COMMUNICATIONS

Fate of C^{14}-Maleic Hydrazide in Rats

The fate of 1-C^{14}-maleic hydrazide (C^{14}-MH) was determined following administration of a single oral dose to rats. Little, if any (<0.001%), radioactivity was detected in tissues or blood samples after 3 days. Expired $C^{14}O_2$ accounted for 0.2% of the radioactivity administered. The radioactivity is eliminated rapidly via the urine, 65% in 12 hours. Recovery of administered radioactivity amounted to 77% in the urine and 12% in the feces in 6 days. The products excreted in the urine are MH (92 to 94%) and a conjugate of MH (6 to 8%).

For several years, maleic hydrazide has been widely used as a plant growth regulator and inhibiter. In 1957, Barnes *et al.* reported MH excreted as the benzylamine salt after administration to rabbits. Williams (1959) quoted El Masri as finding 60% of a 100 mg. per kg. oral dose of MH to rabbits unchanged in the urine. No hydrazine, conjugated glucuronic acid, or ethereal sulfates were found. The purpose of the present investigation was to determine the metabolic fate, tissue accumulation, and excretion patterns in rats receiving C^{14}-labeled MH.

MATERIALS AND METHODS

Sprague-Dawley strain rats weighing 150 to 200 grams were used in this study. Female rats were used to minimize self-contamination. Aqueous solution of C^{14}-MH, (purchased from Tracerlab, Waltham, Mass., 4.12 mc. per mmole), at a concentration of 27 mg. (1.0 mc.) in 20 ml. was prepared by dissolving the compound in 0.1% sodium hydroxide. No radiochemical impurity was revealed by paper chromatography or thin-layer chromatography. The plant growth regulator was administered by stomach tube at dose levels of 10 μc. (0.2 ml., 0.27 mg.) and 25 μc. (0.5 ml., 0.68 mg.) per animal. A dose of 100 μc. was used for urine chromatographing. The oral LD_{50} for MH is about 7000 mg. per kg. of body weight (Tate, 1949–1957). After dosing, the animals were placed individually in metabolism cages (Roth *et al.*, 1948), and the urine and feces were collected periodically. Expired CO_2 was trapped in a solution of 1 part monoethanolamine and 2 parts 2-ethoxyethanol which was changed at 2- and 8-hour intervals and analyzed for radioactivity for 3 days following C^{14}-MH administration. Collection efficiency was 100% for CO_2.

Radioanalysis. The radioactivity in all samples was measured in a Packard Tricarb liquid scintillation spectrometer Model 3003. Two counting formulations were used: solution A for counting water-soluble samples, consisting of 1 part xylene, 3 parts *p*-dioxane, 3 parts 2-ethoxyethanol, 1.0% PPO (2,5-diphenyloxazole), 0.05% dimethyl POPOP [1,4-bis-(4-methyl-5-phenyloxazolyl)-benzene], and 8.0% naphthalene; and solution B for counting the CO_2 trapping solution, consisting of 1 part toluene, 1 part 2-ethoxyethanol, 0.8% PPO, and 0.01% dimethyl POPOP. Fifteen milliliters of solution B and 3 ml. of the CO_2 trapping solution comprised the counting solution. All counted samples were fortified with C^{14}-benzoic acid internal standard and recounted to determine the counting efficiency which was greater than 50% for all samples giving statistically significant count rates. Appropriate aliquots of urine were counted in low-potassium counting vials containing 15 ml. of solution A. The radioactivity of the feces was determined by repeatedly extracting dried, homogenized 5-gram samples with water and counting 3 ml. of the extract in 15 ml. of solution A. Water was the most effective solvent and extractions were continued until an equivalent of less than 0.001% of the total dose was obtained by each 12-hour extraction. Extraction proved more efficient for feces analysis than suspension, oxygen flask combustion, or Hyamine treatment. Rat tissue was analyzed for radioactivity by solubilizing 100- to 200-mg. samples at 60° C. in Hyamine-hydroxide, prepared according to Bruno and Christian (1960), and bleaching with 30% hydrogen peroxide. The samples were acidified with 1 ml. of glacial acetic acid and counted with 15 ml. of solution A. The rat carcass was homogenized to obtain representative samples.

Thin-Layer Chromatography and Autoradiography. Aqueous C^{14}-MH or urine samples were spotted on 250-micron silica gel G–(Brinkmann Instruments Inc., Westbury, L.I., N.Y.) or 1000-micron Adsorbosil-1–(Applied Science Laboratories, Inc., State College, Pa.) coated plates and developed using ethanol–acetone–6*N* HCl (120:30:10) or water–2-propanol (30 to 120) as a developing solvent. Known C^{14}-MH was also chromatographed on each plate. Autoradiograms were made from each plate by exposing medical x-ray film for a period of time calculated to allow 10^8 radioactive disintegrations in order to observe the presence of radioactive spots as small as 0.5% of the total radioactivity placed on the chromatogram.

RESULTS AND DISCUSSION

Excretion Patterns of MH. A study on two rats showed 0.12 and 0.18% of a 25-μc. oral dose of C^{14}-MH was expired as $C^{14}O_2$ during a 3-day period. Radioactivity in the tissues of rats sacrificed after the 3-day

Table I. Average Per Cent Recovery of Radioactivity in Urine of Rats Fed 10 μc. (0.27 mg.) of C^{14}-MH

Hours after Dose	Recovered, %[a]	Range[b]
0–12	65.4	52.13–77.99
12–24	6.0	4.23– 8.46
24–36	2.0	1.14– 3.26
36–48	1.4	0.72– 2.50
48–60	0.7	0.25– 1.13
60–72	0.5	0.21– 1.08
72–96	0.6	0.25– 1.38
96–120	0.4	0.12– 0.77
120–144	0.3	0.12– 0.60
0–144	77.3	66.33–88.66

[a] Mean of 14 rats.
[b] Range of 14 rats.

period was below statistically significant counting levels in all samples but carcass (<0.001% of the administered dose). Tissues analyzed included kidney, liver, spleen, stomach, heart, lungs, brain, muscle, fat, blood, and carcass. About 1% of the dose remained in the entire carcass. Radioactivity excreted in the urine of 14 animals each having received a 10-μc. oral dose amounted to a mean of 65% of the administered radioactivity in the first 12 hours and dropped rapidly thereafter. A mean of 77.3% of the dose was recovered in the urine in a 6-day period (Table I). Total radioactivity in the pooled 6-day feces sample amounted to 12.4% of the total administered dose. About 91% of the dose was recovered.

Metabolites in the Urine. Thin-layer chromatography was used to determine the nature of radioactivity in the urine. Aliquots were chromatographed one dimensionally in two solvent systems. In both cases, two radioactive spots were apparent on autoradiograms. The spots were quantitated by scraping into counting vials and counting with 15 ml. of solution A. The major spot (92 to 94%) was shown to be MH by co-chromatography with authentic C^{14}-MH. The minor spot (6 to 8%) which moved farther than MH in both systems was shown to be a conjugate of MH by the change of R_f value upon acid hydrolyzing and re-chromatographing. No attempt was made to identify the conjugate. Metabolites in feces could not be observed in extracts because of the low specific activity. Further study of the relative concentrations of MH and conjugated MH in the urine at various time intervals was not undertaken because of the rapidity of elimination. Similarly, tissue distribution studies at shorter time intervals were omitted. The experimental data show close similarity between the excretion patterns in rats and that reported in rabbits (Williams, 1959). Accumulation in rat tissue was not observed following a single oral dose and would not be expected from cautious environmental application of MH.

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