The authors are grateful to Prof. H. Matsumura of this university for his encouragement throughout this work. Thanks are also due to Mrs. S. Matsuba and Mr. M. Shirōzu for the elemental analyses and to Messrs. H. Yano, H. Matsui and K. Hikita for the spectral measurements. This work was supported by the Grant-in-aid for Scientific Research provided by the Ministry of Education, to which they are also grataful.

Summary

The reaction process of DHA, when it reacted with an excess of methyl-, ethyl-, benzyl- or phenethylamine under a mild condition, was clarified as follows: The primary reaction product is Schiff's base, the secondly product 2,6-bis(alkylamino)-2,5-heptadien-4-one, and the final product lutidone derivative. In the case of the reaction of DHA with an excess of ammonia, two compounds, lutidone and lutidonecarboxylic acid, were obtained as final products. But lutidonecarboxylic acid seems not to be the intermediate to lutidone under these mild conditions.

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72. Hisashi Nogami, Manabu Hanano,[*1] and Hideo Yamada[*2]: Studies on Absorption and Excretion of Drugs. IV.[*3] Absorption of Various Sulfonamides from the Rat Small Intestine by the Perfusion Method *in vivo*.

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A large number of studies on gastrointestinal absorption of sulfonamides have been published. But there have been few kinetic studies on relationship between the absorption rate of sulfonamide and its concentration in the intestine.

The penetration of various sulfonamides across the intestinal barrier *in vitro* was reported in the preceding paper of this series.[1)]

The present paper describes the observations with disappearance rate of sulfonamides from the perfusion solution through the rat small intestine *in vivo* and with the effects of pH of the solution on the disappearance rate, together with discussion of these results.

When the various forms of a given drug in the solution ini the ntestinal lumen (*e.g.* ionic, nonionic, etc.) are in equilibrium under the fixed conditions, the concentration of one form (*e.g.* i-th form) to the total drug must be constant,

$$\frac{C_i}{C}=K_i \qquad (1)$$

where C_i is the concentration of i-th form, C is the total drug concentration and K_i is constant. If the amount of the drug molecules in the i-th form passing across the unit

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1) H. Nogami, M. Hanano, J. Watanabe: This Bulletin, **10**, 1161 (1962).

area of intestinal wall from the lumen into the plasma in unit time, q_i, is proportional to its concentration in the lumen, C_i, then

$$q_i = p_i C_i = p_i K_i C \tag{2}$$

where p_i is the permeability coefficient. The total amount of the drug molecules passing across the unit area of the wall into the plasma per unit time, q, is

$$q = \sum q_i = [\sum (p_i K_i)] C = pC \tag{3}$$

where p is $\sum (p_i K_i)$, i.e. the permeability coefficient for the drug from the intestinal lumen into the plasma.

Similarly, the total amount of the drug molecules passing in the reverse direction per unit area per unit time, q', is proportional to the plasma concentration, C',

$$q' = p'C' \tag{4}$$

where p' is the permeability coefficient from the plasma into the intestinal lumen.

Consequently, the net amount of the drug molecules passing from the lumen into the plasma across the unit area of the wall in unit time, $\hat{q}$, is from equation (3) and (4),

$$\hat{q} = q - q' = pC - p'C' \tag{5}$$

When the effective area of the intestinal wall is A, absorption rate from the intestine is $A\hat{q}$. On the other hand, $A\hat{q}$ is expressed with the drug concentration, C, and the volume of the drug solution, V, as follows :

$$A\hat{q} = -\frac{d(VC)}{dt} = A(pC - p'C') \tag{6}$$

When the volume of the drug solution is constant,

$$-\frac{dC}{dt} = \frac{A}{V}(pC - p'C') \tag{7}$$

By integration,

$$\log\left(\frac{C}{C_0}\right) = -0.434 \times \frac{Apt}{V} + \log\left(1 + \frac{Ap'}{VC_0}\int_0^t C' \exp\frac{Apt}{V} dt\right) \tag{8}$$

where C_0 is the initial concentration of the drug in the intestinal lumen.

When $pC \gg p'C'$, from equation (7),

$$-\frac{dC}{dt} = \frac{ApC}{V} \tag{9}$$

or

$$\log\left(\frac{C}{C_0}\right) = -0.434 \times \frac{Apt}{V} = -kt \tag{10}$$

where k is constant.

Under the experimental condition that $C \gg C'$, when $p \approx p'$ or $p > p'$, there is the possibility that a semilogarithmic plot of $\frac{C}{C_0}$ *vs.* time gives a straight line. Furthermore, when the linear relationship on the graph extends until the blood level equals to the concentration in the intestinal lumen, it may be suggested that the permeability coefficient of the drug from the intestinal lumen into the plasma, p, is much larger than that from the plasma into the intestinal lumen, p'.

Experimental

Experimental Procedure——The recirculating perfusion method based on that of Schanker *et al.*[2] was as follows : Male rats (Donryu; 250 to 350 g.) were fasted for about 20 hr. prior to the experiments but were allowed free access to H_2O. The animals were anesthetized by the intraperitoneal injection of 0.25% pentobarbital sodium parenteral solution (0.5 ml./100 g. body wt.). The small intestine was exposed by a midline abdominal incision and cannulated at the duodenal and ileal ends with polyethylene cannulae having inside diameters of 2.5 mm. and outside diameters of 3.5 mm. The intestine was replaced in the abdomen, the incision was closed and these cannulae were joined to a perfusion pump.

The small intestine was first cleared of particulate matter by perfusion with 100 ml. of 0.9% NaCl solution maintained at 37°. Then, 100ml. of the solution containing drug was recirculatingly perfused, from duodenum to ileum.

The perfusion solution (pH 6.0～6.4) was consisted of 1 m*M* of sulfonamide together with the following compounds per liter : KH_2PO_4 9.2 g.; Na_2HPO_4 4.4 g.; NaCl 5 g.; phenol red 100 mg. The solution was maintained at 37° and was perfused with a pump at a rate of 2 ml. per min. The samples (0.5 ml.) were taken out at intervals of 15 min. and analyzed. The experiments were repeated four times.

The blood samples were collected at intervals of 30 min. by amputation of tail.

The pH-effect was observed in the perfusion solution which contained KH_2PO_4-Na_2HPO_4 buffer system by Sörensen, phenol red as a volume indicator, NaCl for giving isotonicity and sulfonamide. The pH values were continuously checked with a pH meter (Toadenpa Co., Ltd.; model HM-5A) equipped with microelectrodes. When pH value was unstable, it was kept constant by means of addition of *N* NaOH or *N* HCl. Also in these cases, the volume changes were practically negligible. The operative technique for animals (female Donryu rats weighed 190 to 250 g.) and the perfusion method was the same as above.

Drugs—— Five sulfonamides used were sulfanilamide (SA); sulfaguanidine (SG); sulfathiazole (ST); sulfamethoxypyridazine (SM); sulfisomezole *i.e.* N^1-(5-methyl-3-isoxazolyl)sulfanilamide (SI). The abbreviations in the parentheses represent these drugs, respectively.

Analytical Method——Phenol red and sulfonamides were determined colorimetrically. A sample (0.5 ml.) taken out of the perfusion solution was pipetted into a 20 ml. measuring flask and the volume made up to 20ml. with 0.1*N* Na_2CO_3 solution. A part of this solution (4 ml.) was transferred to a 10 ml. measuring flask for sulfonamide determination and the rest of the solution was used for phenol red determination immediately. The sulfonamide determination was performed as follows : 4*N* HCl (1 ml.) was added to the sample solution in a 10 ml. measuring flask mentioned above. Then 0.2% $NaNO_2$ (3 drops) was added. Ten min. later, 10 % $NH_4SO_3NH_2$ (2 drops) was added. Five min. later, furthermore, 0.2% N-(2-diethylaminoethyl)-1-naphthylamine solution (4 drops) was added. Then, the volume was made up to 10 ml. with EtOH and this solution was used for sulfonamide determination.

To blood sample (about 100 mg.) was added distilled H_2O (3 ml.) and it was treated with 15% trichloroacetic acid (2 ml.). After filtration, 4*N* HCl (0.5 ml.) was added and heated at 100° for 75 min. Then, it was cooled and 0.2% $NaNO_2$, 10% $NH_4SO_3NH_2$ and 0.2% N-(2-diethylaminoethyl)-1-naphthylamine were added in the same way as to the sample from the perfusion solution. The volume was made up to 10 ml. with EtOH. This solution was used for blood sulfonamide determination.

Optical density was read on a spectrophotometer (Hitachi Co., Ltd. model EPU-2) at 553 mμ. for sulfonamides and at 558 mμ. for phenol red.

Results and Discussion

In Fig. 1 the observed values of residual ratio of sulfonamide in perfusion solution (pH 6.0～6.4) are plotted *vs.* time. These pH values are within the normal range in intestinal lumen.

Changes in blood concentration are shown in Fig, 2.

After circulation through the intestine for 3 hours, recovery of the phenol red was 98% on the average, which agreed with the value by Schanker *et al.*[2] Thus, the negligible absorption of phenol red permits its use as an indicator of volume change. Therefore, the reciprocals of phenol red concentration ratio to the initial permit to show apparent volume ratio of perfusion solution. These values are given in Table I.

2) L. S. Schanker, *et al.* : J. Pharmacol. Exptl. Therap., **125**, 275 (1958).

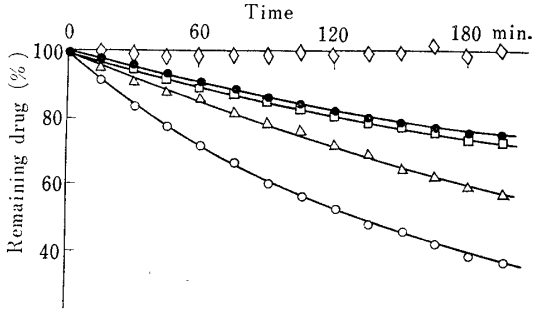

Fig. 1. Percentage of Remaining Sulfonamides in Isotonic Phosphate Buffered Solution (pH 6.0~6.4) Perfused through Rat Small Intestine

◇—◇ sulfaguanidine
●—● sulfathiazole
□—□ sulfanilamide
△—△ sulfamethoxypyridazine
○—○ sulfisomezole

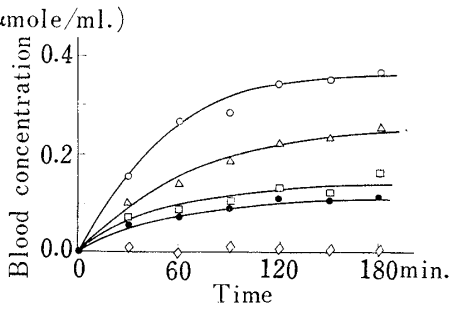

Fig. 2. Changes in Blood Concentration during Perfusion of Sulfonamide Solutions through Rat Small Intestine

◇—◇ sulfaguanidine
●—● sulfathiazole
□—□ sulfanilamide
△—△ sulfamethoxypyridazine
○—○ sulfisomezole

Table I. Apparent Volume Ratio of Isotonic Phosphate Buffered Solutions (pH 6.0~6.4) Containig Sulfonamide during Perfusion through Rat Small Intestine

Time (min.)	SG	ST	SA	SM	SI
0	1.00	1.00	1.00	1.00	1.00
15	1.01	1.01	1.01	1.02	1.01
30	1.01	1.02	1.01	1.01	1.01
45	1.01	1.02	1.01	1.02	1.00
60	1.01	1.01	1.02	1.02	1.01
75	1.00	1.02	1.01	1.01	1.01
90	1.02	1.03	1.03	1.02	1.01
105	1.02	1.02	1.01	1.02	1.01
120	1.03	1.02	1.02	1.01	1.01
135	1.00	1.02	1.01	1.01	1.00
150	1.00	1.02	1.02	1.01	1.01
165	0.99	1.02	1.01	1.01	1.00
180	1.00	1.02	1.03	1.01	1.01

SG : sulfaguanidine, ST : sulfathiazole, SA : sulfanilamide,
SM : sulfamethoxypyridazine, SI : sulfisomezole.

From Table I, it is clear that the volume of the perfusion solution is constant.

When the percentage of remaining sulfonamide in perfusion solution is replotted in the logarithmic scale, a straight line was obtained, as shown in Fig. 3.

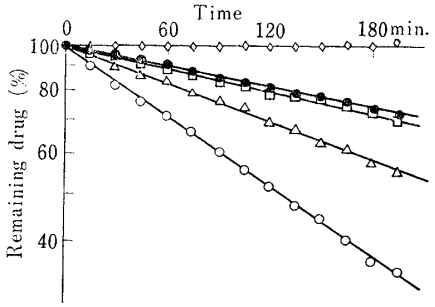

Fig. 3.
Linear Relationship between Percentage of Remaining Sulfonamide in Perfusion Solution plotted in Logarithmic Scale and Time

◇—◇ sulfaguanidine
●—● sulfathiazole
□—□ sulfanilamide
△—△ sulfamethoxypyridazine
○—○ sulfisomezole

The slope corresponds to the constant, k, in equation (10).

These slopes are shown in Table II.

It was so difficult to know the effective area of intestinal mucosa, A, in every animal

that, a intestine is regarded as a cylinder, its apparent area, A_a, was measured. This is obtained from equation (11), where v is the volume of intestinal lumen used and l is its length.

$$A_a = 2\sqrt{\pi l v} \tag{11}$$

The volume v and the length l were obtained as follows : After perfusion experiment, the rat intestinal lumen was filled with saline containing phenol red (100 mg./L.) under the constant water pressure (under 50 cm. column of water). Then the saline was collected by washing of the intestinal lumen and phenol red was determined colorimetrically. From the amount of phenol red, the volume of saline in the intestinal lumen was calculated and regarded as volume of the lumen v. Furthermore, the intestine was taken off and hung up, the length l was measured. The average apparent area of intestinal mucosa, A_a, of male Donryu rats (300±50 g.) was 170 cm^2 and A_a of female Donryu rats (220±30 g.) was 120 cm^2. Of course, the true effective area, A, owing to villous structure, should be much larger than this value. When, instead of A, A_a is employed in equation (10), apparent permeability coefficient, p_a, can be calculated. The results are given in Table II.

Table II. Slope, k, and Apparent Permeability Coefficient, p_a

Sulfonamide	k	p_a (cm./min.)
Sulfaguanidine	0.0×10^{-3}	0.0×10^{-3}
Sulfathiazole	0.72×10^{-3}	0.9×10^{-3}
Sulfanilamide	0.76×10^{-3}	1.0×10^{-3}
Sulfamethoxypyridazine	1.26×10^{-3}	1.7×10^{-3}
Sulfisomezole	2.36×10^{-3}	3.2×10^{-3}

In the case of SI solution at pH 6.0~6.4, when p is equal to p', then $\log(C/C_o)$ in equation (8) is calculated from the concentration in perfusion solution and in plasma, as shown in Fig. 4 (split line). This curve differs from the observed values (circles shown in Fig. 4). Apparently, it seems that equation (10) applies to this case. It suggests that p is much larger than p'.

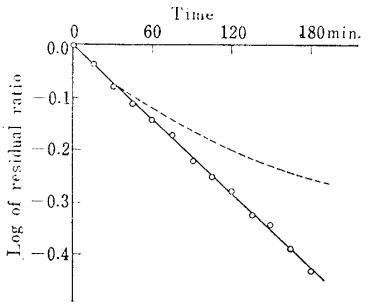

Fig. 4.

Observed Values and Calculated Curves with Sulfisomezole

○ : observed value
------ : calculated curve when $p=p'$
—— : calculated curve when $p \gg p'$

Further experiments were carried out, 1.42M SI-Na solution (0.5 ml.) was injected from femoral vein. After 30 minutes the intestinal lumen was cleared off and 100 ml. of isotonic phosphate buffered solution (pH 6.0~6.4) without sulfonamide was perfused through the intestinal lumen. Then, the concentration of SI in perfusion soiution was measured at intervals of 15 minutes. This experiment was repeated two times. The average of these results is shown in Fig. 5.

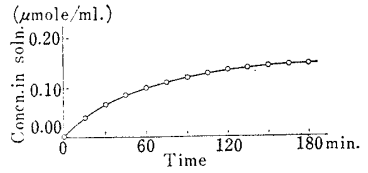

Fig. 5.

Concentration Changes in Perfusion Solution after Intravenous Injection of Sulfisomezole

SI was found in the lumen. The initial slope of this curve $\left(\frac{dC}{dt}\right)_{t=o}$ was 0.003 μmole/ml./min., as shown in Fig. 5. In this case, the initial plasma concentration, C_o', was 5.70 μmole/ml. Therefore, p_a' was calculated approximately from equation (12) with 170 cm^2 for A_a and 100 ml. for V,

$$p_a' = \frac{V}{C_0' A_a}\left(\frac{dC}{dt}\right)_{t=0} \qquad (12)$$

The value obtained for p_a' was about 3×10^{-4} cm./min. The Permeability coefficient of SI from the perfusion solution at pH 6.0~6.4 into the plasma is much larger than that from the plasma into the perfusion solution. The pH in plasma was 7.5~7.6.

There is the possibility that the composition of the solution in the intestinal lumen affects the absorption rate. For the purpose of the investigation of the pH effect on p_a, the p_a-pH diagrams were obtained by perfusion of the phosphate buffered sulfonamide solutions. The results are shown in Fig. 6 to 9.

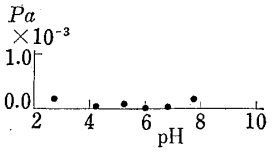

Fig. 6. Relation between Apparent Permeability Coefficient, p_a, for Sulfaguanidine and pH in Phosphate Buffered Perfusion Solution through Rat Small Intestine

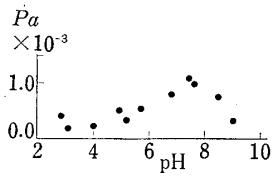

Fig. 7. Relation between Apparent Permeability Coefficient, p_a, for Sulfathiazole and pH in Phosphate Buffered Perfusion Solution through Rat Small Intestine

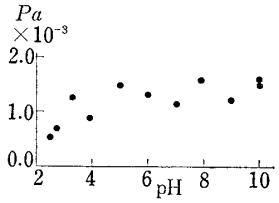

Fig. 8. Relation between Apparent Permeability Coefficient, p_a, for Sulfanilamide and pH in Phosphate Buffered Perfusion Solution through Rat Small Intestine

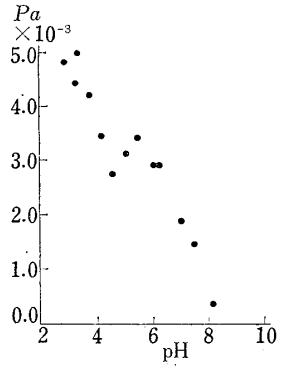

Fig. 9. Relation between Apparent Permeability Coefficient, p_a, for Sulfisomezole and pH in Phosphate Buffered Perfusion Solution through Rat Small Intestine

From Fig. 6, it is clear that p_a for SG is negligibly small over a wide pH range. It seems from Fig. 7 that p_a for SA is independent of the pH ranging from 4 to 10. In Figs. 8 and 9, these diagrams are very complicated. The results with SG and SA may be explained on the theory by Brodie *et al.*[3] but further studies may be necessary for the general interpretation of these phenomena.

From Fig. 9, it seems possible that owing to difference of pH value between in intestinal solution and in plasma, p is larger than p'.

The investigations of the effects of co-existing substances on intestinal absorption rate are being carried out.

3) B.B. Brodie, *et al.* : J. Pharm. Pharmacol. 8, 345 (1956).

Summary

1. While the isotonic phosphate buffer (pH 6.0～6.4) was recirculatingly perfused through the rat small intestine for 3 hours, volume of the solution was constant.

2. When the isotonic phosphate buffered solution (pH 6.0～6.4) containing sulfonamide was recirculatingly perfused through the rat small intestine *in vivo*, the logarithm of residual ratio of the drug *vs.* time curve was a straight line.

3. From the slope of the straight line, apparent permeability coefficient, p_a, was obtained. The constants were : Sulfaguanidine (0.0×10^{-3} cm./min.); Sulfathiazole (0.9×10^{-3}); Sulfanilamide (1.0×10^{-3}); Sulfamethoxypyridazine (1.7×10^{-3}); Sulfisomezole 3.2×10^{-3}).

4. It may be suggested that the permeability coefficient of sulfisomezole from intestinal lumen (isotonic phosphate buffered solution at pH 6.0～6.4) into plasma is much larger than that from plasma into intestinal lumen.

5. The p_a-pH diagrams of four sulfonamides were obtained.

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