Excretion and Tissue Distribution of Uniformly Labeled ^{14}C-Pentachlorophenol in Rats

ROBERT V. LARSEN, LEE E. KIRSCH, STANLEY M. SHAW, JOHN E. CHRISTIAN, and GORDON S. BORN▲

Abstract □ The excretion and tissue distribution of pentachlorophenol were studied in rats by administration of oral doses of 99.5% radiochemically pure, uniformly labeled ^{14}C-pentachlorophenol dissolved in olive oil. After oral administration, less than 0.04% of the administered radioactivity was detected in the respired air in a 24-hr. period. The recovery of pentachlorophenol and/or metabolites in the urine was greatest at the 16-hr. collection period. The average total percent of the administered activity recovered in the urine after 24 hr. was 50.2%, with between 9.2 and 13.2% being recovered in the feces after 10 days. The tissues containing the highest levels of activity were the liver, kidney, and blood, respectively. All other tissues contained less activity than the blood.

Keyphrases □ Pentachlorophenol, radiolabeled—excretion and tissue distribution studies, rats □ Radiolabeling—^{14}C-pentachlorophenol excretion and tissue distribution studied in rats

In a review article, Bevenue and Beckman (1) reported that pentachlorophenol has been used as a fungicide and/or bactericide in the processing of starches, proteins, leather, paints, cellulose products, and oils and has been widely used in the preventative treatment of new lumber for the control of termites. The compound also has been used as a preservative in wooden crates used for packaging raw agricultural products. Blair (2) reported the use of sodium pentachlorophenate as a molluscacide and as a weedkiller in pineapple farms and sugar plantations. Due to the wide variety of industrial and agricultural applications, pentachlorophenol poses a potential environmental hazard; in fact, a number of deaths has been reported due to acute pentachlorophenol intoxication (3, 4).

Few studies have been done to determine the biological disposition and excretion of pentachlorophenol. The work of Deichmann *et al.* (5) indicated the urine is the principal route of excretion of the compound. Small amounts of pentachlorophenol were also detected in the feces and in all tissues analyzed. In a study done by McGavack *et al.* (6), subacute chronic doses of pentachlorophenol were administered to experimental animals, with the results indicating the possibility of a cumulative toxicity existing at dosage levels below the minimum lethal dose for single applications. The majority of the biological studies to date employed the detection of pentachlorophenol based on the spectrophotometric determination of the color produced by the action of nitric acid. This study was undertaken to determine the excretion and storage patterns of pentachlorophenol and/or metabolites using radiotracer techniques.

Table I—Percent of Administered Activity Detected in Urine after Oral Administration of Uniformly Labeled ^{14}C-Pentachlorophenol to Female Rats

Time	Mean Activity Excreted[a], Percent of Dose	Standard Error of the Mean	Cumulative Mean Excretion, Percent of Dose
8 hr.	8.55	2.39	8.55
12 hr.	15.12	5.70	23.67
16 hr.	17.40	2.00	41.07
20 hr.	5.71	2.55	46.78
24 hr.	3.41	1.38	50.19
30 hr.	6.88	1.65	57.07
36 hr.	3.47	0.90	60.54
42 hr.	2.03	0.53	62.57
48 hr.	0.80	0.26	63.37
60 hr.	1.03	0.24	64.40
72 hr.	0.77	0.28	65.17
4 days	1.34	0.70	66.51
5 days	1.07	0.47	67.58
6 days	0.38	0.13	67.96
7 days	0.18	0.10	68.14
8 days	0.08	0.03	68.22
9 days	0.07	0.03	68.29
10 days	0.05	0.02	68.34

[a] Mean of five female rats.

MATERIALS AND METHODS

Reagents and Apparatus—The 99.5% radiochemically pure, uniformly labeled ^{14}C-pentachlorophenol used in this study was obtained from the synthesis as reported by Rogers *et al.* (7). The specific activity of the compound was determined to be 0.27 μc./mg.

All radioactivity measurements were made with a liquid scintillation spectrometer[1], using a scintillation phosphor prepared by dissolving 2,5-diphenyloxazole (0.4%) in a mixture of toluene–2-ethoxyethanol (1:1). ^{14}C-Toluene was used as an internal standard for quench corrections.

Radiorespirometry—To determine if ^{14}C-pentachlorophenol was metabolized to $^{14}CO_2$ and expired *via* the lungs, a female rat was given an oral dose of ^{14}C-pentachlorophenol equivalent to 59 mg./kg. (3.4 μc.). This dose corresponds to approximately three-fourths of the reported LD_{50} (8). After dosing, the animal was maintained in a controlled-ventilation glass metabolism cage[2] for 24 hr. Expired carbon dioxide was collected and analyzed for activity.

Urine and Feces—Five female rats, weighing between 120 and 146 g., were given oral doses of ^{14}C-pentachlorophenol ranging from 37 to 41 mg./kg. Females were chosen to eliminate self-contamination problems. Immediately after dosing, the animals were placed in stainless steel metabolism cages with food and water available *ad libitum*. Urine samples were collected at 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, and 72 hr. and daily thereafter for 7 additional days. Urine samples were analyzed for activity by placing 0.2 ml. of urine in 15 ml. of scintillator.

Feces samples were removed daily, and the total 10-day output for each animal was analyzed for activity. Each sample was exhaustively extracted with benzene in a soxhlet extraction apparatus, dried, and then reextracted with water.

Tissue Samples—Eight rats, four males and four females, ranging in weight from 174 to 227 g., were given oral doses of ^{14}C-penta-

[1] Tri-Carb model 2002, Packard Instrument Co., Downers Grove, Ill.

[2] Delmar Scientific Laboratories, Inc., Maywood, Ill.

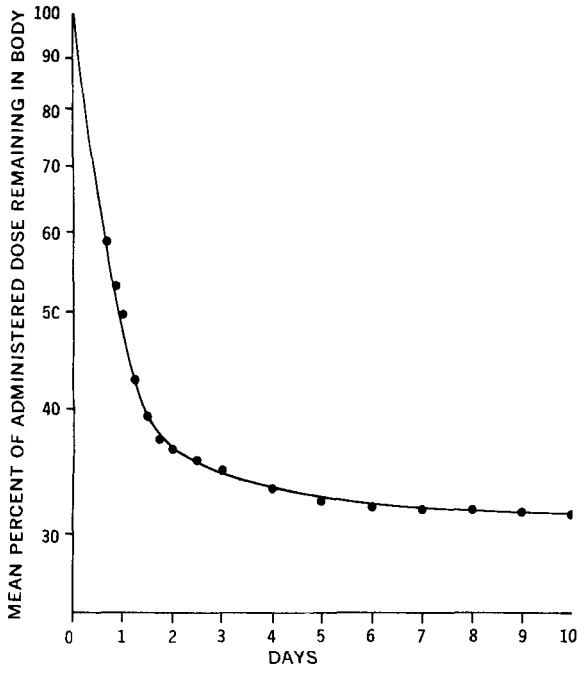

Figure 1—*Retention of orally administered, uniformly labeled ^{14}C-pentachlorophenol. (Fecal excretion was assumed constant with time.)*

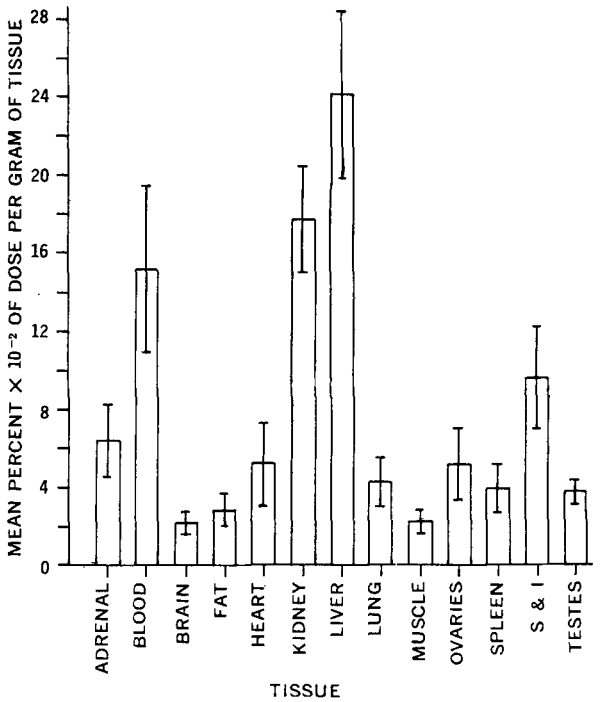

Figure 2—*Tissue distribution of ^{14}C-pentachlorophenol and/or its labeled metabolites 40 hr. after oral administration of uniformly labeled ^{14}C-pentachlorophenol to rats.*

chlorophenol equivalent to 31–40 mg./kg. Inasmuch as stress has been shown to alter drug metabolism (9), an additional four females were given oral doses of ^{14}C-pentachlorophenol but were subjected to hind-leg ligation for 2.5 hr. immediately after dosing. The animals were placed in metabolism cages for 40 hr. with food and water *ad libitum*. They were then sacrificed by decapitation and the following organs were perfused with normal saline to remove any residual blood: lungs, liver, heart, and kidneys. These organs plus the following tissues were analyzed for activity: adrenal, brain, spleen, muscle, testes, ovaries, stomach, and intestines (minus contents), and abdominal fat. All tissue samples were prepared by dissolving 100 mg. of tissue in 1 ml. of sample solubilizer[3], heating to 70° for 2 hr., cooling to room temperature, neutralizing excess sample solubilizer with glacial acetic acid, and adding 15 ml. of scintillator.

Blood—Blood samples were collected at the time of sacrifice from the animals used in the tissue study. The samples were separated into serum and cellular fractions through the use of centrifugation and normal saline washes. The serum activity was determined by placing a 0.1-ml. aliquot directly in 15 ml. of scintillator. The activity present in the cellular fraction was determined by dissolving an aliquot in 1 ml. of sample solubilizer and subsequent decolorization with hydrogen peroxide prior to the addition of scintillator.

RESULTS AND DISCUSSION

The amount of activity detected in the respired air of the rat in a 24-hr. period was less than 0.04% of the administered activity, which was within the purity limits of the labeled compound. This activity could represent metabolism of the impurities rather than metabolism of the parent compound.

The data in Table I show the percent of administered activity detected in the urine of the female rats over 10 days. The average total percentages of administered activity recovered in the urine after 24, 48, and 72 hr. were 50.2, 63.4, and 65.2%, respectively, with the greatest amount of activity being detected in the 16-hr. collection period. The average total recovery of activity in the urine after 10 days was 68.3% of the administered activity, indicating the urine is the principal route of excretion of ^{14}C-pentachlorophenol and/or its metabolites after ingestion.

The percent of activity remaining in the animals (assuming no fecal excretion) was calculated, and these values are represented in Fig. 1. The appearance of this curve suggested a two-component urinary excretion. The curve was resolved into two components by fitting the data to an exponential equation of the form:

$$BB(t) = B_1e^{-B_2t} + B_3e^{-B_4t} \qquad \text{(Eq. 1)}$$

The first component had a half-life of 10 hr., with the second component yielding a half-life of 102 days.

Pooled 10-day feces samples collected during the excretion study were analyzed for activity. The activity recovered during the benzene extraction ranged from 6.5 to 8.2% of the dose, while the activity extracted using water ranged from 1.2 to 5.1% of the administered dose. The total activity recovered ranged from 9.2 to 13.2% of the administered dose.

The tissue concentrations of pentachlorophenol for the male, female, and stressed females were statistically compared using the F test as described by Downie and Heath (10). No significant difference was detected between any of the groups ($p < 0.05$), so the results of all three groups were pooled; Fig. 2 represents the mean percent of administered activity per gram of tissue plus or minus the standard error of the mean. No differences in tissue concentration were noted between sexes, and stress did not significantly alter the tissue distribution of ^{14}C-pentachlorophenol and/or its metabolites after oral administration. The high levels of activity detected in the kidney could be due to the rapid elimination of the compound *via* the urine, with the high levels present in the liver due to the detoxification and/or conjugation of the compound prior to elimination.

Blood samples separated into serum and cellular fractions indicated that the serum contained greater than 99% of the total activity detected in the blood. This would indicate that the ^{14}C-pentachlorophenol and/or metabolites present in the blood are not bound to the cellular constituents.

[3] Soluene, Packard Instrument Co., Downers Grove, Ill.

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▲ To whom inquiries should be directed.

Biosynthesis of Tropic Acid in *Datura innoxia* Root Tissue

N. W. HAMON▲ and J. L. EYOLFSON

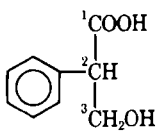
Abstract □ DL-Phenylalanine-1-^{14}C, phenylacetic acid-1-^{14}C, DL-tryptophan-(2-indolyl)-^{14}C, DL-tryptophan-(benzene ring)-U-^{14}C, L-serine-3-^{14}C, and formic acid-^{14}C were all utilized by *Datura innoxia* root tissue as precursors of tropic acid. All of these compounds were incorporated in a specific manner.

Keyphrases □ *Datura innoxia*—biosynthesis of tropic acid, studied using various radiolabeled precursors □ Tropic acid—biosynthesis in *Datura innoxia* root tissue, studied using various radiolabeled precursors □ Alkaloid biosynthesis—*Datura innoxia* root tissue, tropic acid pathways studied

Tropic acid (I) is a constituent of the alkaloids scopolamine (hyoscine) and hyoscyamine. Phenylalanine, phenylacetic acid, and tryptophan have been demonstrated to be the most efficient precursors for this acid. In contrast to phenylalanine (1–7), phenylacetic acid (1) and tryptophan (8) have received relatively little attention by subsequent investigators.

It is obvious that phenylacetic acid requires the addition of one carbon atom (Scheme I) for incorporation into tropic acid. Previous attempts (1) to clarify the origin of the one-carbon fragment using sodium bicarbonate-^{14}C or sodium formate-^{14}C resulted in random labeling of tropic acid. These experiments were, however, conducted on whole plants. The results probably were due to photosynthetic fixation of these compounds into precursors of the aromatic amino acids, resulting in randomization of the label. It is known that sodium formate rapidly degrades to carbon dioxide and water *in vivo*.

In the present investigation, sources of "active formate" were generated within the plant. These sources were serine-3-^{14}C and tryptophan-(2-indolyl)-^{14}C, which produce formate during their normal metabolic degradation (Scheme II). Since tryptophan could also be incorporated into tropic acid *via* another route (8) (Scheme III), it was expected to yield tropic acid labeled in positions C_1 and C_3. Formic acid-^{14}C was also investigated as a source of the C_1 of tropic acid. Vacuum infiltration of root tissue of *Datura innoxia* was used to eliminate the problem of photosynthesis and to lessen the required metabolism time. The biogenesis of the alkaloids occurs primarily in root tissue. Degradation to determine the position of the label was done by known methods, and liquid scintillation counting was employed to determine the activity of the degradation products.



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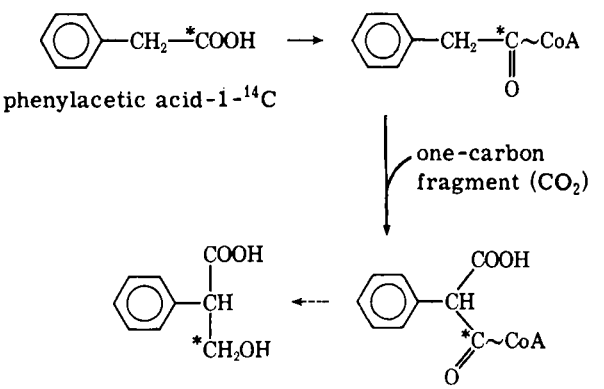
EXPERIMENTAL

Growth Conditions—*D. innoxia* plants were germinated and grown in a controlled-environment room which maintained them at 29.4° (85° F.) for 16 hr. under 1200 ftc. of light followed by 18.3° (65° F.) for 8 hr. in darkness. Relative humidity was 60 and 75%, respectively.

Isotope Administration—Plants (90 days old) were removed from their pots. The root tissue was removed and washed carefully with tap water followed by sterile distilled water, and the secondary roots were weighed into 2-g. samples. Two separate 2-g. samples



Scheme I—Incorporation of phenylacetic acid into tropic acid