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Intestinal Absorption Characteristics of Buformin and Phenformin in Rats

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Absorption characteristics of buformin and phenformin, the hypoglycemic biguanides, were studied in the *in situ* rat small intestine. It was suggested that the small intestinal absorption of these biguanides is probably mediated by passive process. The absorption of these biguanides from the ileum was more rapid than that from the jejunum, and such the difference was not associated with the accumulation characteristic of the drugs in the intestinal tissues. From observations on the release of the components such as membrane phosphorus, lipid phosphorus, and protein from the jejunum and ileum, it was suggested that the more rapid absorption of these biguanides from the ileum would be attributable to that the release of membrane components from the ileum was much larger than from the jejunum, resulting in a much increased permeability of the ileum to the drugs.

Some biguanides such as buformin, metformin, and phenformin have been commonly known as oral blood sugar-lowering agents. A number of investigations[2-6] have been concerned with the excretion and metabolism of biguanides in man and animals. From findings on the urinary excretion following the oral administration of biguanides, some investigators[7-10] have also suggested that the drugs are moderately rapidly absorbed from gastrointestinal tract. However, little work has been done on the gastrointestinal absorption characteristics of the biguanides.

The purpose of the present study is to clarify the characteristics of the *in situ* small intestinal absorption of buformin and phenformin in rats.

Experimental

Materials and Equipment——Buformin hydrochloride and phenformin hydrochloride were prepared according to the methods of Shapiro, *et al.*,[11,12] mp 177—178° and mp 172—175° respectively. Other chemicals were of reagent grade.

A Shimadzu QV-50 spectrophotometer and a Hitachi-Horiba F-5 pH meter were utilized.

Preparation of Sample Solutions——The components of isotonic phosphate buffer solutions used as the medium in an *in situ* experiment are listed in Table I. The initial concentrations of buformin and phenformin were 100—1000 μg/ml.

Test Animals——Male Wistar rats weighing 180—250 g were used. The rats were fasted about 20 hr with drinking water *ad libitum* prior to the experiment. They were housed in cages having wide mesh floors to prevent coprophagy.

Analytical Procedure——To 0.1—0.2 ml of sample solution were added 1 ml of 20% sodium chloride and 0.5 ml of 5 N sodium hydroxide. The mixture was extracted with 8 ml of chloroform–methanol (85: 15) by

1) Location: *5–1 Oehon-machi, Kumamoto, 862, Japan.*
2) G. Debry and F.P. Cherrier, *Therapie*, **20**, 351 (1965).
3) R. Beckmann, *Arch. Int. Pharmacodyn. Therap.*, **160**, 161 (1966).
4) H. Hall, G. Ramachander, and I.M. Glassman, *Ann. N. Y. Acad. Sci.*, **148**, 601 (1968).
5) P.J. Murphy and A.N. Wicks, *J. Pharm. Sci.*, **57**, 1125 (1968).
6) S.S. Fajans, J.A. Moorhouse, H. Doorenbos, L.H. Louis, and J.W. Conn, *Diabetes*, **9**, 194 (1960).
7) B. Elpern, *Ann. N. Y. Acad. Sci.*, **148**, 577 (1968).
8) R. Beckmann, *Ann. N. Y. Acad. Sci.*, **148**, 820 (1968).
9) R. Beckmann, *Diabetologia*, **3**, 368 (1967).
10) R. Beckmann, *Diabetologia*, **5**, 318 (1969).
11) S.L. Shapiro, V.A. Parrino, and L. Freedman, *J. Am. Chem. Soc.*, **81**, 2220 (1959).
12) S.L. Shapiro, V.A. Parrino, and L. Freedman, *J. Am. Chem. Soc.*, **81**, 3728 (1959).

Table I. Preparation of Isotonic Phosphate Buffer Solutions

	pH	$NaH_2PO_4 \cdot 2H_2O$ g/liter	Na_2HPO_4 g/liter	NaCl g/liter
Isotonic phosphate buffer	6.0[a]	12.880	—	4.500
	7.0	7.025	19.675	2.250

a) adjusted to pH 6.0 with 2N NaOH solution

shaking on a mixer for 2 min. After centrifugation at 3000 rpm for 5 min, 5 ml of the lower organic solvent layer was evaporated to dryness *in vacuo*. The resulting residue was dissolved in 5—10 ml of 0.01 M phosphate buffer solution, pH 6.0, and the optical density was determined at 232 nm for buformin and 233 nm for phenformin. Recoveries of known amounts of buformin and phenformin from sample solutions were 92.0± 0.1% and 96.8±2.8%, respectively.

***In Situ* Rat Experimental Procedure**——The procedure for studying drug absorption in the *in situ* rat small intestine was carried out according to the method of Doluisio, *et al.*[13] The loops used were the whole small intestine, jejunum, and ileum. For experiments on the jejunum, the first 20-cm portion of the intestine approximately 15 cm distal to the pylorus was used. The ileal region was cannulated at the ileo-cecal junction and 20 cm proximally. The bile duct was ligated in all experiments.

The volumes of sample solutions used in the absorption experiments of the drugs from the whole small intestine, jejunum, and ileum were 10 ml, 4 ml, and 4 ml, respectively. Phenol red was dissolved in the sample solutions to indicate any volume change.

Determination of Partition Coefficients——Partition coefficients were determined by the procedure reported in the paper from this laboratory.[14]

Determination of Biguanide Content in *in Situ* Rat Small Intestinal Tissue——At the end of the absorption experiments of the biguanides in the jejunum or ileum, the luminal solution was withdrawn as completely as possible and the lumen was flushed with about 100 ml of distilled water. After sacrificing the rat, the jejunum or ileum was removed and homogenized in 4 ml of 1/15 M phosphate buffer, pH 7.2. The homogenizer was washed with 3.5 ml of the buffer and then with ethanol. The homogenate and subsequent washings were added to a 25 ml volumetric flask and brought to volume with ethanol. The entire homogenate was centrifuged at 10000 rpm for 15 min. The supernatant was decanted and filtered into a 100 ml volumetric flask. The precipitate was washed with 70% ethanol, the washings added to the filtrate and the filtrate brought to volume with 70% ethanol. An aliquot of 5 ml of the filtrate was evaporated to dryness *in vacuo* and the residue was dissolved in 1 ml of 20% sodium chloride. The resulting solution was basified with 0.5 ml of 5 N sodium hydroxide and extracted with 8 ml of chloroform–methanol (85: 15). Five milliliters of the organic solvent layer was evaporated to dryness and the residue was dissolved in 5 ml of ethanol. The optical densities of the resulting solution were measured at 2 wavelengths, one the wavelength of maximum extinction (236 nm for buformin; 237 nm for phenformin), and the other the wavelength of low extinction (270 nm). Since the optical density due to blank materials was the same at all wavelengths between 236 and 270 nm, subtraction of the optical density at 270 nm from that at 236 or 237 nm gave an optical density proportional to buformin or phenformin concentration only. Recoveries of known amounts of buformin and phenformin from rat intestinal homogenate were 104.0±0.5% and 106.1±0.3%, respectively.

***In Vitro* Binding Affinities of Biguanides to Rat Small Intestinal Mucosa**——The binding of buformin and phenformin to rat small intestinal mucosa was determined according to a minor modification of the method of Kakemi, *et al.*[15] Rats were sacrificed, and the entire small intestine was removed. Both the mucosal and serosal sides of the intestine were rinsed well with cold physiologic saline. A length of approximately 20 cm of jejunum or ileum was cut open and carefully blotted with filter paper to remove adhering moisture. The mucosa was removed with a slide glass. A portion of the mucosa was dried at 105° for the determination of dry weight/wet weight ratio. The remaining mucosa was homogenized in a phosphate buffer solution (ionic strength 0.05), pH 6.0, with a teflon pestle glass homogenizer, and the concentration was made up to 1% (dry weight). The binding of the biguanides was estimated by the equilibrium dialysis method of Klotz, *et al.*[16] Four milliliters of the mucosal homogenate was pipetted into the bag of a seamless cellulose tubing (24/32 in size).[17] The bag was inserted to a test tube (20×130 mm) containing 8 ml of the drug solution. The apparatus was kept in a cold chamber of approximately 5° for 72 hr to attain to an equilibrium between

13) J.T. Doluisio, N.F. Billups, L.W. Dittert, E.T. Sugita, and J.V. Swintosky, *J. Pharm. Sci.*, **58**, 1196 (1969).
14) S. Kojima, T. Tenmizu, T. Shin-o, and M. Cho, *Chem. Pharm. Bull.* (Tokyo), **22**, 952 (1974).
15) K. Kakemi, T. Arita, R. Hori, and R. Konishi, *Chem. Pharm. Bull.* (Tokyo), **15**, 1883 (1967).
16) I.M. Klotz, F.M. Walker, and R.B. Pivan, *J. Am. Chem. Soc.*, **68**, 1486 (1946).
17) Visking Company.

the solutions inside and outside the dialysis bag. Percent of binding was calculated from the difference between the concentration of free drug in the outer fluid in the presence of mucosa and that in the absence of mucosa.

Measurement of Membrane Components released from *in Situ* Rat Small Intestine——Four milliliters of 0.9% saline at initial pH 6.0 or 0.1% buformin saline solution at initial pH 6.0 was introduced into the *in situ* rat jejunum or ileum preparation provided by the *in situ* rat experimental procedure described above. After 2 hr, the luminal solution was withdrawn, and the lumen was flushed with about 40 ml of distilled water. The washings were combined to the luminal solution, and the combined solution was submitted to the determination of total phosphorus, phospholipid phosphorus, and protein.

Total phosphorus was assayed according to the Feldman, *et al.*[18] modification of the method of Bartlett.[19] Phospholipids were extracted according to the modified procedure[20] of the method of Folch, *et al.*,[21] and lipid phosphorus was assayed according to the determination procedure of total phosphorus mentioned above. Protein determination was made by the method of Lowry, *et al.*[22]

Result and Discussion

Absorption of Buformin and Phenformin from *in Situ* Rat Whole Small Intestine

The absorption of buformin and phenformin in a phosphate buffer solution at pH 6.0 was examined in the *in situ* rat whole small intestine preparation, and the obtained results are represented in Fig. 1. The rate constant of disappearance of each drug was calculated from the slope of the straight line on the semilogarithmic plots of drug concentration in the rat intestinal lumen *vs.* time. The results are listed in Table II. In cases of both drugs, there was no effect of the drug concentration in the luminal solution on the rate constant of disappearance. Also the intestinal absorption of phenformin was examined using a phosphate

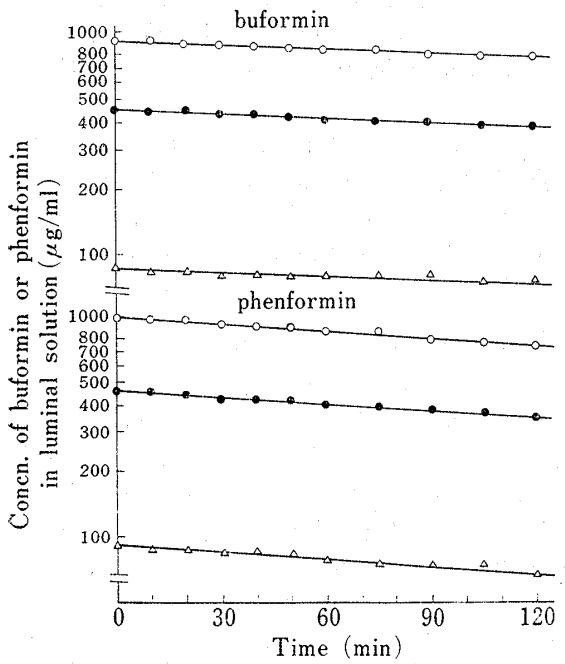

Fig. 1. Absorption of Buformin and Phenformin from Rat Whole Small Intestine

Each value is expressed as the mean of 3 or 4 animals.
initial concentration
—○—: 1000 μg/ml, —●—: 500 μg/ml, —△—: 100 μg/ml

Table II. Disappearance of Buformin and Phenformin from the *in Situ* Rat Whole Small Intestine at Various Initial Concentrations

Drug	pH	Initial concn. (μg/ml)	Rate constant of disappearance[a] (hr^{-1})
Buformin	6.0	100	0.107±0.007
	6.0	500	0.111±0.008
	6.0	1000	0.105±0.014
Phenformin	6.0	100	0.158±0.017
	6.0	500	0.154±0.009
	6.0	1000	0.168±0.023
	7.0	1000	0.171±0.021

a) The values represent the mean ± standard deviation for 3 or 4 animals.

18) S. Feldman, M. Reinhard, and C. Willson, *J. Pharm. Sci.*, **62**, 1961 (1973).
19) G.R. Bartlett, *J. Biol. Chem.*, **234**, 466 (1959).
20) R.A. Muesing and T. Nishida, *Biochemistry*, **10**, 2952 (1971).
21) J. Folch, M. Lees, and G.H. Sloane-Stanley, *J. Biol. Chem.*, **226**, 497 (1957).
22) O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

Table III. Percent of Ionized Forms and Partition Coefficients of Buformin and Phenformin

Drug	pK_a	Percent ionized form[a] pH 6.0	Partition coefficient[b]	
			Chloroform pH 6.0	*n*-Octanol pH 6.0
Buformin	11.3	100	0.011	0.001
Phenformin	11.0	100	0.029	0.033

a) calculated using the Henderson-Hasselbalch equation
b) values between chloroform or *n*-octanol and phosphate buffer at pH 6.0, 37°

Table IV. Disappearance of Buformin and Phenformin from the *in Situ* Rat Jejunum and Ileum at pH 6.0

Drug	Initial concn. (μg/ml)	Rate constant of disappearance[a] (hr^{-1})	
		Jejunum	Ileum
Buformin	1000	0.087±0.005	0.131±0.016[b]
Phenformin	1000	0.113±0.008	0.211±0.017[b]

a) The values represent the mean ± standard deviation for 3 or 4 animals.
b) significantly different from corresponding value in the jejunum, $p<0.01$

buffer solution at pH 7.0. The first-order rate constant of disappearance of phenformin did not appreciably change between the buffer solutions at pH 6.0 and 7.0 (see Table II). These results suggest that the absorption of buformin and phenformin from the rat small intestine is probably mediated by passive process.

The percent of ionized forms and the partition coefficients of buformin and phenformin at pH 6.0 are listed in Table III. These data suggest that these drugs exist mostly as the ionized forms in the buffer solution at pH 6.0 and have extremely poor lipid-solubility. Thus, it is demonstrated that buformin and phenformin penetrate through the aqueous-filled pores or channels in the epithelial membrane of small intestine at relatively slow rate.

Absorption of Buformin and Phenformin from *in Situ* Rat Jejunum and Ileum

The site specificity of the intestinal absorption of buformin and phenformin was examined in the *in situ* rat jejunum and ileum. As can be seen in Table IV, the disappearance of these drugs from the ileum was significantly more rapid than that from the jejunum. In cases of both drugs, the rate constants of disappearance in the whole small intestine were intermediate between those in the jejunum and ileum. These results suggest that the absorption of these biguanides is more rapid at the lower site of the small intestine than at the upper site.

To clarify the difference of absorption characteristics of the biguanides between the jejunum and ileum, the relationship between the disappearance of the drugs from the jejunal and ileal lumens and the contents of the drugs in the intestinal tissues was examined. The results are shown in Table V. In the case of buformin, although the percent of the drug disappeared from the ileal lumen was greater than that from the jejunal lumen, no difference between the contents of the drug in both tissues was observed. Similar result was also obtained in the case of phenformin. These results suggest that the difference in disappearance of buformin and phenformin between the jejunum and ileum was not associated with the accumulation of the drugs in the intestinal tissues. The percent of absorption of each biguanide obtained from the difference between the percent of disappearance from the lumen and that of tissue accumulation was greater in the ileum than in the jejunum as shown in Table V. These findings suggest that the rate constants of absorption of these biguanides are, in fact,

Table V. Absorption of Buformin and Phenformin from Rat Jejunum and Ileum during 2 hr

Drug		Disappeared from lumen (%)	Content in tissue (%)	Absorbed (%)
Buformin	jejunum	15.7±1.1	11.7±1.9	4.1±1.8
	ileum	21.0±1.1	9.8±1.7	11.2±2.7
Phenformin	jejunum	27.0±1.5	20.0±2.0	7.0±0.5
	ileum	36.2±2.9	18.4±0.5	17.7±3.4

The values represent the mean standard deviation for 3 to 5 animals.
The initial concentration of each drug was 1000 μg/ml.

Table VI. Binding of Buformin and Phenformin to Mucosa of Rat Jejunum and Ileum *in Vitro*

Drug	Concn. (μg/ml)	Bound[a] (%)	
		Jejunum	Ileum
Buformin	500	3.3±0.7	2.4±1.4
	1000	2.7±0.3	2.5±1.6
Phenformin	500	2.3±0.1	2.7±0.4
	1000	3.4±0.3	3.8±0.8

a) The values represent the mean ± standard deviation for 3 or 4 determinations.

smaller than those of disappearance of the drugs presented in Table II. In addition, to understand such a phenomenon that approximately half the amount of each drug disappeared from the lumens remained in the intestinal tissues, the binding characteristics of the drugs to the mucosa of rat small intestine were investigated. The homogenates of the jejunal and ileal mucosa were used in the equilibrium dialysis with the drugs. As shown in Table VI, the results showed that these drugs were bound to extremely less extent to the mucosa, and that no clear distinction between the binding extents in the jejunal and ileal mucosa was observed. This finding suggests that buformin and phenformin exist almost unbound in rat small intestinal mucosa.

Subsequently, the effect of buformin, the chelating agent, on the release of the components such as phosphorus, phospholipid, and protein from the *in situ* rat jejunal and ileal membranes was investigated. The results are summarized in Table VII. In cases of either the saline or buformin solution, comparison of each amount of the components released during 2 hr revealed that the amount of each component released from the ileum was significantly larger than that from the jejunum, although in the case of the saline solution, the amount of protein released from the ileum was somewhat smaller than in the jejunum. However, the contents of such membrane components in the intestinal tissues were not changed significantly between the jejunum and ileum, but the details will be reported elsewhere. The data presented in Table VIII show the ratio of the total amount of membrane component released during a 2 hr exposure to buformin solution compared to the amount released in the saline. The release of membrane phosphorus, lipid phosphorus, and protein was significantly increased in the presence of buformin. The release of protein from the intestine was accelerated to greater extent by buformin. Bloch, *et al.*[23] reported that phenformin seemed to increase the extrusion of absorptive cells at the tips of the villi of rat small intestine. From findings on the influence of chelating agents such as ethylenediaminetetraacetic acid and tetracycline upon the rat

23) R. Bloch, H. Menge, W.D. Schaarschmidt, H. Gottesbüren, E. Schaumloffel, H. Goebell, and E.O. Riecken, *Klin. Wschr.*, **51**, 235 (1973).

Table VII. Protein, Total Phosphorus, and Phospholipid Phosphorus released from the *in Situ* Rat Jejunum and Ileum during 2 hr

Solution		Protein[a] (mg)	Total phosphorus[a] (μg)	Phospholipid phosphorus[a] (μg)
Saline	jejunum	3.29±0.88	47.7± 8.2	21.7± 7.7
	ileum	1.83±0.25	131.8±47.0[b]	45.5± 5.3[b]
Buformin	jejunum	20.30±3.14	90.8±22.6	51.6± 8.4
(1000 μg/ml)	ileum	26.21±1.62	266.8± 9.4[c]	115.5±28.6[b]

a) The values represent the mean ± standard deviation for 3 animals.
b) significantly different from corresponding value in the jejunum, $p<0.05$
c) significantly different from corresponding value in the jejunum, $p<0.001$

Table VIII. Ratio of Amount of Membrane Component released after Exposure to Buformin Solution (1000 μg/ml)

	Ratio (buformin/saline)		
	Protein	Total phosphorus	Phospholipid phosphorus
Jejunum	6.17[a]	1.90[b]	2.34[b]
Ileum	14.32[c]	2.02[a]	2.54[b]

a) significantly different from the value in the saline, $p<0.01$
b) significantly different from the value in the saline, $p<0.05$
c) significantly different from the value in the saline, $p<0.001$

intestinal mucosa, Nadai, *et al.*[24,25] suggested that the histological change of the intestinal tissue caused by the chelating agents would be the primary cause of the enhancement in the absorption of drugs which penetrate the intestinal membrane very slowly or not to an appreciable quantities. Thus, it appears that buformin alters the composition of the *in situ* rat intestinal membrane by producing an efflux of membrane components from the intestinal tissue, resulting in an appreciable loss of structural integrity of the intestinal membrane and increasing the permeability of the intestine to the drug itself. In addition, the fact that the absorption of buformin and phenformin from the ileum is more rapid than that from the jejunum appears to be attributable to a large release of membrane components from the ileum.

24) T. Nadai, R. Kondo, A. Tatematsu, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 1139 (1972).
25) T. Nadai, M. Kume, A. Tatematsu, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 543 (1975).