Studies on the Absorption of Practically Water-Insoluble Drugs following Injection VIII: Comparison of the Subcutaneous Absorption Rates from Aqueous Suspensions in the Mouse, Rat, and Rabbit

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Abstract □ Subcutaneous drug absorption rates from aqueous suspensions were measured in the mouse and the rabbit by a local clearance method and compared with those in the rat. A plot of the cube root of the residual fraction (W/W_0) of the drug at the injection site *versus* time (t) gave a good linear relationship for the former two species. This implied that the kinetic equation for the absorption process in the rat, $(W/W_0)^{1/3} = 1 - jt$, could be applied to them. In addition, the absorption rate constants (j) of the mouse and rabbit were close to that of the rat when the drug concentration (C_0) in the suspension and the injection volume (V_0) were fixed. These results led to the presumption that the correlation between j and C_0 or V_0 in the mouse and rabbit might be similar to that in the rat, and therefore, drug plasma levels for the former two at any dose might be roughly predictable even from one j value of the rat. The validity of this assumption was confirmed by comparison of the observed and predicted plasma concentrations after different subcutaneous doses of aqueous suspensions of N^1-acetylsulfamethoxazole in the mouse and rabbit. These findings strongly supported the idea that for the subcutaneous administration of the drug in aqueous suspension under fixed dose per body weight, the rate of bioavailability should decrease with animal size, and therefore, the plasma drug level in larger species is not likely to be as high as that expected, from the data obtained for smaller species.

Keyphrases □ Absorption—studies of practically water-insoluble drugs following injection, comparison of subcutaneous absorption rates from aqueous suspensions, mouse, rat, rabbit □ Aqueous suspensions—studies on the absorption of practically water-insoluble drugs following injection, comparison of subcutaneous absorption rates, mouse, rat, rabbit

Previous reports (1, 2) showed that the absorption rate process after intramuscular or subcutaneous administration of practically water-insoluble drugs in aqueous suspensions was not linear and had a complicated dose dependency. Therefore, single administration of such drug preparations led to nonlinear response of the plasma–drug concentration, that is, a curvilinear increase of its peak and retardation of the time at which the peak occurred with increasing dose (3).

In earlier testings of pharmacological activities and dispositions of new drugs under development in laboratory animals, the dose elevation experiment is often done, not only when examining the dose response in one animal species, but also when maintaining the same or a similar dose per body weight for scale-up in various animal species. Accordingly, the problem arises that the rate of bioavailability at the same dose per body weight may vary for different species; that is, the rate of bioavailability for larger animals given a comparatively large amount of the drug may be lower compared with that for smaller animals. Since such a phenomenon, in some cases, may result in significant species differences in the drug potency, clarification of this problem is important.

In the present report, three laboratory animals, having different body weights, the mouse, rat, and rabbit, were selected as representatives to examine this problem. First, subcutaneous absorption time courses were compared among the three species using the local clearance method to determine whether a significant species difference existed in the absorption rate. Next, plasma concentrations after subcutaneous doses were followed to confirm the validity of the above comparison using N^1-acetylsulfamethoxazole in aqueous suspension. It is indicated that there is a "pitfall of dose per kilogram" in interpreting the results obtained from subcutaneous administration experiments of aqueous suspensions in various animal species.

EXPERIMENTAL

Materials—Suspensions of *p*-aminoazobenzene, *p*-hydroxyazobenzene, *o*-aminoazotoluene, and N^1-acetylsulfamethoxazole were used as models for practically water-insoluble drugs as reported previously (2). The sodium salt of sulfamethoxazole was used for the intravenous injection preparation as reported previously (3). For the fluorometry of sulfamethoxazole in plasma, fluorescamine[1] was used. All other chemicals were of reagent or analytical grade.

Preparation of Test Suspensions and Solutions—The same vehicle[2] described in the previous paper (3) was used to prepare the test aqueous suspensions. All suspensions were prepared according to the controlled preparation method reported previously (1). Their colloidal properties[3]

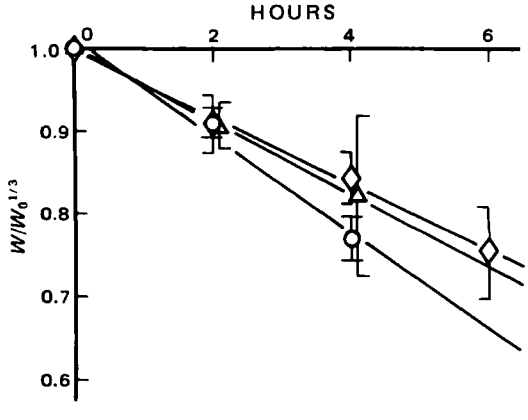

Figure 1—*Comparison of absorption rates following subcutaneous injections of* p-*aminoazobenzene aqueous suspension among three animal species. Each plot represents the mean and standard deviation of three or four animals.* C_0, *5 mg/ml;* V_0, *0.5 ml. Key:* ⊟ *mouse;* ⊕ *rat;* ⊟ *rabbit.*

[1] F. Hoffmann-La Roche and Co., A. G. Diagnostica, Basel, Switzerland.
[2] 0.5% (w/v) Methylcellulose (Metolose SM-15) + 0.005% (w/v) polysorbate 80 + 0.9% (w/v) NaCl.
[3] Mean particle diameter, 3.6–4.2 μm; distribution constant, 2.6–2.7; sedimentation volume, 1.7–2.8 cm^3/g.

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Table I—Comparison of Subcutaneous Absorption Rate Constants (j) from Various Aqueous Suspensions among Three Animal Species

Compound	C_0, mg/ml	V_0, ml	j, hr^{-1} Mouse[a]	Rat[a]	Rabbit[a]
p-Hydroxyazobenzene	50	0.05	0.022 (0.004)	0.018 (0.002)	0.026 (0.005)
			NS	NS	
			NS		
p-Hydroxyazobenzene	5	0.5	0.048 (0.004)	0.043 (0.002)	0.042 (0.004)
			NS	NS	
			NS		
p-Aminoazobenzene	5	0.5	0.043 (0.009)	0.057 (0.004)	0.040 (0.004)
			NS	$p < 0.05$	
			NS		

[a] Each j value was obtained from the data shown in Figs. 1 and 2 by the least-squares method and is given with the standard error in parentheses. A t test was performed between j values of two animals each. NS = not significant ($p > 0.1$).

were similar to those reported previously (2). In addition, sulfamethoxazole solutions were prepared by the same method described in the previous paper (3) and used for the intravenous administration experiments.

Animal Experiments—*Animals*—ICR Jcl strain mice (male, 26–34 g), Wistar albino rats (male, 250–305 g), and mongrel rabbits (male, 2.6–4.0 kg) were used. During the experiments, they were kept in cages with free access to water and food.

Absorption Experiment Procedure—All the test suspensions were administered into the subcutaneous region near the center of the shorn dorsum of the animals, and their absorption rates were measured by the local clearance method described previously (4). In the mouse and rat experiments, each animal received a single injection. In the rabbit experiments, three successive injections were given at designated time intervals into different sites sufficiently apart from each other but within the dorsum of each animal; the amount of the drug remaining at each injection site was measured separately. When the experimental period ended, the mice and rats were sacrificed by exsanguination, and the rabbits were killed by intravenous administration of sodium pentobarbital[4]. The drug remaining in the injection site was recovered from the excised tissues including the depot by extraction with ethyl acetate, and its amount was determined. A group of three to six animals was used in each experiment.

Plasma Drug Level—All the aqueous suspensions of N^1-acetylsulfamethoxazole were administered subcutaneously to mice or rabbits in the manner described above. Blood samples from the mice were withdrawn from the heart at set time intervals and from the rabbits, from an ear vein periodically (serial sampling). One-hundred microliters of plasma was collected for the following analysis. *In vitro* incubation of N^1-acetylsulfamethoxazole with fresh plasma at 37° showed that this compound was very quickly transformed into sulfamethoxazole. The following intravenous administration experiments using sulfamethoxazole instead of N^1-acetylsulfamethoxazole were done in mice and rabbits to examine postabsorptive pharmacokinetic characteristics of N^1-acetylsulfamethoxazole subcutaneously administered. Test solutions of sulfamethoxazole were administered *via* a tail vein for mice (0.05 ml) and an ear vein for rabbits (2.5 ml), then blood samples were taken from the heart for the former and from another ear vein for the latter. For each rabbit, the above subcutaneous and intravenous administration experiments were performed alternately every 7–10 days for a 2-month period.

Analytical Method—The remaining amounts of *p*-aminoazobenzene, *p*-hydroxyazobenzene, and *o*-aminoazotoluene from the injection site were analyzed colorimetrically after extraction, as described previously (2). The plasma concentrations of sulfamethoxazole after subcutaneous injections of N^1-acetylsulfamethoxazole and intravenous injections of sulfamethoxazole in mice or rabbits were determined according to a fluorometric method with fluorescamine as described in the previous report (3).

Prediction of Plasma Drug Concentration—Sulfamethoxazole-plasma concentrations after different subcutaneous doses of the controlled suspension of N^1-acetylsulfamethoxazole in mice or rabbits were predicted from their postabsorptive pharmacokinetic parameters (elimination rate constant and volume of distribution) and one basic absorption rate constant $j(1)$ in rats using the equations[5] presented in the previous report (3). The postabsorptive pharmacokinetic parameters were estimated from the plasma concentration–time curves after different intravenous doses of sulfamethoxazole in mice and rabbits. The experimental value of 0.17 hr^{-1} (N^1-acetylsulfamethoxazole concentration in the test suspension, 5 mg/ml; injection volume, 0.5 ml) from a previous report (2) was used as $j(1)$.

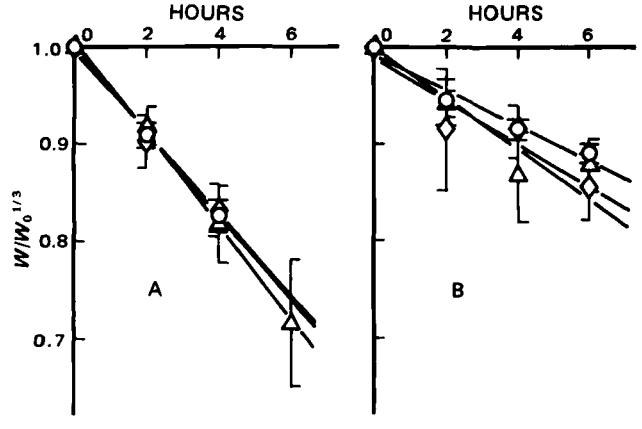

Figure 2—*Comparison of absorption rates following subcutaneous injections of* p-*hydroxyazobenzene aqueous suspensions among three animal species. Each result is given as the mean and standard deviation of three or four animals. (A):* C_0, *5 mg/ml;* V_0, *0.5 ml. (B):* C_0, *50 mg/ml;* V_0, *0.05 ml. Key:* mouse; rat; rabbit.

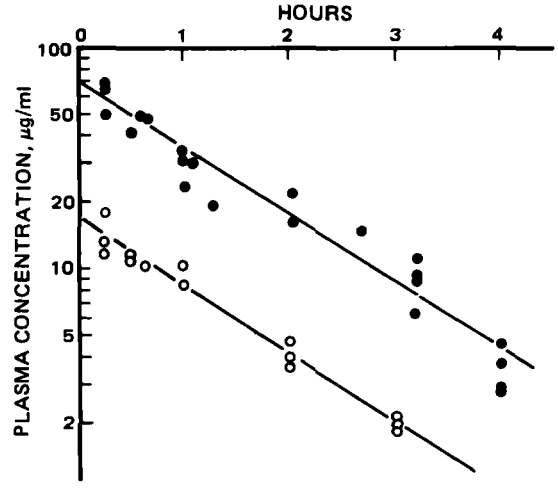

Figure 3—*Plasma concentration of sulfamethoxazole after intravenous doses in mice (26–34 g). Key: (●) 0.502 mg/mouse; (○) 0.103 mg/mouse.*

[4] Pitman-Moore, Inc., N.J.

[5] Equation 7 or 8 and Eq. 21 in the previous report (3).

Table II—An Example for Comparison of Subcutaneous Absorption Rate Constants (j) at Fixed Dose per Body Weight among Three Animal Species

Animal	Body Weight kg	C_0, mg/ml	V_0, ml	Dose, mg/kg	j/j(rat)[a]
Mouse	0.03	5	0.05	8.3	2.1
Rat	0.3	5	0.5	8.3	1.0
Rabbit	3	50	0.5	8.3	0.29

[a] Estimated from Eq. 2 (using $g = -0.66$ and $h = -0.32$).

RESULTS AND DISCUSSION

Comparison of Subcutaneous Absorption Rates among Three Animals—Previous studies (5, 6) have reported the species difference in the absorption rates of solid drugs implanted subcutaneously. However, few detailed investigations have been done on the species difference in subcutaneous absorptions from aqueous suspensions as well as from other conventional dosage forms. Therefore, the difference in the rate of bioavailability among various animal species has been discussed little until now. The subcutaneous absorption rates of practically water-insoluble drugs in aqueous suspension were compared directly among three popular laboratory animal species, the mouse, rat, and rabbit, using a local clearance method. For this comparison, the dorsal subcutaneous region of each animal was selected as the model injection site.

Figure 1 shows the results for subcutaneous administrations of the controlled aqueous suspension of p-aminoazobenzene. In this comparison, the initial concentration (C_0) of p-aminoazobenzene in the suspension and the injection volume (V_0) were fixed as given in the legend. The cube root of the residual function (W/W_0) at the injection site for each animal species was plotted against time. A previous report (2) indicated that such a plot gave a good linear relationship for rats. This appeared to be also true for mice and rabbits, as indicated in Fig. 1. It was surprising that the slopes of these absorption time curves were close to each other. These tendencies were further examined by similar experiments using other test suspensions of p-hydroxyazobenzene. Figure 2 shows the results. The comparisons under the two conditions of different C_0 and V_0 also showed tendencies similar to those shown in Fig. 1. These results suggested that the kinetic equation for rats should also be applicable for describing the subcutaneous absorption process from aqueous suspensions in other animals such as mice and rabbits; that is:

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

where j was defined as an absorption rate constant (2).

Table I summarizes the absorption rate constants with their standard errors estimated from the data in Figs. 1 and 2 by the least-squares method. This table shows that the difference in j among these three species is not very significant and this tendency may be true for other suspensions with variable drug concentrations and injection volumes. This also implies that the following relationship derived from the rat

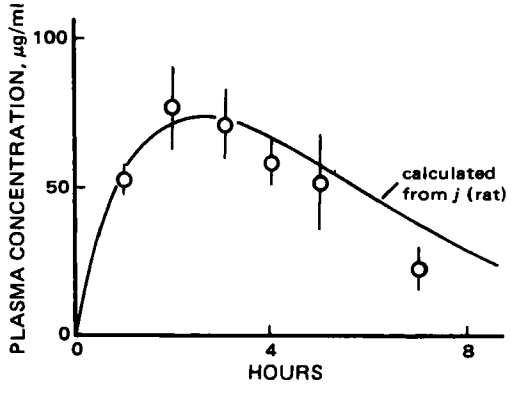

Figure 4—*Observed and predicted plasma concentrations of sulfamethoxazole after subcutaneous administration of* N^1*-acetylsulfamethoxazole aqueous suspension in mice (30.6 ± 1.6 g). Each observed value represents the mean and standard deviation of three mice.* C_0 = *51.6 mg/ml,* V_0 = *0.05 ml. The predicted value (solid line) was obtained using the following parameters:* k_{el}, *0.711 hr*$^{-1}$; V_d, *7.19 ml; j(1) (at* C_0 = *5 mg/ml and* V_0 = *0.5 ml in rats), 0.17 hr*$^{-1}$.

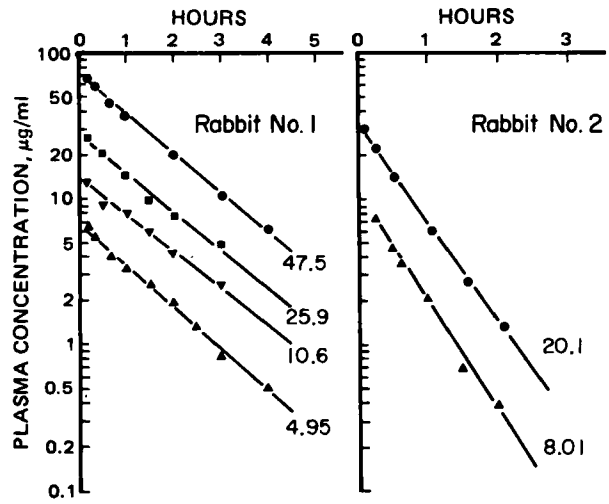

Figure 5—*Plasma concentration after different intravenous doses of sulfamethoxazole in rabbits. The value next to each curve shows the dose of sulfamethoxazole (mg/rabbit). Mean body weights of the rabbits during the experiments: No. 1, 3.96 kg; No. 2, 2.70 kg.*

experiments may also be applied to the subcutaneous absorption in mice and rabbits:

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

where g and h are the constants determined empirically [for rats, the experimental values of g and h were -0.66 and -0.32, respectively (2)]. The term f represents a constant that depends on such biological factors as the injection site and its physiological or histological state when the drug and suspension are the same. Such factors would influence the *in vivo* dispersion and dissolution of the injected particles and, hence, the absorption rate. Accordingly, the above results seem to show that the difference in the apparent *in vivo* dissolution rates of the drug among the three species may not be very large.

Comparison of Predicted and Observed Plasma Drug Concentrations in Mouse and Rabbit—It has been shown (3) that Eqs. 1 and 2, which had been derived empirically based on the results obtained from the local clearance method, reflected the true absorption of a drug into the systemic circulation in rats. The check, from the same point of view, was also performed in mice and rabbits, as described below.

The results shown above indicate that the absorption rate did not vary considerably for the mouse, the rat, and the rabbit when the drug concentration and injection volume were fixed, and that Eqs. 1 and 2 might be applicable for animals other than rats. These results also presented a possibility that the absorption rate constant j for the rat can be used, in rough approximation, as a substitute for that used for the mouse and rabbit. To confirm the validity of this presumptive evidence from the absorption experiments by the local clearance method, plasma drug concentration after different doses of the controlled aqueous suspension of N^1-acetylsulfamethoxazole were followed in the mouse and the rabbit, then compared with those predicted from their individual postabsorptive pharmacokinetic parameters and the absorption rate constant of the rat.

Mouse—Prior to subcutaneous administration experiments of N^1-acetylsulfamethoxazole (I) aqueous suspension, intravenous administration experiments of sulfamethoxazole (II) aqueous solution were undertaken to examine the postabsorptive pharmacokinetic characteristics of I. The reason for use of II instead of I was explained in detail in *Experimental*. Figure 3 shows semilogarithmic plots of plasma concentrations of II *versus* time after two intravenous doses (0.103 and 0.502 mg/mouse) of II in mice. Although the experimental values were slightly scattered, the plasma concentration–time curve for each dose could be approximated as a straight line. The plasma level appeared to be in proportion with the dose. These results suggested that the analytical treatment by a one-compartment model should be approximately possible for II in mice under the present experimental conditions. From the data in Fig. 3, the estimates (standard error, SE) of the apparent elimination rate constant (k_{el}) and volume of distribution per body weight (V_d') were calculated by the least-squares method to be 0.711 (0.020) hr^{-1} and 0.235 (0.047) ml/g, respectively. These parameters were used to predict plasma concentrations of II after the subcutaneous administration of I in aqueous suspension.

Table III—Predicted and Observed Percent Absorbed of *o*-Aminoazotoluene at 7.5 hr after Subcutaneous Administration of Its Aqueous Suspension under Fixed Dose per Body Weight in Three Animal Species

Animal	Percent Absorbed[a]		
	Known	Predicted	Observed[b]
Mouse[c]	—	65.7	60.9 ± 12.5
Rat[d]	36.9[e]		
Rabbit[f]	—	9.2	12.2 ± 4.0

[a] At the same C_0 and V_0 as shown in Table II. [b] Mean ± *SD* of three to six animals. [c] Body weight, 29–32 g. [d] Body weight, 290–305 g. [e] Reported previously (2). [f] Body weight, 2.85–3.20 kg.

Figure 4 compares the observed plasma level of II with the predicted level after the subcutaneous dose of I in aqueous suspension in mice (concentration of I in the suspension, 51.6 mg/ml; injection volume, 0.05 ml). The predicted plasma concentrations were calculated from the above estimated k_{el} and V_d (V_d' × mean body weight) in mice and one subcutaneous absorption rate constant in rats, $j(1)$, which had been reported in a previous paper (2), according to the procedure[5] described in the previous report (3). As is evident from Fig. 4, the observed plasma concentrations were in fair agreement with the predicted plasma-concentration curve. These results suggest the applicability of the above rough approximation in which the subcutaneous absorption rate constant of a drug from aqueous suspension in rats can be used as a substitute for that in mice.

Rabbit—Similar examinations were also performed with rabbits. Figure 5 shows time courses of the sulfamethoxazole (II) plasma concentration on a semilogarithmic scale after different intravenous doses of II in two rabbits (Nos. 1 and 2). The plasma concentration of II appeared to be nearly proportional to the dose for each rabbit. Within a short period after injection (0–20 min), some curvatures showing the distribution phase were observed in the plasma drug concentration–time curves and this was similar to results reported previously (7). However, these curvatures were minute under the present experimental conditions, so each time profile was regarded as a straight line. This allowed use of the one-compartment model for treatment of the data in Fig. 5. The apparent elimination rate constant, k_{el} and volume of distribution per body weight, V_d', were estimated for each rabbit. Their estimated values (*SE*) were 0.584 (0.042) hr^{-1} and 0.199 (0.007) ml/g for rabbit 1 and 1.50 (0.09) hr^{-1} and 0.285 (0.057) ml/g for rabbit 2. Since differences in the parameters k_{el} and V_d' were very large between these two rabbits, individual data for these parameters were used below to predict the plasma drug concentration in each rabbit.

Figure 6 compares the observed and predicted plasma concentrations of II after different subcutaneous doses of N^1-acetylsulfamethoxazole (I) in aqueous suspension in each rabbit. In rabbit 1, the plasma concentrations after three doses with a fixed injection volume (1.0 ml) were followed and in rabbit 2, those after two doses with a fixed concentration of I (50 mg/ml) were examined. The predicted plasma concentrations were obtained using one absorption rate constant $j(1)$, which was the same as that used in the mouse experiments, and k_{el} and V_d values for each rabbit are represented by the solid line in Fig. 6. The experimental plasma concentrations, on the whole, appeared to be close to the predicted curves for both rabbits, although slight deviations were occasionally seen. These results suggest that the rough approximation, in which the subcutaneous absorption rate constant in rats is used as a substitute for that in rabbits, is possible.

The above findings in mice and rabbits support the prediction obtained from the local clearance method, that the subcutaneous absorption rate of a drug in aqueous suspension may not vary considerably among the mouse, rat, and rabbit under the same administration conditions and that Eq. 2 may be approximately applicable for the mouse and rabbit as well as the rat.

Problem in Animal Scale-up: The Pitfall of Dose per Kilogram—The action, metabolism, and disposition of new drugs under development are usually examined in various animal species under similar dose per kilogram schedules and species differences are often discussed. This may be adequate in most cases. However, for the case of subcutaneous administration of drugs in aqueous suspension, such discussions should be handled with caution.

The results mentioned above suggest that Eq. 2 originally derived in the rat might be approximately applicable for different animal species (at least for mice and rabbits). This implies that an unexpected phenomenon for early screening tests may occur in animal scale-up: we call

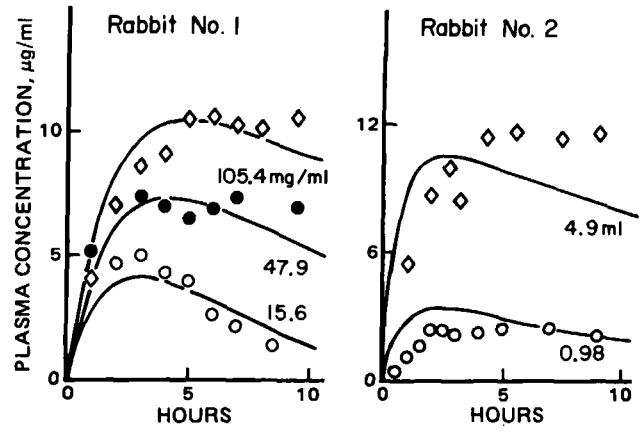

Figure 6—*Observed and predicted plasma concentrations of sulfamethoxazole after subcutaneous administrations of* N^1*-acetylsulfamethoxazole aqueous suspensions in rabbits. The predicted value (solid line) was obtained using the following parameters:* k_{el} *and* V_d, *0.584* hr^{-1} *and 789 ml (Rabbit No. 1,* V_0 *= 1.0 ml), 1.50* hr^{-1} *and 770 ml (Rabbit No. 2,* C_0 *= 50 mg/ml); j(1) (at* C_0 *= 5 mg/ml and* V_0 *= 0.5 ml in rats), 0.17* hr^{-1}.

this the pitfall of dose per kilogram. This pitfall is concretely demonstrated by the following example. Let us consider a case in which the same dose per kilogram of a drug in aqueous suspension is administered subcutaneously to three animal species with different body weights: the mouse, rat, and rabbit. In this case, the amount of drug administered (W_0) must differ among these animal species. Table II shows an example of such an administration schedule. Here, the drug concentration C_0 in the suspension or the injection volume V_0 for each animal is appropriately modified to fix the dose per kilogram. As estimated from Eq. 2, the subcutaneous absorption rate constants in these three animal species under given administration conditions differ from each other. The ratio of the rate constant in the mouse or rabbit to that in the rat, j/j(rat), which can be estimated from Eq. 2 using $g = -0.66$ and $h = -0.32$, are also listed in Table II. Comparisons of these ratios show that the j value in the mouse is about sevenfold as large as that in the rabbit.

The validity of the above predictions was confirmed experimentally using the controlled aqueous suspension of another compound *o*-aminoazotoluene, which has a measured subcutaneous absorption rate constant for rats (2). The absorption rate constants (j) in the mouse and the rabbit after subcutaneous administration of this test suspension under the conditions (C_0 and V_0) shown in Table II can be readily predicted from the j/j(rat) ratios. Then the absorbed fractions (W_{ab}/W_0) of *o*-aminoazotoluene in the mouse and the rabbit at any time (t) are calculated from their individual j values using the equation $W_{ab}/W_0 = 1 - (1 - jt)^3$. The calculation showed that 60–70% of the dose is absorbed at 7.5 hr after administration in the mouse but only ~10% in the rabbit (Table III). Experimental results for the mouse and the rabbit agreed well with the calculated values, as shown in Table III. This ascertains the validity of the predictions in Table II.

The results mentioned above strongly suggest that the absorbed amount of the drug per kilogram for a period after the subcutaneous administration of the drug in aqueous suspension differs considerably among animal species when the same dose per kilogram is given to them: the rate of bioavailability decreases with animal scale-up. This means that the plasma drug level in large animal species may not be as high as expected from the data in small animal species if the elimination rate constant and the apparent volume of distribution per kilogram for the former species are not much smaller than those for the latter. Such a phenomenon may emerge more significantly and seriously for drugs with a slower absorption rate, due to a lower water solubility, for example. This can be understood from the simulation study in our previous report (3).

Accordingly, in the case where species-specific differences in the drug action and disposition are discussed based on the data generated by subcutaneous administration of a drug in aqueous suspension, attention should be paid to the difference in the rate of bioavailability among the species. And it should be noted that the phenomenon called the "pitfall of dose per kilogram" may occur not only for the system presented here but also for drug administration in other heterogeneous dosage forms or for injections into tissues other than the subcutis (e.g., intramuscular administrations). In addition, caution should be exercised against this

pitfall when extrapolating the data from small animal species to humans.

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Determination of Salicylamide and Five Metabolites in Biological Fluids by High-Performance Liquid Chromatography

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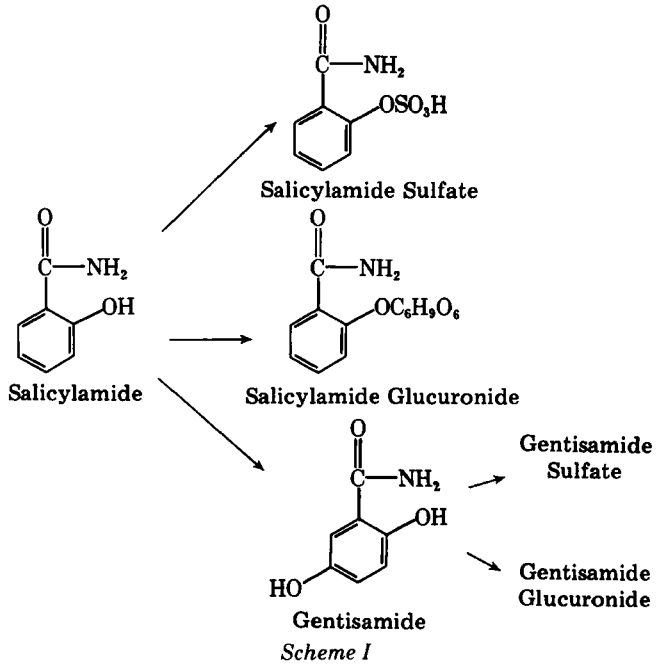
Abstract □ Two high-performance liquid chromatographic (HPLC) assay procedures were developed for the determination of salicylamide and its metabolites in serum, urine, and saliva. One method involves reverse-phase ion-pair chromatography and UV detection, and is used to determine salicylamide, salicylamide glucuronide, and salicylamide sulfate. The other method, with a different mobile phase and without the *ion-pairing reagent, is used to* determine gentisamide (the hydroxylated metabolite of salicylamide), gentisamide glucuronide, and gentisamide sulfate. The assays are performed by direct injection of the sample after protein precipitation with ethanol containing the internal standard. Increased sensitivity for the determination of low concentrations of salicylamide is obtained by organic extraction of this drug from serum or saliva. Calibration curves for the conjugates of salicylamide and gentisamide were obtained, in the absence of authentic standards, by partial enzymatic hydrolysis, using the decrease of the conjugate peaks and the concomitant increase of free salicylamide or gentisamide concentrations to determine peak area ratio–concentration relationships. Application of the HPLC assay procedures to the determination of salicylamide excretion products in the urine of three normal human subjects resulted in 98.6% (range: 97.1–100.1%) recovery of a 1-g oral dose of the drug. All five metabolites of salicylamide were found in urine, but only salicylamide glucuronide, salicylamide sulfate, and gentisamide glucuronide were found consistently and in appreciable quantities. Salicylamide and all of its metabolites except gentisamide sulfate were found in human and rat serum, and unconjugated salicylamide as well as gentisamide were found in human saliva.

Keyphrases □ High-performance liquid chromatography—assay for salicylamide and metabolites in biological fluids, drug conjugate calibration curves in the absence of authentic standards □ Salicylamide—determination in biological fluids, high-performance liquid chromatography □ Metabolites—salicylamide, determination in biological fluids, high-performance liquid chromatography

Salicylamide has analgesic, antipyretic, and hypnotic activities (1–3) but its clinical effectiveness is limited (4–6) due to extensive presystemic biotransformation after oral administration (7, 8). The drug is, however, a valuable research tool for the exploration of drug conjugation reactions (9, 10), drug absorption (11–14) and metabolism interactions (15, 16), route of administration effects on drug disposition (17, 18), effects of disease on drug disposition (19–21), drug concentration–effect relationships (22), product inhibition (23), fetal development (24), and the clinical assessment of metabolic immaturity and disorders (25–27).

Salicylamide is eliminated almost entirely by biotransformation (Scheme I). Many assay methods are available for the determination of this drug in biological fluids (9, 14, 22, 28–36), but none of these provide for the direct determinations (*i.e.*, without prior hydrolysis) of salicylamide conjugates. Apparently only an indirect colorimetric method (9) and a qualitative TLC method (37) have been used for the determination of gentisamide, the hydroxylated metabolite of salicylamide. To facilitate future pharmacokinetic studies with salicylamide, we have developed high-performance liquid chromatographic (HPLC) procedures for the direct determination of salicylamide, gentisamide, and their glucuronide and sulfate



Scheme I

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