method as described above afforded 0.08 g. (12%) of 4-(1-nitroethyl)quinazoline (V) as pale yellow needles, m.p. 142° from H_2O. *Anal.* Calcd. for $C_{10}H_9O_2N_3$ (4-(1-nitroethyl)quinazoline) : C, 59.12; H, 4.46, N, 20.68. Found : C, 59.15; H, 4.29; N, 20.47.

The author expresses his deep gratitude to Prof. Emeritus E. Ochiai of the University of Tokyo, to Dr. T. Ukai, Dean of this College, and to Prof. E. Hayashi of this College, for their unfailing guidance and encouragement throughout the course of this work. The author is indebted to Miss. Y. Saito of this College for microanalytical data.

The expenses for this work was partly defrayed by Grant-in-Aid of Scientific Research for 1961 from the Ministry of Education, which is gratefully acknowledged.

Summary

The reactions of 4-quinazolinecarbonitrile (I) with active methylene compounds were carried out in order to elucidate the chemical properties of (I).

In the presence of sodium amide, reactions of (I) with ethyl acetoacetate, diethyl malonate, or ethyl cyanoacetate give ethyl 4-quinazolineacetate (II), and ethyl α-cyano-4-quinazolineacetate (III), respectively.

In the presence of potassium carbonate, reactions of (I) with nitromethane, or nitroethane give 4-nitromethylquinazoline (IV), and 4-(1-nitroethyl)quinazoline (V), respectively.

The foregoing results showed that the 4-position of (I) was very reactive to anionoid reagents, as was already demonstrated in Parts I, II, and III of this series.

(Received September 30, 1961)

UDC 612.386[615.7]-84

170. Hisashi Nogami and Tai Matsuzawa*[1] : Studies on Absorption and Excretion of Drugs. II.*[2] Kinetics of Penetration of Basic Drug, Aminopyrine, through the Intestinal Barrier *in vitro*.*[3]

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In the previous paper,*[2] a penetration of drug through the rat small intestine was investigated from the physico-chemical standpoint and the theoretical equations were derived from the assumption that the absorption of foreign organic electrolytes was carried out by a simple diffusion process with each specific permeability coefficient for both undissociated and dissociated forms. A previous study on the penetration of salicylic acid demonstrated that its penetration rate was dependent on the degree of dissociation of salicylic acid and the penetration process was thoroughly explainable from the basis of the above assumption. The present report describes the intestinal penetration of aminopyrine (pKa : 5.00) as a representative of basic drugs. Generally, the amount of the drug, *C*, appeared in the outer solution from the inner solution at

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*2 Part I : H. Nogami and T. Matsuzawa : This Bulletin, **9**, 532 (1961).

*3 Presented before the Kanto Local Meeting of the Pharmaceutical Society of Japan, Tokyo, February, 1961.

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any time t was given by the following equation as described previously.*2

$$ln[\{(1-\alpha)P_u+\alpha P_i\}C_0-\{(2-\alpha-\beta)P_u+(\alpha+\beta)P_i\}C] = -\frac{l}{V}\{(2-\alpha-\beta)P_u+(\alpha+\beta)P_i\}t+ln[\{(1-\alpha)P_u+\alpha P_i\}C_0] \quad (1)$$

Where P_u and P_i represent the respective permeability coefficients per unit length of the intestinal segment for the undissociated and dissociated forms of the drug, α and β the respective degrees of dissociation of the drugs in the inner and outer solutions, C_0 the initial drug concentration in the inner solution, l the length of intestinal segment used, and V the volume of the inner and outer solutions, respectively. In the case of penetration of aminopyrine from the intestine, $\beta \fallingdotseq 0$, since Krebs-Ringer bicarbonate solution was employed as an outer solution and its pH value was about 7.4 to 7.6. Then, equation (1) can be written.

$$ln[\{(1-\alpha)P_u+\alpha P_i\}C_0-\{(2-\alpha)P_u+\alpha P_i\}C] = -\frac{l}{V}\{(2-\alpha)P_u+\alpha P_i\}t+ln[\{(1-\alpha)P_u+\alpha P_i\}C_0] \quad (2)$$

In the special case where $\alpha \fallingdotseq 0$, namely, aminopyrine is almost totally undissociated in the inner solution, equation (2) becomes

$$ln(C_0-2C) = -\frac{2l}{V}P_u \cdot t+lnC_0 \quad (3)$$

On the other hand, when aminopyrine is almost completely dissociated, $\alpha \fallingdotseq 1$. Equation (2) is given by the following form,

$$ln\left\{C_0-\left(\frac{P_u+P_i}{P_i}\right)C\right\} = -\frac{l}{V}(P_u+P_i)\cdot t+lnC_0 \quad (4)$$

The penetration process of aminopyrine was investigated based on application of the above equations.

Experimental

All the experimental methods, the rat intestine preparation, the circulation apparatus, the constitution of the inner and outer solutions, and the experimental procedure, are the same as those reported in the previous paper.*2

Determination of Aminopyrine——Aminopyrine was added to the inner solution to give the concentration of about 500~1000 mg./L.*5 Aminopyrine was determined by spectrophotometry described by Brodie, *et al.*[1] Analytical method is as follows: Pipette 1 cc. of sample at the outer solution*6 into 50 cc. glass-stoppered bottle. Add 0.5 cc. of N NaOH and 20 cc. of ethylene dichloride. Shake for 30 min. and centrifuge. Remove the aqueous phase by aspiration. Transfer 15 cc. of the solvent phase to a 50 cc. glass-stoppered bottle containing 5 cc. of 0.1N HCl. Then, shake for 15 min. and centrifuge. Transfer about 5 cc. of the aqueous phase to a cuvette and determine the optical density at 260 mμ with the spectrophotometer.

Results and Discussion

When pH values of inner solution were varied, the amount of aminopyrine appeared in the outer solution was determined as a function of time, t. The samples of

*5 The solution containing 1000 mg./L. of aminopyrine was employed in the case of relative rapid penetration.

*6 When the concentration of the inner solution was determined, each sample was diluted properly with the buffer used.

1) B.B. Brodie and J. Axelrod: J. Pharmacol. Exptl. Therap., **99**, 171 (1950).

TABLE I. Penetration of Aminopyrine at Various pH Values

$$y(\%)=\frac{\text{Concentration of aminopyrine } (C) \text{ at time } (t)}{\text{Initial concentration of aminopyrine } (C_0)}\times 100$$

pH (α)	t_1 (min.)	y_1(%) Observed value	y_1(%) Calcd. value	t_2 (min.)	y_2(%) Observed value	y_2(%) Calcd. value	t_3 (min.)	y_3(%) Observed value	y_3(%) Calcd. value	t_4 (min.)	y_4(%) Observed value	y_4(%) Calcd. value
3.54	10	0.12		15	0.22		20	0.31		25	0.51	
(1.00)	10	0.11		15	0.17		20	0.31		25	0.50	
	10	0.10		15	0.18		20	0.33		25	0.47	
	10	0.05		15	0.17		20	0.31		25	0.44	
	mean	0.10	0.14	mean	0.18	0.22	mean	0.32	0.29	mean	0.48	0.36
4.17	10	0.23		20	0.56		30	0.90		40	1.33	
(0.871)	10	0.25		20	0.60		30	0.94		40	1.34	
	10	0.39		20	0.64		30	0.94		40	1.31	
	mean	0.29	0.32	mean	0.60	0.63	mean	0.93	0.93	mean	1.33	1.24
4.54	10	0.50		20	1.14		30	1.67		40	2.31	
(0.734)	10	0.49		20	1.00		30	1.54		40	1.98	
	10	0.53		20	1.03		30	1.57		40	2.09	
	10	0.54		20	1.05		30	1.67		40	2.27	
	mean	0.52	0.50	mean	1.06	0.99	mean	1.61	1.47	mean	2.16	1.95
4.80	10	0.51		20	1.08		30	1.69		40	2.28	
(0.613)	10	0.77		20	1.48		30	2.11		40	2.99	
	10	0.66		20	1.31		30	2.00		40	2.67	
	10	0.56		20	1.08		30	1.69		40	2.43	
	10	0.64		20	1.40		30	2.37		40	3.21	
	mean	0.63	0.66	mean	1.27	1.31	mean	1.97	1.94	mean	2.72	2.56
5.08	10	0.92		20	1.74		30	2.62		40	3.52	
(0.454)	10	0.83		20	1.78		30	2.60		40	3.50	
	10	0.84		20	1.72		30	2.51		40	3.35	
	10	0.74		20	1.48		30	2.18		40	2.99	
	10	0.86		20	1.77		30	2.85		40	3.87	
	mean	0.84	0.87	mean	1.70	1.73	mean	2.55	2.56	mean	3.45	3.37
5.32	10	0.81		20	1.70		30	2.50		40	3.45	
(0.324)	10	0.87		20	1.92		30	2.90		40	3.87	
	10	1.03		20	2.12		30	3.14		40	4.50	
	10	1.21		20	2.46		30	3.82		40	5.02	
	10	1.04		20	2.23		30	3.11		40	4.26	
	mean	0.99	1.04	mean	2.09	2.06	mean	3.09	3.06	mean	4.22	4.02
5.62	10	1.18		20	2.37		30	3.46		40	4.65	
(0.194)	10	0.98		20	2.17		30	3.39		40	4.72	
	10	1.29		20	2.66		30	3.81		40	5.44	
	10	0.95		20	1.85		30	3.02		40	4.12	
	10	1.01		20	2.35		30	3.77		40	5.06	
	mean	1.08	1.22	mean	2.28	2.40	mean	3.49	3.55	mean	4.80	4.67
6.35	10	1.13		20	2.48		30	4.03		40	5.33	
(0.043)	10	0.87		20	2.25		30	4.40		40	5.33	
	10	2.53		20	2.80		30	4.22		40	5.51	
	10	1.39		20	2.84		30	4.27		40	5.94	
	10	0.68		20	1.72		30	3.24		40	4.90	
	10	1.21		20	1.99		30	3.20		40	5.16	
	mean	1.30	1.42	mean	2.35	2.79	mean	3.71	4.13	mean	5.36	5.43
7.01	10	1.84		20	3.21		30	4.34		40	5.84	
(0.00)	10	2.20		20	3.55		30	5.22		40	6.66	
	10	1.43		20	3.06		30	5.05		40	6.66	
	10	1.17		20	2.88		30	4.48		40	5.93	
	10	1.53		20	3.27		30	4.88		40	7.00	
	mean	1.63	1.47	mean	3.19	2.91	mean	4.79	4.30	mean	6.42	5.64

inner and outer solutions were taken after 5 minutes of the perfusion to give each initial concentration. This period was enough to establish the steady state in the intestinal barrier, because the penetration rate of aminopyrine was considerably slow. The observed concentration ratios C/C_0 have been listed in the column of the observed value in Table I.

y_1, y_2, y_3, and y_4 are the values of C/C_0 at the time t_1, t_2, t_3, and t_4, respectively. From these experimental data obtained, verification of the assumption that penetration process of aminopyrine proceeds according to equation (1) is confirmed by the following three manners. First evidence that the change in aminopyrine concentration may be defined by equation (1) was obtained from equation (3) and the data at pH 7.01. On plotting $\log(1-2C/C_0)$ against t, the straight lines were obtained and there was no marked difference between their slopes each other. Consequently, the permeability coefficient for the undissociated form, P_u, was derived from the slopes of these curves. An example of the curves is given in Fig. 1.

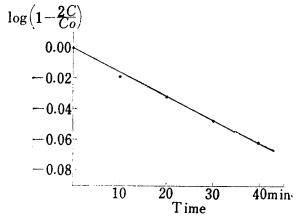

Fig. 1. Curve illustrated the Linear Relationship between the Logarithmic Function and Time at pH 7.01

P_i, the permeability coefficient for the dissociated form, was also determined graphically by substituting the above P_u value into equation (4) and using the data obtained at pH 3.54.

The second evidence was obtained from the following statistical adjustment of the observed values. By substituting the above estimated values of P_u and P_i into equation (2) and using the respective mean values of y_1, y_2, y_3, and y_4 at the all pH levels, P_u and P_i at each series of y have been statistically determined by means of the method of least squares. The values of P_u and P_i obtained here are given in Table II.

Table II. Values for P_u and P_i obtained by the Method of Least Squares (mean ± 95% confidence limit)

	$P_u \times 10^3$ (cc./cm./min.)	$P_i \times 10^4$ (cc./cm./min.)
y_1	2.61 ± 0.22	1.90
y_2	2.59 ± 0.24	2.59
y_3	2.63 ± 0.25	2.61
y_4	2.79 ± 0.16	2.60
mean	2.66	2.42

The statistical treatment of each series of y was used to give a reason for supposing that no distinct physiological change in the intestine which had influence on the penetration rate was recognized during the experimental period. It is evident from Table II that there is no significant difference between each value of the parameters obtained at the respective periods. The calculated values in the column of Table I have been calculated by substituting the mean values of P_u and P_i in Table II into equation (2). Fig. 2 illustrates a relationship between the observed and the calculated values and shows that there is an obvious correlation between them.

Fig. 3 also shows that good agreement exists between the calculated curves defined by equation (2) and the experimentally observed values.

Relationship between the degree of dissociation and the rate of penetration at each period is illustrated in Fig. 4, which demonstrates that the penetration rate of aminopyrine decreases as its degree of dissociation becomes near unity.

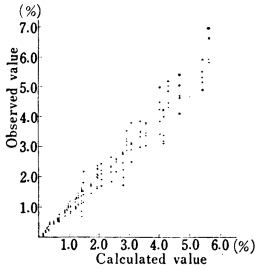

Fig. 2. Diagram illustrated the Relationship between Observed and Calculated Values

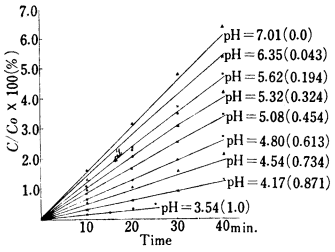

Fig. 3. Diagram illustrated the Relationship between the Theoretical Curves and the Observed Values

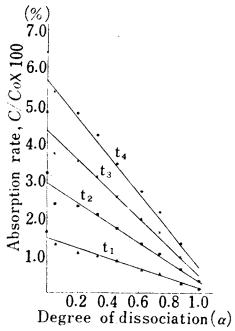

Fig. 4. Diagram illustrated the Relationship between Penetration Rate and Degree of Dissociation after 10, 20, 30, and 40 min.

The more direct evidence was obtained from the experiment which was designed to investigate the penetration mechanism at equilibrium state. At equilibrium state.

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

Then, in the case of penetration of aminopyrine the concentration ratio C/C' is given by the following equation as mentioned previously,*1

$$\{(1-\alpha)P_u+\alpha P_i\}C'-P_uC=0$$

$$\frac{C}{C'}=\frac{(1-\alpha)P_u+\alpha P_i}{P_u} \qquad (5)$$

where C' is the concentration of aminopyrine in the inner solution at t. By substituting the mean values of P_u and P_i into equation (5), the ratio can be determined at any pH level. In the case where $P_u=0.00266$ (cc./cm./min.), $P_i=0.000242$ (cc./cm./min.), and $\alpha=0.448$(pH=5.09), the ratio becomes 0.593 and the transfer of aminopyrine does not occur, since equilibrium has been established. The concentration changes in both inner and outer solutions were investigated by employing the outer solution containing 0.593 times as that in inner and the result is given in Table III.

Table III. Result obtained from the Experiment at Equilibrium State

Exp. No.	Concentration of inner solution C' (γ/cc.)		Concentration of outer solution C (γ/cc.)			
	0 (min.)	30 (min.)	0 (min.)	10 (min.)	20 (min.)	30 (min.)
1	191	190	109	106	108	108
2	183	182	105	102	106	102
3	191	192	111	108	109	109
4	187	186	106	103	108	106

The data in Table III indicate that each aminopyrine concentration in the both solutions was almost kept constant eventually though it only slightly increased or decreased during the course of the experiment and therefore demonstrate the validity of estimated values for P_u and P_i.

The preceding evidences support the conclusion that the penetration process of aminopyrine as well as salicylic acid would proceed at the rate defined by equation (1). In other words, aminopyrine penetrates the intestinal barrier by simple diffusion with

each specific coefficient for both undissociated and dissociated forms of it and the penetration rate is governed by the dissociation of aminopyrine and by the pH value of the intestinal contents. Furthermore, as the ratio of P_u to P_i for aminopyrine is about 11:1, it may be concluded that aminopyrine is absorbed largely in the undissociated form, whereas the absorption of its dissociated form is negligible. It seems that this fact is consistent with Overton's hypothesis that a lipoid layer separates living cells from their environment. In this respect, absorption of aminopyrine differs from that of salicylic acid. Finally, the result presented in this paper also shows that aminopyrine which has high lipid solubility and is largely in the undissociated form at the pH of the intestinal contents is poorly absorbed in comparison with salicylic acid and that it is interesting that a similar conclusion as to the poor absorption of aminopyrine has been reached by Hogben, *et al.*[2)]

The authors wish to thank to Dr. M. Hanano for his advices on the statistical treatment and to Mr. J. Watanabe for his technical assistance in the experiment. This work was supported by a Grant-in-Aid for Scientific Research provided by the Ministry of Education, to which the authors are also grateful.

Summary

1) The intestinal absorption of aminopyrine as a representative of the basic drugs was investigated to prove the validity of equation (1) described previously.

2) From the three evidences, that is, a good fit of the data at pH 7.01 to equation (3), the statistical treatment of all the data, and the experiment at equilibrium state, it is concluded that aminopyrine penetrates the intestinal barrier with each specific coefficient, P_u and P_i, according to equation (1).

3) Respective values of the coefficients were statistically determined and the estimated value of P_u was 0.00266 (cc./cm./min.) and that of P_i 0.000242 (cc./cm./min.). From the ratio of P_u/P_i, it may be expected that aminopyrine is absorbed in its undissociated form according to Overton's hypothesis.

4) A fact that aminopyrine slowly penetrates the intestine is consistent with the result reported by Hogben, *et al.*

(Received September 8, 1961)

2) C. A. M. Hogben, *et al.* : J. Pharmacol. Exptl. Therap., **125**, 275 (1959).