Bioavailability in Rats of Bound and Conjugated Plant Carbamate Insecticide Residues

Thomas C. Marshall and H. Wyman Dorough*

The bioavailability of bound (unextractables) and conjugated (water solubles) carbamate insecticide residues from plants was investigated by administering the ^{14}C residues orally to female rats. After 2 days, 11.5 and 1.3% of the bound residues of carbofuran and carbaryl from bean plants were eliminated in the urine, while 85.1 and 98.1% were voided in the feces. Only 1.4 and 0.4% of the bound residues were detected in the bile. Croneton bound residues from sorghum exhibited a similar excretion pattern. Urinary excretion of conjugated metabolites of carbaryl, aldicarb, Croneton, and carbofuran was 87.7, 87.5, 81.2, and 64.8% of the respective dose after 36 h. Excretion in the feces did not exceed 10% of the administered water solubles while the bile contained 13 to 21% of the dose. These data demonstrated that bound residues of the carbamate compounds were very poorly absorbed from the gastrointestinal tract of rats, while the conjugated ones were readily absorbed.

Most residue methods utilize apolar solvents for extraction and are designed to detect the parent compound and certain apolar metabolites. However, many plant metabolism studies using radiolabeled compounds have revealed that a considerable portion of the pesticide residue may be of a polar and/or bound nature (Casida and Lykken, 1969; Kuhr and Dorough, 1976; Shimabukuro et al., 1971). Recently, more attention has been directed toward evaluating the residual nature of the bound and conjugated residues, and in considering the possible toxicological significance of such residues (Dorough, 1976).

Bound and polar metabolites consumed by animals must be available for absorption to express toxic action. Only a few studies have been reported which have examined this parameter in mammals. Based on urinary excretion, the water-soluble metabolites of carbaryl-*naphthyl*-^{14}C from bean plants were shown to be bioavailable after oral administration to rats (Dorough and Wiggins, 1969). Paulson et al. (1975) also concluded that the water-soluble products of profam-*phenyl*-^{14}C from alfalfa plants were bioavailable when fed to rats, but that the bound residues were not. The latter was based on the fact that 86% of the dose was eliminated in the feces. Eighty-nine percent of a dose of propanil-*phenyl*-^{14}C bound residues from rice plants given to rats was eliminated in the feces and 11.5% in the urine (Sutherland, 1976). Only trace amounts of the dose appeared in the bile of mice and dogs, and, therefore, the bound residues of propanil were considered to be of little toxicological concern because of their limited bioavailability.

A need exists for additional studies which include the role of biliary excretion in determining the bioavailability of bound and conjugated residues of pesticides. This paper reports the results of such investigations in rats using residues from plants treated with [^{14}C] labeled carbamate insecticides. Throughout this text the terminology defined by Dorough (1976) is followed. Water-soluble metabolites and conjugates are used interchangeably, as are unextractable metabolites and bound residues. Endocon refers to that portion of a conjugate derived from an endogenous compound, and exocon to that portion derived from an exogenous or foreign compound. Bioavailable is used to denote that a residue is absorbed from the gastrointestinal tract following oral administration to animals.

Department of Entomology and Graduate Center for Toxicology, University of Kentucky, Lexington, Kentucky 40506.

Table I. Radioactive Insecticides Used in the Treatment of Plants

Chemical identity and position of carbon-14	Designation	Sp act., mCi/mmol
1-Naphthyl-^{14}C *N*-methylcarbamate	Carbaryl-*ring*-^{14}C	19.70
2,3-Dihydro-2,2-dimethyl-7-benzo-^{14}C-furanyl *N*-methylcarbamate	Carbofuran-*ring*-^{14}C	2.85
2-Ethylthiomethylphenyl-^{14}C *N*-methylcarbamate	Croneton-*ring*-^{14}C	7.05
2-Ethylthiomethylphenyl *N*-methylcarbamate-^{14}C	Croneton-*carbonyl*-^{14}C	5.88
2-Methyl-2-(methyl-^{14}C-thio)propionaldehyde *O*-(methylcarbamoyl)-oxime	Aldicarb-*thiomethyl*-^{14}C	2.03

MATERIALS AND METHODS

Chemicals. The radiochemicals used in this study were carbaryl-*ring*-^{14}C carbofuran-*ring*-^{14}C, Croneton-*ring*-^{14}C, Croneton-*carbonyl*-^{14}C, and aldicarb-*thiomethyl*-^{14}C. Chemical names, position of the radiocarbon, and specific activities of these carbamates are shown in Table I. The insecticide metabolite standards used for TLC were from existing stocks in our laboratory. β-Glucosidase and β-glucuronidase were purchased from Sigma Chemical Company.

Radioassay. A Packard Model 3380/544 scintillation spectrometer was used in quantifying radioactivity. Direct scintillation counting was accomplished using a commercial scintillation cocktail (type 3a70B, Research Products International). Solid samples were combusted in a Packard Model 306 sample oxidizer and the evolved [^{14}C]carbon dioxide trapped and radioassayed.

Chromatography. Thin-layer chromatography (TLC) was carried out using Merck silica gel F-254 chromatoplates. Carbofuran-^{14}C metabolites were analyzed by developing the TLC plates two dimensionally, with the first solvent a 7:3 mixture of benzene and ether, and the second a 4:1 mixture of dichloromethane and acetonitrile. Croneton-^{14}C metabolites were analyzed two dimensionally in a 3:1 ether–acetone mixture and 6:5 acetone–hexane. Radioactive spots were located by autoradiography while

the nonradioactive compounds were viewed under ultraviolet light.

Generation of Plant Metabolites. Bean plants, 10 days old, were stem injected with 35 μL of a 4:1 water–acetone solution containing the radioactive carbamate. The plants were treated with 25 μg of carbaryl-*ring*-^{14}C, 175 μg of carbofuran-*ring*-^{14}C, 40 μg of Croneton-*carbonyl*-^{14}C or Croneton-*ring*-^{14}C, and 100 μg of aldicarb-*thiomethyl*-^{14}C. All plants were grown in a mixture of peat moss and soil containing plant nutrients and were maintained in a greenhouse for 20 days after treatment. Then, each plant was cut at the soil level, sealed in a plastic bag, and stored in a freezer until extracted.

Two plants from each treatment were divided into epicotyl leaves, the remaining leaves, and the stems, and these plant parts extracted separately to determine the distribution of radioactivity within the plants. If the radiocarbon was located primarily in one portion of the plant, only that portion of the remaining plants was used in subsequent extractions. The plant material was cut into small pieces and homogenized thoroughly in 100 mL of acetone. This was filtered and the solids extracted again with acetone and then with chloroform. The filtrates were combined in a separatory funnel containing 25 mL of water and 300 mL of chloroform. After shaking the flask and separating the phases, the organic solvent layer was drained into a clean separatory funnel and washed twice with 15-mL portions of water. Aliquots of the organosoluble and water-soluble fractions were radioassayed. The plant solids were then subjected to Soxhlet extraction overnight with methanol, the solvent centrifuged to remove particulate material, and the amount of unextractable ^{14}C determined by combustion of 100 mg of the plant solids. Sorghum plants treated with Croneton-*ring*-^{14}C (by scientists of the Chemagro Agricultural Division of Mobay Chemical Corporation as part of another study made in cooperation with that group) were used as a source of unextractable metabolites. The insecticide, having a sp act. of 3.63 mCi/mmol, was applied as an emulsifiable spray at a rate of 227 g of AI/acre five times at 10-day intervals. Samples were collected 21 days after the last treatment and the unextracted ^{14}C residues obtained as described above.

Treatment of Rats and Collection of Samples. Rats (Cox-SD variety) weighing 200 g were housed one animal/cage in Delmar metabolism cages which separated urine and feces, and allowed the collection of expired gases. Initially, one animal was treated with the concentrated water solubles of five untreated 20-day-old bean plants and found to exhibit no signs of toxicity. Before treatment with the carbamate metabolites, four rats for each compound were placed in the cages and deprived of feed overnight but given water ad libitum. Water solubles of carbaryl-*ring*-^{14}C (1.0×10^6 dpm), carbofuran-*ring*-^{14}C (1.0×10^6 dpm), Croneton-*ring*-^{14}C (1.0×10^6 dpm), Croneton-*carbonyl*-^{14}C (1.1×10^5 dpm), and aldicarb-*thiomethyl*-^{14}C (1.0×10^6 dpm) were lyophilized to dryness and administered orally via a stomach tube in an aqueous solution that did not exceed 2 mL.

The unextracted residues of carbaryl-*ring*-^{14}C (1.53×10^6 dpm) and carbofuran-*ring*-^{14}C (6.5×10^5 dpm) from bean plants, and Croneton-*ring*-^{14}C (3.0×10^5 dpm) unextractables from sorghum were fed to rats and the fate of the radiocarbon examined. For each treatment, four rats were deprived of feed for 18 h, and, then for 2 days, each was fed 5 g of commercial rat chow mixed with 0.5 g of dried, nontreated bean plant solids/day. On the third day, plant solids containing the radioactive unextractables were mixed with the feed and given to the rats. Using this procedure, the feed was consumed completely within 10 to 15 min.

Respiratory carbon dioxide was trapped in a 1.0 N KOH solution which was changed every 12 h. Samples from the trap were taken every 6 h and assayed for radiocarbon content. Urine was collected every 6 h and 0.5-mL samples assayed by direct counting. Feces also were collected every 6 h if available, and a 0.5-g aliquot combusted for radioassay.

Cannulation of the bile ducts of animals treated identically to that of intact rats was performed and the bile collected on an hourly basis using an Isco Model 1200-Pup fraction collector. Sham-operated animals were carried through all surgical manipulations with the exception of cutting and ligating the bile duct. A cannulated and sham-operated rat was treated with one of the following: carbaryl-*ring*-^{14}C water soluble (3.2×10^5 dpm), carbofuran-*ring*-^{14}C water solubles (3.0×10^5 dpm), aldicarb-*thiomethyl*-^{14}C water solubles (5.8×10^5 dpm), Croneton-*ring*-^{14}C water solubles (1.2×10^6 dpm), Croneton-*ring*-^{14}C unextractables from sorghum (3.0×10^5 dpm), carbaryl-*ring*-^{14}C unextractables from bean plants (5.4×10^5 dpm), and carbofuran-*ring*-^{14}C unextractables from bean plants (3.6×10^5 dpm).

Metabolite Analysis. The water-soluble fractions from bean plants treated with carbofuran and Croneton-*ring*-^{14}C were lyophilized to dryness and the radioactivity recovered by washing the solids repeatedly with methanol until no radioactivity was detected in the wash. Portions of the polar and unextractable metabolites were placed in 1 N HCl and heated in a boiling water bath for 2 h. The polar metabolites also were incubated with glucosidase, 20 units, in acetate buffer (pH 5.6) for 24 h at 37 °C in a shaking water bath. Exocons released by acid and enzyme were extracted with ether and applied to silica gel TLC plates. Urine collected from rats treated with the carbofuran and Croneton metabolites was extracted with chloroform, and the polar phase treated with glucosidase and acid as described for the bean plant water solubles. In addition, the polar urinary radiocarbon was subjected to glucuronidase treatment (Dorough et al., 1974). The feces of rats fed unextractable ^{14}C residues were extracted with acetonitrile and the extract separated into an aqueous and organic solvent phase by the addition of chloroform.

RESULTS AND DISCUSSION

Metabolism in Plants. The epicotyl leaves of bean plants treated with carbofuran-*ring*-^{14}C, Croneton-*ring*-^{14}C, and aldicarb-*thiomethyl*-^{14}C contained 80 to 90% of the radiocarbon injected into the stems 20 days earlier. Since the unextracted and water-soluble residues were to be administered to rats, preparations of these metabolites high in specific activity and low in plant material were preferred. Therefore, only the epicotyl leaves of plants treated with these four carbamates were extracted and used in this study. Carbaryl-*ring*-^{14}C treated plants had a radiocarbon distribution of 21% of the dose in the epicotyl leaves, 18% in the remaining leaves, and 61% in the stems. Therefore, the entire bean plant was extracted to isolate the water-soluble and unextracted residues.

Organosoluble metabolites in the plants ranged from 14% of the injected radiocarbon for Croneton-*carbonyl*-^{14}C to 60% for aldicarb (Table II). Soxhlet extraction removed about one-half of the carbaryl and carbofuran organosoluble metabolites following direct extraction of the plant material via blending. With the other treatments, the blending procedure appeared to more effectively extract the apolar metabolites since the Soxhlet extract

Table II. Extraction and Partitioning Characteristics of Radiocarbon in Bean Plants 20 Days after Stem Injection of ^{14}C-Labeled Carbamate Insecticides

Nature of radiocarbon	% of injected radiocarbon				
	Carbaryl-*ring*-^{14}C	Carbofuran-*ring*-^{14}C	Aldicarb-*thiomethyl*-^{14}C	Croneton-*ring*-^{14}C	Croneton-*carbonyl*-^{14}C
Organosolubles Extracted by blending	9.3	8.9	55.8	43.0	13.1
Soxhlet extracted	9.8	6.1	2.1	2.8	0.7
Total	19.1	15.0	57.9	45.8	13.8
Water solubles[a]	30.5	66.6	29.3	40.7	2.6
Unextracted[a]	50.6	7.0	2.2	3.3	1.3
Total recovered	100.2	88.6	89.4	89.8	17.7

[a] Portions administered orally to rats for bioavailability determinations.

Table III. Acid Treatment of Unextracted Metabolites from Bean Plants Injected with ^{14}C-Carbamate Insecticides[a]

Effect of acid treatment	% of Unextracted radiocarbon				
	Carbaryl-*ring*-^{14}C	Carbofuran-*ring*-^{14}C	Aldicarb-*thiomethyl*-^{14}C	Croneton-*ring*-^{14}C	Croneton-*carbonyl*-^{14}C
Rendered organosoluble	0.0	9.0	13.4	8.0	13.8
Rendered water soluble	3.3	7.7	23.0	7.4	9.3
Total released	3.3	16.7	36.4	15.4	23.1
Remained in plant solids	96.7	83.3	63.6	84.6	76.9

[a] Plant solids were placed in HCl and heated in boiling water bath for 2 h.

removed very little radioactivity. These metabolites were not further analyzed in the present investigation.

Water-soluble metabolites, which were subsequently administered to rats, constituted 30% or more of the injected radioactivity in bean plants treated with all compounds except Croneton-*carbonyl*-^{14}C. With the latter material, about 3% of the dose was as water solubles and the total recovery 20 days after treatment was only 18% of that injected into the plants. This loss with the carbonyl-^{14}C label and a recovery of 90% of the ring-^{14}C label, demonstrated that the ester linkage was hydrolyzed and that the carbonyl carbon escaped from the plant, probably as [^{14}C]carbon dioxide. The degree of ester hydrolysis of Croneton by plants indicated by these data is much larger than that reported for the other carbamates used in this study. Aldicarb may be hydrolyzed by 20% or more by plants (Coppedge et al., 1967; Bartley et al., 1970) while only slight hydrolysis occurs with carbofuran (Dorough, 1968) and almost none with carbaryl (Abdel-Wahab et al., 1966; Kuhr and Casida, 1967; Dorough and Wiggins, 1969).

Radiocarbon remaining in the plant solids after blending and Soxhlet extraction was classified as unextracted or bound residues (Table II). This fraction from the carbofuran and carbaryl treated plants was fed to rats to determine the fate of the unextracted residues in animals. The quantity of unextracted residues formed with the other carbamates by bean plants was too low to allow similar evaluation. However, unextracted metabolites of Croneton in field-treated sorghum were of sufficient magnitude for feeding to rats and this source of residues was used in lieu of unextractables from bean plants.

The extraction procedure used to remove the radioactive residues from bean plants was as rigorous, if not more so, than those commonly employed in residue analysis. Therefore, the unextracted residues were properly designated insofar as practical methodology is concerned. To assist in evaluating the bioavailability data obtained in later studies, an attempt was made to extract even more of the residues from the plant solids by acid treatment (Table III). The carbaryl unextracted residues were most resistant to acid treatment, with only 3.3% of the radiocarbon released. All of the released residues were of a water-soluble nature. Identical treatment of the other unextracted residues resulted in radioactivity in both the water and chloroform phases, and in combined quantities ranging from 15 to 36% of the ^{14}C content of the plant solids. It was assumed that the acid-released residues were less tightly bound than those remaining in the plant matrix, and, consequently, that they had a higher potential for being bioavailable. However, the unextracted residues were not treated with acid prior to feeding them to rats since such treatment could modify the chemical nature of the residues in the plant solids.

Fate of Residues in Rats. *Bound Residues.* When the unextracted residues of carbaryl and carbofuran were administered to intact rats as a dietary supplement, 98.1 and 85.1% of the doses were eliminated in the feces within 48 h, while the urinary excretion amounted to 1.3 and 11.5% respectively (Figure 1). Only 0.3 and 1.4% of an identical dose was excreted in the bile of fistulated rats. A similar pattern of elimination was seen when the unextractables of Croneton from sorghum was fed to rats. After 2 days, 90.8% of the dose was voided in the feces and 16.4% in the urine; the bile contained 2.5% of the dose.

Monitoring biliary radiocarbon was a vital aspect of these studies since the relative quantities of urinary and fecal elimination of foreign compounds may not accurately demonstrate the degree of absorption of a compound from the gut (Klaassen, 1975). Houston et al. (1975) demonstrated that 6 h after a single intravenous dose of carbaryl-*carbonyl*-^{14}C to rats, up to 40% of the administered radiocarbon was excreted into the bile. With intact rats, only 3% of the dose was eliminated in the feces. Obviously, some metabolites in the bile are readily reabsorbed and excreted by the kidney. On the other hand, unpublished data from our laboratory show that only 14% of an oral dose of α-endosulfan-^{14}C to rats was eliminated in the urine after 48 h, while 35% was voided via the feces. While these data might suggest that endosulfan is poorly absorbed from the gastrointestinal tract, it was observed that up to 60% of the dose was present in the bile of cannulated rats within the same time period. The study clearly demonstrated that fecal elimination of a material does not necessarily mean that the substance was not

Table IV. Radioactive Residues in the Urine and Feces of Rats Treated with ^{14}C-Unextractable Plant Metabolites of Carbofuran and Carbaryl

Route of excretion and nature 0-72 h	% of radiocarbon administered	
	Carbofuran-*ring*-^{14}C unextractables	Carbaryl-*ring*-^{14}C unextractables
Urine		
Chloroform extractable	0.2	0.0
Acid released	4.9	0.5
Remaining in water	6.4	0.8
Total	11.5	1.3
Feces		
Chloroform solubles	0.9	0.6
Water solubles	0.3	0.1
Remaining in solids	83.9	97.4
Total	85.1	98.1
Total excreted	96.6	99.4

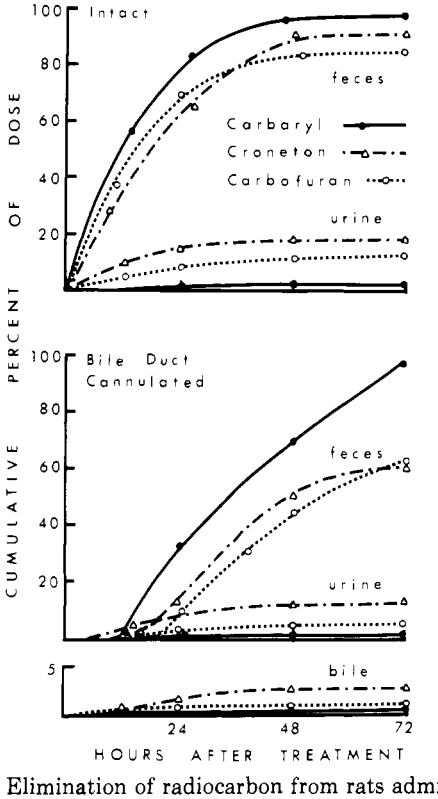

Figure 1. Elimination of radiocarbon from rats administered bound (unextractables) residues formed from ^{14}C carbamate insecticides by plants.

absorbed. Other examples of this phenomena are the polycyclic hydrocarbons, 1,2-benzanthracene, and 3,4-benzopyrene, which undergo extensive biliary excretion but are eventually eliminated in the feces (Falk, 1963; Boyland and Sims, 1964; Levine, 1970).

Urinary and/or biliary excretion of a compound or its metabolites signifies that the material is bioavailable. Quantitative fecal elimination without biliary excretion similarly demonstrates that a material is not bioavailable. The low degree of bioavailability of the unextracted carbamate insecticide plant residues in rats was apparent in that they were excreted predominantly in the feces and only very small amounts appeared in the bile. Urinary excretion of the carbofuran and carbaryl unextractables, 11.5 and 1.3% of dose, and their partitioning characteristics (Table IV) were very similar to that distribution of radiocarbon observed when the plant solids were treated with acid (Table III). It is likely that the acid-released metabolites were responsible for the radiocarbon in the urine and bile while those remaining in the plant solids were excreted in the feces. Extraction of the feces removed

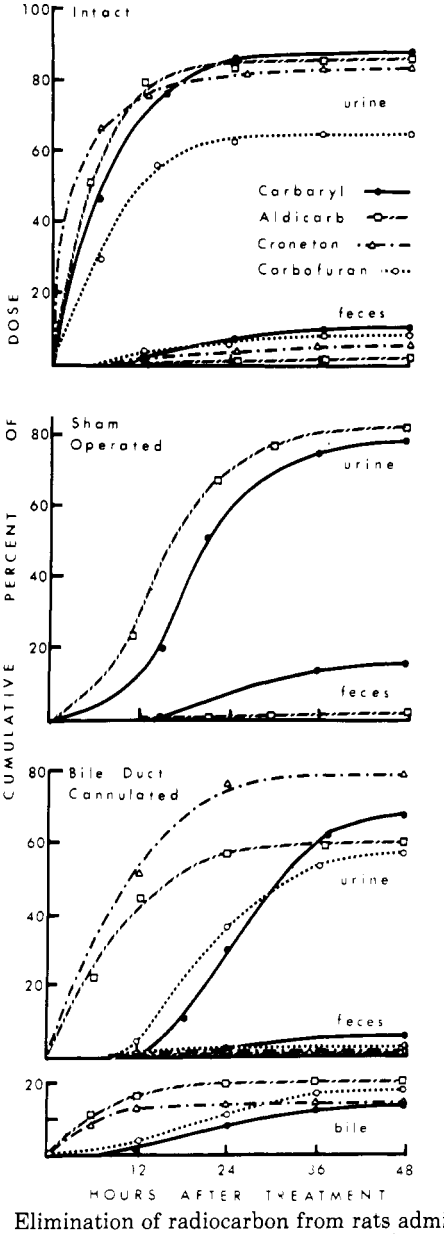

Figure 2. Elimination of radiocarbon from rats administered conjugated (water solubles) residues formed from ^{14}C carbamate insecticides by plants.

only trace amounts of residue (Table IV), thereby indicating that the radiocarbon remained in a tightly bound form.

Conjugated Residues. Intact rats administered a single oral dose of ^{14}C water-soluble metabolites of carbamates from bean plants generally eliminated 80 to 90% of the

Table V. Elimination of Radiocarbon from Rats Treated Orally with Croneton-*carbonyl*-^{14}C Bean Plant Water Soluble Metabolites

Hours after treatment	Cum. % of dose			
	$^{14}CO_2$	Urine	Feces	Total
6	23.1	31.2	0.1	54.4
12	30.0	34.5	0.6	65.1
18	30.0	37.5	3.9	71.4
36	30.0	39.0	6.2	75.2

radiocarbon in the urine (Figure 2). With the carbofuran water solubles, excretion by this route was only 65% of the dose, but this was clearly an exception and there was no indication by the other excretion data, bile and feces, as to the reason for the low recovery. Fecal elimination of the water soluble metabolites did not exceed 10% of the dose in any of the experiments. Biliary excretion was evident at higher levels than those in the feces, especially with the aldicarb residues where the collected bile contained 21% of the dose and intact rats excreted only 1% in the feces. Enterohepatic circulation obviously occurred in intact rats to some extent with the water-soluble carbamate metabolites since their final disposition was so much greater in the urine than in the feces. The excretion pattern of the water-soluble metabolites from rats unquestionably demonstrates that they are readily absorbed from the gastrointestinal tract and must be considered highly bioavailable.

According to Aguiar (1975), there are two main processes which govern the absorption of foreign compounds from the gastrointestinal tract. One is the build-up of an effective concentration of the chemical in solution at the absorption site, and the other is the rate of permeation of the solubilized substance through the gastrointestinal barrier. These processes, in turn, are influenced by many factors including gastrointestinal motility, physiological and metabolic properties of the intestinal epithelia, and the microfloral content of the gut (Kurz, 1975). When a compound is consumed in a solid form, absorption is dependent upon dissolution of the solid and then the accumulation of an effective concentration at the site of absorption. It was obvious that this did not occur with the unextractable carbamate insecticide residues (Figure 1). When compounds are consumed as solutions, the permeation rate is assumed to be the controlling process in their absorption rates (Aguiar, 1975). The data in Figure 2 show that the water-soluble residues, or their derivatives, permeated the gastrointestinal barrier without difficulty.

Being bioavailable, it is likely that the water-soluble carbamate metabolites underwent considerable bioalteration after they were administered to the rats. In vivo cleavage of 1-naphthyl glucoside (Dorough et al., 1974) and the glucosides of 4- and 5-hydroxycarbaryl (Dorough, 1976) has been demonstrated experimentally. The toxicological significance of such cleavage is dependent upon the toxic properties of the released exocons and their ability to withstand enzymatic degradation to innocuous products. With the carbaryl glucosides just mentioned, cleavage of both the glucoside linkage and the carbamate ester occurred rapidly; the resulting naphthol and hydroxynaphthols were then conjugated as glucuronides and sulfates and eliminated from the body primarily via the urine.

In the present study, in vivo metabolism of the water solubles was evidenced by the fate of the radiocarbon when Croneton carbonyl-[^{14}C] metabolites were given to rats (Table V). The carbonyl carbon was detected in the respiratory gases as radioactive carbon dioxide in quantities equivalent to 30% of the dose. This was suggestive of carbamate ester hydrolysis as was shown earlier with the glucosides of 4- and 5-hydroxycarbaryl (Dorough, 1976). The pattern of elimination of radiocarbon from rats treated with the Croneton-*carbonyl*-^{14}C water solubles was similar to that resulting from the administration of the parent compound radiolabeled in the carbonyl position (Nye et al., 1976). It would appear, therefore, that the water-soluble Croneton metabolites containing the carbamate moiety are degraded just as effectively as the apolar parent carbamate.

Table VI. Nature of ^{14}C-Carbamate Bean Plant Water Soluble Metabolites and Their Fate in Rats as Compared to the Parent Carbamate Insecticide

	Nature of radiocarbon in				
	Bean plant water soluble metabolites		Urine of rats fed water soluble metabolites		Urine of rats fed parent carbamate[a]
	% of total ^{14}C in water fraction		% of total ^{14}C voided in the urine		% of total ^{14}C voided in the urine
Insecticide and metabolites	Glucosidase treatment	Acid treatment	Glucuronidase treatment	Acid treatment	Acid treatment
Carbofuran-*ring*-^{14}C			(65% of dose voided in urine		(91% of dose voided in urine)
Organosolubles			3.8	3.8	9.0
Water solubles					
3-Hydroxycarbofuran	52.1	81.7	0.5	6.9	13.5
Carbofuran phenol	0	1.2	0.6	0	19.0
3-Hydroxyphenol	2.6	3.0	1.1	8.6	1.2
3-Ketophenol	1.6	1.0	10.0	73.3	45.5
Remaining in water	43.7	13.1	84.0	7.4	7.3
Croneton-*ring*-^{14}C			(92% of dose voided in urine)		(97% of dose voided in urine)
Organosolubles			1.1	1.1	37.3
Water solubles					
Croneton sulfoxide	0	4.1	0	0	0
Croneton sulfone	0	2.2	0	0	0
Sulfoxide phenol	33.6	45.7	11.5	26.8	19.1
Sulfone phenol	47.5	36.4	10.3	59.2	22.8
Remaining in water	18.9	11.6	77.1	12.9	18.8

[a] Carbofuran data from Dorough, 1968; Croneton data from Nye et al., 1976.

Additional information relative to the nature of the water-soluble metabolites and their fate in rats is shown in Table VI. In this case, the water solubles from carbofuran-*ring*-^{14}C and Croneton-*ring*-^{14}C treated bean plants were exposed to glucosidase and acid, and the identity of the major exocons released was ascertained by TLC analysis. The same type of analyses were performed on the urinary metabolites voided by rats treated with the water solubles. The latter metabolites, it should be noted, were not treated with enzyme or acid prior to administering them to rats. For purposes of comparison, the major exocons released by acid treatment of urine from rats treated orally with the ring-^{14}C-labeled parent carbamates are also given in Table VI.

Carbofuran-*ring*-^{14}C water solubles from plants consisted largely of conjugated 3-hydroxycarbofuran. Acid treatment yielded 82% of the radiocarbon as this free exocon and small amounts, 1 to 3%, as carbofuran phenolic derivatives. While cleavage with glucosidase was not as effective, the pattern was the same as with the acid and indicated that the exocons existed in the plant as glucoside conjugates. When voided in the urine of rats, the major exocon released by glucuronidase and acid treatment of the polar metabolites was the 3-ketophenol derivative of carbofuran (Table VI). This same exocon was the predominant component of the urine of rats treated with carbofuran-*ring*-^{14}C. In fact, the only appreciable difference in the nature of exocons in the urine was the presence of carbofuran phenol when the animals were dosed with the parent carbamate, but absence when the water solubles were administered. This finding was consistent with the observance that the water solubles contained no precursor to carbofuran phenol and that the metabolite, per se, was a minor constituent of the water-soluble materials.

Since 3-hydroxycarbofuran glucoside accounted for the vast majority of the bean plant water solubles, the data resulting from treatment of the rats with this plant fraction actually reflects the fate of the glucoside in animals. The results show that the animal system cleaved the glucoside linkage, hydrolyzed the carbamate ester, and further oxidized the 3-hydroxy derivative to the 3-keto form. As a result of hydrolysis, free hydroxyl groups were generated, and these were again conjugated, at least in part, as glucuronides and voided from the animal in the urine. That the glucoside linkage was cleaved in the animal was supported by the finding that glucosidase treatment of the urinary polar metabolites did not release any aglycones.

Croneton-*ring*-^{14}C water-soluble metabolites were metabolized in rats in a manner similar to that of the carbofuran materials (Table VI). However, the polar plant residues of Croneton were almost entirely conjugates of the sulfoxide and sulfone derivatives of Croneton phenol. Only 6% occurred as carbamates and none of this survived the hydrolytic processes in the animal. The phenolic glucosides were cleaved in the animal and reconjugated as glucuronides, and possibly as other derivatives. Total elimination of the dose in urine, 92%, was about the same as with the parent compound, 97%, and the acid-released exocons were similar in both cases. The difference in the two treatments was that 1% of the urinary metabolites was organosoluble when the animal received the Croneton water solubles but was 37% following treatment with the parent carbamate. Nye et al. (1976) showed that the latter was composed chiefly of Croneton sulfoxide, a product which accounted for only 4% of the water soluble metabolites from plants.

Effects of Surgical Manipulations. As previously pointed out, it is essential that the bile be monitored if bioavailability of certain compounds is to be adequately determined. In those instances where compounds are excreted primarily in the feces, such as the unextractable plant carbamate insecticide residues, bioavailability can be determined only if the bile is monitored. To accomplish this, the bile duct must be cannulated and special precautions taken with the animals to insure that the cannula remains intact. It is possible that the surgery and special handling of the animals may alter the fate of the compound under investigation. Moreover, and possibly more pertinent to bioavailability determinations, is the likelihood that preventing the bile from entering the intestine influences gastrointestinal absorption. While not all effects of bile collection were considered in this study, urinary and fecal elimination of the carbamate residues were determined in animals having the bile duct cannulated and in sham-operated animals. The data are presented in Figures 1 and 2 along with results from the intact rats.

Bile duct cannulated rats dosed with unextracted metabolites exhibited a much slower rate of radiocarbon elimination as compared to the intact animals (Figure 1). After 48 h, the decrease in total excretion was as much as 50%. However, when the cannulated rats treated with carbaryl unextractables were held for 72 h, total elimination reached 99% of the dose. This same delayed pattern of elimination was demonstrated by sham-operated and cannulated rats administered water-soluble metabolites (Figure 2). The excretion of the aldicarb water solubles from the rats was more similar to the intact animals than was that of the other carbamate residues. This occurred because the aldicarb water solubles were not administered until 4 h after surgery to determine if the state of recovery affected excretion rates. Since the rates approached those of intact animals, it appears that slower elimination by surgically manipulated animals was only a transient manifestation of the operation and was not caused by factors irreversibly altering normal absorption mechanisms. Possibly, the decreased elimination results from decreased absorption caused by a temporary loss of gastrointestinal motility following surgery (Parsons, 1975).

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Determination of Total Arsenic Residues in Chicken Eggs

Nuhad J. Daghir* and Nour N. Hariri

Two levels (50 and 100 ppm) of 3-nitro-4-hydroxyphenylarsonic acid (3-nitro) were fed to different groups of White Leghorn layers for a period of 15 weeks. Arsenic residues in eggs laid by these hens were determined all through the experimental period at almost weekly intervals by a spectrophotometric method sensitive to 0.05 ppm. Contrary to the steadily increased drug intake during the experiment, residues in eggs did not show a continuous accumulation but rather an increase up to a certain level after which it gradually decreased. Arsenic residues determined 2 weeks after the withdrawal of the drug from the feed were negligible.

The organoarsenical compounds such as arsanilic acid and 3-nitro are usually administered in modern-day poultry rations for growth promotion, improvement of feed conversion, better pigmentation, increased egg production, and less mortality. Medication with these compounds could produce residues in edible food products which are usually measured as elemental or inorganic arsenic that might sometimes, and especially when not used at the levels recommended by the Food and Drug Administration (FDA), be a hazard to the public health and thus demands considerable attention.

In this study residual arsenic was determined in eggs laid by hens fed different levels of 3-nitro for a 15-week period and 2 weeks after withdrawal of the drug from the feed.

MATERIALS AND METHODS

Experimental. Forty-eight White Leghorn layers housed in individual wire-floored cages with individual feeders and waterers were used in this experiment. The layers were divided into 12 groups, each group with four birds. The groups were then allocated to three treatments having four groups for each treatment. Medication with 3-nitro was given at the 50 and 100 ppm level in a standard layer type ration. The level permitted by the FDA for layers is 25–50 ppm. The remaining four groups of birds were the control group which received the nonmedicated ration. The feeding of the drug continued for a period of 15 weeks and egg samples from each group were collected at weekly intervals, mainly from those laid on the first 3 consecutive days of the week. About three eggs from each group were pooled for sampling.

Arsenic Analysis. Representative samples of three or four eggs from each group were homogenized in a Waring blender. The homogenate was then left standing for 30 min at 10–15 °C in order to allow escape of the entrapped air. A 20-g sample was then taken for analysis and determination of residual arsenic using the method of George et al. (1973) for dry ashing for the determination of total arsenic in animal tissue.

Table I. Recovery of Arsenic Added to Control Egg Samples[a]

Determinations	ppm As added				
	0	0.1	0.2	0.5	1
1	0.016	0.101	0.192	0.462	0.866
2	0.012	0.089	0.193	0.518	0.898
3	0.003	0.093	0.185	0.472	0.920
4	0.010	0.085	0.190	0.462	0.845
5	0.003	0.095	0.192		0.937
6	0.009				
7	0.006				

[a] Slope = 0.0445; reciprocal slope = 22.5.

Preparation of the Standard Curve. Twenty-gram samples of eggs were spiked with 1 mL of the following arsenic working standard solutions: 0 μg, 2 μg, 4 μg, 10 μg, and 20 μg/mL to get samples containing 0, 0.1, 0.2, 0.5, and 1 ppm As. These were then processed through the dry ashing and distillation as described by the above named procedure (George et al., 1973). The best fitting straight line from ≥4 sets of determinations was obtained for each level of the above concentrations by the method of least squares (AOAC, 1970). The arsenic concentration of an unknown sample was calculated by multiplying the absorbance (540 nm) by the reciprocal slope of the line, discarding the y intercept term.

RESULTS AND DISCUSSION

Recovery of arsenic added to egg samples assayed are presented in Table I. Calculation of residual arsenic in the manner described by using the reciprocal slope of the

Faculty of Agricultural Sciences, Department of Animal Science, American University of Beirut, Beirut, Republic of Lebanon.