ferent from one another. The distribution of the minimum number of radioactive components in the ether, anionic, and neutral fractions was 3, 4, and 4, respectively. The distribution of radioactivity in the cation fraction (developed by two-dimensional chromatography) (Table VIII) indicated that almost all amino acids were labeled with C^{14}, with highest labeling in asparagine and the combination histidine plus glutamine. These amino acids might be expected to have lower turnover numbers, hence higher labeling could result because of less dilution during growth.

Table IX summarizes R_f values of major radioactive peaks found in several of the extracts, as well as some standard values for compounds suspected to be present in the extracts.

Because the anion fraction contained so much of the total radioactivity (36%), it was further analyzed by using gradient elution procedures. Three radioactive organic acids were found in this fraction, with 85% of the radioactivity associated in one fraction (tube 22). This cut was cochromatographed with lactic acid and glycolic acid, and the results are shown in Table X.

Since lactic acid appeared to be present as one of the major metabolic components, it was of interest to determine whether this material was formed by direct breakdown of the 2-chlorallyl grouping or whether it was formed indirectly, perhaps by the liberation of $C^{14}O_2$ and reincorporation of CO_2 into lactate. With the isotope dilution technique, 0.5 ml. of 85% aqueous lactic acid was added to tube 22, which did not contain sufficient acidity to estimate the amount of labeled lactate that might be present. The lactic acid was distilled using a molecular still, and the *p*-bromophenacyl derivative was then made. The derivative was recrystallized several times to constant specific activity. The melting point of the ester was identical with that reported in the literature (112°–113° C.). By making the correction for the estimated isotope dilution (8500), a specific radioactivity of the original lactic acid was calculated to be 3.5×10^5 disintegrations per minute (d.p.m.) per mg. This figure was within a factor of 8 of the original specific activity of the labeled material used, suggesting that lactic acid may be formed directly from the 2-chloroallyl moiety. This is particularly significant in that the specific activities of all the other fractions mentioned previously were of the order of 10^3 d.p.m. per mg. compared to the anion fraction which had a specific activity 100 times greater.

Discussion

The data presented illustrate the ability of resistant crop plants to metabolize the CDAA- and CDEC-type herbicide rapidly and extensively to naturally occurring products. The results further indicate that plants have a ready ability to metabolize allylic-containing material even when chlorinated, although at present the exact mechanism by which this metabolic degradation occurs is not known. Probably this rapid metabolic degradation leads to the high degree of resistance shown by the crop species toward these chemicals. Thus not only are desirable plants able to detoxify the chemicals completely, but degradation is carried out to the extent that residues of the parent compound or its component parts are nonexistent.

The general trend for selectivity of pesticidal activities may reside in the ability of plants to metabolize and detoxify the compounds at different rates rather than by inhibition of enzyme systems unique to certain species of plants. This aspect of selectivity, while studied to some extent, requires additional intensive study as to the kinetics of detoxifying biochemical reactions particularly as a springboard toward the better design of selective pesticides.

Acknowledgment

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METABOLISM OF HERBICIDES

The Metabolism of *S*-Propyl-1-C^{14} *n*-Butylethylthiocarbamate (Tillam-C^{14}) in Rats

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SEVERAL thiolcarbamates have been introduced recently as pre- and postemergence herbicides on forage legumes and certain vegetable and field crops. Their mechanism of action is not fully understood. Since some carbamates are active as weed killers and cause nuclear changes in cells, thiolcarbamates might be toxic to cells also. The widespread use of these compounds in weed control undoubtedly raises a residue problem in foods. The solution to this problem requires information on the quantities of the herbicide present in the crops, and on whether or not a given rate of intake of herbicides is injurious to human health. To learn how quickly an animal can detoxify and eliminate the ingested herbicide from the body will also provide information relating to health hazard. Tillam-C^{14} is easily degraded by plant cells (*2*), and the radioactivity is incorporated in the respiratory CO_2. The purpose of this investigation was to characterize the excretory pattern of orally administered Tillam-C^{14} in the rat, and to study tissue residues following administration.

Experimental

Tillam-C^{14}, 3.11 mc. per mmole, was first dissolved in ethanol to give 6.57 mg. of Tillam per ml. of stock solution. No chemical or C^{14} impurities of this Tillam-C^{14} sample were detected by gas liquid chromatography. Twenty per cent alcohol solutions containing various amounts of Tillam-C^{14} were prepared immediately prior to administration. Adult rats of the Wistar strain (4 to 6 months of age) were fed orally a given amount of Tillam-C^{14} by means of a

The metabolism of Tillam-C^{14} in adult rats after dosing with an amount ranging from 0.16 to 1.95 mg. per dose was examined. Approximately 55% of an oral dose was found in the expired air as carbon dioxide, 23% was excreted in the urine, and less than 5% in the feces. Most of the radioactivity was recovered during the first 24 hours. The accumulation of radioactivity in the internal organs after 3 days of dosing from animals receiving either multiple doses at weekly intervals or a single dose never exceeded 1% of the dose. This herbicide was readily degraded in rats to yield no persisting residue in the tissues. Extensive labeling was observed in the urine constituents, such as urea and amino acids, in addition to many unknown metabolites.

stomach tube and then immediately placed in glass metabolism cages. The expired carbon dioxide was continuously trapped through a gas scrubber containing 2*N* NaOH solution which was changed periodically at predetermined intervals. The carbonate was precipitated as $BaCO_3$ and filtered through a glass-fiber filter paper disk. After drying, the weight of $BaCO_3$ was determined and its activity was counted. The radioactivity of the $BaCO_3$ was corrected for self-absorption and background. The urine and feces were collected daily. After the urine was drained, the separator was rinsed thoroughly, first with alcohol and then with water. Both urine and washings were combined and made up to 50 ml. For the determination of Tillam-C^{14} residue in the urine sample, 5 ml. of urine was added to 100 ml. of water and boiled immediately for 3 to 5 hours in an apparatus described previously (*3*). The volatile Tillam-C^{14} which distilled over with the steam was condensed and continuously extracted in a column of isoöctane, while the steam condensate was returned to the boiling flask. Duplicate aliquots of the isoöctane solution containing Tillam-C^{14} were dried over a circle of filter paper which was coated with a layer of activated carbon, and the radioactivity was measured. For the determination of total activity in urine sample, 0.5-ml. aliquots were plated directly on stainless steel planchets, dried, and counted. Feces samples were extracted with a sufficient amount of ethanol, and aliquots of ethanol extract were submitted to Tillam-C^{14} residue determination as well as total radioactivity. A thin mica end window (1.4 mg. per sq. cm.) G-M counter with a manual sample changer was utilized in making all radioactivity measurements.

Dosing was repeated three to five times at weekly intervals to the same rat to determine whether or not a similar excretory pattern may be maintained by the same animal. Finally, the rats were sacrificed and various tissues were carefully separated and freeze dried. The dry tissues were weighed and finely ground. Duplicate aliquots of 0.1 gram dry sample were weighed in planchets and counted directly. Rats No. 7 and 8 were sacrificed after a single oral dose.

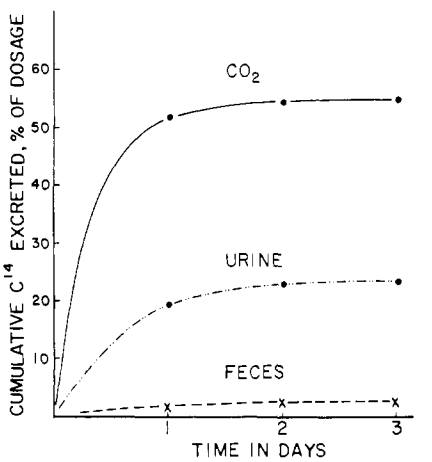

Figure 1. Average cumulative recovery of radioactivity excretion following oral administration of Tillam-C^{14} to rats

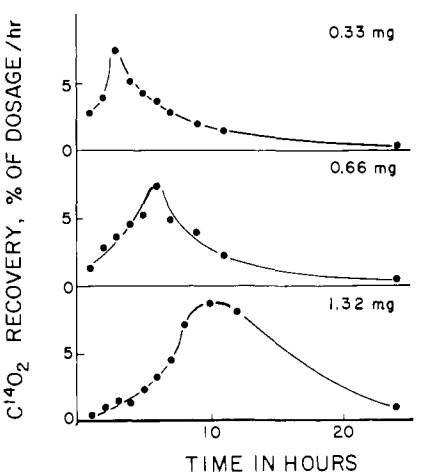

Figure 2. Average hourly recovery of radioactivity of respired $C^{14}O_2$ following oral administration of various doses of Tillam-C^{14} to rats

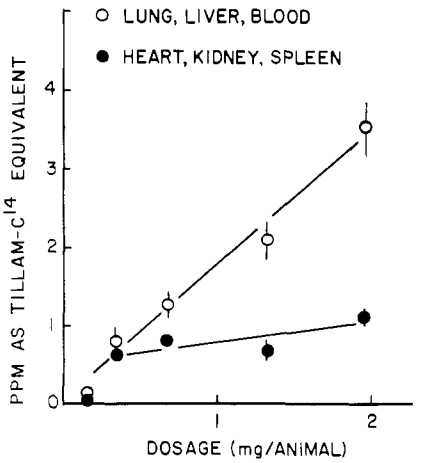

Figure 3. Accumulation of radioactivity in internal organs and tissues as related to the dosage of Tillam-C^{14}

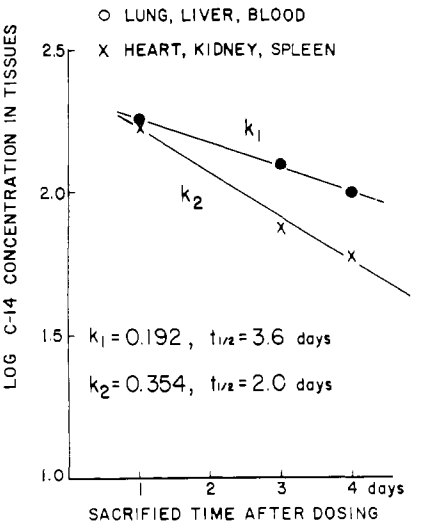

Figure 4. In vivo kinetics of radioactivity elimination in some internal organs of rats following a single oral dose of Tillam-C^{14}

To define the chemical nature of the radioactivity of the urine, small aliquots of these samples were chromatographed either one dimensionally or two dimensionally. Both direct scanning and radioautographic techniques were utilized. Some urine samples were also submitted to hydrolysis with strong acid and then chromatographed to learn whether or not radioactive metabolites can be broken down by hydrolysis. The acid-hydrolyzed urine was also subjected to steam distillation and subsequent extraction of the steam distillate with isoöctane in order to separate the Tillam-C^{14}-like compound.

Table I. Rate of Radioactivity Elimination in Rats Following Oral Administration of Tillam-C^{14}

Rat No.	Sex	Body Weight, Grams	Dosage, μg.	Daily Recoveries, % of Dosage: CO_2 1st Day	CO_2 2nd Day	CO_2 3rd Day	Urine 1st Day	Urine 2nd Day	Urine 3rd Day	Feces 1st Day	Feces 2nd Day	Feces 3rd Day	Total Recovery, %
1	F[a]	195	164	35.8	6.0	0.6	13.5	4.2	0.2	2.3	1.8	...	64.4
2	M[a]	300	657	58.3	1.0	0.1	21.0	3.4	0.3	0.3	0.2	0.1	84.7
3	F	215	329	63.8	1.8	0.7	16.7	1.3	0.3	1.0	0.1	Trace	85.7
4	F	225	329	54.6	2.2	0.5	17.3	2.1	0.2	0.9	0.1	0.1	78.0
5	M	248	657	57.3	1.2	0.4	17.6	8.4	0.6	1.1	0.2	Trace	86.8
6	M	279	657	57.3	2.2	0.3	19.2	3.5	0.2	2.9	0.8	0.1	86.5
7	F	225	1314	63.5	6.7	...	20.5	3.0	...	0.8	0.4	...	94.9
8	F	183	1950	21.2	2.7	0.8	27.8	2.4	0.2	4.2	0.3	Trace	59.6
Av.				51.5	2.7	0.5	19.2	3.5	0.3	1.7	0.5	Trace	80.1

[a] F = female; M = male.

Table II. Accumulation of Radioactivity in Internal Organs from Rats Receiving Tillam-C^{14}

Week	Dosage, μg. No. 1 F[a]	No. 2 M[a]	No. 3 F	No. 4 M	No. 5 M	No. 6 M	No. 7 F	No. 8 F
1st	164	657	657	657	657	657	1314	1950
2nd	164	657	657	657	657	657		
3rd	164	657	657	329	657	657		
4th			329		657	657		
Total	492	1971	2300	1643	2628	2628	1314	1950
	Radioactivity Recovered, %; Sacrifice Time after Dosing, Days							
Organ	1	4	1	4	3	3	2	3
Heart	0	0.002	0.007	0.004	0.006	0.010	0.015	0.011
Lung	0.006	0.005	0.018	0.011	0.010	0.012	0.063	0.033
Liver	0.028	0.040	0.083	0.077	0.162	0.152	0.410	0.386
Kidney	0.003	0.003	0.018	0.015	0.021	0.025	0.036	0.029
Spleen	0	0.001	0.004	0.005	0.005	0.004	0.008	0.006
Intestine	0	0.033	0.034	Trace	0.036	0.070	0.305	0.324
Stomach	0.112	0.011	0.021	0	0.012	0.008	0.093	0.035
Testis	...	0.003	...	...	0.011	0.011	...	...

[a] F = female; M = male.

Results and Discussion

Following oral administration, Tillam-C^{14} was rapidly eliminated from rats. Based on the study with eight adult rats, excretion in the urine accounted for 18 to 30% of the administered dose; the average value was 23.0% (Table I). The greater part of the radioactivity in the urine was excreted during the first 24-hour period. After the third day, no measurable amount of radioactivity was detected in all excreta (CO_2, urine, feces). The radioactivity in the feces samples was generally low, indicating that Tillam-C^{14} was readily absorbed from the gastrointestinal tract and was rapidly oxidized.

The variation in rate among the eight rats did not appear to be related to sex. The same animal which received multiple doses of Tillam-C^{14} at weekly intervals frequently showed a slight variation in excretory pattern. The average cumulative recoveries, in terms of percentage of dosage, in the excreta of eight rats are presented graphically in Figure 1. The maximum hourly recovery of radioactive CO_2 in the exhaled air occurred between 3 to 10 hours after administration. Among various dosages of Tillam-C^{14} used, the peak of $C^{14}O_2$ activity occurred sooner with the lower dosage (Figure 2). Unfortunately, in all runs, the experimental procedure used did not permit the recovery of unchanged Tillam-C^{14} which may be eliminated by the lungs.

Table II shows the accumulations of radioactivity in various internal organs and tissues. Only aliquots of blood, abdominal muscle, fat, skin, and hair were removed for counting, and the radioactivity in the remaining carcasses was not determined. The radioactivity in the tissues was generally low, and the total amount never exceeded 1% of the administered dose. The lung, liver, and blood had the highest concentration. Because of the low activity, no attempt was made to define the chemical nature of the radioactivity remaining in the tissues. The accumulation of radioactivity in various internal organs (excluding the stomach and intestine) and the blood seemed to be dependent on the dosage as well as on the time when the animal was killed after the last dosing. A higher dosage resulted in a greater accumulation in the tissues. The accumulation rate was greater in the lung (12 to 610 c.p.m. per 0.1 gram of dry tissue), liver (21 to 653 c.p.m.), and blood (0 to 530 c.p.m.) than in the other organs (average values for heart, kidney, and spleen were 2 to 193 c.p.m.). This relationship is approximately linear between the dosages used (Figure 3).

Results from animals which received 657 μg. of Tillam-C^{14} and were killed 1, 3, and 4 days after dosing revealed that the C^{14} concentration in five internal organs (heart, lung, liver, kidney, and spleen) and blood decreased progressively with time. The semilog plot of concentration *vs.* time is shown in Figure 4. Abdominal muscle, fat, skin, and hair also accumulated some activity, and this activity appeared not to be correlated with the dosage.

In the urine, a small portion of the radioactivity was the unchanged Tillam-C^{14} residue. The average value was 1.2 ± 0.4% (ranging from 0.3 to 2.8%) for the first-day samples, and reduced to 0.3% (from 0 to 1.3% for the second-day following dosing). Paper chromatographic and radioautographic results of the first-day urine samples revealed an extensive labeling in the urine constituents. A total of 20 to 22 radioactive spots was noted on the two-dimensional chromatograms, which were developed first with phenol-H_2O and followed with *n*-butanol–acetic acid–H_2O solvents. The general labeling pattern was quite similar among eight individual rats, and no significant difference was observed between either male or female rats or those receiving different dosages. Hydrolysis of the urine with 2*N* HCl or NaOH for 24 hours only increased the

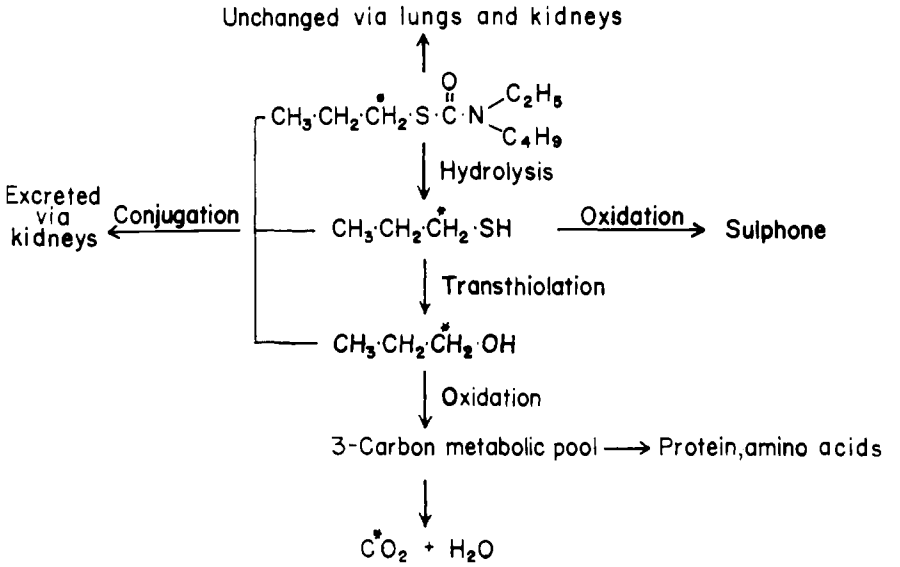

Figure 5. Possible metabolic pathways of Tillam-C^{14} in rats

steam-volatile radioactivity by approximately 2%. When the urine was refluxed with 6*N* HCl for 24 hours, 14% of the radioactivity was hydrolyzed and changed to steam-volatile, isoöctane-soluble metabolites. This observation indicates that Tillam-C^{14} has also undergone conjugation. The nature of this conjugate formation is now being investigated.

In nonhydrolyzed urine, two ninhydrin-reactive spots near the origin were lightly labeled and were presumably protein or peptide. After acid hydrolysis, these two spots disappeared with the appearance of six to eight lightly labeled ninhydrin-reactive spots. Among them were aspartic acid, glutamic acid, glycine, cystine, arginine, and histidine. Urea was highly labeled. Many radioactive metabolites remained unidentified. Since the labeling from Tillam molecule is found in urea as well as many amino acids, the thiolcarbamate molecule probably is hydrolyzed at the ester linkage to form *n*-propylmercaptan-1-C^{14} and then converted to propanol by a transthiolation as shown in Figure 5. The propanol may be oxidized to C-3 acid and (or) further breakdown to C-2 unit before entering into metabolic pool. Canellakis and Tarver (*1*) examined in vivo breakdown of $C^{14}H_3$—SH in rats and found that about 40% of the activity was eliminated as respiratory CO_2 in 6 hours. C^{14} also appeared as the beta-carbon of serine and in the methyl group of methionine, choline, and creatine. This suggests that the metabolism of the methyl group of methyl mercaptan is similar to that of methyl alcohol which might arise from the mercaptan by transthiolation. Snow (*4*) studied the metabolism of *S*-ethyl thiolbenzoate in mice and guinea pigs and detected the presence of ethyl methyl sulphone as one of its metabolites. This finding indicates a second metabolic pathway for the SH compound, which is methylated to yield sulphide and then oxidized to sulphone.

Whether or not the metabolism of Tillam-C^{14} in rats would follow these suggested pathways requires further investigation. However, the authors believe that the thiol moiety of Tillam molecule has undergone extensive breakdown and a fraction of the carbon unit is incorporated into tissue constituents such as protein and amino acids, and the remainder may be completely oxidized and eliminated as respiratory CO_2. This observation suggests that the ingestion of small amounts of Tillam residue from foodstuffs, which may arise from the use of this chemical in weed control, probably would not contribute to health hazard.

Acknowledgment

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METABOLISM OF HERBICIDES

The Halogenated Aliphatic Acids

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There is one characteristic which is common to many groups of herbicides—although various members of the group have been known as chemicals for many years, their use as herbicides is recent. The halogenated aliphatic acids are no exception. Patent citations of chlorinated aliphatic acids as herbicides were made by Bousquet (*7*) for trichloroacetic acid (TCA), by Barrons (*1, 2*) for 2,2-dichloropropionic acid (dalapon, trademark of The Dow Chemical Co. in certain foreign countries) and for 2,2,3-trichloropropionic acid, and by Toornman (*35*) for 2,2-dichlorobutyric acid. Norman *et al.* (*28*) published a comprehensive review on herbicides in 1950. Only four references related to halogenated aliphatic acids. All referred to TCA, and the earliest was a 1948 reference. In subsequent reviews, Blackman *et al.* (*5*), Crafts (*9*), and Woodford *et al.* (*37*) cited nearly 50 additional papers.

In 15 years, literature covering use of halogenated aliphatic acids as herbicides has increased to the point that it is nearly impossible for an individual to cover it all. A recent literature search revealed over 700 papers on dalapon between 1958 and 1962.

Only a few of the chemical derivatives appear in the literature as herbicides. The major ones, in order of their appearance are TCA, dalapon, 2,2,3-trichloropropionic acid, 2,2-dichloro-