The Metabolism of Carbaryl in Man, Monkey, Pig, and Sheep

J. B. Knaak,[1] M. J. Tallant, S. J. Kozbelt, and L. J. Sullivan

The metabolic fate of carbaryl in the pig, sheep, man, and monkey was investigated. The over-all recovery of the naphthyl-C^{14} and methyl-C^{14} label of carbaryl in the pig was, respectively, 85 and 71% of dose, while in the sheep the recovery of the naphthyl and methyl label was, respectively, 74.8 and 67.8% of dose. 1-Naphthyl methylimidocarbonate *O*-glucuronide and 4-(methylcarbamoyloxy)-1-naphthyl glucuronide were the major urinary metabolites excreted by the pig. In addition to these, the sheep excreted 4-(methylcarbamoyloxy)-1-naphthyl sulfate, 1-naphthyl glucuronide, and sulfate. The over-all recovery of carbaryl equivalents in the urine of man using the fluorometric method (26 to 28%) was lower than that obtained with the colorimetric method (37.8%). The metabolites identified in urine were 4-(methylcarbamoyloxy)-1-naphthyl glucuronide, 1-naphthyl glucuronide, and sulfate. The monkey excreted carbaryl primarily as 1-naphthyl methylimidocarbonate *O*-glucuronide and 4-(methylcarbamoyloxy)-1-naphthyl glucuronide.

In the rat and guinea pig, carbaryl is metabolized and excreted as 1-naphthyl methylimidocarbonate *O*-glucuronide, 4-(methylcarbamoyloxy) 1-naphthyl glucuronide, 1-naphthyl glucuronide, 4-(methylcarbamoyloxy)-naphthyl sulfate, and 1-naphthyl sulfate (Knaak *et al.*, 1965). Recently the metabolic products of carbaryl in the urine of the dog were examined (Knaak and Sullivan, 1967) by ion exchange chromatography and fluorescence analysis. Glucuronides and sulfates of 4-hydroxycarbaryl and 1-naphthol present in the urine of the rat and guinea pig were not found in dog urine. The analytical procedures involving the colorimetric determination of 1-naphthol have been used to analyze the urines of the dog and rat (Carpenter *et al.*, 1961), man (Best and Murray, 1962), and cow (Dorough, 1967; Whitehurst *et al.*, 1963) after exposure or administration of carbaryl. The colorimetric method accounted for only 22% of the carbaryl administered to the rat (Carpenter *et al.*, 1961) and 16 to 28% of the radioactive products in the urine of the cow (Dorough, 1967). At the present time the colorimetric method for 1-naphthol is being used to quantitate the exposure of man or his animals to carbaryl without knowledge of the nature of the urinary metabolites and the extent to which they are excreted.

The following studies were conducted to provide information on the metabolism of carbaryl in the pig, sheep, monkey, and man. The man and monkey studies were undertaken in cooperation with J. H. Wills, Albany Medical College of Union University, Albany, N. Y., while the pig and sheep studies were carried out entirely at Mellon Institute. Urine samples supplied by Wills were used by this laboratory to investigate the metabolites of man and the monkey by fluorometric and radiometric methods. For a comparison study in man, R. E. Peele, Union Carbide Corp., South Charleston, W. Va., analyzed a composite urine sample for 1-naphthol by a colorimetric method (Best and Murray, 1962).

Carnegie-Mellon University, Mellon Institute, Pittsburgh, Pa. 15213

[1] Present address, Niagara Chemical Division, FMC Corp., 100 Niagara St., Middleport, N. Y. 14105

METHODS

Chemicals. Carbaryl-methyl-C^{14} (0.93 mc. per mmole) and carbaryl-naphthyl-C^{14} (0.97 mc. per mmole) were prepared by T. E. N. Steele, Union Carbide Corp., Sterling Forest Laboratories, Tuxedo, N. Y., as described by Knaak *et al.* (1965). The samples were examined by gas chromatography (Sullivan *et al.*, 1967) as their *N*-acetyl derivatives and were 99% carbaryl-C^{14}.

Nonlabeled carbaryl was supplied by Union Carbide Chemicals and Plastics Division, South Charleston, W. Va.

Treatment and Handling of Men and Animals. The following work was carried out under the supervision of J. H. Wills, Albany Medical College of Union University, Albany, N. Y.

Two men (Clinton Prison Hospital, New York State Prison, Dannemora, N. Y.) weighing 81.5 and 86.5 kg. were orally administered carbaryl in gelatin capsule at 2.0 mg. per kg. Urines were collected periodically during the first day and pooled to give 0- to 4-, 4- to 8-, 8- to 12-, 12- to 16, and 16- to 24-hour samples. After the first day, urine was collected and pooled on a 24-hour basis to give 2-, 3-, and 4-day urines. Control urines (24 hour) were obtained prior to the administration of carbaryl.

A female rhesus monkey weighing 4.59 kg. was orally administered carbaryl-C^{14} at 300 mg. per kg. The monkey was first treated with naphthyl-C^{14} carbaryl (11.1 μc. per mmole) and then, in a subsequent experiment 4 days later, with methyl-C^{14} carbaryl (11.1 μc. per mmole). The monkey was housed in a metabolism cage for a period of 2 days to facilitate the separate collection of urine and feces.

The total urines collected from the monkey and aliquots of the urines collected from the two men were frozen and shipped in dry ice to Mellon Institute for analysis.

Two young female pigs weighing 14.5 and 18.0 kg., respectively, were orally administered either methyl-C^{14} carbaryl (11.3 μc. per mmole) or naphthyl-C^{14} carbaryl (14.7 μc. per mmole) in gelatin capsules at 25 mg. per kg. The pigs were housed separately in metabolism cages to facilitate the complete and separate collection of urine and feces over a 5-day period. During this period the animals were maintained on a commercial grain ration.

A ewe weighing 42 kg. was orally administered carbaryl-C^{14} in gelatin capsules at 25 mg. per kg. The ewe was

first given naphthyl-C^{14} carbaryl (35.0 μc. per mmole) and then in a subsequent experiment 2 weeks later she was given methyl-C^{14} carbaryl (71.5 μc. per mmole). The ewe was catheterized and held in a metabolism stall to allow for the total separation and collection of urine and feces over a 4-day period. During the study the ewe was fed a daily ration of grain and hay.

C^{14} Analysis of Urine and Fecal Samples. The urines from the monkey and the individual urine and fecal samples from the two pigs and the ewe were analyzed for C^{14} using liquid scintillation counting techniques. The analytical procedure as reported by Knaak *et al.* (1965) was used.

Chromatographic Analysis of the Urinary Metabolites. The individual urines collected from the men and the 24-hour urines (naphthyl and methyl labels) from the ewe, monkey, and pigs were used in the following chromatographic studies.

Ion Exchange Chromatography. Two-milliliter volumes of urine containing either C^{14}-labeled or -nonlabeled metabolites were individually absorbed and chromatographed on a 1.5 × 24 cm. column of diethylaminoethyl (DEAE)-cellulose according to the method of Knaak *et al.* (1965). Three hundred 4.0-ml. fractions were collected and 1.0 ml. of every fifth fraction was analyzed for radioactivity using liquid scintillation counting techniques. Fractions containing the nonlabeled metabolites from man were analyzed in an Aminco-Bowman spectrophotofluorometer using a xenon lamp (American Instrument Co., part 416-992). All fractions were read at a fluorescence excitation setting of 285 mμ. and a fluorescence emission setting of 335 mμ.

A standard curve (fluorescence intensity *vs.* micrograms of metabolite per milliliter of solution) was prepared for naphthyl glucuronide (Pierce Chemical Co., Rockford, Ill.) and naphthyl sulfate (Sigma Chemical Co., St. Louis, Mo.). In addition, 100 μg. of the glucuronide and sulfate were added to 2.0 ml. of control urine and chromatographed under conditions used in the analysis. A curve, internally compensated for background and quench, was obtained as a function of the micrograms,—i.e., 0 to 100 μg.—of metabolite applied to the column and the fluorescence intensity i.e., 0 to 8 intensity units for naphthyl glucuronide—of the peak tube. The volume (ml.) of buffer in this case was considered to be constant. For comparison purposes the fractions underneath each peak were pooled, read in the fluorometer, and the concentration in micrograms per milliliter was obtained directly from the standard curve. This value (μg./ml.) when multiplied by the total number of milliliters of solution gave the total quantity of glucuronide or sulfate in the 2.0 ml. of urine applied to the top of the column. These values were then corrected for background by subtraction of control urine values obtained in the same way. A quench correction was not made. The two methods compared favorably.

Because a standard sample of the glucuronide of 4-hydroxycarbaryl (a metabolite found in both the rat and guinea pig) was not available, this compound was assumed to have a fluorescence intensity reading of 1.0 μg. per ml. equivalent to that of naphthyl glucuronide based on C^{14} and fluorescence analysis (Knaak *et al.*, 1965). The microgram quantities of naphthyl glucuronide and sulfate and the glucuronide of 4-hydroxycarbaryl found in the urines examined were then expressed in terms of carbaryl.

Colorimetric Analysis of Human Urine. The 0- to 4-, 4- to 8-, 8- to 12-, 12- to 16-, and 16- to 24-hour urines from the men were pooled separately to make a composite 24-hour sample (1st-day urine) for each man. The 1st-day urine from each man was then used along with his 2nd-, 3rd-, and 4th-day urines to make a 4-day composite sample. One hundred and twenty milliliters of each of the 4-day composite urines (from the two men) were pooled and 100-ml. samples were sent to R. E. Peele, Union Carbide Corp., South Charleston, W. Va., for analysis of 1-naphthol using the method of Best and Murray (1962). Three milliliters of this urine were chromatographed on DEAE-cellulose and analyzed qualitatively and quantitatively by fluorescence as previously described. Three milliliters of control urine were analyzed in a similar manner and the results were used to correct background fluorescence.

RESULTS

C^{14} Analysis of Urine and Fecal Samples. The results of the excretion studies are given in Figure 1 for the pigs and ewe administered methyl-C^{14}- and naphthyl-C^{14}-labeled carbaryl. The pig excreted 83.4 and 1.6%, respectively, of the naphthyl label in urine and feces while 70 and 1.0%, respectively, of the methyl label was excreted in urine and feces. The ewe excreted 71.4 and 3.4%, respectively, of the naphthyl label in urine and feces while the methyl label was excreted to the extent of 62.4 and 5.4%, respectively, in urine and feces.

Analysis of the Urinary Metabolites. Figure 2*A* gives the chromatographic results obtained with methyl and naphthyl labeled metabolites from the pig on a 1.5 × 24 cm. column of DEAE-cellulose. The pig excreted two major metabolites (D and F) possessing the C—O—C(O)-N—C structure, neutrals (A), and a metabolite (G) pos-

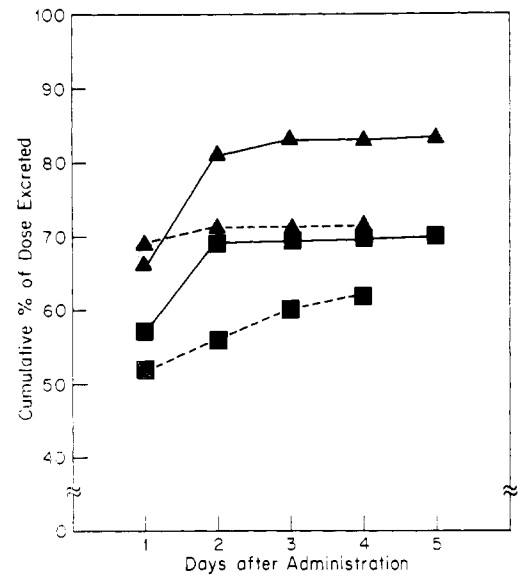

Figure 1. Excretion of carbaryl-C^{14} equivalents in the urine of the sheep and pig

A female pig (———) and ewe (- - - -) were orally administered ▲ naphthyl-C^{14} and ■ methyl-C^{14} carbaryl at 25 mg./kg.

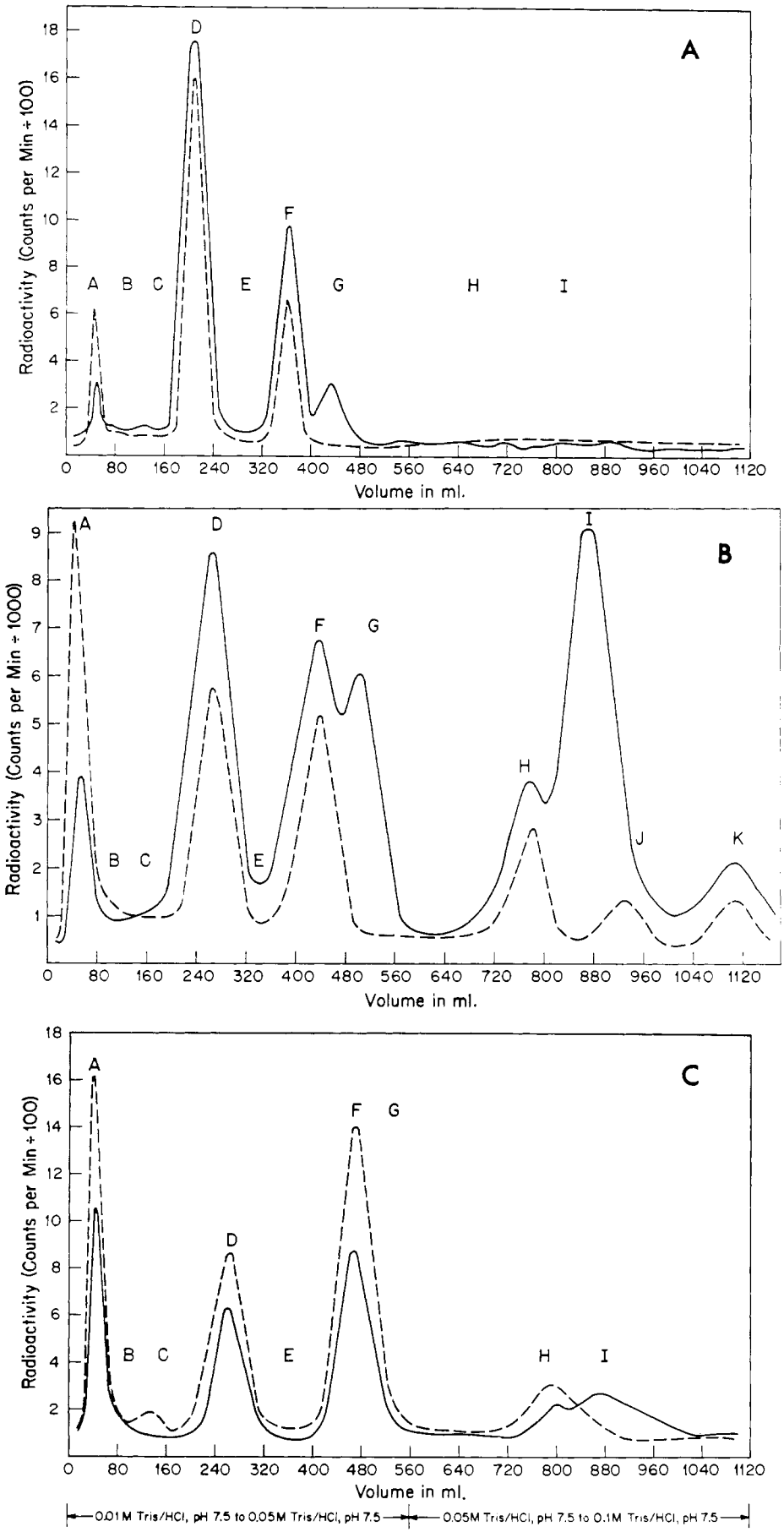

Figure 2. DEAE-cellulose chromatogram of the urinary metabolites of carbaryl-C^{14} excreted

A. Pig
B. Ewe
C. Monkey
——— Naphthyl-C^{14} label
- - - - Methyl-C^{14} label

A 1.5 × 24 cm. column of DEAE cellulose was used

Metabolites identified in Table I

sessing only the naphthyl label. The metabolites are identified in Table I as the glucuronides of the enol form of modified carbaryl (D) (Knaak *et al.*, 1965), 4-hydroxycarbaryl (F), and 1-naphthol (G). The percentage of each of these metabolites as C^{14} recovered from the ion exchange column is given in Table I. Column recoveries averaged 85%.

Figure 2*B* gives the chromatographic results obtained with the methyl- and naphthyl-labeled metabolites in the urine of the ewe. The ewe excreted three major metabolites (D, F, and H) possessing the intact C—O—C(O)N—C structure and two major metabolites (G and I) possessing only the naphthyl label. Neutrals (A) were also excreted in the urine of the ewe. The metabolites are identified in Table I and the percentage of each of these metabolites as C^{14} recovered from the column is given. The metabolites are lettered according to the chromatograms published by Knaak *et al.* (1965) for the rat and guinea pig metabolites of carbaryl. Metabolites J and K possess the intact C—O—C(O)N—C structure of carbaryl. These metabolites were not found in the urines of the rat and guinea pig by Knaak *et al.* (1965). Naphthyl sulfate (I), 1-naphthyl methylimidocarbonate *O*-glucuronide (Knaak *et al.*, 1965; Knaak and Sullivan, 1968) (D), and the glucuronide of 4-hydroxycarbaryl (F) were the major metabolites of carbaryl in the ewe.

Figure 2*C* shows the chromatographic results obtained with the methyl- and naphthyl-labeled metabolites from monkey urine. The monkey excreted carbaryl as neutrals (A) and as two major metabolites (D and F) possessing the intact C—O—C(O)N—C structure. The metabolites are identified in Table I and the percentage of each of these metabolites as a percentage of the total C^{14} recovered from the column is given. Small quantities of naphthyl sulfate (I) and the sulfate of 4-hydroxycarbaryl (H) were excreted by the monkey.

Figure 3 shows a typical chromatogram obtained with a 4-hour urine from men orally administered carbaryl at 2.0 mg. per kg. Metabolites A, F, G, and I were the only fluorescent products found at pH 7.5 (285 mμ, 335 mμ) related to carbaryl. Metabolites A are neutral products while F, G, and I were identified, respectively, as 4-(methylcarbamoyloxy)-1-naphthyl glucuronide, 1-naphthyl glucuronide, and sulfate (Knaak *et al.*, 1965). The fractions between metabolites A and F were made pH 11 and allowed to stand overnight. These fractions were read in the fluorometer at an excitation setting of 330 mμ and an emission setting of 465 mμ. A small fluorescent peak chromatographing in the region of metabolite D (Figures 2, 3, and 4) was detected but could not be quantitated. This metabolite is believed to be 1-naphthyl methylimidocarbonate *O*-glucuronide (Knaak *et al.*, 1965; Knaak and Sullivan, 1968).

Figure 4 gives the quantitative chromatographic results for the excretion of 4-(methylcarbamoyloxy)-1-naphthyl glucuronide, 1-naphthyl glucuronide, and sulfate in the urines of two men at the indicated hours after the administration of carbaryl. The 2nd-, 3rd-, and 4th-day urines were also examined. Small quantities of these metabolites were present in 2nd-day urine, but could not be quantitated with any degree of certainty. No metabolites were found in 3rd- and 4th-day urines. Table II gives the individual chromatographic results for these metabolites as percentage of dose in 24-hour urines (determined from 0- to 4-, 4- to 8-, 8- to 12-, 12- to 16-, and 16- to 24-hour urines). These results are compared to the values obtained for the rat (Knaak *et al.*, 1965).

Colorimetric Analysis of Human Urine. R. E. Peele, Union Carbide Corp., South Charleston, W. Va., reported finding 0.81 mg. of 1-naphthol per 100 ml. of urine. On the basis of the administered carbaryl, this value amounted to 37.8% of the dose. The 4-day composite and control

Table I. Urinary Metabolites of Carbaryl Excreted by the Monkey, Pig, and Ewe

Metabolites[a]	Metabolites Expressed as % of Total[b] C^{14} Recovered from Column					
	I	II	III	IV	V	VI
A. Unidentified neutrals	16.5	16.9	10.0	22.8	13.8	3.4
C. Unidentified metabolite	0.0	5.2	0.0	0.0	0.0	0.0
D. 1-Naphthyl methylimidocarbonate *O*-glucuronide	16.1	18.0	46.0	38.4	26.1	41.8
F. 4-(Methylcarbamoyloxy)-1-naphthyl glucuronide	31.6	37.4	15.2	16.0	13.6	23.4
G. 1-Naphthyl glucuronide	0.0	0.0	5.5	0.0	11.9	0.0
H. 4-(Methylcarbamoyloxy)-1-naphthyl sulfate	25.4[c]	14.2	0.0	0.0	4.5	12.1
I. 1-Naphthyl sulfate		0.0	0.0	0.0	25.2[d]	0.0
J. Unidentified metabolite	0.0	0.0	0.0	0.0		6.9
K. Unidentified metabolite	0.0	0.0	0.0	0.0	3.2	8.4

[a] Listed in order of elution.
[b] Monkey studies I. Carbaryl-naphthyl-C^{14}
II. Carbaryl-methyl-C^{14}
Pig studies III. Carbaryl-naphthyl-C^{14}
IV. Carbaryl-methyl-C^{14}
Sheep studies V. Carbaryl-naphthyl-C^{14}
VI. Carbaryl-methyl-C^{14}
Metabolites B and E were not present in the urines examined.
[c] % for H and I.
[d] % for I and J.

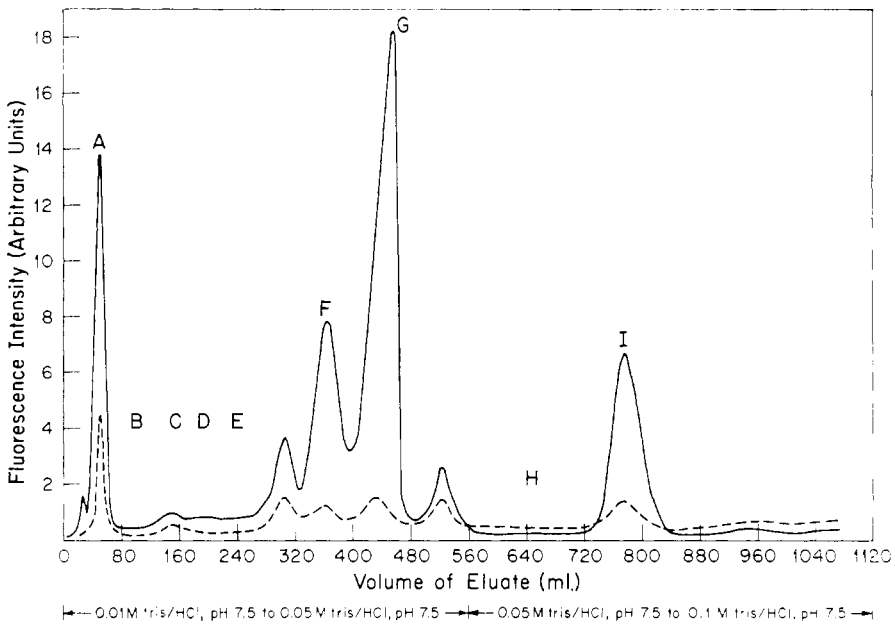

Figure 3. DEAE-cellulose chromatogram of the urinary metabolites of carbaryl excreted by man

——— Carbaryl at 2 mg./kg., 4-hour urine
- - - - Control urine

A 1.5 × 24 cm. column of DEAE-cellulose was used

Metabolites identified in Table II

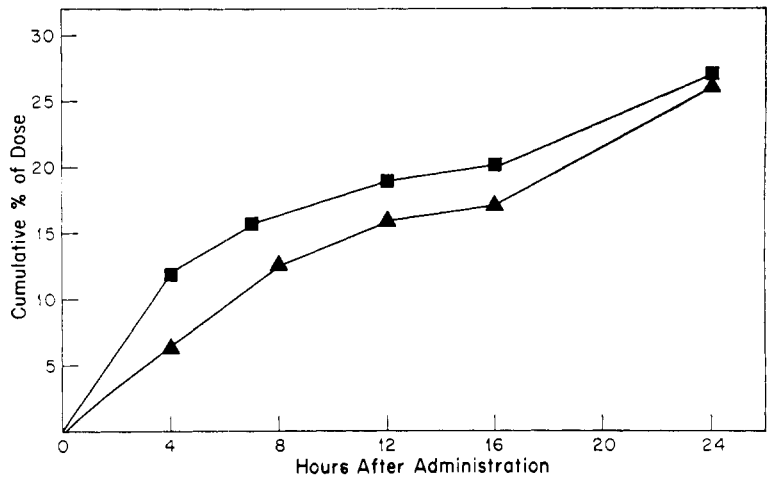

Figure 4. Excretion of quantitated metabolites expressed as carbaryl equivalents in the urine of man

▲ Man No. 1
■ Man No. 2

Carbaryl was orally administered to men at 2.0 mg./kg. See text for analytical procedure

Table II. Urinary Metabolites of Carbaryl Excreted by the Rat and Man

Metabolites[a]	Metabolites Expressed as %[b] of Dose in 24-Hour Urines		
	I	II[c]	III[c]
A. Unidentified neutrals	9.2	[d]	[d]
C. Unidentified metabolite	2.9	[d]	[d]
D. 1-Naphthyl methylimidocarbonate *O*-glucuronide	18.2	[d]	[d]
F. 4-(Methylcarbamoyloxy)-1-naphthyl glucuronide	7.2	4.3	5.8
G. 1-Naphthyl glucuronide	11.2	10.4	15.5
H. 4-(Methylcarbamoyloxy)-1-naphthyl sulfate	5.0	0.0	0.0
I. 1-Naphthyl sulfate	16.2	11.0	6.1

[a] Listed in order of elution.
[b] I. Carbaryl-naphthyl-C^{14} studies in the rat.
II. Carbaryl-fluorescence studies in man No. 1.
III. Carbaryl-fluorescence studies in man No. 2.
[c] Determined from 0- to 4-, 4- to 8-, 8- to 12-, 12- to 16-, and 16- to 24-hour urines. Metabolites B and E were not present in these urines.
[d] Fluorescence material, but no quantitative data.

urines were examined in the fluorometer after being separately chromatographed on DEAE-cellulose. The analysis of the 4-day composite accounted for 25% of the dose (336 mg.) administrated the two men. The 4-day results were equivalent to the cumulative percentage of dose (Figure 4) obtained for the 0- to 4-, 4- to 8-, 8- to 16-, and 16- to 24-hour urine samples from the men.

DISCUSSION

Carbaryl is metabolized and excreted by the monkey and pig as conjugates of intact 4-hydroxycarbaryl and carbaryl, while carbaryl is metabolized in the ewe to conjugates of 1-naphthol, 4-hydroxycarbaryl, and carbaryl. In man, carbaryl is hydrolyzed to 1-naphthol and excreted along with 4-hydroxycarbaryl as conjugates of glucuronic or sulfuric acid. Evidence was also obtained by fluorometry for the presence of 1-naphthyl methylimidocarbonate *O*-glucuronide. Carbaryl is metabolized in mammals as reported in this study and in the rat and guinea pig (Knaak *et al.*, 1965) by a similar route. The major difference between the animal species studied was the extent to which

carbaryl was hydrolyzed to yield 1-naphthol. Little or no hydrolysis of carbaryl occurred in the monkey and pig as opposed to the ewe and man.

Naphthyl-labeled carbaryl is excreted to the extent of 83.4 and 71.4%, respectively, in the urine of the pig and ewe with very little of the label appearing in feces. Table II compares the results for man (% of dose in 24-hour urines) with the results from the rat. The percentage of the dose being excreted as 4-(methylcarbamoyloxy)-1-naphthyl glucuronide, 1-naphthyl glucuronide, and sulfate by man compares favorably to that obtained with the rat.

This study was undertaken to compare the metabolic pathway for carbaryl in several species including man in order to determine principal species variations. For this reason, a detailed study of the mixture of materials designated neutrals was not considered essential.

LITERATURE CITED

Best, E. M., Jr., Murray, B. L., *J. Occupational Med.* **4,** 507 (1962).
Carpenter, C. P., Weil, C. S., Palm, P. E., Woodside, M. W., Nair, J. H., III, Smyth, H. F., Jr., J. AGR. FOOD CHEM. **9,** 30 (1961).
Dorough, H. W., J. AGR. FOOD CHEM. **15,** 261 (1967).
Knaak, J. B., Sullivan, L. J., J. AGR. FOOD CHEM. **15,** 1125 (1967).
Knaak, J. B., Sullivan, L. J., Carnegie-Mellon University, Pittsburgh, Pa., unpublished data, 1968.
Knaak, J. B., Tallant, M. J., Bartley, W. J., Sullivan, L. J., J. AGR. FOOD CHEM. **13,** 537 (1965).
Sullivan, L. J., Eldridge, J. M., Knaak, J. B., J. AGR. FOOD CHEM. **15,** 927 (1967).
Whitehurst, W. E., Bishop, E. T., Critchfield, F. E., Gyrisco, G. G., Huddleston, E. W., Arnold, H., Lisk, D. J., J. AGR. FOOD CHEM. **11,** 167 (1963).

Received for review August 31, 1967. Accepted March 1, 1968.

Correction

ABSORPTION SPECTRA OF COPPER AND ZINC COMPLEXES OF PHEOPHYTINS AND PHEOPHORBIDES

In this article by I. D. Jones *et al.* [J. AGR. FOOD CHEM. **16,** 80 (1968)], in Figure 3, the two curves on the extreme right should be designated as Copper (II) Pheophorbide a rather than as Zinc Pheophorbide a.