Johnson, Ph. D.
Secretary
Technical Task Group on
Vinyl Chloride Research
Manufacturing Chemists Association
1825 Connecticut Avenue, N.W.
Washington, D. C. 20009

Dear Doctor Johnson:

Study of the two feasibility study proposals - one by Food and Drug Laboratories, Inc., and one by Foster D. Snell, Inc. - leaves me with the feeling that we have only one proposal; i.e., Foster D. Snell, Inc. In the case of Food and Drug Research, they simply took our suggestion on technic and returned it to us with elaboration. Foster D. Snell apparently gave the study consideration in depth and, as a result, would consider three alternatives. I have no direct knowledge of Foster D. Snell's performance on studies of this type but they talk a good game and would get my vote.

Developments in the vinyl bottle and coating areas recently raise a question as to whether we want to consider a rat-feeding study at all except as it may apply to a metabolism study. The S. P. I. is currently proposing to F. D. A. and others that the free VC monomer in resins used for food and liquor packaging be low enough that materials contained in those packages contain no detectable free VC monomer by the best available analytical t chnic. While not a specific part of the proposal, bottles made from PVC resin containing less than 25 ppm free monomer cause no measurable contamination of liquor. The best available analytical technic appears to be sensitive to 50 ppb. Based on this data, I think there is serious question regarding the need for an animal-feeding study.

For general information, I am attaching a copy of an article by Dr. C. P. Carpenter, et al on in vitro metabolism studies.

Very truly yours,

R. N. Wheeler, Jr.

RNWJr/ra

Attachment

(Distribution - see attached)

UCC
000714

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TOXICOLOGY AND APPLIED PHARMACOLOGY 22, 161–174 (1972)

In Vitro vs in Vivo Chromatographic Profiles of Carbaryl Anionic Metabolites in Man and Lower Animals[1]

L. J. SULLIVAN, B. H. CHIN, AND C. P. CARPENTER

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Received January 23, 1971

In Vitro vs in Vivo Chromatographic Profiles of Carbaryl Anionic Metabolites in Man and Lower Animals. SULLIVAN, L. J., CHIN, B. H., and CARPENTER, C. P. (1972). *Toxicol. Appl. Pharmacol.* **22**, 161–174. An in vitro technique for metabolic studies utilizing liver tissue has been developed based upon successful organ maintenance methods and chromatographic profiles. This technique qualitatively reflects in vivo metabolic processes of animals, including man, under prescribed conditions without resorting to the risk of dosing human beings. This was accomplished by the utilization of the published literature on in vivo metabolism of carbaryl in rat, dog, guinea pig and man. Under the required conditions, which simulate a dose level comparable to a chronic no-ill-effect feeding level in the rat, the variations between these in vitro and the previous in vivo results are substantially less than between species.

The prime objective of these studies was to develop a practical method to determine the metabolism of a chemical in man without direct human dosing and its attendant risks. It was hypothesized that an in vitro technique which would duplicate as faithfully as possible the conditions within the human body would best serve this objective. The assumption that metabolites formed in the liver (Parke, 1968) would predominate in the urine made it the organ of choice for this investigation. Its availability as a result of surgical procedure and diagnostic biopsy made its use practical. Therefore, the establishment of a suitable organ maintenance method for such tissue "explants" was attempted. The role of other organ tissues, e.g., kidney, lung and blood, in the total metabolism of a chemical has not been discounted or overlooked but must be left for future investigation.

For the purposes of this study, the anionic metabolites were chosen for profile analysis because they not only reflect the complex redox systems in metabolism but include the conjugating systems as well. The important neutral or nonconjugated materials also will be treated species by species in later studies.

The pesticide carbaryl was selected as the prototype because of the extensive metabolic studies (Knaak *et al.*, 1965, 1968; Knaak and Sullivan, 1967; Sullivan *et al.*, 1970) that have been made in man and other species. Any other compound with a comparable body of information on anionic metabolites would have served the purpose equally well.

[1] Paper 28, Division of Pesticide Chemistry, 160th American Chemical Society National Meeting, Chicago, Illinois, September 13–18, 1970.

METHODS

1-Naphthyl methyl-^{14}C-carbamate (carbaryl) of specific activity 11.3 μCi/mg, 1-naphthyl-^{14}C-methylcarbamate of specific activity 5.8 μCi/mg and 3,4-dichlorobenzyl-^{14}C-methylcarbamate of specific activity 5.2 μCi/mg were used.[2] The carbogen used was the commercially available 95% oxygen:5% carbon dioxide mixture. Trowell T8 medium was employed.[3] Organ tissues from the following species were used: 120–150 g male Harlan-Wistar rats, 6 kg male beagle dogs, mature male guinea pigs and liver biopsy from a human female.

Culture methods. Trowell (1959) T8 medium was oxygenated by passing carbogen at the rate of 100 ml/min through a 10 ml aliquot in a 15 ml beaker for 30 min. Aseptically procured liver was gently rinsed in oxygenated medium until free of blood. Tissue, 500 mg, was weighed on the sterilized cover of a 60 × 15 mm petri dish and then cut freehand with a No. 10 scalpel blade into 2 mm cubes under oxygenated medium using aseptic technique. The cubes were then transferred to an open petri dish containing 3 ml of oxygenated Trowell T8 medium and placed in a 9-l vacuum desiccator. Again, this entire unit was flushed with carbogen at 1.5 l/min for 30 min. The chamber was sealed and maintained at room temperature for 90 min; the labeled compound, dissolved in 5–10 μl of 95% ethanol was then added dropwise from a 10 μl syringe, while gently swirling the dish. The dish was returned to the desiccator and again flushed with carbogen, sealed, placed in an incubator at 37°C for the desired incubation time and then analyzed.

Analytical methods. Prior to doing an analysis, the distribution of the applied radioactivity in the medium, in the tissue and on the physical apparatus was determined in the manner outlined below:

1. At the termination of incubation, a 0.05 ml aliquot of the medium was counted using liquid scintillation. The remaining medium was pipetted from the culture dish; if in excess of 2.5 ml, it was concentrated by vacuum distillation to that volume and subjected to diethylaminoethyl (DEAE) cellulose column chromatography to develop the profile.
2. The tissue was placed in a Büchner funnel and washed thoroughly with 100–150 ml of water; an aliquot was assayed for radioactivity.
3. All glassware was thoroughly washed, the volume of the combined washings was measured and an aliquot was taken for assay by liquid scintillation counting.
4. The washed tissue was homogenized in a minimum volume of 50% ethanol and centrifuged. The supernatant was withdrawn, the solids were resuspended with 50% ethanol and the process was repeated. Finally, all supernatants were combined, the volume was measured and an aliquot was taken for radioactive assay.

In an experiment using guinea pig liver, metabolic profiles were developed from the total tissue and medium and from the medium alone for comparison. The tissue, the medium after incubation and the washings were pooled, adjusted with absolute ethanol to make a 50% ethanol solution and then homogenized and centrifuged. The supernatant was removed and the solids were resuspended in 50% ethanol; the process was

[2] Prepared by T. E. N. Steele, Union Carbide Corporation, Tuxedo, New York and W. J. Bartley, Technical Center, Union Carbide Corporation, South Charleston, West Virginia.
[3] Procured from Microbiological Associates, Bethesda, Maryland.

repeated twice. All glassware was washed with distilled water; these washings and the combined supernatants were transferred to a distillation flask, and the total volume was reduced to 2 or 3 ml by vacuum distillation. During the reduction process it was necessary to transfer the concentrate to a smaller distillation flask with the corresponding washings. A 0.05 ml aliquot of concentrated sample was assayed by liquid scintillation counting, and a measured volume was added to the DEAE-cellulose column for profile development.

The basic method published by Knaak *et al.* (1965) was adapted for DEAE-cellulose chromatography. A modification, utilizing an ammonium formate buffer system, gave improved resolution of some components. The formate columns were prepared by adding 6 g of DEAE-cellulose to 150 ml of 0.005 N formic acid at pH 4.0, stirring for 15 min and packing this slurry into a 1.5 × 24 cm column. The column was then washed with 750 ml of 0.005 N formic acid, pH 4.0, and equilibrated by washing with 6 l of 0.005 N ammonium formate, pH 6.5. The elution gradients for these columns consisted of 0.005 to 0.01, 0.01 to 0.05, 0.05 to 0.1 N ammonium formate; 300 ml of each concentration was used per gradient. Fractions (4 ml) were collected, and every fifth fraction was analyzed by liquid scintillation counting techniques.

RESULTS

In all the explant studies 100 μg of carbaryl per 500 mg of tissue was used. For the rat, this is roughly equivalent to an in vivo dose of approximately 7 mg/kg of body weight. The profiles reported are those obtained from analysis of the medium, because studies in the guinea pig using concentrates of homogenates of tissues and medium resulted in the same distribution of components as was found in the medium alone.

Figure 1 illustrates the direct comparison of typical naphthyl-^{14}C carbaryl DEAE-cellulose profiles for both an in vitro and in vivo study in the rat (Sullivan *et al.*, 1970). In most instances the identity of the peak component in Table 1 is that assigned by Knaak *et al.* (1965); any exceptions will be noted.

Peaks A and B are considered to be neutral components to the DEAE-cellulose anion exchange system, and no conjugates have been found in this area. Peak C is an unknown, weakly acidic metabolite not always completely resolved from peak D. Usually when resolution has been obtained a much larger % of methyl-^{14}C metabolites than of naphthyl-^{14}C is found. Peak D in the rat was determined (Sullivan *et al.*, 1970) to be a group of metabolites, the major component of which was the 5,6-dihydro-5,6-dihydroxycarbaryl glucuronide. Peak F has been shown to be a glucuronide of hydroxycarbaryl. In order to determine which hydroxy isomer of carbaryl has been conjugated, the peak must be hydrolyzed and the aglycon identified. Peak G in the rat is naphthyl glucuronide. Peak H is an unknown metabolite of minor importance in all species studied to date. Peak I is the sulfate conjugate of hydroxycarbaryls. Again, each isomer must be identified as in the case of the glucuronide. Peak J is naphthyl sulfate. The remaining peaks, unknown and unlettered, are found in several species and represent small % of the dose in all cases studied.

The in vitro results in the rat for naphthyl-^{14}C carbaryl are related qualitatively to results obtained in vivo and provide evidence for the presence of similar major and minor metabolic components, except for naphthyl sulfate. This component was produced by rat liver in vitro in approximately 3 times the amount found in vivo. Part of

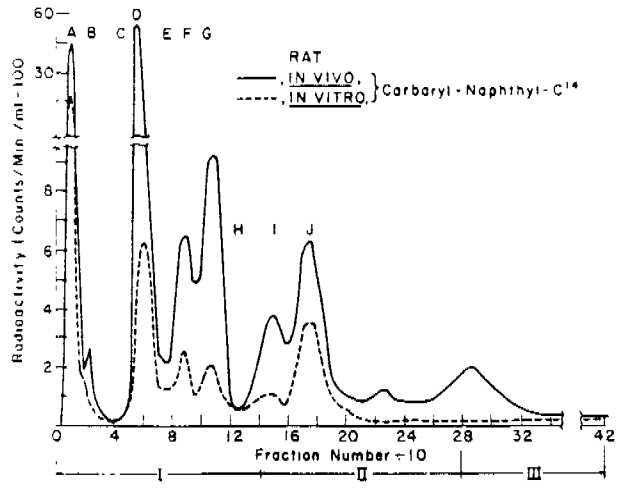

FIG. 1. DEAE-cellulose chromatogram of in vivo and in vitro rat metabolites of carbaryl-naphthyl-^{14}C. Gradient elution program: (I) 0.01 M Tris·HCl buffer, pH 7.5, to 0.05 M Tris·HCl buffer, pH 7.5; (II) 0.05 M Tris·HCl buffer, pH 7.5, to 0.1 M Tris·HCl buffer, pH 7.5; (III) 0.1 M Tris·HCl buffer, pH 7.5, to 0.5 M Tris·HCl buffer, pH 7.5.

TABLE 1

PEAK IDENTITY AND FLUORESCENCE[a]

Peak	Identification	Fluorescence
A, B	Neutrals	Indeterminate
C	Unknown	Unknown
D	5,6-Dihydro-5,6-dihydroxycarbaryl glucuronide + unknown glucuronides[b]	No
E	Unknown	Yes
F	Hydroxycarbaryl glucuronide	Yes
G	Naphthyl glucuronide	Yes
H	Unknown	Unknown
I	Hydroxycarbaryl sulfate	Yes
J	Naphthyl sulfate	Yes

[a] 285 nm activation and 335 nm emission.
[b] Identity established for the rat, Sullivan *et al.* (1970).

this higher level of naphthyl sulfate is undoubtedly caused by nonenzymatic hydrolysis of carbaryl. The compound is relatively stable at room temperature and at pH values of less than 7.5, but appreciable hydrolysis occurs at 37°C. (Chin *et al.*, 1971). The pH of the medium gradually reaches 7.2 in an 18 hr tissue study. Nonenzymatic hydrolysis as determined in buffered systems at pH 7.2 and 37°C without tissue produces an estimated 10% nonenzymatic hydrolysis. Failure to find appreciable amounts of 1-naphthol in the tissue studies from ether extracts of the neutrals indicates that this material is conjugated with either glucuronic or sulfuric acid.

Sullivan *et al.* (1970) reported in vivo values of 12% and 28% for the naphthyl-^{14}C and methyl-^{14}C neutrals, respectively. As illustrated in Tables 2 and 5, values obtained from the in vitro method were approximately twice the in vivo results for each label. Continuous ether extraction of the neutrals obtained in vitro compared favorably on a % basis with the in vivo studies in the rat (Sullivan *et al.*, 1970). From both in vivo and in vitro systems a greater % of naphthyl-^{14}C than of methyl-^{14}C was extractable. Thin-layer analysis of the neutrals in the ether–hexane thin-layer system used by Knaak *et al.* (1965) detected only trace amounts of unmetabolized carbaryl. Extractions of peaks A and B (Fig. 1) from an 18 hr incubation of tissue were chromatographed on silica gel by the method of Sullivan *et al.* (1970). The in vitro method gave qualitatively the same ratio of components as reported in vivo. The major ether soluble neutral chromatographed as 5,6-dihydro-5,6-dihydroxycarbaryl. Only a trace of carbaryl was found.

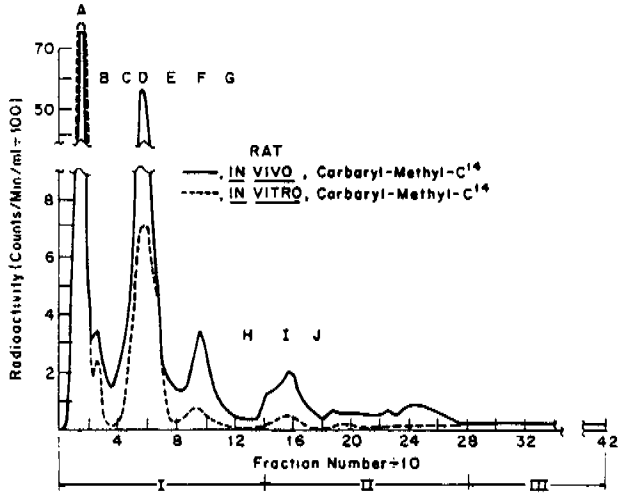

FIG. 2. DEAE-cellulose chromatogram of in vivo and in vitro rat metabolites of carbaryl-methyl-^{14}C. Gradient elution program as in Fig. 1.

Comparative results for methyl-^{14}C carbaryl are given in Fig. 2. The comparison of the in vitro and in vivo profiles in this instance is striking. A higher % of neutrals was obtained than in the in vivo studies. CO_2 was not measured in this particular experiment, but in other studies being conducted CO_2 production was comparable to that obtained from a feeding study. The principal conjugated metabolite again was metabolite D. Although the % neutrals in the in vitro study are higher than in the in vivo, with this label there are significant fractions in both.

To evaluate the reproducibility of the in vitro technique, profiles of metabolites of naphthyl-^{14}C carbaryl obtained from livers of 5 male Harlan-Wistar rats were compared (Table 2). The use of these animals from the weekly output of our breeding colony eliminated variables which might be caused by age, sex and strain. If the means and SD of peaks A, D, EF, G, I and J are considered, all peaks show good reproducibility. Variations are within the limits of experimental error estimated from duplicate

TABLE 2

VARIATION OF IN VITRO RAT LIVER METABOLITES OF CARBARYL (NAPHTHYL-^{14}C)[a]

	Tentative identification by chromatographic position										
	A	B	C	D	E	F	G	I	J		
Rat no.	Neutrals to DEAE	Unknown		Dihydro-dihydroxy-carbaryl glucuronide, major aglycon	Unknown	Hydroxy-carbaryl glucuronide	Naphthyl glucuronide	Hydroxy-carbaryl sulfate	Naphthyl sulfate	Pooled fractions[b]	% Recovered, corrected
	Naphthyl-^{14}C, carbaryl										
1	26	0	0	19	← 4.2 →		7.5	0.4	20	5.8	83
2	21	0	0	23	← 5.0 →		6.0	1.7	21	4.2	84
3	25	0	0	27	← 2.7 →		6.3	Trace	20	4.5	89
4	29	0	0	23	← 4.5 →		6.3	Trace	14	13.5	90
5	25	0	0	18	← 5.8 →		5.8	Trace	24	4.2	83
Means±	25.2±			22.0±	← 4.44± →		6.38±		19.8±	6.44±	85.8±
SD	2.9			3.6	1.15		0.66		3.6	4.00	3.4

[a] Calculated as % of ^{14}C applied on the column.
[b] Combined fractions from areas of chromatogram not containing a distinguishable peak.

determinations on 1 rat liver. "Pooled fractions" represent the combined areas of chromatographic profiles not containing distinguishable peaks. In the case of rat No. 4, the pooled fractions deviated markedly from the others because of poor resolution of column peaks. This was not seriously detrimental to the overall profile analysis and can be minimized by improvement in analytical methodology.

In order to estimate the incubation time required by the in vitro method, experiments were designed utilizing rat liver to determine a practical incubation period. Results of these studies are given in Tables 3 and 4. The 18 hr data are the mean values from Table 2. The 6 and 18 hr times are comparable for the anionic metabolites as illustrated in Table 3. The major difference found was with the neutral fractions. Comparative % of carbaryl, 5,6-dihydro-5,6-dihydroxycarbaryl (the major in vivo neutral metabolite), ether extractable neutrals and of the neutrals per se are given in Table 4. The % of total

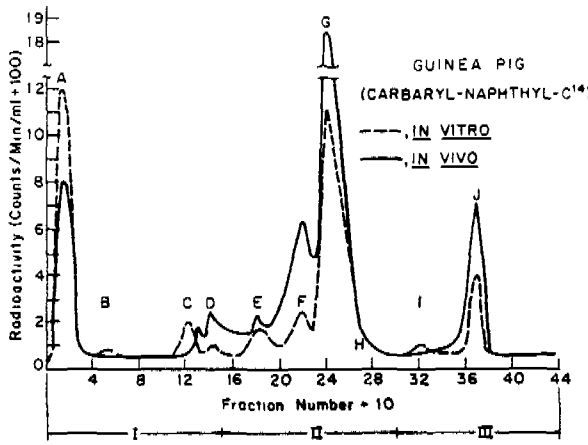

FIG. 3. DEAE-cellulose chromatogram of in vivo and in vitro guinea pig metabolites of carbaryl-naphthyl-^{14}C. Gradient elution program as in Fig. 1.

radioactivity increased in the medium with time, reaching an average of 80% in 18 hr. The % of neutrals, ether extractables and unmetabolized carbaryl all decreased with time. Only the 18 hr incubation data approach that found in vivo for any of these parameters. The major in vivo metabolite, 5,6-dihydro-5,6-dihydroxycarbaryl, was found to increase as a % of recovered activity with time and approached the in vivo results at 18 hr. Based upon profile analysis of the medium, in all probability a 6 hr incubation period is adequate for study of anionic metabolites. Where in-depth studies are needed, longer incubation times are suggested. Because specimens are procured and must be handled on the same day, the 18 hr incubation period provides a practical laboratory procedure. Studies in progress are designed to complete the evaluation of tissue viability as a function of incubation time.

It was found that data from ip dosing of the guinea pig could not be compared quantitatively with data from low dose single po intubation or dietary inclusion studies. Therefore, a study was undertaken using a single po dose of 30 mg/kg body weight of carbaryl. The solid line in Fig. 3 was obtained from this study. The dashed line represents the in

TABLE 3

IN VITRO METABOLIC PROFILES FOR VARIOUS INCUBATION PERIODS[a]

	Tentative identification by chromatographic position										
	A	B	C	D	E	F	G	H	I	J	
Incubation (hr)	Neutrals to DEAE		Unknown	Dihydro-carbaryl glucuronide, major aglycon	Unknown	Hydroxy-carbaryl glucuronide	Naphthyl glucuronide	Unknown	Hydroxy-carbaryl sulfate	Naphthyl sulfate	Pool fractions
3	56.4	0	0	17.1	← 4.2 →		0.9		1.4	11.0	1.9
6	38.6	0	0	21.6	← 10.5 →		4.6		← 15.9 →		3.8
18	25.2	0	0	22.0	← 4.4 →		6.4		Trace	19.9	6.4

[a] All values as % of applied radioactivity.

TABLE 4

OPERATIONAL PARAMETERS EXPRESSED AS PERCENTAGE OF CARBARYL EQUIVALENTS

Incubation (hr)	Naphthyl-^{14}C in the medium	Neutrals from DEAE	Neutrals extracted by ether	Carbaryl[a] in ether phase	Carbaryl in medium	5,6-Dihydro-5,6-dihydroxy-carbaryl[b] in ether phase
3	51	56	82	74.7	30	9.8
6	60	39	69	56.3	15	10.6
18	80	25	40	Trace	Trace	56.7

[a] As % of activity recovered from a silica gel column.

[b] Based upon silica gel column chromatographic position of 5,6-dihydro-5,6-dihydroxycarbaryl, a major neutral metabolite, found in vivo in the rat and expressed as % of recovered radioactivity.

vitro results. All peak designations (Table 1) are the same as for the rat. In the case of isomeric aglycons chromatographing as conjugates, the individual isomers have not been isolated and identified for verification of these designations. In vitro results show a higher % of unconjugated neutrals than in vivo. The major metabolite from the guinea pig under these conditions was naphthyl glucuronide from both in vivo and in vitro, and the peak had the proper fluorescence spectra. Hydrolysis by β-glucuronidase followed by extraction and thin-layer chromatography confirmed the presence of 1-naphthol. The major difference in the in vivo results reported here vs those reported by Knaak *et al.* (1965) is the change in the relative concentrations of metabolite D and naphthyl glucuronide. Because the original study was by ip dosing, the indications are that formation of metabolite D is sensitive to the degree and/or route of insult to the

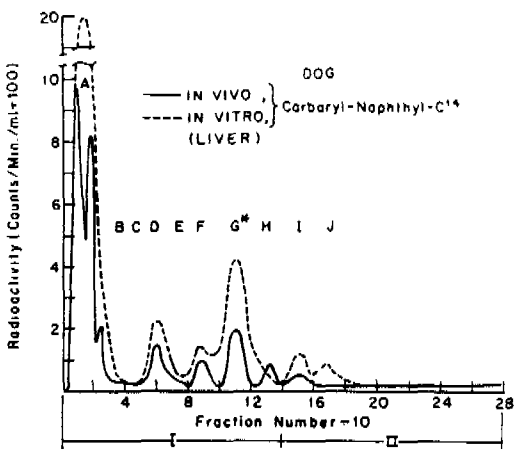

FIG. 4. DEAE-cellulose chromatogram of in vivo and in vitro dog metabolites of carbaryl-naphthyl-^{14}C. Gradient elution program as in Fig. 1.

animal. Although the profiles from the 2 studies are not quantitatively exact, the correlation of major and minor metabolites verifies the rat results, viz. that the in vitro method reflects the in vivo metabolites of carbaryl identified in 24 hr urine specimens after a po dose.

Figures 4 and 5 represent the results found with studies in dogs using naphthyl-^{14}C and methyl-^{14}C carbaryl, respectively. Both in vivo and in vitro neutrals comprise the major metabolites accounting for approximately 50% of the radioactivity. Carbaryl metabolism in the dog is an excellent example for comparison of in vivo and in vitro techniques. Peak G in the in vivo studies of Knaak and Sullivan (1967) was reported to chromatograph on DEAE-cellulose as if it were naphthyl glucuronide (peak G), but it proved to be nonfluorescent. In the profile obtained for the in vitro metabolism of carbaryl with dog liver the same results were obtained. Thus, the in vitro technique in this species not only gave profiles of similar character but reproduced this particular unique metabolite. Qualitatively the profiles from in vivo and in vitro studies for both labels compare favorably.

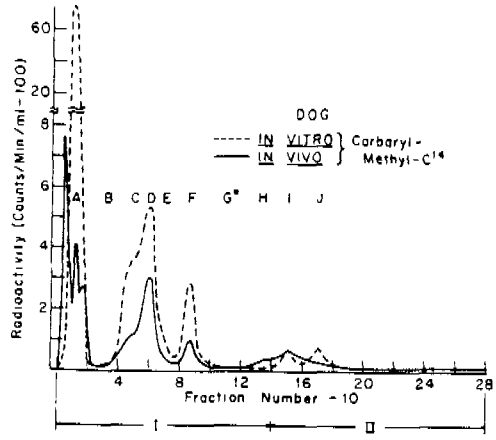

FIG. 5. DEAE-cellulose chromatogram of in vivo and in vitro dog metabolites of carbaryl-methyl-^{14}C. Gradient elution program as in Fig. 1.

Knaak *et al.* (1965, 1968) published in vivo results on human beings using the DEAE-cellulose profile and fluorescent analysis. Figure 6 illustrates the results obtained with naphthyl-^{14}C carbaryl in vitro in liver tissue obtained during surgical procedure on a 40 yr old female, when plotted with the in vivo results of Knaak *et al.* (1968). The quali-

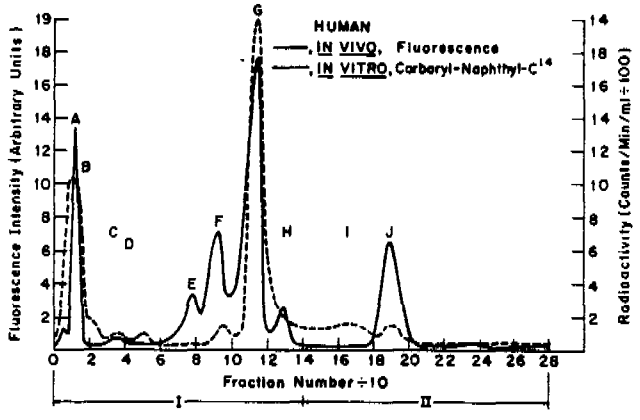

FIG. 6. DEAE-cellulose chromatogram of in vivo and in vitro human metabolites of carbaryl. Gradient elution program as in Fig. 1.

tative agreement of the profiles is extremely good. The minor peak D, absent in vivo, was demonstrated to be a nonfluorescent metabolite in the rat (Knaak *et al.*, 1965). The major difference in the 2 profiles is a substantially greater % of naphthyl sulfate in vivo and a greater % of naphthyl glucuronide in vitro. Reasons for this disagreement are not currently known.

Figure 7 illustrates results from a less precise early study of the in vitro metabolism of 3,4-dichlorobenzyl methylcarbamate in the rat. Knaak and Sullivan (1968) published the in vivo results and designated peak A as the neutrals and peak B as the glucuronide of 3,4-dichlorobenzyl alcohol; peak C was postulated to be a glucuronide of 3,4-dichlorobenzoic acid. Peak D was identified as 3,4-dichlorohippuric acid, and peak E as 3,4-dichlorobenzoic acid. Because 3,4-dichlorohippuric acid was the major metabolite in vivo, this study was conducted to investigate the ability of the in vitro technique to make this metabolite. Again, quantitative comparisons were not obtained. However, peak D in vitro was the major metabolite and was confirmed to be 3,4-dichlorohippuric acid by isolation and gas chromatography of the methylated derivative. Other studies have been conducted which indicate successful reduction of aromatic nitro groups and oxidation of sulfur to sulfoxides where in vivo studies have shown these mechanisms to operate.

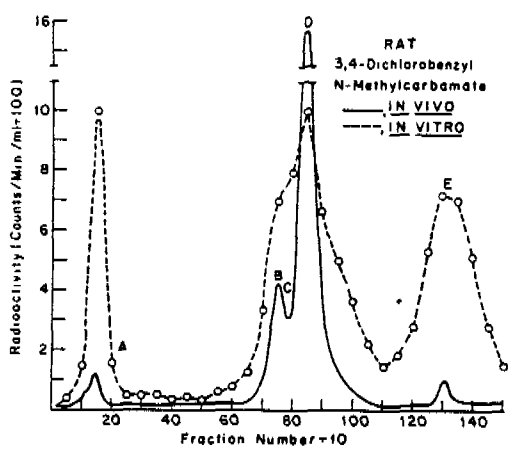

FIG. 7. DEAE-cellulose chromatogram of in vivo and in vitro rat metabolites of 3,4-dichlorobenzyl *N*-methylcarbamate. Gradient elution program: as (I) in Fig. 1.

Histologic changes reported after tissue was incubated in the nutritive medium for 18 hr at 37°C are predictable: degeneration and shrinkage of nuclei, condensing of chromatin and, ultimately, rupture and death of cells. However, fresh tissue that has been subjected to 18 hr incubation under the prescribed conditions of this test is histologically in a state similar to portions of the same tissue stored at 5°C for 18–24 hr in physiological saline.

The quantitative results obtained from the in vitro metabolism of carbaryl by liver tissues of man, rat, guinea pig and dog, respectively, are given in Table 5. These data are given as % of radioactivity recovered from the column. The individual labels can be compared by species but cannot be directly compared for the 2 labels in 1 species, except where the major metabolite contains both labels. Generally speaking, a higher % of dose will be found in the neutral fraction from these in vitro studies than would be found in vivo in the same species. There are several factors which can contribute to this

TABLE 5

IN VITRO DERIVED METABOLITES OF CARBARYL IN FOUR SPECIES[a]

		Tentative identification by chromatographic position										
		A	B	C[c]	D[c]	E	F	G	H	I	J	
		Neutrals to DEAE		Unknown	Dihydro-dihydoxy-carbaryl glucuronide, major aglycon	Unknown	Hydroxy-carbaryl glucuronide	Naphthyl glucuronide	Unknown	Hydroxy-carbaryl sulfate	Naphthyl sulfate	Pool fractions[f]
Man	I[b]	40	0.4	0.3	5	0	3	31	← 10 →		1	5
	II[c]	73.2		← 11.4 →		0	6.7	0	0	0	0	2.9
Guinea pig	I	26	0.6	5	3	4	6	33	0	2	12	5
	II	45	0	11	15	2.4	1.6	0	0	1.6	0	5
Rat	I	25.8	0	0	19	← 4.2 →		7.5	0	0.4	20	5.8
	II	51	0	← 17 →		0	0.8	0	0	2	0	5
Dog	I	29	0	← 22 →		0	5	16[d]	0	3.4	2.5	6
	II	66	0	← 19 →		0	5	0	0	1	1	4

[a] Calculated as % of ^{14}C applied on the column.
[b] Naphthyl-^{14}C carbaryl (I).
[c] Methyl-^{14}C carbaryl (II).
[d] Chromatographs as naphthyl glucuronide but does not fluoresce as this compound should. Identity unknown.
[e] With the methyl label some components which did not contain naphthyl-^{14}C chromatographed in these areas.
[f] Combined fractions from areas of chromatogram not containing a distinguishable peak.

result, including the physical parameters of diffusion, a static vs a dynamic system and time. A further complication arises from the possible interaction of the kidney and other organs with the in vivo liver metabolites. The higher % of neutrals found with methyl-^{14}C than with naphthyl-^{14}C reflects the results obtained in vivo. Man metabolized a higher % of carbaryl to neutrals than did the other species under scrutiny. Extracts of these neutrals in man were found to be 30% of the dose for the naphthyl and 31% for the methyl labels. Analysis showed that no more than 6% of the dose could be unreacted carbaryl. The remainder of the neutrals was found to be a mixture of metabolites; they have not as yet been isolated and identified.

Based upon chromatographic and fluorometric analysis, naphthyl glucuronide was the most significant anionic metabolite from man and the guinea pig, and a dihydrodihydroxycarbaryl glucuronide from the rat and dog (Sullivan *et al.*, 1970). The other significant anionic found in the dog was the nonfluorescent material cochromatographing with naphthyl glucuronide as reported by Knaak and Sullivan (1967).

DISCUSSION

Studies comparing the results of the in vitro metabolism of carbaryl and of 3,4-dichlorobenzyl methylcarbamate with in vivo results have confirmed that the in vitro organ maintenance technique can be a useful method of demonstrating comparative metabolism. The in vitro results closely paralleled in vivo metabolism of the compounds as derived from analysis of 24 hr urine samples. The metabolic products found in vitro are species specific and not dependent upon the addition of cofactors for their production. The method is qualitatively correct but semiquantitative in its present form. In vitro profiles were reproducible, and the individual variation was not significantly different in 5 rat livers. The anionic metabolites were found to have an SD of 0.7–4% depending upon the fraction analyzed. The SD as % of ^{14}C applied to the chromatographic column was generally of the order of magnitude of the quantity present for minor metabolites, a finding which precludes quantitative comparison of the importance of differences between species. However, with major metabolites in man, the value of the standard deviation as determined from rat data was such that direct quantitative comparison could be made.

The in vitro organ maintenance technique thus offers promise as a method to determine metabolism in man utilizing radioactivity of high specific activity without direct dosing of human subjects. In-depth studies of comparative metabolism of man and animals would then fulfill the concepts proposed by Frazer (1970) in regard to selection of animals for toxicologic studies: ". . . if two or more different metabolic patterns are found in animals investigated, it becomes necessary to discover which of these patterns occurs in man. When this is known, animals with a similar pattern should be chosen for further toxicological studies."

It also offers a developmental approach to the needs foreseen by the Committee on Problems of Drug Safety of the Drug Research Board, National Academy of Sciences—National Research Council (1969): "More research is required to establish quantitative criteria to indicate how different species resemble and differ from one another in matters of drug metabolism. These criteria would facilitate the selection of the best species for toxicity studies."

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