an absorption band in the 400–550-nm region appeared within minutes after irradiation of the sample disk with long wavelength UV light of 366 ± 40 nm.

SUMMARY

Compound IV exhibited photochromism in the solid state. This light-induced color conversion process was typical of many hydrazone derivatives and did not affect the chemical nature or purity of the compound in solution. Conditions of elevated temperature, dissolution, and prolonged storage in the dark at ambient temperatures restored the original color of the compound. An action spectrum in the 300–400-nm range induced the change in color. A structure–photochromic activity relationship among several analogs of IV was observed, from which a possible mechanism for the photochromic conversion of IV from a stable to a metastable solid state was proposed.

The kinetics of photochromism of IV were apparently zero order for the return of the metastable (yellow) state to the stable (colorless) state. The energy of activation for this process was about 19 kcal/mole. The appearance and disappearance of an absorption band in the visible region, with a concomitant change in the color of the compound after exposure to long wavelength UV light, were evidence for the presence of a metastable state, which reverts back to the stable state of IV with heat treatment. These studies allowed novel and direct application of absorption spectrophotometry for determining the kinetics of photochromism in the solid state.

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Absorption, Distribution, Metabolism, and Excretion of Furosemide in Dogs and Monkeys I: Analytical Methodology, Metabolism, and Urinary Excretion

GERALD J. YAKATAN ˣ, DALE D. MANESS, JEAN SCHOLLER, WM. J. NOVICK, Jr. *, and JAMES T. DOLUISIO

Abstract □ ^{35}S-Furosemide was administered to beagle dogs and rhesus monkeys in an oral solution on a single and a 20 repeated 5-mg/kg/day dosing regimen. Following the single dose, 25.0% (dogs) and 24.0% (monkeys) of the dose were excreted in the urine in 24 hr. TLC analysis demonstrated that both species had similar excretory patterns; *i.e.*, over 80% of the amount excreted in the urine was present as unchanged furosemide and the remainder was composed of a known metabolite, saluamine, and an as yet unidentified metabolite(s). The repetitive dosing regimen did not appear to alter significantly either the total amount recovered in the 24-hr urine or the excretion pattern. Studies in dogs showed that only 50–60% of furosemide was absorbed from oral solution. A significant biliary secretion elimination pathway for furosemide also was observed.

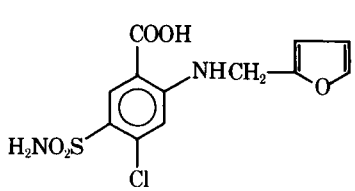
Keyphrases □ Furosemide and metabolite—TLC analysis, urine, dogs and monkeys □ TLC—analysis, furosemide and metabolite, urine, dogs and monkeys □ Excretion—furosemide, TLC analysis, urine, dogs and monkeys □ Pharmacokinetics—furosemide, dogs and monkeys compared □ Diuretics—furosemide, TLC analysis, urine, dogs and monkeys

Despite the widespread clinical use of diuretics in the treatment of edematous states and essential hypertension, there exist little published data on the comparative pharmacokinetics of these agents. This laboratory is involved in a major effort to elucidate the pharmacokinetic profiles of various diuretics and to compare the absorption, distribution, metabolism, and excretion patterns of these agents in several animal species including humans. The present work reports some findings with one diuretic, furosemide, in beagle dogs and rhesus monkeys.

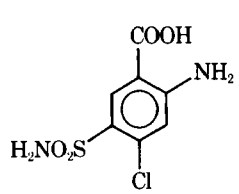
Furosemide, 4-chloro-*N*-furfuryl-5-sulfamoylanthranilic acid (I), is a potent, orally effective diuretic. It exerts its major effect by inhibiting sodium reabsorption in the proximal convoluted tubule and the loop of Henle (1).

4-Chloro-5-sulfamoylanthranilic acid (saluamine) (II) was reported to be the major metabolite of furosemide in humans and dogs (2). Saluamine also was identified as a metabolite in a study on the distribution and urinary excretion of furosemide in the rat (3).

The purpose of the present studies was to determine



I



II

the absorption, distribution, metabolism, and excretion patterns of furosemide following single and repeated 5-mg/kg doses in dogs and monkeys. The generated data could then be compared to the work reported in the rat (3). This initial report describes the experimental design, analytical methodology, and application of this methodology to evaluate the metabolism and urinary excretion of furosemide.

EXPERIMENTAL

Materials—Radiochemically pure ^{35}S-furosemide (specific activity of 7.5 mCi/g initially), unlabeled furosemide, and its known metabolite, saluamine, were used as received[1]. All other chemicals used were analytical reagent or USP grade. Furosemide was administered in an aqueous sorbitol solution containing 5 mg/ml of drug, of which 1 mg/ml was labeled furosemide. The solution was stable over the period of its use.

All materials were protected from light during the preparation of the furosemide solution to prevent potential photolytic degradation. After preparation, the solution was flushed for 5 min with a stream of nitrogen and dispensed in an amber bottle with a nitrogen blanket. After each use, the furosemide solution was purged and blanketed with nitrogen.

Animal Studies—Male beagle dogs[2], 7–8 months old and weighing approximately 10 kg, were dewormed and given immunizations prior to shipment. Young, adult, male rhesus monkeys[3], 2–4 kg, were tuberculin tested prior to shipment.

The animals were individually housed in cages having stainless steel metabolism pans and were randomly assigned to experimental or control groups. Control body weights and temperatures were obtained. Water was allowed *ad libitum* throughout the experiment. Food was given daily, although no solid food was allowed for 4 hr prior to dosing. All doses of furosemide were administered by oral intubation at a level of 5.0 mg/kg. Body weights were determined weekly. Control animals received a solution identical to the one given the experimental groups except that it contained no furosemide.

Blood samples, drawn with a sterile heparinized needle and syringe, were obtained from the cephalic or saphenous veins. The samples were immediately centrifuged to obtain plasma, and the plasma was placed in a foil-wrapped tube and immediately frozen. Hematocrits were checked periodically.

Urine samples were collected in the metabolism cages at specified intervals. The volumes were measured, and an aliquot was stored frozen in a foil-wrapped tube. Urine samples were protected from light as much as possible at all times (even in metabolism cages).

All animals were sacrificed with an overdose of pentobarbital, and the liver, kidneys, and testes were removed immediately. Sections of each tissue were frozen in foil-wrapped vials, and the remainder was fixed in 10% buffered formalin for histopathological studies. The tissues were examined with hematoxylin and eosin, iron, periodic acid–Schiff, and calcium stains.

All biological specimens were stored in coded containers. The code was not broken until all samples had been assayed for radioactivity or examined by the pathologist.

Single-Dose Studies—A single 5-mg/kg dose was administered orally to four groups of dogs and monkeys. Blood, urine, and stool specimens were collected periodically from each animal. The animals were sacrificed (in groups of three) 0.25, 3, 6, and 9 days after dosing, and tissue samples were collected.

In a separate study, a single 5-mg/kg dose was administered orally to a group of three dogs. Blood, urine, and stool specimens were collected at each sampling time from each animal. These animals were not sacrificed. Two of these dogs later received single 5-mg/kg iv doses of furosemide, and blood, urine, and stool specimens were collected.

Multiple-Dose Studies—Groups of animals were given 5 mg/kg/day of furosemide for 20 days. Blood specimens from one group of three animals were obtained immediately prior to, and 2 hr following, the daily dose of drug. Urine collections from these animals were made daily. Blood specimens also were obtained from a group of six animals over 12 hr following the last dose of drug on Day 20.

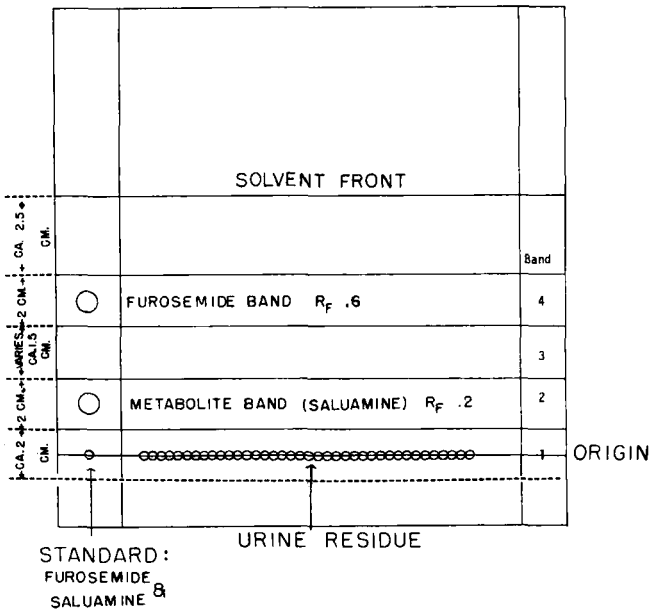


Figure 1—*TLC plate utilized for the analysis of urine samples obtained from dogs and monkeys. Each designated band was scraped and assayed for radioactivity. The band containing the* R_f *0.2 metabolite was saluamine.*

Groups of three animals were sacrificed 24 hr after doses 5, 10, 15, and 20 on a 5-mg/kg/day regimen to determine if kidney accumulation of drug and/or metabolites, or if histopathological changes, occurred. Three additional groups of animals were sacrificed and studied 3, 6, and 9 days after the 20-day dosing regimen.

TLC Analysis of Urine and Plasma Samples—The frozen urine and plasma samples were allowed to thaw, and 0.5-ml duplicate aliquots were sampled into 2-dram vials and lyophilized in the absence of light. The urine residues were extracted three times with 100-μl volumes of hot methanol. The methanolic extract from each sample was spotted on a 20 × 20-cm cellulose TLC plate[4] together with furosemide and saluamine standards, and the plates were eluted with 2-propanol–butyl acetate–water–concentrated ammonia (50:30:15:5 v/v).

Figure 1 shows a representative developed TLC plate. Excellent separation of furosemide (R_f 0.6) from saluamine (R_f 0.2) was obtained. The plates were developed in the dark after the TLC chambers had been equilibrated with the developing solvent. After development, the bands corresponding to furosemide and saluamine were visualized under UV light, marked by comparison to standards, and scraped into scintillation vials for counting of radioactivity. Three additional bands on the cellulose plate were collected to investigate and quantitate the possible existence of unknown furosemide metabolites.

The sizes of the scraped bands were chosen for assay convenience after radiochromatographic scanning and scraping of smaller bands indicated that the radioactivity on the TLC plate would be confined to the areas shown in Fig. 1. To each vial, 15 ml of a scintillation cocktail was added [80 g of naphthalene, 10 g of 2,5-diphenyloxazole, and 0.5 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene dissolved in 1 liter of xylene–dioxane–2-ethoxyethanol (1:3:3 w/v)]. The vials were placed in a box and shaken for 5 min in a horizontal position on an automatic sieve shaker[5]. Then the vials were placed in a liquid scintillation spectrometer[6] and allowed to equilibrate overnight at 6° before analyzing for sulfur-35 activity.

Experiments were performed that demonstrated that: (*a*) radioactivity corresponding to furosemide was totally extracted from the cellulose by the procedures employed; (*b*) the cellulose, which settled to the bottom of the vial, did not alter the counting efficiency; (*c*) 90%

[1] Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876
[2] Marshall Research Animals.
[3] Laboratory of Aerospace Medicine, San Antonio, Tex.
[4] Celplate-22, Brinkmann Instruments, Westbury, N.Y.
[5] Model RX-21, W. X. Tyler Co., Mentor, Ohio.
[6] Tri-Carb model 3385, Packard Instrument Co., Downers Grove, Ill.

Table I—Percentages of the Administered Dose Appearing in the 24-hr Urine of Monkeys and Dogs as Unchanged Furosemide and Metabolites after Single Doses and a 20-Dose Regimen

Regimen	Band 1[a]	Band 2[a]	Band 3[a]	Band 4[a]	Total
		Monkeys			
Single dose[b]	1.50 ± 0.79 (6.1)	1.74 ± 0.85 (7.2)	0.82 ± 0.31 (3.5)	20.0 ± 7.7 (83.2)	24.0 ± 9.2
20 doses[c]	1.73 ± 1.08 (7.5)	1.75 ± 1.06 (7.6)	0.76 ± 0.50 (3.5)	17.9 ± 7.5 (81.4)	22.2 ± 9.7
		Dogs			
Single dose[c]	1.25 ± 0.41 (4.9)	1.78 ± 0.78 (6.9)	0.66 ± 0.23 (2.5)	22.4 ± 4.6 (86.0)	26.0 ± 5.1
20 doses[c]	1.27 ± 0.70 (4.7)	2.40 ± 0.82 (8.9)	0.41 ± 0.19 (1.5)	23.9 ± 9.0 (84.9)	28.0 ± 9.8

[a] The bands correspond to the areas on the TLC plates as shown in Fig. 1. The number in parentheses represents the percent of the amount excreted that appeared in that form. [b] Each value is the mean ± *SD* for nine animals for the 24-hr period following the dose (5 mg/kg). [c] Each value is the mean ± *SD* for 12 animals for the 24-hr period following the dose (5 mg/kg).

of the radioactivity was extracted from the biological sample; (*d*) reproducibility of the procedure was excellent, and processing of duplicate samples was not needed; and (*e*) total recovery of furosemide radioactivity from extraction through separation was 80%.

Thirty plasma samples, selected to represent various sampling times throughout the protocol, were assayed by essentially the same procedure used for the urine specimens. Due to the larger amount of residue after lyophilization, the extraction procedure was modified slightly by initially wetting the residue with 500 μl of hot methanol prior to three extractions with the 100-μl volumes of hot methanol. The results of these studies demonstrated that only about 1% of the total radioactivity on the plate was due to furosemide metabolites. Subsequently, all plasma samples in the study were assayed for total radioactivity only.

Preparation of Plasma Samples for Assay of Total Radioactivity—The frozen plasma samples were allowed to thaw, and 0.5-ml aliquots were added to 15 ml of a scintillation cocktail containing 15% (v/v) of a solubilizer[7], 8 g of 2-(4′-*tert*-butylphenyl)-5-(4″-biphenylyl)-1,3,4-oxadiazole, and 0.5 g of 2-(4-biphenylyl)-6-phenylbenzoxazole/liter of scintillation grade toluene and assayed for radioactivity. The samples were equilibrated at 6° overnight prior to counting.

Preparation of Tissue Samples for Assay of Total Radioactivity—A weighed sample (~100 mg) of lyophilized kidney, liver, or gonad was placed in a scintillation vial. One milliliter of 2 *N* NaOH was added, and the sample was heated in a sand bath maintained at 130°. Heating periods of up to 2–3 hr were required to digest the tissues. Careful dropwise addition of water was made as required to prevent the samples from going to dryness.

After dissolution, the sample was held at reaction temperature for 30 min, removed from the sand bath, and allowed to cool to room temperature. Where necessary, the solution was decolorized by the dropwise addition of 30% hydrogen peroxide. This addition was followed by the addition of 2.75 ml of a solubilizing agent[7], and then the samples were allowed to stand for approximately 30 min. A 10-ml volume of scintillation cocktail then was added, and the samples were mixed[8] and assayed for radioactivity.

Preparation of Feces Samples for Assay of Total Radioactivity—A weighed sample (~100 mg) of lyophilized feces was combusted by the oxygen flask method (4), using a trapping solution containing 10 ml of distilled water and 10 drops of hydrogen peroxide. A 0.1-ml aliquot of the trapping solution was counted for radioactivity in the same scintillation cocktail used for the plasma samples. Recovery experiments with "spiked" feces samples showed that 83% of the radioactivity present was recovered by this procedure.

Counting Data—All samples were assayed for total sulfur-35 activity with a counting error of 5% or less. An automatic external standardization technique was utilized to correct for sample quenching. All samples also were corrected for radioactive decay. The counting data obtained as sulfur-35 activity were converted to concentration or amount of furosemide and/or furosemide equivalents in each sample.

[7] Bio-Solv BBS-2 and BBS-3, Beckman Instruments Co., Fullerton, Calif.
[8] Vortex mixer.

RESULTS AND DISCUSSION

The TLC procedures utilized to assay the urine samples provided excellent separation of furosemide (band 4 in Fig. 1) and its major known metabolite, saluamine (band 2 in Fig. 1). Analysis of other areas on the thin-layer plate showed that significant radioactivity remained in the area of the origin (band 1 in Fig. 1). Since standards of furosemide and saluamine in spiked urine samples carried through the analytical procedures did not show significant radioactivity in band 1, this activity was assigned to the presence of a furosemide metabolite(s) of unknown structure. Preliminary data on the band 1 radioactivity utilizing alternative TLC systems and radiochromatographic scanning indicate the presence of at least two metabolites. Work is continuing to elucidate the structures.

The percentages of the administered furosemide excreted in the urine as unchanged drug and metabolite(s) in the 24-hr period following the single- and 20-dose regimens are shown in Table I. In dogs, 26% of the administered furosemide was recovered in the 24-hr urine following a single oral 5-mg/kg dose. The vast majority (86.0%) of the dose excreted in the urine was unchanged furosemide. Saluamine, the known metabolite (2), accounted for 6.9% of the amount excreted, while 4.9% was recovered as a possible metabolite(s) of unknown structure. Almost all (97%) of the radioactivity found in the urine was excreted in the first 24 hr.

The urinary excretion patterns for dogs in the 24-hr period following the last dose of the 20-dose regimen appear to be qualitatively and quantitatively similar to the single-dose data. Again, less than 30% of the total dose was recovered in 24 hr, and unchanged furosemide accounted for 85% of the drug excreted in the urine. Thus, there was no significant change in the rate or urinary elimination pattern on repetitive oral dosing in dogs.

The monkey eliminated furosemide in much the same fashion as the dog (Table I). Following an oral 5-mg/kg dose, 24.0% of the administered radioactivity was recovered in the 24-hr urine, of which 83% was unchanged furosemide. Saluamine represented 7.2% of the total radioactivity excreted, and 6.1% was recovered as a possible metabolite(s) of unknown structure.

In the 24 hr following the last dose of the 20-dose regimen, 22.2% of the administered dose was found in the urine of the monkeys (Table I). The distribution pattern for the urinary radioactivity was again similar to that observed after a single oral dose (unchanged furosemide, 81.4%; saluamine, 7.6%; unknown metabolite, 7.5%), indicating that no significant changes were occurring as a result of repetitive dosing.

In a study of the distribution and urinary excretion of ^{35}S-furosemide in rats, Seno *et al.* (3) reported recovery of 23.6% of a single oral dose in the 24-hr urine. In a metabolism study limited to the use of two rats, these investigators separated five to seven labeled compounds using TLC on urine samples collected at various time intervals. The overwhelming majority (90%) of the radioactivity resided in three major bands, which were assigned to unchanged furosemide (40–47%), saluamine (30%), and an unknown compound (20%). Schmidt (5) reported urinary recovery of 16–34% of orally administered doses of furosemide in rats and 25–45% in dogs.

Thus, the findings of these investigators regarding urinary excretion

Table II—Percentages of the Administered Dose Appearing in the 24-hr Urine of Monkeys as Unchanged Furosemide and Metabolites after Daily 5-mg/kg Doses[a]

Dose	Band 1[b]	Band 2[b]	Band 3[b]	Band 4[b]	Total
1	2.25	2.36	1.10	26.4	32.11
2	0.72	1.01	0.53	11.0	13.26
3	1.32	1.27	1.40	27.5	31.49
4	1.53	1.34	0.66	26.1	29.63
5	1.28	1.32	0.79	16.4	19.79
6	1.15	0.83	0.74	18.5	21.22
7	1.69	1.92	0.93	23.3	27.84
8	1.29	1.59	0.57	16.1	19.55
9	1.58	1.74	0.77	20.0	24.09
10	1.45	1.57	0.42	18.4	21.84
11	1.77	2.01	0.65	24.1	28.53
12	1.82	2.18	0.78	30.7	35.48
13	1.51	1.71	0.70	17.3	21.22
14	2.20	2.62	0.72	31.4	36.94
15	1.37	1.48	0.57	15.5	18.92
16	2.41	2.30	0.55	26.1	31.36
17	2.68	2.65	0.71	23.4	29.44
18	1.40	1.30	0.75	16.8	20.25
19	1.84	1.50	0.84	18.2	22.38
20	1.62	1.80	0.63	19.5	23.55
20	1.50	1.31	0.50	14.8	18.11
Mean ± *SD*	1.64 ± 0.45	1.70 ± 0.50	0.73 ± 0.22	21.0 ± 5.5	25.1 ± 6.3

[a] Each value is the mean for three animals. [b] The bands correspond to the areas on the TLC plates as shown in Fig. 1.

patterns of furosemide in various animal species are consistent with the data reported in the present study; *i.e.*, the total dose of furosemide is not recovered in the urine after oral administration, furosemide excreted in the urine is primarily in the unchanged form, and furosemide is metabolized to a minor extent to saluamine and a structurally unidentified metabolite.

Tables II and III give the percentage of the daily administered dose of furosemide excreted as unchanged drug and metabolites in the urine of monkeys and dogs for the 24-hr period after each dose. These data are qualitatively and quantitatively consistent with both the single-dose data and the data obtained following the last dose of the multiple dosing regimen (Table I). The mean recovery of the dose in the daily 24-hr urine was 25.1 ± 6.3% for the monkeys and 24.0 ± 6.7% for the dogs. The distribution of the recovered dose was 83.7% as unchanged furosemide, 6.8% as saluamine, and 6.5% as the unknown metabolite(s) for the monkeys and 85.9% as unchanged furosemide, 7.3% as saluamine, and 4.7% as the unidentified metabolite(s) for the dogs.

Although the data obtained in these studies were consistent with the few reported studies on furosemide, the reasons for the low urinary recovery of the administered dose were a cause of concern, particularly in relation to potential bioavailability problems. Two prominent possibilities to explain this problem were: (*a*) incomplete absorption of furosemide in the GI tract, and (*b*) a nonrenal elimination pathway for furosemide and/or its metabolites. The assay of feces specimens collected after oral administration showed a considerable amount of furosemide and/or metabolites present.

The answers to both possibilities dictated that furosemide be administered intravenously. A lack of sufficient ^{35}S-furosemide limited the intravenous studies to two dogs. After administration of a 5-mg/kg iv dose, the urinary excretion of radioactivity in the two dogs was 49.5 and 41.9% of the administered dose. Assay of the feces specimens demonstrated that 52.4 and 50.4% of the intravenous dose were in the feces of the same dogs. Thus, biliary secretory mechanisms appear to play a major role in the elimination of furosemide and/or metabolites in the dog.

That furosemide is not completely absorbed in dogs even from aqueous solution can be observed by comparing the total radioactivity recovered in the urine after intravenous and oral administrations in the same animals. The dog excreting 49.5% of the dose after intravenous administration excreted only 25.2% after receiving the same dose orally; the dog excreting 41.9% after intravenous administration ex-

Table III—Percentages of the Administered Dose Appearing in the 24-hr Urine of Dogs as Unchanged Furosemide and Metabolites after Daily 5-mg/kg Doses[a]

Dose	Band 1[b]	Band 2[b]	Band 3[b]	Band 4[b]	Total
1	1.30	1.54	0.63	25.0	28.5
2	1.25	1.68	0.54	23.4	26.9
3	0.97	1.31	0.82	13.5	16.6
4	1.83	1.93	0.89	32.3	37.0
5	2.07	2.31	0.82	38.8	44.0
6	1.47	1.92	0.41	28.4	32.2
7	1.76	2.55	0.56	35.7	40.6
8	1.80	2.36	1.36	23.6	29.1
9	1.45	1.78	0.58	19.0	22.8
10	1.59	2.15	0.49	27.7	31.9
11	2.13	2.20	0.68	28.2	33.2
12	1.40	2.44	0.72	25.6	30.2
13	0.91	2.38	0.57	21.1	25.0
14	0.99	2.53	0.56	21.4	25.5
15	0.96	2.06	0.41	18.3	21.7
16	0.91	1.98	0.39	22.3	25.6
17	1.18	2.53	0.71	30.5	34.9
18	0.79	1.96	0.59	20.8	24.1
19	0.62	1.40	0.41	17.1	19.5
20	2.13	2.21	0.29	25.5	30.2
20	0.99	3.24	0.41	24.4	29.0
Mean ± *SD*	1.36 ± 0.46	2.12 ± 0.45	0.61 ± 0.23	24.9 ± 6.1	29.0 ± 6.7

[a] Each value is the mean for three animals. [b] The bands correspond to the areas on the TLC plates as shown in Fig. 1.

creted 23.9% after the oral dose. Thus, based on urinary excretion of total radioactivity in dogs, it appears that only 50–60% of furosemide is absorbed from an oral solution.

The plasma and tissue levels and the pharmacokinetic parameters of furosemide in dogs and monkeys following 5-mg/kg oral and intravenous doses will be described in a subsequent paper. These data in dogs confirm the lack of complete bioavailability of furosemide from an oral solution dosage form. Less than 1% of radioactivity in the plasma of dog and monkey is attributable to metabolites.

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* Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876

[x] To whom inquiries should be directed.

Interaction of Sodium Alkyl Sulfates with Everted Rat Small Intestinal Membrane

STUART FELDMAN [x] and MARK REINHARD

Abstract □ The effect of sodium alkyl sulfates (C_6–C_{14}) on the loss of protein from the everted rat small intestine was measured. At a surfactant concentration of 10 m*M*, a peak effect on protein release was noted with sodium decyl sulfate (C_{10}). Measurement of protein release as a function of sodium lauryl sulfate (C_{12}) concentration resulted in the observation that the effect appears to be due to the micellar phase of the surfactant solution. At concentrations of C_{12} above the CMC, the loss of protein from the intestinal preparation increased as the concentration of surfactant was increased. There may be a maximum amount of protein that can be released from the everted rat small intestinal sacs by surface-active agents. At equivalent micellar concentrations of C_8–C_{14} (12.5 times the CMC), there was no difference in the amount of protein released in the presence of the individual alkyl sulfates. The effect appears to be due to the micellar phase of the alkyl sulfate solutions. Other studies on salicylate transfer across the everted rat small intestine indicate that permeability changes occur with anionic and cationic surfactants but not with nonionics.

Keyphrases □ Sodium alkyl sulfates—effect on release of protein from everted rat small intestine □ Membranes, biological—everted rat small intestine, effect of sodium alkyl sulfates on release of protein □ Surfactants—sodium alkyl sulfates, effect on release of protein from everted rat small intestine □ Structure–activity relationships—sodium alkyl sulfates, effect on release of protein from everted rat small intestine

A previous report (1) indicated that sodium taurodeoxycholate, a physiological surface-active agent, accelerates the release of total phosphorus, lipid phosphorus, and protein from the everted rat small intestine. The results indicate that the interaction of the surfactant with the biological membrane accelerates the loss of structural integrity of the preparation and increases membrane permeability. The increase in membrane permeability to phenolsulfonphthalein could be correlated to the increased release of the membrane component in the presence of the physiological surface-active agent.

In view of these findings, it was of interest to examine the effect of a homologous series of anionic surfactants on the everted rat small intestinal membrane to determine the effect of the chain length of the surfactant on the biological membrane. A series of sodium alkyl sulfates from C_6 to C_{14} was chosen. The release of membrane protein was the membrane component investigated.

EXPERIMENTAL

Materials—Sodium hexyl sulfate (C_6), sodium octyl sulfate (C_8), sodium decyl sulfate (C_{10}), sodium lauryl (dodecyl) sulfate (C_{12}), and sodium tetradecyl sulfate (C_{14}) were certified as 99%+ pure by TLC analysis by the supplier[1] and were used as received. All other reagents were analytical grade and were used as received.

Modified Krebs bicarbonate buffer, pH 7.4, with no potassium dihydrogen phosphate included, was prepared as described previously (1). In all cases, the sodium-ion concentration was adjusted to 150 m*M* by the proper addition or omission of sodium chloride.

Preparation of Everted Rat Small Intestinal Sacs—Male, Sprague–Dawley-descent rats[2], 250–350 g, were fasted for 20–24 hr (water allowed *ad libitum*) and then anesthetized with ether. The sacs were prepared as described previously (1). Four consecutive 5-cm segments filled with 1 ml of buffer at pH 7.4 were incubated in a mucosal solution at 37°. The mucosal solution, consisting of 20 ml of buffer alone or buffer with various concentrations of the sodium alkyl sulfates, was oxygenated continuously with a mixture of 95% oxygen–5% carbon dioxide. One-milliliter samples were taken at 30-min intervals for 2 hr.

Protein Determinations—Protein concentrations were determined using the method of Lowry *et al.* (2). The solutions were read[3] at 750 nm against an appropriate blank. The amount of protein in the mucosal samples was calculated as bovine serum albumin equivalents.

Critical Micelle Concentrations (CMC)—The CMC's of the alkyl sulfates were determined by a method (3) based upon spectral changes of a dye in the presence of surfactant micelles. Solutions of each surfactant were prepared in modified Krebs bicarbonate buffer

[1] Schwarz/Mann, Orangeburg, N.Y.

[2] Huntingdon Farms, West Conshohocken, Pa.

[3] Spectronic 20, Bausch & Lomb.