time from pool acetate to expired CO_2 as 60 minutes which is slightly shorter than the half life from the carboxyl carbon of acetate to CO_2 obtained in this investigation of 76.5 minutes. This result following equilibration during an infusion period is probably a better figure than that obtained following a single injection. The longer half life (135 min) of conversion from the methyl carbon of acetate to pool CO_2 found when 2-C^{14} acetate was infused, emphasizes the difference in the pathway of oxidation of the methyl carbon and of the carboxyl carbon of acetate.

The biological half lives, as calculated from the fall of specific activity of body pool bicarbonate and from expired CO_2, are in excellent agreement. Thus, blood data may be used for studying the oxidative rates of acetate, eliminating the turnover time of the bicarbonate pool which otherwise masks the picture of oxidation in the animal body.

Summary. The rates of oxidation of acetate to CO_2 were estimated by using 1-C^{14} and 2-C^{14} labeled acetate and measuring both blood and expired $C^{14}O_2$. Following 1-C^{14} labeled acetate infusion, the half life of blood and expired $C^{14}O_2$ was about 76 minutes indicating a mean turnover time of acetate carboxyl to pool CO_2 of about 110 minutes. The half lives of 2-C^{14} labeled acetate were 135 minutes and 195 minutes respectively. The half life of the pool acetate was 1.2 minutes; turnover time 1.7 minutes; turnover rate 3.4 meq per hour and pool size 6.0 meq per sheep.

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Absorption and Excretion of Furosemide-S^{35} in Human Subjects. (31391)

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Furosemide (Lasix^R) is an anthranilic acid derivative that is at least as efficient as organomercurial agents in provoking water and sodium diuresis(1-3). It has the advantage over the commonly used mercurial diuretics that it is effective when given by mouth and that its action is independent of alterations in acid-base balance(4,5). The absorption, distribution and elimination of S^{35}-labeled furosemide has been studied in several species of animals(6). The present study was undertaken to investigate some of these parameters in human subjects.

Material. This investigation comprised 20 individual acute studies which were conducted during a 6-month period in a group of 16 subjects; these included 14 healthy human volunteers between 22 and 59 years of age and weighing between 54 and 99 kg and 2 patients over 55 years of age who were free of any discernible cardiorenal disease.

The initial specific activities of the 2 batches of furosemide-S^{35}* ($t_{1/2}$ = 87.1 days) were 8 and 40 mc/g, and the purity was ascertained

* Hoechst Pharmaceuticals, Inc., Cincinnati, Ohio.

by radio-paper and thin layer chromatography. Individual doses of furosemide and of radioactivity, sometimes adjusted with carrier, administered by the various routes were: 1 to 60 mg (35 to 120 μc) intravenously, 50 mg (100 and 200 μc) orally and 4 mg (140 μc) intramuscularly. The initial specific activity of chlorothiazide-3-C^{14}† was 0.8 mc/g and hydrochlorothiazide-3-H^3‡ was 1.3 mc/g.

Solutions for parenteral administration were freshly prepared by dissolving the compounds in NaOH, diluting with pyrogen-free physiologic saline to 2 ml, and sterilizing by filtration through a type GS 0.22 μ millipore filter.

Methods. All experiments were started between 8:30 and 11:30 A.M. with the subjects in the fasting or post-absorptive state. This compound was studied during non-hydration, moderate hydration and maximal water diuresis. The 3 moderately hydrated subjects received an oral load of 1000 ml tap water to prevent dehydration, 20 minutes before each study was started. Maximal water diuresis was produced in 3 other subjects with an oral load of 1000 ml water 20 minutes before each study with additional volumes of tap water equal to the volumes of urine and saliva collected at each collection period. In normal subjects, this latter procedure induced a state of sustained maximal water diuresis or physiological diabetes insipidus with an absence of ADH(7). Furosemide-S^{35} was given intravenously to 16 subjects, orally to 2 non-hydrated subjects, and intramuscularly to 1 non-hydrated subject. Except for one subject who received 3 sequential intravenous doses when the urinary flows approximated control levels, all received single doses. Venous blood samples were collected at 1, 5, 15, 30, 60, 120, 180, and 240 minutes after intravenous administration; 1/4, 1/2, 3/4, 1, 2, 2 1/2, 3 and 4 hours after oral administration; 1/4, 1/2, 1, 1 1/2, 2, 3, 3 1/2, and 6 1/4 hours after intramuscular administration. Subjects were instructed to empty their bladders at each voiding. Otherwise, an indwelling Foley catheter was used to collect the urine continuously without rinsing the bladder between collection periods, and the subjects were in the recumbent position. Stool samples were collected during the first 24 hours after oral administration of furosemide-S^{35} in 1 non-hydrated subject. In 3 subjects who received intravenous furosemide-S^{35} saliva was collected during spontaneous flow, or flow stimulated by sucking lemon-flavored lozenges, by placing a plastic suction cup over the orifice of Stensen's duct. Samples were collected continuously, and the 10-minute volumes were measured and assayed for radioactivity.

One non-hydrated patient received a single intravenous mixture of radioactive (100 μc) chlorothiazide-3-C^{14} and hydrochlorothiazide-3-H^3, and plasma levels and urinary excretion patterns were studied.

Plasma, urine, and saliva samples were assayed for radioactivity without special preparation. Fecal specimens were prepared for S^{35} assay by oxidizing with Pirie's solution. Paper chromatographic studies, using Whatman No. 1 Paper, were performed with the n-butanol: ethanol: water (4:1:1) solvent system on some furosemide-S^{35} urine specimens which were concentrated 90% with a Rinco rotating vacuum-type evaporator. The solvent was allowed to descend for 20 hours, and the chromatograms were then air-dried for 1 hour and radioassayed. They were then placed between standard x-ray film for 6 weeks in order to obtain autoradiograms. Radioactivity of various samples was determined with a Tri-Carb Model 314FX liquid scintillating counting system, using counting limits of 100 minutes and 1×10^6 CPM, and a Baird-Atomic radiochromatogram deluxe scanner. Urine osmolarities were determined with a Fiske Mark III osmometer. Glomerular filtration rates were determined in 7 studies by using the colorimetric method of Alving, Rubin and Miller(8) for analyses. A priming dose of inulin was administered, and a sustaining infusion of inulin in physiological saline was maintained at a constant rate of 1 ml/min throughout the experiment.

Results. Biological half-lives of furosemide-S^{35}, as measured by the plasma levels of

† Merck Sharp & Dohme, West Point, Pa.

‡ Ciba Pharmaceutical Co., Summit, N. J.

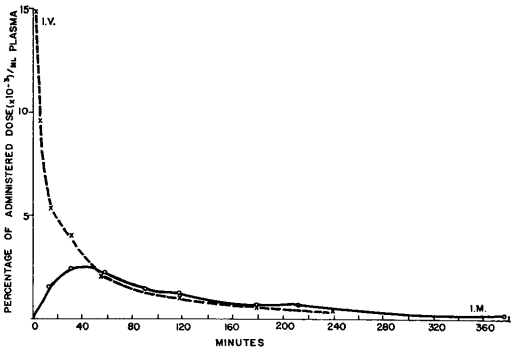

FIG. 1. Comparative plasma levels of furosemide-S^{35} following various routes of administration in non-hydrated subjects. I.V. curve represents a composite of 2 subjects and I.M. curve represents a single subject.

radioactivity (Fig. 1) were quite short. Following intravenous administration in a moderately hydrated subject (Fig. 2), the half-lives were estimated to be 7 and 70 minutes. During maximal water diuresis, there was a more rapid disappearance of radioactivity from the plasma (Table I). When furosemide-S^{35} was given to the non-hydrated subjects intramuscularly, peak plasma levels of radioactivity were found at 30 minutes. Orally, peak plasma levels of radioactivity developed at 60 minutes in 2 other non-hydrated subjects. In one, the maximal concentration of the administered dose per ml was 2.3 $\times$ 10^{-4}% and in the other it was 4.4 $\times$ 10^{-3}%. The respective 24-hour urinary recoveries were 26 and 54% of the administered doses. Following intravenous injections of single doses of furosemide-S^{35} renal clearance of S^{35} began within 10 minutes. However, in one moderately hydrated subject no radioactivity was detected within the first 10 minutes, and then the renal clearance grossly paralleled the slow component of plasma concentration (Fig. 2). There was no significant correlation in the 24-hour recoveries following

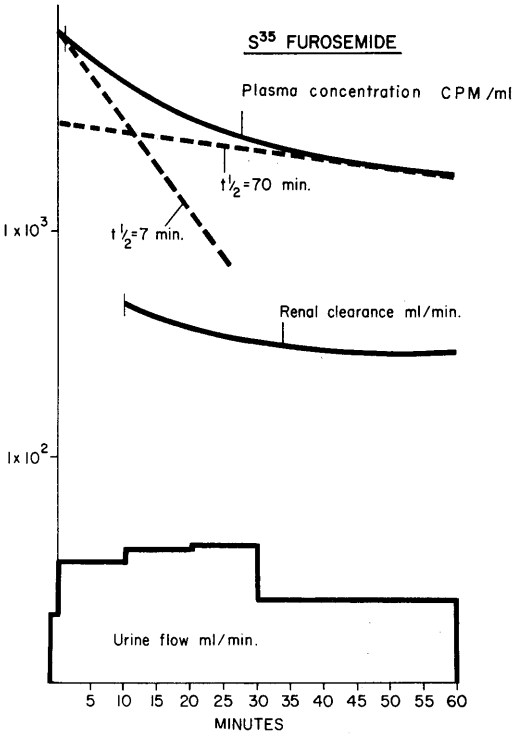

FIG. 2. Comparison of plasma levels, renal clearance and diuretic action of a single I.V. dose of furosemide-S^{35} in a hydrated normal subject. (Subj. H.R.)

I.V. furosemide-S^{35} and the state of hydration, i. e., non-hydration, moderate hydration, and the physiologically induced diabetes insipidus state.

TABLE I. Effect of 40 mg (100 μc I.V. Furosemide-S^{35} on Renal Function Studies During Maximal Water Diuresis (Subject N.S.).

Time (min)	Furosemide-S^{35} plasma conc (mg/100 cc)	Urine vol (ml/min)	GFR* (ml/min)	Furosemide-S^{35} (UV/P) clearance (ml/min)	Furosemide clearance/inulin clearance (ml/min)
0	0	8.8	87.6	0	0
10	.71				
0–20		27.5	91.3	70.5	.77
30	.25				
20–40		35.0	76.6	155.6	2.03
50	.17				
40–60		26.2	62.2	97.1	1.56
70	.15				
60–80		25.0	62.4	81.6	1.30

* Clearance calculated on a basis of a 1.73 square meter surface area.

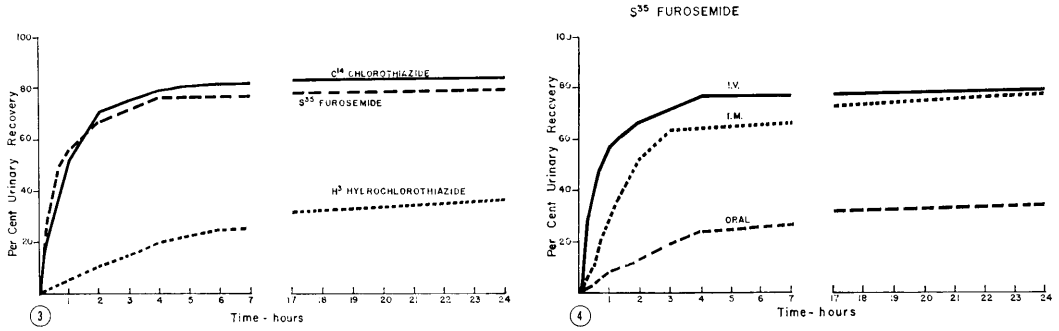

FIG. 3. Comparison of cumulative urinary recoveries of furosemide-S^{35} (average of 5 subjects) chlorothiazide 3-C^{14}, and hydrochlorothiazide-3-H^{3} following single I.V. administrations.

FIG. 4. Comparison of cumulative urinary recoveries of furosemide-S^{35} administered as a single dose by various routes.

The mean 24-hour urinary recovery of intravenously injected furosemide-S^{35} was approximately 80%, with a range of 51 to 94%; a mean 77% of the dose administered was excreted within the first 4 hours (Fig. 3), as compared with 79% urinary recovery of chlorothiazide-C^{14} and approximately 20% recovery of hydrochlorothiazide-3-H^{3} in the same time period. The relationship between urinary excretion of furosemide-S^{35} and renal function (Table I) during sustained maximal water diuresis indicated a greater clearance of this material than inulin. The ratio of the furosemide clearance/inulin clearance was greater than 1.0 as the plasma concentration of furosemide-S^{35} decreased, suggesting that this compound is actively transported by the tubules. This phenomenon might explain the short diuretic effect and rapid excretion of furosemide. As can also be seen, there was a very slight increase in GFR during the first 20 minutes, followed by a decrease.

The 24-hour urinary recovery of intramuscularly injected furosemide-S^{35} approximated 78%. Approximately 2.1% was recovered in the 24-hour stool sample, following oral administration. Comparison of the rates of urinary excretion (Fig. 4) revealed that the major portions (approx. 69-97%) of the recovered activity appeared during the first 4 hours regardless of route of administration.

In the multiple-dose study, three 20 mg (40 μc) doses of furosemide-S^{35} were given intravenously. The second and third injections were made after the urinary minute-volume had returned to previously determined control levels. In this non-hydrated subject urinary excretion rates of radioactivity varied in the same direction as urinary minute-volumes, and both varied indirectly as urine osmolarities (Fig. 5).

Radiochromatographic studies on the urine fractions collected from 1 subject between 3 and 11 hours following I.V. furosemide-S^{35} administration revealed a major peak at a mean R_f 0.354 and a minor peak at R_f 0.154. These corresponded to the peaks obtained with a control which was the same furosemide-S^{35} solution as used in these studies. Since the peak at R_f 0.354 corresponded to the single spot obtained on the autoradiograms

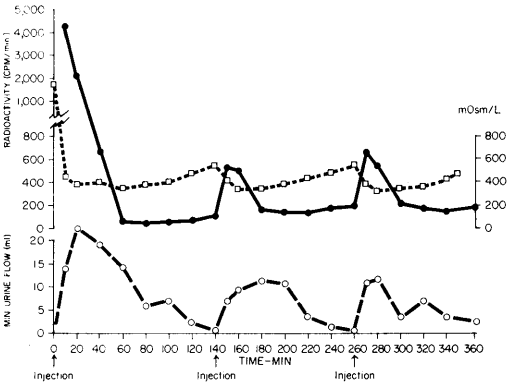

FIG. 5. Urinary studies following three I.V. 20 mg doses of furosemide-S^{35} (40 μc/dose) in a non-hydrated normal subject (J.B.). Rates of excretion of radioactivity and urine flow were calculated for each collection period. Points are plotted at end of each collection period.

□—□ = osmolarity (mOsm/l); ●—● = excretion rate of radioactivity (CPM/min); ○—○ = minute urine flow (ml).

TABLE II. Effect of 40 mg (100 μc) Intravenous Furosemide-S^{35} on Salivary Flow from Parotid Gland. The spontaneous flow was collected during sustained maximal water diuresis, and the stimulated flows were collected from the same non-hydrated subject without (control) and with (treated) diuretic on separate days.

Spontaneous flow (Subject N.S.)		Stimulated flow (Subject C.J.)		
Time (min)	Vol (ml) Treated	Time (min)	Vol (ml) Control	Vol (ml) Treated
—15	1.5	—20	6.0	5.2
— 5	1.5	—10	4.0	9.5
		Furosemide-S^{35} I.V.		
10	1.4	10	4.0	4.5
20	3.0	20	5.0	9.5
30	2.5	30	5.0	10.0
40	1.0	40	7.5	9.0
50	.8	50	7.0	7.0
60	1.3	60	8.0	1.6
70	1.5	70	6.0	.7
80	1.7	80	5.0	.9

during the 6-week exposure, the minor peak probably represented a weak radioactive contaminant.

A review of the data obtained on salivary flow (Table 2) revealed an initial increase within 20 to 30 minutes after I.V. administration of this compound, followed by a secondary decrease, which developed 30 minutes later regardless of the state of hydration. No radioactivity was detected in any of the samples tested.

Discussion. The data obtained in this investigation are in reasonably good agreement with Schmidt's(6) findings in animals. Following intravenous administration of single doses to rats and dogs, this investigator reported 24-hour urinary recoveries between 55 and 78% of the dose given. After intramuscular injection of single doses in sheep, however, the 24-hour urinary recoveries were between 91 and 94%. The quantities recovered in the urine following oral administration of single doses to rats and dogs amounted to 16-34% and 25-45%, respectively, of the doses given. As in the present investigation, curves relating plasma concentrations of furosemide-S^{35} to time after single intravenous injections were biphasic in the studies done by Schmidt in animals. The biological half-lives were 15 and 31 minutes in the experiments performed upon animals, as compared with 7 and 70 minutes in human subjects.

In the present study, chlorothiazide-3-C^{14} was not detectable in the plasma after the first hour following a single intravenous injection of a mixture containing chlorothiazide-3-C^{14} and hydrochlorothiazide-3-H^3, (Fig. 6), yet radioactive material was excreted in the urine over a period of at least 24 hours. The 24-hour urinary recoveries of radioactivity were approximately 84% and 37%, respectively, as compared to the 24-hour complete recoveries in normal subjects previously reported for these benzothiadiazine diuretics (9,10). The 24-hour urinary recoveries of orally administered furosemide-S^{35} (26% and 54%) obtained in the present investigation are comparable to those reported for chlorothiazide-3-C^{14} (33%-58% given by mouth, but somewhat lower than the range of values (61% to 66%) reported for orally administered hydrochlorothiazide-3-H^3.

Because of the rapid onset of diuresis, and the radiochromatographic and autoradiographic findings, furosemide-S^{35} probably did not undergo biotransformation in the body, and the radioactivity assayed represented the intact molecule. Depending on the route of administration, the 24-hour retention of furosemide-S^{35} varied between approximately 20 and 74%. This has been reported for benzothiadiazines(11). In addition, since the 24-hour fecal recovery was only 2.1%, it appeared that the material was sequestered somewhere in the body to be slowly released during subsequent days.

The data suggest that this diuretic produced similar changes on both salivary and urinary flow rates. Since the increase in both flow

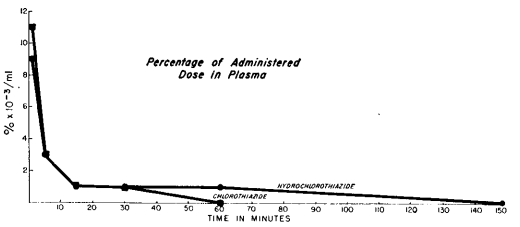

FIG. 6. Plasma levels of chlorothiazide 3-C^{14} and hydrochlorothiazide-3-H^3 administered as a single I.V. mixture to a non-hydrated patient.

rates developed without detectable radioactivity in assay of the saliva samples and during the first 10-minute urine sample in one subject, is might be speculated that this agent was initially attached to certain receptor sites to effect a change in functional activity. Subsequently, changes in the rates of excretion of this agent approximated the change in rates of fluid output in the non-hydrated subject. This time-intensity relationship between diuresis and furosemide-S^{35} excretion was also demonstrated with the diuresis associated with mercaptomerin-Hg^{203} (12,13).

Since the renal clearance of furosemide-S^{35} was greater than inulin, it appeared that the renal clearance of this diuretic must require tubular secretion as well as glomerular filtration. The mechanism whereby furosemide was excreted in excess of glomerular filtration presumably involved that utilized by hydrochlorothiazide(14).

Summary. Furosemide labeled with radioactive sulphur (S^{35}) was given intravenously, intramuscularly or orally to adult subjects without any detectable cardiorenal disease. Peak plasma levels of radioactivity were found at 30 minutes and at 60 minutes after intramuscular and oral administration, respectively, of single doses. Approximately 80% of the dose given was recovered in the urine in 24 hours after intravenous or intramuscular injection of single doses. Following oral administration of single doses to 2 subjects, the 24-hour urinary recoveries were 26% and 54%, and fecal recovery was 2.1%. Comparison of rates of urinary excretion revealed that between 69% and 97% of the recovered activity was excreted in the first 4 hours after the drug was given, regardless of route of administration.

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Differential Disc Electrophoresis of Serum Proteins.*† (31392)

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The Ornstein-Davis polyacrylamide gel electrophoresis procedure permits the separation of extremely complex ionic mixtures. Human serum proteins, in particular, have been extensively examined by separating in the standard 7% gel(1) and more than 20

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