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Metabolism of Piromidic Acid, a New Antibacterial Agent. V.[1] Pharmacokinetics of Piromidic Acid in Humans

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The pharmacokinetic studies of piromidic acid (PA, 5,8-dihydro-8-ethyl-5-oxo-2-pyrrolidinopyrido[2,3-*d*]pyrimidine-6-carboxylic acid=2-pyrrolidino-PPA) were performed in human subjects after oral administration. A compartment model involved thirteen parameters was adopted, in which PA compartment was connected to each compartment in branched metabolic pathway. Each parameter was determined by mathematical method, and a trial and error procedure using the experimental data on blood levels and urinary excretion in man receiving oral PA. Calculated urinary excretion of PA or its metabolites was found to be reasonable.

Combined amounts calculated for biological active materials, PA, M-II (2-(2-hydroxypyrrolidino)-PPA) and M-V (2-(3-hydroxypyrrolidino)-PPA) were found to be in good agreement with those by bioassay, demonstrating that this pharmacokinetic model is valid to lead to better understanding of chemotherapeutic effect of PA.

Keywords——piromidic acid; antibacterial agent; pharmacokinetics; urinary excretion; metabolic pathway; kinetic constant in man

The previous paper[1] revealed that a pharmacokinetic model containing six compartment and eleven parameters was an acceptable and simplified description of piromidic acid (PA, 5,8-dihydro-8-ethyl-5-oxo-2-pyrrolidinopyrido[2,3-*d*]pyrimidine-6-carboxylic acid) metabolism in rats after intravenous administration.

The metabolism of PA in man has been also revealed to be comparable with those in rats,[3] therefore, a study of the pharmacokinetic profile of PA and its metabolites in man, by applying the model in rats, has been performed for better understanding of chemotherapeutic effect of PA.

This paper deals with determination of kinetic parameters for the pharmacokinetic description of PA in man, based on evaluation of blood levels and urinary excretion data, which were reported in the previous paper,[4] after oral administration of PA to man. The results obtained were discussed in comparison with those in rats.

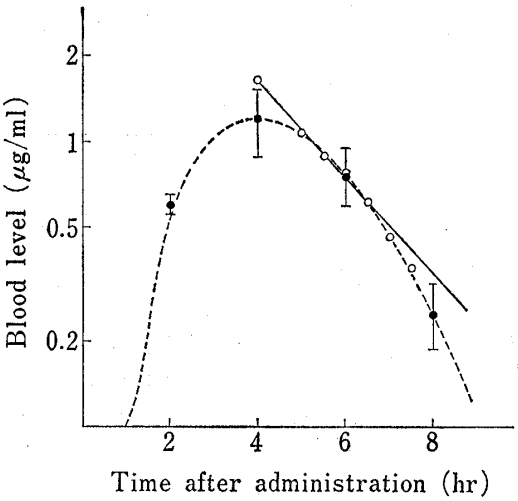

Fig. 1. Semilogarithmic Plots of PA in Blood of Four Volunteers receiving Oral PA at a Dose of 1 g

Plotting: ●, mean of four men ± S.E.
○, mean of two men in a separate experiment.

Pharmacokinetic Model of PA in Man

The elimination of PA from blood can be assumed to be monoexponential, because the

1) Part IV: Y. Sekine, M. Miyamoto, M. Hashimoto, and K. Nakamura, *Chem. Pharm. Bull.* (Tokyo), **24**, 1462 (1976).
2) Location: *33–94 Enoki-cho, Suita, Osaka, 564, Japan.*
3) Y. Sekine, M. Miyamoto, M. Hashimoto, and K. Nakamura, *Xenobiotica*, **6**, 185 (1976).
4) Y. Sekine, M. Miyamoto, M. Hashimoto, and K. Nakamura, *Chem. Pharm. Bull.* (Tokyo), **24**, 1433 (1976).

blood level-time curves of unchanged PA in man receiving oral PA have obtained as shown in Fig. 1. In addition to this, the previous works[1,3] on pharmacokinetics of PA in rats together with urinary and biliary metabolic patterns of rats and humans receiving oral PA led to a kinetic model of PA in humans as shown in Chart 1, which simulates the behavior of PA in a body after oral administration. In the model, compartments 7, 8, 9, 10, 11 and 12 each contain both unconjugated and conjugated metabolites to simplify the model. Lag

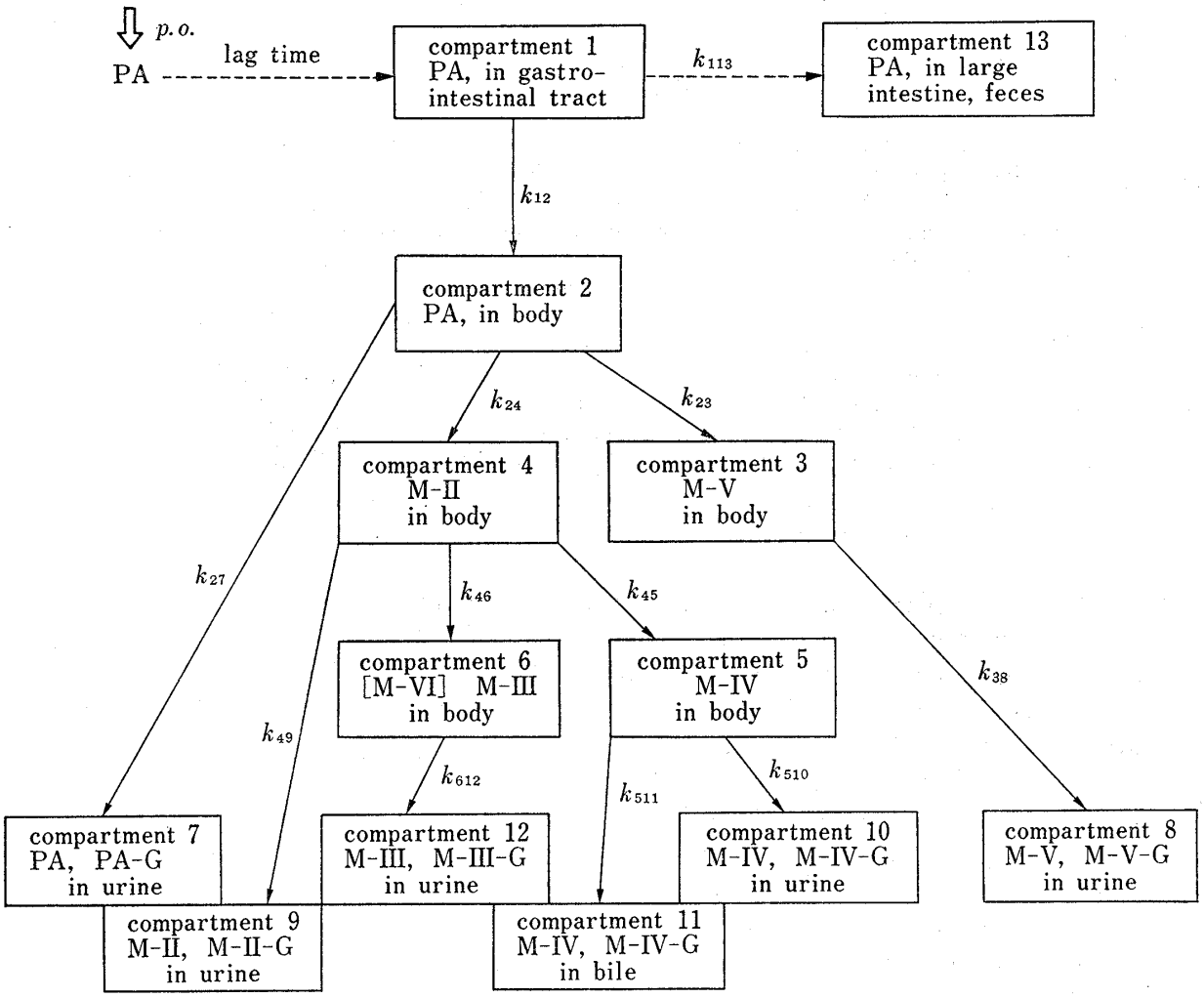

Chart 1. Schematic Representation of PA and Its Metabolites in Body as Linear Compartment Model in Man after Oral Administration

M-II: 2-(2-hydroxypyrrolidino)-PPA, M-III: 2-amino-PPA; M-IV: 2-(3-hydroxycarbonylpropylamino)-PPA, M-V: 2-(3-hydroxypyrrolidino)-PPA, G: glucuronide, PPA: 5,8-dihydro-8-ethyl-5-oxo-pyrido[2,3-*d*]pyrimidine-6-carboxylic acid.

time is defined as the time interval between ingestion of PA and appearance of PA in blood, since PA, which was administered orally in a powder form to man, was found to be very slightly soluble in acidic solution, and the absorption of PA from stomach is likely to be negligible. Compartment 13 can be assumed to be feces and large intestine compartment. PA in compartment 13 indicates the amount of PA excreted into feces without further absorption. Compartment 1 can be assumed to be PA in duodenum, jejunum and ileum. The transit of PA from compartment 1 to compartment 13 is also assumed to require 3 hr, from 6 to 9 hr after administration, based on the transit experiment of charcoal in gastrointestinal tract of rats,[5] that charcoal was found to require 6 hr to reach large intestine and 9 hr to pass

5) Y. Sohji, "unpublished."

completely ileum after oral administration. In mice receiving ^{14}C–PA,[6] levels of radioactivity in gastrointestinal contents were consistent with those of the charcoal experiment. Since a part of PA in ileum can be assumed to pass into large intestine (compartment 13) with first order rate constant, k_{113} can be calculated as a constant of 1.54 hr^{-1} according to the definition described above.[7] Compartment 11, which can be assumed to be M–IV (see the legend in Chart 1) excreted into bile, was added to the model, since M–IV was found to be excreted into bile in high concentrations and virtually not reabsorbed from intestinal tract in rats.[1] The amount of M–IV excreted into bile is defined as the amount which was obtained by subtracting the total urinary amount of PA and its metabolites from the absorbed PA.

Experimental

Blood Levels and Urinary Excretion of Unchanged PA and Its Metabolites in Humans receiving Oral PA——The data of blood levels and urinary excretion of PA and its metabolites in man receiving oral PA were reported in the previous paper.[4] The levels of PA and its metabolites in blood were found almost entirely to be unconjugated (more than 95%).

Analysis of the Kinetic Model——In the model, the amounts of drug in each compartment at any time t after lag time are given as $X_1, X_2, \ldots X_i, \ldots$ and X_{13}, respectively. The equations describing the urinary excretion of the metabolites were derived in the same way as described in the previous paper.[1] The apparent volumes of distribution (V_i) are designated as $V_1 = V_2 = \ldots = V_{13}$ for thirteen compartments. k_{ij} indicates the rate constant from the compartment i to j, and all the rate processes are assumed to be first-order. The variation of the amount X_i in the compartment i at any time t can be written as the equation described in "Appendix" (Eq. (1A)). The amounts of PA in blood and PA, M-II, M-III, M-IV and M-V excreted in the urine can be written as Eq. (2A) to Eq. (9A) ($t \leqq 5$, see "Pharmacokinetic Model of PA in Man").

Blood level and urinary excretion data, prior to calculation, were corrected from the time taken as new zero, since the lag time is an hour[8] (see Fig. 1) after oral administration of PA by the definition described above.

Firstly, the absorption rate constant (k_{12}) and the elimination rate constant ($k' = k_{23} + k_{24} + k_{27}$, see "Appendix") of PA were calculated. Blood levels of PA (c μg/ml, $t \leqq 5$) can be written as the following equation (see Eq. (2A)):

$$c = A(e^{-k_{12}t} - e^{-k't}) \tag{1}$$

where A is a constant containing the rate constants of k_{12} and k'. The constants k_{12}, k' and A can tentatively be calculated from blood level of PA by a trial and error procedure. Secondly, the constant k_{27} can be calculated from Eq. (2A), since the ratio of k_{27} to k' can be assumed to be equal to the ratio of excretion of conjugated and unconjugated PA to PA absorbed:[1]

$$k_{27}/k' = X_{7\infty}/\sum_{i=7}^{12} X_{i\infty} \tag{2}$$

where $X_{7\infty}$ is the total amount of conjugated and unconjugated PA excreted into urine (compartment 7) after lag time. Therefore, the constant k_{27} may be calculated from $X_{7\infty}$, PA absorbed (equal to $\sum_{i=7}^{12} X_{i\infty}$) and the constant k'. Similarly, the constant k_{23} and k_{24} can be calculated from Eq. (3) and (4):

$$k_{23}/k' = X_{8\infty}/\sum_{i=7}^{12} X_{i\infty} \tag{3}$$

$$k_{24}/k' = \sum_{i=9}^{12} X_{i\infty}/\sum_{i=7}^{12} X_{i\infty} \tag{4}$$

The constant k_{38} can be calculated from Eq. (5A) and the urinary excretion data.[1] The constant k_{49} can be calculated by the same estimation as of k_{38}. Therefore, the constants k_{45} and k_{46} can be calculated by using

6) M. Shimizu, Y. Sekine, H. Higuchi, H. Suzuki, S. Nakamura, and K. Nakamura, *Antimicrob. Ag. Chemother.*, **1970**, 123; H. Suzuki, "unpublished."

7) The calculation is based on the assumption that 99% of the drug in ileum pass into large intestine in 3 hr. From the constants k_{113} and k_{12} (see Table I), the unabsorbed PA to the dose administered was calculated as 10.3%, corresponding roughly to the 24 hr fecal excretion of antibacterial active material(s) (13.5%, see "Discussion" in the text).

8) In two of four volunteers receiving oral PA at a dose of 1 g, mean blood levels of PA at 0.5, 1, 1.5 and 2 hr after dosing were assayed and found to be trace, trace, 0.45 and 0.65 μg/ml, respectively. Therefore, lag time was assumed as an hour after oral administration.

Eq. (5), (6) and (7). The constant k_{510} and k_{612} can be calculated from Eq. (7A) and (9A) by using the data for urinary excretion of M-IV and M-III.[1)]

$$k_{49}/k'' = X_{9\infty}/\sum_{i=9}^{12} X_{i\infty} \quad (5)$$

$$k_{45}/k_{49} = (X_{10\infty}+X_{11\infty})/X_{9\infty} \quad (6)$$

$$k_{46}/k_{49} = X_{12\infty}/X_{9\infty} \quad (7)$$

The constant k_{511} is assumed to be proportional to k_{510} and therefore can be calculated from Eq. (8):

$$k_{510}/k_{511} = X_{10\infty}/X_{11\infty} \quad (8)$$

The calculation of the amount in each compartment from 5 hr after lag time was made as follows: (a) Compartment 13 was introduced to the model. (b) Each equation representing the amount of metabolite in the compartment i was derived. (c) A shift in zero time was again made and observed drug excretion was corrected for by substracting of amount excreted in 5 hr period. (d) The calculations were similarly made as mentioned above. (e) Total amount of each compartment i ($7 \leqq i \leqq 12$) can be given as the amount calculated in (d) plus each amount in 5 hr after lag time.

Table I. Rate Constants in Kinetic Model of PA in Man

Rate constant	k (hr^{-1})	Rate constant	k (hr^{-1})
k_{12}	4.07×10^{-1}	k_{49}	4.50×10^{-2}
k_{23}	6.78×10^{-2}	k_{510}	1.30
k_{24}	6.24×10^{-1}	k_{511}	2.51
k_{27}	1.71×10^{-2}	k_{612}	5.00
k_{38}	1.00		
k_{45}	8.17×10^{-1}	k_{113}	1.54
k_{46}	2.03×10^{-2}		
Lag time 1 hr			

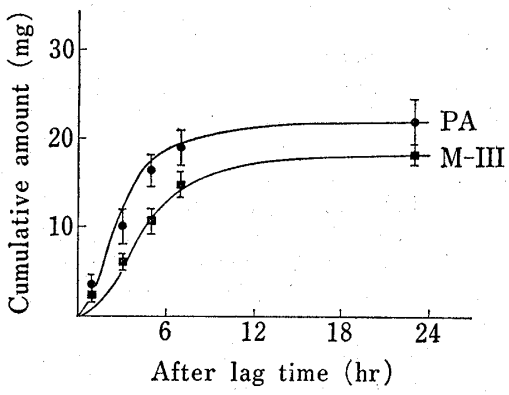

Fig. 2. Cumulative Urinary Excretion Plots and Its Calculated Curves of PA and M-III in Man receiving Oral PA at a Dose of 1 g
(mean of four men ± S.E.)

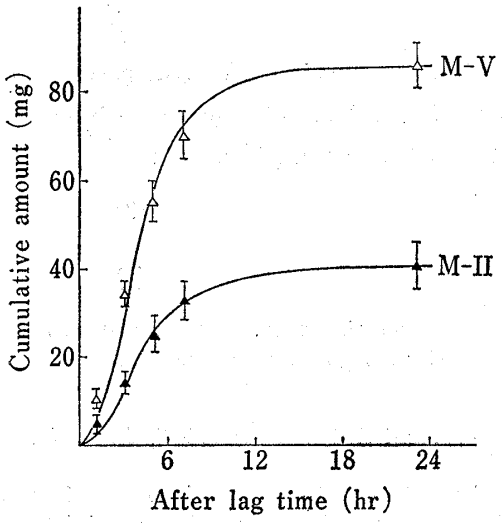

Fig. 3. Cumulative Urinary Excretion Plots and Its Calculated Curves of M-II and M-V in Man receiving Oral PA at a Dose of 1 g
(mean of four men ± S.E.)

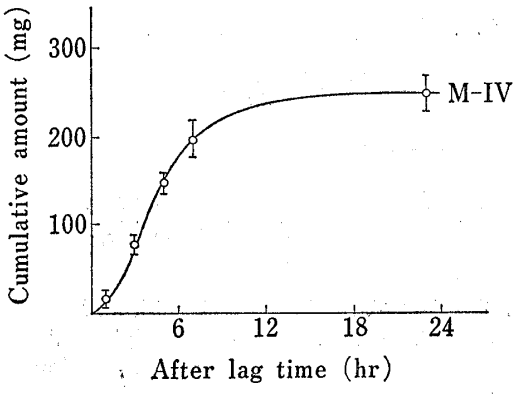

Fig. 4. Cumulative Urinary Excretion Plots and Its Calculated Curve of M-IV in Man receiving Oral PA at a Dose of 1 g
(mean of four men ± S.E.)

Results

The twelve rate constants together with lag time in the pharmacokinetic model of PA in Chart 1 were calculated from the observed data on blood levels and urinary excretions of PA and its metabolites after oral administration of PA to man. The parameters are listed in Table I.

Substitution of these parameters in Eq. (4A), (5A), (6A), (7A) and (9A) afforded cumulative urinary excretion curves of PA, M–V, M–II, M–IV and M–III, respectively, after lag time in humans receiving oral PA. These calculated urinary excretion curves are depicted in Fig. 2, 3 and 4. Fitness was reasonable for unchanged PA and its metabolites.

Discussion

The pharmacokinetic model shown in Chart 1 was found to be a useful and simplified description of PA metabolism in man after oral administration. In the model, the following two assumptions are made in regard to the absorption of PA in gastrointestinal tract. (a) Lag time is defined as the time interval between ingestion and appearance of PA in blood. (b) The movement of PA in compartment 1 to compartment 13 is defined to require 3 hr, from 6 to 9 hr after administration (5 to 8 hr after lag time). The former assumption is likely to be closely related to the gastric emptying time, since PA was found to be very slightly soluble in acidic solution (about 3 μg/ml in buffer solution at pH 4.9, at 25°), and the absorption of PA from stomach is likely to be negligible. The latter assumption is consistent with the results that the blood level of unchanged PA apparently decreased at 8 hr after administration (see Fig. 1) and that antibacterial active material excreted into feces was 13.5% of the dose.[9]

In compartments 7 to 12, each amount X_i consists of both unconjugated and conjugated metabolites. Therefore, two urinary excretion processes with and without conjugation as shown in Chart 2 are given as a rate constant k_{ij} to simplify the model, though each amount of unconjugated metabolite in urine, except for PA, was found to be predominant. Since the calculated urinary excretion curves are in agreement with the observed data as shown in Fig. 2 to 4, two urinary excretion rates with and without the conjugation process are likely to be nearly equal. The ratio (f_i) of the total amount of glucuronide to amount $X_{i\infty}$ can be calculated from the observed data, therefore, the urinary amounts of glucuronide at any time t in compartment i ($7 \leqq i \leqq 12$) can roughly be given as the amount $f_i X_i$. The calculated amounts are in agreement with the observed data as shown in Fig. 5.

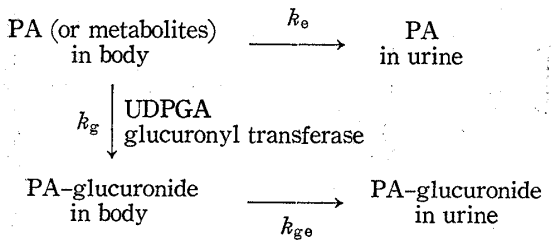

Chart 2. Excretion Model of PA (or Metabolites) and Its Glucuronide into Urine

k_e: excretion rate constant, k_g: metabolic rate constant, k_{ge}: glucuronide excretion rate constant

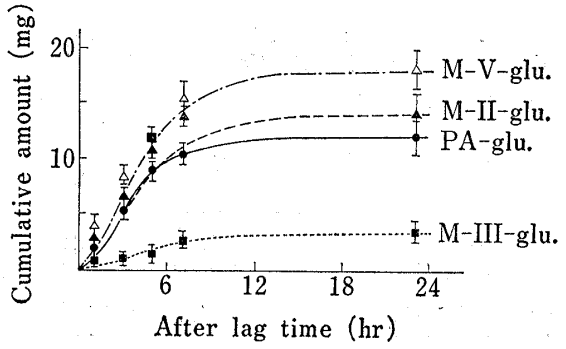

Fig. 5. Cumulative Urinary Excretion Plots and Its Calculated Curves ($f_i X_i$) of Glucuronides in Man receiving Oral PA at a Does of 1 g

(mean of four men ± S.E.)

9) M. Shimizu, Y. Takase, S. Nakamura, and N. Kurobe, "unpublished."

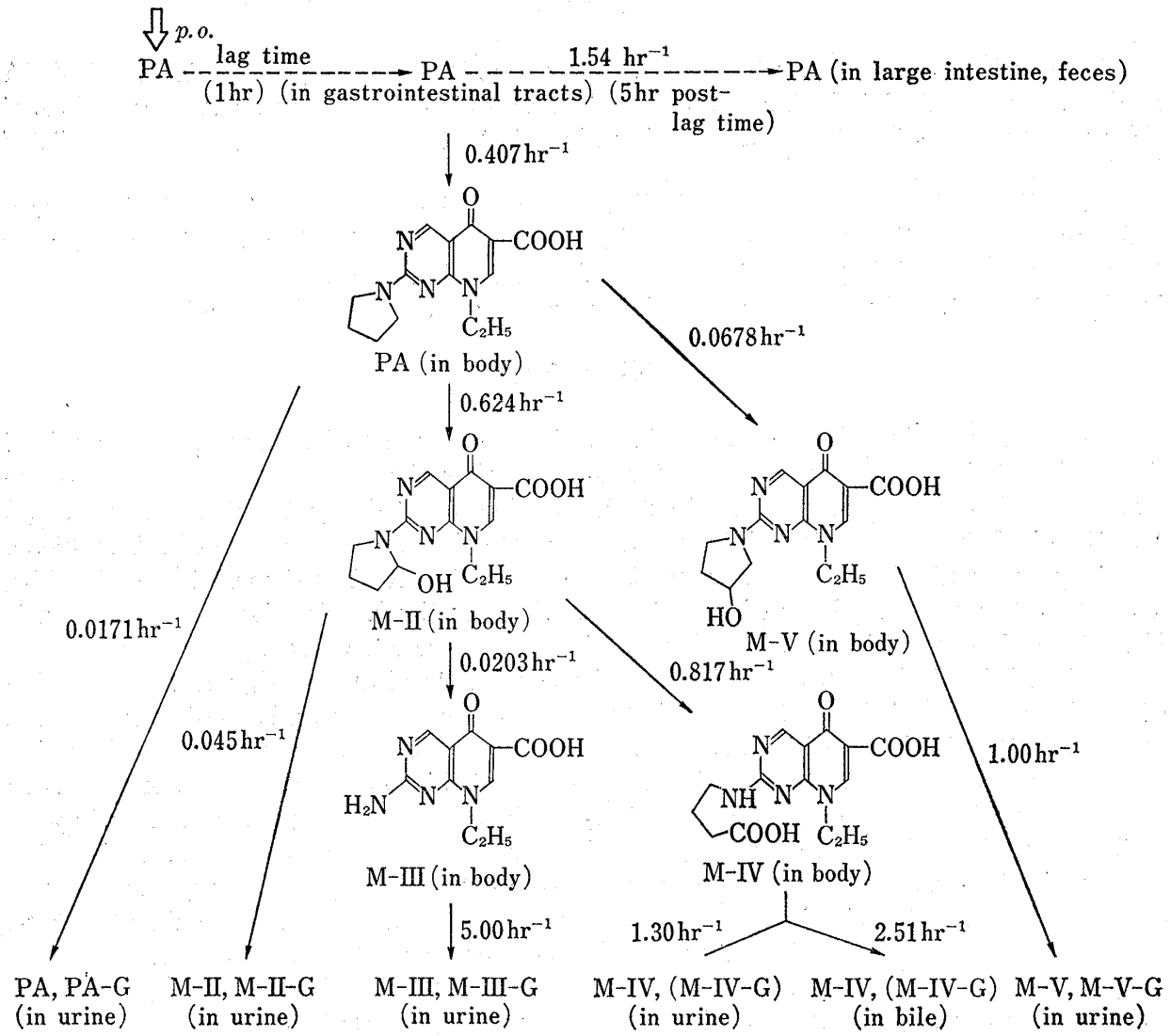

Chart 3. Kinetic Pathway of PA in Man after Oral Administration

Table II. Comparison with Rate Constants of Man and Rats in Kinetic Model of PA

Rate constant	k (hr^{-1}) Man	Rats[a]
k_{23} (PA→M-V)	0.07	0.91
k_{24} (PA→M-II)	0.62	8.16
k_{27} (PA excretion)	0.02	0.05
k_{38} (M-V excretion)	1.00	1.40
k_{45} (M-II→M-IV)	0.82	1.52
k_{46} (M-II→M-III)	0.02	0.14
k_{49} (M-II excretion)	0.05	0.05
$k_{510}+k_{511}$ (M-IV excretion)	3.81	5.10
k_{612} (M-III excretion)	5.00	1.20

a) Taken from Y. Sekine, M. Miyamoto, M. Hashimoto, and K. Nakamura, *Chem. Pharm. Bull.* (Tokyo), 24, 1462 (1976).

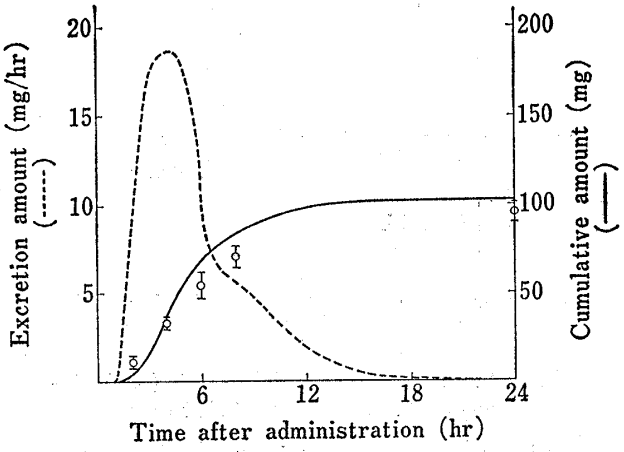

Fig. 6. Calculated Urinary Excretion Curves of Antibacterial Substances (Unconjugated PA, M-II and M-V) and Excretion Plots obtained by Bioassay in Man receiving Oral PA at a Dose of 1 g
(mean of four men ± S.E.)

The kinetic pathway of PA in man receiving oral PA is summarized in Chart 3. Each rate constant of PA metabolism in humans was compared with that of rats (Table II). Marked differences between man and rats were seen on the constants k_{23}, k_{24} and k_{46}, which indicate the rate constants of oxidation processes by the mixed-function oxidase on PA-metabolism.[10] However, the ratios of the constant k_{23} to k_{24} were 1/9.2 in man and 1/9.0 in rats, with the latter being in good agreement with an *in vitro* microsomal conversion ratio in rats.[10] This indicates that *in vivo* hydroxylation ratio of 2- and 3-positions in pyrrolidine ring of PA is nearly equal in both species. On the contrary, k_{45}, the constant associated with the aldehyde dehydrogenase,[10] was nearly equal in both species. Therefore, the relative activities of both enzymes in humans and rats possibly result in the difference of M–III and M–IV formation.

A two-hundred fold exceeding elimination rate as compared with the formation rate was found for M–III leading to its markedly low blood levels in man.

The urinary antibacterial activity after administration of PA is given as the combined amount of unconjugated PA and active metabolites, M–II and M–V,[4] as indicated that the antibacterial activity of M–II and M–V was equal to that of PA.[3] Therefore, the combined amount excreted into urine is useful for understanding of clinical effects of PA, which chiefly given to the patients with urinary tract infections.[11] Substitutions of the parameters in Eq. (4A), (5A) and (6A) afford urinary amounts of PA, M–V and M–II (X_7, X_8 and X_9) in man, respectively, so the products $(1-f_i)X_i$ ($i=7, 8, 9$) give the urinary unconjugated amounts of PA, M–V and M–II. Fig. 6 shows the calculated urinary excretion amounts of antibacterial substances in man receiving oral PA at a dose of 1 g. These values were found to be agreement with the amounts obtained by bioassay.[6,12]

This pharmacokinetic model may be concluded from the above results to be relevant for better understanding of chemotherapeutic effect of PA.

Appendix

The variation of the amount X_i of drug in the compartment i can be written as Eq. (1A):

$$dX_i/dt = \sum_{j=1}^{n}(k_{ji}X_j) - (\sum_{j=1}^{n}k_{ij})X_i \qquad (1A)$$

$$i, j = 1, 2, \cdots, 13$$

Therefore, the amount of unchanged PA in the body (X_2) are given as Eq. (2A) by the same way as described previously:[1]

$$X_2/x_0 = k_{12}\exp(-k_{12}t)/(k'-k_{12}) - k_{12}\exp(-k't)/(k'-k_{12}) \qquad (2A)$$

where x_0 is the amount of PA in the compartment 1 at zero after lag time, which is regarded as the dose of PA, and k' is given as Eq. (3A):

$$k' = k_{23} + k_{24} + k_{27} \qquad (3A)$$

Similarly, the amounts of PA, M–V, M–II, M–IV and M–III excreted into urine, and of M–IV excreted into bile are given as follows:

$$X_7/x_0 = -k_{27}\exp(-k_{12}t)/(k'-k_{12}) + (-k_{12}k_{27})\exp(-k't)/k'(k_{12}-k') + k_{27}/k' \qquad (4A)$$

$$X_8/x_0 = -k_{23}k_{38}\exp(-k_{12}t)/(k'-k_{12})(k_{38}-k_{12}) + (-k_{12}k_{23}k_{38})\exp(-k't)/k'(k_{12}-k')(k_{38}-k') + (-k_{12}k_{23})\exp(-k_{38}t)/(k_{12}-k_{38})(k'-k_{38}) + k_{23}/k' \qquad (5A)$$

10) Y. Sekine, M. Miyamoto, M. Hashimoto, and K. Nakamura, *Chem. Pharm. Bull.* (Tokyo), **24**, 437 (1976).
11) J. Ishigami, S. Hara, T. Mita, and S. Kamidono, *Chemotherapy* **19**, 625 (1971).
12) M. Shimizu, S. Nakamura, Y. Takase, Y. Sekine, H. Suzuki, and K. Nakamura, *Chemotherapy*, **19**, 387 (1971).

$$X_9/x_0 = -k_{24}k_{49}\exp(-k_{12}t)/(k'-k_{12})(k''-k_{12}) + (-k_{12}k_{24}k_{49})\exp(-k't)/k'(k_{12}-k')(k''-k') + (-k_{12}k_{24}k_{49})\exp(-k''t)/k''(k_{12}-k'')(k'-k'') + k_{24}k_{49}/k'k'' \quad (6A)$$

$$X_{10}/x_0 = -k_{24}k_{45}k_{510}\exp(-k_{12}t)/(k'-k_{12})(k''-k_{12})(k'''-k_{12}) + (-k_{12}k_{24}k_{45}k_{510})\exp(-k't)/k'(k_{12}-k')(k''-k')(k'''-k') + (-k_{12}k_{24}k_{45}k_{510})\exp(-k''t)/k''(k_{12}-k'')(k'-k'')(k'''-k'') + (-k_{12}k_{24}k_{45}k_{510})\exp(-k'''t)/k'''(k_{12}-k''')(k'-k''')(k''-k''') + k_{24}k_{45}k_{510}/k'k''k''' \quad (7A)$$

$$X_{11}/x_0 = -k_{24}k_{45}k_{511}\exp(-k_{12}t)/(k'-k_{12})(k''-k_{12})(k'''-k_{12}) + (-k_{12}k_{24}k_{45}k_{511})\exp(-k't)/k'(k_{12}-k')(k''-k')(k'''-k') + (-k_{12}k_{24}k_{45}k_{511})\exp(-k''t)/k''(k_{12}-k'')(k'-k'')(k'''-k'') + (-k_{12}k_{24}k_{45}k_{511})\exp(-k'''t)/k'''(k_{12}-k''')(k'-k''')(k''-k''') + k_{24}k_{45}k_{511}/k'k''k''' \quad (8A)$$

$$X_{12}/x_0 = -k_{24}k_{46}k_{612}\exp(-k_{12}t)/(k'-k_{12})(k''-k_{12})(k_{612}-k_{12}) + (-k_{12}k_{24}k_{46}k_{612})\exp(-k't)/k'(k_{12}-k')(k''-k')(k_{612}-k') + (-k_{12}k_{24}k_{46}k_{612})\exp(-k''t)/k''(k_{12}-k'')(k'-k'')(k_{612}-k'') + (-k_{12}k_{24}k_{46})\exp(-k_{612}t)/(k_{12}-k_{612})(k'-k_{612})(k''-k_{612}) + k_{24}k_{46}/k'k'' \quad (9A)$$

where k'' and k''' are given as Eq. (10A) and (11A):

$$k'' = k_{45} + k_{46} + k_{49} \quad (10A)$$

$$k''' = k_{510} + k_{511} \quad (11A)$$

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