The Effects of Synthetic Surfactants on Intestinal Permeability to Glucose *In Vitro*[1] (35742)

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(Introduced by W. S. Platner)

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Although natural (1–3) and synthetic surfactants (2, 4) were shown to increase intestinal permeability to a variety of materials, there are no reports of these agents influencing permeability to nutrient substances. There is evidence of depression of intestinal glucose uptake in the presence of synthetic surfactants (5–7) and increased intestinal permeability could, in part, be responsible for this effect.

Materials and Methods. Male golden hamsters (*Microcoetus auratus*) weighing 75–120 g were fasted 24 hr prior to the preparation of intestinal segments. The everted small intestine was divided into three 7–10-cm segments designated as upper, middle, or lower regions to determine whether there was a variation in permeability dependent upon the region of intestine.

A modification of the Crane and Wilson (8) apparatus was utilized with a mucosal volume of 45 ml and serosal volume of 1 ml.

Serosal solutions consisted of 0.6 mg/ml inulin in Krebs–Ringer (pH 7.4). The mucosal solution was Krebs–Ringer containing 56 mmoles/liter glucose, 5×10^{-4} moles/liter phloridzin, and varying concentrations (10–1000 ppm) of the detergents examined. Detergents utilized in this study were: alkylbenzenesulfonate (ABS),[3] linear alkylate sulfonate (LAS),[4] cetyltrimethylammonium bromide (CTAB),[5] and Triton X-100[6]. Serosal and mucosal solutions were maintained at isotonicity (308 mOsm/kg) by adjustment of sodium chloride concentration of the mucosal solutions. Segments were incubated at 37 ± 0.2° for 30 min.

Initial and final mucosal and serosal concentrations of glucose and inulin were determined using Auto-Analyzer (Technicon Instrument Corp.) procedures. Glucose concentration was determined by the Hoffman method (9) and inulin by the procedure of Galli and Jeanmarie (10).

Segments were emptied, dried 24 hr at 100° and data were computed on the basis of dry segment weights in the following manner:

1. Inulin ratio (R) = (initial serosal inulin concentration)/(final serosal inulin concentration).

2. Glucose diffusion rate, μM/ g-hr = final serosal glucose concentration $\times$ R $\times$ 2/dry weight of the segment.

Histologic examination was made of segments demonstrating alteration in permeability.

Results. A comparison of upper, middle, and lower segments of 12 control hamsters

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[3] Obtained as a mixture with sodium sulfate from the American Association of Soap and Detergent Manufacturers, New York City, New York. Average chain length = 12 carbons (range 10–14); mol wt = 347; CMC = 1.2 mmole/liter. Sodium sulfate was removed from the mixture by precipitation in 80% ethanol.

[4] Donated by Chevron Research Company, San Francisco, California. Average chain length = 12 carbons (range 10–14); mol wt = 346; CMC = 1.2–1.6 mmole/liter.

[5] Obtained from K and K Laboratories; mol wt = 364.5, CMC = 0.82–1.0 mmole/liter.

[6] Obtained from Winthrop Laboratories, New York City, New York, mol wt = 646, CMC = 0.9–3 mmoles/liter.

TABLE I. Final Glucose *M/S* Ratio *in vitro.*

ppm	Phloridzin control (P)	P + ABS	P + LAS	P + CTAB	P + Triton
$0_{\bar{x}}$	56.36				
SEM	5.04				
n^a	36				
$10_{\bar{x}}$			70.80		
SEM			3.27		
n			6		
$25_{\bar{x}}$			57.37	72.27	
SEM			5.52	3.26	
n			18	9	
$50_{\bar{x}}$		39.68	26.18	36.74	41.15
SEM		2.36	.958	1.09	5.92
n		6	18	18	6
$100_{\bar{x}}$		61.28	15.54	20.56	71.43
SEM		3.99	.593	1.17	5.31
n		6	18	18	18
$250_{\bar{x}}$		24.04	11.58	13.07	22.85
SEM		1.54	.352	.199	.600
n		18	18	18	18
$500_{\bar{x}}$		9.80	6.21	7.75	16.10
SEM		.228	.192	.210	.250
n		18	18	18	18
$1000_{\bar{x}}$					15.36
SEM					.510
n					18

[a] The number of segments in each series.

revealed no significant difference in glucose transfer in the absence of detergent (upper 11.9 μM/g-hr, middle 15.6 μM/g-hr, lower 11.4 μM/g-hr, $p > 0.90$). Similarly, a comparison of surfactant effect at each concentration revealed no difference in transfer rates among the three groups of segments ($p >$ .50). These results made it possible to compare statistically all control with experimental segments.

Table I contains the mucosal/serosal ratios of glucose and indicates that the phloridzin concentration used was sufficient to prevent an uptake of glucose against a concentration gradient. Further, in all of the experiments the concentration remained higher in the mucosal than serosal solutions insuring a driving force for passive transport of glucose.

Table II shows the increase in passive transport of glucose as the detergent concentrations were increased to 1000 ppm. The dose-response curves are indicated in Fig. 1 with detergent concentrations expressed in moles/liter. LAS and CTAB exposed segments at concentrations of 25 and 50 ppm, respectively, exhibited an increased passive glucose transfer rate (Table II). Triton and ABS did not produce an increase in permeability until concentrations of 250 ppm were attained.

A comparison of the surfactant relative capacities to increase glucose permeability (Table III) indicates that there was a greater effect of LAS and CTAB than of ABS or Triton.

High levels of anionic and cationic surfactants produced gross tissue alterations which were observed microscopically. Segments treated with CTAB and ABS did not demonstrate histologic abnormalities until a concentration of 250 ppm was attained. In contrast, segments in LAS at 100 ppm were abnormal

TABLE II. Mucosal-to-Serosal Glucose-Transfer Rate *in vitro*.

ppm	Phloridzin control (P) (μM/hr-g)	P + ABS (μM/hr-g)	P + LAS (μM/hr-g)	P + CTAB (μM/hr-g)	P + Triton X-100 (μM/hr-g)
$0_{\bar{x}}$	13.01				
SEM	0.875				
n	36				
$10_{\bar{x}}$			12.50		
SEM			1.165		
n			6		
$p \leq$			0.90		
$25_{\bar{x}}$			18.40	10.31	
SEM			1.184	0.590	
n			18	9	
$p \leq$			0.005	0.50	
$50_{\bar{x}}$		12.63	32.39	20.54	12.19
SEM		1.142	1.717	0.548	1.003
n		6	18	18	6
$p \leq$		0.90	0.001	0.001	0.90
$100_{\bar{x}}$		12.08	50.59	50.14	10.94
SEM		0.411	4.634	4.147	0.723
n		18	18	18	6
$p \leq$		0.90	0.001	0.001	0.90
$250_{\bar{x}}$		33.46	63.71	55.29	33.82
SEM		3.039	4.634	1.550	0.844
n		18	18	18	18
$p \leq$		0.001	0.001	0.001	0.001
$500_{\bar{x}}$		51.08	80.74	64.85	46.02
SEM		4.704	8.216	3.209	0.901
n		18	18	18	18
$p \leq$		0.001	0.001	0.001	0.001
$1000_{\bar{x}}$					88.91
SEM					3.758
n					18
$p \leq$					0.001

while Triton at 1000 ppm produced no gross destructive changes. The abnormalities noted were irregular striated borders of the mucosal epithelium, with the suggestion of loosening of intercellular junctions.

Of the surfactants tested, all produced intestinal permeability changes at levels lower than the critical micellar concentration (CMC). Although the CMC of CTAB was exceeded prior to noticeable destructive effects, ABS and LAS produced histologic changes at concentrations lower than the CMC and Triton did not produce observable damage even at levels higher than the CMC. It is, therefore, apparent that permeability effects were elicited by the monodisperse form of the surfactants and histologic changes were not directly related to the production of micelles for all surfactants.

Discussion. Phloridzin has been shown to block competitively the active transport of glucose without significant alteration of tissue permeability or endogenous glucose metabolism (11, 12). The procedure utilized in the present investigation provided a system with which it was possible to examine the effects of synthetic surfactants on intestinal permeability to glucose in the absence of a functioning active transport mechanism.

The permeability to glucose of intestinal

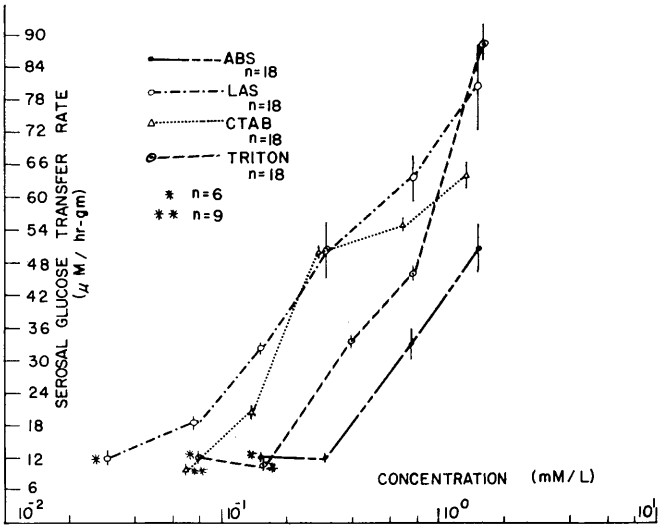

FIG. 1. *In vitro* effects of anionic, cationic, and nonionic surfactants (in mmoles/liter) on mucosal to serosal glucose transfer rates.

segments increased at concentrations of 25 and 50 ppm with LAS and CTAB treatment, respectively, while ABS and Triton X-100 exerted no effect until a concentration of 250 ppm was attained. Webb (13) demonstrated that CTAB, sodium lauryl sulfate, and polyoxethylene sorbitan monolaurate had no effect on passive ionic permeability of short-circuited frog skin until concentrations of 2.0 mmoles/liter or more were attained. The results of this study are in agreement with respect to the levels required for permeability increases produced by ABS and Triton. However, CTAB (0.136 mmole/liter) and LAS (0.07 mmole/liter) produced significant increases in intestinal permeability to glucose at much lower concentrations.

TABLE III. Comparison of Synthetic Surfactant Effects on Mucosal-to-Serosal Glucose-Transfer Rates.

ppm	ABS vs LAS (μM/g-hr)	ABS vs CTAB (μM/g-hr)	ABS vs Triton (μM/g-hr)
$50_{\bar{X}_1-\bar{X}_2}$	—19.75	—7.90	—0.45
$p \leq$	0.01	0.025	0.90
$100_{\bar{X}_1-\bar{X}_2}$	—38.50	—38.06	—1.1
$p \leq$	0.01	0.001	0.90
$250_{\bar{X}_1-\bar{X}_2}$	—30.34	—21.91	—0.45
$p \leq$	0.001	0.001	0.90
$500_{\bar{X}_1-\bar{X}_2}$	—29.66	—13.77	—5.06
$p \leq$	0.001	0.025	0.25

The greater effectiveness of LAS (a linear chain anionic detergent) than ABS (branched chain anionic detergent) to produce increased permeability of intestinal segments may be related to the work of Ponder (14) who demonstrated that the length and linearity of the alkyl chain increased the effects of synthetic detergents.

Triton, a nonionic surfactant, produced effects that did not differ from ABS. This finding is in agreement with chloroplast permeability (15) and recent toxicity (16) studies, but is contrary to the observations of the nonreactivity of nonionic agents with other tissue preparations (17).

Levine *et al.* (18) recently examined the integrity of isolated intestinal segments and demonstrated progressive histologic damage with time during incubation in Krebs–Henseleit buffer. If low concentrations of detergents (even below those producing increased permeability) caused irreversible changes in membrane structure, then destructive processes would have been accelerated. They were not. Further, depression of glucose uptake *in vivo* occurs quite rapidly, within 5 min, and is reversible at low concentrations

(7 and unpublished results). Therefore, the permeability changes induced by low concentrations of detergents were not irreversible changes of membrane structure and a threshold concentration was necessary for the production of tissue damage.

These results may shed some light on a mechanism of detergent depression of glucose uptake by the intestine. Since the permeability of intestinal tissue to glucose is increased by detergents, it is possible that the glucose gradient established by the transport system is diminished or abolished by the "leak" produced by increased permeability, thus passive diffusion of glucose can proceed to both the serosal and luminal sides of the mucosal cell.

Summary. Two anionics, a cationic and a nonionic synthetic surfactant, were examined in an *in vitro* phloridzinized preparation for their effects on passive intestinal permeability to glucose. These agents increased intestinal permeability to glucose in a dose-related manner with the anionic surfactant, linear alkylate sulfonate, producing this effect at lower concentrations than any of the other surfactants tested. Histologic observations demonstrated that the mucosal epithelium was not altered by low surfactant concentraions which increased intestinal permeability. Increased glucose permeability could account, in part, for decreased glucose uptake by the intestine when exposed to detergents due to the production of a "leak" at the mucosal surface preventing a glucose gradient to be established between the mucosal cell and the serosal border.

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