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Studies on the Absorption of practically Water-insoluble Drugs following Injection. II.[1] Intramuscular Absorption from Aqueous Suspensions in Rats

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The absorption characteristics and kinetics of practically water-insoluble drugs following intramuscular injection of their aqueous suspensions were investigated by the local clearance method in the *m. gastrocnemius* of the rat. The plot of the cube root of the residual fraction of the drug in the injection site *versus* time gave a good straight line. Between the absorption rate constant (j) and the initial drug concentration (C_0) or injection volume (V_0), the relationship $j \propto C_0^g V_0^h$ was observed experimentally ($g=-0.55$ and $h=-0.32$). Comparison of j values among various compounds having different solubilities in saline (C_s') but similar colloidal properties (particle size distribution and sedimentation volume) showed that a plot of log j against log C_s' gave a linear relationship with a slope close to 0.5. The absorption rate constant (j) increased with decreasing particle size on condition that the other colloidal properties changed little. This increase was remarkable in the region of mean particle diameter (D_{ss}) less than 2—3 μm, while it was gradual or slight in the region above this. The results could be explained by a kinetic model in which the dissolution (diffusion) process from the particle agglomerate formed by injection was assumed to be a rate-limiting step for the drug absorption. The effect of the type or concentration of the macromolecular dispersing agent was also examined in detail.

Keywords——drug absorption kinetics; intramuscular injection; local clearance method; practically water-insoluble drug; aqueous suspension; initial drug concentration; injection volume; solubility; particle size; suspension vehicle

In a previous paper[1] we discussed the intramuscular absorption behavior of practically water-insoluble drugs from water-immiscible oil solutions. Like oily solutions, aqueous suspensions are one of the most popular and useful parenteral dosage forms for such drugs and are commonly administered intramuscularly, subcutaneously or intraperitoneally to laboratory animals.

A few investigators[2] have described the effect of particle size on plasma drug level following intramuscular injection of aqueous suspensions. However, except for this, very little is known about the intramuscular absorption characteristics and kinetics of practically water-insoluble drugs from aqueous suspensions because of the lack of systematic and detailed investigations.

The present study was undertaken to clarify the above problems. Several azo dyes, two sulfa drugs and a steroid were used as models for practically water-insoluble drugs and the absorption characteristics were examined by the local clearance method in the *m. gastrocnemius* of intact rats. The effect of initial drug concentration, injection volume, drug solubility, particle size and the type or concentration of the macromolecular dispersing agent on the drug absorption are discussed here, as well as the kinetics, on the basis of a possible absorption model.

Experimental

Materials——Azo dyes [*p*-aminoazobenzene (PAAB), *p*-hydroxyazobenzene (PHAB), *o*-aminoazotoluene (OAAT) and 1-phenylazo-2-naphthylamine (PANA)], a steroid [2α,3α-epithio-5α-androstan-17β-ol (epitiostanol, ES)], and sulfa drugs [N^1-acetylsulfamethoxazole (AcMS) and sulfamethoxazole (MS)] were used as

model compounds for practically water-insoluble drugs. These azo dyes and the steroid were the same as reported previously.[1] AcMS and MS were synthesized in our laboratory and were of medicinal grade. Methylcellulose (MC), sodium carboxymethyl cellulose (CMC), acacia (Ar-G) and polysorbate 80 (Ps-80), selected as dispersing agents, were obtained commercially: MC, commercially named Metolose SM-15, Shinetsu Kagaku Kogyo Co., Ltd. (Tokyo); CMC, JP grade, Nissho Iwai Co., Ltd. (Osaka); Ar-G, JP grade, Fujisawa Pharmaceutical Co., Ltd. (Osaka); Ps-80, Kao Atlas Co., Ltd. (Tokyo). They were used without further purification. All other chemicals used were of analytical or reagent grade.

Preparation of Test Suspensions——All suspension vehicles contained 0.9% (w/v) NaCl for isotonization and were used for formulations after being presaturated with the drug to be dispersed then filtered through a Millipore membrane filter HA (Millipore Corporation, Massachusetts) or glass filter G3. Unless otherwise mentioned, a vehicle composed of 0.5% (w/v) MC, 0.005% (w/v) Ps-80 and 0.9% (w/v) NaCl was used because of its good dispersing power and crystal growth retardation characteristic for all the model compounds used. All test suspensions, in order to regulate their particle size, were prepared by fractionation techniques using natural or centrifugal sedimentation. Unless otherwise mentioned, the suspension formulated according to the following controlled preparation method was used as a 'controlled' suspension; it was intended to contain particles of about 4 μm in average diameter. About 300 mg of crystals was micronized and made into a paste in an agate mortar, then dispersed in about 15 ml of the vehicle mentioned above. After excluding the coarse and nonwet particles from this dispersed solution, it was transferred into a sedimentation tube (cross section area, 5 cm^2) and allowed to settle for 2 hr. Next, the upper layer containing unsedimented particles was collected and 20 ml of this was transferred into another sedimentation tube. The particles sedimented by centrifugation at 1000 rpm for 10 min were collected then redispersed in the same fresh vehicle. After adjustment of the drug concentration, it was stored at 25° until use. All suspensions other than the 'controlled' suspension were formulated by similar methods with appropriate modifications.

Procedure of Absorption Experiment——Male Wistar albino rats weighing 250—300 g were used in all absorption experiments. The test suspension (50 μl, unless otherwise mentioned) was injected into the *m. gastrocnemius* of intact rats and the drug absorption properties were examined by the local clearance method. The procedure was identical to that reported in the previous paper.[1] *In vitro* incubation experiments of all the test compounds in aqueous suspension used here with freshly removed muscle tissues demonstrated that the metabolic changes at the injection site were slight enough to be negligible and hence that the absorption experiment adopted did reflect the true absorption phenomena of the systems of interest. The syringeability for all the test suspensions was good for the same needle (25G × 1″, Terumo Co., Ltd., Tokyo) and syringe as used in the absorption experiment.

Solubility (C_s')——An excess amount of sample powder was shaken well at 37° with 0.9% NaCl (saline) or pH 7.25 phosphate buffer (Na_2HPO_4–KH_2PO_4, 1/15 M) isotonized with NaCl until the dissolution was complete. The undissolved crystals were removed by filtration with a glass filter G5 or by centrifugation, and the resulting filtrate or supernatant was analyzed for solubility determinations. The solubilities of PANA and ES, which were too low to be determined accurately, were further checked with the nephelometric method.[3] Except for MS, the solubilities of test compounds in saline were not significantly different from those in pH 7.25 isotonic phosphate buffer.

Dissolution Rate——The sample powder was compressed at 150 kg/cm^2 under a vacuum using a Riken Power model P1OB (Riken Seiki Co., Ltd., Japan) to make a disc 1 cm in diameter and about 1 mm thick. The relative dissolution rate of this disc was measured at 37° using the continuous flow, column-type dissolution apparatus described by Tingstad *et al.*[4] under the following conditions: dissolution chamber, 25 mm Millipore ultrafiltration cell; dissolution fluid, saline; flow rate, 5.90 or 9.86 ml/min. Just before the dissolution test, the disc was kept in contact with a suspension vehicle for 3—5 sec to improve its wettability by the dissolution fluid. The amount dissolved was obtained by comparing the weights of the disc before and after the dissolution test, and the relative dissolution rate (D_{iss}) was represented as the dissolved amount per unit time and surface area of the disc (mg/hr/cm^2). The relative dissolution rate constant (k') was calculated from the equation $k' = D_{iss}/C_s'$.

Density (ρ)——The density of the crystal of each test compound was measured with a Beckman air comparison pycnometer, model 930 (Toshiba Beckman Co., Ltd., Tokyo).

Sedimentation Volume (V_{sed})——The sedimentation volume of the test suspension was measured at 37° in a sedimentation tube of 3.5 or 5.5 mm internal diameter and 20 cm long. The equilibrium value was obtained after the tube had been allowed to stand for 7—10 days.

Particle Size Analysis——The particle size distribution of the test suspension was analyzed with a Coulter counter model TA (Coulter Electronics, Inc., Florida), equipped with a 50 or 100 μm aperture tube. As size calibrators of this instrument, polyvinyltoluene latex of 2.03 μm mean diameter (Dow Chemical Co., Indianapolis) and pollen of 19 μm (Coulter Electronics, Ltd., Brit.) were used for the 50 and 100 μm aperture tubes, respectively. Isoton II (Coulter Electronics, Inc., Florida) or saline, which had been presaturated with the compound to be analyzed and then filtered three times with a Millipore membrane filter GS prior to analysis, was used as the electrolyte solution for the measurement. The data of cumulative volume size

distribution obtained above were plotted on a Rosin-Rammler graph to yield the mean particle diameter (D_{ss}) and distribution constant (n).

Viscosity (η)——The viscosity of the disperse medium was measured at 37° according to the method reported previously.[1]

Analytical Method——(i) Determination of Drug Concentration in Test Suspensions: The concentration of the compound in a test suspension was assayed spectrophotometrically with a Perkin Elmer UV–VIS spectrophotometer (Hitachi Co., Ltd., Tokyo) as follows: for PAAB, PHAB, OAAT, PANA and ES at 375, 349, 389, 450 and 262 nm, respectively, after dilution with EtOH; for MS and AcMS at 267 and 289 nm, respectively, after dilution with H_2O–EtOH (1: 1, v/v).

(ii) Absorption Experiment: The remaining amounts of PAAB, PHAB, OAAT and PANA in the injection site were determined colorimetrically as described previously,[1] and that of ES was analyzed by the GLC method reported previously.[1] AcMS and MS were analyzed colorimetrically after being diazotized in the following manner. A portion of the ethyl acetate extract of AcMS or MS (containing 0—75 μg each) was transferred into a volumetric flask (20 ml) and evaporated to dryness with a flow of N_2 gas. The residue was dissolved in 250 μl of acetone or N,N-dimethylformamide, then 1 ml of 3 N HCl and 0.3 ml of 0.2% (w/v) $NaNO_2$ were added, and mixed well. After this mixture had stood for 5 min, 0.3 ml of 1.0% (w/v) NH_4OSO_2-NH_2 was added and the whole was stirred vigorously for about 5 min. Next, 0.3 ml of 0.2% (w/v) Tsuda's reagent (N-1-naphthyl-N'-diethyl-ethylenediamine oxalate) was added and mixed thoroughly.[5] The resulting coloured mixture was made up to 20 ml with acetone. From 20 to 60 min after the reaction, the optical density was measured at 541 and 544 nm for AcMS and MS, respectively, with a UV-VIS spectrophotometer using acetone as a reference.

(iii) Solubility Measurement: The sample solutions (the filtrate or supernatant) of PAAB, PHAB, OAAT, PANA, AcMS and MS were assayed spectrophotometrically as follows: for PAAB and PHAB at 376 and 348 nm, respectively, after dilution with EtOH; for OAAT and PANA at 379 and 470 nm, respectively, after 10/9-fold dilution with EtOH; for AcMS and MS at 289 and 267 nm, respectively, after dilution with EtOH–H_2O (1: 1, v/v). The sample solution for ES was analyzed by the GLC method reported in the previous paper[1] after extraction with ethyl acetate.

Results and Discussion

State of the Depot and Drug Absorption Kinetics

Our careful observation of the depot formed by intramuscular injection of a dye suspension showed that the remaining suspension particles were confined to the fibrous or membraneous tissues between muscle fibers and formed a very loose agglomerate, while their surrounding aqueous vehicle almost disappeared by penetration into the tissues adjacent to the depot or by absorption. Shaffer,[6] using a radiopaque substance, first observed in detail the depot of an intramuscularly injected suspension and reported that the suspension particles spread to their final locations almost as soon as the injection procedure was completed. The tissue fluid colored by the dissolution of a dye was largely limited to the region near the surface of the particles or their agglomerate except for a short time period immediately after the injection. This situation remained unchanged as these particulates became smaller with time due to their dissolution.

From the images of the depot mentioned above, the absorption model illustrated in Fig. 1 may be proposed. A drug molecule must first become dissolved by diffusion in the body fluids appearing in the intercellular space of muscle fibers or connective tissues, and then pass through cell membranes of vascular tissues, *etc.*, until it enters the blood or lymph stream. In this model, the first dissolution (or diffusion) process is assumed to be rate-limiting in the whole transport process of absorption, and direct transport by fine particles into the vascular systems is not involved.

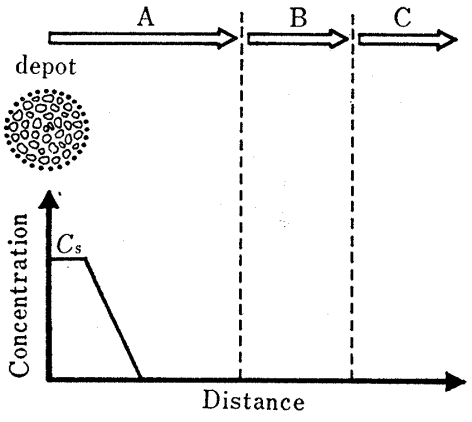

Fig. 1. Model for Drug Absorption from Aqueous Suspension following Intramuscular Injection

A: intercellular space of muscle fiber or connective tissue.
B: cell membranes of vascular tissues, *etc.*
C: blood or lymph.

For the drug, the major part of which remains as a solid form at the injection site, the kinetic process for absorption, similar to that for dissolution, can be written as:

$$dW/dt = -kC_sS \quad \text{(Eq. 1)}$$

where W and C_s are the remaining amount of the drug at any time t and the solubility of the drug in the body fluid at the injection site (*in vivo* solubility), respectively, and S represents the effective surface area for dissolution of the particle agglomerate. The parameter k is defined as a term related to the *in vivo* dissolution rate constant which depends on the diffusibility of the drug molecule and the flow or agitation of the body fluids in the injection site. Using the density of the suspension particle, ρ, the following relationship between S and W is introduced:

$$S = \varepsilon(W/\rho)^{2/3} \quad \text{(Eq. 2)}$$

where ε is a constant which is determined by the degree of agglomeration of the suspension particles and the shape of the resulting agglomerate. The parameter ε is an important factor, since it determines the effective area for *in vivo* dissolution.

For a suspension with monodispersed spherical particles, the magnitude of ε is expected theoretically to fall within the following range:

$$(36\pi)^{1/3} < \varepsilon < (36\pi N_0)^{1/3}$$

where N_0 is the number of particles injected. The left-hand term of this inequality relationship corresponds to the case in which the suspension particles agglomerate so tightly that only one particle seems to have been injected, while the right-hand term represents the case in which each particle (diameter, D) is dispersed effectively enough to become dissolved independently. For the latter case, the particle size reflects most strongly the parameter ε, since $N_0^{1/3}=(6W_0/\rho\pi)^{1/3}/D$ where W_0 is the dose. If the parameter ε takes either of the above limit values, the mathematical treatment of Eq. 1 becomes easy, but this is not the case for usual injections. Therefore, the theoretical evaluation of the parameter ε may be difficult for common cases. The parameter ε is controlled by many factors such as (1) particle size (D) and dispersibility of the suspension particles (that is, the cohesive property of particles), (2) initial volume concentration (C_0/ρ) and injection volume (V_0), (3) hydrodynamic factors (injection speed and pressure) and (4) histological and physiological states at the injection site. A good controlled absorption experiment can eliminate the contribution of factors (3) and (4) to ε and thus,

$$\varepsilon = F(D,\ C_0/\rho,\ V_0,\ U) \quad \text{(Eq. 3)}$$

where the term U represents the contributions of factors other than D, C_0/ρ and V_0. Substitution of Eq. 2 for S in Eq. 1 yields

$$dW/dt = -k\varepsilon C_s(W/\rho)^{2/3} \quad \text{(Eq. 4)}$$

Assuming that the parameter ε may be determined by the initial injection conditions and remains nearly constant with time, Eq. 4 can be readily integrated as follows:

$$(W/W_0)^{1/3} = 1 - jt \quad \text{(Eq. 5)}$$

$$j = k\varepsilon C_s\rho^{-2/3}/(3W_0^{1/3}) \quad \text{(Eq. 6)}$$

where j is defined as an absorption rate constant. This constant (j) is directly related to the *in vivo* dissolution rate constant (k), as shown by Eq. 6.

Equation 5 is similar to the cube root-type dissolution equation of Hixon and Crowell.[7] Ballard and Nelson[8] proposed a kinetic equation similar to Eq. 5 for the absorption following subcutaneous implantation of a spherical solid drug, but there has been no paper describing such an equation for aqueous suspension systems. In this paper, an attempt to gain greater understanding of the unknown parameter ε through experiments will be presented later.

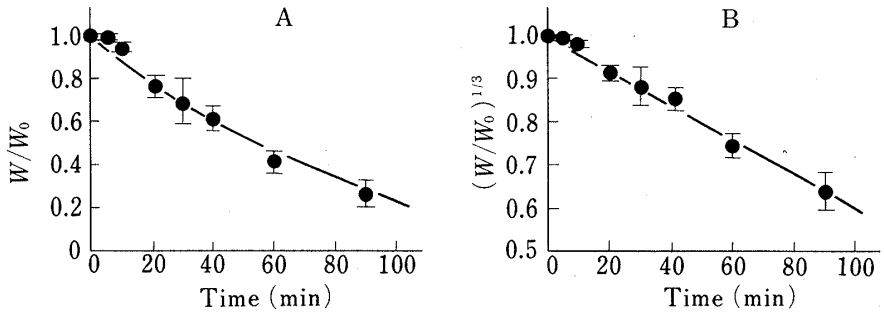

Fig. 2. Time Course of AcMS Absorption from the Injection Site

Each data point represents the mean of 4 or 5 experiments. The vertical bar shows the standard deviation.
Initial drug concentration (C_0), 5 mg/ml; injection volume (V_0), 0.05 ml.

Time Course of Drug Absorption

Figure 2 shows a plot of the residual fraction (W/W_0) of AcMS in the injection site *versus* time following intramuscular injection of its 'controlled' suspension. The initial drug concentration (C_0) and injection volume (V_0) of this experiment are given in the legend. In the plot of Fig. 2B, which shows the cube root of W/W_0 as a function of time, a good linear relationship was observed, while in Fig. 2A, where W/W_0 is plotted against time, an upward curvature was seen. This tendency was common through all the following absorption experiments (the correlation coefficient obtained by the least-squares method from the cube root plot was always larger than that from the linear plot). These results indicated that Eq. 5 could be experimentally accepted. Since the standard deviations, as shown in Fig. 2, were not unusually large, the contribution of factors (3) and (4) to the parameter ε mentioned above appeared not to be significant under the present experimental conditions. Therefore, it is evident that the above-mentioned considerations may be reasonable, and that absorption rates among various experimental conditions and systems can be compared in terms of the j value.

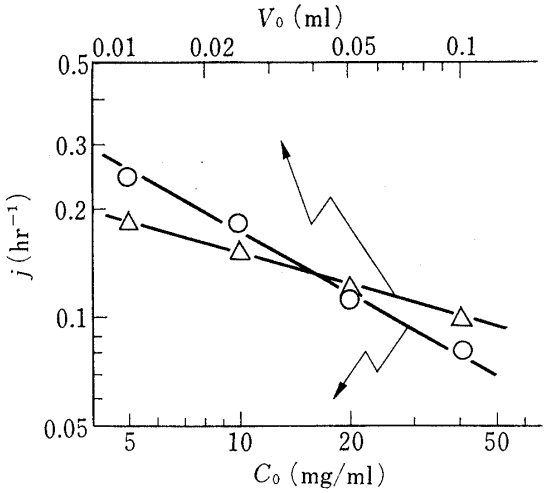

Fig. 3. Relation between Initial Drug Concentration (C_0) or Injection Volume (V_0) and Absorption Rate Constant (j) for AcMS 'Controlled' Suspension

Key: -○-, V_0 (0.05 ml); -△-, C_0 (20 mg/ml). The absorption rate constant (j) was estimated by the least-squares method from the cube root plot of the data in the absorption experiment.

Effect of Initial Drug Concentration and Injection Volume

The absorption rate constant (j), as shown by Eq. 6, is a function of the dose W_0 and the parameter ε. W_0 is the product of the initial drug concentration (C_0) and the injection volume (V_0). The parameter ε, as shown by Eq. 3, is expected to depend on C_0 and V_0 but their correlation is not apparent. Accordingly, the quantitative relationship between j and C_0 or V_0 is also not apparent.

To investigate this relationship, the intramuscular absorption time courses for AcMS 'controlled' suspensions of different C_0 (5—40 mg/ml) and V_0 (0.0125—0.1 ml) were followed, then their absorption rate constants (j) obtained from the cube root plot were compared. Figure 3 shows these results. Absorption rate constants (j) were plotted against the initial drug concentration (C_0) at a fixed injection volume (0.05 ml) or against the injection volume

(V_0) at a fixed initial drug concentration (20 mg/ml) on a log-log scale. From the good linear relationships of both plots, the following tentative approximation can be made:

$$j = fC_0{}^g V_0{}^h \qquad \text{(Eq. 7)}$$

where f is a constant which depends on the suspension and the physiological conditions at the injection site. The experimental values of g and h were estimated by multiple regression analysis from the data shown in Table. I and are tabulated in the bottom row of the left-hand side of this table. To confirm the generality of these values, further absorption experiments were done using 'controlled' suspensions of PHAB, and these results are summarized in the right-hand side of Table I. The experimental values, g and h, obtained for PHAB were not significantly different from those for AcMS. The average g and h values of both compounds were −0.55 and −0.32, respectively.

Table I. List of Data (C_0, V_0 and j for AcMS and PHAB Suspensions[a]) for Estimation of the Parameters g and h, and the Values estimated[b]

AcMS			PHAB		
C_0 (mg/ml)	V_0 (ml)	j (hr^{-1})	C_0 (mg/ml)	V_0 (ml)	j (hr^{-1})
5	0.050	0.24	20	0.0125	0.094
10	0.050	0.18	10	0.025	0.14
20	0.050	0.11	5	0.050	0.17
40	0.050	0.08	2.5	0.100	0.15
20	0.0125	0.18	10	0.010	0.18
20	0.025	0.15	10	0.030	0.11
20	0.050	0.12	10	0.050	0.10
20	0.100	0.097	10	0.100	0.084
			10	0.150	0.068
$g=-0.537(0.028)$			$g=-0.557(0.077)$		
$h=-0.309(0.028)$			$h=-0.334(0.047)$		

a) 'Controlled' suspension.
b) The values of g and h in each suspension were estimated by multiple regression analysis and are given together with the standard errors in parentheses.

If these g and h values are applicable to other suspensions, the following consideration can be developed. Experimentally, we have

$$j = fC_0{}^{-0.55} V_0{}^{-0.32} \qquad \text{(Eq. 8)}$$

Using the relationship $W_0 = C_0 V_0$, Eq. 6 can be rewritten as:

$$j = (k\rho^{-2/3} C_s \varepsilon/3) C_0{}^{-0.33} V_0{}^{-0.33} \qquad \text{(Eq. 9)}$$

From the equivalency between Eqs. 8 and 9, ε must satisfy the following relationship:

$$\varepsilon = F(D,\ C_0/\rho,\ V_0,\ U) = \delta(C_0/\rho)^{-0.22} V_0{}^{0.01} \qquad \text{(Eq. 10)}$$

where δ is a term which depends on the remaining factors D and U. Eq. 10 means that the parameter ε depends little on the injection volume (V_0) but decreases with increasing initial drug concentration (C_0). Substitution of Eq. 10 for ε in Eq. 9 finally yields

$$j = (kC_s\delta/3)\rho^{-0.45} C_0{}^{-0.55} V_0{}^{-0.32} \qquad \text{(Eq. 11)}$$

Despite the fact that this equation was derived on the basis of the experimental results, it should be very important and useful since it gives a relationship between the dose and the intramuscular absorption rate constant from aqueous suspensions, about which nothing has previously been known.

Comparison of Absorption Rate among Various Compounds

According to Eq. 11, the absorption rate constant (j) depends on C_s, k and ρ values, when

the other factors are fixed. As defined previously, ρ, k and C_s represent the density of the compound, the *in vivo* dissolution rate constant and *in vivo* solubility in the injection site, respectively. Of these factors, only the value of ρ can be obtained from an *in vitro* experiment; the other two cannot be completely estimated from such an experiment. From a practical point of view, we attempted to define the quantitative relationship between j and these three factors (or their first approximations).

Using suspensions of seven test compounds which were formulated according to the controlled preparation method, the intramuscular absorption rates were compared at fixed C_0 and V_0 values. Of these compounds, MS was used as the test material for the upper limit of solubility. The mean particle diameter (D_{ss}), distribution constant (n) and sedimentation volume (V_{sed}), given in Table II, showed that these colloidal properties of all the suspensions used here were similar to each other and therefore the variation in δ in Eq. 11 among such suspensions was expected to be sufficiently small. Figure 4 showed the results of the absorption experiments with these suspensions. All the seven cube root plots gave good linear time profiles with different absorption rates.

Table II. Particle Size (D_{ss}), Distribution Constant (n) and Sedimentation Volume (V_{sed}) of Aqueous Suspensions of Various Compounds tested[a]

Compound	D_{ss}(μm)[b]	n[c]	V_{sed}(cm^3/g)
MS	4.2	3.5	1.7
AcMS	2.9	2.5	1.8
PAAB	3.9	2.7	2.0
PHAB	4.1	2.5	2.9
OAAT	4.2	2.7	2.3
PANA	4.0	2.4	2.2
ES	4.1	2.3	1.7

a) Prepared by the controlled preparation method described in the text.
b) Mean particle diameter based on specific surface area.
c) This value indicates the narrowness of the particle size distribution.

In an attempt to gain greater understanding of the factors which resulted in the different rates, molecular weight (M), solubility in saline (C_s'), crystal density (ρ), *in vitro* relative dissolution rate constant (k') and observed absorption rate constant (j) were compared (Table (III). Diffusion coefficients for compounds of molecular weight (M) ranging from 6 to 500 are known to be inversely proportional to the square root of M.[9] The dissolution rate constant *in vivo* (k) in Eq. 11 is considered to be proportional to the diffusion coefficient, like that *in vitro*.[10] Thus, Eq. 11 can be rewritten as follows:

$$j = (\theta\delta/3)C_s M^{-0.5}\rho^{-0.45}C_0^{-0.55}V_0^{-0.32} \qquad \text{(Eq. 12)}$$

where θ is a constant. The values of $M^{-0.5}\ \rho^{-0.45}$ are also listed in Table III as the correction term for the different M and ρ values of the compounds. However, these values were similar to each other for the compounds presented here. Further, the *in vitro* relative dissolution rate constant (k'), which was represented as D_{iss}/C_s' (for details, see the Experimental section) and could be a relative measure of the *in vivo* dissolution rate constant (k), appeared to depend little on the compounds tested.

In contrast, the *in vitro* solubility (C_s') had a positive relation with the absorption rate constant (j). Equation 11 means that a plot of j against *in vivo* solubility (C_s) on a log-log scale should give a straight line with a slope of unity when other factors are the same. Figure 5 shows this plot using C_s' instead of C_s. The plot of this figure is nearly linear with a slope close to 0.5 (regression equation, $\log j = 0.521 \log C_s' - 0.143$; correlation coefficient, 0.963). The deviation of the slope from unity observed in the above regression line may be attributed to (a) a gradual increase in the ratio C_s/C_s', (b) a gradual increase in the parameter k in Eq.

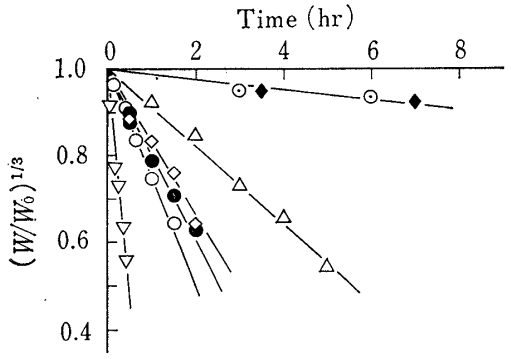

Fig. 4. Comparison of Absorption Rate among Various Compounds following Intramuscular Injection of 'Controlled' Aqueous Suspensions

Key: -⊙-, PANA; -◆-, ES; -△-, OAAT; -◇-, PHAB; -●-, PAAB; -○-, AcMS; -▽-, MS. C_0, 5 mg/ml; V_0, 0.05 ml. Each data point represents the mean of 4 or 5 experiments.

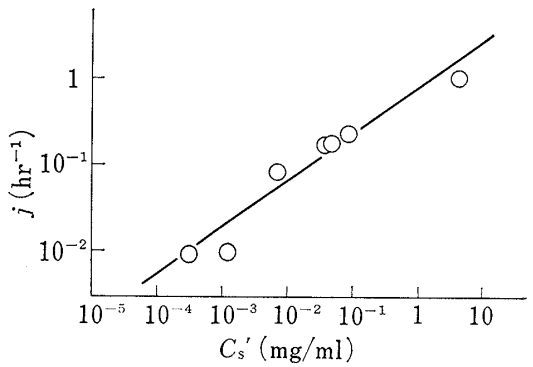

Fig. 5. Relationship between Absorption Rate Constant (j) and Solubility in Saline (C_s')

The solid line was obtained by the least-squares method. $\log j = 0.521 \log C_s' - 0.143$ ($r = 0.963$).

Table III. Comparison of Absorption Rate Constant (j) with Physicochemical Properties, Molecular Weight (M), Solubility in Saline (C_s'), Density (ρ) and *in Vitro* Relative Dissolution Rate Constant (k')

Compound	M	C_s'(mg/ml)[a]	ρ(g/cm^3)	$M^{-0.5}\rho^{-0.45}$[b]	k'[c]	j (hr^{-1})[d]
MS	253	0.61(5.7)[e]	1.49	0.0525	1.5	1.099(0.072)
AcMS	295	0.076	1.38	0.0504	1.2	0.244(0.009)
PAAB	197	0.049	1.19	0.0659	1.3	0.184(0.011)
PHAB	198	0.034	1.38	0.0615		0.171(0.011)
OAAT	225	0.0070	1.21	0.0612	1.2	0.0932(0.0054)
ES	311	0.0012	1.89	0.0426		0.0103(0.0015)
PANA	247	0.0003	1.28	0.0569		0.0093(0.0006)

a) Solubility in saline at 37°.
b) This term is explained in detail in the text.
c) $k' = D_{1ss}/C_s'$ (ml/cm^2/hr).
d) Each value was estimated by the least-squares method from the data shown in Fig. 4 and is listed together with the standard error in parentheses.
e) The value in parentheses shows the solubility in pH 7.25 isotonic phosphate buffer at 37°.

11, or (c) the increasing participation of other transport mechanism such as direct absorption of fine particles with decreasing *in vitro* solubility (C_s'). However, the second factor (b) seems unlikely since the *in vitro* relative dissolution rate constant (k') depended little on C_s', as shown in Table III, and the contribution of the third (c) may be very slight except for exceedingly fine particles. On the other hand, the first factor (a) may be operating because the protein component of body fluids raises the solubility of the practically water-insoluble compounds such as cholesterol[11] and some oil-soluble dyes.[12] We have also observed this phenomenon for the compounds tested here.[13] The linear relationship shown in Fig. 5 is expected to be applicable for the rough prediction of absorption rates of other compounds from similar suspension systems.

Effect of Particle Size on Absorption

The parameter δ in Eq. 11 contains the factor of particle size. Hence, the effect of particle size on the absorption rate constant (j) was investigated.

In the case in which suspension particles at the injection site are dispersed too widely to aggregate with each other and their dissolution process governs the rate of drug absorption, the particle size effect on the absorption appears most strongly. In such a case, theoretically,

the absorption rate constant (j) is independent of the dose and inversely proportional to the particle size. However, this is expected to be very rare in practice because of agglomerate formation *in vivo*, except in an exceedingly dilute suspension with very fine particles or in administration sites such as the gastrointestinal tract where there are large amount of space, body fluids, and effective agitation.[14] The dose dependency of j shown in Fig. 3 and Table I supports the above expectation.

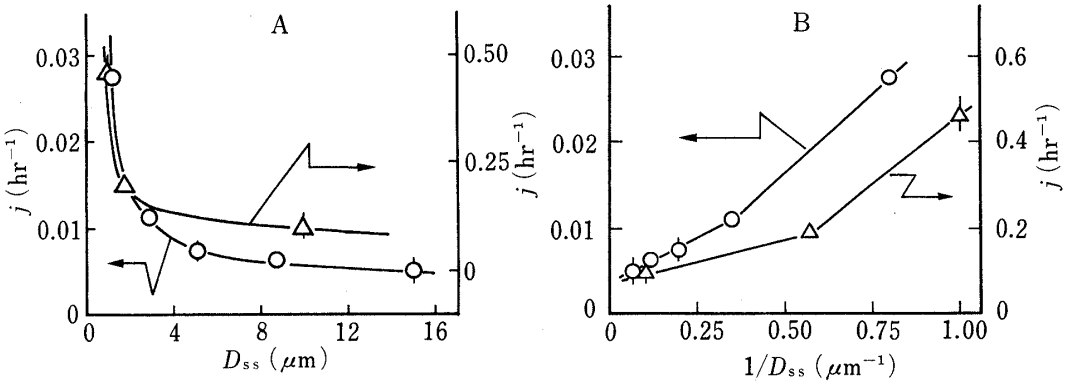

Fig. 6. Relation between Absorption Rate Constant (j) and Particle Size (D_{ss})

Key: -○-, ES (C_0, 5 mg/ml; V_0, 0.05 ml); -△-, AcMS (C_0, 10 mg/ml; V_0, 0.05 ml). The absorption rate constant (j) was estimated by the least-squares method from the data obtained in the absorption experiment. The vertical bar represents the standard error.

Previous investigators,[2] in order to evaluate the particle size effect on parenteral drug absorption, have mainly used the plasma drug concentration data, though they are influenced by disposition characteristics other than absorption. To examine this effect in detail, a direct comparison by the local clearance method was done by using five ES and three AcMS suspensions of different particle sizes (D_{ss}, 1.2—15.0 μm for ES and 1.1—9.9 μm for AcMS) but with similar distribution constants (n) and dispersibilities (n, 2.2—2.7 for ES and 2.2—2.6 for AcMS; V_{sed}, 1.4—1.7 cm^3/g for ES and 1.7—1.9 cm^3/g for AcMS). Figure 6 shows the relation between the observed absorption rate constant (j) and particle size (D_{ss}). For both compounds, j increased with decreasing D_{ss} (Fig. 6A) but the j *versus* $1/D_{ss}$ plot did not give a good linear relationship (Fig. 6B). The increase of j in the region of D_{ss} less than 2—3 μm was larger than that in the region above this. The particles smaller than 2—3 μm seemed to pass more easily through the network of the fibrous tissues accompanying the spreading of the dispersion medium during injection, and to form a looser agglomerate. This may be responsible for the larger change of j in the range of D_{ss} less than 2—3 μm.

The results presented here demonstrate a qualitative relationship between δ and D although they do not offer a clear quantitative correlation. In any event, it is evident that these phenomena are of histological interest and practical importance.

Effect of the Type or Concentration of Macromolecular Dispersing Agent on Drug Absorption

In the examinations hitherto described, only a dispersion medium (vehicle) composed of 0.5% (w/v) MC, 0.005% (w/v) Ps-80 and 0.9% (w/v) NaCl was used because of its good dispersing power and crystal growth retardation characteristic for all the model compounds tested. Other macromolecular dispersing agents such as sodium carboxymethyl cellulose (CMC), acacia (Ar–G) and kalaya gum are also used commonly with or without nonionic surfactants. Nonionic surfactants alter the proportion of dissolved drug in a suspension. They also modify the intramuscular absorption rate of a drug in aqueous solution, as noted by previous investigators.[15] For this reason, only the effect of the type or concentration of the macromolecular dispersing agents (MC, CMC and Ar–G) on drug absorption was examined here using vehicles in which the surfactant concentration was fixed.

The dispersing agent often alters to a great extent the dispersibility of the particles. Figure 7 shows an example of this. This figure compares two vehicles with different dispersibilities for particles with respect to the absorption curve of PHAB. Suspension B was prepared by using 0.5% CMC+0.01% Ps-80+0.9% NaCl as the vehicle in place of the vehicle (0.5%MC+0.01% Ps-80+0.9% NaCl) in suspension A; both suspensions had particles of the same size (D_{ss} of primary particles, 1.2 μm) but the particles in suspension B aggregated with each other much more strongly than those in suspension A, as inferred from the considerably larger V_{sed} value of the former (these values are given in the legend to Fig. 7).[16] The comparison indicated that in this case, a better dispersed system would give a larger absorption rate. The dispersibility, especially for the systems of very fine particles, appears to have a significant effect on the initial spreading of particles at the injection site, as does the particle size.

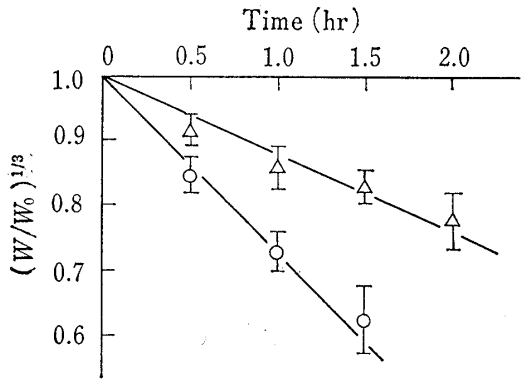

Fig. 7. Comparison of PHAB Absorption between Two Vehicles with Different Dispersibilities

Key: -○-, suspension A (vehicle, 0.5% MC+0.01% Ps-80+0.9% NaCl; V_{sed}, 3.2 cm³/g); -△-, suspension B (vehicle, 0.5% CMC+0.01% Ps-80+0.9% NaCl; V_{sed}, 6.0 cm³/g). C_0, 5 mg/ml; V_0, 0.05 ml; D_{ss} (primary particle), 1.2 μm. Each data point represents the mean of 4 or 5 experiments and the vertical bar shows the standard deviation.

Table IV compares the effect of different concentrations of dispersing agent, MC or CMC, on AcMS absorption and Table V compares the effect of different dispersing agents (MC, CMC

Table IV. Effect of Concentration of the Dispersing Agent (MC or CMC) on AcMS Absorption following Intramuscular Injection of an Aqueous Suspension[a]

Dispersing agent	Concentration of Dispersing agent (%, w/v)	V_{sed}(cm³/g)	% Remaining[b] Mean±S.D.
MC[c]	0.5	1.8	54.3±9.2 (at 1 hr)
	3.0	>1.7	54.0±6.1 (at 1 hr)
CMC[d]	0.5	1.6	44.9±4.8 (at 1.5 hr)
	2.0	2.4	46.9±6.3 (at 1.5 hr)

a) C_0, 10 mg/ml; V_0, 0.05 ml.
b) The percent of the drug remaining in the injection site at 1 or 1.5 hr after injection. Each value represents data from 4 or 5 experiments.
c) To each, 0.005% Ps-80 and 0.9% NaCl were also added; D_{ss} (n), 6.7 μm (2.2); viscosity (25°), 1.4 cP for 0.5% MC and 22.4 cP for 3.0% MC.
d) To each, 0.1% Ps-80 and 0.9% NaCl were also added; D_{ss} (n), 4.7 μm (2.2).

Table V. Effect of Type of Dispersing Agent on AcMS or PHAB Absorption following Intramuscular Injection of an Aqueous Suspension

Compound	Dispersing agent[a]	V_{sed}(cm³/g)	% Remaining[b] Mean±S.D.
AcMS[c]	MC	1.8	58.4±2.7 (at 1 hr)
	CMC	1.6	54.6±5.7 (at 1 hr)
	Ar–G	1.7	59.4±4.2 (at 1 hr)
PHAB[d]	MC	5.4	52.0±6.2 (at 1.5 hr)
	CMC	5.2	59.7±9.7 (at 1.5 hr)
	Ar–G	6.2	57.0±5.2 (at 1.5 hr)

a) Concentration of dispersing agent: 0.5%. To each, 0.1% Ps-80 and 0.9% NaCl were also added.
b) Each value represents data from 4 or 5 experiments.
c) C_0, 10 mg/ml; V_0, 0.05 ml; D_{ss} (n), 6.7 μm (2.2).
d) C_0, 5 mg/ml; V_0, 0.05 ml; D_{ss} (n), 7.0 μm (2.1).

and Ar–G) on AcMS or PHAB absorption, using suspensions with particles of size ranges commonly used. Here, each comparison was conducted at a constant Ps-80 concentration using a group of test suspensions with similar colloidal properties (particle size distribution and sedimentation volume, *i.e.*, dispersibility) and having almost equal proportion of undissolved drug in suspension. In this case, in contrast to the results in Fig. 7, no significant difference was observed in the percentage of the drug remaining in the injection site at a set time after administration, that is, in the absorption rate, within each group. These results suggest that the drug absorption rate is not much affected by the type and concentration (if it is not too high) of the macromolecular dispersing agent, if the dispersibility of the particles and the proportion of undissolved drug in suspension are modified little by these conditions. The rapid drainage of the vehicle from the agglomerate, compared to the drug absorption, and the successive displacement of body fluids for the vehicle may minimize the effect of the type and concentration of the macromolecular dispersing agent.

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16) According to microscopic observation of a sample droplet laid on a slide glass without a cover glass, the size of secondary particles in suspension B approached about 10—20 μm, while the particles in suspension A aggregated little under the same conditions.