suggested that five of the minor fecal metabolites were a result of nonmetabolic breakdown of the robenidine which occurred during the extraction process. Chromatography in solvent system I of the radioactivity extracted from the feces with acidified ethanol revealed that all of the radioactivity remained near the origin, while chromatography in solvent system II resolved the radioactivity into ten radioactive spots, none of which was identified. Since more than 60% of the radioactivity found in feces was identified as unmetabolized robenidine, this suggests that robenidine was incompletely absorbed from the digestive tract and does not undergo extensive breakdown in the body of the animal.

Tissues. In order to establish the relevance of the urinary metabolites to those occurring in tissues, the radioactivity in selected tissues was isolated by extraction and chromatographed in solvent system I. Benzene extracts of fat and skin, containing 93–95% of the total radioactivity in these tissues, revealed that robenidine was the only compound present. Ethanol extracts of liver, kidney, and muscle containing 87–97% of the total radioactivity in these tissues revealed the presence of p-chlorohippuric acid, p-chlorobenzoic acid, and robenidine as tissue residues.

CONCLUSION

Since p-chlorobenzoic acid is excreted as p-chlorohippuric acid in dogs (Novello et al., 1926) and rabbits (Bray et al., 1952) and these compounds have been identified as urinary metabolites of robenidine, this evidence indicates that the scheme shown in Figure 1 represents a major route for the metabolism of robenidine in the rat. No 1-amino-3-(p-chlorobenzylidineamino)guanidine, postulated to be a potential metabolite, was found.

It is speculated that this aminoguanidine fragment may be a transient metabolite. It could be split by the rat to yield p-chlorobenzoic acid and 1,3-diaminoguanidine, as suggested from the mammalian metabolism of the related aldehyde derivatives of thiosemicarbazide (Williams, 1959). Since there is little literature on the known fate of guanidine in mammalian metabolism, no proposals can be made to suggest the possible fate of the aminoguanidine moiety.

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Metabolism of the Herbicide Methazole in Cotton and Beans, and Fate of Certain of Its Polar Metabolites in Rats

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Methazole, 2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione, was metabolized by cotton and beans to 3-(3,4-dichlorophenyl)-1-methylurea and 3-(3,4-dichlorophenyl)urea. These metabolites occurred in the free form and as polar products which were converted to the free form by acid treatment. ^{14}C residues in the solids after acetone extraction were removed by heating in 1 N HCl and extracting with ethyl acetate. The methylurea and urea derivatives of methazole were the major components of the ethyl acetate extract. When polar acetone-extractable metabolites from cotton and beans were administered orally to rats, 70 to 80% of the radioactivity was excreted within 24 hr. Residues were low, 0.13 ppm maximum, in tissues of animals sacrificed 12 hr after treatment orally for 14 days with polar methazole metabolites at a rate of approximately 1 µg of methazole-^{14}C equivalents/animal/day.

The general metabolic fate of methazole, 2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione, in cotton has been reported (Jones and Foy, 1972). It was demonstrated that the herbicide was metabolized to 1-(3,4-dichlorophenyl)-3-methylurea, 1-(3,4-dichlorophenyl)urea, and to significant quantities of more polar materials which remained unidentified. In addition, large quantities of methazole-^{14}C equivalents, over 30% in leaves, could not be extracted from the treated plants with methanol.

Similar pathways have been described for monuron, 3-

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(4-chlorophenyl)-1,1-dimethylurea, and diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (Frear and Swanson, 1972; Onley et al., 1968; Smith and Sheets, 1967; Swanson and Swanson, 1968). Frear and Swanson (1972) found that after 24 hr, 20 to 25% of the methanol-extractable monuron equivalents from excised cotton leaves treated with monuron was as β-D-glucosides of 3-(4-chlorophenyl)-1-hydroxymethyl-1-methylurea and 3-(4-chlorophenyl)-1-hydroxymethylurea. Subsequent studies showed that the hydroxymethyl intermediate was unstable but would react with methanol to form 3-(4-chlorophenyl)-1-methoxymethylurea, which was stable (Tanaka et al., 1972).

The methoxymethylurea derivative of methazole was detected in methanol solutions of the herbicide after exposure to ultraviolet light (Ivie et al., 1973). It was not a
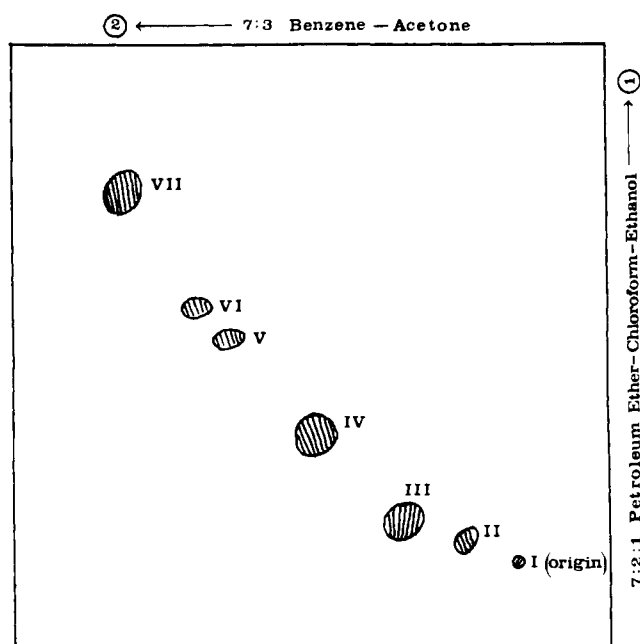
Figure 1. Silica gel tlc of acetone extractable ^{14}C residues from cotton and bean leaves treated with methazole-phenyl-^{14}C or methazole-3-^{14}C.

Table I. Chemical Names and Abbreviated Nomenclature of Methazole Derivatives Mentioned in Text

Chemical name	Designation in text[a]
2-(3,4-Dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione	Methazole, met VII
3-(3,4-Dichlorophenyl)-1-methylurea	Methylurea, met IV, P-III
3-(3,4-Dichlorophenyl)urea	Urea, met III
3-(3,4-Dichlorophenyl)-3-hydroxy-1-methylurea	3-N-Hydroxymethylurea
3-(3,4-Dichlorophenyl)-1-hydroxy-methylurea	Hydroxymethylurea, met VIII
3-(3,4-Dichlorophenyl)-1-methoxy-methylurea	Methoxymethylurea, P-IV
6,7-Dichloro-1-methyl-2-benzimidazolinone	P-I
5,6-Dichloro-1-methyl-2-benzimidazolinone	P-II

[a] Authentic standards available except for hydroxymethylurea (met VIII) derivative.

photoproduct of methazole when exposed to sunlight in water for 7 days. However, two other photoproducts were formed in both methanol and water solutions of methazole. These were identified as 6,7-dichloro-1-methyl-2-benzimidazolinone and 5,6-dichloro-1-methyl-2-benzimidazolinone.

The present study was undertaken to evaluate the metabolism of methazole in cotton and bean plants, with particular emphasis on determining the nature of the polar metabolites and products which could not be extracted with methanol. Attempts also were made to determine if the hydroxymethyl derivative of methazole occurred as a metabolite in plants and to compare the photolytic pathway of methazole to its metabolic pathway. Finally, polar methazole metabolites from cotton and beans were administered orally to rats and their excretion rates determined.

METHODS AND MATERIALS

Chemicals. Three radiocarbon preparations of methazole were used in these studies. The methazole phenyl-^{14}C (sp act., 13.5 mCi/mmol) and methazole-3-^{14}C (7.7 mCi/mmol) were purified to greater than 97% by thin-layer chromatography. Methazole-5-^{14}C, 15.7 mCi/mmol, was over 99% pure and required no additional purification.

Twenty derivatives of methazole were available for comparison with metabolites detected in the various experiments. The chemical names and designations used in the text for those pertinent to the current investigation are given in Table I.

Thin-Layer Chromatography. For routine tlc analysis, silica gel F_{254} precoated chromatoplates (0.25 mm, Merck AG, Darmstadt, Germany) were developed one-dimensionally in a 7:2:1 mixture of petroleum ether–chloroform–ethanol (Jones and Foy, 1972). To establish cochromatography of an unknown metabolite and one of the metabolite standards, two-dimensional chromatography was utilized (Figure 1). Chromatoplates and the first solvent system were the same as just mentioned. The second solvent system consisted of a 7:3 mixture of benzene–acetone. Two-dimensional tlc adequately resolved all radioactive metabolites and/or standards except the methylurea and the methoxymethylurea. The latter products were separated on aluminum oxide F_{254} chromatoplates (0.25 mm, Merck AG) developed in a 9:1 mixture of ethyl acetate-

isopropyl alcohol. R_f values for methazole and certain of its derivatives were: methazole, 0.65; methylurea, 0.44; methoxymethylurea, 0.36; and urea, 0.11.

Another tlc system used for isolation of metabolites was silica gel chromatoplates developed in a 1:1 mixture of chloroform–acetonitrile. With this system, metabolites III through VII (Figure 1) were moved together near the solvent front and metabolites I and II moved as one spot with an R_f of approximately 0.5. No radioactive metabolites remained at the origin in the 1:1 solvent system.

Treatment of Plants. Cotton "Deltapine 16" and beans, *Phaseolus* sp., were maintained under greenhouse conditions. Excised leaf experiments were conducted by removing leaves, including petioles, from cotton 2 to 3 months old and from beans which were approximately 4 weeks old. The freshly cut leaves were placed immediately in 1 ml of treatment solution contained in a 2-ml shell vial. The treatment solution was prepared by adding the appropriate radioactive preparation of methazole, about 1.5×10^6 dpm in 50 μl of acetone, to a shell vial containing 1 ml of water. Two 50-μl aliquots were removed after thoroughly mixing the acetone and water and radioassayed to determine the quantity of methazole-^{14}C in the treatment solution. The leaves were left in the solution for 24 hr, where they adsorbed from 70 to 80% of the methazole-^{14}C. They were then transferred to distilled water, where they remained until harvested for analysis.

To determine the fate of methazole-^{14}C when applied to intact cotton leaves, an aqueous solution of the herbicide was applied to the surface of leaves on plants which were 2 months old. Each leaf received 5.0×10^5 dpm of either methazole-phenyl-^{14}C or methazole-3-^{14}C by placing small drops of the solution on the leaf surface with a microsyringe and then spreading evenly with the needle of the syringe. Plants used in this phase of the study were placed outdoors in June, 1 week prior to treatment, and remained there throughout the entire experimental period.

Treatment of Rats. The more polar metabolites of methazole in cotton and beans, metabolites I and II (Figure 1), were administered orally in water to rats in order to determine their fate in animals. Female albino rats weighing 250 g were treated with a mixture of the metabolites, approximately 1:1 ratio, and placed in metabolism cages which allowed the separate collection of the feces and urine.

In the case of the metabolites from beans, four rats received a single oral dose, 6.4×10^5 dpm, of metabolites I and II from plants treated with methazole-phenyl-^{14}C or with methazole-3-^{14}C. The same was true for the cotton

metabolites, except that daily oral doses, 8.0×10^5 dpm, were administered to the rats for 14 days. Urine and feces were monitored for radioactivity for 6 days after the single oral dose of bean metabolites and for 8 days following the last treatment of cotton metabolites. Tissue samples were removed for radioassay 12 hr and at 8 days after the last of the cotton metabolite treatments. Two animals were sacrificed at each of these times.

Extraction. Two leaves were extracted three times with mortar and pestle in 30-ml portions of acetone. The extracts were combined, concentrated, and applied directly to tlc plates. Plant solids, after extraction, were placed in 20 ml of 1 N HCl solution and heated 30 min at 90°. The mixture was cooled and filtered, and the aqueous phase extracted three times with ethyl acetate. Acid treatment of the plant solids and extraction of the water layer was repeated and the ethyl acetate extracts were combined and analyzed by tlc. Radioactivity in the acetone, ethyl acetate, water, and plant solids was quantitated.

It was desirable to remove methazole and its apolar metabolites prior to acid treatment, since appreciable degradation of these products occurred when exposed to acid in the presence of plant material. Most of these degradation products remain at the origin in the silica gel, 7:2:1 tlc system. Identical acid treatment in the absence of plant material resulted in relatively minor degradation.

Rat urine from each animal treated with methazole metabolites I and II for 14 days was pooled and extracted three times with equal volumes of benzene. The radiocarbon content of both the water and benzene phases was determined and the benzene concentrated and applied to silica gel chromatoplates. The tlc's were developed in 7:2:1 petroleum ether–chloroform–ethanol.

Stability of Methazole and Metabolites. The stability of methazole and certain of its metabolites was evaluated under various conditions to which they were exposed during this investigation. Of primary concern was their stability during extraction from plant material, under acid and basic condition, and during routine recovery from tlc. Methazole-^{14}C and its radioactive metabolites previously extracted from plants were exposed to these conditions and then reexamined by tlc analysis.

Enzyme Studies. Radioactive methazole metabolites I and II from cotton were incubated with β-glucosidase in citrate–phosphate buffer, pH 5.0, at 37° for 4 hr. The incubation mixtures were extracted with ethyl acetate and the quantity of ^{14}C materials in the water and organic solvent phases was determined. Both layers were concentrated and examined by tlc analysis.

Evidence for Existence of Hydroxymethylurea, Metabolite VIII. Based on the general assumption that N-demethylation is preceded by the formation of an N-hydroxymethyl derivative and that such does occur with herbicides of similar structures to that of methazole (Frear and Swanson, 1972; Tanaka et al., 1972), attempts were made to establish the presence or absence of the hydroxymethylurea in cotton plants. Because the compound, if formed, would probably be unstable (Tanaka et al., 1972), an indirect approach to the problem was necessary. A rather conclusive method of proving the existence of the hydroxymethylurea and/or its conjugate in plants would be to isolate methoxymethylurea from plants extracted with methanol. Methoxymethylurea is a stable compound which can be separated from the methylurea by tlc on aluminum oxide plates but not on silica gel.

A duplicate set of cotton leaves treated with methazole was simultaneously extracted with acetone and methanol and the extracts were analyzed by aluminum oxide tlc. Radioactivity corresponding to the position of the known methoxymethylurea derivative on the chromatograms was extracted with acetone and isotopic dilution analyses were performed. Approximately 2.3×10^5 dpm were added to 20 mg of authentic methylurea and to 20 mg of methoxy-

methylurea. The known products were recrystallized six times from ethyl acetate by the addition of hexane. After each recrystallization, the specific activity, dpm/mg, was determined. A similar experiment was conducted on excised cotton leaves treated with methylurea-^{14}C to determine if this material served as an intermediate to the hydroxymethylurea metabolite.

In connection with these studies, tests were conducted to determine if the hydroxymethyl metabolite of methazole might be formed by photolysis under natural conditions, and if certain photoproducts might be formed metabolically. Methazole-^{14}C and methylurea-^{14}C were exposed to sunlight and to ultraviolet light in both water and methanol (Ivie et al., 1973) and the photoproducts compared to metabolites of methazole formed in the cotton plant.

Radioassay and Detection of Reference Standards. All quantitative radioassays were performed on a Packard Tri-Carb model 3380/544 scintillation counter. Aliquots of liquid samples were placed directly into vials containing 15 ml of scintillation fluid. Solid samples such as green plant material, plant solids after extraction, rat feces, etc., were combusted in a Beckman Biological Materials Oxidizer and the trapped $^{14}CO_2$ was quantitated. Radioautography with Kodak no-screen medical X-ray film was used to detect radioactive areas on tlc plates, while the nonradioactive standards were visualized under ultraviolet light.

Radioactive CO_2 was collected from plants treated with methazole-5-^{14}C by placing the plants in a glass container equipped with an opening for air to enter and an exhaust port from which the air was drawn through a carbon dioxide trap. The trap solution consisted of a 2:1 mixture of 2-methoxy ethanol–2-ethanolamine. Four milliliters of the trap solution was radioassayed.

RESULTS AND DISCUSSION

Stability. Metabolites typically extracted from cotton and bean plants treated with methazole are shown in Figure 1. However, it was noted early in this study that a similar pattern could be produced from methazole by a number of different methods. This instability seriously complicated the study of the metabolism of methazole and great care was required to distinguish degradation products from true metabolic products.

Methazole-phenyl-^{14}C and methazole-3-^{14}C contained 1 to 2% of products V and VI when received from the manufacturer. Attempts to purify the compounds by tlc on silica gel in the 7:2:1 solvent system were not entirely successful. The process of extracting the material from the gel with acetone and concentrating it to a usable volume resulted in the appearance of these same impurities. These products cochromatographed with reference standards P-I and P-II (Table I). In some cases they accounted for only 0.5 to 1%, but often were present at levels of 2 to 3% of the total recovered radioactivity. In addition, it was not uncommon to see trace amounts of what appeared to be methylurea (IV).

Attempts to purify methazole using the aluminum oxide tlc system were even less rewarding. Methazole decomposed rapidly to materials consisting primarily of photoproducts P-I and P-II which remained at the origin after the tlc was developed. Because of this, methazole was purified on silica gel tlc and was used only when the purity was at least 97%.

Methylurea (IV) and urea (III) were stable on silica gel and aluminum oxide tlc. The products could be extracted with acetone or methanol without the production of impurities. However, recovery of the urea was low, especially if allowed to stand on the gel for several days.

Metabolites I and II yielded small quantities of products with R_f values identical to methylurea and urea when rechromatographed after isolation from tlc. The

Table II. Extraction Characteristics of Radiocarbon Following Incubation of Methazole-¹⁴C under Acid and Base Conditions for 18 Hr at Room Temperature

	% of total ¹⁴C in water following benzene extraction	
pH of incubation	Extracted directly	Adjusted to pH 7 and extracted
2	0.4	0.3
5	0.7	0.4
9	46.4	5.4
12	94.8	4.7

greatest difficulty with these products was their affinity for the gel. Unless extracted from the gel with methanol immediately after developing the tlc, the metabolites could not be recovered. Even waiting until the chromatoplates were thoroughly dry resulted in poor extraction of the materials from the gel. When these products were required for further study, plant extracts were applied as a band to a silica gel plate and developed in the 7:2:1 solvent system. Immediately, the gel containing metabolites I and II was scraped from the plates directly into methanol. Only in this manner could metabolites I and II be isolated for use in experiments designed to further evaluate their identity and/or to determine their fate when administered orally to rats.

Rechromatography of metabolites I and II on silica gel using acetonitrile as a solvent system indicated that the combined metabolites consisted of as many as 15 components. When these components were placed in 1 N HCl for 1 hr at 90°, the methylurea and urea were the predominant products formed. This suggested a situation which has been reported for 1-naphthol (Paulson and Zehr, 1972) and observed many times in our own laboratory. 1-Naphthol in the presence of biological material forms a number of different salts which have vastly different R_f values. Acid treatment of the 1-naphthol salts yields free 1-naphthol. Since metabolites I and II could be resolved into so many components, and these components converted largely to methylurea and urea, it appeared quite possible that they consisted, at least in part, of various salts of the methylurea and urea derivatives of methazole.

Further evidence for salt formation was obtained by incubating methazole in water solutions having pH's of 2, 5, 9, and 12 (Table II). While there was some degradation under acid conditions, only small quantities of water-soluble products were formed. Under basic conditions, methazole was almost completely degraded and there was considerable formation of water solubles (Table II). When the water phase was neutralized and again extracted with benzene, only 4 to 5% of the radioactivity remained in the water. Analysis of the benzene extractables by tlc gave the same series of products, including products corresponding to the urea and methylurea derivatives, which were obtained by benzene extraction before neutralization. This type of behavior is typical of salt formation and demonstrates that the urea and methylurea, if present as salts, could possess the polar characteristics exhibited by metabolites I and II and, likewise, could be converted to the free form when treated with acid.

Another methazole derivative, 3-N-hydroxymethylurea, was also very unstable under acid conditions. Interest in this product stemmed from the fact that acid treatment of the plant solids produced a product which appeared just above methylurea on silica gel tlc. It never exceeded 1% of the total radiocarbon in the hydrolyzate and usually could not be detected at all. 3-N-Hydroxymethylurea also chromatographed just above methylurea and may have, in fact, been the same as the unknown. It was evident that small quantities of the 3-N-hydroxymethylurea, if present in the plant, would be degraded by acid treatment to as many as six components, none of which would be in detectable concentrations.

Table III. Nature of Radioactive Residues in Excised Cotton Leaves Treated with Methazole-¹⁴C[a]

Extraction characteristics and metabolite number	% of applied dose/days after treatment[b]				
	0	1	3	6	12
Acetone extractables					
I	2.2	7.5	12.0	18.2	25.2
II	2.5	11.3	15.3	11.0	6.7
III	3.9	15.4	17.6	18.7	22.0
IV	14.3	17.6	9.6	8.0	2.5
V	1.5	2.1	1.1	0.7	0.4
VI	1.6	1.6	1.5	1.3	0.5
VII	66.1	31.4	21.3	10.9	8.0
Total	92.1	86.9	78.4	68.8	65.3
Acid extractables					
I	0.3	1.4	1.6	1.3	1.5
III	1.9	4.9	5.6	9.3	14.7
IV	2.1	4.6	5.1	6.7	9.3
Total	4.3	10.9	12.3	17.3	25.5
Unextracted	0.8	1.7	2.2	1.5	1.4
Total recovery	97.2	99.5	92.9	87.6	92.2

[a] Excised leaves placed in water solutions of methazole-¹⁴C for 24 hr and then placed in fresh water until analyzed. Data represent averages from treatment with methazole-phenyl-¹⁴C (sp act., 1.1 × 10⁵ dpm/μg) and heterocyclic ring-methazole-3-¹⁴C (sp act., 6.4 × 10⁴ dpm/μg), since all metabolites were present with both labeled batches of methazole. [b] Each leaf contained approximately 7.5 × 10⁵ dpm when removed from treatment solution.

Metabolism in Cotton and Beans. Methazole-5-¹⁴C treatment of beans and cotton yielded only the parent compound and a small amount of material which remained at the origin on tlc. The origin material could not be converted to benzene-extractable products by treatment with acid. These data demonstrated that none of the methazole metabolites contained the no. 5 carbon atom and that hydrolysis to yield CO_2 was the initial step in the metabolism of methazole by cotton and beans. This was confirmed by the fact that 78.5% of the methazole-5-¹⁴C taken up by the leaves was recovered as ¹⁴CO₂ during the first 24 hr after being removed from the treatment solution.

Methazole-phenyl-¹⁴C and methazole-3-¹⁴C were metabolized identically by the plants (Tables III and IV). Therefore, all metabolites contained the phenyl-N-C moiety intact. The metabolism of methazole by cotton (Table III) followed the same pathway as described by Jones and Foy (1972). Methylurea (IV) constituted the major metabolite shortly after treatment but dissipated gradually thereafter. The urea (III) increased in concentration as the time after treatment increased.

Metabolites I and II were not identified intact but were identified following incubation in 1 N HCl. Metabolite I yielded the methylurea and the urea, the ratio being 1:1 in plants analyzed 1 day after treatment and increasing to about 1:4 by 12 days. Small amounts of origin materials, 1 to 3%, were also produced. Metabolite II yielded only the methylurea. Neither metabolite was altered by treatment with β-glucosidase. These two metabolites in their original form are comparable to the "origin" materials reported by Jones and Foy (1972).

Metabolites V and VI occurred in very small quantities and were unstable on tlc. Their tlc characteristics were very similar to photoproducts P-I and P-II (Table I); however, good cochromatographic data were not obtained because of their low concentrations.

Acid treatment of the cotton plant solids after acetone extraction removed almost all of the remaining radioactivity (Table III). Because prolonged exposure of the metabolites caused considerable degradation to origin materials, several 30-min incubations, followed by ethyl acetate ex-

Table IV. Fate of Methazole-14C in Excised Bean Leaves[a]

Metabolite	% of applied dose/days after treatment[b]			
	1	3	6	12
Acetone extractables				
I	6.2	12.8	17.7	30.6
II	12.6	10.5	9.5	8.6
III	1.7	4.5	3.2	5.1
IV	61.7	62.4	51.9	37.9
V	0.8	0.3	0	0
VI	0.7	0	0	0
VII	2.1	1.5	0.5	0
Total	85.8	92.0	82.8	82.2
Unextracted	4.6	4.2	4.5	3.7
Total recovery	90.4	96.2	87.3	85.9

[a] Excised leaves placed in water solution of methazole-14C for 24 hr and then transferred to fresh water until analyzed. [b] Each leaf contained approximately 5×10^5 dpm when removed from treatment solution.

Table V. Fate of Methazole in Cotton When Applied as a Leaf Surface Treatment[a]

Metabolite	% of applied dose/days after treatment						
	1	3	7	14	21	28	46
I							
Surface	2.0	2.8	2.3	1.8	0.5	0.5	0.3
Internal	1.9	3.9	9.5	11.2	13.6	14.8	14.9
Total	3.9	6.7	11.8	13.0	14.1	15.3	15.2
II							
Surface	1.5	3.2	1.5	0.6	0.2	0.2	0.2
Internal	3.1	5.9	8.5	3.5	3.2	3.8	1.7
Total	4.6	9.1	10.0	4.1	3.4	4.0	1.9
III							
Surface	3.1	6.1	5.3	4.3	2.8	2.9	2.3
Internal	0.7	3.0	7.0	4.2	4.6	4.2	4.1
Total	3.8	9.1	12.3	8.5	7.4	7.1	6.4
IV							
Surface	18.8	11.5	1.5	0.7	0.5	0.8	0.7
Internal	3.4	2.7	7.0	1.9	0.7	2.0	0.4
Total	22.2	15.2	8.5	2.6	1.2	2.8	1.1
V							
Surface	4.2	1.3	0.2	0.1	0.2	0.4	0.2
Internal	0.5	1.0	0.5	0.2	0.2	0.1	0.1
Total	4.7	2.3	0.7	0.3	0.4	0.5	0.3
VII							
Surface	29.0	0.7	0.1	0.1	0.2	0.2	0.4
Internal	2.7	3.6	1.6	0.3	0.1	0.6	0.3
Total	31.7	4.3	1.7	0.4	0.3	0.8	0.7
Totals							
Surface	58.6	25.6	10.9	7.6	4.4	5.0	4.1
Internal	12.9	23.2	41.0	29.8	33.1	38.9	35.7
Extractable	12.3	21.1	34.1	21.3	22.4	25.5	21.5
Unextracted	0.6	2.1	6.9	8.5	10.7	13.4	14.2
Loss	29.1	51.2	48.1	62.6	62.5	56.1	60.2

[a] Cotton plants approximately 10 weeks old and held outdoors for duration of experiment.

tractions each time, were conducted. Heating the solids in 1 N HCl for 30 min converted approximately 50% of the 14C material to benzene extractables. An almost equal amount was converted after the second 30 min of heating, while only trace amounts were removed by heating for a third time. Methylurea (IV) and urea (III) were the major components released by acid extraction, while radioactivity at the tlc origin constituted from 1 to 2% of the total methazole-14C equivalents in the leaves when harvested.

Total recovery of the radioactivity in the cotton leaves was usually in excess of 90%. Total 14C residues were determined by direct combustion of a portion of leaves after being cut into small pieces and by summing the radioactivity in the acetone extract and that in the plant solids after extraction. Both methods yielded similar results.

Although detailed analyses of the bean plant solids were not attempted, the data indicate that the general metabolic fate of methazole in beans was the same as in cotton. Quantitative differences did occur, however. Methazole (VII) was metabolized much faster in beans, with a resulting higher accumulation of methylurea (IV) (Table IV). Further metabolism of the methylurea was slower in beans than in cotton, especially the formation of urea (III) and unextracted 14C residues. This indicates that the cotton plant more efficiently formed the N-hydroxymethyl derivative which, in turn, is converted to the urea.

Intact cotton leaves metabolized methazole-phenyl-14C and methazole-3-14C in the same manner as excised leaves (Table V). In addition to indicating the metabolism of methazole in cotton plants under natural environmental conditions, these experiments also reflected the penetration and dissipation rates of methazole when applied to the leaf surface. Dissipation of the 14C deposits occurred quite rapidly after treatment, with only 50% of the applied material recovered after 3 days. Almost no dissipation occurred between the 14th and 46th days after treatment. Since most of the residues were inside the leaf during this period, it was evident that little loss of methazole-14C equivalents occurred once methazole and/or its metabolites entered the plant tissue. Unextractable 14C material accumulated steadily as absorption into the leaf increased and accounted for about one-third of the total internal residues after absorption reached a maximum. These materials were not subjected to acid treatment and subsequent tlc analysis, as was done in the excised leaf experiments.

Hydroxymethylurea. Assuming that hydroxymethylurea (Table I, metabolite VIII) would be unstable unless reacted with methanol to form the methoxymethylurea (P-IV), this product would not be present in acetone extracts of plants. This was shown to be the case when ex-
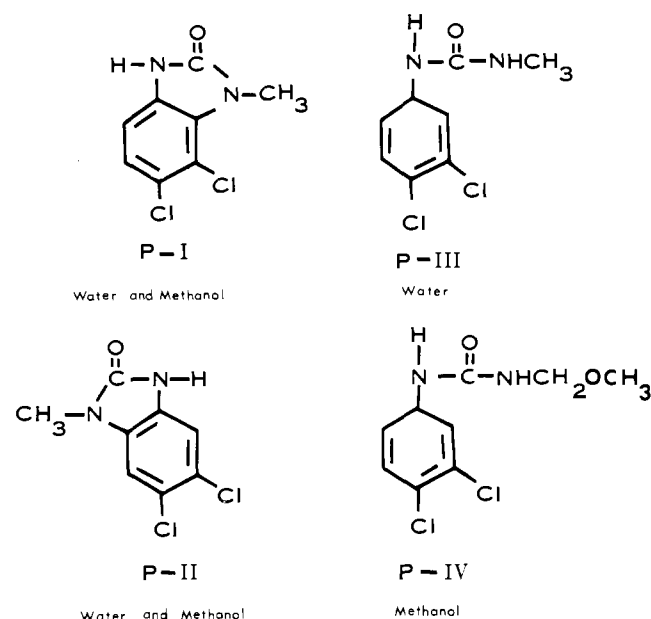
Figure 2. Photoproducts of methazole formed in water when exposed to sunlight and in methanol when exposed to ultraviolet light.

cised cotton leaves (6 days after treatment with methazole-phenyl-14C) were extracted with acetone and the extract was analyzed on aluminum oxide tlc. There was no radioactivity corresponding to the position of authentic methoxymethylurea. However, when duplicate leaves were
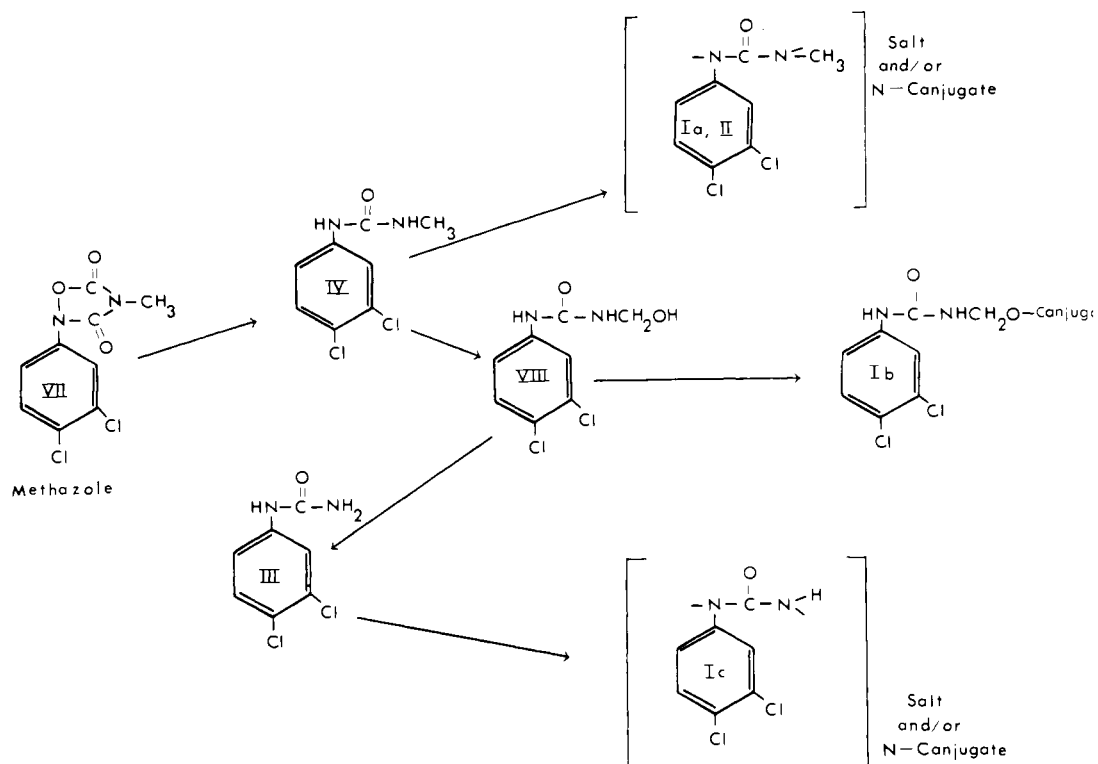
Figure 3. Metabolic pathway of methazole in cotton and bean plants. Methazole (VII), methylurea (IV), urea (III), polar methylurea derivatives (Ia, Ib, II), polar urea derivatives (Ic), and hydroxymethylurea (VIII).
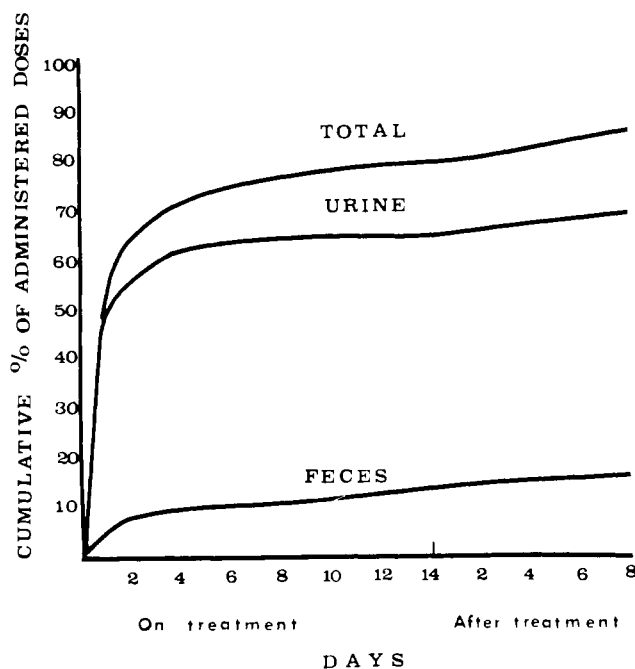

Figure 4. Excretion of radiocarbon from rats treated daily with an oral dose, 8.0 × 10^5 dpm, of methazole-^{14}C cotton metabolites I and II. Metabolites from excised cotton leaves 3 days after treatment with methazole-^{14}C.

extracted with methanol and likewise analyzed, radioactive methoxymethylurea was detected. Its identity was confirmed by isotopic dilution techniques. Based on this study, at least one-third of the urea in the acetone extract of these leaves (Table III) resulted from the degradation of hydroxymethylurea and/or its conjugate during extraction. The remaining two-thirds was formed *in vivo*, presumably from the same hydroxymethylurea intermediate.

It was determined that the methylurea could serve as a precursor to hydroxymethylurea in the cotton plant (Table VI). Leaves treated with methylurea-phenyl-^{14}C

and extracted with acetone contained the urea but no methoxymethylurea. The latter compound was evident when the leaves were extracted with methanol.

Ivie *et al.* (1973) reported that the methylurea was not converted to methoxymethylurea when exposed to ultraviolet light in the presence of methanol. This was confirmed in the present study, as was the formation of other photoproducts formed in water and methanol (Figure 2). Water solutions of methazole-phenyl-^{14}C exposed to sunlight for 7 days contained P-I, P-II, and P-III or methylurea. Since no urea was detected, it was clear that no hydroxymeth-

Table VI. Metabolism of 3-(3,4-Dichlorophenyl-^{14}C)-1-methylurea by Excised Cotton Leaves[a]

Metabolite[b]	% of total ^{14}C in leaves when leaves extracted with[c]	
	Acetone	Methanol
Methylurea	28.2	16.8
Urea	43.8	56.7
Methoxymethylurea	0	2.3
I (origin material)	4.7	8.4
Unextracted	23.3	15.8

[a] Cotton leaves placed in water solution of methylurea-^{14}C for 6 days. [b] Metabolites resolved on aluminum oxide plates with 9:1 ethyl acetate–isopropyl alcohol. See Table I for metabolite identification. [c] Only those ^{14}C materials extracted directly with acetone or methanol evaluated. Acid extraction of the plant solids was not attempted.

ylurea was formed under these conditions and, therefore, probably would not be a product of methazole photolysis under normal environmental conditions.

Methazole-phenyl-^{14}C exposed to germicidal lamps (Ivie et al., 1973) for 24 hr when dissolved in methanol produced products P-I, P-II, and P-IV or methoxymethylurea (Figure 2). Again, no urea was formed. The identity of methoxymethylurea was based on the cochromatograph of P-IV with authentic methoxymethylurea on aluminum oxide and by isotopic dilution techniques. Also, a constant specific activity was achieved when ^{14}C-P-IV was recrystallized with the methoxymethylurea but not when recrystallized with the methylurea.

Based upon identification of the acetone extractable radioactivity from cotton and bean plants treated with methazole-^{14}C, the identification of the ethyl acetate extractables after treatment of cotton plant solids with acid and the confirmation of hydroxymethylurea as a metabolite, a metabolic pathway for methazole in cotton and beans, is proposed (Figure 3). Although all metabolites were not identified as they exist in the plant, it is important to note that with proper acid treatment the metabolites in Figure 3 can be converted to known compounds. Then, almost all products would be in the form of either methazole, the methylurea, or the urea.

Animal Studies. Since results of studies using methazole-phenyl-^{14}C and methazole-3-^{14}C plant metabolites were the same, they will be discussed collectively in this section.

A 1:1 mixture of ^{14}C metabolites I and II from bean or cotton plants, when given orally to rats, was excreted from the body via both the urine and feces. Nearly 50% of a single oral dose of the bean metabolites was in the urine and 11% in the feces 1 day after treatment (Table VII). Continued collection of the excreta for 6 days showed that 86% of the dose was eliminated. Three-fourths of the total excreted radioactivity was in the urine. No further analyses were conducted on the bean metabolite feeding study.

In a more complete study, cotton ^{14}C metabolites I and II were administered orally to rats for 14 days. The excretion pattern of radiocarbon during the time the animals were on treatment and for 8 days thereafter is shown in Figure 4. Residue levels in the urine and feces reached a near plateau after 3 days and then increased only slightly through the remaining 14-day feeding period. During the latter period, from 75 to 80% of the daily doses was eliminated during the next 24-hr period. Total excretion and the relative quantities of ^{14}C residues in the urine and feces were similar to that observed after administering a

Table VII. Excretion of Radiocarbon from Rats Treated with a Single Oral Dose of Methazole-^{14}C Bean Metabolites I and II[a]

Days after treatment	Cumulative % of administered dose		
	Urine	Feces	Total
1	47.6	11.3	58.9
2	55.3	17.5	72.8
3	60.4	20.8	81.2
4	62.2	21.6	83.8
5	63.3	22.1	85.4
6	63.6	22.4	86.0

[a] Metabolites I and II, 6.4×10^5 dpm, from excised bean leaves 3 days after treatment with methazole-^{14}C.

Table VIII. Methazole-^{14}C Equivalents in Tissues of Rats Treated Orally for 2 Weeks with Methazole Cotton Metabolites I and II

Tissues	ppm of methazole equivalents/time after last treatment	
	12 hr	8 days
Fat	0.128	<0.014
Kidney	0.006	0.032
Liver	<0.004	0.016
Muscle	0.011	<0.004
Brain	0.014	<0.004

single oral dose of the bean metabolites. During the 8 days following the last treatment, total excretion of the administered radioactivity was only 5% greater than that excreted daily while the animals were on treatment.

Benzene extraction of the pooled urine, 0–14 days, removed 25% of the radiocarbon. Silica gel tlc of the benzene extract using the 7:2:1 solvent system separated eight radioactive areas from this mixture. No cochromatographic analyses were performed with these components, but side-by-side chromatography with reference standards suggested that 38% of the benzene extractables was the methylurea, 36% was the urea, and 6% was methazole.

Radioactive residues in the tissues of rats treated with methazole metabolites I and II were very low (Table VIII). Fat of rats sacrificed 12 hr after the last treatment contained the highest level of methazole-^{14}C equivalents, 0.13 ppm. Even here, the concentration was not great enough to allow characterization of the ^{14}C residues.

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