UDC 612.386[615.778.25]-084

191. Hisashi Nogami, Manabu Hanano, and Jun Watanabe: Studies on Absorption and Excretion of Drugs. III.*1 Kinetics of Penetration of Sulfonamides through the Intestinal Barrier *in Vitro*.*2

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The absorption of sulfonamides from the alimentary tract has been discussed by many investigators from a clinical point of view but few reports on the absorption mechanism of sulfonamides have been found.

The present report deals with the penetration of sulfonamides through the rat small intestinal barrier from the physicochemical standpoint. It has been already observed that the dissociated form of a drug (salicylic acid[1] or aminopyrine*1), the undissociated form of which is lipid-soluble, also penetrates through the rat intestinal barrier *in vitro*. In these cases, however, the penetration rate of the dissociated form is relatively smaller than that of the undissociated form. Generally sulfonamides are slightly soluble in organic solvents, and so it is doubtful that the penetration rate of undissociated form of some sulfonamides should be larger than that of dissociated form. Therefore, it is interesting to investigate whether the absorption mechanism for lipid-soluble drugs is applicable to sulfonamides or not.

In the present experiment, (a) the penetration mechanism of sulfathiazole was investigated by applying the kinetic consideration resembling that in the previous papers of this series,[1] and (b) the penetration rates of five sulfonamides (sulfanilamide, sulfathiazole, sulfaguanidine, sulfamethoxypyridazine, sulfisomezole) were compared at the same pH value.

Sulfathiazole, an amphoteric compound ($Ka=7.6\times10^{-8}$, $Kb=2.3\times10^{-12}$),[2] dissociates in both acid and alkaline solutions and if it penetrates through the intestinal barrier in three forms (alkaline, acid and undissociated forms) at the respective penetration

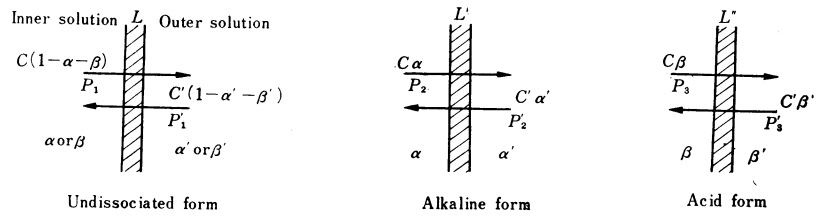

Fig. 1. Distribution of Drug between Inner and Outer Solution Separated by the Intestinal Barrier

C : concentration of sulfathiazole in inner solution
C' : concentration of sulfathiazole in outer solution
α : degree of dissociation of sulfathiazole as alkaline form in inner solution
α' : degree of dissociation of sulfathiazole as alkaline form in outer solution
β : degree of dissociation of sulfathiazole as acid form in inner solution
β' : degree of dissociation of sulfathiazole as acid form in outer solution
L, L', L'', P_1, P_2, P_3, P_1', P_2', P_3' : see the text

*1 Part II : This Bulletin, **9**, 1055 (1961).
*2 Presented before the Kanto Local Meeting of the Pharmaceutical Society of Japan, Tokyo, January, 1961.
*3 Hongo, Tokyo (野上 寿, 花野 学, 渡辺 淳).
1) H. Nogami, T. Matsuzawa : This Bulletin, **9**, 532 (1961).
2) P.H. Bell, R.O. Roblin : J. Am. Chem. Soc., **64**, 2905 (1942).

rates, the following theoretical equations are introduced to the penetration of sulfathiazole through the rat small intestinal barrier *in vitro*.

Fig. 1 shows an imaginary scheme of the penetration layers in the intestinal barrier, in which L, L', and L'' represent each layer which determines the respective penetration rates of undissociated and dissociated forms of the drug. Of course, there may be cases where L, L', and L'' are equal. The permeability coefficients per unit length of the intestinal segment are represented as P_1, P_2, P_3 (from mucosal to serosal side), P_1', P_2', and P_3' (from serosal to mucosal side) respectively for undissociated, alkaline, and acid forms. C is the concentration of drug solution circulating through the lumen (inner solution), and C' the concentration of drug which appeared in solution bathing the exterior of the intestine (outer solution). α is the degree of dissociation of alkaline form of the drug in the inner solution and α' in the outer solution, and β is the degree of dissociation of acid form of the drug in the inner solution and β' in the outer solution. Then each concentration of the drug on the respective sides of the intestinal barrier will be illustrated as shown in Fig. 1. If the penetration of the drug through the intestinal barrier occurs in the steady state under the experimental condition, the flux of the undissociated form from inner to outer solution, q_1, is defined as follows

$$q_1=\{P_1(1-\alpha-\beta)C-P_1'(1-\alpha'-\beta')C'\}\cdot l \tag{1}$$

where l is the length of the intestinal segment used. Similarly, for the flux of the alkaline form,

$$q_2=(P_2\alpha C-P_2'\alpha'C')\cdot l \tag{2}$$

and for the flux of the acid form,

$$q_3=(P_3\beta C-P_3'\beta'C')\cdot l \tag{3}$$

when the following assumption is satisfied,

$$P_1=P_1',\ P_2=P_2',\ P_3=P_3' \tag{4}$$

overall flux of the drug, q, is expressed by

$$\begin{aligned} q&=q_1+q_2+q_3 \\ &=l\cdot[P_1\{C(1-\alpha-\beta)-C'(1-\alpha'-\beta')\}+P_2(C\alpha-C'\alpha')+P_3(C\beta-C'\beta')] \end{aligned} \tag{5}$$

Matsuura studied the penetration of several sulfonamides and from the results[3] he suggested that sulfonamides do not penetrate actively through the rat small intestinal barrier *in vitro*. So equation (4) will be reasonable.

The total amount of the drug in outer solution, Q, is given by

$$Q=V'\cdot C' \tag{6}$$

where V' is the volume of the outer solution. When V' change is negligible in experiments,

$$q=\frac{dQ}{dt}=V'\frac{dC'}{dt} \tag{7}$$

Substituting for q in equation (5) gives

$$\begin{aligned} V'\frac{dC'}{dt}=l\cdot[&P_1\{C(1-\alpha-\beta)-C'(1-\alpha'-\beta')\}+P_2(C\alpha-C'\alpha') \\ &+P_3(C\beta-C'\beta')] \end{aligned} \tag{8}$$

3) H. Matsuura: Shikoku Acta Medica, **15**, 738 (1959).

If dC'/dt, C, C', and $\Delta C'$ are given in the following approximate forms

$$\frac{dC'}{dt}=\frac{\Delta C'}{\Delta t},\quad C=\frac{C_{30}+C_{60}}{2}=C_{av},\quad C'=\frac{C'_{30}+C'_{60}}{2}=C'_{av},$$
$$\Delta C'=C'_{60}-C'_{30},\quad \Delta t=30\ (\text{min.}),\quad V'=75\ (\text{cc.}) \qquad (9)$$

in the approximation, the equation (8) may be written

$$V'\frac{\Delta C'}{\Delta t}=l\cdot[P_1\{C_{av}(1-\alpha-\beta)-C'_{av}(1-\alpha'-\beta')\}+P_2(C_{av}\alpha-C'_{av}\alpha')$$
$$+P_3(C_{av}\beta-C'_{av}\beta')] \qquad (10)$$

where C_{30} or C_{60} is the value of C at $t=30$ or 60 (min.), and C'_{30} or C'_{60} is that of C'.

When U, X, Y, and Z are defined as follows

$$U=V'\cdot\frac{\Delta C'}{\Delta t},\quad X=l\cdot\{C_{av}(1-\alpha-\beta)-C'_{av}(1-\alpha'-\beta')\},$$
$$Y=l\cdot(C_{av}\alpha-C'_{av}\alpha'),\quad Z=l\cdot(C_{av}\beta-C'_{av}\beta') \qquad (11)$$

equation (10) now becomes

$$U=P_1X+P_2Y+P_3Z \qquad (12)$$

Then, P_1, P_2, and P_3 which satisfy the linear form, equation (12), will be statistically determined by means of the method of least squares from experimental data.

Experimental

Animals, outer solution, and a circulation apparatus were the same as described in the previous papers of this series.*[1,1)]

Inner Solution——Isotonic buffer solution for a warmblooded animals, consisting of 0.263M citric acid and 0.123M Na_2HPO_4, or consisting of 2.33% KH_2PO_4 and 1.44% $NaHCO_3$ was used. To that was added sulfonamide drug to give the drug concentration of about 100~500 mg./L.

Experimental Procedure——The experimental procedure was almost the same as described in the previous papers.*[1,1)]

Time, t, was regarded zero when 80 cc. of inner solution was poured into the upper chamber. To measure pH values and the drug concentrations, the aliquots of 5 cc. were taken out from inner and outer solutions at $t=30$ (min.). Then the aliquots of 1 cc. were taken out at $t=60$ (min.) and analyzed.

Analytical Method——After 5% trichloroacetic acid solution was added to the aliquots of 1 cc., and protein in the aliquots was removed by centrifuging, the concentrations of sulfonamides in the adequately diluted samples were determined colorimetrically according to the method of Tsuda.[4)] The optical density at 550 mμ was determined with Shimazu Spectrophotometer Model DF-II.

When the concentration of sulfathiazole in the intestinal tissue should be measured, protein was removed by centrifuging after 10% trichloroacetic acid solution was added to the homogenate of the intestinal tissue which has been weighed.

Results and Discussion

Figs. 2~4 suggest that some assumptions used in the above equations are not unreasonable. Fig. 2 shows that the concentration of sulfathiazole in outer solution is proportional to that in inner solution at the same pH value at any time when t is greater than 30 (min.).

In Fig. 3, the influence of pH value of inner solution on the penetration rate of

4) K. Tsuda, *et al.*: Yakugaku Zasshi, **62**, 362 (1942).

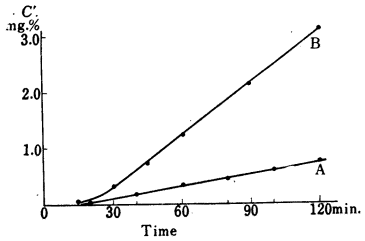

Fig. 2. Diagram Illustrating the Proportionality of the Drug Concentration in Outer Solution, C' (mg.%), to That in Inner Solution.

A: Isotonic buffer solution (pH 7.0) containing 10 mg.% sulfathiazole was used as inner solution
B: Isotonic buffer solution (pH 7.0) containing 50 mg.% sulfathiazole was used as inner solution

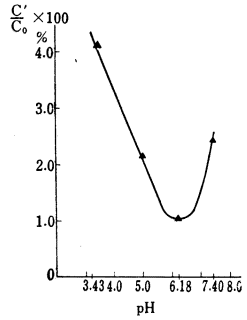

Fig. 3. Curve Illustrating the Relationship between Penetration Rate ($C'/C_0 \times 100$) of Sulfathiazole and pH Value in Inner Solution at $t=60$ (min.)

C': Concentration of sulfathiazole in outer solution
C_0: Initial concentration of sulfathiazole in inner solution

sulfathiazole is found, and the penetration of the drug can be considered to occur in both undissociated and dissociated forms.

Fig. 4 shows that the concentration of sulfathiazole in the small intestinal segment is constant after $t=30$; this is taken to indicate that experiments were carried out in the steady state.

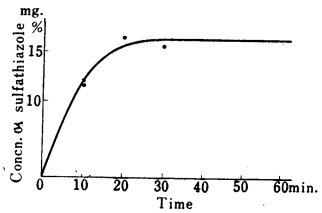

Fig. 4. Diagram Illustrating the Relationship between the Concentration of Sulfathiazole in Intestinal Tissue and Time

The relative values of C_{av}, C'_{av}, and $\Delta C'$ at the various pH values of inner solution, that is, at the various degrees of dissociation of sulfathiazole, are listed in Table I. From the observed values, U, X, Y, and Z were calculated by applying equation (11).

Then, P_1, P_2, and P_3 were statistically determined by means of the method of least squares.[5] The values of P_1, P_2, and P_3 ($\pm 95\%$ confidence limit) are $(6.40\pm1.53)\times10^{-4}$, $(9.79\pm1.50)\times10^{-3}$, and $(1.32\pm0.20)\times10^{-3}$ (cc./cm./min.), respectively. That is to say, when the other conditions are equal, sulfathiazole in alkaline form penetrates the intestinal barrier about fifteen times faster than in undissociated form, and about seven times faster than in acid form. Thus the penetration of sulfathiazole is different from that of lipid-soluble durg (salicylic acid or aminopyrine) of which the dissociated form penetrates through rat's small intestine more slowly than the undissociated form. But further investigations will be necessary to find out whether the penetration mechanism of sulfathiazole is different from that of lipid-soluble drug or not. From equation (12) values of U were calculated by using the values of P_1, P_2, and P_3. A relationship between the calculated and observed values of U is illustrated in Fig. 5, which shows the obvious correlation between them.

TABLE I. Original Data and Calculated Values

Rat No.	Inner solution C_{av}	pH	β	Outer solution C'_{av}	pH	β'	$\Delta C'$	$U_{calcd.}$		$U_{obsd.}$
1	20.04	7.97	0.875	0.445	7.94	0.867	0.490	1.093		1.225
2	19.40	8.11	0.906	0.470	7.85	0.843	0.440	1.074	±0.157	1.100
3	19.48	8.25	0.930	0.191	8.04	0.891	0.342	1.114		0.855
4	19.18	8.30	0.937	0.246	7.88	0.851	0.372	1.092		0.930
5	19.38	7.29	0.595	0.298	7.40	0.655	0.392	0.899		0.980
6	19.54	7.31	0.610	0.334	7.41	0.662	0.424	0.914	±0.107	1.060
7	18.78	7.69	0.790	0.311	7.94	0.867	0.418	0.979		1.045
8	19.22	6.60	0.232	0.140	7.69	0.789	0.168	0.683		0.420
9	20.10	6.62	0.240	0.152	7.71	0.796	0.184	0.719		0.460
10	19.28	6.66	0.258	0.128	7.94	0.867	0.216	0.701	±0.093	0.540
11	18.80	6.64	0.249	0.238	7.81	0.829	0.238	0.673		0.595
12	18.68	6.64	0.249	0.275	7.90	0.792	0.350	0.667		0.875
13	18.68	6.44	0.175	0.201	7.87	0.848	0.314	0.628		0.785
14	19.20	6.42	0.169	0.210	7.88	0.850	0.334	0.642	±0.095	0.835
15	18.74	6.43	0.172	0.209	7.95	0.870	0.358	0.627		0.895
16	19.54	6.53	0.206	0.087	8.11	0.906	0.174	0.669		0.435
			α			β'				
17	19.50	4.80	0.004	0.223	7.75	0.810	0.238	0.481		0.595
18	18.06	4.90	0.003	0.121	7.94	0.867	0.242	0.474	±0.132	0.605
19	18.22	4.82	0.004	0.060	7.98	0.877	0.120	0.503		0.300
20	18.68	3.07	0.164	0.537	6.89	0.370	0.666	1.710		1.665
21	18.86	3.04	0.173	0.505	7.20	0.545	0.678	1.763		1.695
22	18.84	3.03	0.176	0.548	7.24	0.567	0.696	1.768	±0.164	1.740
23	17.72	3.09	0.158	0.592	6.78	0.317	0.696	1.568		1.740
24	16.72	3.01	0.184	0.518	7.40	0.655	0.588	1.598		1.470

Values for P_1, P_2, and P_3 (±95% confidence limit)
$P_1=0.00064\pm0.00015$, $P_2=0.00979\pm0.00150$, $P_2=0.00132\pm0.00020$ (cc./cm./min.)

Fig. 6 shows a relationship between the penetration rates of sulfathiazole and pH values of inner solution, since the values of U represent the relative penetration rates of sulfathiazole in various cases. All the observed values of U are included in the 95% confidence band.

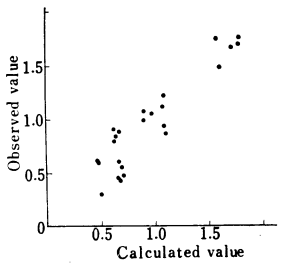

Fig. 5. Relationship between Observed and Calculated Values of U

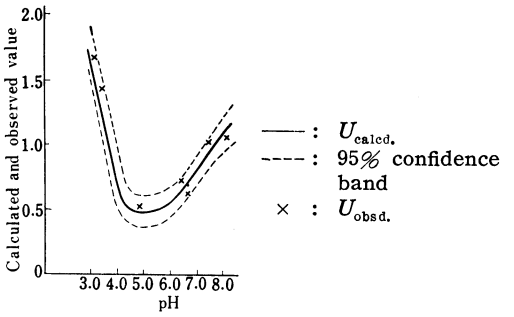

Fig. 6. Curve Illutrating the Relationship between the Penetration Rates of Sulfathiazole and pH Values of Inner Solution

Furthermore, in order to examine the validity of equation (8), the following experiment was attempted. From condition for the equilibrium state, equation (7) gives

$$\frac{dQ}{dt}=0$$

Therefore, from equation (8)

$$P_1\{C(1-\alpha-\beta)-C'(1-\alpha'-\beta')\}+P_2(C\alpha-C'\alpha')+P_3(C\beta-C'\beta')=0 \quad (13)$$

$$\frac{C}{C'}=\frac{P_1(1-\alpha'-\beta')+P_2\alpha'+P_3\beta'}{P_1(1-\alpha-\alpha)+P_2\alpha+P_3\beta} \quad (14)$$

When an equilibrium system is accomplished, the concentration ratio is described as equation (14). Accordingly, if the experiment is made under the condition of $P_1=0.00064$, $P_3=0.00132$, $\beta'=0.786$ (pH=7.68), $\beta=0.129$ (pH=6.29), $C=16.1$ mg. %, and $C'=10.0$ mg. %, the concentration ratio of 1.61 will be kept unchanged. Similarly, when the experiment is carried out under the condition of $P_1=0.00064$, $P_2=0.00979$, $P_3=0.00132$, $\beta'=0.093$ (pH=6.13), $\alpha=0.194$ (pH=2.98), $C=10.0$ mg. %, and $C'=34.3$ mg. %, the concentration ratio of 0.2912 will be maintained at the same value throughout the experiment.

The concentration change of the drug in the outer solution was investigated under the condition mentioned above. The results are given in Table II.

Table II. Results obtained from the Experiment at Equilibrium State

Inner solution ($C=16.1$ mg. %, pH : 6.29, $\beta=0.129$)
Outer solution ($C'=10.0$ mg. %, pH : 7.68, $\beta'=0.786$)

Exptl. No.	Concn. of outer solution $(C'/C'_0)\times 100$		
	30	60	90
1	98.2	100.0	101.0
2	98.5	98.5	99.8
3	99.8	101.8	101.2

Inner solution ($C=10.0$ mg. %, pH : 2.98, $\alpha=0.194$)
Outer solution ($C'=34.3$ mg. %, pH : 6.13, $\beta'=0.093$)

Exptl. No.	Concn. of outer solution $(C'/C_0')\times 100$		
	30	60	90
4	93.9	92.5	86.6
5	97.2	93.4	91.3
6	97.8	93.8	90.6

As shown in Table II, the concentration of sulfathiazole in the outer solution was kept almost constant when the experiment was made under the condition of $\beta'=0.786$, $\beta=0.129$, $C=16.1$ mg. %, and $C'=10.0$ mg. %. But the satisfactory result was not obtained in the experiment under the condition of $\beta'=0.093$, $\alpha=0.194$, $C=10.0$ mg. %, and $C'=34.3$ mg. %. This result means probably that the intestinal wall was not maintained at the constant condition because of the low pH value (2.98) of the inner solution. Especially it may be meaningless to carry out the experiment after $t=30$ (min.) under these conditions.

Secondly, the penetration rates of five sulfonamides were investigated at the same pH value. The pH value of inner solution was 6.0, and that of outer solution was 7.2. The results are illustrated in Fig. 7.

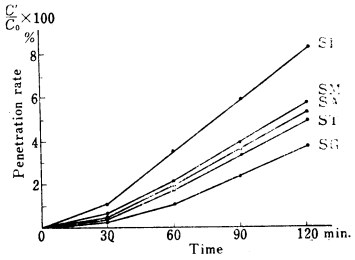

Fig. 7. Diagrm Illustrating the Penetration Rates for Five Sulfonamides

SI : Sulfisomezole
SM : Sulfamethoxypyridazine
SA : Sulfanilamide
ST : Sulfathiazole
SG : Sulfaguanidine

As shown in Fig, 7, the penetration rates for five sulfonamides are in the following order : sulfisomezole > sulfamethoxypyridazine > sulfanilamide > sulfathiazole > sulfaguanidine. Sulfaguanidine was more permeable than expected.

5) W.E. Deming : "Statistical Adjustment of Data" (1946). John Wiley & Sons, Inc., New York.

Generally sulfaguanindine does not seem to penetrate through the small intestine *in vivo*; thus the above results suggest that there may be some differences between studies on the penetration *in vitro* and these on the absorption *in vivo*.

The authors thank Dr. T. Matsuzawa for his kind advices on the experimental technique and Mr. H. Toguchi for his technical assistance in the experiment. This work was supported by the Grant-in-Aid for Scientific Research provided by the Ministry of Education, to which they are also grateful.

Summary

1. The penetration of sulfonamides through the rat small intestine was investigated from the physicochemical standpoint *in vitro*.

2. Theoretical equations for the penetration mechanism of sulfathiazole were derived from the assumption that it penetrates through intestinal barrier in its three forms at the respective penetration rates.

3. From the data obtained, the respective permeability coefficients for the undissociated, alkaline and acid forms of sulfathiazole, P_1, P_2, and P_3 were statistically determined. The estimated values of P_1, P_2, and P_3 were 0.00064, 0.00979, 0.00132 (cc./cm./min.), respectively.

4. The experiment at the equilibrium state was attempted in order to examine the validity of the theoretical equation. Satisfactory results are obtained for P_1 and P_3, but not for P_2.

5. The penetration rates of five sulfonamides were compared at the same pH value. The penetration rates were in the following order : sulfisomezole>sulfamethoxypyridazine>sulfanilamide>sulfathiazole>sulfaguanidine.

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192. Takeo Ueda, Shigeshi Toyoshima, Tadakazu Tsuji, and Sumiko Watanabe : Synthesis and Antiviral Effect of Hexahydro-*s*-triazines.

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The antiviral activity of several triazine derivatives on Japanese encephalitis and influenza viruses has been examined by our research group.[1,2)]

In connection with these studies, hexahydro-*s*-triazine derivatives were synthesized for the purpose of examining their antiviral activities.

This paper concerns with the synthesis and the antiviral properties of alkyl derivatives of 2-iminohexahydro-*s*-triazine, tetrahydro-*s*-triazine-2(1*H*)-thione and tetrahydro-*s*-triazin-2(1*H*)-one.

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1) T. Tsuji : This Bulletin, **2**, 403 (1954).
2) I. Nakata, T. Ueda : Yakugaku Zasshi, **80**, 1068 (1960).