Unchanged via lungs and kidneys

Figure 5. Possible metabolic pathways of Tillam-C¹⁴ in rats

steam-volatile radioactivity by approximately 2%. When the urine was refluxed with 6N HCl for 24 hours, 14% of the radioactivity was hydrolyzed and changed to steam-volatile, isoöctane-soluble metabolites. This observation indicates that Tillam-C¹⁴ 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-C¹⁴ 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 C¹⁴H₃—SH in rats and found that about 40% of the activity was eliminated as respiratory CO₂ in 6 hours. C¹⁴ 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-C¹⁴ 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 CO₂. 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.

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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-

Several of the chlorinated aliphatic acids have become well known as herbicides within the past 15 years. Alpha chlorination appears to be a major requirement for activity, with trichloroacetic acid, 2,2-dichloropropionic acid, and 2,2-dichlorobutyric acid being the most active compounds as herbicides. Higher plant systems have little effect on these acids, but the acids disrupt a number of important plant processes. Several species of soil organisms have been shown to decompose these materials.

butyric acid, and 2,3-dichloroisobutyric acid. The unsubstituted aliphatic acids, including formic, acetic, acrylic, butyric, and oleic (among others), are inactive as herbicides.

Structure–Activity Relationships

Within the group of chlorinated aliphatic acids are two kinds of phytotoxicity. Some chlorinated aliphatic acids are capable of producing an acute contact burn (chloroacetic acid, TCA, or dalapon) probably because they are both strong acids and protein precipitants. Another type response, a systemic effect which can result in the selective control of grasses, apparently results from lower concentrations, and the effect shows primarily in meristems or growing points.
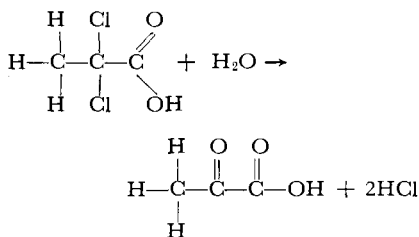
Chloroformic acid, chloroacetic acid, and dichloroacetic acid are all inactive as selective grass control herbicides. Within the propionic acid and the butyric acid series, alpha chlorination results in herbicidal activity with the 2-chloro compounds being highly active. Chlorination in other positions alone does not result in activity and, in combination with alpha chlorination, may weaken the effects of the alpha chlorination.

Increasing chain length reduces activity, even with alpha chlorination. The 2,2-dichlorovaleric acid is only weakly active, and the 2,2-dichlorohexanoic acid is inactive. The substitution of other halogens for chlorine generally reduces activity throughout the series.

Effect of Higher Plants on Dalapon

The chemistry of such compounds as TCA and dalapon is deceptively simple. The compounds are easily prepared, and the products undergo standard reactions. In the laboratory, alpha chlorinated acids can undergo dehydrochlorination, yielding (according to reaction conditions) hydroxy acids, amino acids, or cyano acids. These reactions apparently do not take place in plant tissue.

In water solutions, these acids decompose at room temperature. Tests using C^{14}-TCA have shown that TCA breaks down in solution to form chloroform and carbon dioxide. Solutions of dalapon undergo the following reaction:

$$H-\overset{\displaystyle H}{\underset{\displaystyle H}{\vphantom{|}C}}-\overset{\displaystyle Cl}{\underset{\displaystyle Cl}{\vphantom{|}C}}-\overset{\displaystyle O}{C\!\!\diagup}\overset{\diagdown}{\vphantom{|}}\;OH + H_2O \rightarrow$$

$$H-\overset{\displaystyle H}{\underset{\displaystyle H}{\vphantom{|}C}}-\overset{\displaystyle O}{\overset{\|}{C}}-\overset{\displaystyle O}{\overset{\|}{C}}-OH + 2HCl$$

The reaction does not take place readily under acid conditions and proceeds very slowly at temperatures below 20° C. Here again, the reactions are simple, but take place only to a very limited degree in higher plant tissue, if indeed they go at all. This is not too surprising, since plant tissues are ordinarily quite acid.

Research results reported by a number of workers indicate that the chloroaliphatic acid herbicides are not readily metabolized by plants. Barrons and Hummer (3) and Tibbetts and Holm (34) demonstrated the presence of TCA in plants grown in TCA-treated soil. Blanchard (6) treated pea and corn plants with C^{14}-labeled TCA, extracted the sap, and found only a single radioactive spot which cochromatographed with labeled TCA. Foy (12, 13), using both Cl^{36}-dalapon and C^{14}-dalapon and working with both resistant and susceptible plants, demonstrated that the herbicide moves into the plant, translocates throughout the plant, and in some cases, is even exuded from roots as dalapon. Apparently higher plants do not do much to the chlorinated aliphatic acids.

Effect of Soil Systems on Dalapon

The situation in soil systems is quite different from that in plants. Loustalot and Ferrer (25) reported that the disappearance of TCA from soil was favored by warm, moist conditions, and Thiegs (32), as well as Holstun and Loomis (17), demonstrated the same thing for dalapon. Thiegs also reported that subsequent additions of dalapon to soil were decomposed more rapidly than the initial application. Hirsch and Alexander (16) isolated and characterized the strains of *Pseudomonas* and five strains of *Nocardia* which decomposed dalapon, liberating 90 to 100% of the halogen in periods of 3 weeks. Other workers have reported

additional species which have decomposed chlorinated aliphatic acids. Magee and Colmer (26) reported *Agrobacterium*, Thiegs (33) reported *Flavobacterium*, and Jensen (19, 20) added *Penicillium*, *Trichoderma*, *Clonostachys*, and *Arthrobacter*.

Thiegs (33), using both 1-C^{14}-dalapon and 2-C^{14}-dalapon, reported the recovery of labeled CO_2 in both experiments, and concluded that the decomposition of dalapon in soil was complete to CO_2. The degradation of 2,2-dichlorobutyric acid in soil follows the same pattern as do dalapon and TCA, but proceeds much more slowly.

A simple but rather dramatic experiment in the Dow research greenhouse demonstrated the extremely rapid breakdown which can occur when populations of the appropriate soil organisms build up. Pots were treated at weekly intervals with dalapon at 50 pounds per acre applied in sufficient water to wet the soil to a depth of 3 inches. The pots were kept moist between treatments with routine greenhouse watering but were never allowed to be leached.

After 6 weeks, the pots were seeded to Japanese millet, wild oats, radishes, and cranberry beans, and immediately treated again with a pre-emergence application of dalapon at 50 pounds per acre. The seedlings all grew normally in these pots which had received a total of 350 pounds of dalapon per acre within a period of less than 7 weeks. In pots treated for the first time, the effects of the herbicide were drastic on all four species, indicating that a considerable change took place within the soil system during the test.

Cultures of *Pseudomonas* and *Flavobacterium* which were isolated from treated pots were inoculated into pots of sterilized soil and caused very rapid breakdown of added dalapon.

Other pots in this test which were pretreated with dalapon for different lengths of time showed that this change occurred within 3 weeks. Applications of dalapon made after that time were ineffective, and the herbicide presumably broke down completely under the conditions employed.

Kutschinski (23) reported that the sodium salt of dalapon will decompose in soil independently of biological breakdown but that this is much slower than biological breakdown. Under con-

ditions of high soil moisture and temperature, sodium dalapon hydrolyzes to pyruvate and HCl. In neutral or acid soils, the HCl would produce the acid from the sodium salt, and some losses due to volatility of the acid could occur.

Effect of Animal Systems on Dalapon

There are certain similarities between the behavior of dalapon in plant and soil systems and its behavior in animal systems. When fed to animals, dalapon is quickly excreted (as dalapon) in herbicidal concentrations in the urine. There is some limited evidence that the decomposition of the relatively small amounts remaining in the animal system proceeds along the same general lines as in soil systems.

Kutschinski (24) reported that the dalapon residue level in milk was much less than 1% of the amount ingested in the feed. Redemann and Hamaker (29) reported that only two labeled compounds were found in milk from a cow whose feed contained Cl^{36}-labeled dalapon. These were dalapon and chloride ion, and the chloride ion was present in much larger quantities than was the dalapon. It is postulated that the dalapon hydrolyzes to pyruvate, which then breaks down to acetate and carbon dioxide.

Effect of Dalapon on Higher Plants

Mechanisms. Although the effects of plants on the chlorinated aliphatic acids are almost nil, these compounds exhibit a wide range of effects on plant systems. TCA, absorbed by grass roots, causes formative effects which indicate rather profound disturbances. Dalapon causes similar formative effects through either leaf or root absorption and definitely exhibits a growth regulator-type activity typical of plant hormones.

Fawcett et al. (10) reported that certain concentrations of various chlorinated aliphatic acids stimulated growth in the wheat cylinder and oat coleoptile bioassays, but that the results were not typical of auxin responses. He suggested that they might be due to changes in membrane permeability. Ingle and Rogers (18) showed that although the chlorinated aliphatic acids did cause slight elongation in the wheat cylinder test, they were inactive in the pea stem test and had little effect on oxygen uptake by mitochondria. They concluded that these compounds do not interfere with the production of metabolic energy but rather with its utilization. Wilkinson (36) reasons that since auxin-type growth stimulation seems to require both a ring structure and an acidic side chain and the chlorinated aliphatic acids are definitely growth regulators but not auxin-type stimulators, the ring portion of auxin-like compounds may be re-

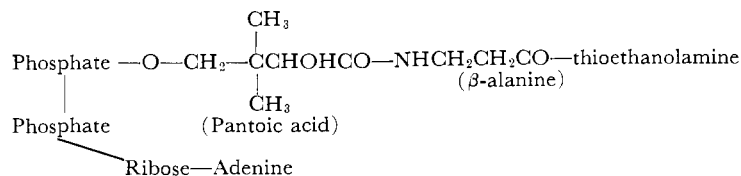quired for stimulation but not for all growth regulator responses.

Foy (11) pointed out that the point of attack on an enzyme-substrate system may be on the production of an essential substrate, on enzymes that bring about the release of energy from a substrate, or on enzymes required in the essential utilization of this energy. Several sites have been suggested. Redemann and Meikle (30) suggested that pyruvic acid metabolism was involved, either competitively or noncompetitively, but their results were indicative, not conclusive. Redemann also suggested the precipitation of essential protein complexes. Hilton et al. (15) proposed that the synthesis of pantothenic acid was involved, either by a noncompetitive reaction involving β-alanine or by a reaction competitive with pantoic acid. However, the addition of β-alanine or pantoic acid will only partially reverse the inhibition of barley seedling growth by dalapon.

In one test conducted at the Dow Research Center, seeds treated with calcium pantothenate at the rate of 2 ounces per bushel were planted in the same pot with untreated seeds (one row each), then treated with dalapon. Results after 3 weeks are given in Table I.

Other similar tests have only slightly reduced the visible symptoms of dalapon applications, and pantothenate sprays have been ineffective in reducing the effect of postemergence sprays of dalapon.

If dalapon disrupts pyruvate metabolism or competes with either β-alanine or pantoic acid, the synthesis of pantothenic acid would be disrupted and the supply of functional co-enzyme A reduced. Since Co-A is involved in energy transfers in carbohydrate, nitrogen, and fat metabolisms, disruption of Co-A production could seriously upset the plant, and this could be manifested in many ways.

The structure of coenzyme-A:

$$CH_3$$
$$Phosphate\ —O—CH_2—CCHOHCO—NHCH_2CH_2CO—thioethanolamine$$
$$CH_3\ \ \ \ \ \ (\beta\text{-alanine})$$
Phosphate   (Pantoic acid)

Ribose—Adenine

shows the possibility for dalapon to interfere in regions of structural similarity.

Specific Responses

A number of growth responses have been ascribed to the chlorinated aliphatic acids. Both TCA and dalapon cause a marked reduction in the amount of surface wax produced on the leaves of various plants (21, 22), but no mechanism for this response was suggested.

The composition of plant parts can be altered by applications of these acids,

Table I.   Results of Dalapon Treatments on Calcium Pantothenate-Treated Seeds as Compared with Untreated Seeds

Dalapon Treatment, Pounds per Acre	Pantothenate Treatment, Ounces per Bushel	Herbicidal Rating[a]	Plant Height, Inches
SOYBEANS			
10	2	0	8–10
	Untreated	20	7–8
20	2	30	8–10
	Untreated	80	2–3
JAPANESE MILLET			
10	2	0	12–14
	Untreated	30	9–11
20	2	0	12–14
	Untreated	80	3–4
COTTON			
10	2	0	5–6
	Untreated	0	5–6
20	2	0	5–6
	Untreated	30 (stunt)	4–5

[a] Herbicidal rating on scale of 0 = no dalapon effect; 100 = kill.

even though no formative effects or growth suppression are noted. Corns (8) and Miller et al. (27) showed that both TCA and dalapon decreased the moisture content of sugar beet seedlings and increased both sugar and dry-matter content, but had no effect on either water-soluble nitrogen or total nitrogen content.

One striking effect (either as a direct or as an indirect result of the changes in leaf composition) was the increased cold resistance of treated seedlings. Only 17% of control seedlings survived a cold treatment of 10 minutes' exposure to −10° C., but the survival of seedlings treated with TCA and dalapon ranged from 41 to 57% when subjected to the artificial frost 1 week after the chemical treatment. In similar tests, IPC, 2,3,6-TBA, and sodium chloride had no effect.

An extremely interesting effect of these acids is their action as a selective gametocide in certain plants. Scott (31), working with cotton, reported that both dalapon and 2,3-dichloroisobutyric acid induced nondehiscent anthers when applied at rates of 100 to 500 μg. per plant. Plants thus affected were male-sterile and readily produced hybrid

cottonseed when cross-fertilized. No other effects of treatment were noted, either on the treated plants or in their hybrid progeny. The application of either pantothenic acid or D-ribose partially reversed the effect of the chlorinated acids.

Susceptibility to dalapon may be under partial genetic control. Scott reported rather widely differing results in his gametocide experiments with several varieties of cotton. Funderburk and Davis (14) reported that hybrid varieties of corn differed in their susceptibility to dalapon, and Buchholz at Wisconsin as well as Behrens (4) studied a number of inbred lines of corn which differ widely in tolerance to dalapon.

Although higher plant systems seem to have little effect on the various chlorinated aliphatic acids, these acids have varied and profound effects on higher plants. Obviously a number of plant processes are affected, and it is likely that more than a single pathway is inhibited. The evidence points to multiple pathways and to more than one site of action.

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End of Symposium

ANIMAL METABOLISM OF HERBICIDES

The Fate of 2,4-Dichlorophenoxyacetic Acid in Sheep

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THE EFFECTIVENESS of 2,4-dichlorophenoxyacetic acid (2,4-D) and related compounds as plant growth regulators has been recognized for a number of years (1, 4). The herbicidal activity of 2,4-D has been attributed to its hormonelike activity rather than to direct dehydration or necrosis of plant tissues (3). Although previous studies have shown that phenoxyacetic acid is excreted unchanged by man and dogs (6, 8) and almost quantitatively in urine by rats and rabbits within 24 hours (2), no work has been done on the metabolism of 2,4-D or related compounds in ruminants. Previous observations have shown that sheep

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can tolerate rather large quantities of 2,4-D salts and esters for extended periods of time (7). However, whether 2,4-D is metabolized, stored, or excreted unchanged by the sheep has not been established.

Apparatus

The instrument used for carbon-14 quantitation was a thin-window (mica) Geiger-Müller tube enclosed in a Tracerlab SC-59S Shielded Manual Sample Changer and attached to a Tracerlab "Versamatic II" Scaler.

Reagents

Chromotropic acid (4,5-dihydroxy-2,7-naphthalenedisulfonic acid, disodium salt, dihydrate): 0.05% in concentrated sulfuric acid.

2-Phenoxyethanol–silver nitrate reagent: 1.7 grams of silver nitrate in 5 ml. of water. Add 20 ml. of 2-phenoxyethanol and dilute to 200 ml. with acetone. If the solution darkens, 1 to 5 drops of 30% hydrogen peroxide may be added.

Electrophoresis buffer: dissolve 5.4 grams of KH_2PO_4 and 0.93 gram of NaH_2PO_4 in 1 liter of water and adjust pH to 6.0 with Na_3PO_4.

Procedure

Administration of 2,4-D and Sampling Procedure. A gelatin capsule containing 539.6 μc. in 106.3 mg. of 2,4-dichlorophenoxyacetic acid-2-C^{14} (Tracerlab, Inc.) in 95% ethanol was administered orally to a yearling ewe (weight 26.6 kg.). The dose of 4.0