[Chem. Pharm. Bull. 26(3) 915—924 (1978)]

UDC 615.31.011.4.033.076.9 : 612.3-06

Generality in Effects of Transmucosal Fluid Movement and Glucose on Drug Absorption from the Rat Small Intestine

Shikifumi Kitazawa,[1a] Hajime Ito (the late), Ikuo Johno, Terutaka Takahashi, and Hideo Takenaka[1b]

Department of Pharmacy, Kyoto University Hospital,[1a] and Gifu College of Pharmacy[1b]

(Received August 22, 1977)

The generality in effects of transmucosal fluid movement and glucose on drug absorption from the rat small intestine was investigated using twenty three drugs having different charges in the physiological pH of the intestine of the animal with the *in situ* recirculating perfusion method. All of the regression lines representing the relation between ratio of the transmucosal fluid movement on the vertical axis and intestinal absorption of respective drugs on the horizontal axis were not perpendicular to the horizontal axis but have some inclinations without exception. These evidences did support the concept that intestinal drug absorption was subtly affected by the transmucosal fluid movement and thus the generality in effect of the movement was apparently demonstrated.

Concerning the glucose effect, two regression lines, one was of sodium chloride and another was of glucose in the perfusate, were obtained in all of the drugs. However, these two regression lines were overlapped in the cases of unionized drugs. On the other hand, in the cases of cationic drugs the regression lines of glucose were always shifted significantly to the right hand side of those of sodium chloride and in the cases of anionic drugs the regression lines of glucose were shifted to the left hand side without any exception. These evidences demonstrated that glucose increased the absorption of cationic drugs and decreased that of anionic drugs and, moreover, these findings were supported by investigating blood level of respective drugs in the subjected animal. Thus the generality in the glucose effect was apparently demonstrated.

Moreover, the glucose effect was also recognized when two drugs were coexisted simultaneously in the perfusate. The glucose effect in drug absorption might be one of mechanisms of drug interactions which have been observed in clinical medicine.

Keywords——glucose; glucose effect; intestinal drug absorption; partition coefficient; ratio of fluid movement; recirculating perfusion method; transmucosal fluid movement

In the course of studies investigating effects of the transmucosal fluid movement on drug absorption from the rat small intestine using an *in situ* recirculating perfusion technique,[2] peculiar fluctuations in absorption of ionized drugs were observed when sodium chloride in the perfusate was replaced to D-glucose (glucose). Glucose apparently increased absorption of metoclopramide, a cation in the experimental condition, and decreased absorption of sulfisoxazole, an anion, and did not influence absorption of sulfanilamide, an unionized compound.[3] These peculiar effects caused by the presence of glucose in the perfusate on the drug absorption were difficult to be understood even if the concept of the transmucosal fluid movement was taken into considerations in analyzing the absorption results, and these findings could not be explained not only by the transmucosal fluid movement but also by any findings and hypotheses[4] which have been established in the studies of drug absorption. These peculiar effects of glucose was termed arbitrarily the glucose effect for the sake of convenience in the previous study.[3]

1) Location: *a*) *Kawara-cho, Shogoin, Sakyo-ku, Kyoto 606, Japan*; *b*) *Mitahora, Gifu 502, Japan.*
2) L.S. Schanker, D.J. Tocco, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **123**, 81 (1958).
3) S. Kitazawa, H. Ito, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 1856 (1975).
4) T.H. Wilson, "Intestinal Absorption," W.B. Saunders Co., Philadelphia, 1962, pp. 40—68; T.B. Binns, "Absorption and Distribution of Drugs," E. & S. Livingstone Ltd., London, 1964, pp. 1—15.

Along with development in the study concerning the effect of the transmucosal fluid movement on drug absorption, some evidences relating to the glucose effect were also accumulated gradually in our laboratories.[5] Based on the findings, it was elucidated that the glucose effect was also observed in *in vivo* experiment,[5a] and that the segment of the alimentary tract of the animal in which the glucose effect was dominantly observed was the upper part of the small intestine.[5b] These lines of evidences might be clues in elucidating mechanism of the glucose effect and encouraged authors to accumulate more efforts in the elucidation of mechanism. However, before driving our studies further in advance, it should be necessary to confirm that the glucose effect might also be observed in other drugs than those which have been used in the previous studies.[3,5]

In the present study, many drugs including anions, cations, and unionized compounds in the experimental conditions were selected and generality in the effects of transmucosal fluid movement and glucose was investigated.

After certifying that the generality was observed in all cases of drugs employed in the present study. Further attempts were undertaken to demonstrate whether the glucose effect was observed when two drugs were coexisted in the perfusate. As the results of these trials, the glucose effect was observed consistently even in such complicated systems in the perfusate.

Experimental

Drugs and Their Analytical Procedures——Drugs that the intestinal absorption was demonstrated to obey the first-order kinetics in the preliminary experiment were selected. They were aminopyrine,[6] barbital,[7] caffeine,[8] chloramphenicol,[9] isoniazid,[10] paramidine,[11] phenylbutazone,[12] pyrazinamide,[13] sulfanilamide,[3] theophylline,[8] thiopental,[14] triamterene,[15] chlorpheniramine maleate,[16] diphenhydramine,[17] ephedrine·HCl,[18] metoclopramide,[3] quinine·HCl,[19] salicylic acid,[20] *p*-aminobenzoic acid,[3] *p*-aminosalicylic acid,[20] sulfamethizole,[3] sulfamethoxazole,[3] and sulfisoxazole.[3] They were purchased from commercial sources in reagent grade and were used in the experiment without further purifications. Analytical procedures for these drugs were followed to the method presented in each reference as shown above. These analytical procedures were found to be utilized even though glucose presents in the perusate.

Perfusion Procedures——Male albino rats of Wistar strain weighing about 150 g were purchased. The *in situ* recirculating perfusion experiment was followed just as the same manner as described in full in the previous report.[3] All the recirculating perfusions conducted in the present study used the entire small intestine of the animal from a proximal end of the duodenum to a distal end of the ileum.

Thirty milliliters of perfusion solution were recirculated in order of the proximal end to the distal end of the small intestine at a rate of 5 ml per minute. Ten minutes after the beginning of the perfusion, an initial sample of the perfusate was pipetted out from the reservoir which was located in the closed circuit of the recirculating system. Almost of the recirculating perfusions were for one hour, however, in cases of drugs which have been found well absorbed in the preliminary experiments, the periods of the perfusion

5) *a*) S. Kitazawa, H. Ito, and M. Iinuma, *Chem. Pharm. Bull.* (Tokyo), **23**, 2128 (1975); *b*) S. Kitazawa, H. Ito, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **25**, 19 (1977).
6) S. Ono, R. Onishi, M. Tange, K. Kawamura, and T. Imai, *Yakugaku Zasshi*, **85**, 245 (1965).
7) L.R. Goldbaum, *Anal. Chem.*, **24**, 1604 (1952).
8) I. Sugimoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 1098 (1962).
9) K. Kakemi and S. Ohashi, *Yakugaku Zasshi*, **82**, 338 (1962).
10) K. Kakemi, T. Arita, H. Sezaki, M. Nakano, and T. Kiriyama, *Yakugaku Zasshi*, **82**, 195 (1962).
11) H. Mima, T. Matsuzawa, K. Terada, and T. Kondo, *Ann. Rep. Takeda Res. Lab.*, **24**, 9 (1965).
12) J.J. Burns, R.K. Rose, T. Chenkin, A. Goldman, A. Schulert, and B.B. Brodie, *J. Pharmacol. Exptl. Therap.*, **109**, 346 (1953).
13) K. Kakemi, T. Uno, and H. Sezaki, *Yakugaku Zasshi*, **76**, 273 (1956); *idem*, *ibid.*, **76**, 906 (1956).
14) B.B. Brodie, L.C. Mark, E.M. Papper, P.A. Lief, E. Bernstein, and E.A. Rovensteine, *J. Pharmacol. Exptl. Therap.*, **98**, 85 (1950).
15) K. Lehmann, *Arzneim.-Forsch.*, **15**, 812 (1965).
16) K. Sato and K. Tonooka, *Yakugaku Zasshi*, **87**, 231 (1967).
17) H. Koike, K. Ohashi, M. Matsuo, and A. Kawasaki, *Bunseki Kagaku*, **17**, 603 (1968).
18) M.S. Kawawya, S.K. Wahba, and A.R. Kozman, *J. Pharm. Pharmacol.*, **19**, 332 (1967).
19) L.S. Schanker, *J. Pharmacol. Exptl. Therap.*, **126**, 283 (1954).
20) G.J. Cosmides, F.W. Stemler, and T.S. Miya, *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 16 (1956).

were shortened to 15 or 30 minutes depedning on the extent of absorption of the subjected drug. After ceasing the perfusion, a final sample was pipetted.

Blood samples were collected during and after the perfusion experiment, if necessary, into a small heparinized beaker by cutting off the end of the tail of the animal[21] at a given interval. These samples were stored in a refrigerator until analysis. Analytical procedures were followed as the same manner reported previously.[3]

Perfusion Solution——Perfusion solution used in the recirculating perfusion experiment consisted of either sodium chloride or glucose in concentration to make at least three levels in tonicity of hypertonic, isotonic, and hypotonic. Physiological isotonic concentration of sodium chloride and glucose are 0.9% and 5.0%, respectively. Perfusion solutions having concentrations more than these isotonic concentrations of the solute were regarded as hypertonic perfusion solution, and solutions having less than the isotonic concentration were nominated as hypotonic solution in the present report. All the perfusion solutions contained phenol red in an appropriate concentration as a nonabsorbable indicator.

Perfusion solutions contained 1 mM of the drug as a rule, however, the concentration of the drug having high sensitivity in the determination was decreased to an appropriate concentration so that both of the initial and the final concentrations of the drug in the perfusate were able to determine without any dilution.

Determination of the Drug Absorption and Evaluation of the Glucose Effect——According to the recirculating perfusion method which was devised by Schanker and his co-workers,[2] amount of the drug disappeared in the perfusate was regarded as the amount absorbed. Taking into considerations the transmucosal fluid movement which was determined by measuring the concentration change of the nonabsorbable indicator during the perfusion, drug absorption from the small intestine of the animal was determined following an equation:

$$\text{drug absorption } (\%) = 100 - 100\left(\frac{C_{\text{drug final}}}{C_{\text{drug initial}}} \times \frac{C_{\text{indicator initial}}}{C_{\text{indicator final}}}\right)$$

where C is the concentration of the drug or phenol red in the perfusate and $C_{\text{indicator initial}}/C_{\text{indicator final}}$ is estimated as the transmucosal fluid movement or ratio of fluid movement in the present study.

Individual results of absorption were obtained following the method mentioned above, however, the results were fluctuated depending on the ratio of the transmucosal fluid movement. To obtain the most proper result in absorption which might be indifferent to the fluid movement, the protocol in determining the drug absorption which was presented in the previous report was applied.[22]

The absorption of the drug from the perfusates having various tonicities with sodium chloride or glucose were obtained and all the results were plotted in an illustration which had the ratio of fluid movement on the vertical axis and the absorption in percent on the horizontal axis. After confirming that a straight regression line of the scattered plots might be obtained, an extent of the absorption at an intercept of the regression line and a horizontal line at 1.0 in the ratio of the fluid movement was regarded as the drug absorption which was indifferent to the fluid movement.

These procedures were conducted both of the perfusates having sodium chloride and glucose and the two regression lines were compared statistically in all cases. When the significant difference was observed, it was concluded that the effect of glucose on the absorption of the subjected drug was found.

Determination of Partition Coefficient——Drugs were dissolved in an isotonic buffer solution of pH 6.5 containing a half volume of an isotonic phosphate buffer of which the components were KH_2PO_4 and $Na_2HPO_4 \cdot 12H_2O$ and a half volume of an isotonic solution of sodium chloride or glucose. After adding the equivolume of chloroform or isoamyl acetate, partition ratio was determined following regular manner[23] at 37°. Three levels in concentration (0.1, 1.0, 10.0 mM) of respective drugs were conducted in determining partition ratio and after ensuring that the ratios were found to be approximate same values, the partition coefficient was calculated as an average of these ratios.

Results

Generality in Effects of Transmucosal Fluid Movement and Glucose

Twenty three drugs were classified into unionized drugs, cationic drugs, and anionic drugs with considerations of their pK_a values and the physiological pH[24] of the small intestine of the animal. All the data obtained, when the respective drugs were subjected in the perfusate, were listed in Table I and in three of these having different charges in the pH the relation

21) H.C. Grice, *Lab. Animal Care*, **14**, 483 (1964).
22) S. Kitazawa, M. Ishizu, and E. Arakawa, *Chem. Pharm. Bull.* (Tokyo), **24**, 3169 (1976).
23) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 413 (1964).
24) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 421 (1964).

Table I. Molecular Weight, pK_a Values, and Glucose Effect on Intestinal Absorption of Drugs

Drugs	M.W.	pK_a	Regression equation sodium chloride				Drug absorption (%)	Regression equation glucose				Drug absorption (%)	Drug concentration (mM)	Perfusion time (min)
			$n^{a)}$	$r^{b)}$	$A^{c)}$	$B^{c)}$		$n^{a)}$	$r^{b)}$	$A^{c)}$	$B^{c)}$			
Unionized drugs														
Aminopyrine	231.3	5.0[d]	10	−0.857	−0.0026	1.1226	47.2	12	−0.891	−0.0024	1.1205	50.2	1.0	15
Barbital	184.2	7.8[d]	10	−0.957	−0.0153	1.9031	59.0	9	−0.878	−0.0119	1.6614	55.6	0.5	60
Caffeine	194.2	0.8[e]	9	−0.988	−0.0081	1.3625	44.8	8	−0.949	−0.0064	1.2357	36.8	1.0	30
Chloramphenicol	323.1	Undissociated[f]	9	−0.952	−0.0108	1.5054	46.8	9	−0.954	−0.0119	1.5605	47.1	1.0	60
Isoniazid	137.2	1.50, 3.50[g]	10	−0.967	−0.0129	1.6572	50.9	12	−0.981	−0.0131	1.6336	48.4	1.0	60
Paramidine	266.3	4.4, 13.9[h]	9	−0.968	−0.0095	1.6357	66.9	8	−0.973	−0.0088	1.5404	61.4	1.0	30
Phenylbutazone	308.4	4.4[d]	10	−0.968	−0.0066	1.2269	34.4	8	−0.943	−0.0052	1.1369	26.3	1.0	15
Pyrazinamide	123.1	0.5[i]	13	−0.918	−0.0155	1.8171	52.7	10	−0.977	−0.0215	2.1402	53.0	1.0	60
Sulfanilamide	172.2	2.36, 10.43[j]	21	−0.959	−0.0125	1.6028	48.2	16	−0.955	−0.0124	1.5958	48.0	1.0	60
Theophylline	180.2	0.7[d]	9	−0.970	−0.0172	2.0668	62.0	8	−0.926	−0.0137	1.8246	60.2	1.0	60
Thiopental	242.3	7.6[d]	6	−0.955	−0.0069	1.3933	57.0	8	−0.882	−0.0054	1.3340	61.9	0.1	30
Triamterene	253.3	6.2[k]	11	−0.917	−0.0158	1.7154	45.3	8	−0.931	−0.0126	1.5147	40.8	1.0	60
Cationic drugs														
Chlorpheniramine maleate	390.9	10.35[l]	11	−0.959	−0.0126	1.5956	47.3	9	−0.963	−0.0104	1.6624	63.7	1.0	60
Diphenhydramine	255.4	8.98[m]	10	−0.914	−0.0098	1.3600	36.7	8	−0.995	−0.0100	1.5785	57.9	1.0	60
Ephedrine·HCl	201.7	9.6[e]	11	−0.915	−0.0140	1.2986	21.3	11	−0.935	−0.0154	1.8274	53.7	1.0	60
Metoclopramide	299.8	8.97[n]	13	−0.942	−0.0129	1.2886	22.4	13	−0.997	−0.0133	1.5430	40.8	1.0	60
Quinine·HCl	396.9	8.4[d]	12	−0.957	−0.0105	1.3990	38.0	14	−0.817	−0.0074	1.5195	70.2	1.0	60
Anionic drugs														
p-Aminobenzoic acid	137.1	4.65, 4.80[i]	13	−0.949	−0.0097	1.5600	57.7	14	−0.950	−0.0083	1.3071	37.0	1.0	30
p-Aminosalicylic acid	153.1	3.25[i]	12	−0.924	−0.0163	1.4842	29.6	6	−0.966	−0.0197	1.0707	3.6	1.0	60
Salicylic acid	138.1	3.0[d]	13	−0.953	−0.0266	2.9958	75.0	11	−0.956	−0.0213	2.4601	68.5	1.0	60
Sulfamethizole	270.3	2.20, 5.45[j]	11	−0.742	−0.0156	1.3651	23.4	11	−0.906	−0.0278	1.2216	8.0	1.0	60
Sulfamethoxazole	253.3	1.76, 5.8[j]	11	−0.928	−0.0145	2.0738	74.1	21	−0.911	−0.0127	1.5647	44.5	1.0	60
Sulfisoxazole	267.3	1.55, 5.10[j]	11	−0.978	−0.0155	1.7472	48.2	12	−0.912	−0.0180	1.4782	26.6	1.0	60

a) Number of experiments; *b*) Coefficient of correlation; *c*) The regression equation between intestinal absorption of drugs on the horizontal axis and ratio of fluid movement on the vertical axis under each experimental condition was obtained by the least squares method and represented as following equation: $y=Ax+B$. P values of the t test for coefficient of correlation was less than 0.01 in all cases; References of pK; *d*) P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **119**, 361 (1957); *e*) L.S. Shanker, P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **120**, 528 (1957); *f*) R. Okada, *Personal communication*; *g*) S. Inoue, *Yakugaku Zasshi*, **87**, 883 (1967); *h*) H. Mima, Y. Asahi, I. Terada, T. Matsuzaki, E. Mizuta, and H. Izumi, *Ann. Rep. Takeda Res. Lab.*, **24**, 1 (1965); *i*) Merck Index, 8th Ed.; *j*) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 413 (1964); *k*) Data by: Smith, Kline and French, Philadelphia, Pa. 19101; *l*) S. Kitazawa and H. Ito, *Personal communication*, *m*) W.A. Ritshel, "Applied Biopharmaceutics I," Hamilton, Illinois, 1969, pp. 169; *n*) S. Kitazawa, H. Ito, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 1856 (1975).

between ratio of fluid movement and intestinal absorption of drugs was depicted in Fig. 1—3. As presented in Table I and Fig. 1—3, all the regression lines representing the rela-

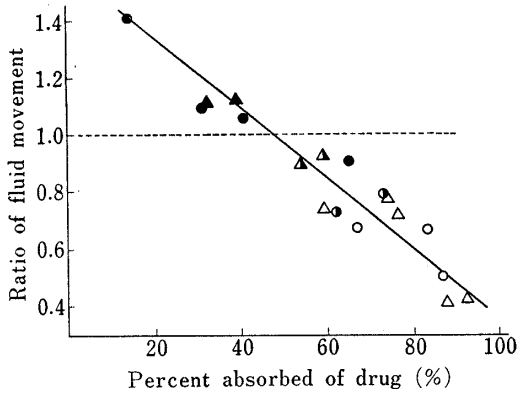

Fig. 1. Relationship between Percent Absorbed of Chloramphenicol and Fluid Movement

Key: Component and tonicity of the perfusate
▲: glucose hypertonic,
▲: glucose isotonic,
△: glucose hypotonic,
●: sodium chloride hypertonic,
◑: sodium chloride isotonic,
○: sodium chloride hypotonic.

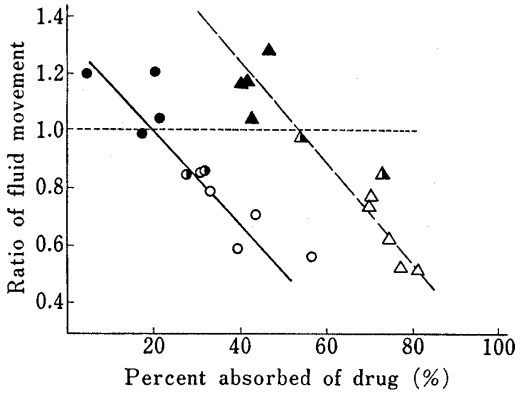

Fig. 2. Relationship between Percent Absorbed of Ephedrine and Fluid Movement

Key: component and tonicity of the perfusate
▲: glucose hypertonic,
▲: glucose isotonic,
△: glucose hypotonic,
●: sodium chloride hypertonic,
◑: sodium chloride isotonic,
○: sodium chloride hypotonic.

tion between ratio of fluid movement on the vertical axis and intestinal absorption of each drug on the horizontal axis obtained with the perfusion solution containing sodium chloride or glucose demonstrated fairly straight and p values of the t test for their correlation coefficients were found to be $p<0.01$. Moreover, evidences that all the regression lines including of sodium chloride and of glucose had certain inclination did indicate that the absorptions of these drugs were influenced by the transmucosal fluid movement.

Differences in the regression lines obtained with both the sodium chloride perfusate and the glucose perfusate in each drug were established statistically[25] and p values less than 0.05 were considered to be significant in this paper. In the cases of unionized drugs, although there were observed some fluctuations and exact coincidences were hardly obtained, based on the results obtained from both of the perfusates, it could not be found significant differences between the regression line obtained with the sodium chloride perfusate and that obtained with the glucose perfusate ($p0>0.05$). The evidence led to the conclusion that the effect of glucose was not observed in the absorption of these unionized drugs employed in the present study. In the cases of cationic drugs, the absorptions brought about in the presence of

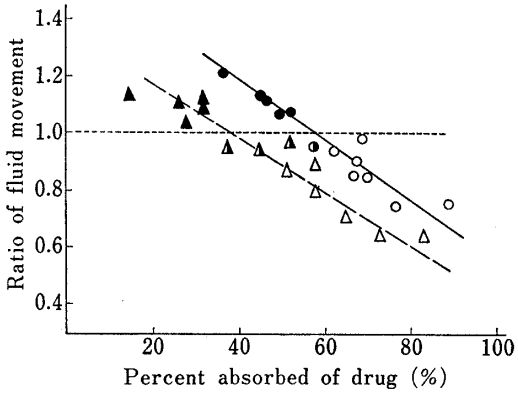

Fig. 3. Relationship between Percent Absorbed of p-Aminobenzoic Acid and Fluid Movement

Key: component and tonicity of the perfusate
▲: glucose hypertonic,
▲: glucose isotonic,
△: glucose hypotonic,
●: sodium chloride hypertonic,
◑: sodium chloride isotonic,
○: sodium chloride hypotonic.

25) "Documenta Geigy Scientific Tables," 7th ed., ed. by K. Diem and C. Lentner, Pub. by J.R. Geigy S.A., Basle, Switzerland, 1970, pp. 178—179.

glucose were exceeded than those of sodium chloride without exceptions, and the difference in these absorptions were more evident than those observed in the cases of unionized drugs. The significant differences between the regression line obtained with the sodium chloride perfusate and that obtained with the glucose perfusate were observed ($p<0.005$) in all cases. Based on these results, it might be possible to conclude that the effect of glucose was observed in all of the cationic drugs employed in the present study. Contrary to the cases of cationic drugs, the absorptions of anionic drugs in the presence of glucose were always inferior to those obtained in the presence of sodium chloride. The two regression lines obtained from the sodium chloride and glucose perfusates did not also coincide statistically ($p<0.02$). These evidences supported the conclusion that the effect of glucose was apparently demonstrated in these cases of anionic drugs used in the present study.

Time Course Study of the Glucose Effect

Phenomenal evidences of the glucose effect have been pursued. However, to promote better understanding the glucose effect, it might be necessary to investigate the mode of absorption under these phenomena. Along with these purposes, time course observations of the drug in both the perfusate and blood of the animal during the perfusion experiment were examined. All the drugs showed essentially the same pattern in absorption, so the results obtained by metoclopramide, as one of the example of cationic drugs, and by sulfisoxazole, as one of the cases of anionic drugs, were illustrated in Fig. 4 and Fig. 5.

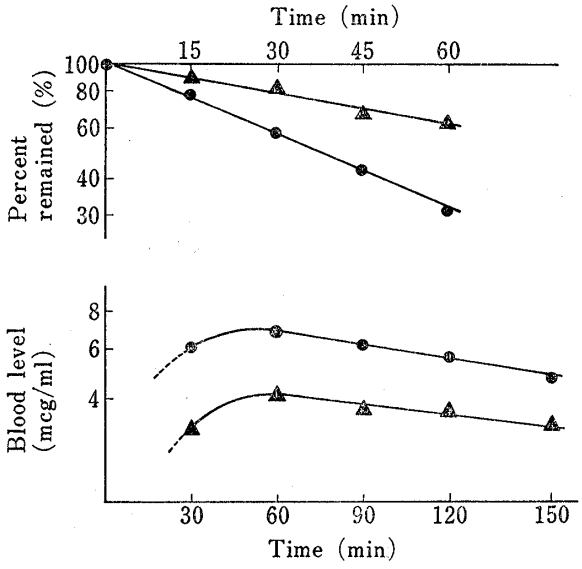

Fig. 4. Time Course Study of Blood Level and Percent Remained of Sulfisoxazole

Key: ▲: glucose in perfusate,
●: sodium chloride in perfusate.

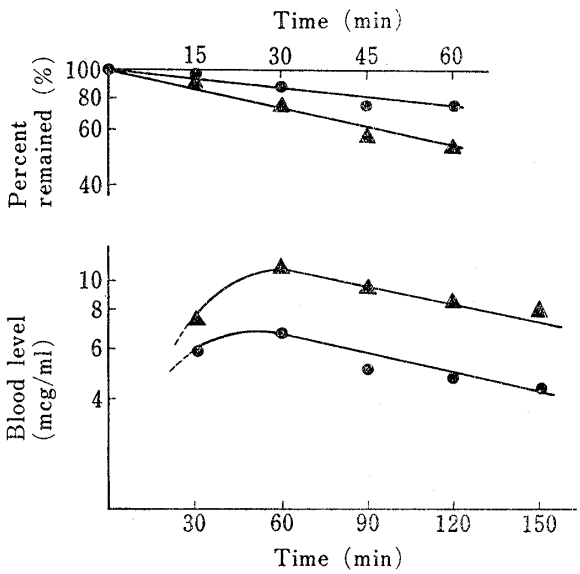

Fig. 5. Time Course Study of Blood Level and Percent Remained of Metoclopramide

Key: ▲: glucose in perfusate,
●: sodium chloride in perfusate.

As depicted in Fig. 4, both of the modes of absorption of metoclopramide from the isotonic perfusates of sodium chloride and glucose apparently obeyed the first-order kinetics, however, the rate of absorption from isotonic glucose solution was demonstrated faster than that from sodium chloride. Reflecting these results in the perfusate, the drug levels in blood of the animal obtained during and after application of glucose perfusate were apparently higher than those of sodium chloride perfusate. These results did indicate that the glucose effect was observed not only in the drug disappearance in the perfusate but also in the blood levels of the drug which might be important in determining bioavailability and, moreover, development pharmacological effect of respective drugs.

Quite reverse results were obtained in the case of sulfisoxazole. Although the modes of absorption of the drug in both the isotonic sodium chloride and the isotonic glucose perfusates indicated the first-order kinetics, which were similar to those of metoclopramide, the rate of absorption from sodium chloride perfusate was observed to be always exceeded to that of glucose. These results were reflected directly to the drug levels in blood of the aniaml.

Consistency in Appearance of the Glucose Effect in the Presence of Two Drugs in the Perfusate

Investigations so far proceeded were concerned to the glucose effect observed in single drug system, in other words, the perfusate contained only one drug. Possibilities were still remained that the glucose effect might be modified when two drugs or more than two drugs were in the perfusate. Attempts were undertaken to elucidate the consistency or the modification of the glucose effect in such a double drug system with drugs of cationic–anionic, anionic–anionic, and cationic–cationic in combination. After confirming that the analytical procedures of one drug were not interfered by the presence of another drug, combinations of following drugs were decided, ephedrine–*p*-aminosalicylic acid, sulfisoxazole–salicylic acid, and metoclopramide–chlorpheniramine maleate.

Before starting the experiment of recirculating perfusion, partition coefficients of these drugs were determined individually and in combinations between organic solvents of chloroform or isoamyl acetate and aqueous solutions containing either sodium chloride or glucose. The results were listed in Table II. As were apparent from Table II, partition coefficients

Table II. Partition Coefficients of Drugs between An Isotonic Aqueous Solution of pH 6.5 and Chloroform or Isoamyl Acetate at 37°

Drugs	Chloroform		Isoamyl acetate	
	Sodium chloride[a)]	Glucose[a)]	Sodium chloride[a)]	Glucose[a)]
Ephedrine	0.4	0.5	0.4	0.3
p-Aminosalicylic acid	0.1	0.1	0.1	0.1
Ephedrine[b)] +	0.3	0.6	0.3	0.2
p-Aminosalicylic acid[b)]	0.1	0.1	0.1	0.1
Sulfisoxazole	0.2	0.1	0.7	0.6
Salicylic acid	0.1	0.1	0.1	0.1
Sulfisoxazole[b)] +	0.2	0.1	0.7	0.6
Salicylic acid[b)]	0.1	0.1	0.1	0.1
Metoclopramide	1.9	2.0	0.2	0.3
Chlorpheniramine maleate	49.7	41.7	4.6	4.2
Metoclopramide[b)] +	2.6	2.2	0.2	0.2
Chlorpheniramine maleate[b)]	50.5	44.1	4.4	3.8

All values were raised at two places of decimals.

a) Sodium chloride or glucose was added to a half isotonic phosphate buffer solution of pH 6.5 of which the components were KH_2PO_4 and $Na_2HPO_4 \cdot 12H_2O$ and an isotonic buffer solution was prepared.

b) It suggests the respective partition coefficients when two drugs were coexisted in the aqueous phase.

of these drugs were not affected by the replacement of sodium chloride to glucose. These lines of results indicated decisively that glucose did not affect lipid solubilities of these drugs not only in the single drug system but also in the double drug system.

Moreover, partition coefficients of respective drugs in the double system consistently demonstrated the same values as those obtained in the single system, in other words, the partition coefficient of one drug did not change even when the other drug was introduced in the system. This evidence supported speculation that these drugs might not interact each other to form such as hydrophobic bounding or ion pair complex which might influence considerably the drug absorption.

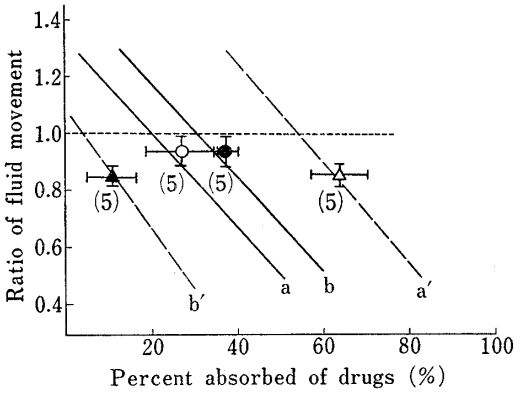

Fig. 6. Effect of Glucose on Absorption of Drugs Coexisted (*p*-Aminosalicylic Acid and Ephedrine)

Numbers in parentheses indicate number of experiments.
Key: ▲: *p*-aminosalicylic acid in glucose isotonic, ●: *p*-aminosalicylic acid in sodium chloride isotonic, △: ephedrine in glucose isotoniec, ○: ephedrine in sodium chloride isotonic.
a: regression line of ephedrine obtained by the perfusate containing sodium chloride calculated with the least squares method.
a′: regression line of ephedrine obtained by the perfusate containing glucose calculated with the least squares method.
b: regression line of *p*-aminosalicylic acid obtained by the perfusate containing sodium chloride calculated with the least squares method
b′: regression line of *p*-aminosalicylic acid obtained by the perfusate containing glucose calculated with the least squares method.
⊥: mean ±S.D.

After the results of preliminary experiment such as partition coefficient were presented, authors' attentions were turned to the recirculating perfusion experiment employing perfusion solutions of the double drug system.

Figure 6 shows the results obtained using the isotonic perfusate containing equimillimoles of *p*-aminosalicylic acid and ephedrine simultaneously. A straight line which is indicated as (a) represents the regression line of ephedrine in the sodium chloride perfusate which has been obtained from the experiment using the perfusate of the single drug system as depicted in Fig. 2, and broken regression line (a′) was obtained when sodium chloride in the perfusate was replaced to glucose. The same relationship in the regression lines of (b) and (b′) was obtained when *p*-aminosalicylic acid was subjected in the experiment. The results representing the relation between the absorptions of respective drugs and the transmucosal fluid movement obtained from the double drug system containing isotonic sodium chloride were plotted just on the respective regression lines of sodium chloride and when sodium chloride in the perfusate was replaced to glucose, the plots shifted to respective directions and appeared just on the respective regression lines of glucose. These results demonstrated that the glucose effect was consistently observed not only in the single drug system but also in the double drug system.

The modifications in absorption of drugs in the case of a combination of anionic–anionic drug were illustrated in Fig. 7. The absorptions of respective drugs of sulfisoxazole and salicylic acid from the isotonic perfusate containing sodium chloride were appeared approximately on the respective regression lines obtained when respective drugs were subjected for the absorption study in the single drug system containing sodium chloride. However, the absorptions of the respective drugs in the double drug system were shifted to the same direction of decreasing when sodium chloride was replaced to glucose, and appeared just on the respective regression lines of glucose obtained when these drugs were studied in the single drug system containing glucose.

The same inclination in nature was observed in the case of a combination of cationic–cationic drug. The results were illustrated in Fig. 8. The absorptions of metoclopramide and chlorpheniramine maleate in the glucose perfusate were shifted to the right hand side of the respective regression lines obtained in the single drug system containing sodium chloride, and increasings in absorption were demonstrated, however, these increasings were obeyed to the rule of the glucose effect, since respective plots were appeared on the respective regression lines when these drugs were studied in the single drug system using the perfusate containing glucose.

These lines of results obtained so far apparently demonstrated that the glucose effect did take place even in such conditions of the double drug system in completely similar manner as was observed in the single drug system. Thus the consistency of the glucose effect was clearly demonstrated.

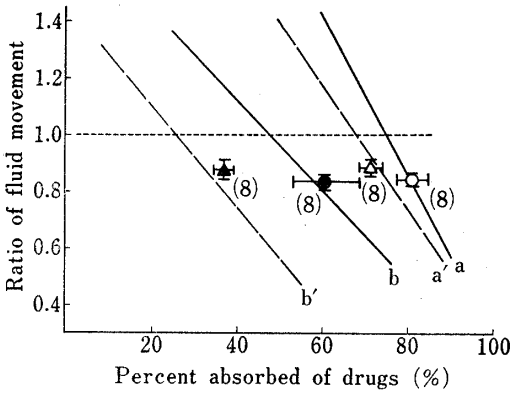

Fig. 7. Effect of Glucose on Absorption of Drugs Coexisted (Sulfisoxazole and Salicylic Acid)

Numbers in parentheses indicate number of experiments.
Key: ▲: sulfisoxazole in glucose isotonic, ●: sulfisoxazole in sodium chloride isotonic, △: salicylic acid in glucose isotonic, ○; salicylic acid in sodium chloride isotonic.
a: regression line of salicylic acid obtained by the perfusate containing sodium chloride calculated with the least squares method.
a′: regression line of salicylic acid obtained by the perfusate containing glucose calculated with the least squares method.
b: regression line of sulfisoxazole obtained by the perfusate containing sodium chloride calculated with the least squares method.
b′: regression line of sulfisoxazole obtained by the perfusate containing glucose calculated with the least squares method.
I: mean ±S.D.

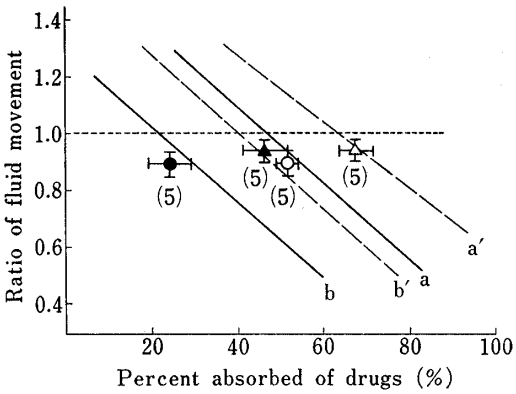

Fig. 8. Effect of Glucose on Absorption of Drugs Coexisted (Metoclopramide and Chlorpheniramine)

Numbers in parentheses indicate number of experiments.
Key: ▲: metoclopramide in glucose isotonic, ●: metoclopramide in sodium chloride isotonic, △: chlorpheniramine in glucose isotonic, ○: chlorpheniramine in sodium chloride isotonic.
a: regression line of chlorpheniramine obtained by the perfusate containing sodium chloride calculated with the least squares method.
a′: regression line of chlorpheniramine obtained by the perfusate containing glucose calculated with the least squares method.
b: regression line of metoclopramide obtained by the perfusate containing sodium chloride calculated with the least squares method.
b′: regression line of metoclopramide obtained by the perfusate containing glucose calculated with the least squares method.
I: mean ±S.D.

Discussion

Up to twenty three drugs were investigated in detail to demonstrate the glucose effect which was originally disclosed in our laboratories.[3] The absorption of these drugs were affected subtly in the presence of glucose in the perfusate. The absorption of cationic drugs was enhanced and that of anionic drugs was decreased and, on the other hand, that of unionized drugs was not affected when sodium chloride in the perfusate was replaced to glucose. The generality of the glucose effect was clearly demonstrated in all of the drugs employd in the present study without any exception (Table I and Fig. 1—3).

However, detailed surveys of these results brought another finding of importance. Metoclopramide and sulfisoxazole have been selected as only the example of cationic and anionic drugs in the previous report.[3] Although increasing and decreasing in the absorption of these drugs were observed when sodium chloride was replaced to glucose, the difference of these increasing and decreasing was always seemed to be constant and the extent was approximately 20%. This evidence suggests that the effect of glucose on drug absorption might be characterized not only by qualitative property but also by quantitative property, that is, direction of the variations in drug absorption brought about by the replacement of sodium chloride to glucose was determined by positive or negative charge of drug molecule in the perfusate and the differences were always fixed in approximate 20%.

However, the results obtained in the present study suggested that the difference of variations was not fixed, since the differences at 1.0 in the ratio of fluid movement were varied from 6.5% in the case of salicylic acid to 32.4% in the case of ephedrine hydrochloride (Table I). This finding did strongly support the quantitatively variable characteristic of the glucose

effect and might be one of clues and might open the way of investigating mechanism of the glucose effect.

Similar finding in analysis of the difference in drug absorption was turned to the results obtained by these unionized drugs employed in the present study. The respective regression lines obtained by the perfusates of sodium chloride and glucose were observed to be completely overlapped when sulfanilamide, an example of unionized drugs, was subjected in the perfusion experiment as reported in the previous publication.[3] Essentially the same results were obtained in the case of sulfanilamide and the extent in difference in the absorption was 0.2%, that was able to be regarded as the same absorption (Table I). However, these results were not always observed in all of unionized drugs employed in the present study. In the cases of phenylbutazone and caffeine, the differences in absorption were revealed to be 8.1% and 8.0%, respectively (Table I). These differences were apparently greater than the case of salicylic acid (6.5%) of which the absorption was regarded to be decreased when sodium chloride was replaced to glucose in the perfusate.

The comparison of respective regression lines obtained with the sodium chloride perfusate or the glucose perfusate in each drug was carried out statistically. Although the significant difference between the respective regression lines could not be found in the cases of unionized drugs, the significant differences were observed in the cases of ionized drugs. It seems reasonable to conclude that the glucose effect is not simply defined by the extent of difference in the drug absorption at 1.0 in the ratio of fluid movement, but is defined by the difference between the respective regression lines.

To examine further in detail the glucose effect, the time course studies of drug levels in both the perfusates and the blood of the animal during the course of the perfusion experiment were undertaken and the glucose effect observed in the perfusate reflected directly to the drug levels in blood of the animal (Fig. 4 and 5). Moreover, the consistency of the glucose effect in the double drug system was also demonstrated in the present study (Fig. 6—8). Evidences relating modification in intestinal absorption of one drug in the presence of another drug were presented in fields of clinical medicine and pharmaceutical sciences.[26] These evidences have been explained in terms of interaction between drugs in the gastrointestinal tract, and mechanisms of such interaction were not revealed completely yet. Any interactions between drugs were not observed in the combination of two drugs used in the present study.

Dietary carbohydrate is 50—60% of the American mixed diet and in many countries is a larger percentage. Carbohydrate intake ranges from 250 to 800 g per day and the major dietary form of carbohydrate is plant starch composed of straight and branched chains of glucose.[27] Hence there are enough evidences that glucose exists in the intestinal tract of both of animal and man as the results of digestive enzyme and digestive fluids. As one of clinical drug interactions in the gastrointestinal tract, nutrients may enhance or impair the intestinal absorption of drugs.[28] The glucose effect in drug absorption might be one of the mechanisms which might explain such interactions in the drug absorption which were observed in clinical medicine.

26) L. Lasagna, "Clinical Effects of Interaction between Drugs," ed. by L.E. Cluff and J.C. Petrie, Excerpta Medica, Amsterdam, American Elsevier Pub. Co., Inc., New York, 1974, pp. 1—8.
27) H.W. Davenport, "Physiology of the Digestive Tract," 3rd ed., Year Book Medical Pub., Inc., Chicago, 1971, p. 183.
28) A. Bartelink, "Clinical Effects of Interaction between Drugs," ed. by L.E. Cluff and J.C. Petrie, Excerpta Medica, Amsterdam, American Elsevier Pub., Co., Inc., New York, 1974, pp. 103—116.