Studies on the Absorption of Practically Water-Insoluble Drugs following Injection V: Subcutaneous Absorption in Rats from Solutions in Water Immiscible Oils

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Abstract □ To elucidate the kinetics and mechanisms of subcutaneous absorption of practically water-insoluble drugs in oily solutions, the absorption behaviors of select azo dyes and other prototype agents were investigated by a local clearance method in the dorsum in intact rats. The absorption of the drug components appeared to be first-order. The first-order rate constant (k) was inversely proportional to the cube root of the injection volume. In more limited studies, essentially the same behavior was observed in the rat abdomen, and the difference in k between the dorsal and abdominal injections was slight. The comparison of k of a given compound from different oily vehicles showed that k was governed predominantly by the distribution coefficient (K) between the oily vehicle and the aqueous subcutaneous medium and depended little on the viscosity of the vehicle. This distributioning relationship was shown through correlation of the rate constants with *in vitro* distribution coefficients. A plot of log k *versus* log K for all the compounds tested was linear with a slope of ~−0.7. This linear relationship allows adequate prediction of absorption rates of other drugs from oily vehicles. The observed subcutaneous absorption rates and behaviors are compared with previous results involving the intramuscular route.

Keyphrases □ Absorption, subcutaneous—from solutions in water immiscible oils, rats □ Water-insoluble drugs—subcutaneous absorption from solutions in water immiscible oils, rats □ Drug clearance—local clearance method following subcutaneous injection of practically water-insoluble drugs, drug absorption kinetics from oily solution, rats.

A previous study (1) investigated the intramuscular absorption characteristics of practically water-insoluble drugs in oily solutions in rats and considered the major factors governing the absorption kinetics. In parenteral drug administration, the subcutaneous route is as useful and important as the intramuscular route for early screening and preclinical testing of drugs in laboratory animals. The extensiveness of the subcutaneous tissue and its porosity and loose attachment allow injections of larger volumes than can be easily given intramuscularly. Thus, subcutaneous administration is applicable for dose–response experiments and multiple dosing experiments in small animals.

Since the publication of Schou's review (2) of drug absorption from subcutaneous connective tissue, there has been an increasing interest in quantitatively evaluating the subcutaneous absorption rate of drugs (3–6). However, very little work has been done on the absorption characteristics of marginally water-soluble drugs relative to highly water-soluble drugs in aqueous solution. The absorption rate and the resulting pharmacological responses of hydrophobic drugs are dependent primarily on the dosage form or physicochemical state of the preparation, and thus, it is very important to clarify the relationship of the kinetics of release of such drugs to the physical form in which they were administered.

The present study was undertaken to clarify the absorption mechanisms and kinetics attending subcutaneous injection of practically water-insoluble drugs in oily solutions and to compare them with intramuscular injection. Several azo compounds and testosterone were used as model drugs and a variety of water-immiscible oils were used as model vehicles. Subcutaneous absorption rates of these model drugs were measured in intact rats by a local clearance method first reported by Secher-Hansen *et al.* (3). Rates were compared across diverse experimental systems and conditions to evaluate the contribution of physicochemical factors to the kinetic process.

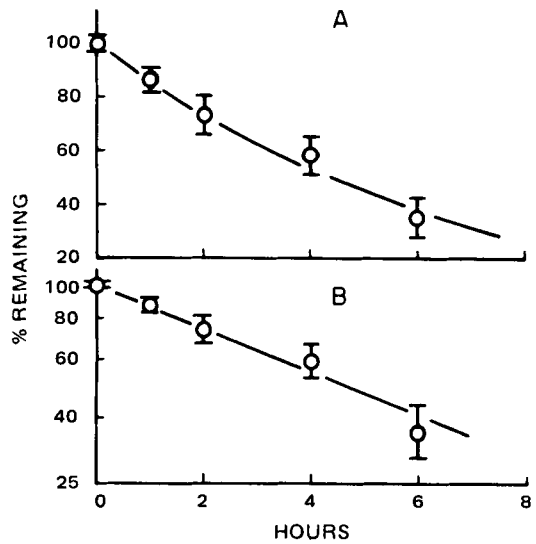

Figure 1—*Absorption time course of* p-*hydroxyazobenzene in sesame oil solution after subcutaneous injection into the intact rat. Initial drug concentration (C_0), 1 mg/ml; injection volume (V_0), 0.5 ml. Each data point represents the mean of four or five experiments and the vertical bar indicates the standard deviation.*

EXPERIMENTAL

Materials—Azo dyes (*p*-hydroxyazobenzene[1], *p*-aminoazobenzene[2], *o*-aminoazotoluene[3], 1-phenylazo-2-naphthylamine[2]), and testosterone[2] were selected as model compounds. These compounds are unionized under physiological conditions and are the same as used in a previous study (1). Sesame oil[4], medium-chain (C_8–C_{12}) triglycerides[5], isopropyl myristate[6], diethyl sebacate[6], and simethicone[7] were selected for their wide ranging viscosities and solubilizing powers. Other than simethicone, the oils were the same as used previously (1). All other chemicals used in this study were of analytical or reagent grade.

Preparation of Test Solutions—Test injection solutions for the absorption studies were prepared by dissolving the drugs in the oily media and then clarifying the media by passing the solutions through membrane filters (1). All test solutions of the azo dyes and testosterone were ascer-

[1] Eastman Kodak Co., New York.
[2] Tokyo Kasei Kogyo Co., Ltd., Japan.
[3] Ishizu Pharmaceutical Co., Ltd., Japan.
[4] Maruishi Pharmaceutical Co., Japan.
[5] Miglyol 812, Chemische Werke Witten, West Germany.
[6] Nikko Chemicals Co., Japan.
[7] KF 96 (20), Shinetsu Kagaku Kogyo Co., Japan.

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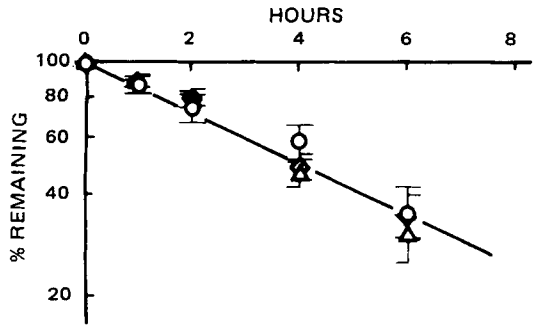


Figure 2—*Effect of initial concentration (C_0) on the subcutaneous absorption of* p-*hydroxyazobenzene in sesame oil (V_0 = 0.5 ml). Each data point represents the mean of at least three experiments and the vertical bar indicates the standard deviation. Key: (C_0) ⊖, 1 mg/ml; ⏃, 5 mg/ml; ◇, 20 mg/ml.*

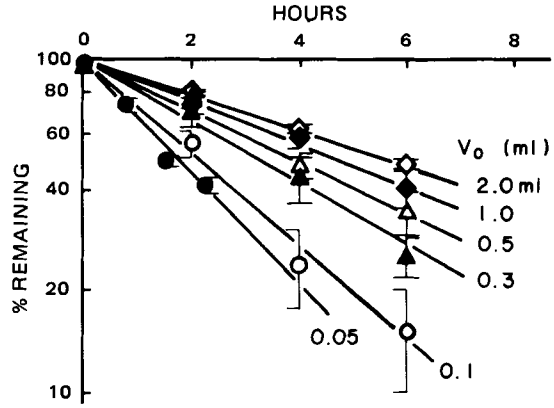


Figure 3—*Effect of injection volume (V_0) on the subcutaneous absorption of* p-*hydroxyazobenzene in sesame oil (20 mg/ml). Each data point represents the mean of at least three experiments and the vertical bar indicates the standard deviation.*

tained to be chemically and physically stable over the experimental period.

Procedure for Subcutaneous Absorption—Male Wistar albino rats (260–280 g) were used in all absorption experiments. Prior to an experiment, the hair in the center region (area, 5 × 5 cm^2) of the dorsum (or abdomen) of the rat was removed with an electric clipper[8]. With the rats under light anesthesia with ether, a given volume of test solution was injected into the subcutaneous space near the center of the shorn area using a calibrated syringe connected to a 0.50 × 25-mm needle[9] at an injection speed between 0.04 and 0.4 ml/sec. Immediately after withdrawal of the needle, a rapidly drying adhesive[10] was applied to the insertion site to prevent leakage. During an absorption experiment, each rat was housed in a cage and allowed freedom of movement and easy access to water and food. At various intervals postinjection, each rat was decapitated and bled, and the tissues around the injection site containing the oily depot were excised as completely as possible and then minced. Drug remaining at the injection site was extracted from the minced tissues with 20 ml of ethyl acetate, and the amount of drug was determined. Most of the test compounds recovered from the tissue were within the oily deposits and there appeared to be little drug recovered from the surrounding tissues. Decomposition and metabolism of the model compounds at the injection site were presumed to be negligible; thus, their clearance in the absorption experiments is mainly attributed to removal by blood and lymphatic flows. Three to five rats were used for each time interval in all the absorption experiments.

Measurement of Absolute Viscosity—The kinematic viscosity of each oily solvent was determined with a viscometer[11] and converted into the absolute viscosity using its density, which was measured with a calibrated flask[12]. These determinations were done at 37°.

Determination of the Distribution Coefficient—The distribution coefficient (K) of a model compound between an oily solvent and 0.9% (w/v) NaCl in water (saline) was determined by equilibrating an oily solution with saline and computing K from the equilibrium saline concentration and the initial concentration in the oil phase (1).

Analytical Method—In the absorption experiments extracts containing the azo dyes were adequately diluted with ethyl acetate and analyzed colorimetrically (1). Extracted testosterone was assayed by a gas chromatographic method (1). Three-milliliter samples were shaken with 0.6 g of Al_2O_3–silica gel (1:1) to remove interfering substances. The mixture was centrifuged for 5 min at 3000 rpm and 1 ml of the supernatant liquid was mixed with 0.1 ml of the internal standard (3β-acetoxy-5α-androstan) in ethyl acetate. Two microliters of these solutions was injected using the same instrument settings reported previously (1). For determination of the distribution coefficients, test compounds in saline equilibrated with oily solutions of known initial concentration were assayed by direct spectrophotometric methods (1).

RESULTS

Time Course of Drug Absorption and Effect of Initial Drug Concentration—It was reported (5) that benzyl alcohol, which is relatively hydrophobic, was absorbed from an aqueous medium in the rat subcutaneous region according to first-order kinetics. However, the kinetic process for subcutaneous absorption of practically water-insoluble drugs from oily solutions has not been explored.

The time course of subcutaneous absorption in a typical experiment is illustrated in Fig. 1, which shows the profile for *p*-hydroxyazobenzene absorbed from a sesame oil solution. The initial drug concentration (C_0) and the injection volume (V_0) are given in the legend. The fraction (percent) of *p*-hydroxyazobenzene remaining at the injection site was plotted against time on arithmetic and logarithmic scales. The linear relationship shown in Fig. 1B indicates this compound is absorbed according to first-order kinetics. To verify this, the effect of the initial drug concentration (C_0) on the absorption rate was examined. Figure 2 shows the absorption time courses of *p*-hydroxyazobenzene from solutions initially of 1, 5, and 20 mg/ml on a semilogarithmic scale. All three profiles are approximately linear with the same slope. Similar results were obtained with other compounds such as *p*-aminoazobenzene and *o*-aminoazotoluene. These findings suggest that subcutaneous absorption of practically water-insoluble and unionized drugs from oily solutions behaves as a first-order process.

Effect of Injection Volume—It has been noted that the injection volume of aqueous (7, 8) or oily (9) solutions might be an important factor influencing intramuscular absorption rates of drugs. A previous report (1) revealed the quantitative relationship between the absorption rate constant and the injection volume for intramuscular administration of practically water-insoluble drugs administered as oily solutions. However, for subcutaneous administration, the effect of the injection volume has not always been clear (10, 11).

Figure 3 compares the absorption time courses of *p*-hydroxyazobenzene injected in different volumes on a semilogarithmic scale. Each absorption profile is linear and the slopes decrease with increased injection volume. Figure 4 demonstrates the relation between the observed first-

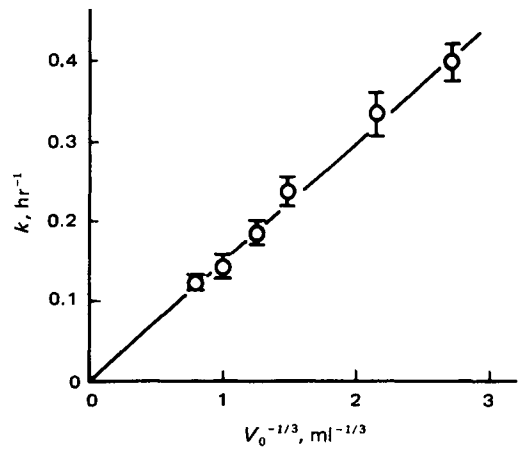


Figure 4—*Relationship between the first-order absorption rate constant (k) and injection volume (V_0). The values of* k *were estimated from the data shown in Fig. 3 by the least-squares method and are plotted together with their standard errors.*

[8] Thrive SNB-25, Daito Electric Mfg. Co., Japan.
[9] Terumo Co., Ltd., Japan.
[10] Aron Alpha, Toa Gousei Kagaku Co., Ltd., Japan.
[11] Ubbelohde, Kaburagi Kagaku Kikai Kogyo Co., Japan.
[12] Cassia, Shibota Kagaku Kikai Kogyo Co., Japan.

Table I—Values of Absolute Viscosity of Oil (η), Distribution Coefficient (K), and First-Order Absorption Rate Constant (k)

Oil	η(cps)	*p*-Hydroxyazobenzene		*p*-Aminoazobenzene		*o*-Aminoazotoluene	
		K[a]	k, hr^{-1} [b]	K[a]	k, hr^{-1} [b]	K[a]	k, hr^{-1} [b]
Simethicone	16.0	17	3.00 (0.41)	29	1.32 (0.17)	310	0.43 (0.04)
Isopropyl myristate–simethicone (1:9, v/v)	13.2	54	1.66 (0.12)	—	—	—	—
Isopropyl myristate–simethicone (2:8, v/v)	10.7	160	0.98 (0.08)	170	0.70 (0.06)	1700	0.21 (0.02)
Isopropyl myristate–simethicone (4:6, v/v)	7.6	510	0.30 (0.01)	550	0.34 (0.03)	5400	0.13 (0.02)
Sesame oil	35	1300	0.21 (0.01)	1200	0.12 (0.009)	13400	0.034 (0.004)
Isopropyl myristate	3.6	2900	0.13 (0.01)	1700	0.11 (0.008)	20000	0.023 (0.001)
Medium chain triglycerides	15	3500	0.084 (0.007)	2900	0.061 (0.003)	27000	0.017 (0.002)
Diethyl sebacate	3.9	15000	0.029 (0.003)	9900	0.030 (0.003)	75000	0.011 (0.001)

[a] Distribution coefficient; the ratio of the drug concentration in oil to that in 0.9% NaCl aqueous solution (saline) at equilibrium (37°). [b] Estimated by the least-squares method and given with the standard error (SE) in parentheses.

order absorption rate constant (k) and injection volume (V_0). It was evident from this figure that k was inversely proportional to the cube root of V_0.

Comparison of Absorption Rates between Dorsal and Abdominal Subcutaneous Injections—A significant difference in the absorption half-life of ^{131}I-labeled insulin injected subcutaneously into the human arm and thigh was noted (12). Given this difference, and considering the fact that subcutaneous drug injections to animals are not always limited to the dorsal site, the abdomen was selected as a second subcutaneous injection site to draw site-to-site comparisons. Drug absorption time courses from this site with different injection volumes were evaluated, and again first-order absorption rate constants were obtained from semilogarithmic plots. The values are compared in Fig. 5 with those obtained for the dorsal injections. The relationship between the rate constant of absorption and the volume of material injected found by dorsal subcutaneous injection also held for the subcutaneous abdominal injections. Remarkably, differences in the absorption rate constants between these two injection sites were negligible. These findings showed that, at least with these substances and under these experimental conditions, absorption behaviors of the two sites are virtually identical.

Comparison of Absorption Rates from Various Oily Solvents—Up to this point, sesame oil was used as the oily solvent since it is commonly used as a parenteral solvent. To clarify possible influences of the oily medium on absorption kinetics, the absorption time courses from different oily vehicles were evaluated. Figure 6 shows absorption time profiles of *p*-hydroxyazobenzene from six oily vehicles, diethyl sebacate, medium chain triglycerides[5], isopropyl myristate, sesame oil, and two isopropyl myristate–simethicone systems (40:60 and 20:80, v/v), all administered at the same volume. The semilogarithmic plots are linear but each has a different slope. Similar results were obtained for *p*-aminoazobenzene and *o*-aminoazotoluene. The first-order absorption rate constants obtained from these experiments are summarized in Table I along with the viscosities (η) of the oil solvents and the distribution coefficients (K, oily vehicle/saline). Clearly, the distribution coefficient is the predominant factor controlling the absorption rates from these oily vehicles.

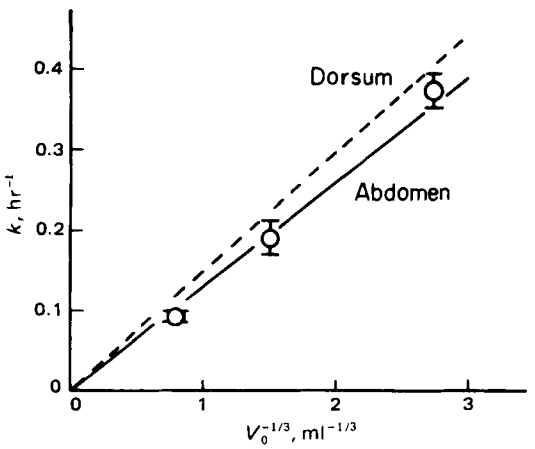


Figure 5—*Comparison of the subcutaneous absorption rate constants (k) of p-hydroxyazobenzene in sesame oil at various injection volumes (V_0) between injection sites on the dorsum and abdomen of rat (C_0, 5 mg/ml). The k values were estimated by the least-squares method and plotted together with their standard errors. Key: (—○—) abdomen; (- - -) dorsum (same as shown in Fig. 4).*

DISCUSSION

Shape of Depot and Drug Absorption Kinetics—It is observed that when a given volume of water-immiscible oil is injected subcutaneously, the major part of it is confined to the injection site by the connective tissues and possibly other surrounding tissues. Also the depot formed in such a manner tends to take the shape of a flattened oblate or prolate spheroid. These observations are similar to those described previously (13), where w/o emulsions containing radioactive substances were used to define the local distribution. The shape of the oily depot, which determines the surface area of the oily phase exposed to the body fluids, is thought to be a critical factor affecting drug absorption. The geometry of the oily depot may depend on the injection volume, the fluidity of the oily solution, hydrodynamic factors such as the injection speed, the available space at the injection site, and/or body movements. The deposit configuration is undoubtedly time-dependent. Visual observation confirmed geometrical change of the depots caused by spreading. These were initially abrupt but became very slight after a short time. Water-immiscible oils such as sesame oil apparently disappear much more slowly from the subcutaneous site than do the drug components they contain (14). A previous study (1) showed that sesame oil was also very slowly absorbed from the m. gastrocnemius of the rat during the first several hours after injection. Therefore, it seems reasonable to regard the surface area of the oily depot as a constant during the absorption process except for a short period immediately after injection. The state of the oily depots and the first-order absorption characteristic of the rate process strongly suggest that the absorption behavior of drugs in oily solutions deposited subcutaneously is very similar to that of the intramuscular route (1). Therefore, the kinetic treatment previously proposed for intramuscular absorption (1) seems equally applicable to the subcutaneous situation.

The kinetics of absorption from an intramuscular, oil deposit were developed for a physical model in which mass transfer was strictly controlled by diffusion through the interface of the depot with the tissue.

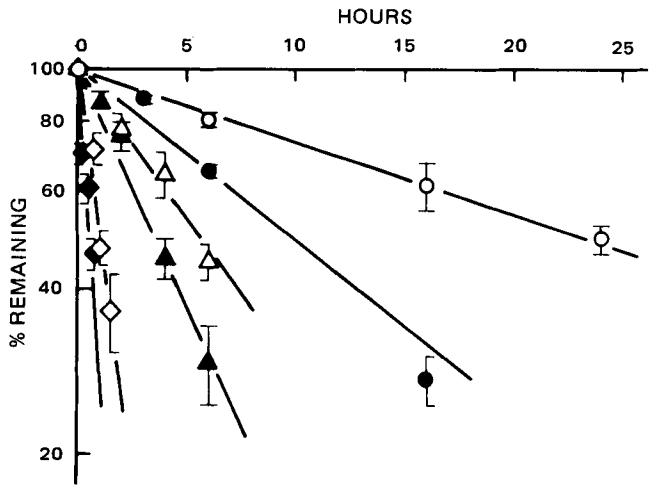


Figure 6—*Time courses of the subcutaneous absorption of p-hydroxyazobenzene from various oily vehicles (C_0, 0.5–5 mg/ml; V_0, 0.5 ml). Each data point represents the mean of at least three experiments and the vertical bar indicates the standard deviation. Key: (○) diethyl sebacate; (●) medium chain triglycerides; (△) isopropyl myristate; (▲) sesame oil; (◇) isopropyl myristate–simethicone (40:60, v/v); (◆) isopropyl myristate–simethicone (20:80, v/v).*

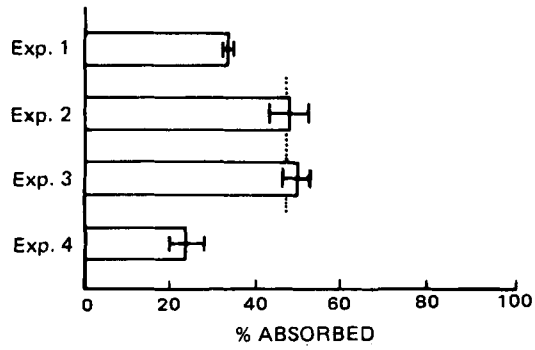


Figure 7—*Comparison of the subcutaneous absorption of* p-*aminoazobenzene among various injection methods at a constant dose. The dotted line shows the value for Exps. 2 and 3 predicted from the results of Exp. 1. Each data point shows the mean of at least four experiments. Key: Exp. 1, 4 mg/ml–0.5 ml; Exp. 2, 20 mg/ml–0.1 ml; Exp. 3, 4 mg/ml–0.1 ml, 5 injections at different points; Exp. 4, 4 mg/ml–0.5 ml (o/w emulsion).*

The interface was seen as a series barrier, possibly with significant diffusional resistances on each side of the interface, *i.e.*, in the boundary layer in the depot and in the tissue immediately in contact with the depot. Mass transfer coefficients (permeability coefficients) k_o and k_a describe the diffusive velocities in the depot's boundary and the tissue, respectively. It is implicit that the depot acts as a well-stirred phase everywhere except at its interface. It was also assumed in developing the model that: (*a*) over the full course of drug release the depot's interfaces and surface area remain essentially constant; (*b*) the distribution coefficient of a drug between the depot and the tissue fluids strictly governs the relative concentrations on each side of the interface; (*c*) the depot acts as a reservoir for the drug; (*d*) the body of the animal acts as a diffusional sink; and (*e*) there is no assimilation of the oily vehicle by the body over the course of drug release. With these assumptions the release process becomes a first-order depletion of a reservoir into a sink by means of diffusion across an oil–water boundary. The following equations were derived for the quasi-steady-state (period after establishment of the gradient across the boundary):

$$\ln (W/W_0) = -kt \qquad \text{(Eq. 1)}$$

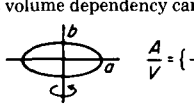
and

$$k = \frac{k_o A}{V_0\left(1 + \frac{k_o}{k_a} K_i\right)} \qquad \text{(Eq. 2)}$$

where W_0 and W represent the dose and the remaining amount of the drug at any time t, respectively; V_0 and A are the injection volume and the surface area of the oily depot, respectively; k_o and k_a are the diffusive velocities of the drug in the oily and aqueous tissue boundaries, respectively; K_i is the distribution coefficient at the interface; and k is the first-order absorption rate constant (1). Equation 1 describes the expected first-order behavior, while Eq. 2 characterizes the rate constant for the series barrier situation.

Comparison of Absorption Rates among Various Injection Methods—Equation 2 contains the ratio, A/V_0, which describes the geometrical aspects of the absorption process. The dependency of drug-release rate on the injected volume centers around the interdependency of area and volume. When the shape of the injected mass is spherical or near spherical, the area–volume ratio is simply $3/r$ or three times the reciprocal radius of the equivalent sphere. This is directly proportional to the reciprocal cube root of the volume[13]. Therefore, for an injection acting as a near spherical (or spheroidal) mass, it is expected that the absorption rate constant of an incorporated drug will be proportional to the $V_0^{-1/3}$, a dependency on volume which was closely followed in the subcutaneous administrations as evidenced in Figs. 4 and 5.

[13] When the injected mass takes the shape of a spheroid or a near spheroid and retains the same shape (dimensions) for alterations in injection volume, the same cube root volume dependency can also be derived mathematically as follows:

Spheroid 

$$\frac{A}{V} = \left\{\frac{3}{2\beta}\left(\frac{3}{4\pi\beta}\right)^{-\frac{1}{3}}\left(1 + \frac{\beta^2}{2\sqrt{1-\beta^2}} \log \frac{1+\sqrt{1-\beta^2}}{1-\sqrt{1-\beta^2}}\right)\right\} V^{-\frac{1}{3}}$$

$(a > b,\ b/a = \beta)$

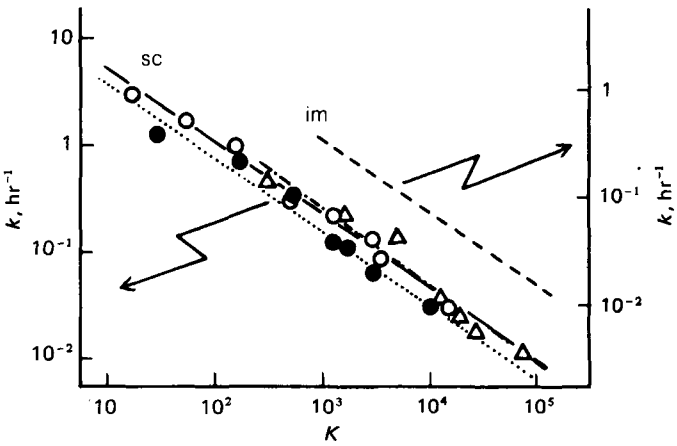


Figure 8—*Plots of* k versus K *on log–log scale for three model compounds. Key: (—○—) p-hydroxyazobenzene; (·····●·····) p-aminoazobenzene; (-·-▵-·-) o-aminoazotoluene; (sc) data for subcutaneous injection at* C_0 = *0.5–5 mg/ml and* V_0 = *0.5 ml; (im) data for intramuscular injection m. gastrocnemius) at* C_0 = *5 mg/ml and* V_0 = *0.05 ml reported previously (1).*

The preceding discussion indicates that the absorption rate constant is dependent on injection volume and not on the initial drug concentration. This means that the absorption rate can be manipulated by the injection method even if the drug, solvent, and dose are fixed. This was demonstrated experimentally using *p*-aminoazobenzene in sesame oil solution. Figure 7 shows the percentage of this drug absorbed 3 hr after injection of a fixed dose, 2 mg/rat, by four differing injection protocols. In Exp. 1 a single, 0.5-ml injection of solution containing 4 mg/ml was given; in Exp. 2 a single, 0.1-ml injection of solution containing 20 mg/ml was given; in Exp. 3 five injections were given at different sites, each with 0.1 ml of solution containing 4 mg/ml; and in Exp. 4 a single, 0.5-ml injection of an o/w emulsion [equal volumes of sesame oil containing 8 mg/ml of *p*-aminoazobenzene and saline containing 2% (w/v) polysorbate 80; emulsion particle diameter, 2–10 μm] was given. The theory was tested as follows. The first-order absorption rate constant (k_1) was determined in Exp. 1. Using this k_1 value, the value of the percentage absorbed for Exp. 2 can be estimated by the following equation derived from Eq. 1 and the cube root volume dependency:

$$\text{Percent absorbed} = 100\{1 - \exp[-k_1(V_1/V_2)^{1/3}t]\} \qquad \text{(Eq. 3)}$$

The dotted line in Fig. 7 shows the value estimated by substitution of the calculated value, 0.12 (hr^{-1}), for k_1, of 5 for V_1/V_2 (the actual injection volume ratio), and of 3 (hr) for t in Eq. 3. In Exp. 3 the absorption at each injection site will proceed independently and to the same extent; thus, the dotted line also shows the estimate for the third case as the injection volume at each injection point is equal to that in Exp. 2. The value calculated from the results of Exp. 1 agrees well with the observed values of Exps. 2 and 3. Accordingly, it is concluded that the reciprocal cube root volume dependency expressed in Eq. 3 will be generally applicable. These findings imply that faster absorption occurs when the injection volume per one site is minimized either by increasing the number of injections or by using more concentrated solutions.

Addition of surface-active agents and emulsifiers to injected oily solutions of drugs has been reported to enhance the absorption of the oily vehicle (9) or practically water-insoluble drugs contained therein (15). Recently, emulsion systems have been promoted as novel drug delivery systems for some anticancer agents (16). Figure 7 shows that the absorption of *p*-aminoazobenzene from the emulsion system (Exp. 4) would be slower than that from oily solution (Exp. 1). However, so many new variables are introduced when the administration system is changed to an emulsion that it is impossible to even qualitatively interpret this result.

Correlation among the Absorption Rate Constant, Viscosity, and the Distribution Coefficient—It was shown previously (17) that a certain correlation exists between the viscosity of vaccines (aluminum monostearate paraffin gels) injected subcutaneously into rabbits and guinea pigs and the degree of enhancement and prolongation of the antitoxin levels. However, as is evident from Table I, no distinguishable relationship was observed between the viscosity (η) of the vehicle and the absorption rate constant (k) under our experimental conditions (η, 3.6–35 cps). This difference is not surprising when it is realized that the

Table II—Predicted and Observed Subcutaneous Absorption Rate Constants (k) [a]

Test Compound	M.W.[b]	K[c]	k, hr^{-1} Predicted[d]	k, hr^{-1} Observed (SE)
Testosterone	288	1.30×10^2	0.79	0.64 (0.09)
1-Phenylazo-2-naphthylamine	247	3.82×10^5	0.0035	0.0053 (0.0003)

[a] Sesame oil solution; C_0, 2.5 mg/ml for testosterone and 5 mg/ml for 1-phenylazo-2-naphthylamine; V_0, 0.5 ml. [b] Molecular weight. [c] Distribution coefficient (sesame oil/saline) at 37°. [d] Calculated using the regression equation obtained from all sc data in Fig. 8: $\log k = -0.68 \log K + 1.33$

gelled systems of Coles *et al.* (17) could hardly act as well-stirred phases. Moreover, smaller mass transfer coefficients (k_o) due to the increased viscosity are expected to elevate the role in rate control played by the vehicle's boundary layer.

If the term $(k_o/k_a)K_i$ in Eq. 2 is $\ll 1$, Eq. 2 is approximated by:

$$k = Ak_o/V_0 \qquad \text{(Eq. 4)}$$

This equation signifies that diffusion in the oily phase is the rate limiting process in drug absorption as appears to be the case for the gels of Coles *et al.* (17). However, this relationship does not appear representative of the behavior of the present systems as k, according to Eq. 4, depends upon k_o, which in turn depends on viscosity of the oily vehicle, but not on K_i. On the other hand, when the term $(k_o/k_a)K_i$ is $\gg 1$, which seems very likely due to the large distribution coefficients (K_i) of the drugs between the phases considered here, Eq. 2 is approximated by:

$$k = Ak_a/(V_0K_i) \qquad \text{(Eq. 5)}$$

This equation signifies that k is independent of k_o; that is, independent of the viscosity of the oily vehicle and dependent on K_i. Given the partitioning dependency of the process (Table I), the present results seem to favor the relationship as written in Eq. 5 on two counts. Partitioning and diffusion across a tissue boundary also appear to control rates of intramuscular absorption (1).

Taking the logarithm of both sides of Eq. 5 gives:

$$\log k = \log (Ak_a/V_0) - \log K_i \qquad \text{(Eq. 6)}$$

The term $\log (Ak_a/V_0)$ in this equation is governed by the injection volume and the permeability of the drug in the connective tissues adjacent to the oily depot. According to Eq. 6, a plot of $\log k$ *versus* $\log K_i$ should give a single straight line with a slope of -1 for compounds having a similar tissue permeability as long as the injection volume is maintained constant. Figure 8 shows this plot for the three compounds presented in Table I, all of which have similar molecular weights. In this plot the *in vitro* bulk distribution coefficient (K, oily solvent/saline) is used instead of the *in vivo* distribution coefficient (K_i) which is not measurable. The plot indicates linear relationships over a wide range of K values for all three compounds. The regression equations ($x = \log K$, $y = \log k$) and their correlation coefficient (r) for the individual compounds are as follows: for *p*-hydroxyazobenzene, $y = -0.686x + 1.398$ ($r = -0.994$); for *p*-aminoazobenzene, $y = -0.689x + 1.256$ ($r = -0.982$); for *o*-aminoazotoluene, $y = -0.733x + 1.594$ ($r = -0.974$). From these separate results, the following experimental equation relating the rate constant to the *in vitro* distribution coefficient is evident:

$$\log k = \log (Ak_a/V_0) - \delta \log K \qquad \text{(Eq. 7)}$$

The resultant values for δ are of the order of -0.7, whereas perfect *in vivo–in vitro* distributioning correlation would yield a value of -1.0. This slight deviation might be attributable to fundamental, systematic differences in partitioning sensitivities between the *in vivo* and *in vitro* systems, to a not totally negligible diffusional resistance in the vehicle's boundary layer, which itself precludes exact adherence to Eq. 5, or to the coexistence of another absorption mechanism not accounted for in Eq. 2, *i.e.*, absorption of the drug as the result of assimilation of the injected medium by the body. In any case, the experimental equation can be put in the following, general phenomenological form:

$$k = \gamma/V_0{}^mK^\delta \qquad \text{(Eq. 8)}$$

where γ is a coefficient lumping together all intangible experimental parameters. This relationship seems to have practical value for the estimation of absorption rates of diverse drugs from diverse oily vehicles.

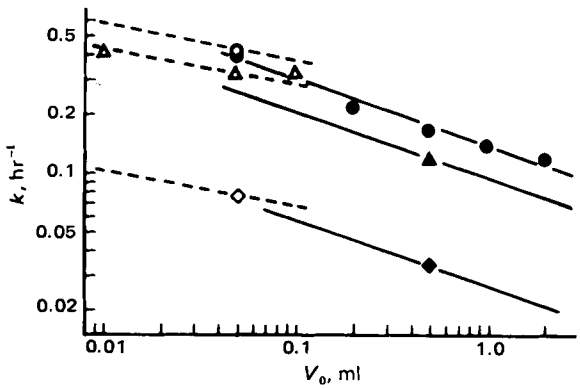


Figure 9—*Comparison of the absorption rate constants (k) at various injection volumes (V_0) between subcutaneous (dorsum) and intramuscular (m. gastrocnemius) injections in intact rats. The straight lines for subcutaneous injection of* p-aminoazobenzene *and* o-aminoazotoluene, *and those for intramuscular injection of* p-hydroxyazobenzene *and* o-aminoazotoluene *are depicted to pass through one data point with a slope of −0.33 and −0.14, respectively. The subcutaneous data are shown elsewhere in this paper and those for intramuscular injection are quoted from a previous work (1). Key: (—●—) (sc), (---○---) (im),* p-hydroxyazobenzene; *(—▲—) (sc), (---△---) (im),* p-aminoazobenzene; *(—◇—) (sc), (---◇---) (im),* o-aminoazotoluene.

Figure 8 also shows with a broken line the corresponding regression line for the intramuscular absorptions of the same three compounds (1). The parallelism of the regression lines suggests a strong similarity in distributioning behavior at the injection's interface between the subcutaneous and intramuscular routes.

Prediction of Absorption Rates of Other Drugs—Figure 8 shows the regression lines of three separate compounds to be essentially inseparable when the injection volume was fixed. Their composite regression equation is: $\log k = -0.68 \log K + 1.33$ ($r = -0.98$). It was anticipated through this colinearity that this relationship might provide a means of estimating absorption rate constants (k) of other drugs simply from the K values determined in the *in vitro* saline system. To examine this possibility, testosterone and 1-phenylazo-2-naphthylamine, compounds with molecular weights similar to the three compounds presented in Fig. 8, were selected as additional test substances. Absorption rate constants predicted from their respective K values and the above regression equation were compared with experimental values. The results appear in Table II. For these compounds, the predicted values agree well with the observed values. This satisfactory result suggests the absorption behaviors of other drugs may be similarly predicted.

Comparison of Absorption Rates between Subcutaneous and Intramuscular Routes in Rats—It was found (12) that no significant difference in the absorption rates of ^{131}I-labeled insulin[14] (insulin suspensions) from subcutaneous and intramuscular tissues exists at either the thigh or arm region in humans. No such comparison between sites exists for a drug presented in oily solution. In the present and a previous (1) report, the quantitative relationships between the absorption rate constant (k) and the injection volume (V_0) for subcutaneous and intramuscular routes has been elucidated in rats. By both routes the value of k was proportional to $V_0{}^m$. However, the experimental m values for subcutaneous (dorsum) and intramuscular (m. gastrocnemius) injections in intact rats are apparently different and are -0.33 and -0.14, respectively.

In Fig. 9, k values in intact rats obtained by the subcutaneous route (dorsum) for the three test compounds at various injection volumes (V_0) are compared with those by the intramuscular route (1). Although the data points available for direct comparison are few in this figure, it appears that the values by the two routes may be quantitatively compatible. If this is true, then a change in volume dependency (m) is apparent as the injected volume goes from the very small volumes given intramuscularly to the relatively large subcutaneous volumes.

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Studies on the Absorption of Practically Water-Insoluble Drugs Following Injection VI: Subcutaneous Absorption from Aqueous Suspensions in Rats

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Abstract □ The absorption characteristics and kinetics of practically water-insoluble drugs following subcutaneous injection of their aqueous suspensions were investigated in intact rats by the local clearance method and compared with those following intramuscular injection reported previously. The plot of the cube root of the residual fraction of the drug in the injection site *versus* time gave a good linear relationship under various experimental conditions. The absorption rate constant (j) increased with decreasing particle size. This increase was remarkable in the region of mean particle diameter <2–3 μm, while it was gradual or slight in the region above this. This phenomenon was explained by the fact that the *in vivo* spreading of particles of more than ~3 μm was still more limited by the network of the fibrous tissues. Between j and the initial drug concentration (C_0) or injection volume (V_0), the practically important relationship $j \propto C_0^g V_0^h$ ($g = -0.66$ and $h = -0.32$) could approximately be derived from the experimental results. Comparison of j values among various compounds with different solubility (C_s') in saline but with similar colloidal properties (particle size distribution and sedimentation volume) showed that a log j *versus* log C_s' plot gave a nearly straight line with a slope of ~0.5. All the results observed for the subcutaneous absorption were similar to those for intramuscular absorption and could reasonably be explained by the kinetic model proposed for intramuscular absorption.

Keyphrases □ Absorption, subcutaneous—from aqueous suspensions of practically water-insoluble drugs, rats □ Water-insoluble drugs—subcutaneous absorption from aqueous suspensions, rats □ Aqueous suspensions—subcutaneous absorption of practically water-insoluble drugs, rats

Although subcutaneous administration of aqueous suspensions is very popular for practically water-insoluble drugs in preclinical animal experiments, little has been studied on their absorption mechanisms and kinetics except for the particle-size effect on pharmacological responses (1). Previously investigated (2, 3) were the absorption behaviors of subcutaneously implanted solid drugs (sphere, disk, and cylindrical shapes) and the absorption kinetic process was clarified. The intramuscular absorption characteristics of practically water-insoluble drugs from aqueous suspension were investigated in detail, and a kinetic equation was proposed for the drug absorption, which was obtained empirically and was very useful (4).

In the present work, similar investigations for the subcutaneous route were done with the local clearance method in intact rats. The purpose of the present study was to clarify the relationship between the absorption rate and physicochemical properties (particle size, initial drug concentration, injection volume, drug solubility, *etc.*), to express this relationship in appropriate mathematical terms, and to compare the results with those for the intramuscular route reported previously (4).

The findings obtained in this study will offer a novel and useful guide for more detailed screening and preclinical testing in laboratory animals of new drugs under development.

EXPERIMENTAL

Materials—Azo dyes (*p*-aminoazobenzene, *p*-hydroxyazobenzene, *o*-aminoazotoluene, and 1-phenylazo-2-naphthylamine), sulfa drugs (N^1-acetylsulfamethoxazole and sulfamethoxazole), and a steroid (betamethasone dipropionate[1]) were used as models for practically water-insoluble drugs. These azo dyes and sulfa drugs were the same as those used in a previous study (4). Betamethasone dipropionate was of medicinal grade and its purity was ascertained to be satisfactory through elementary analysis, melting point measurement, and TLC. Methylcellulose[2] and polysorbate 80[3], used as dispersing agents for preparations, were the same as those reported previously (4). All other chemicals were of analytical or reagent grade.

To compare the spreading area of injected particles, the following standard particles of different sizes were also used: two polystyrene latexes (0.721 and 1.305 μm in mean diameter based on number size distribution)[4], polyvinyltoluene latex (2.956 μm)[4], spores (4.94 μm)[5], di-

[1] Schering Corp., Bloomfield, N.J.
[2] Metolose SM-15, Shinetsu Kagaku Kogyo Co., Ltd., Tokyo.
[3] Kao Atlas Co., Ltd., Tokyo.
[4] The Dow Chemical Co., Indianapolis, Ind.
[5] Coulter Electronics, Ltd., Dunstable.

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