Metabolism of Carbofuran Alfalfa Residues in the Dairy Cow

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The metabolism of residues of carbonyl-C^{14} and benzofuranyl-7a-C^{14} carbofuran in alfalfa was investigated in a lactating dairy cow in conjunction with carbofuran and 3-hydroxycarbofuran. In four days the cow excreted 77 and 38%, respectively, of the ring and carbonyl labels in urine. Fecal excretion of these labels amounted to 22 and 18%, respectively, of dose. The principal carbamate residue, the glycoside of 3-hydroxycarbofuran, was excreted in urine as the glucuronides and sulfates of 2,3-dihydro-7-hydroxy-2,2-dimethyl-3-oxobenzofuran and 2,3-dihydro-3,7-dihydroxy-2,2-dimethylbenzofuran. Similar metabolites were observed for 3-hydroxycarbofuran, while the major metabolites of carbofuran in the cow were the sulfate and glucuronide of 2,3-dihydro-7-hydroxy-2,2-dimethylbenzofuran.

The metabolism of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) has been investigated in the rat (Dorough, 1968a), mouse (Metcalf *et al.*, 1968), cow (Ivie and Dorough, 1968), and plant (Dorough, 1968b; Metcalf *et al.*, 1968). Carbofuran undergoes oxidation and hydrolysis in alfalfa to 2,3-dihydro-3-hydroxy-2,2-dimethyl-7-benzofuranyl methylcarbamate, 2,3-dihydro-7-hydroxy-2,2-dimethylbenzofuran, 2,3-dihydro-7-hydroxy-2,2-dimethyl-3-oxobenzofuran, and 2,3-dihydro-3,7-dihydroxy-2,2-dimethylbenzofuran (Knaak *et al.*, 1970). These products are glycosylated and stored in the plant as carbofuran residues. 3-Hydroxycarbofuran glycoside is the major carbofuran residue in alfalfa.

This investigation was undertaken to study the metabolism of these carbofuran residues in the lactating dairy cow. In conjunction with this study, a reexamination of the metabolism and excretion of carbofuran and 3-hydroxycarbofuran was made.

METHODS

Chemicals. Carbonyl-C^{14} (2.7 mCi per mmole) and benzofuranyl-7a-C^{14} (0.145 mCi per mmole) labeled carbofuran (Figure 1) were prepared, respectively, by Mallinckrodt/Nuclear, St. Louis, Mo., and J. F. Start, FMC Corporation, Princeton, N.J.

Carbonyl and ring labeled 2,3-dihydro-3-hydroxy-2,2-dimethyl-7-benzofuranyl methylcarbamate (3-hydroxycarbofuran) were synthesized according to the procedure of Metcalf *et al.* (1968) by S. E. Forman, FMC Corp., Princeton, N.J., using labeled carbofuran. Carbonyl and ring labeled 2,3-dihydro-2,2-dimethyl-3-oxo-7-benzofuranyl methylcarbamate (3-oxocarbofuran) were obtained as additional products during oxidation. 2,3-Dihydro-7-hydroxy-2,2-dimethyl-7a-C^{14}-benzofuran (7-phenol), 2,3-dihydro-3,7-dihydroxy-2,2-dimethyl-7a-C^{14}-benzofuran (3,7-diol), and 2,3-dihydro-7-hydroxy - 2,2 - dimethyl - 3 - oxo - 7a - C^{14} - benzofuran (3-keto-7-phenol) were obtained by NaOH hydrolysis of their respective C^{14} labeled carbamates. The corresponding nonlabeled compounds were made from carbofuran by S. T. Young, FMC Corporation, Middleport, N.Y., using the described procedures. The abbreviated terms for these compounds will be used throughout the manuscript.

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Excretion Studies. Fifty milligrams of carbonyl-C^{14} (2.7 mCi per mmole) and 271.1 mg of benzofuranyl-C^{14} (0.145 mCi per mmole) carbofuran were placed in separate gelatin capsules, size No. 00, and individually administered via rumen fistula to a 545 kg lactating Holstein cow. Three hundred and fifty grams of alfalfa hay containing carbofuran-carbonyl-C^{14} residues (C^{14} equivalent to 27.6 mg of carbofuran) and 530 g of alfalfa hay containing carbofuran-ring-C^{14} residues (C^{14} equivalent to 148 mg of carbofuran) were individually administered via rumen fistula to the cow. In addition to these studies, 60 mg of 3-hydroxy carbofuran-carbonyl-C^{14} (2.5 mCi per mmole) was administered to the cow in a gelatin capsule via rumen fistula. The carbofuran residues in alfalfa were identified by Knaak *et al.* (1970) and are reviewed in the results section. During the metabolism study the cow was fed alfalfa-brome hay *ad libitum* (21 lb per day) and 16 lb per day of a standard herd ration.

Urine was collected over the following time intervals: 0 to 4 hr; 4 to 8 hr; 8 to 24 hr; 24 to 48 hr; 48 to 72 hr; and 72 to 96 hr. Feces were collected and pooled on a 24 hr basis. Milk was obtained over a 96 hr period following administration of pesticide or pesticide residues at the regular milking time in the morning and evening. All urine volumes (ml) and milk and fecal weights (lb) were recorded for the indicated time periods. The analytical procedure for urine and feces were essentially those reported by Knaak *et al.* (1965). Milk samples (25 g) were freeze-dried and 0.5 g samples were combusted in a Paar oxygen bomb, as were the fecal samples. Urine samples were analyzed by direct counting in scintillation liquid.

Analysis of Urinary Metabolites. The 4 to 8 hr urines collected in the excretion studies were used as a source of metabolites for the following studies.

Ion Exchange Chromatography. Five ml of 4 to 8 hr urine containing the cow metabolites of carbofuran (ring and carbonyl labels), the cow metabolites of the carbofuran alfalfa residues (ring and carbonyl labels), and the cow metabolites of 3-hydroxy carbofuran were individually chromatographed on a 1.5 × 24 cm column of diethylaminoethyl (DEAE) cellulose according to the procedure of Knaak *et al.* (1965). The neutrals (A) from the studies involving carbofuran-ring-C^{14}, carbofuran-carbonyl-C^{14}, and carbofuran-carbonyl-C^{14} residues in alfalfa were continuously extracted over a 24-hr period at pH 7.5 with diethyl ether. The ether extracts were dried over anhydrous sodium sulfate, concentrated by distillation to 1.0 to 2.0 ml, and set aside for chromatography on a silica gel column.

β-Glucuronidase Treatment. All urines (50 ml aliquots)

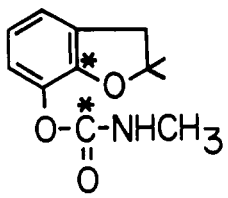

Figure 1. Structural formula of carbofuran and position of C^{14} label

mentioned in the previous section on ion exchange chromatography were adjusted to pH 4.5 with acetic acid. Three ml of 0.1*M* sodium acetate buffer, pH 4.5 and 300 units of β-glucuronidase (Nutritional Biochemicals Corp., Cleveland, Ohio) were added per ml of urine. The urines were incubated overnight with the enzyme at 37° C, adjusted to pH 7.0 with sodium hydroxide, and extracted with diethyl ether. The extracts were dried over anhydrous sodium sulfate, and concentrated by distillation to 1.0 to 2.0 ml for silica gel chromatography and for acetylation prior to gas chromatography.

The carbonyl-C^{14} metabolites (B and C) and the ring-C^{14} metabolites (B, C, and D) from the ion exchange column were hydrolyzed with β-glucuronidase using the procedure described for the whole urines. Fifty mg (3000 units) of β-glucuronidase were used. The aglycones were extracted from neutral solutions with diethyl ether, dried over anhydrous sodium sulfate, and concentrated by distillation to 1.0 to 2.0 ml for silica gel chromatography.

Acid Hydrolysis. The whole urines after β-glucuronidase treatment were refluxed in 0.5*N* HCl for 1 hr, neutralized with sodium hydroxide, and extracted with diethyl ether. The extracts were dried over anhydrous sodium sulfate and concentrated by distillation to 1.0 to 2.0 ml for silica gel chromatography and for acetylation prior to gas chromatography.

The ring-C^{14} metabolites (E and F) from the ion exchange column were hydrolyzed in a similar manner with HCl, neutralized, and extracted with diethyl ether. Extracts were dried over anhydrous sodium sulfate and concentrated by distillation to 1.0 to 2.0 ml for chromatography on silica gel.

Silica Gel Chromatography. The ether extracts (1.0 to 2.0 ml) containing the aglycones from the β-glucuronidase and acid hydrolyzates of whole urines and the aglycones from the individual metabolites separated by ion exchange chromatography were individually absorbed onto 2.0 to 5.0 g of silica gel (Davison Chemical Division, W. R. Grace & Co., Baltimore, Md., Grade 923) and air dried. The gel was added to the top of a 1.5 × 24 cm column of silica gel previously packed as a slurry in hexane and the aglycones were chromatographed using a stepwise gradient of hexane to ethyl acetate. In practice, each step (total of 10 steps) consisted of 50 ml of solvent. The percentage of the starting solvent in these volumes was decreased in the following manner: 100, 90, 80, 70, 60, 50, 40, 30, 20, and 10%. Labeled carbofuran (ring-C^{14} and carbonyl-C^{14}), 3-hydroxycarbofuran (ring-C^{14} and carbonyl-C^{14}), 3-oxocarbofuran (ring-C^{14} and carbonyl-C^{14}), the 7-phenol-C^{14}, the 3-keto-7-phenol-C^{14}, and the 3,7-diol-C^{14} were used as standards for the identification of the metabolites.

Gas Chromatography. A Barber-Coleman 5000 gas chromatograph equipped with a radioactive monitor (RAM) and a hydrogen flame detector (10 to 1 split) was used throughout this study. Glass columns (6 ft × 5 mm i.d.) were packed with 5% SE-30 on Applied Science Gas-Chrom Q (80 to 100 mesh). The column was temperature programmed at 3° C per min with an injection port temperature of 300° C and a detector temperature of 340° C. A helium carrier gas flow of 75 ml per min was used throughout.

The aglycones obtained by diethyl ether extraction of the β-glucuronidase and acid hydrolyzed metabolites in whole urines were acetylated at 100° C using 5.0 ml of acetic anhydride and 1–2 drops of methane sulfonic acid after removal of solvent by distillation. The reaction mixture was stirred for 1.0 hr, poured over crushed ice, and neutralized with 5*N* NaOH. The acetylated products were extracted with chloroform and dried over sodium sulfate. The volume of solvent was reduced to 1.0 to 2.0 ml by distillation and 10 μl of this solution was gas chromatographed. Nonlabeled carbofuran, 3-hydroxycarbofuran, 3-oxocarbofuran, the 7-phenol, the 3-keto-7-phenol, and the 3,7-diol were acetylated as previously described for the aglycones and used as standards.

Nature of the C^{14} Metabolites Excreted in Milk. One hundred ml of first-day milk containing metabolites of carbofuran-carbonyl-C^{14} were freeze-dried. The solids were continuously extracted in a Soxhlet extractor with diethyl ether and then reconstituted with water. The protein was precipitated with ethanol, centrifuged off, and air dried. The remaining aqueous-ethanol phase containing lactose was dried by reduced pressure distillation. The three fractions—fat, casein, and lactose—were counted using liquid scintillation techniques. The lactose fraction was then dissolved in 0.01*M* Tris/HCl, pH 7.5 buffer. A 5.0 ml aliquot was chromatographed on a DEAE-cellulose column as described in the section on ion exchange chromatography. The remaining sample was acid hydrolyzed using sufficient HCl to make the solution 0.5*N*. The sample was extracted using diethyl ether, dried over sodium sulfate, and concentrated by distillation to 1.0 to 2.0 ml. The C^{14} labeled products were absorbed on silica gel and chromatographed on a silica gel column as described for the urinary metabolites of carbofuran in the section on silica gel chromatography.

RESULTS

Excretion Studies. The results of the excretion studies are given in Figure 2 for carbofuran (ring-C^{14} and carbonyl-C^{14}), carbofuran residues (ring-C^{14} and carbonyl-C^{14}) in alfalfa, and 3-hydroxycarbofuran-carbonyl-C^{14}.

Carbofuran (ring-C^{14} and carbonyl-C^{14}) and 3-hydroxycarbofuran (carbonyl-C^{14}) were readily absorbed from the rumen-intestinal tract as less than 3.0% of the dose was excreted in feces, while substantially larger quantities of the administered label (18 to 22% of dose) were excreted in feces in the case of the carbofuran residues (ring-C^{14} and carbonyl-C^{14}).

In urine, 83, 12 and 23.7% of the dosed label was excreted, respectively, for carbofuran-ring-C^{14}, carbofuran-carbonyl-C^{14}, and 3-hydroxycarbofuran-carbonyl-C^{14}. The carbofuran residues were excreted in urine to the extent of 77 and 38% of dose, respectively, for the ring and carbonyl labels. The percent figure for the carbofuran-ring-C^{14} residues is based on almost 100% recovery of the label.

The ring and carbonyl labels of carbofuran were excreted in milk up to 2.5% of dose. The carbonyl label from the residues of carbofuran in alfalfa gave similar results. The ring-C^{14} label from the residues of carbofuran in alfalfa and the carbonyl-C^{14} label from 3-hydroxycarbofuran were excreted to the extent of 0.70 and 1.0%, respectively.

Urinary Metabolites of Carbofuran and Carbofuran Residues in Alfalfa. Figure 3 gives the results obtained with the urinary metabolites of carbofuran (ring-C^{14} and carbonyl-C^{14}) on a DEAE-cellulose column. The approximate percentages

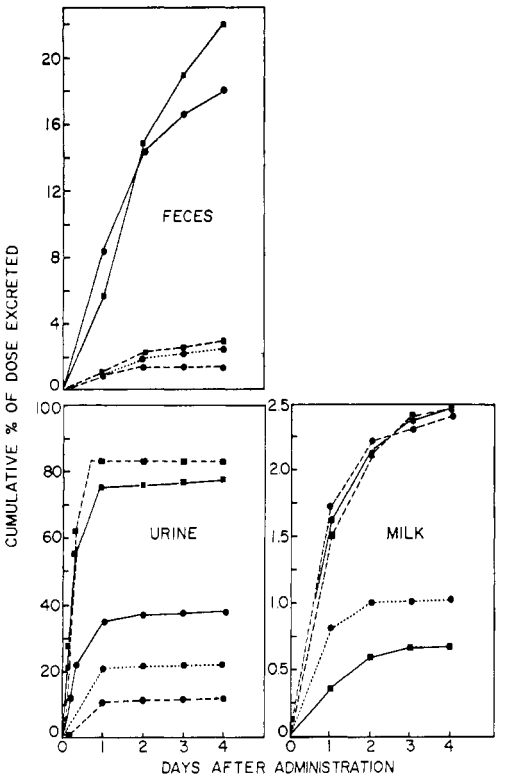

Figure 2. Excretion of C^{14} labeled carbofuran, carbofuran residues in alfalfa, and 3-hydroxycarbofuran

--- Carbofuran
—— Carbofuran residues in alfalfa
- - - - 3-Hydroxycarbofuran
● Carbonyl label
■ Ring label

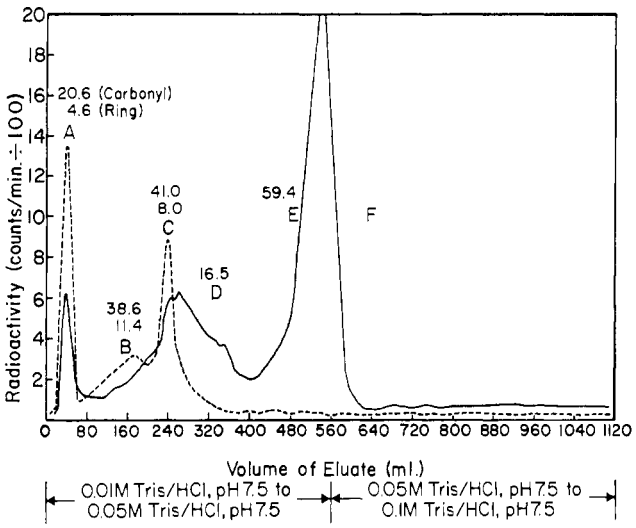

Figure 3. DEAE-cellulose chromatogram of the urinary metabolites of C^{14} labeled carbofuran on a 1.5 × 24 cm column

—— Ring label
- - - - Carbonyl label
Metabolites identified in text

found for each metabolite are given. Neutrals or nonionic products chromatographed under area A, while B, C, D, and E represent anionic metabolites. The ion exchange column did not separate the metabolites. Metabolites B and C appear to possess both the ring and carbonyl labels, while D and E do not. Metabolites B, C, and D chromatograph

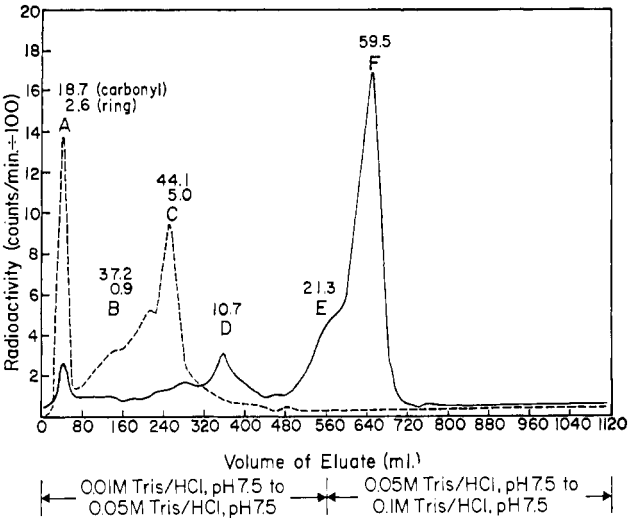

Figure 4. DEAE-cellulose chromatogram of the urinary metabolites of C^{14} labeled carbofuran residues in alfalfa on a 1.5 × 24 cm column

—— Ring label
- - - - Carbonyl label
Metabolites identified in text

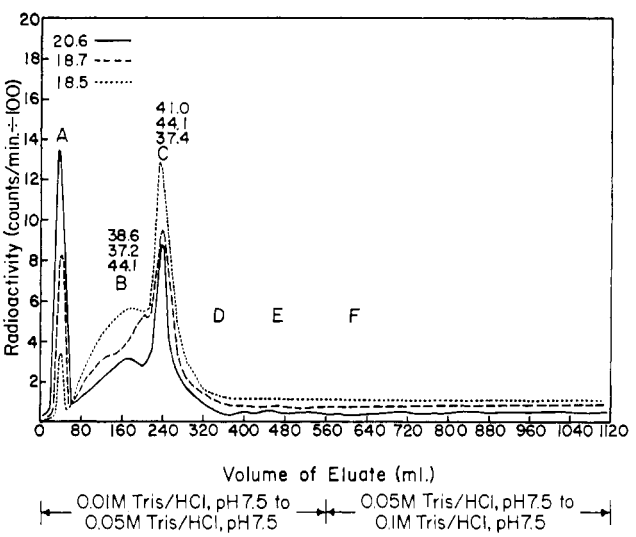

Figure 5. DEAE-cellulose chromatogram of the urinary metabolites of carbonyl-C^{14} labeled carbofuran, carbofuran residues in alfalfa, and 3-hydroxycarbofuran on a 1.5 × 24 cm column

—— Carbofuran
- - - - 3-Hydroxycarbofuran
- - - - Carbofuran residues in alfalfa
Metabolites identified in text

similarly to glucuronides, while E chromatographs like an ethereal sulfate (Knaak *et al.*, 1965).

Figure 4 gives the results obtained with the urinary metabolites of carbofuran residues in alfalfa on a DEAE-cellulose column. The chromatogram differs significantly from that of Figure 3, in that metabolite E accounts for only 21.3% of the radioactivity, while a new metabolite F accounted for 59.5% of the C^{14}. Sulfates which chromatograph in this position are the major metabolic products.

Figure 5 gives the results obtained for carbofuran-carbonyl-C^{14}, residues of carbofuran-carbonyl-C^{14} (3-hydroxycarbofuran glycoside) in alfalfa, and 3-hydroxycarbofuran-carbonyl-C^{14} on a DEAE-cellulose column. A similar chromatographic pattern was obtained for all carbonyl-C^{14}-labeled products investigated.

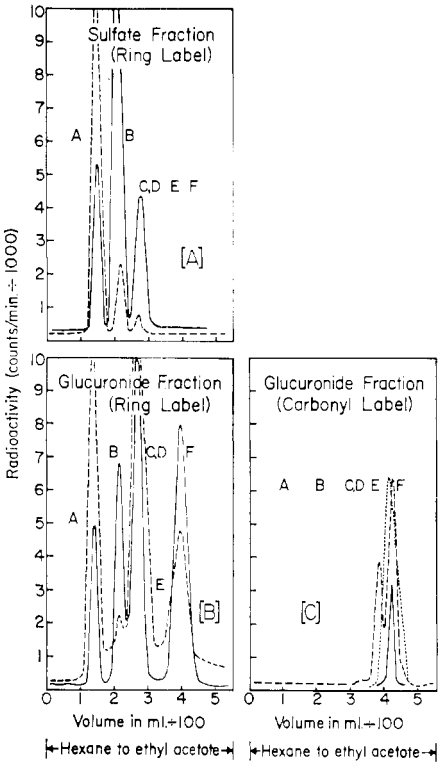

Figure 6. Silica gel chromatograms of the aglycones obtained by β-glucuronidase treatment and acid hydrolysis of the urinary metabolites of carbofuran, carbofuran residues in alfalfa, and 3-hydroxycarbofuran

– – – Carbofuran
—— Carbofuran residues in alfalfa
- - - - 3-Hydroxycarbofuran
Metabolites identified in text

The urinary neutrals off the ion exchange column (Figures 3 and 4) were extracted from water with diethyl ether and chromatographed on a silica gel column. 3-Hydroxycarbofuran was found in the neutrals from the urine of the cow administered carbofuran-carbonyl-C^{14} residues and carbofuran-carbonyl-C^{14}. The 3,7-diol was found in the neutrals, along with a trace of 3-hydroxycarbofuran when carbofuran-ring-C^{14} was administered.

Figure 6 gives the silica gel column results obtained with the aglycones from the glucuronide and sulfate fractions off the DEAE-cellulose column for ring and carbonyl labeled carbofuran, carbofuran residues in alfalfa, and carbonyl labeled 3-hydroxycarbofuran. Similar results were obtained by direct β-glucuronidase treatment of whole urines followed by acid hydrolysis of the sulfates. Figure 6 (A) gives the results obtained with the aglycones-sulfate fraction (ring label) from the carbofuran and carbofuran residue study. The aglycones were characterized by cochromatography with known C^{14} standards as the 7-phenol (A), the 3-keto-7-phenol (B), and the 3,7-diol (C). Figure 6 (B) gives the results obtained with the glucuronide fraction (ring-C^{14} label) from the same urines. 3-Hydroxycarbofuran (F) was found, in addition to the aforementioned aglycones, in the glucuronide fraction. Figure 6 (C) gives the results obtained with the glucuronide fraction (carbonyl-C^{14} label). 3-Hydroxycarbofuran (F) was found in the studies involving carbonyl-C^{14} labeled carbofuran, carbofuran residues in alfalfa, and 3-hydroxycarbofuran. In the carbofuran study, an additional and unknown metabolite (E) was found. Carbofuran (D) when present cochromatographs with the 3,7-diol (C).

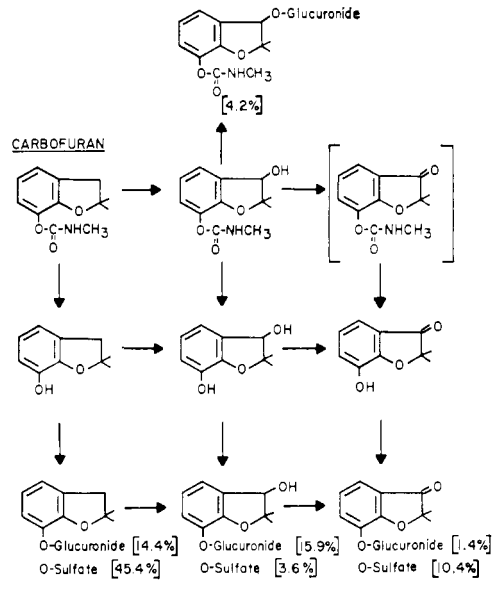

Figure 7. Metabolism of carbofuran in the cow. Metabolites were analyzed by DEAE-cellulose and silica gel chromatography. See text for conditions

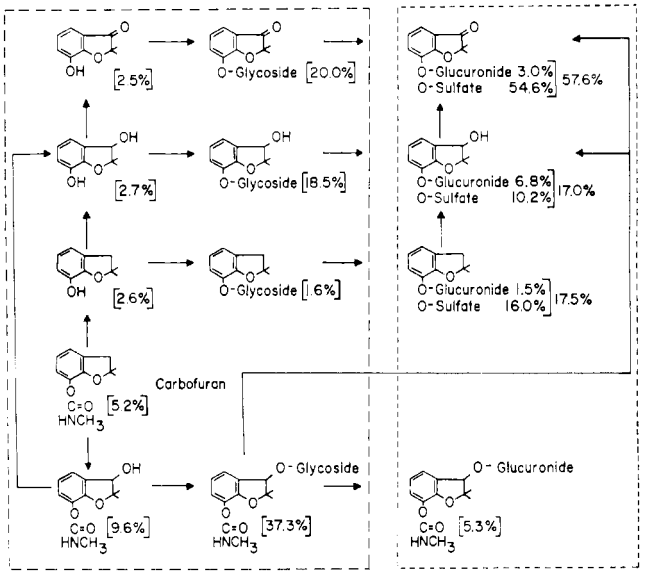

Figure 8. Metabolism of carbofuran alfalfa residues in the cow

– – – Residues of carbofuran in alfalfa
- - - - Urinary metabolites of the carbofuran residues
Metabolites were analyzed by DEAE-cellulose and silica gel chromatography. See text for conditions

Figure 7 gives the metabolic products of carbofuran-ring-C^{14} excreted by the cow in 4 to 8 hr urine. The percentages for each of these metabolites are indicated in the figure. These percentages are based on separations achieved by ion exchange chromatography and then by silica gel chromatography assuming complete hydrolysis of the conjugates. In practice, β-glucuronidase treatment released approximately 50% of the ring-C^{14} aglycones from glucuronic acid, while acid released approximately 95% of the sulfated phenols. In the case of the carbonyl labels, β-glucuronidase released 20, 25, and 50%, respectively, of the C^{14} from the urine of the cow administered carbofuran, carbofuran residues in alfalfa, and 3-hydroxy carbofuran.

Figure 8 gives the metabolic products of the carbofuran-

Table I. Gas Chromatographic Analysis of the Aglycones Obtained from the Urinary Metabolites[a] of Carbofuran-Ring-C^{14} and Carbofuran-Ring-C^{14} Residues

Aglycones[b]	Aglycones Expressed as % of Total C^{14}					
	Carbofuran			Carbofuran residues		
	I	II	III	IV	V	VI
A. 2,3-dihydro-7-hydroxy-2,2-dimethyl benzofuran	15.8	43.0	58.8	3.9	9.4	13.3
B. 2,3-dihydro-7-hydroxy-2,2-dimethyl-3-oxobenzofuran	4.2	8.0	12.2	4.0	65.0	69.0
C. 2,3-dihydro-3,7-dihydroxy-2,2-dimethylbenzofuran	11.8	8.4	20.2	3.1	6.4	9.5
D. 3-hydroxycarbofuran	4.1	0.0	4.1	5.6	0.0	5.6

[a] 4 to 8 hr urine. [b] The aglycones were acetylated prior to chromatography. For conditions see Text and Figure 9. [c] I. Glucuronides, 35.9%, as determined by ion exchange chromatography. II. Sulfates, 59.4%, as determined by ion exchange chromatography. III. Total of I and II. IV. Glucuronides, 16.6%, as determined by ion exchange chromatography. V. Sulfates, 80.8%, as determined by ion exchange chromatog raphy. VI. Total of IV and V.

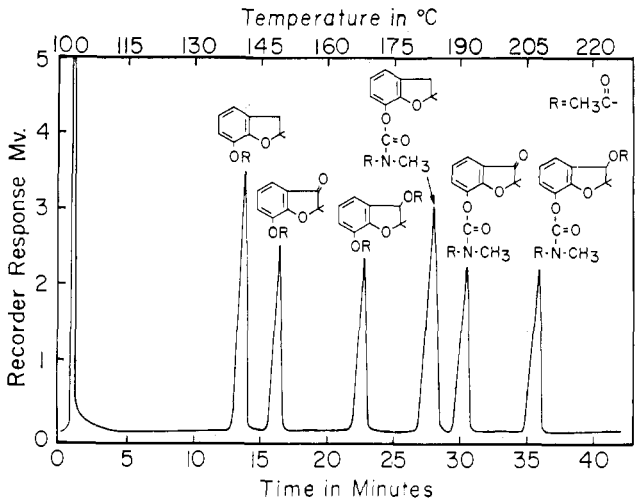

Figure 9. Gas chromatogram of acetylated carbofuran and derivatives on 5% SE-30

Column, 6 ft, 5-mm i.d.; Support, Gas Chrom Q, 80 to 100 mesh; Flow rate, helium, 75 ml/min; Injection port temp., 300° C; Detector temp., 340° C; Temp. program, 3° C/min

ring-C^{14} residues excreted by the cow in 4 to 8 hr urine. The nature of the carbofuran residues and their percentages in alfalfa are indicated in this figure (Knaak *et al.*, 1970).

The aglycones from β-glucuronidase and acid hydrolyzed urines (ring and carbonyl labeled metabolites of carbofuran, carbofuran residues in alfalfa, and carbonyl labeled 3-hydroxycarbofuran) were acetylated and gas chromatographed. Figure 9 is a routine gas chromatogram obtained with a standard mixture of the acetates of the 7-phenol, the 3-keto-7-phenol, the 3,7-diol, and the *N*-acetyl derivatives of carbofuran, 3-oxocarbofuran, and 3-acetoxycarbofuran (3-acetoxy - 2,3 - dihydro - 2,2 - dimethyl - 7 - benzofuranyl methylcarbamate).

Table I gives the results obtained by gas chromatography (C^{14} detection) for the ring-C^{14} carbofuran and carbofuran residues in alfalfa. The results compare favorably with those obtained by silica gel chromatography (Figures 7 and 8). The 7-phenol is the major aglycone found in the urine of the cow administered carbofuran, while the 3-keto-7-phenol is the major metabolite of the carbofuran-ring-C^{14} residues in alfalfa. An unidentified metabolite was found in the urine of the cow administered carbofuran-carbonyl-C^{14} and carbofuran-C^{14} residues in alfalfa. The metabolite gas chromatographed directly after 3-hydroxycarbofuran. In the studies involving carbofuran-carbonyl-C^{14}, the unknown metabolite accounted for 33% of the aglycones released, while in studies involving the carbofuran-carbonyl-C^{14} residues in alfalfa the metabolite accounted for only 6.5%. The presence of a corresponding ring-C^{14} metabolite could not be substantiated. The metabolite, if present, would account for 1.0 to 2.0% of the excreted C^{14} in the carbofuran-ring-C^{14} studies.

Nature of the Carbofuran-Carbonyl-C^{14} Metabolites in Milk. Of the C^{14} metabolites in cow milk, 2% of the label extracted from the dried milk with diethyl ether. The remaining 98% was found equally distributed between casein and lactose. The C^{14} in the lactose chromatographed as a neutral on DEAE-cellulose. Acid hydrolysis of the neutral fraction resulted in the extraction of 18.5% of the radioactivity. On silica gel the radioactivity chromatographed in the position of 3-hydroxycarbofuran. No other products were found.

DISCUSSION

Research papers dealing with the metabolism of glycosides in the dairy cow have been mainly confined to studies involving the cyanogenetic glycosides (Dobson, 1967). These glycosides are hydrolyzed in the rumen with the subsequent release of hydrocyanic acid. The present complex study involving the carbofuran residues, therefore, must be discussed in relationship to the metabolism of carbofuran and 3-hydroxy carbofuran.

Carbofuran is hydrolyzed and excreted in urine primarily as the glucuronide and sulfate of the 7-phenol (58%). Small quantities of the glucuronides and sulfates of the 3,7-diol and the 3-keto-7-phenol were also excreted along with the glucuronide of 3-hydroxy carbofuran (4.1%). 3-Hydroxycarbofuran-carbonyl-C^{14} is hydrolyzed in the cow to almost the same extent as carbofuran *per se*.

The carbofuran-carbonyl-C^{14} residues in milk were examined by extraction and chromatographic methods. The residues in the lactose fraction, neutral, to DEAE-cellulose, were partially (18.5%) extracted from water with diethyl ether after acid hydrolysis. The extractables were identified as 3-hydroxycarbofuran. This suggests that either the carbamate is bound to lactose and can only be released on treatment with acid or that it is present in the lactose fraction as a glycoside. In this case the first assumption is probably more correct, as residues are also associated with the casein fraction and the presence of a glycoside would require direct absorption from the rumen or *in vivo* synthesis. The majority (90%) of the carbonyl-C^{14} residues are not related to carbofuran. These findings are consistent with those of Dorough and Ivie (1967). The milk containing ring-C^{14} residues of low specific activity was not examined in this study.

The carbofuran residues in alfalfa were identified as the glycosides of 3-hydroxycarbofuran (37.3%), the 7-phenol (1.6%), the 3,7-diol (18.5%), and the 3-keto-7-phenol (20.0%). Carbofuran (5.2%) and 3-hydroxycarbofuran (9.6%) were found *per se* along with small quantities of the phenols. Because these products were administered simultaneously, their individual metabolic pathways cannot be defined. The residues as administered were metabolized and excreted as the sulfates of the 3-keto-7-phenol (65.0%), the 7-phenol (9.4%), and the 3,7-diol (6.4%). The glucuronides of these phenols accounted for 11.0% of the total C^{14}. The overall metabolic process involved hydrolysis of the glycosides and carbamates, oxidation of the phenols, and conjugation of the resulting products with sulfuric or glucuronic acid.

Gas chromatography was used in combination with ion exchange and silica gel chromatography to characterize and quantitate the metabolites. The individual metabolites were incompletely resolved on the ion exchange column. The metabolites were separated into three classes—neutrals, glucuronides, and sulfates. These products were examined individually by silica gel chromatography after extraction (neutrals), or after hydrolysis and extraction (glucuronides and sulfates). Complete hydrolysis of the conjugates was not achieved. The aglycones released and extracted are believed to represent all the aglycones present. The percentages of each aglycone in the 4 to 8 hr urines were calculated on the basis of 100% release of the aglycones from their conjugates. Aglycones released by β-glucuronidase treatment were considered to be all conjugates of glucuronic acid, while the aglycones released by subsequent acid hydrolysis were considered to be conjugates of sulfuric acid. Some overlapping did occur, the most noticeable being the presence of small quantities of 3-hydroxycarbofuran in the sulfate fraction.

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