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Methanesulfonic Acid Derivative of Drug. I. Reversible Hydrolysis Rate of Methanesulfonic Acid Derivative of Substituted Aniline and Biopharmaceutical Study on Sodium *p*-Phenetidinomethanesulfonate[1]

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For the elucidation on the mechanism of change of methanesulfonated drug *in vivo*, the rate of hydrolysis was investigated on the derivatives of substituted anilines. In aqueous solution the hydrolysis was found to be reversible between pH 4 and 8 and the rate is pH-independent. The pH-profile of the reverse reaction rate indicated that the formation of methanesulfonic acid derivative occures between molecular aniline and hydroxymethanesulfonate. The electron donating groups facilitate the hydrolysis as well as reverse reaction and the electron attracting groups on the contrarily retard both reactions. Methylation on amino group or C atom of methylene group promotes the hydrolysis significantly.

To see the change in rabbit, Na *p*-phenetidinomethanesulfonate was intravenously administrated and the plasma concentration of intact phenetidine was followed. The curves had maxima which appear within ten minutes. The early maximum would be explained by rapid transference of phenetidine into extravascular fluid or organs. Pharmacokinetic inquiries were given on the processes of the change in blood refering to the data obtained by supplemental studies.

For the purpose to obtaine a soluble form of amine drug, to prepare sodium salt of methanesulfonic acid derivative (MSD) has been known for long time as a common way. Among the medicaments actually used in this form are antipyretics of aniline and pyrazolone derivatives, sulfones, and isoniazide.[3] According to patent literatures, however, solubilization by methanesulfonation has been attempted to many drugs. But most of them have not been used in practice and this indicates that the modification of amino group, which is essential for the pharmacological activities of these drugs, may reduce or lose the activities of the parental drugs. For instance on MSD of sulfonamide diminution of activity was reported. But ethanesulfonic acid derivative of this drug was found not to lose its activity, and the contrast was attributed to the differences of hydrolysis rate.[4,5] These facts arouse interest for further information on this accustomed form of solubilized drug. However, up to the present time accurate study on the rate of hydrolysis of these compounds had not been done except sulpyrine.[6] The aim of this study is to acquir systematic knowledge on these problems and explore the possibility for new form of drug.

As the first step of the study, the relationship between chemical structure and the rate of hydrolysis of MSD was investigated on substituted anilines. Concurrently with the hydrolysis, the formation rates of MSD from substituted anilines and hydroxymethanesulfonate were also determined. Recently, Ono, *et al.* have studied on the hydrolysis of sulpyrine from the

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[1] Main part of this study was presented at the 89th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April 1969.

[2] Location: *3-1, Tanabe-dori, Mizuhoku, Nagoya.*

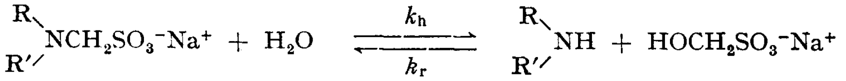
[3] W. Logeman and G.P. Miori, *Arzneimittel Forschung*, **5**, 213 (1955).

[4] H. Bauer, *J. Am. Chem. Soc.*, **61**, 617 (1939).

[5] A.G. Green and M. Coplan, *Chem. Ind.* (London), **1940**, 793.

[6] S. Ono, R. Onishi, and K. Kawamura, *Yakugaku Zasshi*, **86**, 11 (1966); *idem, ibid.*, **88**, 554 (1968).

view point of chemical analysis of this drug.[6] According to their results the drug is hydrolyzed and reversible equilibrium is attained in aqueous solution at neutral pH, which is shown as:



$$\overset{R}{\underset{R'}{\ }}NCH_2SO_3^-Na^+ + H_2O \underset{k_r}{\overset{k_h}{\rightleftharpoons}} \overset{R}{\underset{R'}{\ }}NH + HOCH_2SO_3^-Na^+$$

In preliminary studies, similar equilibrium state was also found for MSDs of substituted anilines between pH 4 and 8. Subsequent to the study *in vitro*, the rates of the hydrolysis and elimination of MSD in rabbit blood were investigated administrating MSD intravenously. The MSD of *p*-phenetidine, which is known as the first commercialized product of this kind, was used for this purpose. For the resultant curve of plasma concentration of intact phenetidine pharmacokinetic inquiries were given refering to the supplemental studies.

Experimental

Preparation of MSD——MSDs were prepared according to the procedure reported by Neelakantan, *et al.*[7]

Determination of Hydrolysis Rate of MSD——The hydrolysis rate was determined in bufferes of 0.1M acetic acid–Na acetate, 0.1M KH_2PO_4–K_2HPO_4, or 0.1M glycine–NaOH system according to pH value intended. The concentration of MSD was from 3 to 4×10^{-3}M, and all determination was carried out at 37°. To determine residual MSD, aniline produced by hydrolysis was extracted with dichloroethane from sample solution. The term of aniline is used in this report to represent aniline and/or substituted aniline without any specification. The extraction was quantitative when the aqueous phase was made approximately pH 7 by the addition of pH 7 buffer composed of 0.5M phosphates. Remaining MSD in aqueous phase was determined by UV spectrophotometry. As this hydrolysis is reversible after some time equilibrium state is attained and amount of MSD becomes constant. This equilibrated amount is reduced as pH decreases, which corresponds to the reduction of the reverse reaction rate. When the reaction vessel is shaken in the presence of dichloroethane continuously during the hydrolysis, the reaction proceeds in typically first order. In these solutions aniline produced is removed into dichloroethane and consequently no reverse reaction takes place. The reaction rate constant determined in this way agreeded with that determined from the initial slope of the disappearance of MSD in the absence of dichloroethane.

Determination of Hydrolysis Rate of Anilinoethanesulfonic Acid Derivative——The hydrolysis of these compounds proceeds so fast that the procedure used for MSD can not be applied. The determination was carried out recording the change of absorbance at an appropriate wavelength. The wavelength was chosen where the largest difference was found between spectrum obtained immediately after the dissolution and that obtained later than 30 minutes. About 0.1 mg of sodium salt of ethanesulfonic acid derivative was weighed in a 1 cm spectrophotometer cell, which will be about 5×10^{-5}M by the dissolution with 4 ml of water. The cell was mounted on the spectrophotometer and the recording was started immediately after the dissolution that was carried out by the injection of 4 ml water into the cell using a syringe. The hydrolysis rate constant can be calculated from the half life of the exponential curve recorded.[8]

Determination of Formation Rate of MSD——Equimolar $HOCH_2SO_3Na$ was dissolved in 8×10^{-3}M aniline solution and at appropriate intervals sample solutions were pipeted out. Remaining aniline was extracted and MSD formed was determined by above procedure. The reaction rate was calculated by equation 2, which was derived from equation 1.

$$\frac{dx}{dt} = k_h(a-x) - k_r x^2 \qquad (1)$$

$$k_h = \frac{2.303}{t} \times \frac{x_e}{a^2 - x_e^2} \log \frac{x_e(a^2 - x x_e)}{a^2(x_e - x)} \qquad (2)$$

In these equations a, k_h, and kr represent the initial concentration of reactants, hydrolysis rate, and formation rate constant respectively. The concentration of formed MSD is represented by x and x_e denotes that in the equilibrium state. When too much time was needed to attained equilibrium state, the reaction rate

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7) L. Neelakantan and W.H. Hartung, *J. Org. Chem.*, **24**, 1943 (1959).

8) A.A. Frost and R.G. Pearson, "Kinetics and Mechanism," 2nd Ed., John Wiley & Sons, Inc., New York, 1963, p. 28.

was calculated from the initial slope of the formation curve of MSD using general equation for second order reaction.

Biopharmaceutical Procedure——Male rabbits unanesthetized and weighing about 3 kg were used. MSD of phenetidine, which could be dissolved up to 5 w/v%, was dissolved in sterile distilled water immediately before use to avoid hydrolysis. For the preparation of intact phenetidine injection, *p*-phenetidine citrate, which could be dissolved up to 2 w/v%, was used to reduce the volume of injection and appropriate amount of NaCl was added to adjust tonicity. The solution was filtered through a sintered glass filter if necessary. The intravenous injection was performed through the ear vein and blood specimen was taken directly from the heart with a heparinated syringe. In advance of urinary excretion tests 50 ml of water was given orally through catheter before the injection.

Determination of Plasma and Urine Concentration of Phenetidine——A modified method of Brodie's procedure[9] was used. To determine the amount of MSD excreted, urinary sample was extracted with benzene containing 1.5% isoamyl alcohol shaking for about 3 hours at 37°, during which practically all MSD is hydrolyzed. Metabolized phenetidine is not extracted in this procedure. The determination of phenetidine was performed after the reextraction from benzene to acid aqueous phase.

Result and Discussion

Hydrolysis Rate in Aqueous Solution

Figure 1 shows the pH-profile of logarithum of hydrolysis rate constant, k_h, at 37°. As is shown the rate constants are pH-independent and their half lives range widely from 20 minutes to 55 days. The reaction rate was found to be not affected by buffer concentration. The electron attracting groups substituted on benzene ring retard the hydrolysis and the electron donating groups facilitate the hydrolysis. This is clearly illustrated in Fig. 2, which is the relationship between log k_h and pK_a of the corresponding anilinium ion.[10] The linear correlation shows that the reaction follows Hammett's rule. MSD of *N*-methylaniline is hydrolyzed significantly faster than the prospect from the linear relationship. These results indicate that the electron density of N atom is the dominant factor for hydrolysis. The remarkable difference among the hydrolysis rates which is ascribable to substituted group, may elucidate

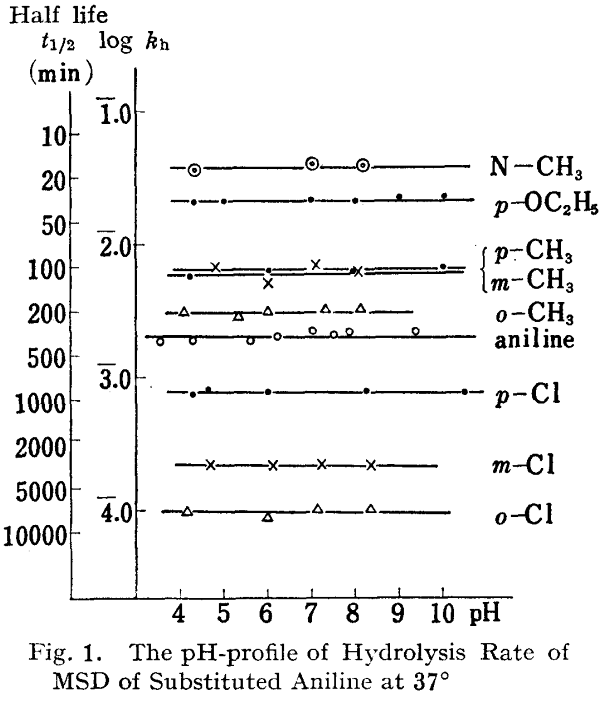

Fig. 1. The pH-profile of Hydrolysis Rate of MSD of Substituted Aniline at 37°

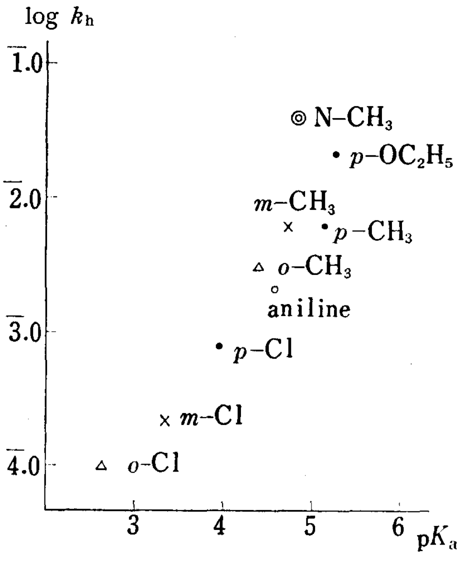

Fig. 2. The Relationship between log k_h and pK_a of the Corresponding Anilinium Ion

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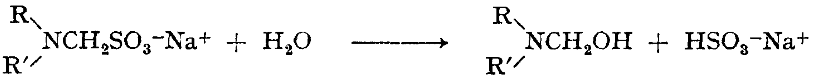
9) B.B. Brodie and J. Axelord, *J. Exptl. Therap.*, **97**, 58 (1949).

10) A. Albert and E.P. Serjant, "Ionization Constant of Acid and Base," Methuen & Co. Ltd., London, 1962, p. 144.

the various results on the activity of MSD. As was pointed out, MSD of phenetidine, which has an electron donating group, had been used as an antipyretic but that of sulfonamide, which has an electron attracting group, lose its activity. For the pharmacological activity of sulpyrine the elctron donating property of pyrazolone nucleus[11] and/or N-CH_3 group seem to be indispensable facilitating the hydrolysis of methanesulfonic acid group. On MSD and related compound of sulfonamide succeeding study will be reported later. The necessity of the fast hydrolysis will be realized also from the considerably fast excretion of MSD, which will be shown in the results of the biopharmaceutical study on MSD of phenetidine.

Below pH 4 it was found that the apparent hydrolysis rate determined as above was higher than that of pH-independent rate at neutral pH. In these reactions the solution colored yellow with time and the UV spectral change indicated that the reaction which can not be explained by simple reversible hydrolysis took place. On the change of sulpyrine in acid solution, Ono, *et al.* revealed that following hydrolysis which is different from the above and produces carbinolamine and bisulfite takes palce.[6]



$$\genfrac{}{}{0pt}{}{R}{R'}\!\!>\mathrm{NCH_2SO_3^-Na^+} + \mathrm{H_2O} \longrightarrow \genfrac{}{}{0pt}{}{R}{R'}\!\!>\mathrm{NCH_2OH} + \mathrm{HSO_3^-Na^+}$$

The carbinolamine produced in this hydrolysis is known to be very reactive and forms numerous complicated substances including methylenebisamine.[12] Recently, McMillan, *et al.* have reported on the complicated equilibria concerning n-$BuNHCH_2SO_3Na$ for the elucidation on the dissociation of sodium colistimethate.[13] They proved the formation of carbinolamine even at neutral pH by means of NMR spectroscopy. Similar complicated equilibria had been discussed previously by Le Hênaff for MSD of ammonia.[14] For sulpyrine the carbinolamine formation was proved in acid solution, but it was disproved at neutral pH by means of NMR spectroscopy.[6] From the reaction schemes presented by Mc Millan and Le Hênaff it may be concluded that the carbinolamine formation and further complicated reaction take place at lower pH where the amino groups of MSD and reaction products are protonated. With respect to sulpyrine pK_a of 4-amino group is not known, but it is supposed to be as large as that of aromatic amine from the results of Ono, *et al.*[6] To the reaction at acid pH more attention should be paid to see the change of MSD in stomach, but further study has not been done yet.

The diversity of the hydrolysis rate was found also above pH 8, *viz.* the hydrolysis was simply first order and reverse reaction was not found. Concerning this finding discussion will be described in the paragraph of the formation rate of MSD.

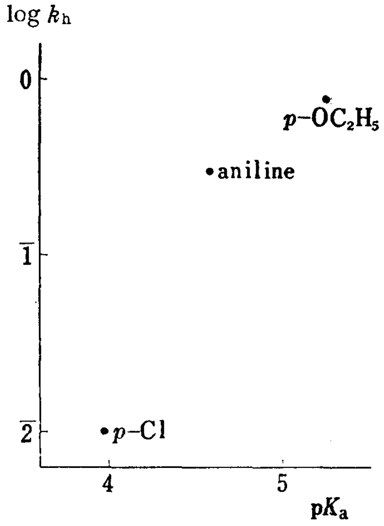
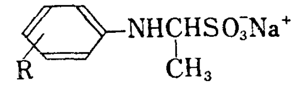


Fig. 3. The Relationship between log k_h and pK_a of Ethanesulfonic Acid Derivative of Substituted Aniline

Hydrolysis Rate of Anilinoethanesulfonate

Figure 3 is the relationship between pK_a and logarithum of the hydrolysis rate of anilinoethanesulfonate which has the following general formula.



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[11] *a*) T. Okano, K. Uekama, and K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **16**, 6 (1968); *b*) *Idem, Yakugaku Zasshi*, **87**, 1231, 1237 (1967).

[12] E.C. Wagner, *J. Org. Chem.*, **19**, 1862 (1954).

[13] F.H. McMillan and I.C. Pattison, *J. Pharm. Sci.*, **58**, 730 (1969).

[14] M.P. LeHenaff, *Compt. Rend.*, **256**, 3090 (1963).

The hydrolysis rate was determined in aqueous solution at room temperature uncontrolled (about 15°) as was described in the section of experiment. These compounds are hydrolyzed so fast that the hydrolysis can not be followed by the procedure for MSD. According to Frankel, *et al.* α-aminoalkylsulfonic acid is further labile than α-aminomethanesulfonate,[15] which is in accordance with our finding. Anilinoethanesulfonate has a slightly higher peak of UV spectrum at about 10 mμ longer wavelength comparing to parental aniline, which is similar with respect to MSD. The UV spectra of solutions of anilinoethanesulfonate change in several minutes at a room temperature towards that of parental aniline. Throughout the change an isobestic point is held and the final spectrum is identical with that of intact aniline. From these facts the spectral change may be attributed to the hydrolysis of ethanesulfonic acid group. The recording of absorbance at certain wavelength showed a simple exponential change, which indicates that the reverse reaction is negligible. This may be admitted from the low concentration of the substance, in order of 10^{-5}M, and also from the instability of hydroxyethanesulfonate[16] in contrast with hydroxymethanesulfonate which is not hydrolyzed at neutral pH.

Formation Rate of MSD

Figure 4 is the pH-profile of logarithum of k_r at 37°, the dimension of which is liter mole^{-1} min^{-1}. In acid range the pH-profile has a slope estimable to be 1, and it is pH-independent at around pH 7—8. This indicates that hydroxymethanesulfonate react with molecular species of aniline. Referring to pK_a of the anilinium ion, the reaction rate at pH 7—8 can be considered as the intrinsic reaction rate between molecular aniline and hydroxymethanesulfonate. Figure

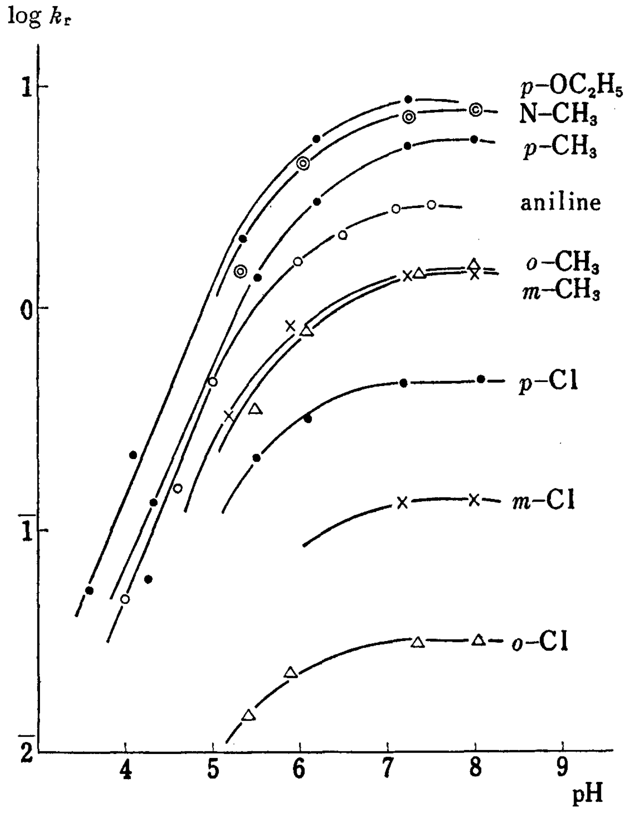

Fig. 4. The pH-profile of the Formation Rate of MSD between Hydroxymethanesulfonate and Substituted Aniline at 37°

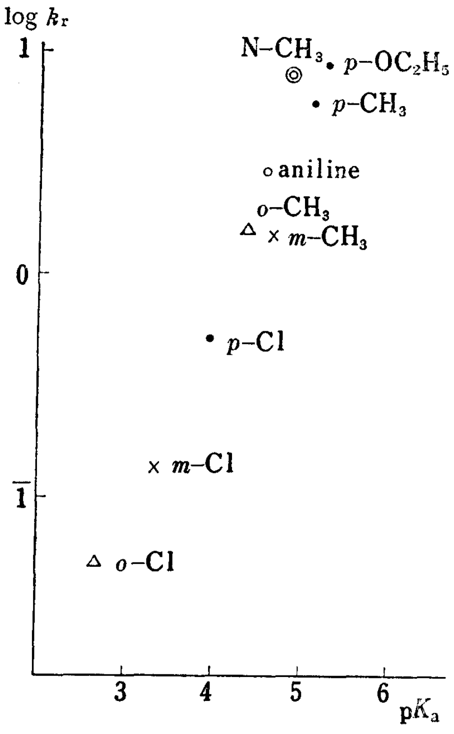

Fig. 5. The Relationship between log k_r at pH 7—8 and pK_a of the Corresponding Anilinium Ion

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15) M. Frankel and P. Moses, *Tetrahedron*, **9**, 289 (1960).
16) D.A. Blackaddar and Sir C. Hinshelwood, *J. Chem. Soc.*, **1958**, 2720.

5 is the relationship between log k_r at pH 7—8 and pK_a of the anilinium ion, which is also linear as that of the hydrolysis rate. As was seen in hydrolysis rate, the higher the electron density of N atom the higher the reaction rate, *i.e.*, the attack of hydroxymethanesulfonate on aniline is electrophilic. The equilibrium constant of the reversible reaction at various pH can be calculated from k_h and k_r.

Above pH 8, it was found that the apparent reaction rate determined by above procedure decreases as pH increases. In these reaction, the MSD formed in the early stage of the reaction was found to diminish gradually. These phenomena may be explained by the decomposition of hydroxymethanesulfonate forming doubly charged ion at alkaline pH, which is shown as[16]

$$\begin{tikzcd} HOCH_2SO_3^- \arrow[r, rightleftharpoons] & {}^-OCH_2SO_3^- + H^+ \arrow[d] \\ & HCHO + SO_3^{2-} \end{tikzcd}$$

This assumption may be approved also from the singly first order hydrolysis of MSD and the absence of reverse reaction at higher pH.

Biopharmaceutical Study of MSD of *p*-Phenetidine

Figure 6 shows the plasma concentration of phenetidine, which was given intravenously in the form of MSD. Although the result is not reproducible as is shown, any curve has maximum that appears within ten minutes. The variance of the result may be partially due to the individual deviation of numerous pharmacokinetic parameters of each subject. But that *i.v.* injection can not be performed instantly may be a serious cause for the variance of the results, because very rapid transference of phenetidine plays an important role in plasma concentration as will be discussed later. It was inevitable to spend several minutes for the *i.v.* injection of more than 20 ml. depending upon condition of vein. Blood specimen at time 0 was taken immediately after the finish of injection.

For the elucidation of the results, some supplemental studies were performed and a tentative scheme was presented for the change in blood as follows.

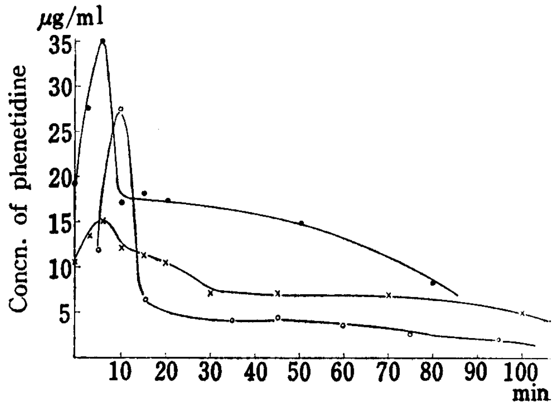

Fig. 6. The Plasma Level of Phenetidine After the Intravenous Administration of Sodium *p*-Phenetidinomethanesulfonate

administrated amount:
●: 1.00 g MSD (0.507 g as phenetidine)
×: 0.90 g MSD (0.455 g as phenetidine)
○: 0.85 g MSD (0.430 g as phenetidine)

$$\begin{tikzcd} \begin{array}{c}\text{A}\\ \text{MSD in blood}\end{array} \arrow[r, "k_1"] \arrow[d, "k_1'"] & \begin{array}{c}\text{B}\\ \text{phenetidine in blood}\end{array} \arrow[r, "k_2"] \arrow[d, shift right, "k_2'"'] & \begin{array}{c}\text{C}\\ \text{excreted or metabolized}\\ \text{phenetidine}\end{array} \\ \begin{array}{c}\text{A}'\\ \text{excreted MSD}\\ \text{unhydrolyzed}\end{array} & \begin{array}{c}\text{B}'\\ \text{phenetidine in}\\ \text{extravascular tissue}\end{array} \arrow[u, shift right, "k'_{-2}"'] & \end{tikzcd}$$

The inquiries on each process of the scheme will be described in the following, where all amounts and concentrations of phenetidine will be represented as intact phenetidine to avoid confusion arisen from various forms of phenetidine.

Figure 7 shows the result of the check on the hydrolysis of MSD of phenetidine if the rate in blood is identical with that in buffer. The abscissa is the part of blood diluted with

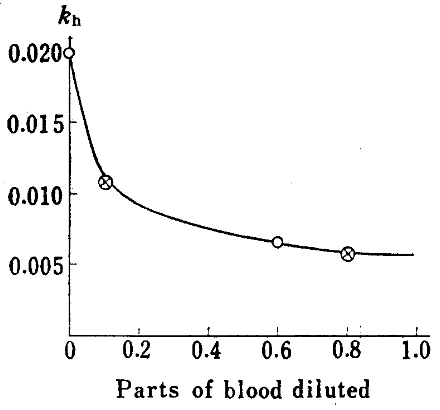

Fig. 7. The Hydrolysis Rate of MSD of Phenetidine observed in Blood diluted with Isotonic NaCl Solution

○: in rabbit blood
×: in man blood

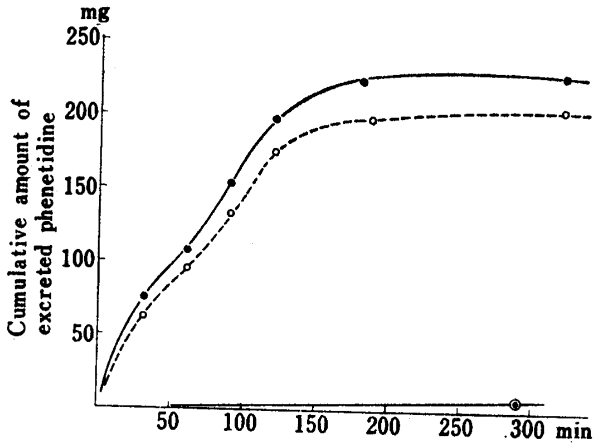

Fig. 8. The Cumulative Amounts of Excreted Phenetidine observed after the Intravenous Injection of MSD and Intact Phenetidine

● and ○: 1 g of phenetidine MSD (0.507 g as phenetidine) was given.
⊙: 0.450 g of phenetidine citrate (0.187 g of as phenetidine) was given.

isotonic saline solution. As is shown, in whole blood the hydrolysis rate decreases to about 1/4 of that in buffer. It seems that some components of blood retard the hydrolysis. The value of k_1 can be estimated to be 0.0051 min^{-1}.

The second inquiry is on the excretion rate of MSD unhydrolyzed, which is suspected to be considerably fast because the renal excretion of polar substance is very rapid in general. Figure 8 shows the cumulative amount of phenetidine excreted in the form of intact phenetidine and MSD unhydrolyzed. The lower creeping line was obtained when 0.450 g of phenetidine citrate (0.187 g as phenetidine) was intravenously given. In 6 hours only 6 mg of phenetidine was recovered from urine. That the portion of phenetidine excreted in intact form is very small had reported previously by Smith, *et al.*, who recovered only 2% when phenetidine was orally given to rabbit.[17] The upper two curves were obtained as the result of the *i.v.* administration of 1 g MSD (0.507 g as phenetidine). As is shown nearly 45% of phenetidine is excreted in about 6 hours and further accumulation is very small. Judging from the low plasma concentration of phenetidine shown in Fig. 6, practically all of the accumulated amount may be regarded as the excretion of unhydrolyzed MSD. Concurrently with the direct excretions of MSD and intact phenetidine some amount is excreted in metabolized forms. But this is not included in the amount determined by the used procedure and hence has no effect on the following calculations. The excretion rate of MSD, k_1', can be calculated assuming that the processes represented by k_1 and k_1' are parallel first order. The cumulative amounts proceeded through each of parallel processes are proportional to reaction rate constant, which can be represented as

$$\frac{[\mathrm{B}]}{[\mathrm{A}']}=\frac{k_1}{k_1'} \qquad (3)$$

where [A′] and [B] represent the total amounts of MSD excreted unhydrolyzed and that eliminated after the hydrolysis. The value of [A′] estimated from the average of the two results shown in Fig. 8 is 0.216 g, and hence [B] is 0.507−0.216=0.291 g. The value of k_1' calculated by equation 3 substituting above k_1 is 0.0038 min.$^{-1}$ The slope of accumulation

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[17] J.K. Smith and R.T. Williams, *Biochem. J.*, **44**, 250 (1949).

curve at initial stage shows an excretory rate something faster than above value, but the plots are sparse for further inquiries. This considerably fast excretion rate of MSD, which is almost same as that of hydrolysis, should be considered as a demerit of MSD. The hydrolysis rate must be fast enough to cover such fast excretion. In this case it may be concluded that 45% is excreted without therapeutic efficiency. On the other hand, the low toxicity, which is sometimes appraised as a merit of these drugs,[3] should be criticized if this is not an outcome from the fast excretion.

Figure 9 shows the disappearance of phenetidine when intact phenetidine was given intravenously. In the two experiments, the administrated amounts were identically 0.640 g of phenetidine citrate (0.267 g as phenetidine). The apparent distribution volumes of phenetidine were 6280 and 9380 ml. respectively. The semilog plots in Fig. 9 indicate that a multicomponental model should be taken into account.[18] For plots A, which is smoother than B, equation 4 was obtained by Perl's method[19] where C_p is the plasma concentration.

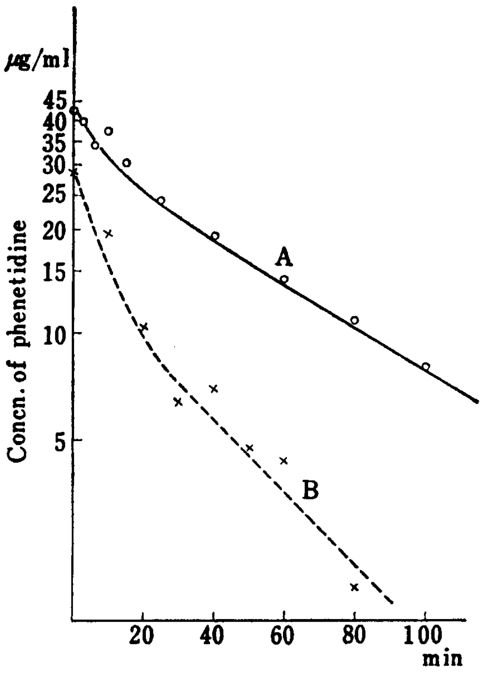

Fig. 9. The Plsama Level of Phenetidine after Intravenous Injection of Intact Phenetidine

Curves A and B were obtained when 0.640 g of phenetidine citrate (0.267 g as phenetidine) was given.

$$C_p = 12.5e^{-0.0800t} + 32.0e^{-0.0136t} \qquad (4)$$

The derivation of further exponential terms was supposed to be insignificant judging from the experimental error. The values of k_2, k_2', and k_{-2}', calculated from the hybrid rate constants in equation 4 are 0.0144, 0.0178, and 0.0613 min^{-1} respectively. As all rate constants involved in the tentative scheme have been estimated, numerical calculation of B/A_0 was tried by equation 5,[20] where A_0 is the administrated amount.

$$\frac{B}{A_0} = C_1e^{-0.0800t} + C_2e^{-0.0136t} + Ke^{-(k_1+k_1')t} \qquad (5)$$

But the calculated curve, which has a gently-sloped plateau and the maximum is at about 90 min, is quite different from the curves in Fig. 6. To account for an early maximum as seen in Fig. 6 the summation of the rates of subsequent processes, k_2+k_2', must be much faster than the preceding rate, k_1. An approximate calculation disregarding the last term of equation 7

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18) *a*) S. Riegelman, J.C.K. Loo, and M. Rowland, *J. Pharm. Sci.*, **57**, 117, 128, 918 (1968); *b*) R. Nagashima, G. Levy, and R.A. O'Reilly, *ibid.*, **57**, 1888 (1968).

19) W. Perl, *Intern. Appl. Radiation Isotop.*, **8**, 211 (1965).

20) Equation 5 was obtained as the solution of the following simultaneous differential equations derived from the tentative scheme.

$$\frac{dA}{dt} = -(k_1+k_1')A \qquad (6)$$

$$\frac{dB}{dt} = k_1A - (k_2+k_2')B + k_2'B' \qquad (7)$$

$$\frac{dB'}{dt} = k_2'B - k_{-2}'B' \qquad (8)$$

C_1, C_2, and K are constants intricately composed of rate constants and A_0. The hybrid rate constants of the first and second terms are identical with those in equation 4.

revealed that for the elucidation of maximum earlier than ten minutes k_2+k_2' must be greater than 0.3 min.$^{-1}$ About such fast transference of phenetidine no literature could be found, but according to Brodie, *et al.* aniline transfers from rabbit blood to cerebrospinal fluid with a rate constant of 0.40 min^{-1}, 1.7 min as half life.[21] In the two compartmental model, generally such fast transference of durg is not dealt with, in other words the hepatoportal system or cerebrospinal fluid, into which drugs transfer so rapidly, are included in the central compartment. However from the facts that phenetidine administrated to rabbit is predominently distributed in the liver[21] and almost portion is excreted in metabolized form,[17] the process of transference into this organ can not be ignored. From the pharmacokinetic point of view, the rapid transference of phenetidine can be considered as an expansion of the distribution volume which accompanies with the transformation from polar MSD to lipophile phenetidine. The so-called tissue compartment is supposed to be composed mainly of the muscle or adipose tissue, and the values of k_2' and k'_{-2} estimated above may be concerned with these tissues.

As has been discussed for the elucidation of the change in blood an improved model as follows would be conceived.

$$\begin{tikzcd}
 & B'' \arrow[r, "k_2'"] \arrow[d, shift left, "k''_{-2}"] & C' \\
A \arrow[r, "k_1"] \arrow[d, "k_1'"] & B \arrow[r, "k_2"] \arrow[u, shift left, "k_2''"] \arrow[d, shift right, "k_2'"'] & C \\
A' & B' \arrow[u, shift right, "k'_{-2}"'] &
\end{tikzcd}$$

In this scheme, B'' represents phenetidine in the so-called well perfused tissues including the liver or cerebrospinal fluid and B is that purely in plasma and C' is the metabolized amount. But for the provement of such scheme it is necessary to device an experimental procedure capable to follow the rapid transference of drug *in vivo.*

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21) B.B. Brodie, H. Kurz, and L.S. Schanker, *J. Pharmacol. Exptl. Therap.*, **130**, 20 (1960).