[Chem. Pharm. Bull. 17(6)1109—1114(1969)]

UDC 615.033 : 615.45.076.9

Studies on Absorption of Drugs. IV.[1] Effects of Surface Active Agents on Intestinal Absorption of Drugs[2]

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(Received June 21, 1968)

Effects of surface active agents (ionic and nonionic) on intestinal absorption of drugs were studied on three method, *i.e.*, (1) circulation with surfactants, (2) preperfusion with surfactant solutions, followed by circulation of drug solution free from surfactants, and (3) feeding experiments of surfactants solutions, followed by circulation of drug solution.

Effects of surfactants were reviewed from two points. One is the effect due to interaction of drugs with surfactants and the other effect due to degeneration of the intestinal mucosa.

It is concluded that the absorption of ionized species of drugs were more decreased than that of unionized species in degenerated intestinal mucosa, except for ionic interaction between drugs and surfactants.

In pharmaceutical preparations, surface active agents, especially a nonionic type, have been used as solubilizer for water insoluble substances. It is generally accepted that the solubilizing action of surface active agents is due to their characteristic to form micelles. So, in some cases, surface active agents inhibit the hydrolysis of drugs[4] and in other cases, they show effects on an availability of drugs.[5–7]

When drugs absorbed through intestinal lumen, effects of surface active agents seems to be too much complicated to be fully explain.

Nissim[6] reported a damaging action of cetyltrimethyl ammonium stearate to the gastro-duodeno-jejunal mucosa following large oral and parenteral doses. Also, he and other authors made toxocological investigations of other surfactants.[8,9]

Kakemi, *et al.*[7] showed various actions of surfactants on intestinal absorption of drugs. Yamada reported the effect of nonionic surfactants in reference to availability of drugs.[10]

The present paper concerns with effects of surface active agents on intestinal absorption of drugs from two viewpoints. One is the effect due to interaction of drugs with surface active agents and the other is the effect presumably due to functional change of the intestinal mucosa.

1) Part III: M. Aoki and H. Fukuchi, *Yakuzaigaku*, **23**, 35 (1963).
2) Part of the present report was presented at the 85th Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, Apr. 1965.
3) Location: *No. 5, Toneyama-6-chome, Toyonaka, Osaka.*
4) H. Nogami, S. Awazu and N. Nakajima, *Chem. Pharm. Bull.* (Tokyo), **10**, 503 (1962); A.G. Mitchell and J.F. Broadhead, *J. Pharm. Sci.*, **56**, 1261 (1967).
5) M. Matsumoto and M. Aoki, *Chem. Pharm. Bull.* (Tokyo), **10**, 252, 261 (1962).
6) J.A. Nissim, *Nature*, **185**, 222 (1960).
7) K. Kakemi, R. Arita, H. Sezaki and I. Sugimoto, *Yakugaku Zasshi*, **84**, 1210 (1964).
8) J.A. Nissim, *Nature*, **187**, 305 (1960).
9) S.L. Hart and J.A. Nissim, *J. Pharm. Pharmacol.*, **18**, 228 (1966).
10) H. Yamada and R. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **13**, 1279 (1965); H. Yamada, T, Ichihashi, F. Kogishi and R. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **14**, 786 (1966).

Sodium lauryl sulfate (anionic surface active agent), benzethonium chloride (cationic surface active agent), polysorbate 80 and polyoxyethylene (10 mole) octyl phenyl ether (nonionic surface active agent) were used in a concentration of 0.5 w/v% in Tyrode's solution (pH 7.8). Sulfamethoxypyridazine, diphenhydramine hydrochloride, salicylic acid, and *p*-hydroxybenzoic acid were tested.

Absorption of drugs not only in the presence of surfactants but after a pretreatment of perfusion or after feeding with surfactant solutions was studied using the rat small intestines *in situ*.

Experimental

Methods——Sprague–Dawley male rats weighing 150 to 200 g were kept fast for about 20 hr prior to the experiment but water was given *ad libitum*. During the fasting period, the animals were kept in cages to prevent coprophagy. They were anesthetized with 2.5 mg of pentobarbital per 100 g body weight and their small intestines were exposed by a mid–abdominal incision. The stomach and cecum were ligated by cannulation below the duodenum and at the upper portion of ileocecum with glass cannulae connected to a polyethylene tube, 2.5 mm in inner diameter and 5.0 mm in outer diameter, following recovery of the intestines in the abdomen.

The intestines were washed with about 1 liter of Tyrode's solution (pH 7.8) at 37°. The washing solution was eliminated to a possible maximum to be replaced by 100 ml of Tyrode's solution containing drug (1 mM/l). The ileal out flow was recirculated at the rate of 32 ml/min (Fig. 1) to the stock solution by aeration. The rate of flow was checked with a micro–flow meter equipped in the middle part of a circulation tube. In an hour, the circulating solution was collected in a 500 ml volumetric flask following washing with drug–free Tyrode's solution to make 500 ml.

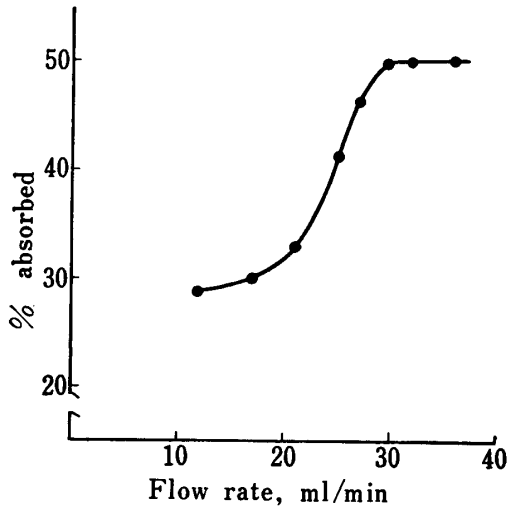

Fig. 1. Effect of Flow Rate on Absorption of Salicylic Acid at pH 7.8 (Tyrode's Soln.)

This procedure was also adopted in the presence of a 0.5% surface active agent. In a pre–perfusion with a surface active agent, Tyrode's solution containing a 0.5% surfactant was circulated for 15 or 60 min, followed by circulation of drug solution after the intestines were washed with 1 liter of Tyrode's solution to keep the intestines free from the surfactant solution.

In feeding experiments, the animals were given aqueous solutions of the following concentrations: 0.5% of polysorbate 80 for 30 days, 0.5% of sodium lauryl sulfate for 10 days and 0.05% of polyoxyethylene (10 mole) octyl phenyl ether (abbreviated as OP–10) for 10 days. The animals did not consume benzethonium chloride solution presumably because of its bitter taste. So, 5 ml of 0.05% benzethonium chloride aqueous solution was administered with a catheter once a day for week. Then, absorption experiments were performed with surfactant–free drug solutions as described above.

In all experiments, the degree of absorption was calculated from the difference of the amounts of drugs in pre– and post–perfusion.

Analytical Methods——Sulfamethoxypyridazine was analysed routinely by diazotizations. Colorimetric determination was not affected by surfactants in a concentration of 0.05% which was purposely diluted for determination.

Diphenhydramine was determined with the auther's previous method.[11] In the presence of surfactants, 0.5 ml of 1 N NaOH was added to 2 ml of sample solution taken into a glass–stoppered centrifuging tube, following cooling with an ice bath. After adding 5 ml of cyclohexane, the mixture was shaken vigorously for 2 min under cooling. It was centrifuged and 3 ml of the cyclohexane solution was pipetted into a separating funnel containing 2 ml of McIlvaine's buffer (pH 5.6), 1 ml of bromcresol green solution and 5 ml of ethylene dichloride, then processed as in case with surfactant–free aqueous solution.

Calibration curves were made on each solution containing polysorbate 80, benzethonium chloride and sodium lauryl sulfate.

Salicylic acid was analysed by the following: five ml of acetate buffer (pH 2.4) was added to 2 ml of sample solution, followed by the addition of ferric solution. The ferric solution was prepared as follows: 6 ml of

11) M. Aoki, Y. Iwayama and N. Yata, *Yakugaku Zasshi*, **82**, 918 (1966).

water and 6 ml of 10% HNO_3 were added to 0.2 g ferric ammonium sulfate, then the solution was boiled. After cooling the solution was filtered and water was added to make 100 ml. The color developed was estimated spectrophotometrically at 530 mμ. Polysorbate 80 and OP-10 did not interfere with the estimation below a concentration of 0.5%.

p-Hydroxybenzoic acid was estimated colorimetrically with a coupling of *p*-hydroxybenzoic acid and a diazotized sulfanilic acid.[12] The analysis was satisfactorily made even in the presence of nonionic surfactants.

Binding of Drugs with Surfactants——Degree of binding of drugs with polysorbate 80 or OP-10 was measured by a dialysing method at 37°. Three kinds of pH were employed at which drugs are found completely unionized or ionized depending upon their p*Ka*. A visking tube containing 5 ml of buffer was immersed into 30 ml of buffer solution containing drug (1 mM/liter) and a surfactant in various concentrations. In 3 hr, equilibriation being achieved, the inner solution was estimated with the above methods and Kostenbauder's plot[13] was made.

Results and Discussion

Effects of Benzethonium Chloride

Absorption of sulfamethoxypyridazine in one hour was decreased by the presence of benzethonium chloride and the effect was also observed in caseof pre-perfusion and feeding experiment (Table I).

Table I. Effects of Benzethonium Chloride on the Instestinal Absorption of Drugs from Tyrode's Solution

	% absorbed in 1 hr	
	Sulfamethoxypyridazine	Diphenhydramine
Control	46 (3)	38 (4)
With	25 (3)	45 (3)
Pretreat	13 (6)	45 (3)
Fed	30 (4)	44 (4)

Parentheses indicate no. of animal.

Table II. Changes of Distribution Ratio ($CHCl_3$/Tyrode's Soln.) in the Presence of Ionic Surfactants

	Without	Benzethonium chloride	Sodium lauryl sulfate
Sulfamethoxypyridazine	0.29	1.40	0.27
Diphenhydramine	35.5	0.94	0.69

On the contrary, absorption of diphenhydramine was increased in all experiments (Table I). It is generally accepted that the degree of absorption of drugs is proportional to their oil and water distribution. But in the present case, the distribution ratio did not reflect drug's absorption in the presence of ionic surfactants (Table II). So the effect on the intestinal mucous membrane should be taken into consideration. It was observed that the intestinal mucous membrane was thined and degenerated. But the absorption of diphenhydramine was increased even in the degenerated membranes. This point will be discussed later in relation to effects of sodium laurylsulfate.

12) M. Aoki, N. Yata, H. Sakaguchi and I. Mimura, *Yakuzaigaku*, **26**, 9 (1966).
13) K.B. Kostenbauder, *Am. Perfumer*, **75**, 28 (1960).

Effects of Sodium Lauryl Sulfate

In the presence of 0.5% sodium lauryl sulfate, the degree of absorption of diphenhydramine or sulfamethoxypyridazine was decreased, the degree being more significant in the former than the later (Table III).

Table III. Effects of Sodium Lauryl Sulfate on the Intestinal Absorption of Drugs from Tyrode's Solution

	% absorbed in 1 hr	
	Sulfamethoxypyridazine	Diphenhydramine
Control	46 (3)	38 (4)
With	29 (4)	10 (3)
Pretreat	31 (3)	57 (3)
Fed	30 (3)	37 (2)

Parentheses indicate no. of animal.

In pre-perfusion with the surfactant solution, diphenhydramine was absorbed 20% more than control and 47% more than with sodium lauryl sulfate, but the absorption of sulfamethoxypyridazine was decreased in a degree almost equal to that in the presence or in the ingestion of the surfactant.

Thus, it was observed that sulfamethoxypyridazine was less absorbed in the presence of ionic surfactants. In case of benzethonium chloride, a cationic surfactant, its inhibitory effect of absorption may be interpreted from two following points: one, interaction with anionic species of sulfamethoxypyridazine and the other, abnormal changes of mucous membrane. Formation of salts or molecular complexes of higher order due to interaction will result in enlarged molecular size. And what is formed will be squeezed out from an absorption route of the ionic species, *i.e.*, pore route. Subsequently absorption will be decreased. But the results of pre-perfusion will offer a problem of abnormal changes of mucous membrane. In case of sodium lauryl sulfate, no interaction would occur because it has a same charge as sulfamethoxypyridazine. Sodium lauryl sulfate's inhibitory effects on the absorption of sulfamethoxypyridazine throughout the experiments seems to be attributable to the degeneration of intestinal mucous membranes (Table III).

Unlike the case of sulfamethoxypyridazine, the absorption of diphenhydramine was increased even in the intestine which was affected either by benzethonium chloride (for all experiments) or by sodium lauryl sulfate (in pre-perfusion or feeding experiments). And the absorption of diphenhydramine in the presence of sodium lauryl sulfate was markedly decreased due to the interaction similar to the case of sulfamethoxypyridazine in the presence of benzethonium chloride.

From these results and the fact that sulfamethoxypyridazine (pK_a 7.05[14]) and diphenhydramine (pK_a 7.56[15]) are found ionized in the persued solution (pH 7.8) 86% and 38% respectively, the following speculation could be made: absorption of ionized species of drug will be more affected than unionized species.

If this speculation is accepted, the following explanation could be made on the increasing effects of benzethonium chloride and of sodium lauryl sulfate on the absorption of diphenhydramine.

It is generally accepted that there are two routes for drug absorption, *i.e.*, lipid route which is a major route for unionized molecules and pore route which is a major route for ionized molecules. When surfactants solution are brought into the intestinal lumen,

14) M. Yamazaki, M. Aoki, A. Kamada and N. Yata, *Yakuzaigaku*, **27**, 37 (1967).
15) A.V. Tolstoouhov, *Trans. N. Y. Acad. Sci.*, **14**, 260 (1952).

endogeneous lipids in the intestinal mucosa will be partially spread on pores on the mucosa due to solubilizing action of surfactants. This will result in an increase of the lipid route as well as a decrease of the pore route. This increase of the lipid route will be reflected on the increased absorption of diphenhydramine and on the decreased absorption of sulfamethoxypyridazine (Table I, III). The difference of effects of benzethonium chloride and sodium lauryl sulfate in feeding experiments on the absorption of diphenhydramine may be explained on the basis of the difference of their chronic toxicity.

Effects of Polysorbate 80 and Polyoxyethylene (10 mole) Octyl Phenyl Ether (OP-10)

Unlike in case of diphenhydramine and salicylic acid, sulfamethoxypyridazine and *p*-hydroxybenzoic acid were less absorbed in pre- and co-perfusion with polysorbate 80 (Table IV). With OP-10, absorption of all the drugs under study were decreased in various degree (Table V).

Table IV. Effects of Polysorbate 80 on the Intestinal Absorption of Drugs from Tyrode's Solution

	% absorbed in 1 hr			
	Sulfamethoxy-pyridazine	Diphen-hydramine	Salicylic acid	*p*-Hydroxy-benzoic acid
Control	46 (3)	38 (4)	50 (7)	25 (8)
With	37 (5)	34 (4)	53 (5)	13 (5)
Pretreat	32 (3)	44 (3)	48 (4)	10 (6)
Fed	47 (4)	50 (2)	—	—

Parentheses indicate no. of animal.

Table V. Effects of Polyoxyethylene(10 mole) Octyl Phenyl Ether on the Intestinal Absorption of Drugs from Tyrode's Solution

	% absorbed		
	Sulfamethoxypyridazine	Salicylic acid	*p*-Hydroxybenzoic acid
Control	46 (3)	50 (7)	25 (8)
With	32 (5)	27 (5)	7 (5)
Pretreat	20 (5)	25 (5)	2 (5)

Parentheses indicate no. of animal.

Effects of nonionic surfactants on the intestinal absorption of drugs seems to be composed of two factors, *i.e.*, one, an interaction of drugs with surfactants and the other, a degeneration of the intestinal mucosa. The former factor being considered negligible in the present study (Fig. 2a, b, c), nonionic surfactants are most likely to be responsible for a degeneration of the intestinal musoca.

Decreasing effects of nonionic surfactants are significant in drugs which are found more ionized in Tyrode's solution (pH 7.8). These effects will result from a decrease of pore route as described in relation to ionic surfactants. An increased absorption of diphenhydramine in pre-perfusion with polysorbate 80 solution may be recognized on the basis of the increase of lipid route.

Nevertheless salicylic acid is assumed to ionize completely in Tyrode's solution, it was not affected by polysorbate 80. This findings are interesting but are left unexplained in this paper.

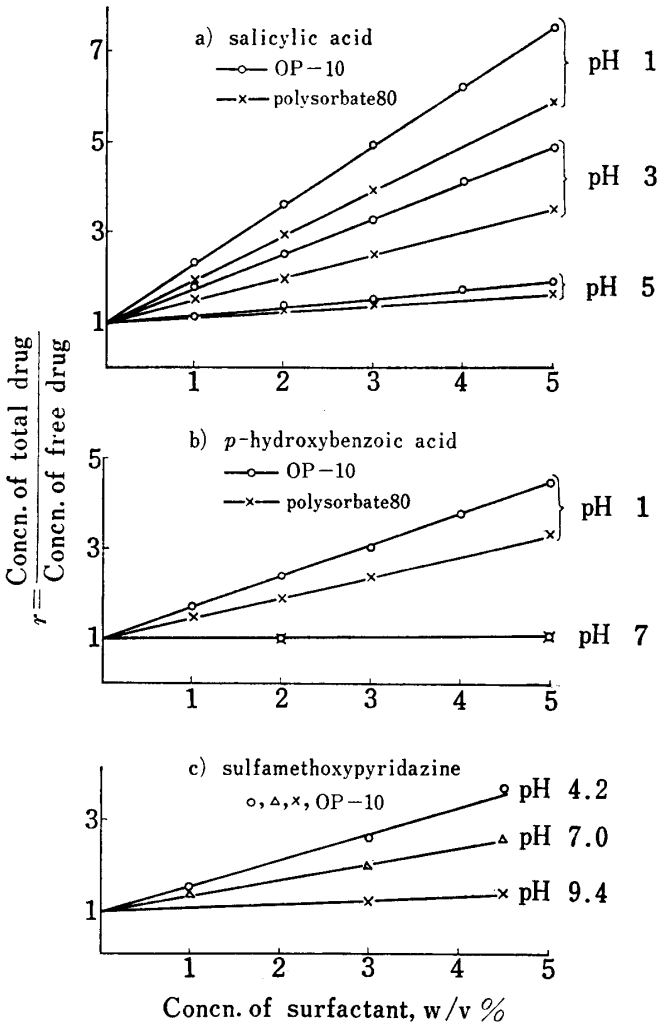

Fig. 2. Interaction of Drugs with Nonionic Surfactants

Thus, the effect of surfactants on the absorption of drugs should be considered as effects on absorption route of two species of drugs, *i.e.*, ionized and unionized species.