Studies on the Absorption of Practically Water-Insoluble Drugs Following Injection VII: Plasma Concentration after Different Subcutaneous Doses of a Drug in Aqueous Suspension in Rats

KOICHIRO HIRANO ˣ and HIDEO YAMADA

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Abstract □ The response patterns of the time profile of plasma drug level to dose elevation after subcutaneous administration of a practically water-insoluble drug in aqueous suspension were investigated. Equations to predict the plasma drug concentration at any time and the time T_{max} at which it reaches the maximum (C_{max}) at different doses were derived using empirical equations describing the subcutaneous absorption kinetic process as an input function of one- and two-compartment models. Computer simulation for the one-compartment model showed that C_{max} tended to increase curvilinearly with dose elevation accompanied by the retardation of T_{max}. It also showed that this tendency was noticeable in dose elevation at fixed injection volume and became more significant for drugs with a smaller absorption rate and/or a larger elimination rate. This suggested that a more effective means of raising the drug plasma level for such drugs would be improvement of the formulation. The validity of the information obtained from the simulation was confirmed by various subcutaneous administration experiments of N^1-acetylsulfamethoxazole aqueous suspensions in rats. Further, the multiple-point injection method was ascertained to be useful for avoiding the nonlinear increase in plasma drug concentration with dose elevation appearing in the subcutaneous administration of aqueous suspensions.

Keyphrases □ Absorption—studies on practically water-insoluble drugs following injection, plasma concentration after different subcutaneous doses of a drug in aqueous suspensions in rats □ Aqueous suspensions—studies on absorption of practically water-insoluble drugs following injection, plasma concentration after different subcutaneous doses in rats

Aqueous suspensions are extensively used as a parenteral dosage form for practically water-insoluble drugs. Despite their usefulness for early screening and preclinical testing of newly developed drugs in laboratory animals, little is known about the patterns of plasma drug level change with time and dose after subcutaneous, intramuscular, or intraperitoneal administration. This is undoubtedly due to insufficient information on the absorption kinetics for aqueous suspensions when using such administration routes.

Drug administration *via* the subcutaneous route is convenient for early animal experiments because it allows injections of a larger volume than possible with the intramuscular route and does not involve the first-pass effect sometimes observed when the intraperitoneal route is used (1). Previous studies on the subcutaneous absorption kinetics of practically water-insoluble drugs in aqueous suspension using the local clearance method in rats (2) gave a kinetic equation which was not of the first order and which differed from that for oily (3) or surfactant micellar solutions[1]. The rate constant, which was empirically derived from the experimental results, was a complex function of the initial drug concentration and injection volume, *i.e.*, the dose (2).

[1] K. Hirano and H. Yamada, unpublished results.

The purpose of the present study was to examine plasma drug levels after different subcutaneous doses of a practically water-insoluble drug in aqueous suspension. First, pharmacokinetic approaches were made using the previously obtained kinetic equation for absorption as an input function of one- and two-compartment models to generate equations describing the time course of the plasma drug level. Next, computer simulations were undertaken to clarify the response patterns of the plasma drug concentration–time curve for the change in dose or absorption rate constant. Then the validity of the information obtained from the simulation was checked experimentally in rats with aqueous suspensions of N^1-acetylsulfamethoxazole. In addition, the usefulness of multiple-point injections for drugs having an exceedingly slow absorption rate was examined experimentally.

THEORETICAL

Previous investigations have shown that the drug absorption process from aqueous suspensions injected subcutaneously (2) or intramuscularly (4) can be represented, if the injected particles loosely agglomerate and if dissolution (or release) of the drug from the surface of the particle agglomerate is the rate-limiting step for overall drug absorption, by:

$$W = W_0(1 - jt)^3 \qquad (0 \le t \le 1/j) \qquad \text{(Eq. 1)}$$

where W_0 and W are the dose and the amount of the drug remaining at the injection site at any time t, respectively, and j is defined as an apparent absorption rate constant[2]. This constant could be related to the initial drug concentration C_0 and the injection volume V_0 by the following empirical equation:

$$j = fC_0^g V_0^h \qquad \text{(Eq. 2)}$$

where the term f is a constant that depends on the drug, preparation

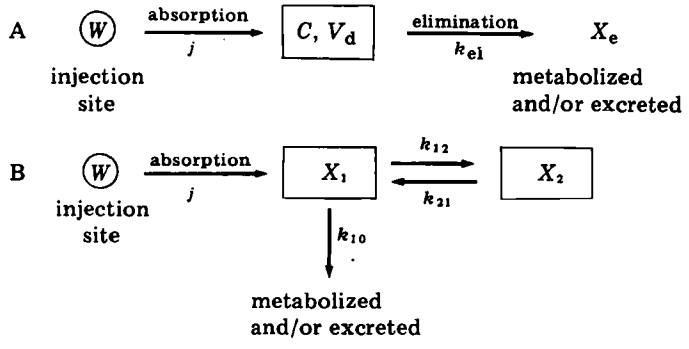

Figure 1—*(A) One- and (B) two-compartment models with nonlinear subcutaneous absorption.*

[2] The term "apparent" is used, since the assumption that Eq. 1 reflects the true absorption of the drug into the systemic circulation was not checked directly by experiments [although its validity was indirectly confirmed by the local clearance method (2, 4)]. The validity will be verified in the latter half of this paper.

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conditions (particle size, *etc.*), injection site, and physiological state of the subject. The exponents g and h in this equation are constants determined experimentally. For the subcutaneous route in rats, the experimental values for g and h are −0.66 and −0.32, respectively (2).

Applying the above kinetic equations as an input function of a one- or two-compartment model, the following pharmacokinetic approaches can be made to derive equations which give the plasma drug concentration as a function of time.

One-Compartment Model—For the one-compartment model (Fig. 1A), the elimination rate of the drug (dX_e/dt) can be defined as:

$$dX_e/dt = k_{el}CV_d \qquad \text{(Eq. 3)}$$

where X_e is the amount of the drug eliminated from time 0 to t and C represents the plasma concentration of the drug. The parameters k_{el} and V_d are the apparent first-order elimination rate constant and the apparent volume of distribution, respectively. Differentiation of Eq. 1 with respect to time gives the rate of absorption of the drug from the injection site:

$$dW/dt = -3W_0j(1 - jt)^2 \qquad \text{(Eq. 4)}$$

And from mass balance:

$$\frac{dCV_d}{dt} = -\frac{dW}{dt} - \frac{dX_e}{dt} \qquad \text{(Eq. 5)}$$

Combining Eqs. 3–5 results in:

$$\frac{dC}{dt} + k_{el}C = \frac{3W_{0j}}{V_d}(1 - jt)^2 \qquad \text{(Eq. 6)}$$

Solving for C gives two relationships between the plasma drug concentration and time.

For $0 \le t \le t^*$:

$$C = P[Q(1 - e^{-k_{el}t}) + Rt + St^2] \qquad \text{(Eq. 7)}$$

and for $t > t^*$:

$$C = C^*e^{-k_{el}(t-t^*)} \qquad \text{(Eq. 8)}$$

The terms t^*, P, Q, R, S, and C^* are written by setting $\gamma = j/k_{el}$ as follows:

$$\left.\begin{aligned} t^* = 1/j, \quad P = 3W_0/V_d, \quad Q = \gamma(1 + 2\gamma + 2\gamma^2) \\ R = -2j\gamma(1 + \gamma), \quad S = j^2\gamma, \\ C^* = P[Q(1 - e^{-k_{el}t^*}) + Rt^* + St^{*2}] \end{aligned}\right\} \qquad \text{(Eq. 9)}$$

When the plasma concentration reaches maximum (C_{max}) at time T_{max} ($0 < T_{max} < t^*$), then $dC/dt = 0$. Therefore:

$$Qk_{el}e^{-k_{el}T_{max}} + R + 2ST_{max} = 0 \qquad \text{(Eq. 10)}$$

T_{max} is given as one of the real solutions of this transcendental equation. (Numerical analysis may be useful for solving this equation.) C_{max} is obtained by substituting T_{max} for t in Eq. 7.

Two-Compartment Model—Analytical treatment for a multicompartment model with a complex input function not having a simple Laplace transform, is not as simple as a one-compartment model. Also, a previous general treatment for linear mamillary models (5, 6) may not be applicable for such a model.

Recently, a valuable partial transformation approach was reported (7), which avoids Laplace transformation of the input function and the use of convolution integrals, and can be used for treatment of a linear multicompartment model with complex input functions in one or more compartments. According to this approach:

$$X = \Phi(t)[X(0) + \int_0^t \Phi(-t)f(t)dt] \qquad \text{(Eq. 11)}$$

where the ith component of vector X or f is the amount X_i at time t or the input function $f_i(t)$ in the ith compartment, respectively, and $X(0)$ is the vector X at time $t = 0$. The ijth component Φ_{ij} of matrix Φ is given by:

$$\Phi_{ij} = L^{-1}(|S_{ij}|/|S|) \qquad \text{(Eq. 12)}$$

where L^{-1} is the inverse Laplace transform operator, $|S|$ is the determinant of the matrix S, and S_{ij} is the cofactor corresponding to the ijth element. Here, the matrix S is defined as:

$$S = -K + \text{diag}(s + E_1, s + E_2, \ldots, s + E_n) \qquad \text{(Eq. 13)}$$

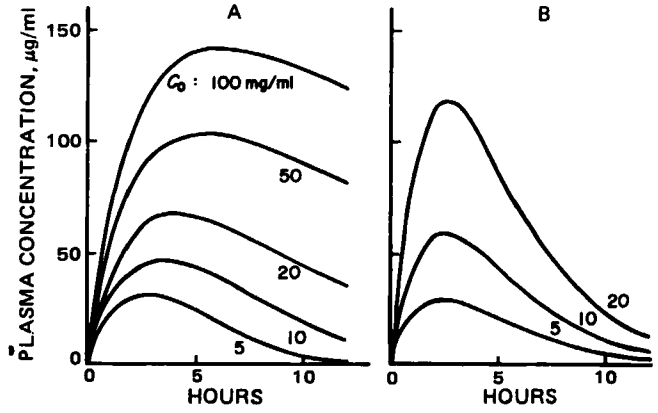


Figure 2—*Computer-predicted plasma levels after different subcutaneous doses of a drug in aqueous suspension (A) and oily solution (B) at fixed injection volume (0.5 ml). Plasma concentrations were calculated from hypothetical parameters:* k_{el}, *0.5 hr*$^{-1}$; V_d, *25 ml*; j*(1) (at* C_0 *= 5 mg/ml and* V_0 *= 0.5 ml), 0.10 hr*$^{-1}$ *(*$t_{1/2}$, *2.06 hr);* k, *0.336 hr*$^{-1}$ *(*$t_{1/2}$, *2.06 hr).*

where the ijth element k_{ij} of the matrix K is the first-order intercompartmental transfer rate constant between the ith and jth compartments, and E_i is the sum of the exit rate constants of the ith compartment. The symbol s represents the Laplace operator and $s + E_i$ is the ith diagonal element of the diagonal matrix, diag$(s + E_1, s + E_2, \ldots, s + E_n)$.

Using Eq. 11 for analytical treatment of the two-compartment model with an input function of Eq. 4 (Fig. 1B) yields the following equations which describe the drug amounts in the central and peripheral compartments (X_1 and X_2, respectively) as a function of time. Thus, for $0 \le t \le t^*$:

$$X_1 = \frac{3(\alpha - k_{21})W_0}{\alpha - \beta}[Q_1(1 - e^{-\alpha t}) + R_1t + S_1t^2] + \frac{3(k_{21} - \beta)W_0}{\alpha - \beta}[Q_2(1 - e^{-\beta t}) + R_2t + S_2t^2] \qquad \text{(Eq. 14)}$$

and:

$$X_2 = \frac{3k_{12}W_0}{\beta - \alpha}[Q_1(1 - e^{-\alpha t}) + R_1t + S_1t^2 - Q_2(1 - e^{-\beta t}) - R_2t - S_2t^2] \qquad \text{(Eq. 15)}$$

For $t > t^*$ (setting t' equal to $t - t^*$):

$$X_1 = \frac{3W_0}{(\alpha - \beta)^2}[\{(\alpha - k_{21})^2 + k_{12}k_{21}\}U_1e^{-\alpha t'} + \{(k_{21} - \beta)^2 + k_{12}k_{21}\}U_2e^{-\beta t'}] \qquad \text{(Eq. 16)}$$

and:

$$X_2 = \frac{3k_{12}W_0}{\beta - \alpha}(U_1e^{-\alpha t'} - U_2e^{-\beta t'}) \qquad \text{(Eq. 17)}$$

where α and β are the real solutions ($\alpha > \beta$) of the equation $y^2 - (k_{10} + k_{12} + k_{21})y + k_{21}k_{10} = 0$. The terms Q_1, Q_2, R_1, R_2, S_1, S_2, U_1, and U_2 are written, using $\gamma_1 = j/\alpha$ and $\gamma_2 = j/\beta$, as follows:

$$\left.\begin{aligned} Q_1 = \gamma_1(1 + 2\gamma_1 + 2\gamma_1^2), \quad Q_2 = \gamma_2(1 + 2\gamma_2 + 2\gamma_2^2) \\ R_1 = -2\gamma_1j(1 + \gamma_1), \quad R_2 = -2\gamma_2j(1 + \gamma_2), \quad S_1 = j^2\gamma_1 \\ S_2 = j^2\gamma_2, \quad U_1 = \frac{2S_1}{\alpha^2} - Q_1e^{-\alpha t^*}, \quad U_2 = \frac{2S_2}{\beta^2} - Q_2e^{-\beta t^*} \end{aligned}\right\} \qquad \text{(Eq. 18)}$$

By introducing the apparent volume (V_c) of the central compartment, the plasma drug concentration (C) is given as:

$$C = X_1/V_c \qquad \text{(Eq. 19)}$$

In addition, T_{max} ($0 < T_{max} \le t^*$) is also given as one of the real solutions of the following transcendental equation:

$$(\alpha - k_{21})(Q_1\alpha e^{-\alpha T_{max}} + R_1 + 2S_1T_{max}) + (k_{21} - \beta)(Q_2\beta e^{-\beta T_{max}} + R_2 + 2S_2T_{max}) = 0 \qquad \text{(Eq. 20)}$$

Thus, plasma concentration C at any time t, T_{max}, and C_{max} can be readily calculated for different doses of a drug when only one absorption rate constant (under certain administration conditions) and other

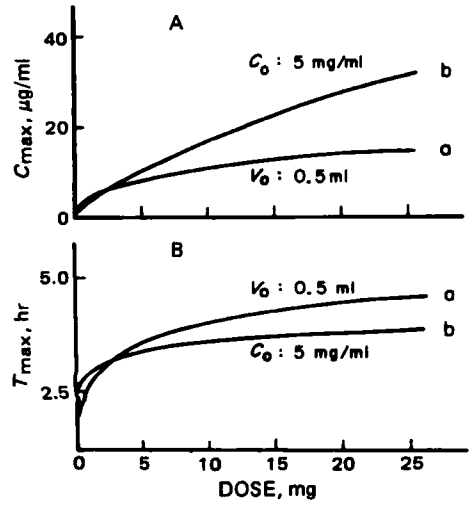


Figure 3—*Relationship between C_{max} (A) or T_{max} (B) and subcutaneous dose of aqueous suspension at fixed injection volume (a) or fixed drug concentration (b). These curves were obtained using hypothetical parameters:* k_{el}, *1.0 hr*$^{-1}$; V_d, *25 ml; j(1) (at* C_0 = *5 mg/ml and* V_0 = *0.5 ml), 0.0238 hr*$^{-1}$.

pharmacokinetic parameters are known; this confers one- or two-compartment characteristics on the body. In the present study, using the one-compartment model as an example, the response pattern of the plasma drug concentration–time curve to a change in dose or absorption rate constant was examined through some simulations and experiments.

EXPERIMENTAL

Calculation of Plasma Concentration—The apparent absorption rate constant j at any drug concentration (C_0) and at any injection volume (V_0), *i.e.*, at any dose, was calculated from one set of known data [$j(1)$ at $C_0(1)$ and $V_0(1)$], assuming the same injection site and an aqueous suspension of the same prescription, using the following equation derived readily from Eq. 2:

$$j = j(1)[C_0/C_0(1)]^g[V_0/V_0(1)]^h \qquad \text{(Eq. 21)}$$

In this study, the experimentally determined values of −0.66 and −0.32 were used for g and h, respectively (2). Drug plasma concentration C at any time t was calculated from Eq. 7 or 8 using values of j obtained from Eq. 21, pharmacokinetic parameters (k_{el} and V_d), and dose W_0. T_{max} was obtained by solving Eq. 10 with the aid of numerical analysis (Newton–Raphson method). For calculation of the sulfamethoxazole plasma concentration after subcutaneous doses of N^1-acetylsulfamethoxazole in aqueous suspension, the experimental value of 0.17 hr^{-1} (C_0 = 5 mg/ml; V_0 = 0.5 ml of N^1-acetylsulfamethoxazole), which was reported in a previous paper (2), was used as $j(1)$. All of the above calculations were carried out with a FACOM 270-20/30 computer.

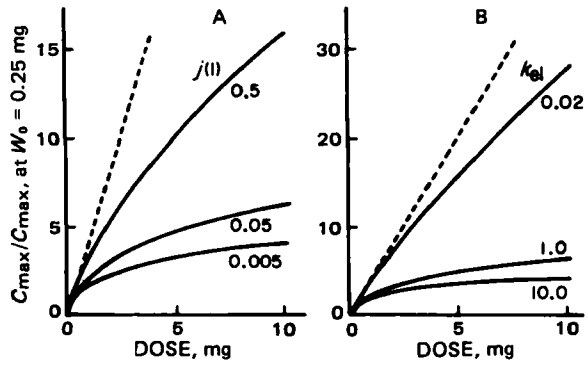


Figure 4—*Curvilinear increase in C_{max} with dose elevation at different j(1) (section A) or k_{el} values (section B) under fixed injection volume (0.5 ml). These curves were calculated from hypothetical parameters:* V_d, *25 ml;* k_{el} *in section A, 1.0 hr*$^{-1}$; *j(1) in section B, 0.05 hr*$^{-1}$. *j(1) shows the* j *value at* C_0 = *5 mg/ml and* V_0 = *0.5 ml.*

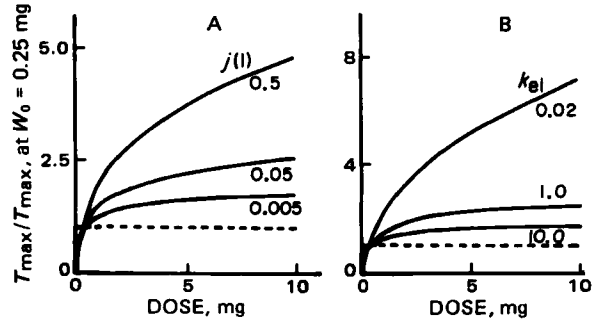


Figure 5—*Retardation of T_{max} with dose elevation at different j(1) (section A) or k_{el} values (section B) under fixed injection volume (0.5 ml). These curves were calculated from the same hypothetical parameters:* V_d, *25 ml;* k_{el} *in section A, 1.0 hr*$^{-1}$; *j(1) in section B, 0.05 hr*$^{-1}$.

Materials and Test Suspensions—N^1-Acetylsulfamethoxazole[3] was selected as a model of a practically water-insoluble drug and aqueous suspensions of the different concentrations (5, 10, 20, 50, and 100 mg/ml), prepared according to the controlled preparation method described in a previous report (4), were used as test suspensions for the subcutaneous administration experiments. N^1-Acetylsulfamethoxazole[3] and a dispersion medium[4] used for these preparations were the same as those reported in the previous paper (2). The mean particle diameter, distribution constant, and sedimentation volume of the test suspensions were 3.7–4.2 μm, 2.4–2.6, and 1.7–1.8 cm^3/g, respectively. These colloidal properties were similar to those reported in the previous paper (2). In addition, sulfamethoxazole solutions (concentration: 1.25, 2.50, and 5.0 mg/ml; medium: saline) were also used for the intravenous administration experiments. These solutions were prepared using the sodium salt[3] of sulfamethoxazole; pH values were adjusted to 8.0 with 1 *N* HCl.

Animal Experiments—Male Wistar albino rats weighing 250–292 g were used in all animal experiments. All test suspensions were administered into the dorsal subcutaneous region of the rats according to the manner described in a previous paper (3). Blood samples were withdrawn periodically through a tail vein. The rats were placed in a conventional cage with free access to water and food, except for a short period during drug administration and blood sampling when they were placed under light anesthesia with ether. One hundred microliters of plasma was collected for the following assay. A group of three to six rats was used in each experiment.

In vitro incubation of N^1-acetylsulfamethoxazole (I) at 37° [concentration of I in medium, 76 μg/ml (below solubility level of I)] with fresh rat plasma or saline shaken with subcutaneous connective tissues and interstitial materials[5], caused >90% of I to be transformed into sulfamethoxazole (II) within a 30-sec period. A similar conversion has been reported (8) for N^1-acetylsulfisoxazole using human plasma. It is inferred from these findings that N^1-deacetylation of I occurs immediately after dissolution in the injection site medium and that II is actually the compound being absorbed. In practice, I could not be detected in blood samples taken at 10 and 20 min and 1, 2, and 5 hr after subcutaneous administration of an aqueous suspension of I (dose: 5 mg/0.5 ml/rat) even in carefully conducted experiments[6].

Although I, when dissolved in the injection site medium, is deacetylated to II, the resultant II disappeared much more rapidly[7] than the dissolution of I. Thus, no significant accumulation of II at the injection site was

[3] Shionogi Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan.

[4] 0.5% (w/v) Methylcellulose (Metolose SM-15) + 0.005% (w/v) polysorbate 80 + 0.9% (w/v) NaCl.

[5] This incubation medium was obtained as follows: The rat was injected at four different points with 0.5 sc ml of saline. Immediately after the injections, the connective tissues containing saline depot were excised, minced, shaken with 2 ml of saline for 30 min, and centrifuged at 3000 rpm for 10 min. The resultant supernatant was used as the medium.

[6] Immediately after blood sampling, 0.2 ml of blood was mixed vigorously with 2 ml of 0.03 *N* HCl kept at 0° and 2 ml of 6% (w/v) $HClO_4$ was added. The mixture was shaken well and centrifuged at 5°. A portion of the clear, deproteinized solution was injected into the high-performance liquid chromatographic (HPLC) system as follows: Shimadzu LC-3A; column, Nucleosil 10C_{18} (4-mm i.d. × 300 mm); mobile phase, pH 6.3 phosphate buffer (50 m*M*)–acetonitrile (3:1, v/v); flow rate, 2 ml/min; detection, UV (290 nm); retention time, 14.1 min for I; detection limit for I (determined using blood samples inactivated with HCl then added with I), 1–2 μg/ml of blood.

[7] For example, the half-life of II in aqueous solution in the injection site was ~8.3 min (dose, 260.4 μg/0.5 ml/rat), while that of I in aqueous suspension was ~70 min (dose, 2.5 mg/0.5 ml/rat).

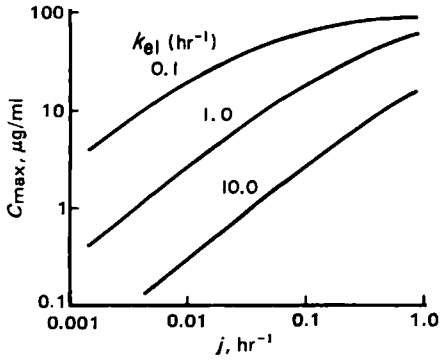


Figure 6—*Relationship between maximum plasma concentration (C_{max}) and absorption rate constant (j) at different k_{el} values under a fixed dose. These curves were obtained using hypothetical parameters: V_d, 25 ml; W_0, 2.5 mg.*

observed under the present administration conditions[8]. Therefore, postabsorptive pharmacokinetic parameters of I were estimated from plasma concentration–time profiles after intravenous administration of II. All test solutions of II were injected into a tail vein, and blood samples were periodically taken from the vein in the other side of the tail.

Analytical Method—Sulfamethoxazole in rat plasma was measured according to the fluorometric method with fluorescamine reported previously (9). To 100 μl of plasma were added 2 ml of pH 4.7 buffer (1 *N* HCl–1 *N* CH_3COONa, 3:7, v/v) and 6 ml of 1,2-dichloroethane. This was shaken well and then centrifuged. Three milliliters was withdrawn from the 1,2-dichloroethane layer, 3 ml of pH 10.6 buffer (0.1 *N* $NaHCO_3$–0.1 *N* Na_2CO_3, 1:9, v/v) was added, and the mixture was shaken and centrifuged. To 2 ml of the buffer layer (after dilution with the same buffer as necessary) were added 5 ml of pH 2.6 buffer (1 *N* HCl–1 *N* CH_3COONa, 1:1, v/v) and 1 ml of fluorescamine[9] reagent (0.2 mg/ml in acetone), and the mixture was shaken vigorously for several seconds. After this had been left standing for ~40 min, its fluorescent intensity was measured with a spectrophotometer[10] at excitation and emission wavelengths of 405 and 490 nm (uncorrected), respectively.

RESULTS AND DISCUSSION

Simulation Study—*Plasma Levels after Different Subcutaneous Doses of a Drug in Aqueous Suspension*—General patterns in the response of plasma concentration to the change in the subcutaneous dose of a drug in aqueous suspension were examined under various administration conditions, using computer simulation with hypothetical data in a one-compartment model. Figure 2A shows computer-simulated time courses of the plasma level after different subcutaneous doses of a drug in aqueous suspension at a fixed injection volume (plasma concentration shown here was calculated from Eq. 7 or 8 using j values generated from Eq. 21). The values of the absorption rate constant $j(1)$ and other pharmacokinetic parameters k_{el} and V_d used for these computations are shown in the legend. For comparison, plasma concentration–time curves for oily solutions are also shown in Fig. 2B as an example of first-order absorption (rate constant, k). Figure 2A shows the typical features of the subcutaneous administration of aqueous suspension, *i.e.*, the curvilinear increase of the maximum plasma concentration and the retardation of T_{max} with dose elevation. It should be noted that these tendencies differ from those of the preparations with first-order absorption characteristics such as oily (3) and surfactant micellar solutions[1] as shown in Fig. 2B. The above two features were more evident in dose elevation with fixed injection volume than with fixed drug concentration, as can be seen from Fig. 3.

Figure 4 compares the degree of deviation from the linear response of the maximum plasma concentration C_{max} to dose elevation among different $j(1)$ values (A) or k_{el} values (B). In this figure, the ratio of C_{max} at any dose to the C_{max} where C_0 = 0.5 mg/ml and V_0 = 0.5 ml was taken as a measure representing such a degree and is plotted against the dose. The downward deviation of this plot from the broken line, which showed

[8] The percentages of the dose disappearing from the injection site at 40, 90, and 180 min after administration of I in aqueous suspension (dose, 2.5 mg/0.5 ml/rat) were ~31, 63, and 75%, respectively, while those for II appearing at the site were ~5, 6, and 4%, respectively (each value was the mean of two experimental determinations).

[9] F. Hoffmann-La Roche Co., A. G., Basel, Switzerland.

[10] Hitachi Model 203, Hitachi, Ltd., Japan.

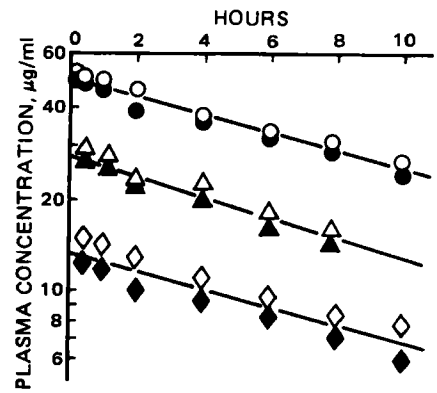


Figure 7—*Plasma concentration–time curves following three intravenous doses of sulfamethoxazole in rats. The data points in the same symbol show the plasma concentrations for each rat. Key (dose per rat): (○, ●) 2.50 mg; (△, ▲) 1.25 mg; (◇, ◆) 0.625 mg.*

a proportional relationship between C_{max} and the dose, demonstrates the phenomenon of deviation from the linear response. The deviation became larger for drugs with smaller absorption rate constants, $j(1)$, and those with larger elimination rate constants, k_{el}. Figure 5 shows the retardation of T_{max} with dose elevation at various $j(1)$ values (A) or k_{el} values (B). Similarly, the ratio of T_{max} at any dose to the T_{max} at C_0 = 0.5 mg/ml and V_0 = 0.5 ml was used as an index for this retardation. This figure indicated that the retardation of T_{max} with dose elevation became more marked the larger the $j(1)$ value and the smaller the k_{el} value.

For the subcutaneous administration of a drug in aqueous suspension, these simulations indicated that the rate of systemic drug availability decreases with increasing dose and that this decrease becomes more significant for drugs having a smaller $j(1)$ and a larger k_{el}.

Relationship Between Peak Plasma Level and Absorption Rate Constant—The parenteral drug absorption rate from an aqueous suspension can be altered by modifying not only preparation conditions such as particle size but also administration conditions such as drug concentration and injection volume (2, 4). The relationship between the absorption rate constant j and the maximum plasma concentration C_{max} at a fixed dose was examined by computer simulation. Figure 6 shows this relationship at various values of the elimination rate constant k_{el} on a log–log scale. This figure demonstrates that the curve representing such a relationship becomes closer to a straight line having a slope of unity with smaller j and larger k_{el}, while it tends to level off with larger j and smaller k_{el}. This also suggests that an attempt to elevate the drug plasma level by particle size reduction is more effective for drugs with a smaller j value [for example, due to a lower water solubility (2)] and a larger k_{el} value.

Comparison of the Results from Simulation Analyses with Experiments—Equation 1 was derived from a model in which injected particles loosely agglomerate and dissolution (or release) of the drug from the surface of the particle agglomerate is the rate-limiting process for overall drug absorption. Equation 2 was derived from the experimental

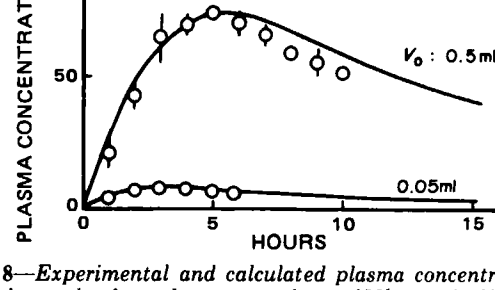


Figure 8—*Experimental and calculated plasma concentrations of sulfamethoxazole after subcutaneous doses of N^1-acetylsulfamethoxazole aqueous suspension at fixed initial drug concentration (10 mg/ml) in rats (mean body weight, 286 g). Each experimental value was given by the mean (open circle) and standard deviation (vertical bar) of three or four rats. Calculated values (solid line) were obtained with the following parameters: k_{el}, 0.072 hr^{-1}; V_d, 49.5 ml; $j(1)$, 0.17 hr^{-1}.*

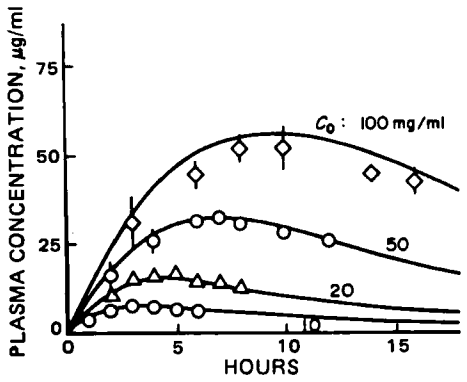


Figure 9—*Experimental and calculated plasma concentrations of sulfamethoxazole after different subcutaneous doses of* N^1*-acetylsulfamethoxazole aqueous suspensions at fixed injection volume (0.048 ml) in rats (mean body weight, 277 g). Each experimental value was given by the mean and standard deviation of four to six rats. Calculated values (solid line) were obtained with the following parameters:* k_{el}, *0.072* hr^{-1}; V_d, *48.0 ml;* $j(1)$, *0.17* hr^{-1}.

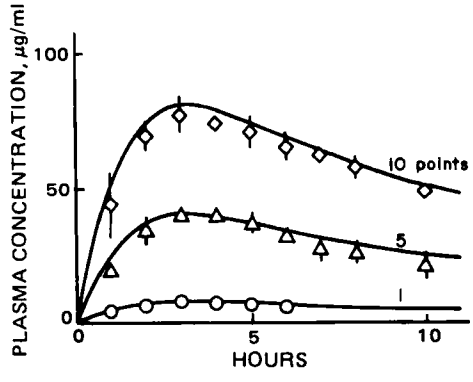
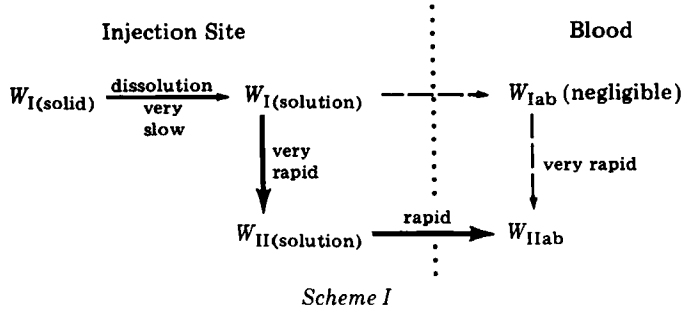


Figure 10—*Experimental and calculated plasma concentrations of sulfamethoxazole after subcutaneous doses of* N^1*-acetylsulfamethoxazole aqueous suspension by the multiple-point injection method in rats (mean body weight, 284 g).* C_0 *and* V_0 *for each injection point were 10 mg/ml and 0.048 ml, respectively. Each experimental value was given by the mean and standard deviation of four to six rats. Calculated values (solid line) were obtained with the following parameters:* k_{el}, *0.072* hr^{-1}; V_d, *49.1 ml;* $j(1)$, *0.17* hr^{-1}.

results by the local clearance method in rats (2, 4). Based on the assumption that these equations reflect the true overall absorption of the drug (or active metabolites) into the systemic circulation, the results obtained by computer simulation should be valid. However, until now the validity of this assumption had not been checked directly by experimentation. To check the feasibility of the above simulation results, plasma drug levels after subcutaneous doses using aqueous suspensions of N^1-acetylsulfamethoxazole (I) were followed in rats and compared with the calculated levels.

As mentioned in *Experimental*, I is very rapidly deacetylated to sulfamethoxazole (II) immediately after dissolution in the injection site medium, inferring absorption as II. However, absorption of II formed at the injection site occurred more rapidly than dissolution of I, and, thus, no significant accumulation of II at the injection site was observed under these injection conditions. This indicates that the rate-limiting process in overall absorption of I from aqueous suspension is the dissolution step and that the disappearance rate of I estimated by the local clearance method can be regarded as approximately equivalent to the overall absorption rate of I.

In this case, the absorption behavior for I is summarized in Scheme I:



Scheme I

The amount of I (W_{ab}) absorbed *via* II (W_{IIab}) can be represented as:

$$\left.\begin{aligned} W_{ab} &= W_{IIab} \\ &= W_0 - [W_{I(solid)} + W_{I(solution)} + W_{II(solution)}] \\ &= W_0 - [W_I + W_{II(solution)}] \end{aligned}\right\} \quad \text{(Eq. 22)}$$

where W_0 is the amount injected. Since $W_I = W_{I(solid)} + W_{I(solution)} \gg W_{II(solution)}$, therefore:

$$W_{ab} \simeq W_0 - W_I \quad \text{(Eq. 23)}$$

By differentiation of this equation with respect to time (t), the following equation was obtained:

$$dW_{ab}/dt \simeq -dW_I/dt \quad \text{(Eq. 24)}$$

Accordingly, the disappearance rate of I (dW_I/dt) is approximately equal to the overall absorption rate of I (dW_{ab}/dt).

The equation for the absorption kinetics (Eq. 1) is based on the premise that dissolution (or release) is the rate-limiting step for overall drug absorption. Compound I satisfies this premise and is adequate as a model compound for this study, although it undergoes site metabolism to II.

First, plasma concentrations of sulfamethoxazole (II) after different intravenous doses were measured to examine the postabsorptive pharmacokinetic characteristics of N^1-acetylsulfamethoxazole (I) in rats. The reason for the use of II instead of I is explained in detail in *Experimental* and in the previous paragraphs. Figure 7 shows semilogarithmic plots of plasma concentrations of II as a function of time for each dose in a rat. From the nearly linear relationship of these curves, with a similar slope and linear response of the plasma levels to the intravenous dose, the pharmacokinetic characteristics of II could be explained approximately by a one-compartment model under the described experimental conditions.

From each plot in this figure, the apparent elimination rate constant k_{el} and distribution volume V_d were estimated by the least-squares method. The mean (standard deviation, *SD*) values of k_{el} and V_d' (equal to V_d per body weight) for these six experiments were 0.072 (0.008) hr^{-1} and 0.173 (0.006) ml/g, respectively. These parameters are used below together with the absorption rate constant $j(1)$, described in *Experimental*, to calculate plasma concentrations of II after different subcutaneous doses of aqueous suspensions of I *in rats*.

Single-Point Injection—Plasma concentrations of II after different subcutaneous doses of I in aqueous suspension were determined in rats and compared with calculated plasma concentrations. Figures 8 and 9 show the results of these comparisons for different doses at fixed drug concentration and fixed injection volume, respectively. These comparisons indicated that the observed plasma concentrations for all the doses

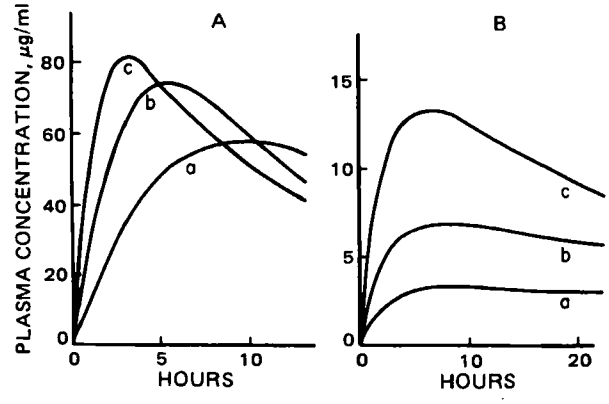


Figure 11—*Comparison of predicted plasma levels after subcutaneous administrations of aqueous suspensions among three injection methods at a fixed dose. The curves in section A were calculated from the parameters of* N^1*-acetylsulfamethoxazole (the same as shown in Fig. 10), and those in section B from hypothetical parameters as follows:* k_{el}, *0.5* hr^{-1}; V_d, *48 ml;* $j(1)$, *0.01* hr^{-1}. *Key:* a, *100 mg/ml–0.05 ml;* b, *10 mg/ml–0.5 ml;* c, *10 mg/ml–0.05 ml (10-point injection).*

examined corresponded with the calculated curves. Also, it should be noted that the curves in Figs. 8 and 9 showed the typical characteristics of subcutaneous administration of aqueous suspensions, *i.e.*, the nonlinear increase in C_{max} and retardation in T_{max} with dose elevation.

These findings appear to support the validity of the aforementioned assumption that Eqs. 1 and 2 reflect the true overall absorption of a drug in aqueous suspension into the systemic circulation.

Multiple-Point Injection—As mentioned above, dose elevation by a single subcutaneous injection of aqueous suspension may result more or less, in a nonlinear increase in plasma drug concentration. In most screening tests or preclinical testing of new drugs in laboratory animals, this phenomenon may be undesirable. It can be overcome by increasing the number of injection points in the neighboring subcutaneous area for dose elevation while keeping the drug concentration and injection volume fixed. In this multiple-point injection method, the drug absorption from each point can be expected to occur independently at nearly equal rates, and, therefore, the plasma drug level would increase in proportion to the number of injection points, *i.e.*, the dose, if the postabsorptive disposition of the drug in the body occurs in a linear manner.

To confirm this expectation, plasma sulfamethoxazole concentrations after 1-, 5-, and 10-point subcutaneous injections of N^1-acetylsulfamethoxazole aqueous suspension (C_0, 10 mg/ml; V_0, 0.048 ml) were followed in rats and compared with calculated plasma concentrations. Figure 10 shows a good agreement between the experimental and calculated values. Therefore, this multiple-point injection method seems to be a simple and convenient way to obtain a high plasma drug level without retardation of T_{max} after subcutaneous administration of a suspension.

Figure 11A compares the predicted plasma concentration–time curves following subcutaneous doses of N^1-acetylsulfamethoxazole aqueous suspension among the three injection methods at a fixed dose: C_0–V_0, (a) 100 mg/ml–0.05 ml; (b) 10 mg/ml–0.5 ml; (c) 10 mg/ml–0.05 ml, 10-point injection. This figure shows that the multiple-point injection is useful for obtaining a higher plasma drug level at a fixed dose. Figure 11B shows similar computer-simulated curves for another drug which has $j(1)$ and k_{el} values one order of magnitude below and above those in Fig. 11A, respectively. The comparison of the two figures suggests that multiple-point injection is especially effective for drugs with a small absorption rate constant and a large elimination rate constant.

The good agreement between the observed and calculated plasma concentrations presented in Figs. 8 and 9 support the validity of the assumption that Eqs. 1 and 2, derived from the experimental results by the local clearance method (2), reflect the true pattern of drug absorption into the systemic circulation. From this finding, it can be predicted that with multiple-point injection, C_{max} should be proportional to the dose without a change in T_{max}, unlike the case of single-point injection; this prediction was verified by the results shown in Fig. 10. In addition, it is indicated that the plasma concentration–time curve should differ considerably even at a fixed dose depending on the administration conditions (Fig. 11). All results of this study confirm the suitability of the analytical treatment chosen for subcutaneous drug absorption from aqueous suspensions.

Information from the simulation study presented here should be useful for predicting the dose-response pattern of plasma concentration–time curves after subcutaneous administrations in aqueous suspensions of other practically water-insoluble drugs with similar pharmacokinetic characteristics. It should also be applicable to intramuscular administrations and to drugs with two- or multicompartmental characteristics in the body.

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