emission was determined at 488 nm using a wavelength of 421 nm for excitation in a Hitachi MPF-4 fluorimeter with tubes with a 1 cm light path.

Calibration curves proved a linear correlation between the concentration of the drugs and the intensity of fluorescence.

The method was calibrated in the range of 0–5 μg ml^{-1} and had a sensitivity of 0·05 μg ml^{-1}.

In two patients receiving 1 mg kg^{-1} tobramycin, serum concentrations were determined every 10 min for 1 h after the injection of tobramycin and hourly up to 8 h. Fig. 2 shows the serum concentration curves.

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Physiological pharmacokinetics of β-lactam antibiotics: penicillin V distribution and elimination after intravenous administration in rats

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To clarify the physiological action and behaviour of a drug in man and to establish a dose scheduling for therapeutics, conventional pharmacokinetics based on the curve fits of the time-course of a drug concentration in blood have been widely used. The two-compartment open model is generally used for β-lactam antibiotics administered to man and other species (e.g. Dittert et al 1970; review by Nightingale et al 1975). However, due to the lack of anatomical or physiological meaning for transfer rate constants derived from this method, the time-course of antibiotic concentration in the particular target organ, under normal and diseased states, appears difficult to predict.

The present communication describes the physiologically based pharmacokinetics for penicillin V to predict tissue concentrations in rats. This approach by physiological perfusion model has been used to describe the pharmacokinetics of several drugs (for examples, Bischoff & Dedrick 1968; Bischoff et al 1971; Benowitz et al 1974; Harrison & Gibaldi 1977; Tterlikkis et al 1977) and has the intrinsic possibility of being scaled up for application to man from animal results.

Scheme 1 represents the flow diagram of various compartments used in the present analysis. This model assumes that (1) each tissue acts as a well-stirred compartment, (2) the antibiotic distribution is limited by the blood flow rate, and (3) tissue-to-blood concentration ratio of penicillin V is independent of the antibiotic concentration. A typical mass balance equation is given for the total drug in the liver:

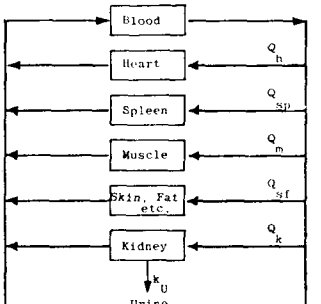
$$V_l\frac{dC_l}{dt} = (Q_l - Q_g)C_b - Q_l\frac{C_l}{K_l} + Q_g\frac{C_g}{K_g} - \frac{T_B(C_l/K_l)}{K_B + (C_l/K_l)} - \frac{T_M(C_l/K_l)}{K_M + (C_l/K_l)} \quad \ldots \quad (1)$$

where $V_l(dC_l/dt)$ is the accumulation of the drug in the liver, $(Q_l - Q_g)C_b$ is the rate of inflow with blood, $Q_l(C_l/K_l)$ and $Q_g(C_g/K_g)$ are the rates of outflow from the liver and inflow from the gut wall, respectively, with blood, and the last two terms represent capacity-limited biliary secretion and metabolism possibly occuring in the liver.

The mass balance equation for the blood is written by:

$$V_b\frac{dC_b}{dt} = M\,I(t) + Q_h\frac{C_h}{K_h} + Q_{sp}\frac{C_{sp}}{K_{sp}} + Q_m\frac{C_m}{K_m} + Q_{sf}\frac{C_{sf}}{K_{sf}} + Q_k\frac{C_k}{K_k} + Q_l\frac{C_l}{K_l} - (Q_h + Q_{sp} + Q_m + Q_{sf} + Q_k + Q_l)C_b \quad \ldots \quad (2)$$

where b, h, sp, m, sf, k, and l signify blood, heart, spleen, skeletal muscle, skin-fat, kidney, and liver, respectively, V_i is the compartment volume, C_i is the total antibiotic concentration bound and unbound to any protein, Q_i is the blood flow rate to the compartment, K_i is the tissue-to-blood partition coefficient, M is the total dose (μg), and I(t) is the injection function, which is a short pulse to simulate an intravenous injec-



Scheme 1. Pharmacokinetic model for distribution and elimination of penicillin V in the rat.

* Correspondence.

tion as expressed by eqn 3 (Bischoff & Dedrick 1968):

$$I(t) = 30\lambda(\lambda t)^2(1 - \lambda t)^2 \quad .. \quad (3)$$

where λ is the reciprocal of the injection time (min).

The mass balance equations can be described similarly for the remaining compartments, but gastrointestinal absorption and renal clearance are assumed to be linear. Nine differential equations were solved simultaneously by the Runge-Kutta-Gill method performed with a digital computer (FACOM M-160, Kanazawa University).

Male albino Wistar rats, 240 ± 6 g, were fasted overnight by giving water freely and used under urethane anaesthesia (1·3 g kg^{-1}, i.p.). For intravenous bolus studies, rats catheterized into the bladder were injected through the femoral vein with a dose of 100 mg kg^{-1} of penicillin V. Blood and urine samples were collected at definite time intervals and the animals were killed. Various tissues were excised, rinsed well with ice-cooled saline, blotted, weighed, and homogenized.

For the infusion studies to determine steady-state blood concentrations and biliary and renal clearance, rats catheterized in both bile duct and bladder were dosed through the femoral vein at a constant rate between 2·0 and 25 mg h^{-1}. Blood, urine, and bile were collected continuously during the infusion. To determine the steady-state tissue-to-blood partition coefficients, rats without cannulation were killed at the end of the infusion and various tissue samples were prepared in the manner similar to that described for bolus injection.

In all experiments, a 0·2 ml aliquot of blood sample was collected and haemolysed with distilled water. Determination of whole blood concentration was made to avoid possible error caused by transport of the antibiotic to erythrocytes. All the samples were assayed using the disk diffusion microbiological technique using *Staph. aureus*. Standards were established for each organ by using identical organs.

Penicillin V concentrations or amounts determined in blood, urine, and various tissues are shown in Fig. 1. Data for blood and urine are the average from four or more rats; for the other tissues each experimental value was plotted. Penicillin V was concentrated more in the kidney and gut wall than in blood, suggesting considerable partition of the antibiotic into these tissues. A substantial amount appeared in gut content, up to about 15% of the dose and then decreased gradually due to intestinal absorption rather than by faecal elimination, indicating that enterohepatic circulation plays a significant role in the disposition of penicillin V in rats.

Table 1 lists physiological model parameters used in the simulation for a 240 g rat. Organ volume and blood

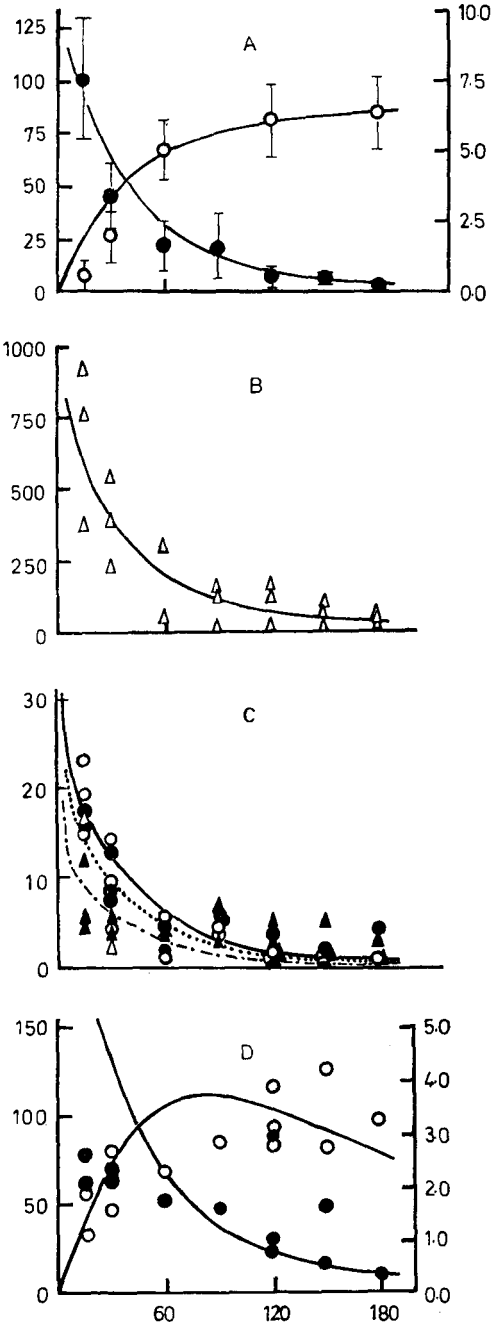


FIG. 1. Predicted (——, · · · · ·, – · – · –) and observed concentrations (ordinate) of penicillin V in blood, urine and various tissues after intravenous dose of 100 mg kg^{-1} to rats as a function of time (abscissa; min). A. Blood concentration (left ordinate, $\mu g\ ml^{-1}$, ●) and cumulative amount (right ordinate, mg, ○) in urine. Vertical bars represent standard deviation. B. Kidney concentration ($\mu g\ g^{-1}$, —△—). C. Concentrations ($\mu g\ g^{-1}$) in heart (—○—), spleen (—●—), muscle (– · –△– · –), and liver (· · · ▲ · · ·). D. Concentration (left ordinate, $\mu g\ g^{-1}$, —●—) in gut wall and amount (right ordinate, mg, —○—) in gut content. Densities of tissues were assumed to be all 1 g ml^{-1}.

flow rate were evaluated from the reported values for the rat. Tissue-to-blood partition coefficient, K_i, for the i-organs was obtained as an average value calculated from a wide range of steady-state blood and tissue concentrations. By comparison of K_i values in Table 1, penicillin V was taken up efficiently by the kidney and gut wall. Other tissues, heart, spleen, muscle, and liver, showed little ability to concentrate penicillin V.

Penicillins are known to be metabolized, depending on the nature of the side-chain structure, probably in the liver (Kind et al 1968; Ryrfeldt 1973; Cole et al 1973). About 20% of penicillin V administered intravenously to rats was found in the present study and previously (Tsuji et al 1978) to be excreted as penicilloic acid after 3 h, in urine and bile. To evaluate metabolic clearance, the steady-state clearance concept was adopted. When a drug is infused into the animal for a sufficiently long time to establish the steady-state blood concentration, the elimination rate by all routes should equal the infusion rate, I_0. Thus,

$$I_0 = \left(\frac{d X_U}{dt}\right)_{ss} + \left(\frac{d X_B}{dt}\right)_{ss} + \left(\frac{d X_M}{dt}\right)_{ss} \quad (4)$$

where $(dX_U/dt)_{ss}$ and $(dX_B/dt)_{ss}$ refer to the amount of steady-state unchanged drug per unit time excreted in urine and bile, respectively, to give steady-state constant rates of R_U and R_B; $(dX_M/dt)_{ss}$ represents the amount of total metabolites in steady-state per unit time formed by all sites of metabolism to give the constant metabolic rate of R_M.

Table 1. Physiological constants and tissue-to-blood partition coefficients for the distribution of penicillin V in the rat.

Tissue	Volume[a] (ml)	Blood flow[b] (ml min^{-1})	Tissue-to-blood partition constant, K_i
Blood	13·8	—	—
Heart	1·3	1·6	0·2
Spleen	0·7	0·6	0·2
Muscle	120	10·6	0·1
Skin, fat, etc.[c]	59·3	19·6	2·0[d]
Kidney	2·3	9·8	6·6
Liver	8·9	14·0	0·2
Gut wall	6·7	10·3	1·9
Gut content	11·7	—	—

a Based on 240-g rat (Dedrick et al 1973; Harrison & Gibaldi 1977).
b Total blood flow = 56·2 ml min^{-1} (Sapirstein et al 1960; Harrison & Gibaldi 1977).
c Miscellaneous compartments for all other body regions.
d Estimated from the sum of the amount of total tissue determined actually and the amount calculated from the total clearance in the steady-state experiment.

Fig. 2 shows typical results from the infusion study by plotting the whole blood concentration, cumulative amount of urinary and biliary excreted unchanged drug, X_U and X_B, against time, t. R_U and R_B were obtained from the corresponding slopes after the achievement of steady-state blood level. R_M can be calculated from equation 5 by the determination of only unchanged drug in urine and bile.

$$R_M = I_0 - R_U - R_B \quad .. \quad .. \quad (5)$$

The plots of R_B and R_M vs the steady-state whole blood concentration, $(C_b)_{ss}$, are shown in Fig. 3, yielding a convex ascending curve to obey the capacity-limited elimination of equations 6 and 7

$$R_B = \frac{T_B(C_b)_{ss}}{K_B + (C_b)_{ss}} \quad .. \quad .. \quad (6)$$

$$R_M = \frac{T_M(C_b)_{ss}}{K_M + (C_b)_{ss}} \quad .. \quad .. \quad (7)$$

where T_B and T_M are the maximum rates and K_B and K_M are the apparent Michaelis-Menten constants, for the biliary secretion and metabolism, respectively. The parameters, T_B, T_M, K_B, and K_M were computed by the non-linear least squares regression of the respective datum sets. Although the renal excretion rate, R_U, tends to depend on the steady-state blood penicillin V concentration $(C_b)_{ss}$, as shown in Fig. 3, linear renal clearance has been approximated in this report to give $k_U = 1{\cdot}2$ ml min^{-1}, because R_U may be a mixed rate including glomerular filtration, tubular secretion, and reabsorption.

The first-order intestinal absorption rate constant, k_a, was evaluated to be 0·1 min^{-1} for 100 cm intestine. This was based on the value of 27·4 ± 1·8% (n = 3) for absorption after 1 h from 1 ml of solution of penicillin V by a 5 cm loop of rat gut prepared by the method of Tsuji et al (1977). The various parameters of penicillin V are listed in Table 2.

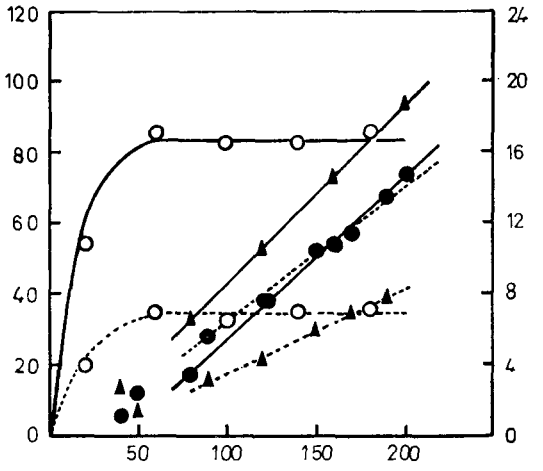


FIG. 2. Typical plots of blood concentration (left ordinate, μg ml^{-1}, ○) and cumulative amounts (right ordinate, mg) in urine (●) and bile (▲) against infusion time (abscissa, min) after femoral vein infusion of penicillin V in rats. Solid lines, infusion rate = 25 mg h^{-1}. Dashed lines, infusion rate = 15 mg h^{-1}.

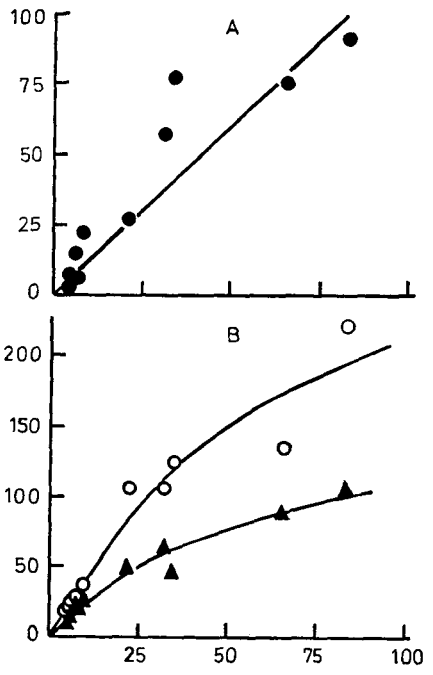


FIG. 3. Plots of steady-state rates (ordinate, μg min^{-1}) for urinary (R_U, ●, A), biliary (R_B, ▲, B) and metabolic (R_M, ○, B) elimination of penicillin V against steady-state blood concentration (abscissa, μg ml^{-1}) in rats.

Predicted penicillin V concentrations or amounts in blood, urine, gut contents and in certain tissues of the rat are given in Fig. 1. These were computed mathematically for the simultaneous differential equations by setting the physiological values and the elimination and intestinal absorption parameters listed in Tables 1 and 2. Most of the predictions agreed reasonably well with the observed values.

The present physiological pharmacokinetic approach to therapeutically useful antibiotics may play an important role for reasonable dosage regimens for infections. When sufficient data for other laboratory animals become available, this goal can be achieved by the scale-up to man by adjusting only the organ blood flow rate, organ volume, and various clearance parameters and the intestinal absorption rate constant.

It is also emphasized that the steady-state clearance method described in this paper is applicable to all animal species including man to evaluate individual elimination clearance.

Table 2. Various parameters for elimination and absorption of penicillin V in the rat.

Parameter		Estimated value	
Renal clearance	k_u	1·2	ml min^{-1}
Biliary secretion	T_B	172	μg min^{-1}
parameter*	K_B	62·2	μg ml^{-1}
Metabolic parameter*	T_M	382	μg min^{-1}
	K_M	78·1	μg ml^{-1}
Intestinal absorption rate constant	k_a	0·1	min^{-1}
Faecal clearance	k_f	0·0	ml min^{-1}

* Capacity-limited elimination where T and K represent the maximum rate of elimination and the apparent Michaelis-Menten constant, respectively (see eqns 6 and 7).

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