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Comparative Pharmacokinetics of Metabolism and Urinary Excretion of Isoxazolylpenicillins in Man

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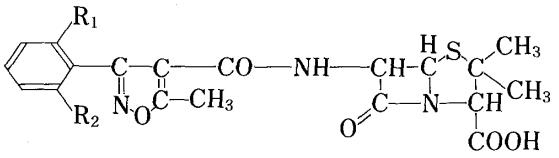
Pharmacokinetic evaluations of cloxacillin and dicloxacillin in man were performed by detailed investigations of the urinary excretion profiles of the unchanged form and three metabolites (penicilloic acid, 5-hydroxymethyl derivative, and penicilloic acid of the 5-hydroxymethyl derivative). The last metabolite was newly found in human urine. The time course curves for excretion of unchanged form and the metabolites were measured by high performance liquid chromatography of urine excreted after oral administration of cloxacillin and dicloxacillin to human subjects. The values of cumulative excretion amount at infinite time (X_u^∞) and of mean residence time (*MRT*) for each species were estimated by moment analysis of excretion rate *vs.* time curves. The metabolic pathways of cloxacillin and dicloxacillin were assessed, and the transfer ratio at each elimination step and the *MRT* value intrinsic to each metabolite were evaluated from the results of moments. On the basis of these and the previous results for oxacillin and flucloxacillin, the pharmacokinetic features of four isoxazolylpenicillins are compared in terms of statistical moments. The extent and rate of total bioavailable activity due to the unchanged form and 5-hydroxymethyl metabolite are also discussed comparatively.

Keywords——oxacillin; cloxacillin; dicloxacillin; flucloxacillin; urinary excretion; metabolism; pharmacokinetics; moment analysis; mean residence time; high performance liquid chromatography

Oxacillin, cloxacillin, dicloxacillin, and flucloxacillin are isoxazolyl analogues of semisynthetic penicillins presently used for clinical chemotherapy (Fig. 1).



oxacillin (OX) : $R_1 = -H$, $R_2 = -H$
cloxacillin (CX) : $R_1 = -Cl$, $R_2 = -H$
dicloxacillin (DX) : $R_1 = -Cl$, $R_2 = -Cl$
flucloxacillin (FX) : $R_1 = -Cl$, $R_2 = -F$

Fig. 1. Isoxazolylpenicillins

These penicillins are known to have activity against Gram-positive bacteria including penicillin G-resistant *Staphylococci*, and their acid stability and effective absorption through the gastrointestinal (G.I.) tract make them suitable for oral use.[1-4] Beside the chemical and physicochemical properties of these drugs, data on their biopharmaceutical characteristics, especially pharmacokinetic behavior, have been accumulated. Gravenkemper *et al.*[1] observed higher serum levels and smaller amounts of urinary excretion of dicloxacillin than cloxacillin and oxacillin for the same doses in healthy subjects. Naumann[2] and Modr *et al.*[5] attributed this difference to the better absorption, slower elimination, and smaller volume of distribution of dicloxacillin than cloxacillin and oxacillin. The absorption of isoxazolylpenicillins through the G.I. tract is known to increase with increasing halogen substitution on the phenyl ring.[2,3]

Rosenblatt *et al.*,[6] comparing the steady-state plasma levels of oxacillin, cloxacillin, and dicloxacillin, concluded that the high serum levels of dicloxacillin were a consequence of a small renal clearance and strong resistance to biodegradation in the liver. Notari[7] reported a detailed pharmacokinetic consideration based on compartment-model analysis, concluding that the higher serum level of dicloxacillin than cloxacillin (dicloxacillin > cloxacillin) is due to decreased elimination of dicloxacillin, and cloxacillin > oxacillin is due to decreased distribution. Similarly, flucloxacillin > cloxacillin was ascribed to slower renal and extrarenal elimination and better absorption.[8] The high serum level of isoxazolylpenicillins was also explained in terms of high degree of binding to plasma protein (>90%). Nauta *et al.*[8-10] and Thijssen *et al.*[11] compared the pharmacokinetic properties of isoxazolylpenicillins in healthy and renal failure subjects. Renal failure, as expected, caused decreased urinary excretion, resulting in prolonged high serum levels.

On the other hand, isoxazolylpenicillins are known to be biotransformed to a considerable extent in man to active metabolites,[14] whose antibiotic activities are of almost the same order of magnitude as those of the parent penicillins.[15] Van Harken *et al.*[16] elucidated the structure of the active metabolite of dicloxacillin as 6-[3-(2,6-dichlorophenyl)-5-hydroxymethyl-4-isoxazolecarboxamido]-penicillanic acid, *i.e.* the 5-hydroxymethyl derivative of the parent compound. Thijssen showed that the active metabolite in the rat is the 5-hydroxymethyl derivative of the parent penicillin for four isoxazolylpenicillins.[17] In the previous papers,[18,19] we reported that a similar metabolism occurs in man, and achieved the isolation and gas chromatography-mass spectrometric (GC-MS) identification of the active metabolites as the 5-hydroxymethyl derivative of the parent penicillins.

Most pharmacokinetic studies of isoxazolylpenicillins have so far been performed on the basis of biological methods. In general, microbioassay is highly sensitive, but is not very suitable for defining the amounts of co-existing active substances. Therefore, for samples comprising active metabolites together with the parent compound, a more specific and precise assay method has been required. High performance liquid chromatography (HPLC), particularly in the reversed phase mode, is suitable for this purpose because of its high specificity, accuracy, and reproducibility with minimum pretreatment.[19-25] Recently, Thijssen[24] described HPLC analysis of isoxazolylpenicillins and obtained pharmacokinetic parameters for cloxacillin and flucloxacillin taking their penicilloic acids and active metabolites into account, but unfortunately the results were obtained with only one subject, and oxacillin and dicloxacillin were not discussed. In our previous studies, we discovered new metabolites (*i.e.* penicilloic acids of the 5-hydroxymethyl derivatives) of oxacillin[25] and flucloxacillin,[19] by the precise HPLC separation of human urine following administrations of oxacillin and flucloxacillin, and discussed their metabolic pathways and pharmacokinetics in man on the basis of moment analysis of urinary excretion rate-time curves.

The aim of the present work was to investigate the metabolism and pharmacokinetics of cloxacillin and dicloxacillin and to compare the pharmacokinetic behavior of four isoxazolylpenicillins in man.

Experimental

Reagents and Materials——Sodium cloxacillin and sodium dicloxacillin used as standard materials were supplied by Meiji Seika Kaisha (Tokyo) and Banyu Pharmaceutical Co. (Tokyo), respectively. Cloxacillin capsules (Orbenin, 250 mg as potency) and dicloxacillin capsules (Staphcillin-A, 250 mg as potency) were obtained from Fujisawa Pharmaceutical Co. (Osaka) and Banyu Pharmaceutical Co., respectively. The 5-hydroxymethyl isoxazolyl derivatives of cloxacillin and dicloxacillin were isolated from human urine after administration of the parent penicillin by means of preparative column chromatography. The procedures were described in detail in the previous paper.[19] Penicilloic acids of cloxacillin and dicloxacillin and penicilloic acids of their 5-hydroxymethyl derivatives were prepared, respectively, by hydrolyses of the parent penicillins and the 5-hydroxymethyl derivatives in aqueous

1 N NaOH solution for 7 min at room temperature followed by neutralization with 0.5 N HCl. Glass-distilled water and methanol were used to prepare the mobile phase of HPLC. Tetrabutylammonium bromide (TBAB) and other chemicals used for HPLC were commercial products of analytical reagent grade.

Drug Administration and Sample Preparation——Five healthy male adults of 22 to 28 years, weighing 55 to 60 kg, participated in this experiment. Each subject, who had been drug-free for at least one week and had fasted overnight, received cloxacillin capsules (250 mg × 2) or dicloxacillin capsules (250 mg × 2) orally with 200 ml of water. Urine was collected from each subject just before and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, and 8.0 h after administration. After measurement of the volume, the urine was filtered through a 0.45 μm pore size membrane filter (Fuji Photo Film Co.) and a 5.0 μl portion of the filtrate was subjected to HPLC analysis under the conditions described below. The remaining portion was used for the isolation of the 5-hydroxymethyl derivatives.

High Performance Liquid Chromatography——A high performance liquid chromatograph (ALC/GPC 204 Waters Assoc.) equipped with an ultraviolet (UV) detector (254 nm, model 440, Waters Assoc.) was used in a reversed phase mode with a stationary phase of LiChrosorb RP-18 (10 μm particle diameter, E. Merck Co.) packed in 25 cm × 4.6 mm i.d. stainless steel tubing. A short pre-column (5 cm × 4.6 mm i.d.) packed with LiChrosorb RP-2 (E. Merck Co.) was used to guard the main column. The mobile phase used was i) for the assays of cloxacillin, an aqueous solution containing 7 mM TBAB, 0.01 M Na_2HPO_4, and 0.01 M KH_2PO_4 (pH = 6.98) mixed with acetonitrile at a volume ratio of 10/3, whose flow rate was maintained at 3.0 ml/min (2000 psi), and ii) for the assays of dicloxacillin, 0.02 M acetate buffer (pH = 5.88) mixed with acetonitrile at a volume ratio of 40/9, whose flow rate was maintained at 3.0 ml/min (1800 psi). The calibration lines were obtained by the peak height method.

Estimation of Statistical Moments——Urinary excretion amount at infinite time (X_u^∞) and mean residence time (MRT) are given by the zero and first normal moments,[26] which are defined as

$$X_u^\infty = \int_0^\infty (dX_u/dt)dt \tag{1}$$

$$MRT = \int_0^\infty t(dX_u/dt)dt \Big/ \int_0^\infty (dX_u/dt)dt \tag{2}$$

where dX_u/dt is a function expressing the urinary excretion rate-time curve. In using these equations, the moments were calculated by trapezoidal integration of the time course curve with extrapolation to infinite time on the basis of a monoexponential equation. The exponential equation was determined by the least-squares method using the last three to seven data points on the time course curve. The details of mathematical operations used to obtain the moments from the experimental data were described in the previous paper.[26] The computations were carried out on a personal computer (PET 2001, Commodore Co.) with programming in BASIC.

Results

HPLC Analysis

Figures 2 and 3 show the chromatograms of urine excreted 2.5 h after administration of cloxacillin and dicloxacillin. Peaks 2,3 and 4 in both chromatograms were assigned, respectively, to penicilloic acid of parent penicillin, the 5-hydroxymethyl derivative and unchanged penicillin by comparing their retention times with those of standard materials. Peak 1 is due to a new metabolite (*i.e.* penicilloic acid of the 5-hydroxymethyl derivative), which was confirmed by enzymatic hydrolysis and alkaline hydrolysis of the corresponding 5-hydroxymethyl derivative. The procedure for hydrolysis has been described previously.[19,25]

Metabolic Pathways

In the previous papers, we investigated the metabolic pathways of oxacillin[25] and flucloxacillin[19] in man, and found that the penicilloic acid of the 5-hydroxymethyl derivative was produced by the *in vivo* hydrolysis of the 5-hydroxymethyl derivative, but not by the hydroxylation of penicilloic acid. In order to check that this is also the case for cloxacillin and dicloxacillin, these penicillins and their known metabolites (their 5-hydroxymethyl derivatives and penicilloic acids) were intraperitoneally dosed to rats. The HPLC separation of the rat urine indicated that penicilloic acid of the 5-hydroxymethyl derivative (5-OH-PA) was formed from the parent penicillin and the 5-hydroxymethyl derivative (5-OH), but not from penicilloic acid (PA). Thus, it follows that the metabolic pathways in man are common to all

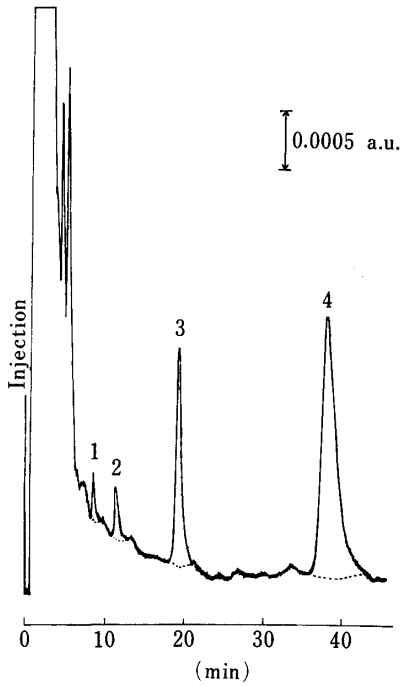


Fig. 2. HPLC Separation of Human Urine Excreted 2.5 h after Oral Administration of Cloxacillin Capsules

Peak 1, penicilloic acid of the 5-hydroxymethyl derivative of cloxacillin; 2, penicilloic acid of cloxacillin; 3, 5-hydroxymethyl derivative of cloxacillin; 4, cloxacillin. HPLC conditions: see the text.

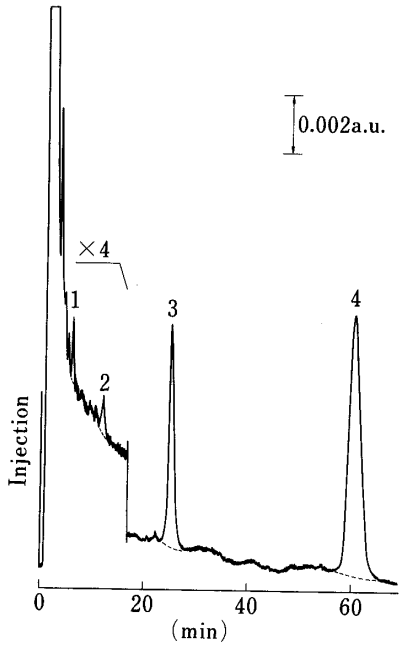


Fig. 3. HPLC Separation of Human Urine Excreted 3 h after Oral Administration of Dicloxacillin Capsules

Peak 1, penicilloic acid of the 5-hydroxymethyl derivative of dicloxacillin; 2, penicilloic acid of dicloxacillin; 3, 5-hydroxymethyl derivative of dicloxacillin; 4, dicloxacillin. HPLC conditions: see the text.

four isoxazolylpenicillins, as depicted in Fig. 4.

Time Course of Urinary Excretion

Figures 5 and 6 illustrate the time course curves of excretion rates for the metabolites and unchanged forms of cloxacillin and dicloxacillin (average of five subjects), where the values for metabolites are given as equivalent to parent penicillin. Figures 7 and 8 show the time course curves of cumulative urinary excretion amounts.

Moment Analysis

The statistical moments were calculated from the excretion rate-time curves according to Eqs. (1) and (2). The results are shown in Table I, where F is the fraction of the dose excreted in urine at infinite time (X_u^∞/D) and t-MRT is the total mean residence time of the excretion rate-time curve for each species. These F and t-MRT values can be allotted to each transfer step involved in metabolism and urinary excretion as shown in Fig. 9.

As is clear from the definition (Eq. (2)), the t-MRT value for a metabolite given in Table I represents the mean time from administration of the parent penicillin to urinary excretion of the metabolite, that is, the mean overall time required for absorption, distribution, metabolism, and excretion. Therefore, in general, the time period intrinsic to a metabolite (i-MRT) is given by the difference in t-MRT value between the metabolite and its immediate precursor, since MRT can be additive in linear systems.[26] For instance, the intrinsic MRT value for penicilloic acid of dicloxacillin (i-$MRT_{DX\text{-}PA}$) is estimated as t-$MRT_{DX\text{-}PA}$ − t-MRT_{DX} = 3.00 h, and similarly those for the 5-hydroxymethyl derivative (i-$MRT_{DX\,5\text{-}OH}$) and penicilloic acid of the 5-hydroxymethyl derivative (i-$MRT_{DX\,5\text{-}OH\text{-}PA}$) are given as t-$MRT_{DX\,5\text{-}OH}$ − t-MRT_{DX} = 0.53 h and t-$MRT_{DX\,5\text{-}OH\text{-}PA}$ − t-$MRT_{DX\,5\text{-}OH}$ = 4.11 h, respectively.

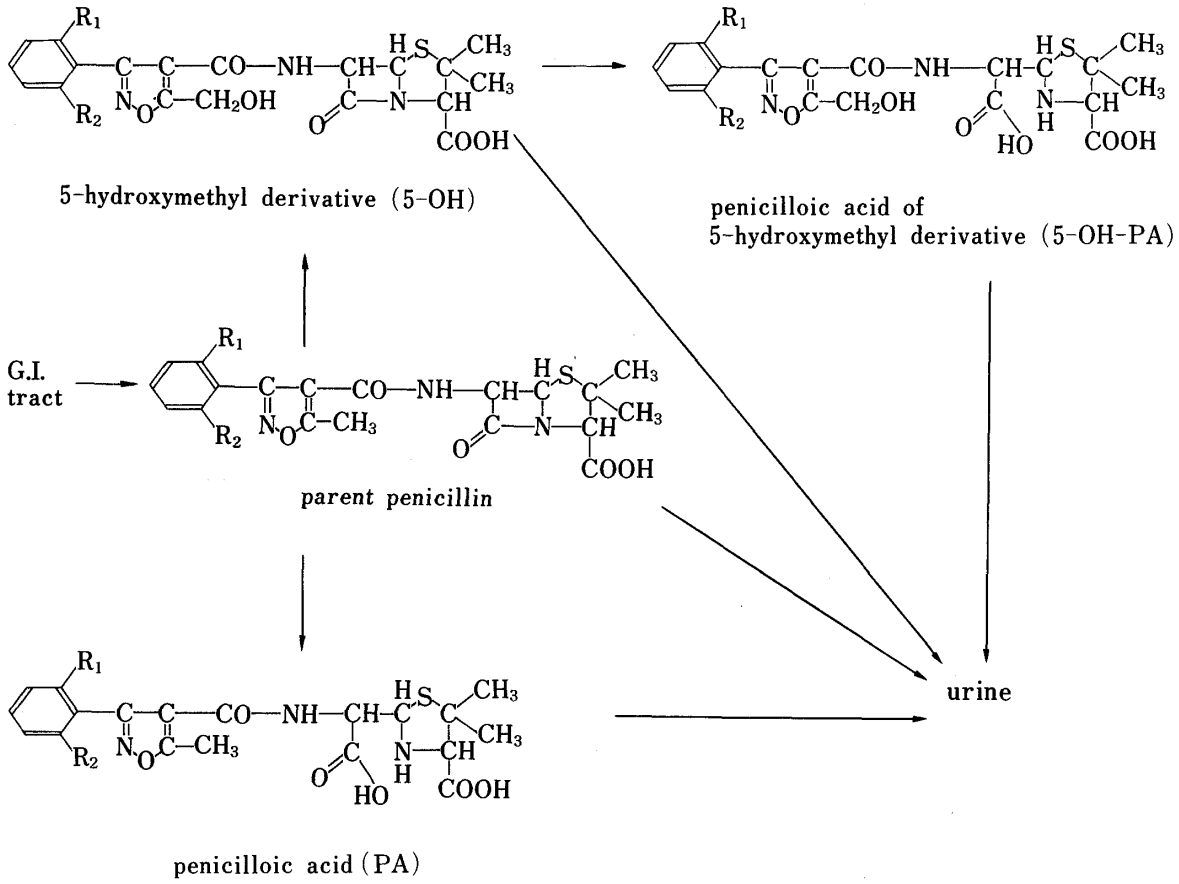


Fig. 4. Metabolic Pathways of Isoxazolylpenicillins

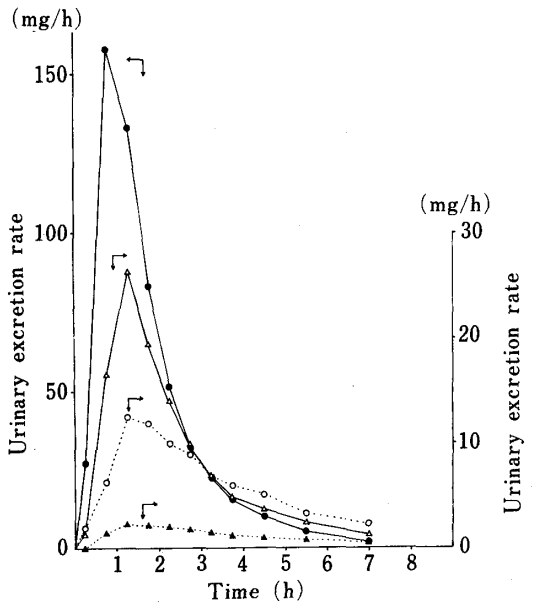


Fig. 5. Time Course Curves of Urinary Excretion Rate of Cloxacillin and Metabolites (Average of Five Subjects)

●, unchanged cloxacillin; △, 5-hydroxymethyl derivative; ○, penicilloic acid; ▲, penicilloic acid of the 5-hydroxymethyl derivative.

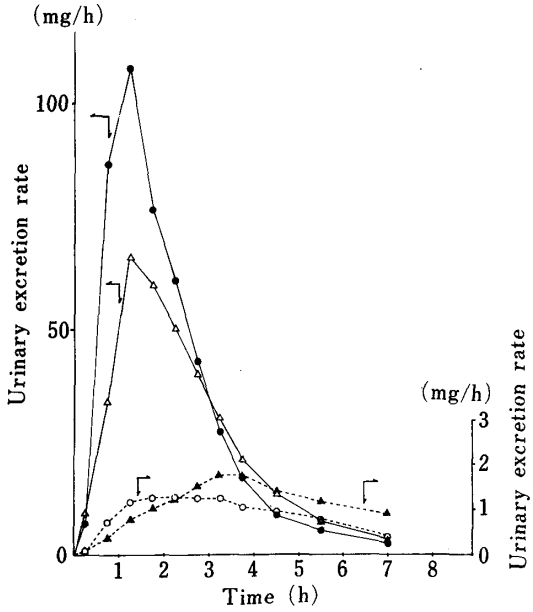


Fig. 6. Time Course Curves of Urinary Excretion Rate of Dicloxacillin and Metabolites (Average of Five Subjects)

●, unchanged dicloxacillin; △, 5-hydroxymethyl derivative; ○, penicilloic acid; ▲, penicilloic acid of the 5-hydroxymethyl derivative.

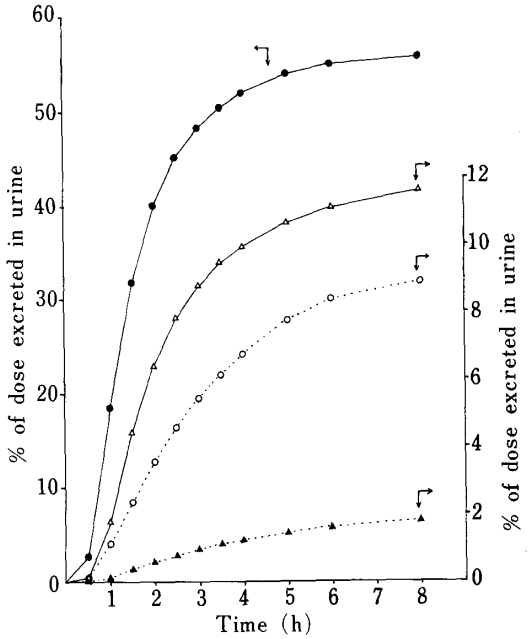


Fig. 7. Time Course Curves of Cumulative Excretion Amounts of Cloxacillin and Metabolites (Average of Five Subjects)

Symbols: see Fig. 5.

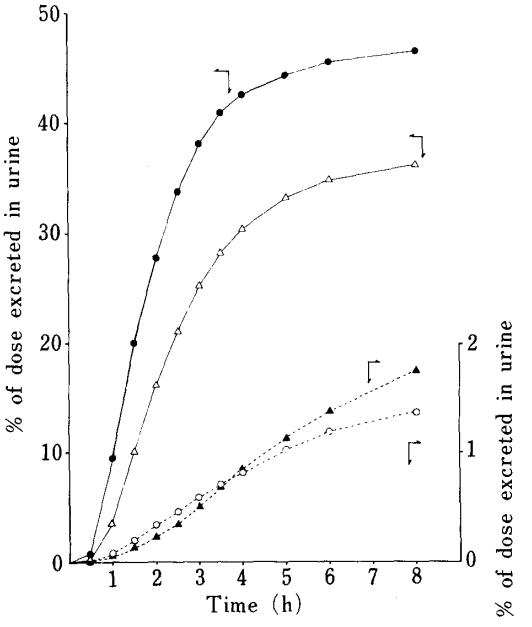


Fig. 8. Time Course Curves of Cumulative Excretion Amounts of Dicloxacillin and Metabolites (Average of Five Subjects)

Symbols: see Fig. 6.

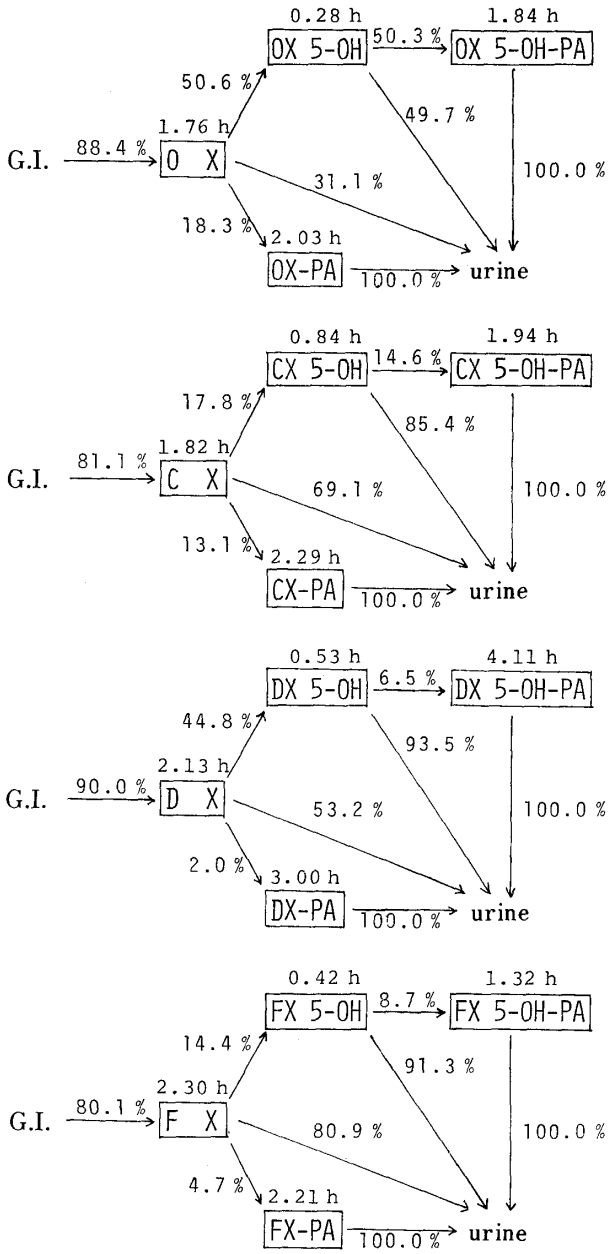


Fig. 9. Flow Diagram for Metabolism and Urinary Excretion of Four Isoxazolylpenicillins

Figures above substances are intrinsic *MRT* values, and those on arrows are intrinsic transfer ratios with respect to the immediate parent compound.

OX, oxacillin; CX, cloxacillin; DX, dicloxacillin; FX, flucloxacillin; 5-OH, 5-hydroxymethyl derivative; PA, penicilloic acid; 5-OH-PA, penicilloic acid of the 5-hydroxymethyl derivative.

On the other hand, the transfer ratio at each successive step (*i.e.* the intrinsic transfer ratio) can be calculated from the *F* value in Table I, and the results are also shown in Fig. 9. For instance, 44.8% of absorbed dicloxacillin is transformed into the 5-hydroxymethyl derivative, 2.0% into penicilloic acid, and 53.2% is excreted in the urine as the unchanged form; further, 6.5% of the 5-hydroxymethyl derivative undergoes hydrolysis of the β-lactam ring to yield penicilloic acid of the 5-hydroxymethyl derivative and the rest (93.5%) is excreted

TABLE I. Values of Fraction Excreted ($F^{a)}$%) and Total Mean Residence Time (t-$MRT^{b)}$ h) of Cloxacillin and Dicloxacillin and Their Metabolites

	Y.M.	M.S.	A.S.	Y.T.	T.H.	Mean	(S.D.)
F_{CX} (%)	60.3	50.9	52.8	62.0	54.1	56.0	(4.34)
$F_{CX\ 5\text{-}OH}$	12.2	11.4	9.4	11.5	16.8	12.3	(2.45)
$F_{CX\text{-}PA}$	8.7	10.6	11.1	8.5	14.3	10.6	(2.10)
$F_{CX\ 5\text{-}OH\text{-}PA}$	2.6	2.0	1.5	2.4	2.5	2.2	(0.40)
F_{total}	83.8	74.9	74.8	84.4	87.7	81.1	(5.29)
t-MRT_{CX} (h)	1.91	1.81	1.45	1.88	2.04	1.82	(0.20)
t-$MRT_{CX\ 5\text{-}OH}$	2.66	2.38	1.84	2.42	4.00	2.66	(0.72)
t-$MRT_{CX\text{-}PA}$	4.11	4.83	3.67	2.89	5.06	4.11	(0.79)
t-$MRT_{CX\ 5\text{-}OH\text{-}PA}$	5.64	3.82	3.30	4.71	5.51	4.60	(0.92)
F_{DX} (%)	47.7	37.6	49.9	58.5	46.0	47.9	(6.72)
$F_{DX\ 5\text{-}OH}$	49.5	26.4	31.6	29.6	51.3	37.7	(10.53)
$F_{DX\text{-}PA}$	1.0	1.1	2.3	3.7	1.0	1.8	(1.06)
$F_{DX\ 5\text{-}OH\text{-}PA}$	2.7	5.6	1.5	1.2	2.0	2.6	(1.58)
F_{total}	100.8	70.8	85.2	93.0	100.3	90.0	(11.17)
t-MRT_{DX} (h)	2.10	1.86	2.10	2.47	2.10	2.13	(0.20)
t-$MRT_{DX\ 5\text{-}OH}$	2.89	2.29	2.59	2.74	2.78	2.66	(0.21)
t-$MRT_{DX\text{-}PA}$	3.92	3.83	3.93	9.43	4.54	5.13	(2.16)
t-$MRT_{DX\ 5\text{-}OH\text{-}PA}$	7.41	8.01	4.96	5.92	7.57	6.77	(1.15)

a) $F=X_u^{\infty}/D$.
b) t-*MRT*; see Eq. (2).
Subscripts: see Fig. 9.

in the urine. The two penicilloic acids thus formed are all excreted in the urine as the final products. The corresponding results for oxacillin[25] and flucloxacillin[19] reported previously are also shown in Fig. 9 for convenience of comparison.

Discussion

The microbiological assay methods so far employed are highly sensitive and specific to active substances, but are essentially incapable of detecting penicilloic acids. The HPLC method, on the other hand, has made possible simultaneous separation and specific determination of isoxazolylpenicillins and their metabolites in biological fluids, which permitted the discovery of a new metabolite[19,25] (*i.e.* penicilloic acid of the 5-hydroxymethyl derivative) and consequent re-evaluation of the pharmacokinetic characteristics in man.

The statistical moment method, which allows model-independent analysis of rate processes, has been useful in estimating the extent and rate of absorption and excretion of β-lactam antibiotics, in separating mean *in vitro* disintegration, absorption, and distribution times, and in correlating *in vivo* and *in vitro* dissolution times of oral drugs.[26-30] In the present study as well as in the previous papers,[19,25] we used the moment method to evaluate the extent and rate of metabolism and urinary excretion of isoxazolylpenicillins.

It has been reported that absorption of isoxazolylpenicillins in man is enhanced by progressive halogen substitution on the phenyl ring.[2,3] However, the results in Fig. 9 indicate that the fractions absorbed (80—90% of the dosed amounts) are higher and less scattered among the four isoxazolylpenicillins than had previously been reported.[1-5,8,10,13,31] This is obviously because the excretion of the new metabolite (penicilloic acid of the 5-hy-

TABLE II. Values of Fraction Excreted ($F^{a)}$%) and Mean Residence Time (t-,[a] and i-$MRT^{b)}$ h) of Four Isoxazolylpenicillins

	OX	CX	DX	FX
$F_{\text{unchanged}}$ (%)	27.4 (4.96)	56.0 (4.34)	47.9 (6.72)	64.8 (2.10)
$F_{\text{5-OH}}$	22.2 (3.66)	12.3 (2.45)	37.7 (10.53)	10.5 (2.44)
F_{PA}	16.1 (6.71)	10.6 (2.10)	1.8 (1.06)	3.8 (0.90)
$F_{\text{5-OH-PA}}$	22.4 (4.10)	2.2 (0.40)	2.6 (1.58)	1.0 (0.43)
F_{total}	88.4 (11.22)	81.1 (5.29)	90.0 (11.17)	80.1 (2.67)
t-$MRT_{\text{unchanged}}$ (h)	1.76 (0.38)	1.82 (0.20)	2.13 (0.20)	2.30 (0.37)
t-$MRT_{\text{5-OH}}$	2.04 (0.35)	2.66 (0.72)	2.66 (0.21)	2.72 (0.37)
t-MRT_{PA}	3.79 (0.81)	4.11 (0.79)	5.13 (2.16)	4.51 (0.38)
t-$MRT_{\text{5-OH-PA}}$	3.89 (0.65)	4.60 (0.92)	6.77 (1.15)	4.04 (0.27)
i-$MRT_{\text{unchanged}}$ (h)	1.76 (0.38)	1.82 (0.20)	2.13 (0.20)	2.30 (0.37)
i-$MRT_{\text{5-OH}}$	0.28 (0.13)	0.84 (0.57)	0.53 (0.18)	0.42 (0.13)
i-MRT_{PA}	2.03 (0.66)	2.29 (0.74)	3.00 (1.99)	2.21 (0.62)
i-$MRT_{\text{5-OH-PA}}$	1.84 (0.81)	1.94 (0.61)	4.11 (1.19)	1.32 (0.38)

a) *F*, t-*MRT*; see Table I. *b*) i-*MRT*; see the text.
Subscripts: see Fig. 9. Figures show mean (±S.D.).

droxymethyl derivative), whose amount is as high as that of penicilloic acid of the parent penicillin, is taken into pharmacokinetic consideration.

The results in Fig. 9 also indicate that the degree of biotransformation of both the parent penicillin and 5-hydroxymethyl derivative into corresponding penicilloic acids is decreased with increasing number of halogen substituted on the phenyl group. The yield of penicilloic acid from parent penicillin is in the same sequence as reported by Cole *et al.*[31] However, the yield of the 5-hydroxymethyl derivative is in the order dicloxacillin > oxacillin > cloxacillin > flucloxacillin. This agrees with Thijssen's result that the metabolic clearance is in the order dicloxacillin > cloxacillin > flucloxacillin.[11] Finally, the total metabolite fraction (%) against absorbed amount is 68.3% for oxacillin, 46.8% for dicloxacillin, 30.9% for cloxacillin, and 19.1% for flucloxacillin (see Table II). This shows that the ease of metabolism of isoxazolylpenicillins in man is not always correlated with the degree of halogen substitution on the phenyl ring.

Pharmacokinetic study of a drug for clinical therapy is usually concerned with its bioavailable activity. The antimicrobial activities of isoxazolylpenicillins depend considerably on their active metabolites (*i.e.* 5-hydroxymethyl derivatives), whose activities against certain bacteria such as *S. lutea* are of almost the same order of magnitude as those of the parent penicillins.[15] Also, the susceptibilities of *S. lutea* to the parent penicillins are almost comparable.[1-5] Therefore, provided that the total activity of isoxazolylpenicillin may be expressed by the sum of activities of unchanged form and 5-hydroxymethyl derivative, and that the activity may be regarded as proportional to dX_u/dt for each active species within a certain limited area, it follows from Eqs. (1) and (2) that

$$MRT_a = \frac{pX^{\infty}_{\text{unchanged}} MRT_{\text{unchanged}} + qX^{\infty}_{\text{5-OH}} MRT_{\text{5-OH}}}{pX^{\infty}_{\text{unchanged}} + qX^{\infty}_{\text{5-OH}}} \quad (3)$$

where MRT_a denotes MRT of the total activity-time curve, p and q denote the proportionality factors relating urinary excretion rate to activity, which are assumed to be time-independent, and $pX^{\infty}_{\text{unchanged}} + qX^{\infty}_{\text{5-OH}}$ represents the area under the activity-time curve, *i.e.* total bioavailable activity. From the results for antimicrobial activities of isoxazolylpenicillins and their 5-

hydroxymethyl derivative referred to above, it may follow that $p=q$. Thus, the extent of bioavailable activity for dicloxacillin is estimated as, $F_{\text{unchanged}}+F_{5\text{-OH}}=85.6\%$, which means that 85.6% of the dosed amount of dicloxacillin is bioavailable for antimicrobial activity, and similarly the value is 68.3% for cloxacillin. The corresponding values for oxacillin[25] and flucloxacillin[19] have been estimated as 49.6% and 75.3%, respectively (see Table II). With the given rate of bioavailable activity, tentative substitution of $p=q$ in Eq. (3) gives $MRT_a=$ 1.97 h for cloxacillin and 2.36 h for dicloxacillin. The corresponding values for oxacillin and flucloxacillin are 1.89 h and 2.36 h, respectively. These results suggest that from the viewpoint of the rate of bioavailable activity, the activities of dicloxacillin and flucloxacillin may be retained in the body longer than those of cloxacillin and oxacillin. This corresponds to the fact that the slower elimination due to lower renal clearance causes higher serum concentrations of flucloxacillin and dicloxacillin than cloxacillin and oxacillin,[5-8,10,32] although these previous results were obtained without taking the active metabolite into account.

Recently, Thijssen *et al.* reported that the plasma half-lives of the 5-hydroxymethyl derivatives of cloxacillin and flucloxacillin are slightly longer than those of the parent penicillins (*e.g.* $t_{1/2\cdot\text{unchanged}}=69$ min, $t_{1/2\cdot 5\text{-OH}}=78$ min for cloxacillin) and that the renal clearances (C) of the former are greater than those of the latter (*e.g.* $C_{\text{unchanged}}=160$ ml/min, $C_{5\text{-OH}}=220$ ml/min for cloxacillin).[11,24] This corresponds to the present results (see Table II) that t-MRT of the 5-hydroxymethyl derivative is slightly longer than that of the parent penicillin. Furthermore, as can be seen from Fig. 9, in all penicillins, the intrinsic MRT of the 5-hydroxymethyl derivative is smallest among those of other substances. This means that the 5-hydroxymethyl derivative remains in the human body for a shorter period of time than penicilloic acids, that is, this active metabolite is a rather eliminatable intermediate. Thijssen also stated that the higher renal clearance of the 5-hydroxymethyl derivative of flucloxacillin is due to lower protein binding than in the case of the parent penicillin.[11] That is, the weaker affinity for plasma albumin causes not only a higher filtration rate but also a weaker competition between the plasma albumin and the tubular secretion carrier protein, which plays a major role in the renal excretion of lipophilic drugs such as isoxazolylpenicillins.[33] The lower protein binding of the 5-hydroxymethyl derivative than the parent penicillin also suggests that dicloxacillin would provide a good tissue distribution of activity, because absorption of dicloxacillin through the G.I. tract and subsequent biotransformation into the 5-hydroxymethyl derivative occur efficiently.

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