[Chem. Pharm. Bull. 22(8)1781—1787(1974)]

UDC 547.29.09 : 615.31.033.076.9

Effect of Short-Chain Fatty Acids on the Intestinal Absorption of Drugs in the Rat[1]

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(Received December 18, 1973)

The effect of propionic, butyric, and caproic acid, and of methyl butyrate on the absorption of various water-soluble drugs from the rat small intestine were investigated using the *in situ* perfusion technique. In preliminary experiment, butyric acid was absorbed rapidly by an active-like transport system. Fatty acids inhibited the absorption of anionic drugs (sulfisoxazole, salicylic acid, *etc.*), but enhanced the absorption of neutral drugs (caffeine, sulfanilamide, *etc.*) and cationic drugs (metoclopramide, quinine, *etc.*) at pH 6.5. In contrast, methyl butyrate enhanced the absorption of drugs in any case. The enhancement of apparent water absorption was observed in the presence of fatty acids and methyl butyrate, and the pH of perfusate containing fatty acids rose slightly after experiments. Butyric acid enhanced the exsorption rate of sulfisoxazole, but inhibited that of metoclopramide. Possible mechanisms of these effects are discussed.

The digestion and absorption of fats and fatty acids have been studied extensively from physicochemical, physiological, and biochemical standpoints, and the pathophysiological information of fat absorption has been presented with the investigation of malabsorption syndromes in various clinical situations.[3]

On the other hand, little specific and detailed information is available on the effect of fatty acids on the intestinal absorption of drugs. Levine[4] has reported that some water-soluble saturated fatty acids increased the absorption of benzomethamine and phenol red from the rat intestine. Levy and Anello[5] have shown that the fatty acid had an enhancing effect on the absorption of secobarbital in goldfish.

The present investigation was carried out to get further insight into the biopharmaceutical role of fatty acids, one of the essential components for biological systems, on the transfer of drugs through the biological membranes. The effect of short-chain fatty acids on the absorption of various water-soluble drugs from the perfused rat small intestine was systematically studied. The drugs were selected on the basis of the difference of ionic nature at physiological pH, and as fatty acids, butyric acid was mainly used and its effect was compared with the behavior of propionic acid, caproic acid, and methyl butyrate.

Experimental

Materials——Metoclopramide, imipramine hydrochloride, and 2-allyloxy-4-chloro-N-(2-diethylaminoethyl)benzamide hydrochloride (A.C.D.B.) were kindly supplied by Fujisawa Pharmaceutical Industry Ltd., Osaka. All other drugs used in these experiments were of analytical grade and were obtained commercially. The column substrate for gas–liquid chromatography was prepared by Nishio Industry Co., Ltd., Tokyo.

1) Presented to the 92nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April 1972.
2) Location: *Yoshidashimoadachi-cho, Sakyo-ku, Kyoto.*
3) J.M. Johnston, "Handbook of Physiology," Section 6, Alimentary Canal, Vol. III ed. by C.F. Code, Am. Physiol. Soc., Washington, D.C., 1968, p. 1353; N.J. Greenberger and T.G. Skillman, *New Eng. J. Med.*, **280**, 1045 (1969); K.Y. Lee, W.J. Simmonds, and N.E. Hoffman, *Biochim. Biophys. Acta*, **249**, 548 (1971); F.A. Wilson and J.M. Dietschy, *Arch. Intern. Med.*, **130**, 584 (1972); R.K. Ockner, J.P. Pittman, and J.L. Yager, *Gastroenterol.*, **62**, 981 (1972); *etc.*
4) R.R. Levine, *J. Pharmacol. Exp. Ther.*, **131**, 328 (1961).
5) G. Levy and J.A. Anello, *J. Pharm. Sci.*, **58**, 494 (1969).

Preparation of Drug Solutions——The composition of isotonic buffer solution used as the perfusion medium for the absorption studies was NaH_2PO_4–Na_2HPO_4 and the experiments were usually carried out at pH 6.5. Fatty acids were dissolved in phosphate buffer and the pH was adjusted to 6.5 with equivalent NaOH following the addition of calculated volume of distilled water for isotonic solution. The tonicity of each perfusate was measured by Beckmann freezing-point apparatus, but they hardly varied before and after perfusion.

Analytical Methods——Sulfonamides,[6] sulfanilic acid,[7] salicylic acid,[8] A.C.D.B.,[6] quinine hydrochloride,[7] imipramine hydrochloride,[7] metoclopramide,[9] pyridine-2-aldoxime methiodide (P.A.M.),[10] and caffeine[8] were estimated spectrophotometrically by the same method as described in the previous reports from this laboratory. Fatty acids used in this study did not interfere with the assay procedure of these drugs. Fatty acid concentrations were determined by gas-liquid chromatography using a Shimadzu GC-5A gas chromatograph with a flame-ionization detector. A glass column, 1.5 m long and 3.0 mm i.d., packed with 10% FFAP (Free fatty acid polyester) on acid-washed, silanized Chromosorb W (60—80 mesh) was used. The column temperature was 145°, and the injection port and detector temperatures were approximately 240°. The flow rates of nitrogen, hydrogen, and air were approximately 40, 40, and 700 ml/min, respectively. Into a 10 ml centrifuge tube were added 5 ml of diluted perfusate containing butyric acid and 1 ml of 25% metaphosphoric acid. After standing for 30 min, the contents were centrifuged at 3000 rpm for 10 min. Caproic acid, as an internal standard, was added to the supernatant, and 2 μl aliquots were injected into the chromatograph set at the fixed range and attenuation. A plot of the area of the butyric acid peak divided by the area of the internal standard peak as a function of the butyric acid concentration was linear and passed through the origin.

Procedure of Absorption Experiment——Male Wistar albino rats weighing 150—180 g were used in all experiments. The procedure of *in situ* absorption experiment from the rat small intestine was the same as reported in the paper from this laboratory.[6] The bile duct was ligated in all experiments. Forty milliliters of drug solution, kept in volumetric cylinder, was perfused at the rate of 5 ml/min. Simultaneously, the volume change of perfusate was measured periodically for the estimation of water absorption, described below. After the definite time, the perfusate was withdrawn as completely as possible, and washed with physiological saline. The washings were combined to the perfusate and made up to 100 ml with physiological saline. The amount absorbed was calculated by the difference in amount of drug between the initial and the final solutions.

Uptake of Drugs into the Intestinal Membrane——The uptake of drugs into the everted intestine was examined by the method described in the report from this laboratory,[11] except that the period of the incubation was one minute.

Procedure of Exsorption Experiment——The exsorption of drugs to the gut was studied by the method described in the report from this laboratory.[12] Sulfisoxazole, 30 mg/0.5 ml in 50% N,N′-dimethylacetamide solution, and metoclopramide, 8 mg/0.5 ml in 50% N,N′-dimethylacetamide solution, were administered intravenously to the rat from the femoral vein, and the small intestine was perfused with pH 6.5 buffer at the rate of 5 ml/min. The perfusion solution was collected every five minutes, and the exsorption rates of the drugs were calculated from the amount of drug in the solution. In some experiments, to investigate the effect of butyric acid and its reversibility, the small intestine was perfused first with pH 6.5 buffer for one hour, and with 50 mM butyric acid for the next one hour, followed by perfusion with the pH 6.5 buffer again.

Relationship between Water Transfer and Drug Absorption——The relationship between water transfer and drug absorption was examined by the method of Kitazawa, and Ito.[13] Apparent water transfer was calculated from the periodical volume change of perfusate in the reservoir, and water transfer ratio was estimated by the ratio of final to initial volume. Water transfer ratio was plotted against the absorption of drugs, and the regression line was determined by its relationship without fatty acids in isotonic and hypotonic buffer solutions at pH 6.5.

Microscopic Observations——After the perfusion, the small intestine was removed, fixed in formalin solution, serially sectioned under freezing, and stained with haematoxylin and eosin.

6) K. Kakemi, H. Sezaki, R. Konishi, T. Kimura, and M. Murakami, *Chem. Pharm. Bull.* (Tokyo), **18**, 275 (1970).
7) T. Kimura, H. Sezaki, and K. Kakemi (the late), *Chem. Pharm. Bull.* (Tokyo), **20**, 1656 (1972).
8) K. Kakemi, T. Arita, R. Hori, R. Konishi, K. Nishimura, H. Matsui, and T. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **17**, 255 (1969).
9) K. Okumura, H. Sezaki, and K. Kakemi (the late), *Chem. Pharm. Bull.* (Tokyo), **20**, 1607 (1972).
10) E. Suzuki, M. Tsukigi, S. Muranishi, H. Sezaki, and K. Kakemi, *J. Pharm. Pharmacol.*, **24**, 138 (1972).
11) T. Kimura, K. Inui, and H. Sezaki, *Yakuzaigaku*, **31**, 167 (1971).
12) K. Kakemi, H. Sezaki, R. Konishi, T. Kimura, and A. Okita, *Chem. Pharm. Bull.* (Tokyo), **18**, 1034 (1970).
13) S. Kitazawa, and H. Ito, "Absorption, Metabolism, and Excretion of Drugs," ed. by K. Kakemi, Hirokawa Publishing Company, Tokyo, 1971, p. 30.

Results and Discussion

Absorption of Short-Chain Fatty Acids

The absorption of short-chain fatty acids by rat small intestine was examined using *in situ* perfusion method. Barry, *et al.*[14] investigated the absorption of short-chain fatty acids from the intestine of the rat and presented evidence to suggest that this was accelerated by an active transport system. In this experiment, the absorptive process of butyric acid was slightly saturable at higher concentration. The time course of 50 mM butyric acid (initial concentration) is illustrated in Fig. 1. It follows apparent first-order kinetics with absorption half-life about 25 min. This is considerably more rapid than the absorption of drugs used in these experiments. To obtain a distinguished effect of short-chain fatty acids, therefore, it seemed more desirable to measure the initial rate of absorption and 30 minutes was mainly selected for the length of absorption study.

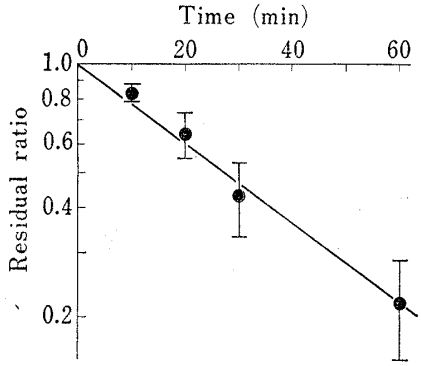

Fig. 1. Time Course of the Intestinal Absorption of Butyric Acid

concentration of butyric acid=50 mM, pH 6.5 Results are expressed as the mean ±S.D. in at least 4 animals.

Effect of Short-Chain Fatty Acids on Drug Absorption

The apparent effect of 50 mM butyric acid (initial concentration) on the absorption of various drugs from the rat small intestine at pH 6.5 is summarized in Table I. In general, butyric acid inhibited the absorption of anionic drugs (sulfisoxazole, salicylic acid, *etc.*) at pH 6.5. In contrast, it enhanced the absorption of neutral drugs (caffeine, sulfanilamide, *etc.*), and that of cationic drugs (metoclopramide, quinine, *etc.*) at pH 6.5. Light microscopy failed to reveal obvious loss of structural integrity of the epithelium with or without butyric acid.

Table I. Effect of Butyric Acid on the Absorption of Drugs from Rat Small Intestine at pH 6.5

Drug	Concn. (mM)	% absorbed in 30 minutes	
		Control	50 mM Butyric acid
Sulfanilic acid	0.1	2.9±1.3	1.1±1.0
Sulfisoxazole	0.1	30.7±2.5	20.6±4.3
Sulfadimethoxine	0.1	58.5±3.6	51.7±7.6
Salicylic acid	2.0	66.3±4.9	58.3±2.1
Sulfaguanidine	0.1	3.6±0.9	2.2±0.4
Sulfanilamide	0.1	28.6±2.7	35.5±3.9
Caffeine	1.0	46.9±5.6	55.4±7.4
Metoclopramide	0.1	12.2±2.8	26.0±3.2
P.A.M.	1.0	13.8±1.6	15.1±1.1
Quinine	1.0	25.8±1.9	46.0±3.4
A.C.D.B.	1.0	28.8±2.0	44.4±0.6
Imipramine	1.0	56.6±3.7	67.3±6.4

Results are expressed as the mean±S.D. in at least 4 animals.

To clarify the mechanism of these varying effects of butyric acid, sulfisoxazole, caffeine, and metoclopramide were chosen as the model drugs due to the difference of their ionic nature at physiological pH. Fig. 2 illustrates the effect of butyric acid concentration on the intestinal

14) R.J.C. Barry, M.J. Jackson, and D.H. Smyth, *J. Physiol.*, **182**, 150 (1966).

absorption of the three drugs. As is evident from the figure, these effects increase with increasing concentration of butyric acid.

There are three possible factors by which fatty acids can affect drug absorption from the rat small intestine; first, physicochemical effect such as formation of a drug-fatty acid complex in the gut lumen; second, the direct action of the fatty acid on the permeability characteristics of the intestinal membrane; third, some physiological effects in the small intestine.

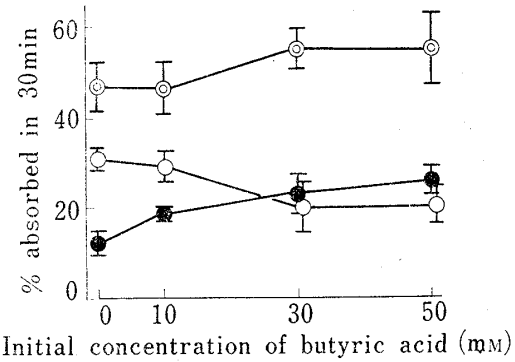

Fig. 2. Effect of Buryric Acid Concentration on the Intestinal Absorption of Drugs

Drug concentrations are the same as in Table I. pH 6.5
—○—: sulfisoxazole
—●—: metoclopramide
—◎—: caffeine
Results are expressed as the mean ±S.D. in at least 4 animals.

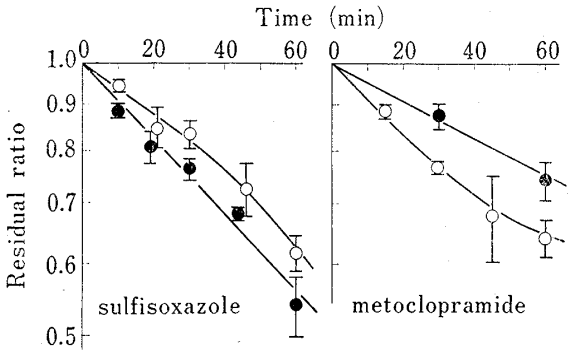

Fig. 3. Time Course of the Intestinal Absorption of Drugs with or without Butyric Acid

Drug concentrations are the same as in Table I. pH 6.5
—●—: control
—○—: with 50 mM butyric acid
Results are expressed as the mean ±S.D. in at least 4 animals.

At first, to elucidate drug-fatty acid interaction in the intraluminal phase, the apparent partition coefficients of the three drugs between chloroform and pH 6.5 phosphate buffer were investigated, but no significant effects by the addition of various fatty acids were observed. In another experiment, butyric acid did not influence on the initial uptake of drugs into the intestinal everted sacs *in vitro*. From these results, there is little possibility that the absorption-enhancing effect of fatty acids is due to the increased affinity to the biological membrane by cationic drug-fatty acid interaction such as ion-pair complex formation and the absorption-inhibiting effect is due to the displacement of anionic drugs with fatty acids on the mucosal surface.

The time course of the intestinal absorption of sulfisoxazole and metoclopramide with or without butyric acid is shown in Fig. 3. The disappearance of these drugs from the intestinal lumen in the presence of butyric acid did not follow apparent first-order kinetics. Absorption-enhancing effect and absorption-inhibiting effect by butyric acid were decreased gradually and their curves approached to their respective control values.

In addition, pretreatment of the intestinal lumen with 50 mM butyric acid hardly affected the absorption of drugs. Considering rapid absorption of butyric acid shown in Fig. 1, it is presumed that a certain existence of butyric acid on the mucosal surface is necessary for these effects of butyric acid.

Physiological effect of butyric acid is further examined, and the effect of its concentration on the intestinal absorption of water for 30 min is shown in Fig. 4. In both cases, butyric acid alone and with drugs, apparent water absorption was increased at high concentration of butyric acid. Methyl butyrate increased apparent water absorption similarly. Pretreatment of the gut with 50 mM butyric acid for 30 min hardly affected water absorption at all. Therefore, relationship between water transfer and drug absorption with and without fatty acids was investigated.

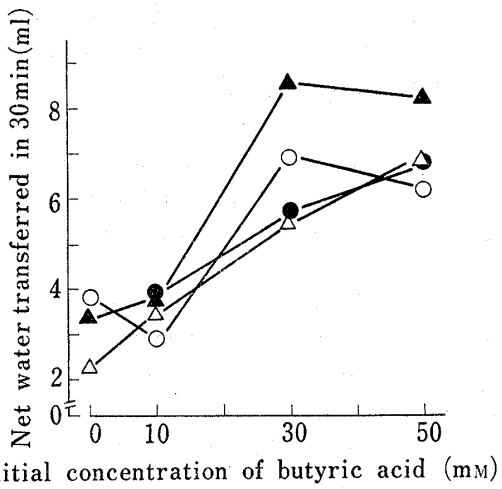

Fig. 4. Effect of Butyric Acid Concentration on the Intestinal Absorption of Water

Drug concentrations are the same as in Table I. pH 6.5
—●—: butyric acid alone
—○—: with sulfisoxazole
—△—: with caffeine
—▲—: with metoclopramide
Each point represents the mean in at least 4 animals.

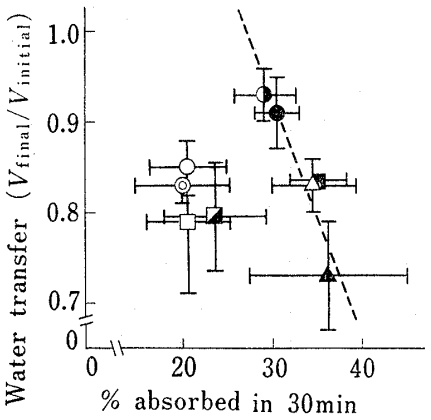

Fig. 5. Relationship between Water Transfer and Sulfisoxazole Absorption with or without Short-Chain Fatty Acids

concentration of sulfisoxazole=0.1 mM, pH 6.5
●: control
○: 50 mM butyric acid
△: 2/3 isotonic
■: 50 mM methyl butyrate
▲: 1/2 isotonic
□: 50 mM propionic acid
◑: 10 mM butyric acid
◪: 50 mM caproic acid
◎: 30 mM butyric acid
Results are expressed as the mean ±S.D. in at least 4 animals.

Fig. 5 shows the relationship between water transfer and the absorption of sulfisoxazole, and the regression line is determined by its relationship without fatty acids in isotonic and hypotonic buffer solutions at pH 6.5. Kitazawa and Ito[13] have demonstrated the linear relationship between water transfer and drug absorption within a limited range. As shown in Fig. 5, propionic, butyric, and caproic acid enhanced the absorption of water, but the absorption of sulfisoxazole was inhibited against expectation from its regression line, that is "water-transfer effect" by Kitazawa, *et al.* In case of methyl butyrate, however, the abso rption of sulfisoxazole was enhanced and happened to fall on the regression line.

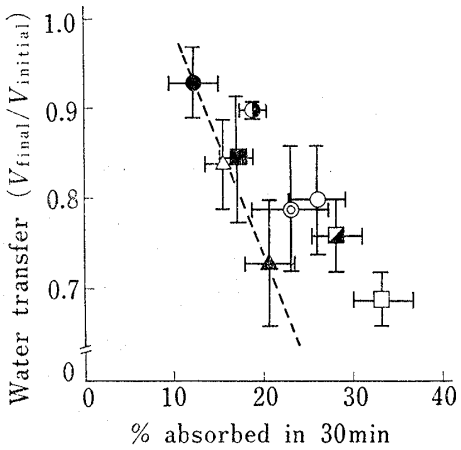

Fig. 6. Relationship between Water Transfer and Metoclopramide Absorption with or without Short-Chain Fatty Acids

concentration of metoclopramide=0.1 mM, pH 6.5
Symbols are the same as in Fig. 5.
Results are expressed as the mean ±S.D. in at least 4 animals.

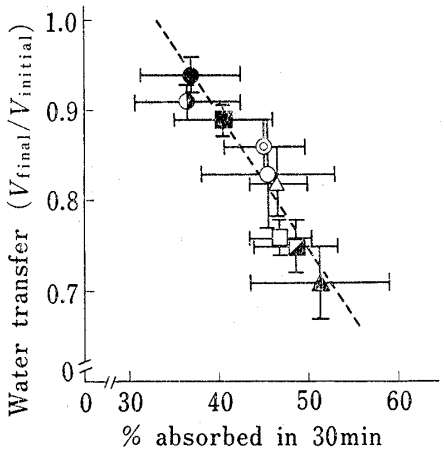

Fig. 7. Relationship between Water Transfer and Caffeine Absorption with or without Short-Chain Fatty Acids

concentration of caffeine=1.0 mM, pH 6.5
Symbols are the same as in Fig. 5.
Results are expressed as the mean ±S.D. in at least 4 animals.

In contrast, as shown in Fig. 6, the addition of propionic, butyric, and caproic acid to the perfusate more enhanced the absorption of metoclopramide than expectation from its regression line obtained by the same way. The point by methyl butyrate was on the regression line as the case of sulfisoxazole.

Fig. 7 shows about caffeine, unionized form at pH 6.5. The linear relationship between water transfer and absorption of caffeine was obtained with various fatty acids and methyl butyrate, and this effect seemed to be caused by the increasing absorption of water with fatty acids.

From these observations of the effect of fatty acids on the absorption of drugs, they seemed to possess the enhancing and inhibiting effects depending on the ionic nature of drugs besides the absorption-enhancing effect due to increasing water absorption. It is well known that methyl butyrate exists on the mucosal surface as ester form and is hydrolyzed to butyric acid within the epithelial cells. Csáky, *et al.*[15] have demonstrated that ethyl acetate stimulates the water transport in the jejunum of rat *in vitro*. It is conceivable, therefore, that the former effect of fatty acids is caused by existence of fatty acids on the mucosal surface.

To examine these effects of fatty acids in detail, the exsorption experiment was performed. The results are shown in Fig. 8. When the perfusion solution was changed from buffer solution to 50 mM butyric acid, the exsorption rate of sulfisoxazole, whose absorption was inhibited by fatty acids, was increased. On the other hand, exsorption rate of metoclopramide, whose absorption was increased by fatty acids, was decreased in the same conditions. It is worthy to note that such effect of the fatty acids was reversible. Because of opposite effects in absorption and exsorption experiments, it is considered that the effects of fatty acids were not caused by alteration in the permeability characteristics of the absorptive membrane. In another exsorption experiment, similar effects were observed by using pH 7.5 buffer solution in place of 50 mM butyric acid solution.

Ash and Dobson[16] have reported that the uptake of fatty acid from the rumen of sheep is accompanied by a consumption of carbon dioxide and production of bicarbonate within the rumen solution, due to the penetration of unionized fatty acid, and consequently the pH in the rumen is increased. In this study, the bulk pH of perfusate never varied by more than 0.2 pH unit before and after each experiment because of using mainly phosphate buffer. In contrast, the bulk pH of perfusate, which consists of unbuffered isotonic mannitol solution containing 50 mM butyric acid at pH 6.5, increased about 0.7 pH unit.

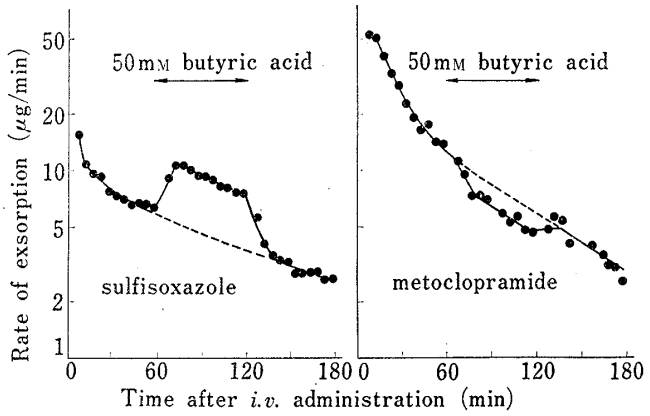

Fig. 8. Effect of Butyric Acid on the Exsorption of Drugs to Rat Small Intestine at pH 6.5

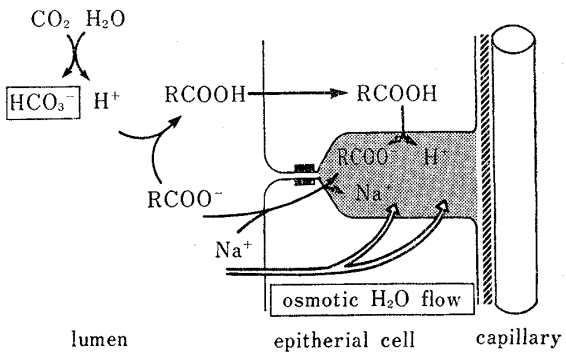

Fig. 9. Schematic Representation of Physiological Action of Short-Chain Fatty Acids in the Intestine

15) T.Z. Csáky, G. Esposito, A. Faelli, and V. Capraro, *Proc. Soc. Exptl. Biol. Med.*, **136**, 242 (1971).
16) R.W. Ash and A. Dobson, *J. Physiol.*, **169**, 39 (1963); A. Dobson and A.T. Phillipson, "Handbook of Physiology" Section 6, Alimentary Canal, Vol. V ed. by C.F. Code, Am. Physiol. Soc., Washington, D.C., 1968, p. 2761.

From the results described above, it appears that the effect of butyric acid was probably due not to the intraluminal interaction nor to the direct actions such as the alteration in the permeability of membrane but to some physiological actions in the small intestine.

Fig. 9 illustrates the most probable physiological effects of short-chain fatty acids that are infered from the results of Ash and Dobson[16] and this study. Fatty acids in the lumen were absorbed as ionized and unionized form through the two pathways respectively, following the increase of water absorption and pH of mucosal surface. That is, fatty acid on the mucosa was absorbed as unionized form by passive transport, and a supply of hydrogen ions, which was necessary to convert the ionized form of fatty acid to unionized one because less than 2% would be unionized at pH 6.5, was maintained by the conversion of carbonic acid to bicarbonate according to the scheme in Fig. 9, and the pH of neighborhood on the mucosa rose due to bicarbonate produced. Therefore, the absorption of cationic drugs was enhanced and that of anionic ones was inhibited.

On the other hand, a part of ionized form of fatty acid was absorbed by active-like transport coupling with the absorption of sodium, and net water absorption occurred as a consequence of net solute transport, and water absorption due to such local osmosis increased drug absorption. This also seemed to be supported by the facts that in sodium-free mannitol solution, fatty acid absorption was decreased and apparent water absorption hardly occurred.

Thus, it is suggested that the absorption of drugs in the presence of short-chain fatty acids may be mainly affected by the physiological factors such as the absorption of water and the alteration of microclimate pH on the absorptive membrane.