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The Disposition of Orally Administered Cholestyramine-C^{14}. (30453)

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Cholestyramine, a strongly basic anion exchange resin, has been shown to be an effective hypocholesteremic agent(1,2) and to be useful in the relief of pruritus associated with biliary cirrhosis(3,4,5). It has been generally assumed that ion exchange resins are not absorbed from the gastrointestinal tract due to their high molecular weight and extreme insolubility. This assumption was supported by the absence of toxic reactions associated with long-term administration of cholestyramine. In the present investigation the question of whether cholestyramine is absorbed from the gastrointestinal tract was studied directly by oral administration of C^{14}-labeled cholestyramine to rats, and subsequent measurement of C^{14} in the blood, tissues, excretions and expired air of the treated animals. The results indicate that cholestyramine resin is not absorbed from the gastrointestinal tract of rats.

Materials and methods. Cholestyramine-C^{14}. The carbon skeleton of cholestyramine is composed of a copolymer of styrene and divinylbenzene; the strongly basic character of the resin is due to the presence of aromatic quarternary ammonium groups. The cholestyramine-C^{14} used in these studies was synthesized by Dow Chemical Co., Midland, Mich., using styrene-8-C^{14} as one of the starting materials. The resin product was ground and washed with dilute HCl and hot distilled water to remove radioactive impurities. It was then lyophilized and ground further to a size small enough to pass through a 200-mesh seive. The specific activity of the labeled resin was approximately 0.14 μc per mg.

Experimental design. The experiments were designed to study the possible absorption of orally administered cholestyramine-C^{14} under two types of experimental conditions: (1)

after a single large dose of resin and (2) after administration for 5 days. In the acute experiment 10 rats were given a single dose of approximately 200 mg (30 μc) of cholestyramine-C^{14} per 100 g body weight. The animals were placed in glass metabolism chambers and respiratory $C^{14}O_2$ collected in 2 N NaOH for 6 hours. Blood samples, the various organs, and the remainder of the carcass were treated as described below. Feces and urine were collected during the 6-hour period. Fecal matter excreted was added to the gastrointestinal sample.

For the subchronic study 10 rats were given approximately one-fifth of the dose administered in the acute experiment (6 μc) each day for 5 days. The animals were housed in stainless steel metabolism chambers and provided food (Purina Laboratory Chow) during the day only. Fecal and urine samples were collected each day. Six hours after the final dose, blood samples were taken, the animals killed and the tissues removed and treated as described below. The fecal samples were also lyophilized and ground.

Female rats from the Mead Johnson Colony (McCollum-Wisconsin strain) were used. All animals were fasted overnight prior to the experiment. Cholestyramine-C^{14} was administered by stomach tube as a suspension in 0.5% methocel-400. Six hours later a sample of blood was obtained by cardiac puncture under chloroform anesthesia. The rats were then killed by cervical dislocation and the liver, kidneys and entire gastrointestinal tract proximal to the stomach to the anal sphincter were removed. The remainder of the carcass was placed in an infusion bottle, which was sealed with a neoprene stopper and an aluminum cap. The carcasses were autoclaved 4 hours at 120°C and homogenized using a Waring blendor. Weighed portions of the homogenate, as well as the individual organs and the blood sample, were separately lyophilized, weighed and ground to a fine powder using a mortar and pestle.

C^{14} determinations. All C^{14} analyses were performed using a Packard Instrument Co. automatic Tri-Carb® liquid scintillation spectrometer, Model 314E. The fluor solution used contained 100 g naphthalene, 7 g 2,5-diphenyloxazole (PPO), 0.5 g 1,4-*bis*-2-(4-methyl-5-phenyloxazolyl) benzene (dimethyl POPOP) and 40 g Cab-O-Sil per liter dioxane. The C^{14} content of the urine and respiratory $C^{14}O_2$ (in 2 N NaOH) was determined by transferring 1-ml aliquots to counting vials and adding 15 ml of the fluor solution.

TABLE I. Recovery of C^{14} in Tissues of Rats Given a Single Oral Dose of Cholestyramine-C^{14}.*

Tissue	Amt analyzed	Observed counting rate,† cpm	Recovery, % of dose
Liver	5 mg	1 ± 2	
Blood	5 "	2 ± 1	
Kidneys	5 "	2 ± 7	
Carcass	5 "	3 ± 11	
Respiratory $C^{14}O_2$	1 ml	6 ± 5	
Urine	1 "	379 ± 138	<.03
Intestine	2 mg	34569 ± 3643	96 ± 6

* Average dose of cholestyramine-C^{14} given was $36.1 \times 10^6 \pm 1.5 \times 10^6$ cpm.

† Counts above background level, mean ± standard deviation.

The C^{14} content of the tissue powders was determined by a Schöniger-type oxidation procedure. The sample, weighing 20 to 50 mg, was wrapped in special filter paper (Schöniger sample wrapper, Arthur H. Thomas Co., Philadelphia, Pa.) and placed in the platinum wire basket of a combustion head. Ten ml of 2 N NaOH were placed in a 1-liter combustion flask; the flask was then flushed with oxygen and sealed with the combustion head. After ignition and combustion, the flask was rotated mechanically for 20 minutes to insure complete absorption of all $C^{14}O_2$. A 1-ml aliquot of base solution, containing the $C^{14}O_2$ from 2 to 5 mg of tissue powder, was transferred to a counting vial and 15 ml of the fluor solution added. All C^{14} determinations were done in duplicate. The counting efficiency of the assay system was about 55%. The samples containing low levels of C^{14} were counted for 30 to 100 minutes; the background count ranged from 19 to 23 cpm.

Results and discussion. The distribution of radioactivity in the tissues of rats given a single large dose of labeled cholestyramine is given in Table I.

The amounts of C^{14} found in liver, blood, kidneys, carcass and respiratory $C^{14}O_2$ were

TABLE II. Recovery of C^{14} in Tissues of Rats Given Daily Oral Doses of Cholestyramine-C^{14} for 5 Days.*

Tissue	Amt analyzed	Observed counting rate,† cpm	Recovery, % of dose
Blood	5 mg	0 ± 2	
Kidney	5 "	1 ± 2	
Liver	5 "	1 ± 3	
Carcass	5 "	47 ± 57	.7 ± .8
Intestine	2 "	6472 ± 437	18 ± 5
Urine	1 ml	12 ± 5	<.01
Feces	2 mg	7663 ± 1245	90 ± 6
Total			109 ± 7

* Average dose of cholestyramine-C^{14} given was $30.3 \times 10^6 \pm 1.4 \times 10^6$ cpm.

† Counts above background level, mean ± standard deviation.

not significantly greater than the background count. The small amount of radioactivity found in the urine and cage washings, amounting to less than 0.03% of the administered dose, is probably the result of contamination from either fecal material or from the small amounts of the administered solution which remained in the oral cavity after tubing. The only tissue examined which contained significant amounts of radioactivity was the gastrointestinal tract; the total recovery of C^{14} from this organ amounted to 96% of the administered dose. It was not possible to quantitatively wash cholestyramine-C^{14} from the intestine. Therefore, the assumption was made that the radioactivity associated with the gastrointestinal tract represents unabsorbed resin.

After the administration of 5 daily doses of resin containing 6 μcuries per 100 g body weight, significant amounts of C^{14} could not be detected in blood, kidneys or liver (Table II). A small amount of C^{14}, equivalent to 0.7% of the administered dose, was measured in the carcass. The source of this radioactivity could have been fecal material, present on the fur and paws of the animals. The small amount of radioactivity found in the urine and pan washings is also presumably the result of fecal contamination. The absence of detectable amounts of C^{14} in blood, liver and kidneys—tissues not likely to become contaminated—supports this conclusion. Recovery of the administered dose was approximately 109%—almost entirely from the gastrointestinal tract and feces. This apparently excessive recovery is due to the difficulty of uniformly suspending the resin and administering it precisely in a small volume of water.

Summary. Groups of rats were given a single large dose, or repeated smaller doses of cholestyramine labeled in the carbon skeleton with C^{14}. No radioactivity could be detected in the blood, liver, kidneys or respiratory CO_2 of these animals. Significant amounts of radioactivity were found only in feces and in the gastrointestinal tract, indicating that cholestyramine is not absorbed from the gastrointestinal tract of the rat.

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