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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ACKNOWLEDGMENTS AND ADDRESSES

Received August 18, 1975, from the *Drug Dynamics Institute, College of Pharmacy, University of Texas at Austin, Austin, TX 78712*

Accepted for publication December 8, 1975.

Presented in part at the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, San Francisco meeting, April 1975.

The authors thank Miss Winifred Ball, Mr. Guy Foster, and Miss Janet Gamble for technical assistance. They also gratefully acknowledge support of this work by grants from Hoechst-Roussel Pharmaceuticals, Inc., and the American Heart Association, Texas Affiliate, Inc.

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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.

Table I—Protein Release from Everted Rat Small Intestinal Sacs as a Function of Time and 10 m*M* Concentration of Sodium Alkyl Sulfates

Solution	Numbers of Sacs	Protein, mg[a] 30 min	60 min	90 min	120 min
Buffer	13	2.30 ± 0.69	3.04 ± 0.74	3.87 ± 0.59	4.93 ± 0.57
C_6	4	1.80 ± 0.25	2.28 ± 0.13	2.61 ± 0.13	3.40 ± 0.36
C_8	4	2.70 ± 0.24	3.61 ± 0.58	4.41 ± 0.29	5.10 ± 0.32
C_{10}	8	6.30 ± 0.61	10.1 ± 0.62	11.7 ± 0.81	12.4 ± 0.60
C_{12}	12	5.20 ± 0.63	8.46 ± 0.58	10.6 ± 0.77	11.5 ± 0.96
C_{14}	8	5.30 ± 0.91	7.60 ± 0.75	10.1 ± 0.77	11.5 ± 0.90

[a] Mean ± *SD*. Calculated as bovine serum albumin equivalents.

containing 0.0005% rhodamine 6G[4]. The solutions were maintained at 37° and were diluted at a constant dye concentration until the orange fluorescent color changed to red and the fluorescence almost disappeared. This end-point was taken as the CMC of the surfactant.

Salicylate Transfer across Everted Rat Small Intestine—Everted rat small intestinal segments, 10 cm in length, were prepared as described by Feldman and Gibaldi (4). The segments were suspended for 1 hr in 80 ml of modified Krebs bicarbonate buffer containing either sodium lauryl sulfate (10 or 50 m*M*), cetrimonium bromide (4 m*M*), polysorbate 80 (1% w/v), or poloxamer 188[5] (1%). Control segments were suspended in buffer for 1 hr.

After incubation, the segments were rinsed on both mucosal and serosal sides and then placed in 80 ml of modified Krebs buffer containing 2 mg of salicylate/ml. Modified Krebs bicarbonate buffer served as the serosal fluid. Serosal samples were withdrawn at appropriate intervals as described previously (4) and assayed for salicylic acid content according to the method of Trinder (5).

RESULTS AND DISCUSSION

The effect of 10 m*M* concentrations of sodium alkyl sulfates on the release of protein from everted rat small intestinal sacs is presented in Table I. At 30, 60, 90, and 120 min after incubation, there was an increase in the amount of protein present in the mucosal solutions. As indicated in Table I, the amount of protein at 120 min after incubation apparently reached a maximum with sodium decyl sulfate.

Figure 1 shows a plot of the ratio of the amount of protein released at 60 and 120 min after incubation in the surfactant solution to that of buffer controls *versus* the number of carbon atoms in the alkyl chain. At the 10 m*M* concentration of surfactant, the maximum ratio occurred at C_{10}. A 10 m*M* solution of sodium hexyl sulfate appeared to offer protection against the deterioration of the membrane because there was an approximate 30% reduction in the amount of protein released. The reason for this apparent stabilization of the intestinal membrane by sodium hexyl sulfate needs further evaluation.

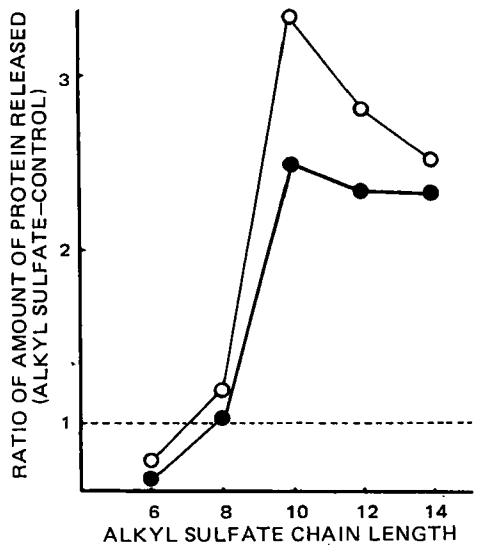

Figure 1—*Plot of the ratio of the amount of protein released in alkyl sulfate solutions to that of controls* versus *alkyl sulfate carbon atom chain length at 60 (○) and 120 (●) min after incubation.*

To investigate the effect of surfactant concentration on the efflux of membrane protein, various concentrations of sodium lauryl sulfate were incorporated into the mucosal solution. The results from this experiment, utilizing surfactant concentrations of 0, 1, 5, 7.5, 10, and 20 m*M*, are presented in Fig. 2. This figure is a plot of the amount of protein in the mucosal solution at 120 min postincubation *versus* surfactant concentration. The results indicate that a relationship exists between surfactant concentration and release of protein from the membrane. With increasing concentrations of surfactant, there was an increase in the amount of protein present in the mucosal fluid.

Inspection of Fig. 2 reveals several interesting features. For example, the largest increase in protein release for a change in surfactant concentration occurred between 0 and 1 m*M* sodium lauryl sulfate concentrations. The CMC of sodium lauryl sulfate in the present buffer system at 37° was 0.6 m*M*. Thus, the increase occurred at surfactant concentrations at or above the CMC. This finding is in agreement with previously reported data (1) on the effect of sodium taurodeoxycholate on the everted rat small intestinal membrane.

A second feature is the relationship of protein release to surfactant concentration at surfactant concentrations above the CMC. The dashed line in Fig. 2 assumes a linear relationship between protein efflux and surfactant concentration. There was an apparent linear relationship between 1 and 10 m*M* sodium lauryl sulfate concentrations, but the 20 m*M* concentration deviated from the extrapolated line. Rather than a linear relationship, the efflux of protein appeared to have asymptotically approached or reached a maximum value at 20 m*M* surfactant concentration. If one assumes that a finite amount of protein can be removed from the membrane by the surface-active agent, then the reaching of a maximum effect on protein loss from the intestinal sacs by the surface-active agent appears to be a reasonable consequence.

Inspection of the data in Table I indicates that approximately 12 mg of protein was released in 2 hr at 10 m*M* concentrations of sodium decyl sulfate, sodium lauryl sulfate, and sodium tetradecyl sulfate, which were at concentrations of 1.67, 16.7, and 83.3 times their CMC's, respectively. A 20 m*M* concentration of sodium lauryl sulfate resulted in approximately 13.5 mg of protein released in 2 hr. In a previous

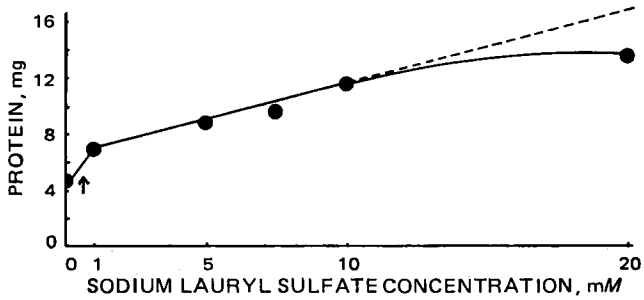

Figure 2—*Plot of the amount of protein released at 120 min after incubation* versus *concentration of sodium lauryl sulfate. See text for details.*

[4] K & K Laboratories, Plainview, N.Y.

[5] Ethylene oxide–propylene oxide polymer of average molecular weight 8350, obtained as Pluronic F-68, Wyandotte Chemicals Corp., Wyandotte, Mich.

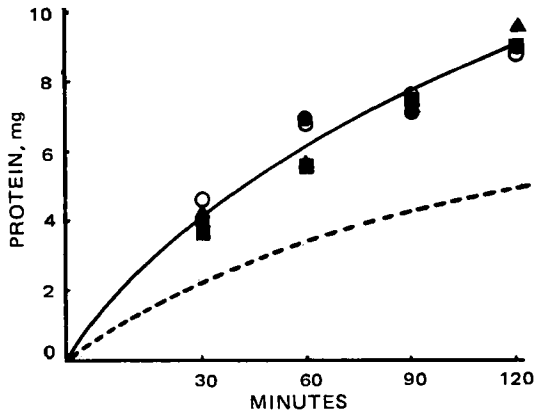

Figure 3—*Plot of the amount of protein released in various sodium alkyl sulfate solutions at a concentration 12.5 times their CMC values* versus *time. Key:* O, C_8; ●, C_{10}; ▲, C_{12}; ■, C_{14}; *and* - - -, *control experiments.*

study (1), 5 and 10 m*M* sodium taurodeoxycholate resulted in approximately 12 mg of protein released in 2 hr. Thus, a limit may exist in the maximum amount of protein that can be released from the small intestinal sacs in the presence of the surfactants.

In view of the apparent effect of concentration of sodium alkyl sulfate on the release of protein from the everted rat small intestinal sacs, the effect of equivalent micelle concentration was investigated. To measure this effect, a 12.5-fold multiple of the CMC's of the C_8–C_{14} surfactants was chosen. Sodium hexyl sulfate does not form micelles. At a constant multiple of the CMC, there should be approximately the same number of surfactant micelles present, assuming equivalent aggregation numbers.

The CMC's of the sodium alkyl sulfates at 37°, as determined in the present investigation, were 41.0, 6.0, 0.6, and 0.12 mmoles/liter for the octyl, decyl, lauryl, and tetradecyl sulfates, respectively. The results from this experiment appear in Fig. 3. Control values are included for reference. The results show that the rate of release of protein from the intestinal membrane was essentially identical for each alkyl sulfate and support the hypothesis that the micelle is responsible for the alteration in membrane structure and, thereby, its permeability.

For example, a 10 m*M* concentration of sodium octyl sulfate, which is below its CMC of 41 m*M*, produced an efflux of protein which, at 120 min, was equal in amount to the protein released in control experiments. However, at a concentration of C_8 of 12.5 times the CMC, there was a 1.83-fold increase in protein released in 120 min compared to control values. The only deviation from this observation occurred with sodium decyl sulfate, where a maximum efflux of protein occurred at a surfactant concentration of 1.7 times its CMC and less of an effect was noted at 12.5 times the CMC.

The present study utilizing anionic surfactants leads to a consideration of the effect of cationic and nonionic surfactants on membrane permeability and integrity. Reports in the literature (6) indicate that anionic and cationic surfactants alter the permeability of the GI membranes while nonionic surfactants do not. Because of cationic and nonionic surfactant interference with the protein assay, the effect of the type of surfactant on the transfer of salicylate across the everted rat small intestine was investigated.

The effect of sodium lauryl sulfate, polysorbate 80, poloxamer 188, and cetrimonium bromide on the transfer of salicylate across the everted intestinal preparation was examined (Table II). There were large increases in salicylate transfer after exposure of the intestinal segments to micellar concentrations of the anionic (sodium lauryl sulfate) and cationic (cetrimonium bromide) surfactants, but no increase was noted after the intestinal segments were exposed to micellar concentrations of two nonionic surfactants (polysorbate 80 and poloxamer 188).

Of interest is the observation (Table II) that, with both sodium lauryl sulfate and cetrimonium bromide, a maximum effect appeared to have been reached in the permeability of the everted intestinal preparation to salicylate. For example, 50 m*M* sodium lauryl sulfate resulted in an increase in salicylate transfer equivalent to the increase noted after exposure of the intestine to 4 m*M* cetrimonium bromide. Both these concentrations are far above their respective CMC's. If 13.5 mg/5 cm represents the maximum amount of protein that can be removed from the intestinal segments by a surface-active agent and if this amount results in a maximum increase in membrane permeability, then 50 m*M* sodium lauryl sulfate should yield a maximum effect while 10 m*M* surfactant should yield a permeability increase slightly below the maximum. Although not statistically significant, the data in Table II support this trend. It should be noted that 10 m*M* sodium lauryl sulfate did not produce a maximum effect on protein loss from the intestinal sacs.

Table II—Percent Change in Salicylate Transfer across the Everted Rat Small Intestine after Preexposure of Segment to Micellar Concentrations of Surfactant

Surfactant	Concentration	Change over Control[a], %
Sodium lauryl sulfate	10 m*M*	+85
	50 m*M*	+95
Polysorbate 80	1%(w/v)	−4
Poloxamer 188	1%(w/v)	+6
Cetrimonium bromide	4 m*M*	+102

[a] Mean of four determinations.

A similar effect was seen between phenolsulfonphthalein transfer and sodium taurodeoxycholate concentration in a previous study (1). In this study, 5 m*M* sodium taurodeoxycholate produced an efflux of 11.7 mg of protein and a 79% increase in phenolsulfonphthalein transfer in 2 hr, while 10 m*M* surfactant produced a protein release of 12.1 mg and a 94% increase in phenolsulfonphthalein transfer.

Based upon previous findings (1) and results of the present study, it appears that the alterations in membrane permeability in the presence of surface-active agents are due to the micellar phase of the surfactant solution as well as the charge on the surfactant molecule. It also appears that a maximum effect of the surfactant on membrane permeability may be achieved, and this maximum may be related to the amount of protein that can be removed from the intestinal segments by the micellar solutions of surface-active agents.

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ACKNOWLEDGMENTS AND ADDRESSES

Received January 19, 1975, from the *Department of Pharmaceutics, College of Pharmacy, University of Houston, Houston, TX 77004*

Accepted for publication December 8, 1975.

Supported in part by a Grant-in-Aid of Research from Temple University.

Part of this work was conducted while S. Feldman was at Temple University School of Pharmacy. The author wishes to acknowledge the support given by that institution. The assistance given by Temple University undergraduate research project students Delores Dobler and Andrew Cuttone is gratefully acknowledged.

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