rocking vibration and lower is C-S stretching vibration in CH_3-S group.

The S-N band near 900 cm^{-1} region of methanesulfonamide and N-monosubstituted compounds shifted to lower wave number about 72~20 cm^{-1} on N-deuteration, as shown in Table II and Fig. 3. The definitely position of S-N stretching vibration can be decided by to examine the spectral shift on N-deuteration of sample.

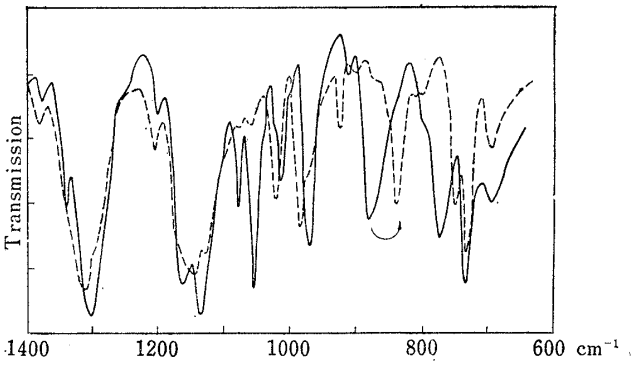

Fig. 3. Infrared Spectra of $CH_3SO_2NHCH_2C_6H_5$ (solid line) and $CH_3SO_2NDCH_2C_6H_5$ (broken line)

The authors wish to thank Prof. T. Uno of Kyoto University for many helpful discussions and suggestions during this work.

Summary

The infrared spectra of methanesulfonamide derivatives and their N-deuterated compounds were measured. The S-N stretching vibrations were recognized between 947~836 cm^{-1} as in benzenesulfonamide derivatives. The spectral shift of N-deuterated compounds were recognized lower wave number region about 72~20 cm^{-1} than ordinary compounds. In addition the S-N bands of N-monosubstituted compounds is located in the lower wave number region than N,N-disubstituted compounds.

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[Chem. Pharm. Bull. 13(7) 861~869 (1965)]

UDC 615.778.25

112. Kiichiro Kakemi, Takaichi Arita, and Shozo Muranishi : Absorption and Excretion of Drugs. XXV.*1 On the Mechanism of Rectal Absorption of Sulfonamides.

(*Faculty of Pharmaceutical Sciences, Kyoto University**2)

It is well known that a number of drugs are readily absorbed through the rectum, and the elucidation of the mechanism of rectal absorption is important for a view-point of dosage schedule. Concerning the rectal absorptions, the reports have been used to evaluate the level of drug in blood[1,2] or urine[3,4] following the rectal administration in rabbit, and the other[5] measured the residual amount in the rectum of rat by an isotope technique. Because most of studies have involved different techniques and various

*1 Part XXIV : This Bulletin, **13**, 551 (1965).

*2 Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto (掛見喜一郎, 有田隆一, 村西昌三).

1) A. F. Cacchillo, W. H. Hassler : J. Am. Pharm. Assoc., Sci. Ed., **43**, 683 (1954).
2) L. Pennati, K. Steiger-Trippi : Pharm. Acta Helv., **33**, 663 (1958).
3) H. Hoffmann, U. H. Hornbogen : Pharm. Zentralhalle, **89**, 369 (1950).
4) J. Büchi, P. Oesch : Pharm. Acta Helv., **20**, 29 (1945).
5) S. Riegelman, W. J. Crowell : J. Am. Pharm. Assoc., Sci. Ed., **47**, 115, 123, 127 (1958).

bases incorporated, however, it is difficult to recognize which properties of drug govern its rates of absorption.

In this study, the absorption of sulfonamides from aquoeus solution was studied, using a recirculation technique *in situ* for the rectum in order to clarify the behavior of rectal membrane. Schanker and Hogben[6~9] suggested that a large number of drugs penetrate the gastro-intestinal blood barrier by a passive diffusion of the unionized drug moiety across a membrane having lipoid characteristics. This paper presents the application of this concept to the rectal absorption.

The results suggest that sulfonamides are readily absorbed in the unionized form through the rectal membrane, which is lipoidal in character and has the slightly acidic zone at the rectal epithelial border like the blood-small intestine and blood-colon barrier.

Experimental

Absorption Experiments——Male rats weighing 200 to 230 g. were anesthetized with sodium pentobarbital by interperitoneal injection in 4.0 to 4.5 mg. per 100 g. The rectum was exposed by a midline abdominal incision, and was cannulated at the end of the colon with polyvinyl tubing (having an outside 5 mm. and a wall 1 mm. thick). Glass cannula was then inserted into the anus, and was secured by a ligature at

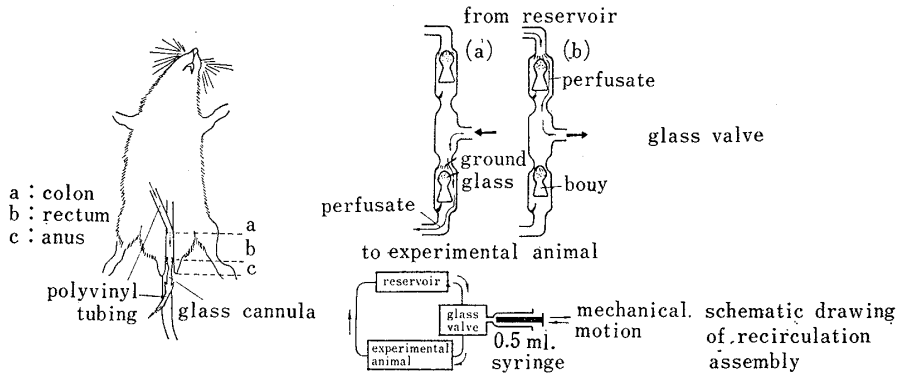

Fig. 1. Diagram showing Position of the Rectum and Cannula, and Recirculation Apparatus

Table I. Salts Components used for Isotonic Buffer in Rat

pH	Citric acid (g.)	$Na_2HPO_4 \cdot 12H_2O$ (g.)	KH_2PO_4 (g.)	$NaHCO_3$ (g.)	Na_2CO_3 (g.)	NaCl (g.)	H_2O
2.1	49.8	4.4	—	—	—	—	
2.5	38.7	13.2	—	—	—	—	
2.9	27.7	22.1	—	—	—	—	
3.3	22.1	26.5	—	—	—	—	
3.8	16.6	30.9	—	—	—	—	
4.8	11.1	35.3	—	—	—	—	to make
5.5	8.3	37.5	—	—	—	—	1000 ml.
6.3	5.5	39.7	—	—	—	—	
6.9	—	—	14.0	5.8	—	—	
7.1	—	—	9.3	8.6	—	—	
7.6	—	—	4.7	11.5	—	—	
9.0	—	—	—	7.6	0.5	1.6	
10.6	—	—	—	0.8	4.8	4.4	

6) L. S. Schanker, D. J. Tocco, B. B. Brodie, *et al.* : J. Pharmacol. Exptl. Therap., **123**, 81 (1958).
7) L. S. Schanker, A. Shore, *et al.* : *Ibid.*, **120**, 528 (1957).
8) A. M. Hogben, L. S. Shanker, *et al.* : *Ibid.*, **120**, 540 (1957).
9) A. M. Hogben, D. J. Tocco, *et al.* : *Ibid.*, **125**. 275 (1959).

the hollow of glass cannula, connecting polyvinyl tubing at the other end (Fig. 1). The length of the rectum was 3 cm. The rectal lumen was washed with saline until the washings were clear and then two times with drug solution warmed to body temperature. The incision was closed and the tubings attached to the inflow and outflow cannulae were then transferred to a flask containing 20 ml. of drug solution. This volume was then continuously circulated through the rectum lumen by the apparatus (shown in Fig. 1) with the circulation flow rate of 5 ml./min. at 37°. The drug solution which contained 0.5 mmole /L. of sulfonamides was prepared with isotonic buffered solution, shown in Table I.

0.5 ml. of the sample solution was pipetted at 20, 40, 60, 90 and 120 min. after zero time. Zero time was set at 10 min. after the start of recirculation. The absorption velocity constant was calculated from a decrease of the concentration in the drug solution.

Phenol red of tetraethylammonium bromide, which were expected to be unabsorbed, were dissolved in the drug solution to indicate any volume change. The concentrations of the drug and indicator were determined at the zero and final times. The concentrations of both indicators did not change in any drug solutions. The pH values of the solution after recirculation remained almost constant or changed less then 0.3 unit. The drug solution below pH 2.0, were not used as the blood and lumen were denatured due to its acidity.

***in vitro* Permeation Experiments**——A circulation apparatus was employed following the Wiseman's[10] so that the rectum 3 cm. long was able to be attached. Male rats weighing 200 to 230 g. was killed by blow on the head, the abdomen was opened by a midline incision, and the rectum was then removed by cutting across the lower end of the colon and the anus. The rectum was washed out twice with saline warmed to body temperature, and this lumen was attached in position of the circulation unit. 20 ml. of pH 7.2 isotonic buffer solution (KH_2PO_4, $NaHCO_3$), containing 0.1% glucose, was transferred into lower chamber (outer fluid), 20 ml. of various pH isotonic buffers, containing 0.1% glucose, was transferred into upper chamber (inner fluid), and this circulation unit was immersed in water bath maintained at 37°. Inner fluid passed down the rectal lumen and was returned to the reservoir by means of gas bubbles (5% CO_2 and 95% O_2). Sulfonamides were dissolved 0.5 mmole/L. in inner fluid. At the end of the experiment period, the outer fluid was determined and permeated amount was calculated. The pH values of drug solution after circulation remained almost constant or changed less then 0.5.

Steady State Experiments——Male rats anesthetized were administered intravenously in the following doses (mg. per kg.); aminopyrine, 75, aniline hydrochloride, 200. After 15 min. the perfusion solutions were started by recirculation similar to those described in absorption experiments, and a concentration of drug was selected which would allow attainment of a steady state. The weakly buffered saline solution (Hogben[9]) was used for perfusion. About 4 ml. of the heart blood sample was taken at the steady state. Protein binding was measured by ultrafiltration technique. After correction for protein binding, the steady state distribution ratio was calculated by dividing the level of drug in the perfusion fluid by that in plasma. Besides, *in vitro* permeation experiments were used for *in vitro* steady state experiments. The weakly buffered saline solution was used for inner fluid, and at the steady state, the drug concentrations of both fluids were measured.

Partition Coefficients——Aquoeus solutions containing 30, 50 and 100 μg./ml. sulfonamides were prepared having the pH of their isoelectric points by means of 0.1*N* NaOH or 0.1*N* HCl. Four ml. portions of the aqueous solution were equilibrated with equal volumes of the organic solvent. These were kept in a constant temperature water-bath at 37° with removal for vigorous shaking twenty times in two hours period. The drug content was determined in the aquoeus phase and partition coefficients were calculated.

Ionization Constants——The ionization constants for basic amino groups of sulfonamides (pKa_1) were determined by the spectrophotometric method, and these for acidic groups (pKa_2) were determined by microtitration method at 25°.

Diffusion Constants——The diffusion constants were determined according to the method of Brooks[11]. The pads composed with 20 filter papers of Toyo No. 3 were pressed under a constant pressure for 24 hr. Diffusion was started by placing a small disc of filter paper wetted with sulfonamides aquoeus solution on the top of the pads in incubator at 37°. After 15, 30, 45, 60 and 90 min. the pads were removed and the filter sheets were rapidly separated. After drying, the filter papers were sprayed with *p*-dimethylaminobenzaldehyde solution. The diffusion constants were obtained by designing of diffusion distance and time.

Analytical Methods——Sulfonamides were diazotized following regular manner, coupled with 2-diethylaminoethyl-1-naphthylamine and their optical densities were determined at 550 mμ, using Shimadzu spectrophotometer type QR-50. Plasma aminopyrine and aniline were determined according to the methods of Brodie and Axelrod.[12,13]

10) G. Wiseman : J. Physiol., **120**, 63 (1953).
11) M.C. Brooks, R.M. Badger : J. Phys. Colloid. Chem., **52**, 1390 (1948).
12) B.B. Brodie, J. Axelrod : J. Pharmacol. Exptl. Therap., **99**, 171 (1950).
13) *Idem* : *Ibid.*, **94**, 22 (1948).

Results and Discussion

Absorption Rate and Drug Concentration of Recirculation Fluid

The absorption rates of sulfisoxazole and sulfaethylthiadiazole were found to be constant over a wide concentration range at a definite pH as shown in Fig. 2, showing

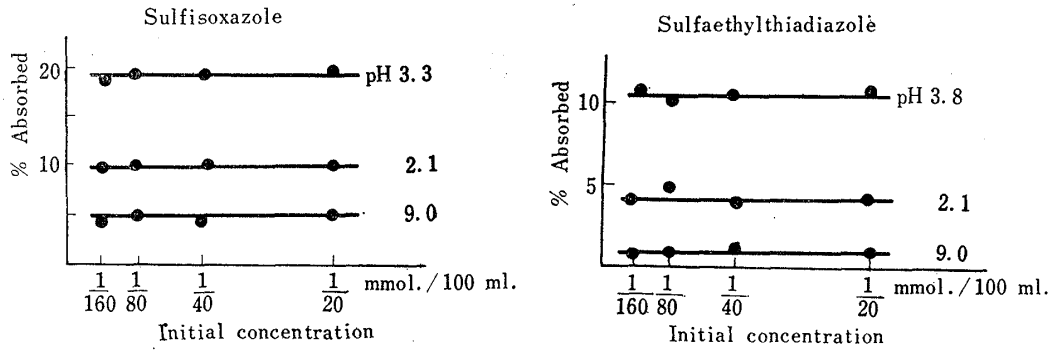

Fig. 2. Absorption Rate of Drugs from Solutions of Various Concentrations (after 1 hour)

The per cent absorbed is expressed as the mean of three animals.

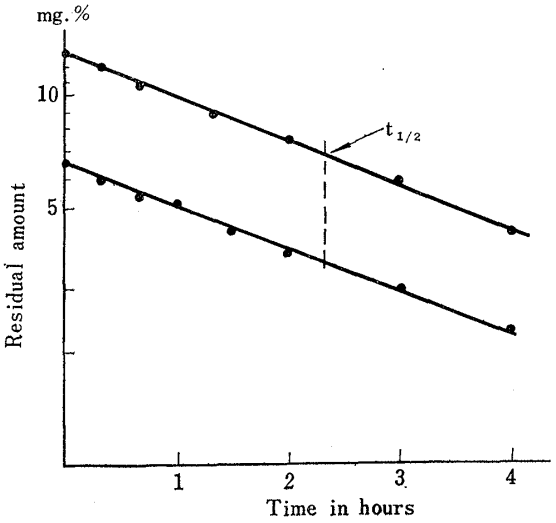

Fig. 3. Logarithmic Plot of Drug Remaining in Recirculating Solution (Solfisoxazole, pH 3.3)

that the absolute amount of drug absorbed increases proportionally to the initial concentrations.

The logarithm of remaining sulfisoxazole was plotted against time. And at a different concentrations the straight lines with the same slope were obtained as shown in Fig. 3. The agreement of both the lines' half lives indicates that the absorption of sulfisoxazole from the rat rectum is first order process, and therefore the absorption velocity constant can be calculated from the slope of the line multiplied with 2.303. These results suggest that the rectal absorption of sulfonamides is a passive transport across the rectal epithelium.

Absorption Rate *vs.* pH Profile

A sulfonamide molecule possesses two ionizing groups, and the absorption rate would be expected to be changed with increasing or decreasing the pH of the solution. Fig. 4 shows the absorption rate *vs.* pH profile on sulfisoxazole and sulfapyridine (the solid line). Krotz[14] reported already that sulfonamide possess no zwitterions in its isoelectric state in aquoeus solution. Accordingly, the theoretical fractions of unionized form are represented as dotted lines in the figure. In both sulfonamides, the absorption rate ascended and descended passing maximum point with increasing pH values. The maximum points of both sulfonamides' absorption rates coincided with those of the fraction of unionized forms, and these points are corresponding to their isoelectric points, suggesting that the absorption of sulfonamide from the rat rectum depends upon its ionization degree and the unionized form is preferentially absorbed.

14) I.M. Krotz, D.M. Gruen : J. Am. Chem. Soc., **67**, 843 (1945).

However, the solid lines are parallel with abscissa in the region of larger pH. This indicates that the ionized form was absorbed to some extent.

in vitro Permeation

The above results suggest that the lipoid barrier permeable to uncharged drug exists in the rectal membrane. Fig. 5 shows the relative rates of permeability of sulfonamides through the rectum using *in vitro* apparatus. Those were not affected by a change in pH in every sulfonamides, and the significant difference of permeability among the sulfonamides are not found. The results suggest that the lipoid barrier selectively permeable to unionized drug exists in the portion separating the rectal lumen from plasma.

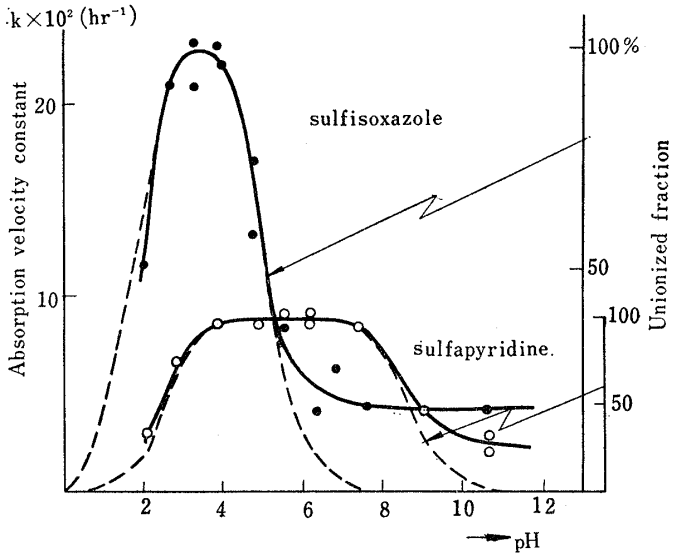

Fig. 4. Absorption Rate of Sulfonamides through the Rectum from Solutions of Various pH *in situ* (after 1 hour)

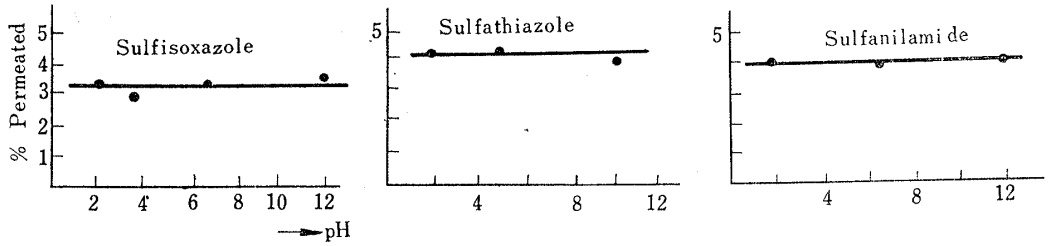

Fig. 5. Permeation Rate of Sulfonamides through the Rectum from Solutions of Various pH *in vitro* (after 1 hour)

The per cent permeated is expressed as the mean of three animals.

Influence of pH Rectal Surface on Absorption

The absorption of sulfonamides from a weakly buffered solution which was used by Hogben,[9] was investigated. Table II shows a comparison between the absorption rates from a weakly buffered solution and a highly buffered solution of the same pH. The pH of both solutions did not change during the experiment. The absorption rates from a weakly buffered solution were larger about one and a half to two times than a highly buffered solution in both sulfonamides. The result suggests that the absorption from a weakly buffered solution is not always depending on the pH of the solution.

Hogben[9] and Schanker[15] reported that there were the slightly acidic zone at the intestinal absorbing surface, which have virtual pH of about 5.3 for the small intestine and about 6.5 for the colon. Accordingly, it is expected that there are also such a slightly acidic zone influencing on the absorption at the rectal surface.

If the barrier between the rectal lumen and plasma is selectively permeable to the unionized form of drug, the steady state distribution between the rectal fluid and plasma will depend upon the difference in pH of the fluids and pKa of the drug. This relation was expressed in the form of an equation by Shore.[16] The following equation gives the steady state distribution ratio of a weak base between the rectal lumen and plasma,

15) L. S. Schanker : J. Pharmacol. Exptl. Therap., **126**, 283 (1959).
16) P. A. Shore, B. B. Brodie : *Ibid.*, **119**, 361 (1957).

$$R=\frac{1+10^{(pKa-pH(rectum))}}{1+10^{(pKa-pH(plasma))}} \quad (1)$$

Aminopyrine and anilne which are readily absorbed, were employed for the steady state experiment. The absorption rates of unionized drugs of aminopyrine and of aniline were much larger than the ionized forms, respectively 21.0 and 29.5%. If the drug absorption is governed by the pH 7.2, which was observed to be the pH of the saline perfusate leaving the rectum, the steady state distribution ratio, R, would be unity for both drugs.

Table II. Comparison of between Absorption Rates from Weakly Buffered Solution and from Highly Buffered Solution at pH 7.1 (after 1 hour)

	Weakly buffered solution (%)	Highly buffered solution (%)
Sulfisoxazole	7.5	4.6
Sulfaethylthiadiazole	3.2	1.6

Each value represents the mean one for 3 animals.

Table III. Steady State Distribution Values

	Protein binding (%)	R	pH (rectum)
Aminopyrine	0	1.41	5.4
Aniline	38.5	1.16	5.4

Each value represents the mean one for 3 animals.

Table IV. Theoretical pH (rectum) Calculated from Equation (2)

Ku/Ki	Aminopyrine	Anilline
∞	5.38	5.39
10	5.32	5.34
5	5.24	5.28

The results obtained are presented in Table III. At the steady state, the concentration of drugs was higher in the rectum than in plasma. The steady state distribution ratios are respectively 1.41 and 1.16, and a virtual pH of the rectum calculated is 5.4.

Equation (1) is based on the assumption that the ionized moiety is not completely absorbed, but the ionized moieties of some drugs is also able to be absorbed to a certain extent. If the permeability coefficients of the ionized and unionized moieties are respectively Ki and Ku for a organic base, the steady state distribution across the rectal epithelium can be written as :

$$R=\frac{1+10^{(pKa-pH(rectum))}}{1+10^{(pKa-pH(plasma))}}\cdot\frac{10^{(pKa-pH(plasma))}+Ku/Ki}{10^{(pKa-pH(rectum))}+Ku/Ki} \quad (2)$$

If Ku/Ki is 5, theoretical pH (rectum) would be 5.24 for aminopyrine and 5.28 for aniline as shown in Table IV.

Furthermore, when the highly buffered solution of pH 5.4 was used for perfusion solution and the drug concentrations in the solution were controled previously for R to be 1.41 on aminopyrine and 1.16 on aniline, the steady state was obtained.

Then *in vitro* steady state experiment was investigated for both drugs, of which unionized forms were preferentially permeable. But the steady state distribution was obtained to be unity for both drugs.

These results suggest that the rectal absorbing surface has a pH of about 5.4 like the intestine, and such a slightly acidic zone exsists only the rectal epithelium border between the lumen and plasma.

Partition Coefficients

The foregoing studies indicate as mentioned above that the lipoid barrier exists between the rectal lumen and plasma. Therefore it is expected that the absorption rates of unionized molecules may be related to their partition coefficients. The ionization constants and the isoelectric points of six sulfonamides were listed in Table V. The highly buffered solutions were used at pH's of their isoelectric points, to determine the absorption rates of unionized sulfonamides. The results of experiment, using three animals for each sulfonamide, was obtained as shown in Fig. 6.

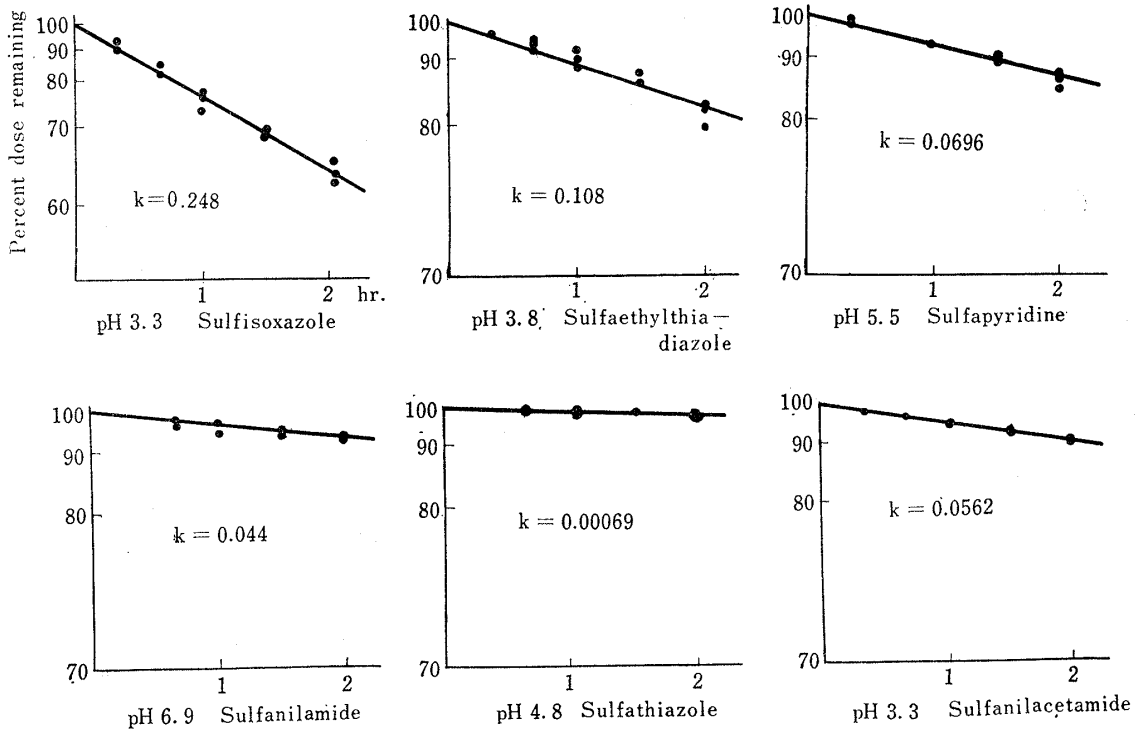

Fig. 6. Logarithmic Plot of Per Cent Drug Remaining in Recirculating Solution

Each straight line was the regression line calculated by least squares and a absorption velocity constant, k, was calculated from the slope of the line multipled with 2.303.

On the other hand, to compare the affinity of drugs for lipoid, partition coefficients were determined at the pH's of isoelectric points of the drugs with three organic solvents : chloroform, isoamylacetate and ethylenedichloride. The partition coefficients obtained are shown in Table VI. The results indicate that the absorption velocity constants are arranged in ascending order of the partition coefficients on the whole, but that their relation is not always proportional, and particularly sulfanilamide and sulfathiazole are arranged opposite in order.

When the drug is absorbed from the rectal lumen, a diffusion of drug through the lipoid barrier should be considered besides partition. Höber[17] reported that a diffusion

17) R. Höber, J. Höber : J. Cell Comp. Physiol., **10**, 401 (1937).

Table V. pKa's and Isoelectric Points of Sulfonamides

	pKa_1	pKa_2	Isoelectric point
Sulfisoxazole	1.65	5.12	3.39
Sulfaethylthiadiazole	1.95	5.40	3.68
Sulfapyridine	2.58	8.65	5.62
Sulfanilamide	2.14	10.43	6.28
Sulfathiazole	2.36	7.30	4.83
Sulfanilacetamide	1.56	5.38	3.47

Table VI. Partition Coefficients and Diffusion Constants

	Partition coefficient, P			Diffusion constant, D	P·D
	Chloroform	Isoamylacetate	Ethylenedichloride		
Sulfisoxazole	3.53	14.1	8.45	29.2	411.7
Sulfaethylthiadiazole	2.68	7.25	5.52	33.6	243.2
Sulfapyridine	0.973	1.90	1.81	48.6	92.3
Sulfanilacetamide	0.094	0.872	0.269	69.4	60.5
Sulfanilamide	0.019	0.353	0.117	76.4	26.9
Sulfathiazole	0.127	0.534	0.367	48.6	25.9

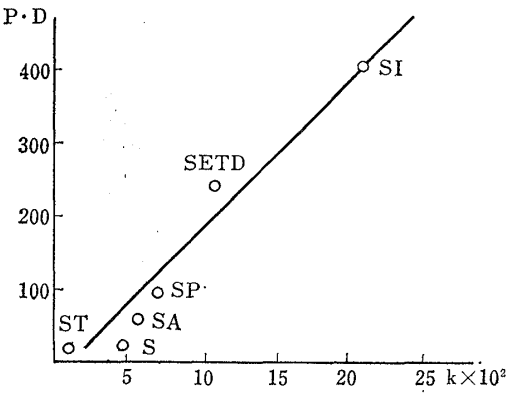

Fig. 7. Relationship between Absorption Velocity Constant and Product of Partition Coefficient and Diffusion Constant

SI : Sulfisoxazole
SETD : Sulfaethylthiadiazole
SP : Sulfapyridine
SA : Sulfanilacetamide
S : Sulfanilamide
ST : Sulfathiazole

constant was also concerned in the intestinal absorption, and Higuchi[18] suggested that the penetration of the drug in the barrier phase was in proportion of the permeability constant which was represented as the product of a partition coefficient and a diffusion constant. The diffusion constant of various sulfonamides in aqueous solution was measured at 37°. The permeability constant was calculated using the diffusion constant and the isoamylacetate-water partition cofficient, since isoamylacetate is close to the dielectric constant of the lipoid layer in membrane[19]. The permeability constants were plotted against the absorption velocity constants as shown in Fig. 7, showing that the good linearity exists among the spots. This suggests that the rectal absorption of sulfonamide depends upon its partition to the lipoid barrier, and is related to its diffusion through the barrier.

This investigation was supported in part by grants-in-aid from the Smith Kline and French Laboratories Fund.

Summary

The rates of absorption of several sulfonamides were measured in the rectum of

18) T. Higuchi : J. Soc. Cosmetic Chemist, **11**, 86 (1960).
19) J. F. Danielli : Lipid transport across cell membranes, Proceedings of an International Symposium on Lipid Transport (1964).

the anesthetized rat. Their absorption was considerably affected by the pH of solution. The unionized form was in general readily absorbed, while the ionized form was more slowly absorbed. Sulfonamides which are highly lipid-soluble were more readily absorbed than those which are poorly lipid-soluble. And there is a slightly acidic zone at the rectum-blood barrier, like the intestinal-blood barrier. The results suggest that sulfonamides are absorbed from the rectum by a passive transport, and that the patterns of absorption in the rectum are similar as a gastro-intestinal absorption of drugs. When the permeability constants were plotted against the absorption velocity constants, the good agreement was obtained among the spots. The diffusion constant as well as lipid-solubility is an important factor for rectal absorption.

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UDC 547.823.07

113. Takeo Naito, Toru Yoshikawa, Fumiyoshi Ishikawa, Sumiro Isoda, Yoshiaki Omura, and Isao Takamura : Synthesis of 3-Pyridinols. I. Reaction of 5-Unsubstituted Oxazoles with Acrylonitrile.*1

(*Central Research Laboratory, Daiichi Seiyaku Co., Ltd.**2)

Recently, diene-synthesis of oxazoles was investigated by Kondrat'eva, *et al.*, and they described that 5-unsubstituted-3,4-pyridinedicarboxylic acids are obtained from 5-unsubstituted oxazoles with maleic anhydride[1] and 5-hydroxy-3,4-pyridinedicarboxylic acids are formed by the same reaction from 5-alkoxyoxazoles.[2] This method was applied to 4-methyl-5-ethoxyoxazole with some dienophiles by Harris, *et al.* to synthesize several 3-pyridinols having pyridoxine-like structure.[3] The present paper deals with a new synthetic method of 3-pyridinols by condensation of 5-unsubstituted oxazoles with acrylonitrile.

4-Methyloxazole was treated with acryronitrile in toluene (method A) or in acetic acid (method B). Reaction by method A gave three crystalline products, *i.e.* compounds (I), (II), and (III), under intensive evolution of ammonia, and that by method B

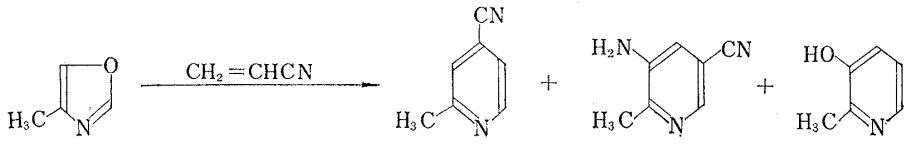

Chart 1.

*1 A part of this paper was presented at the Kanto Branch Meeting of the Pharmaceutical Society of Japan, Tokyo, September 19, 1964.

*2 Minamifunabori-cho, Edogawa-ku, Tokyo (内藤武男，吉川 徹，石川文義，磯田純郎，大村祐章，高村功).

1) G. Ya. Kondrat'eva : Khim. Nauk i Prom., **2**, 666 (1957). *Idem* : Izv. Akad. Nauk, Otd. Khim. Nauk, **1959**, 484.

2) G. Ya. Kondrat'eva, Ch. Huang : Doklady Akad. Nauk S. S. S. R., **141**, 628, 861 (1961).

3) E. E. Harris, R. A. Firestone, K. Pfister, 3rd, R. R. Boettcher, F. J. Cross, R. B. Currie, M. Monaco, E. R. Peterson, W. Reuter : J. Org. Chem., **27**, 2705 (1962).