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Methanesulfonic Acid Derivative of Sulfonamides. I. Hydrolysis Rate *in Vitro* and Pharmacokinetics *in Vivo*[1,2]

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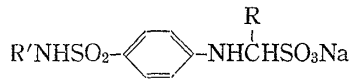
Various methanesulfonic acid derivatives of four sulfonamides, which are represented generally as $R'NHSO_2C_6H_5NHCHRSO_3Na$ wer esynthesized and their hydrolysis rates *in vitro* and pharmacokinetics of blood concentration change in rabbit were investigated. When R is H the hydrolysis rate is so slow that the pharmacological activity of parental sulfonamide can not be expected. When R is alkyl, phenyl or $HOCH_2(CHOH)_4$ the derivative has fast hydrolysis rate which may promise good availability of parental sulfonamide. The effect of R on hydrolysis rate was elucidated by linear free energy relationship. The dissociation of sulfonamide group hastens the hydrolysis. Dynamic process of appearance and disappearance of parental sulfonamide in blood was quantitatively explained by a scheme which assumes two compartment models for both of the derivative and parental sulfonamide respectively.

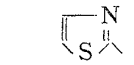
Methanesulfonic acid derivative (MSD)[4] of sulfonamide has been known since 1930's[5] and even in recent years still new patent literatures on the derivative of this kind have been reported. Sulfonamide MSD has the advantages not only that it is highly soluble but also that its aqueous solution is neutral and other drug such as antibiotics can be compounded with sulfonamide in parenteral solution for example. However, on the hydrolysis of sulfonamide MSD, which may be decisively influential in its pharmacological activity, no detailed study has been done hitherto. For the pharmacological activity of parental sulfonamide the hydrolysis is indispensable because MSD is deprived of the original activity of sulfonamide and in addition the water soluble derivative is suspected to be excreted rapidly without efficacy.[2] The hydrolysis can be represented as follows similarly to MSD of substituted aniline which was previously studied.

1) Presented at the 90th Annual Meeting of Pharmaceutical Soceity of Japan, Sapporo July 1970.
2) This report consitutes Part II of the studies entitled "Methanesulfonic Acid Derivative of Drug," Part I of which is in *Chem. Pharm. Bull.* (Tokyo), **18**, 440 (1970).
3) Location: *Tanabe-dori, Mizuhoku, Nagoya.*
4) Methanesulfonic acid derivatives which are substituted some group on methylene group as shown in Table I are included in this abbreviation.
5) W. Lodgeman and G.P. Miori, *Arzneimittel Forsch.*, **5**, 212 (1955).

That the plain sulfonamide MSD (R=H in Table I) is deprived of the parental activity of sulfonamide was reported by Bauer, *et al.*[6] and Green, *et al.*,[7] but the latter authors found that ethanesulfonic acid derivative ($R=CH_3$) retains the parental activity. They attributed such difference to the hydrolysis rate of MSD from the supposition based on the brief observation on excretion, but no further study on this problem has been reported thereafter. In previous study it was revealed that the hydrolysis rate of aromatic amine MSD is retarded markedly by the electron attracting group substituted at *para* position and accelerated by the substitution on methylene group,[2] which verifies Green, *et al.*'s assumption. In this study various sulfonamide MSD were synthesized and the hydrolysis rate *in vitro* and pharmacokinetics on these MSD was investigated. This report refers only to forward hydrolysis reaction which dominates the efficacy *in vivo*. On the reverse reaction, *i.e.* the formation of MSD from sulfonamide and α-hydroxymethanesulfonate, will be reported in the following article of this series. For the pharmacokinetic study on sulfonamide MSDs, these were intravenously administrated in rabbit and time course of blood concentration of sulfonamide which was produced by hydrolysis *in vivo* was followed. Mathematical equations were derived to elucidate the blood concentration change. The abbreviations of the derivatives and sulfonamides studied are tablated in Table I.

Table I. Abbreviation of Sulfonamide MSD and Sulfonamide



Substituted group R′	Substituted group R	Abbrev.	Substituted group R′	Substituted group R	Abbrev.
H– sulfanilamide (Sf)	H–	SfHS	CH_3CO– acetosulfamine (Sa)	H–	SaHS
	CH_3–	SfMeS		CH_3–	SaMeS
	C_2H_5–	SfEtS		C_6H_5–	SaPhS
	n-C_3H_7–	SfPrS	 sulfathiazol (St)	CH_3–	StMeS
	iso-C_3H_7–	SfPr′s		C_6H_5–	StPhS
	n-C_4H_9–	SfBuS			
	C_6H_5–	SfPhS			
	$HOCH_2(CHOH)_4$–	SfGuS	 Sulfisoxazol (si)	CH_3–	SiMeS
				C_6H_5–	SiPhS

Experimental

Synthesis of Sulfonamide MSD——Synthesis was followed to the method of Tillson[8] and Green, *et al.*[7] The purity of the synthesized derivative was determined by the colorimetry of the parental sulfonamide by diazo coupling method which will be described below.

Determination of Sulfonamide and Its MSD in Aqueous Solution——Sulfonamide and its derivatives were determined by direct ultraviolet (UV) spectrophotometry and colorimetry of the product of diazo coupling. By methanesulfonation UV spectral peak of parental sulfonamide shifts bathochromically about 10 mμ and intensity increases. UV spectrophotometry was used mainly for the following of rapid reaction as will be described. Parental sulfonamide and their derivatives are colored equally by the diazo coupling with Tsuda's reagent[9] to the same intensity depending upon their molar concentration. In 10 ml

6) H. Bauer, *J. Am. Chem. Soc.*, **61**, 617 (1939).
7) A.G. Green and M. Coplan, *Chem. Ind.* (London), **1940**, 713.
8) E.W. Tillston, U.S. Patent 2374791 (1945) [*C.A.*, **40**, 1635 (1946)].
9) T. Sakaguchi, "Iyakuhin Teiryo Bunseki," Nankodo, Tokyo, 1959, pp. 34—39.

volumetric flask 1 ml solution of sulfonamide or its MSD (1.0×10^{-4} to 5.0×10^{-4}M) was taken and 0.1 ml of 30% trichloroacetic acid and 0.28 ml of 0.2% $NaNO_2$ were added. Then small amount of water was added to facilitate shaking. After 10 minutes standing for diazoation 0.1 ml of 10% ammonium sulfamate was added. One minute later 1.6 ml of 0.2% Tsuda's reagent was added. The final volume was made 10 ml by the addition of water. The spectral peak of color produced is at 545 mμ.

Separative Determination of Parental Sulfonamide and Its MSD——For the separation of parental sulfonamide and its MSD Amberilte IR-120 column (1 cm diameter, 5 cm length) was used. The resin was prepared as H^+ form before use and 1 ml of test solution was added and distilled water was added successively as influent. Parental sulfonamide is adsorbed on resin and its MSD passes quantitatively. MSD concentration was determined by the colorimetry of effluent. Free sulfonamide concentration was estimated from the difference between the results which were obtained without and with passage through ion exchange resin column.

Determination of Sulfonamide MSD and Hydrolyzate of Blood Sample——In a test tube containing 5 ml 0.1% saponin, 1 ml blood sample was taken and hemolyzed. To 5 ml of the hemolyzed sample, 2 ml of 30% trichloroacetic acid was added and filtered. The filtrate was analyzed by the colorimetry described above. For the separative determination of sulfonamide and its MSD, 5 ml of saponified blood sample was passed through ion exchange resin column with distilled water into 25 ml volumetric flask containing 2 ml of 30% trichloroacetic acid. The effluent was diluted to 25 ml and MSD concentration was determined after filtration. Acetylated sulfonamide passes into effluent and the difference of the amounts in direct sample (without passage through ion exchange resin column) and that in effluent gives free sulfonamide.

Determination of Hydrolysis Rate——Sulfonamide MSD was dissolved in buffer (about 1.0×10^{-4}M) at 37° and remaining MSD was determined at appropriate intervals. The buffer systems used were the same to those in previous study. As the concentration was low, reverse reaction could be disregarded and the reaction rate was calculated as simple first order reaction. For the determination of the hydrolysis rate of *p*-chloroaniline MSD, UV absorbance change was recorded at 250 mμ where absorbance change is largest. The hydrolysis rate is so fast that the hydrolysis during determination can not be ignored. About 0.1 mg of *p*-chloroaniline MSD was taken in 1 cm spectrophotometer cell which was mounted on temperature controlling apparatus kept at 37° and 4 ml buffer at the same temperature was injected with syringe to dissolve MSD in the cell. The recording of absorbance was started immediately after the injection. Reaction rate was calculated from the half life estimated by semilog plot of the exponential curve recorded. It was ascertained on SfMeS as an example that the result obtained from the curve of UV recording agrees with that obtained by the separative determination passing through ion exchanger column.

Biopharmaceutical Study——Male rabbit weighing 2 to 3 kg was used as test animal. Through ear vein about 250 mg/kg weight sulfonamide or its MSD was injected. Blood sample (1.0 or 2.0 ml) was taken directly from heart with syringe. For the preparation of parenteral solution of intact sulfonamide, sodium salt was dissolved with sterilized distilled water. For the preparation of sulfonamide MSD solution, MSD was dissolved immediately before administration to avoid hydrolysis before injection. The volume of distilled water used to dissolve was 5 ml at most, which could be intravenously injected within 2 to 3 minutes.

Result and Discussion

Effect of R on Hydrolysis Rate

The pH-profiles of the hydrolysis rate of various MSD of Sf substituted on methylene group are shown in Fig. 1. As is seen in Fig. 1, when R is alkyl, hydrolysis rate constant is pH-independent from pH 4.0 to 9.0 which is in accord with MSD of substituted aniline (R=H) in previous study. When R is Ph or Gu the hydrolysis rate constant has some pH-dependency, which is not accountable from the result obtained. To confirm Green, *et al.*'s supposition the hydrolysis rates of SfHS and SaHS at pH 7.4 were determined and shown in Fig. 1. As the hydrolysis rates of these MSD are despairingly slow when R=H, *viz.* as low as 2 and 7 thousand minutes in half life for SaHS and SfHS respectively, it is admitted that when R is H sulfonamide MSD is not practically usefull. The hydrolysis rate in blood was found to be somewhat lower than that *in vitro*. The effect of ionic strength on hydrolysis was small as some example shown in Fig. 2, where the data were obtained at pH 7.4 and ionic strength was varied with addition of NaCl. The change of hydrolysis rate due to buffer components was not found. MSD whose R is Gu has been synthesized by a number of investigators because this is expected to be favorable with regard to water solubility and possible toxicity.[5] However, as is seen in Fig. 1 it is not so advantageous with respect

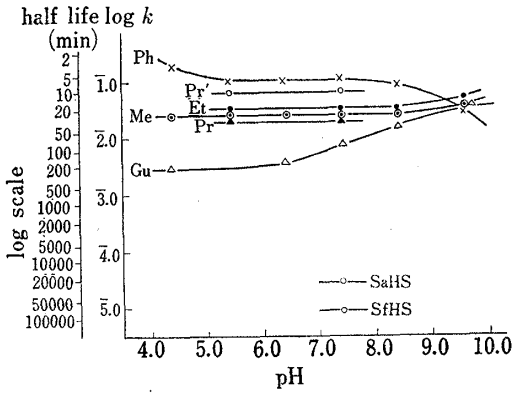


Fig. 1. Hydrolysis Rate Constant versus pH on Sulfanilamide MSD Substituted with Various R at 37°

As the comparison hydrolysis rate constants of SfHS and SaHS are shown.
—○— : SaHS, —○— : SfHS

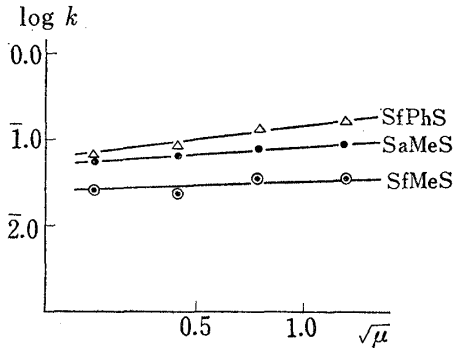


Fig. 2. Effect of Ionic Strength on Hydrolysis Rates of Some Sulfonamide MSD at pH 7.4, 37°

to hydrolysis rate in some pH region.

According to Taft[10] the reactivity is separated into polar and steric effect, *viz.* logarithum of hydrolysis rate constant, log k, is composed of terms concerning to Taft's substituent constant, σ^*, steric substituent constant, E_s, and logarithum of the rate of standard compound, log k_o, as shown in equation 1.

$$\log k = \rho^*\sigma^* + sE_s + \log k_0 \quad (1)$$

Fig. 3 shows the application of equation 1 to the result in this study. Specific coefficients, ρ^* and s, and log k_o were estimated by the least square method and the experimental equations 2 and 3 were obtained respectively for Sf MSD and *p*-chloroaniline MSD which was studied as a comparison.

$$\log k = -6.67\sigma^* + 0.228E_s - 2.14 \quad (2)$$

$$\log k = -5.33\sigma^* + 0.080E_s - 0.60 \quad (3)$$

Although the plots in Fig. 3 are not so many and have some deviation from regression line, there is an acceptable linear correlation between σ^* and log k—sE_s. The deviation from linearity may be eliminated if the correction is made on the effect of number of α-H in substituted group, which was successfully carried out in the case on the hydrolysis of acetal and ketal.[10] The number of plots, however, are too small for the detailed inquiry on this effect. Equations 2 and 3 show that the steric effect of R on hydrolysis is small and the predominant effect is due to polarity of R. When R=C_6H_5 (σ^*=0.6), log k for Sf MSD, which was plotted in Fig. 3 without subtraction of $s\ E_s$, is about 5.0 log unit above from the value estimated by the regression line. This large difference may be explained by the resonance effect due to benzene ring, which was also seen similarly on the hydrolysis of acetal and ketal.[10] Probably the data on α and β-unsaturated groups may be on the assumptive parallel line drawn by dotted line in Fig. 3.

Hydrolysis Rate of MSD of Various Sulfonamides

The difference of the hydrolysis rate owing to parental sulfonamide is shown in Fig. 4. MSD of Sa, St, and Si whose R are CH_3 show some pH-dependency of hydrolysis rate in con-

10) R.W. Taft, Jr., "Steric Effect in Organic Chemistry," ed. by M.S. Newman, John Wiley & Sons, Inc., New York, 1956, chapter 13.

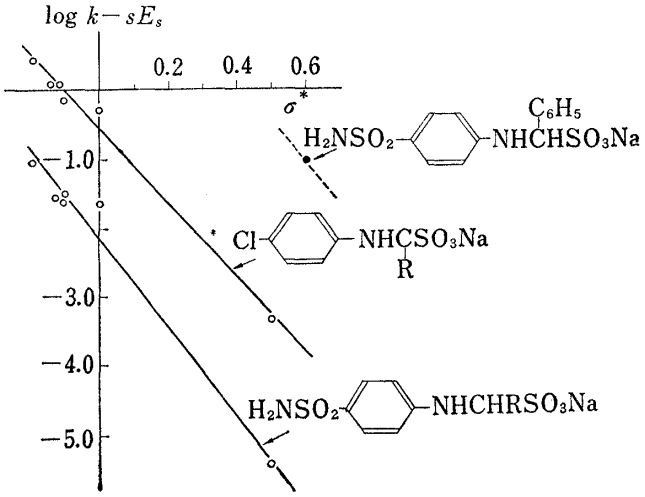


Fig. 3. Linear Free Energy Relationship on Hydrolysis of MSD Illustrated by log k-sE_s versus σ^* at 37°

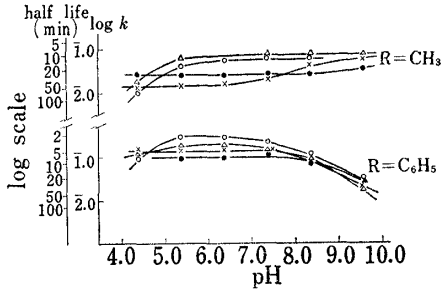


Fig. 4. Hydrolysis Rates of MSD of Various Sulfonamides versus pH (R=CH_3 and C_6H_5) at 37°
●:Sf, ○:Sa, ×:St, △:Si

trast with SfMeS. This difference may be attributed to the dissociation of sulfonamide group, which is presumed from the fact that shift of hydrolysis rate occurs at pH around pK_a value of the corresponding sulfonamide. Values pK_a for Si, Sa, St and Sf are 4.9, 5.4, 7.1 and 10.5 respectively.[11] It is reasonable to suppose that the electron attracting activity of sulfonamide group decreases as the result of dissociation and the hydrolysis rate increases. It is indicated that hydrolysis rate of undissociated sulfonamide is in order of pK_a value of parental sulfonamide. MSD whose R is C_6H_5 also shows some pH-dependency which is unexplainable. For the full study of the hydrolysis of MSD which is substituted some R group further hydrolysis of $HOCHRSO_3^-$ must be taken into account as well as the reverse reaction. This secondary step hydrolysis is known to be also reversible as the first step. On such successively reversible hydrolysis will be reported in the following article of this series.

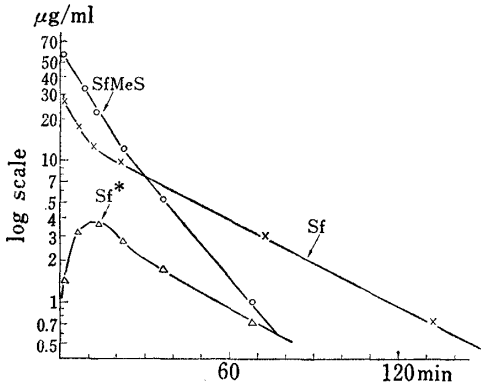


Fig. 5. Blood Concentration Change of SfMeS, Sf* and Sf Administrated Intravenosuly

SfMeS: blood concentration of SfMeS administrated intravenously
Sf*: Sf produced by hydrolysis after intravenous administration
Sf: Sf administrated intravenously as sodium salt solution of intact form

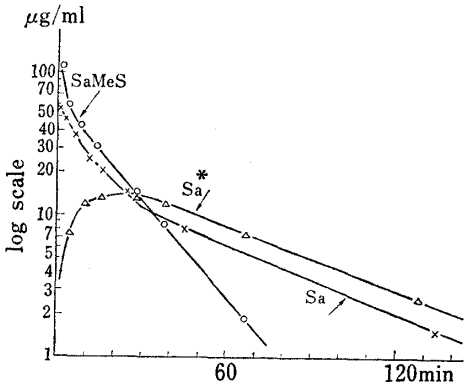


Fig. 6. Blood Concentration Change of SaMeS, Sa* and Sa Administrated Intravenously

SaMeS: blood concentration of SaMeS administrated intravenously
Sa*: Sa produced by hydrolysis after intravenous administration
Sa: Sa administrated intravenously as sodium salt solution of intact form

11) T. Struller, *Drug Res.*, **12**, 389 (1968).

Pharmacokinetics of Sulfonamide MSD

As has been discussed above, substitution of R group is an adequet means to improve the availability of sulfonamide. Figure 5 shows the change of blood concentration of SfMeS and Sf*[12] produced by the hydrolysis of SfMeS when MSD was intravenously administrated. For the determination of pharmacokinetic parameters on parental Sf, sodium salt solution of intact Sf was intravenously administrated and the change of blood concentration was followed, which is shown by the curve denoted by Sf. As was supposed SfMeS is eliminated rapidly and curve of Sf* has a peak. The elimination curves of Sf* and that of intact Sf are parallel as is expected. Figure 6 is the similar plots on SaMeS, Sa*, and intact Sa, which are analogous to those in Fig. 5. For the mathematical representation of the curves in Fig. 5 and Fig. 6 a kinetic model as Chart 1 was postulated, where x_i (i=1 to 6) represents the amount of MSD or sulfonamide as indicated and those are connected by first order kinetic processes with rate constants specified. Solution of simultaneous differential equations based on Chart 1 gives two exponential equation with respect to X_1 and blood concentration of SfMeS, which is denoted by C_{SfMeS}, can be represented in the form of[13]

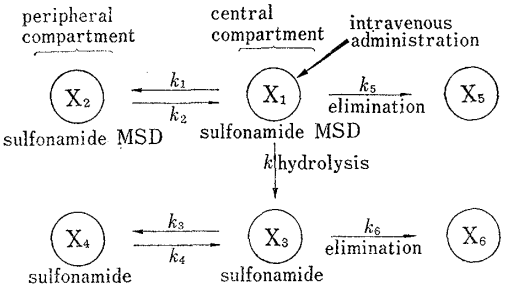


Chart 1

$$C_{SfMeS} = Ae^{-\alpha t} + Be^{-\beta t} \quad (4)$$

From the plot on SfMeS in Fig. 5 following experimental equation is obtained by Perl's method[14] on blood concentration of SfMeS.

$$C_{SfMeS} = 0.407e^{-0.228t} + 0.398e^{-0.0537t} \quad (5)$$

From the coefficients of equation 5, K ($=k+k_5$), k_1 and k_2 can be estimated. As the value of k, hydrolysis rate determined in whole blood may be most pertinent and from K and k, k_5 can be obtained. Values estimated are listed in Table II. The plots obtained by administration of intact Sf gives following experimental equation on the blood concentration of Sf, which is denoted by C_{sf}.

Table II. Pharmacokinetic Parameters Determined

	Rate constant (min^{-1})							Apparent distribution volume (ml)	
	k	k_1	k_2	k_3	k_4	k_5	k_6	Intact sulfonamide	MSD of sulfonamide
Sf	0.0141	0.0545	0.140	0.0388	0.0950	0.0734	0.0478	1380	817
Sa	0.0415	0.161	0.257	0.0173	0.0936	0.0565	0.0458	1130	728

$$C_{Sf} = 0.159e^{-0.152t} + 0.182e^{-0.0300t} \quad (6)$$

By conventional method on ordinary two compartment model k_3, k_4 and k_6 were calculated from the coefficients of equation 6.[15] On the other hand solution of the differential equation

12) To specify the sulfonamide produced *in vivo* by the hydrolysis the abbreviation of sulfonamide is marked with asterisk, *e.g.* Sf* and Sa*.
13) See Appendix.
14) W. Perl, *Intern. J. Appl. Radiation Isotopes*, **8**, 211 (1960).
15) S. Riegelman, J.C.K. Loo and M. Rouland, *J. Pharm. Sci.*, **57**, 117 (1968).

gives four exponential equation 7 with respect to X_3,[13] where X_1° is the initial dose administrated and α and β are same to those in equation 4.

$$\frac{X_3}{X_1^0} = Ce^{-\alpha t} + De^{-\beta t} + Ee^{-\gamma t} + Fe^{-\delta t} \quad (7)$$

As all rate constants, k_i (i=1 to 6) and k, were already estimated as above, which were tablated in Table II, equation 7 can be rewritten in numerical form as follows.

$$\left(\frac{X_3}{X_1^0}\right)_{Sf^*} = -0.0627e^{-0.228t} - 0.0728e^{-0.0537t} + 0.0102e^{-0.152t} + 0.177e^{-0.0300t} \quad (8)$$

Numerical calculation of equation 8 reveals that maximum of this function is at about 10 minutes, which is in good agreement with the peak obtained experimentally. When t is large, only 4th and 2nd terms in equation 8 are significant, *e.g.* at 60 minutes these are 0.0264 and 0.0029 respectively and the other terms are negligible. In these region the declining slope of equation 8 is same to that of C_{sf}, both of those are experimentally in good agreement as seen in Fig. 5.

Similarly pharmacokinetic calculations were carried out on SaMeS and following equations corresponding to equations 5, 6, and 8 were obtained.

$$C_{SaMeS} = 0.665e^{-0.461t} + 0.660e^{-0.0547t} \quad (9)$$

$$C_{Sa} = 0.437e^{-0.207t} + 0.282e^{-0.0207t} \quad (10)$$

$$\left(\frac{X_3}{X_1^0}\right)_{Sa^*} = -0.000685e^{-0.461t} - 0.155e^{-0.0547t} - 0.0327e^{-0.207t} + 0.257e^{-0.0207t} \quad (11)$$

The maximum of equation 11 is at about 15 minutes which is in accord with the peak obtained experimentally.

For the evaluation of MSD values k and k_5 are important among the values listed in Table II. The ratio of the amounts that is hydrolyzed *in vivo* and that directly eliminated is proportional to k: k_5 in the dynamic process shown in Chart 1.

Appendix

Chart 1 is formulized as follows.

$$\frac{dX_1}{dt} = k_2X_2 - (k+k_1+k_5)X_1 \quad (i)$$

$$\frac{dX_2}{dt} = k_1X_1 - k_2X_2 \quad (ii)$$

$$\frac{dX_3}{dt} = kX_1 + k_4X_4 - (k_3+k_6)X_3 \quad (iii)$$

$$\frac{dX_4}{dt} = k_3X_3 - k_4X_4 \quad (iv)$$

(a) Solution with Respect to X_1——From equations i and ii treating similarly to those of typical two compartment model[15] with the exception that outlet of X_1 is composed of two rate processes, $k+k_5=K$, equation v is obtained with respect to X_1 where X_1° is the initial dose administrated.

$$\frac{X_1}{X_1^0} = \frac{k_2-\alpha}{\beta-\alpha}e^{-\alpha t} + \frac{k_2-\beta}{\alpha-\beta}e^{-\beta t} \quad (v)$$

α and β are

$$\alpha \text{ and } \beta = \frac{(K+k_1+k_2) \mp \sqrt{(K+k_1+k_2)^2-4k_2K}}{2} \quad (vi)$$

Consequently blood concentration is represented in the form of equation 4. From the experimentally determined coefficients of equation 4 rate constants are calculated with following relationships.

$$k_2 = A'\beta + B'\alpha \quad \text{(vii)}$$

$$k_1 = A'B'(\beta-\alpha)^2/k_2 \quad \text{(viii)}$$

$$K = 1/(A'/\alpha+B'/\beta) \quad \text{(ix)}$$

In these equations $A'=A/(A+B)$ and $B'=B/(A+B)$. Substituting k value determined experimentally into the relationship $K=k+k_5$, k_5 can be calculated.

(b) Solution with Respect to X_3——By Laplace transformation, solution with respect to X_3 gives equation in the form of equation 7 which is composed of four exponential terms. In equation 7, α and β are same to those of equation 4 and γ and δ are as follows.

$$\gamma \text{ and } \delta = \frac{(k_3+k_4+k_6) \mp \sqrt{(k_3+k_4+k_6)^2-4k_4k_6}}{2} \quad \text{(x)}$$

And C, D, E, and F are as follows.

$$C = \frac{-k(\alpha-k_2)(\alpha-k_4)}{(\alpha-\beta)(\alpha-\gamma)(\alpha-\delta)} \quad \text{(xi)}$$

$$D = \frac{-k(\beta-k_2)(\beta-k_4)}{(\beta-\alpha)(\beta-\gamma)(\beta-\delta)} \quad \text{(xii)}$$

$$E = \frac{-k(\gamma-k_2)(\gamma-k_4)}{(\gamma-\alpha)(\gamma-\beta)(\gamma-\delta)} \quad \text{(xiii)}$$

$$F = \frac{-k(\delta-k_2)(\delta-k_4)}{(\delta-\alpha)(\delta-\beta)(\delta-\gamma)} \quad \text{(xiv)}$$