Excretion and Distribution of Radioactive S^{35}-Cyclamate Sodium (Sucaryl Sodium) in Animals.*† (19129)

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Sodium N-cyclohexylsulfamate (sucaryl sodium) has been introduced recently as a non-caloric sweetening agent. It was shown previously that cyclamate sodium‡ was excreted unchanged to the extent of 80-90% in 12 hours in the urine of rabbits when given intravenously, intraperitoneally, or orally(1). In man given 0.3 g of sucaryl in a preliminary experiment(2) 26% of the dose was excreted the first 24 hours and 79.5% excreted after 7 days. The methods used were insensitive and required relatively large doses. In an attempt to overcome these limitations and to extend our work in animals, sucaryl sodium containing radioactive S^{35} was synthesized.

This paper is concerned with the excretion and distribution of S^{35}-sucaryl§ in animals.

Experimental. Young, adult, white rats were the principal animals studied; their urine and feces were collected separately. In one experiment a male dog was anesthetized with pentobarbital and the carotid artery, bile duct, and the ureters were cannulated. After intravenous injection of S^{35}-sucaryl sodium, samples of bile, blood, and urine were taken at various time intervals up to 5 hours, at which time the dog was killed and samples of tissue were taken for analysis. The analytical method used for the estimation of S^{35}-sucaryl was a modification of that previously described(1,2) and adapted for determination of S^{35} in biological materials. In all of these experiments S^{35}-sucaryl sodium originally containing 7.06 millicuries per g (2.2×10^6 counts/minute/mg) was used through 3 half-lives. One to 4 g of tissue were weighed into a 300 ml Kjeldahl flask and 10 ml of concentrated HNO_3 were added and washed down with 50 ml of water. The contents of the flask were boiled, using a microburner, and slowly evaporated until brown fumes appeared. After cooling, distilled water was added and the solution was filtered through Whatman No. 42 filter paper, washing well with distilled water. The filtrate was made to about 500 ml with distilled water, neutralized to pH 6-8 with 25% NaOH and carrier sulfate added in the form of sulfuric acid or Na_2SO_4 equivalent to about 100 mg of $BaSO_4$. Ten ml of concentrated HCl were added, stirred, and then 10 ml of 10% $BaCl_2$ were added slowly and the mixture allowed to stand 1 hour without stirring. The sample was stirred and heated on the steam bath to digest. The samples were cooled to room temperature and filtered through tared filtering crucibles (Selas No. 3010). The precipitate was washed well with water, then alcohol, and finally dried in an oven at 130°C. After cooling to room temperature, the crucibles were weighed.

Ten to 30 mg of the radioactive $BaS^{35}O_4$ were removed from the crucible and plated on a 1-inch tared Cu disk. The $BaS^{35}O_4$ was spread evenly with the aid of a drop or two of water over a fixed inscribed area of 3.56 cm^2 and dried under a heat lamp. The disc was weighed when cool. From these data the mg/cm^2 was calculated. The disc was counted under an end-window Geiger counter (1.8 mg/cm^2 window). Counts per minute were corrected for decay and for self-absorption using an empirically determined calibration

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‡ Cyclamate sodium has been accepted as the generic name for sucaryl sodium.

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§ In this paper S^{35}-sucaryl sodium will be referred to in some cases as S^{35}-sucaryl because only the anionic portion of the molecule is radioactive and measurable. However, all values are calculated on per cent of dose of sucaryl sodium.

curve. From this value the sucaryl content was calculated, assuming that all the radioactivity was unchanged S^{35}-sucaryl sodium. An assay of the original solution used was performed simultaneously and all values were referred to it. On replicate determination of S^{35}-sucaryl added to urine, feces, and tissues, recoveries of 95±5% (av. dev.) were obtained. An alternative method used on urine was to pipette an aliquot of the suitably diluted urine onto a tared Cu disc. After drying, weighing, and counting, the counts/min. were corrected by referring to an empirically determined curve. This gave values that agreed within 10% of those obtained with the $BaSO_4$ method.

Results and discussion. Preliminary experiments showed that there was no demonstrable exchange between the S^{35} of sucaryl sodium and Na_2SO_4 *in vitro* at room temperature in 24 hours. Experiments on the urine of rats fed S^{35}-sucaryl sodium using radiopaper chromatographic methods showed that less than 0.15% of the 100 mg/kg dose was $S^{35}O_4$ ion. The remainder of the radioactivity was concentrated in a single band having an Rf value comparable to that of pure S^{35}-sucaryl sodium. Cold isotope dilution experiments on rat urine fed S^{35}-sucaryl indicated that at least 95% of the sucaryl was excreted unchanged. The possibility of aromatization of the cyclohexane ring(3,4) was not investigated, but from the isotope dilution experiments and the paperchromatograms it is assumed in this work that sucaryl was excreted unchanged and that the measurement of S^{35} content of any tissue or fluid is also a direct measurement of the unchanged drug.

Excretion. The data on the excretion of S^{35}-sucaryl by the rat given a single dose intraperitoneally is shown in Table I. With a dose of 90-410 mg/kg an average of 60% was found in the urine in 3 hours and 72% in 5 hours. In 1 animal given an intravenous injection of 83 mg/kg, 75 and 77% of the dose was excreted by the kidney in 3 and 5 hours respectively.

TABLE I. Excretion of S^{35}-Sucaryl in Urine of the Rat Injected Intraperitoneally. Animals injected intraperitoneally with 10 ml of .9% NaCl at time of dosing.

Dose, mg/kg	No. of rats	% of original dose excreted in the urine 2 hr	3 hr	4 hr	5 hr
90	1	36	51	55	56
105	1	68	80	83	84
110	1	—	62	85	86
410	1	56	67	67	72
410	1	—	40	40	62
Avg			60	66	72

TABLE II. Excretion of S^{35}-Sucaryl by the Rat Given a Single Dose. Excreta from two rats pooled for analysis.

Route admin.	Dose, mg/kg	% of original dose excreted in urine and feces 1 day	2 days	4 days	Distribution of excreted S^{35}, % Urine	Feces
Oral	100	75		101	54	47
,,	100	58		99	67	33
Subcut.	100	92		94	93	7
I.P.	650	94	106		79	21
,,	1250	71	78		76	24
,,	2450	82	91		87	13
,,	3200	81	86		90	10

Table II shows the excretion of S^{35}-sucaryl over longer periods of time. At the end of 4 days, an average of 98% of the radioactivity was recovered in the urine and feces in 3 experiments on 6 rats. Increasing the intraperitoneal dose about 4 times only slightly lowered the amount excreted the first day. At the largest dose of 3.2 g/kg, 81% of the radioactivity was excreted in 24 hours. In one experiment one kidney was removed and 24 hours later a dose of 1.4 g/kg was administered intraperitoneally. At the end of 24 hours, 70% of the dose was excreted. This amount is not significantly different from the amounts excreted by a rat with both kidneys intact and given a comparable dose. The relative proportion of a single dose of sucaryl excreted by the kidney and the intestines varied depending on the route of administration and the amount of the drug given. Of the total amount excreted about 40% of an oral dose of 100 mg/kg was excreted in the feces in 4 days. Subcutaneous injection of the same dose resulted in a decrease in the

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TABLE III. Excretion of S^{35}-Sucaryl by the Rat Given 5 Repeated Doses. Excreta from two rats pooled for analysis.

Route admin.	Dose, mg/kg/day	% of dose excreted in urine and feces		Distribution of excreted S^{35}, %	
		5 days	9 days	Urine	Feces
Oral	120	95	100	37	63
"	120	78	81	33	67
"	80	81	82	32	68
Avg		84.7	87.7	34	66
I.P.	140	93	94	98	2

TABLE IV. S^{35}-Sucaryl Content of Blood, Bile, and Urine at Various Times in the Dog Injected Intravenously with 9.7 mg/kg Sucaryl.

Time after inj., hr	Blood, mg/ml	Bile		Urine	
		mg/ml	% dose inj.	mg/ml	% dose inj.
.5	13.6	—	—	—	—
1	—	35	.2	4	34.4
1.5	5.9	—	—	—	—
2	—	34	.2	2	17
3	2.6	24	.07	.8	7.6
4	2	16	.03	.4	4
5	.9	8	.02	.2	3.8
Total			.52		66.8

proportion excreted in the feces to 7%. Increasing the dose injected intraperitoneally resulted in a decreased proportionate amount excreted by the intestines over a period of 2 days.

The data in Table II showed that a single dose of sucaryl sodium was almost completely excreted by the rat. Table III summarizes the data on rats given repeated oral doses for 5 consecutive days. An average of 85% was excreted in 5 days and another 3% in the 4 days following the last dose. On intraperitoneal injection, 93% was excreted in 5 days with only 1% eliminated in the 4 days after the last dose. These low recoveries of excreted sucaryl 4 days after the last dose are probably due to accumulated losses during these experiments, as the carcasses of these animals yielded less than 1% of the total dose at this time. About two-thirds of the oral dose was excreted in the feces, the remainder in the urine. On repeated intraperitoneal injection only 2% of the dose was found in the feces. In one experiment rats were fed a diet containing 1% of cold sucaryl sodium for 54 days, following which a single dose of 100 mg/kg of S^{35}-sucaryl sodium was injected intraperitoneally. An average of 77% of this dose was excreted in 24 hours, indicating that feeding of sucaryl sodium for this period of time did not significantly influence its excretion. From these data it was concluded that sucaryl was not stored in the body of the rats to any significant extent.

Table IV shows the data on a dog injected intravenously with 9.7 mg/kg of S^{35}-sucaryl and with the bile duct and ureters cannulated. Only 0.5% of the total dose was excreted into the bile at the end of 5 hours as compared to 66.8% in the urine in the same time. Evidently the bile is not an important route of excretion in the dog. It is probable that the large amounts of sucaryl excreted by the rat in the feces is partially the result of incomplete absorption from the lumen of the intestine and not of re-excretion by way of the bile.

Tissue distribution. Table V summarizes the data on the distribution of sucaryl in the rat, rabbit, and dog. Generally, the animals did not concentrate sucaryl in any one tissue to any significant extent except the kidney, and less regularly in the liver. There was considerable variation from rat to rat 4 hours after injection. This was probably caused by differences in the rate of excretion from animal to animal. At 2.5 hours there was great diversity in concentration of sucaryl among the various tissues; at 24 hours the tissue contents were similar in concentration. This was probably caused by the relatively long time necessary for the equilibrium to develop between blood and the individual tissues. There is practically no sucaryl in brain or cerebrospinal fluid during the first few hours after administration, indicating that the blood-brain barrier is relatively impermeable to sucaryl. From the large amount of sucaryl found in the fetus it is evident that it penetrates the placental membrane. The data also indicate that sucaryl is not evenly distributed throughout the body water, at least during the first 4 hours following injection.

Summary. 1. The excretion and distribution of S^{35}-sucaryl in the tissues of the rat, dog, and rabbit were studied. 2. By means

TABLE V. Distribution of Intravenously Injected S^{35}-Sucaryl in the Tissues of Animals. Values in mg/g of tissue (wet wt).

Species	Rat					Rabbit	Dog
Dose	100 mg/kg					50 mg/kg	9.7 mg/kg
Time, hr	2.5	4	4	4	24	1	5
Tissue							
Blood	104	.18	.37	.7	.13	12.4	.92
Brain	1.5	.05	.05	—	.11	.6	.07
Kidney	50	1.4	3.13	3.5	.34	72	2.77
Liver	159	1.3	.38	—	.10	4.3	1.89
Heart	25	.82	.46	—	.10	—	.86
Lungs	17	.31	.43	—	.07	—	.94
Spleen	7	.30	.41	—	.29	—	.79
Intestine	9	2.70	.31	—	.31	—	.83
Skin	9	—	.25	—	.05	—	.54
Muscle	5	.20	.10	—	.22	11.5	.41
Bone	.3	1.35	.27	—	.37	—	1.11
Testicles	—	.80	—	—	.08	—	.97
Eyeballs	.3	—	—	—	—	—	—
Adrenals	.1	.52	.00	—	.00	—	—
Pancreas	—	—	—	—	—	—	.76
Cerebrospinal fluid	—	—	—	—	—	.5	—
Fetus	—	—	—	3.5	—	—	—

of cold isotope dilution and paperchromatographic methods, it was shown that in the rat at least 95% of the material was excreted unchanged. 3. Single doses up to 3.2 g/kg intraperitoneally were rapidly excreted by the rat in 24 hours. A dose of 1.4 g/kg was excreted with equal rapidity by normal and a unilaterally nephrectomized rat. 4. With repeated oral dosing in rats about two-thirds of the drug was excreted in the feces and one-third in the urine. No significant amounts of S^{35}-sucaryl were found to be stored in the body. 5. In the dog only 0.5% of the injected S^{35}-sucaryl was found in the bile 5 hours after an intravenous injection of 9.7 mg/kg. 6. With the exception of the kidney and perhaps the liver, there was no significant concentration of the drug in the various organs of the rat, rabbit, and the dog. Sucaryl penetrated into the brain with difficulty, but was found in the fetus of the rat.

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Serum Lipase (Tributyrinase) in Hypertension and Arteriosclerosis.* (19130)

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Changes in serum lipase activity have been observed in patients with disturbed pancreatic function and with carcinoma of the gastrointestinal tract(1-4). As far as we can ascertain, no detailed studies have been made of the lipolytic activity of the serum in persons with hypertension or arteriosclerosis. This paper records such a study in normal, hypertensive, and arteriosclerotic individuals.

Methods and materials. The technic em-

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