JOURNAL OF Pharmaceutical Sciences

March 1968 volume 57, number 3

Review Article

Biopharmaceutical Considerations in Subcutaneous and Intramuscular Drug Administration

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THE PURPOSES of this review are to consider some of the factors affecting the absorption rates of drugs administered subcutaneously and intramuscularly, and to consider some of the literature pertaining to their formulation and administration. This review will largely supplement rather than duplicate references contained in earlier reviews on these subjects (1–4). As a result, certain important but previously covered topics may be omitted entirely, or be discussed from different points of view. The parenteral route of drug administration is an important one for the preclinical screening and evaluation of drugs as well as their clinical use in human and veterinary medicine. The rational design of products intended for parenteral use depends upon many factors including the physical, chemical, pharmaceutical, pharmacological, and toxicological properties of the drug and the adjuvants used in its formulation. Certain anatomical or physiological factors, such as injection site and body movement, may influence the absorption rates of some parenteral products.

ENDOTHELIAL TISSUE

Although this review will not go into extensive detail on the anatomy and histology of the microcirculation at the many possible injection sites, a few key points are worth mentioning. At present there are many investigations of the several mechanisms involved in the passage of drugs and other substances through capillary and lymphatic vessels and the ground substance surrounding them. The subcutaneous region is well supplied by capillary and lymphatic vessels (5). Muscle tissue also has a rich supply of capillary vessels. However, it is generally agreed that there are few, if any, lymph vessels in muscle tissue proper (5, 6). There is an abundant supply of lymphatic vessels in connective tissue sheaths and tendons. Lymph vessels usually exist where fascial planes enter muscles, and the fluid moves through spaces along fascial planes between muscle fibers. One important difference among lymphatics in several regions of the body concerns the state of their intercellular junctions. In active regions of the body one junction in two to five may be open, but in motionless regions there may be only one open junction in 50 to 100. Normal lymphatics in motionless regions and normal blood vessels allow very few particles to pass through their junctions. In contrast, lymphatics in regions where there is much movement, injured lymphatics, and injured blood vessels all allow much more material to pass through their frequently opened junctions. Mild trauma near a lymphatic vessel in a motionless region can result in a marked increase in the vessel's permeability, probably due to the opening of many of the normally closed junctions (7).

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This paper was supported by general research support grant FR 05453 from the Division of Research Facilities and Resources, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

The author wishes to dedicate this paper to Dean T. C. Daniels, whose leadership at the University of California has done so much to raise the standards of education and research in the pharmaceutical sciences.

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The passage of various particles and fluid from one to the other side of blood and lymphatic endothelial tissue has been shown to occur in part by means of vesicles. Particles and fluid contacting one endothelial surface enter cells in small vesicles, and then leave the opposite surface from the same organelles. This process has been called cytopemphis (6, 7). There is evidence that these vesicles are not directed in their intracellular passage, but may move and discharge their contents randomly on either side. Cytopemphis permits the net transport of material to be proportional to the concentration difference of the substance on either side of the endothelial tissue. The various paths by which particles of different sizes are thought to traverse the lymphatic endothelium have been summarized by Casley-Smith (7). A classification of the types and sizes of apertures found in normal and injured capillary and venous walls has been compiled by Landis (8). As to particles, there are theoretically only four possible paths that can be taken through endothelial tissue and these include: intercellular, intracellular in organelles, cytoplasmic matrix, or fenestrae paths. It appears that all four of these paths are used to some extent in various endothelial tissues (7). For lipid-soluble molecules there is abundant evidence that they diffuse through capillary walls and can pass through regions of the wall that are relatively impermeable to lipid-insoluble molecules (9).

DIFFUSION

From a macroscopic point of view, many drugs in solution injected into subcutaneous or intramuscular sites behave as if their absorption were taking place passively by diffusion. For most drugs it has not been determined whether the microscopic absorption route is *via* capillaries, lymphatics, or both. Insofar as drug molecules penetrate endothelial tissues rapidly by passive diffusion, the penetration rate of the drug from the injection site can be described by Fick's Law in one direction.

$$dN/dt = \bar{D}A(dC/dx) \qquad \text{(Eq. 1)}$$

where dN/dt is the penetration rate, N is the amount of drug penetrating the tissue, and t is time. In a well-stirred system, the rate is proportional to the mean diffusion coefficient (10) of the drug in the membrane, $\bar{D}$, the area, A, of the absorbing membrane exposed to the solution, and the concentration gradient (dC/dx) of drug across the membrane. Equation 1 may be approximated (11) by:

$$dN/dt = \frac{\bar{D}AK}{\delta}(C_s - C_b) \qquad \text{(Eq. 2)}$$

where the dx term of Eq. 1 has been replaced by δ, the thickness of the thin subcutaneous or intramuscular membrane that is assumed to be constant for each animal and site, and the dC term of Eq. 1 has been replaced by the terms K and $(C_s - C_b)$. The term K is the equilibrium distribution ratio or partition coefficient of the lipid-soluble drug between the membrane lipids and the aqueous phases found at the injection site or body fluids. The term $(C_s - C_b)$ is the difference between the drug concentration at the injection site, C_s, and the drug concentration, C_b, in the body fluids, *e.g.*, blood and lymph, flowing past the absorption site at any time.

The term C_b can usually be ignored, since it can be reasonably assumed that the drug's concentration in the fluids of distribution is negligible compared to its concentration at the absorption site at any time. Thus, Eq. 2 can be written as:

$$dN/dt = \left(\frac{\bar{D}AK}{\delta}\right) C_s \qquad \text{(Eq. 3)}$$

When the volume of the drug solution at the absorption site, V_s, remains nearly constant throughout the experiment, the rate of penetration will be equal to:

$$dN/dt = \left(\frac{\bar{D}AK}{\delta V_s}\right) A_s \qquad \text{(Eq. 4)}$$

where A_s is the amount of drug at the site at any time. Equation 4 can be further reduced to:

$$dN/dt = PA_s \qquad \text{(Eq. 5)}$$

where P is the penetration coefficient which includes all the terms in parentheses in Eq. 4, and has the units of time^{-1}. The penetration coefficient or absorption rate constant has the same magnitude as the clearance constant, but the opposite sign. Thus, it can be seen from Eq. 4 that the absorption rate is directly proportional to $\bar{D}$, A, and K, but is inversely proportional to δ and V_s. The half-life of the drug at the absorption site, $t_{0.5}$, can be calculated from a plot of the logarithm of the fraction of drug remaining at the site at any time *versus* time. The half-life and penetration coefficient are related by:

$$P = \ln 2/t_{0.5} \qquad \text{(Eq. 6)}$$

Area—The local distribution of solutions injected subcutaneously or intramuscularly is of interest, because the penetration rate of the drug depends in part upon the geometry and the resulting area of the depot exposed to the tissue. When the water-immiscible silicone, dimethylpolysiloxane,[1] was injected subcutaneously into animals, most of it was limited to the tissue planes of the subcutaneous region.

[1] Dow Corning 360 medical fluid.

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Pathologic findings in the rat, mouse, and guinea pig following the administration of massive doses of the silicone were essentially similar. In most instances the silicone was contained in innumerable thin-walled spherical or ellipsoidal sacs and several larger pools within the adipose tissue (12, 13). Brown *et al.* (14) studied the disposition of subcutaneous injections of a radiopaque water-in-oil emulsion in guinea pigs at the injection site. The horizontal and vertical aspects of the injection site were X-rayed over a period of days. A cross-section of the tissue showed on the film a lateral spread of the radiopaque material in the subcutaneous tissue that progressed over a period of days.

What is the immediate distribution of substances injected intramuscularly? Shaffer (15) injected radiopaque substances into the gluteus muscle of humans. He found that iodized oil and bismuth salicylate in oil were confined to the planes of fascia or connective tissue surrounding muscles and groups of muscles. Injections of metallic bismuth suspended in isotonic dextrose solution were similarly distributed in the fascial planes of the muscle. Fluoroscopic studies showed that viscous oily solutions, such as iodized oil, tended to form a sphere-shaped deposit about the needle point and then spread out more slowly. It did not spread as extensively along the fascial septums as did the aqueous systems studied. The oily solutions continued to spread from 1 to 5 min. before they became fixed in position. The aqueous suspension of bismuth spread to its final location almost as soon as the injection procedure was completed. Some authors (16) have suggested that the term "intramuscular" is a misnomer and should be called "intermuscular" when it refers to injections in this region, because of the spread of solutions along the fibrous tissue *between* muscle fibers. Gruhzit (17) also noted that water-soluble bismuth tartrate injected intramuscularly was precipitated in the tissues and distributed along the connective tissue fasciculi between the muscle fibers. Zelman (18) has stated that deep, firm massage of the muscle tissue following an intramuscular injection favors the spread of the medication through a wider area of tissue, thus increasing the area for absorption to take place.

From the foregoing examples it can be seen that with the usual needle-injection technique, it is difficult, if not impossible, to control the area of an injected solution, suspension, or emulsion in contact with the tissues. If other factors are equal (*e.g.*, metabolic and excretion rates), one would expect that higher initial serum levels of drug would result when the solution has a larger area exposed to the tissue, since drug penetration rate is directly proportional to this area.

Recently, an experimental technique in animals has been devised (19) to study the *in vivo* subcutaneous absorption rate of about 1% (w/v) benzyl alcohol in normal saline where the area of tissue exposed to the solution is held constant. A medical silicone adhesive is used, which is capable of affixing a cylindrical, hollow glass absorption cell to moist subcutaneous tissue. This adhesive prevents the spread of the solution from the site, and is reported to be completely inert to living tissue and will not cause irritation or sensitization. With this procedure it is possible to keep the solution in the cell constantly stirred, which cannot be conveniently done when needle-injection techniques are used. Furthermore, it is convenient when removing drug samples periodically for analysis.

Volume—The subcutaneous absorption cell just described has another advantage. The volume of solution in the cell, as well as the area exposed, can be held constant. Thus, the penetration coefficient, P, defined in Eqs. 4 and 5 will be constant for fixed values of $\bar{D}$, K, and δ. One would predict that if the volume of the solution in the cell were increased over control values, a decrease in the magnitude of P would occur. If so, the result would be that the penetration rate of the drug would also decrease. Similarly, a decrease in volume over controls should result in an increase in both P and the penetration rate. These predictions have not been verified experimentally using the subcutaneous absorption cell just described, but Sund and Schou (20) have shown that the intramuscular clearance rates of radioactive mannitol and sucrose solutions were inversely proportional to the volume of solution injected.

When radioactive drugs or ions are used in absorption studies, it has been observed that plots of the logarithm of the fraction of drug (*e.g.*, radioactive counts) remaining at the site *versus* time may not be linear (20). The absorption half-life at the beginning of the experiment is often shorter than that calculated at some later time. This may indicate that a bi- or polyexponential equation would fit the data better. A few of the reasons for this phenomenon are: (*a*) blood or lymph flow from the site may be impaired at the later time, (*b*) the solution at the site is not uniformly mixed and there is a longer diffusion path for molecules at the center of the injected solution, or (*c*) the magnitude of the penetration coefficient may not remain constant with time. For example, if the area of tissue exposed to the liquid progressively decreases, or if the volume of solu-

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tion progressively increases at the site, the value for the penetration coefficient will decrease with time to give rise to a longer absorption half-life toward the end of the experiment. Either hydrostatic (*e.g.*, injection pressure), osmotic pressure effects, or both may also be factors in determining the net solvent flow between the fluids at the site and in the vessels.

Concentration—Sund and Schou (20) studied the effects of different drug concentrations on clearance rate. On the one hand, with a pharmacologically inert, neutral, water-soluble substance like sucrose, the clearance rate from rat muscle was independent of drug concentration over the range of 0.19–9.6 mg./ml. This is the predicted result on the basis of Eqs. 4 and 5 when the injection volume is constant as it was in their experiment. On the other hand, the absorption rate of a substance like atropine depends markedly upon its concentration at the injection site. The relative clearance rate for atropine decreases with increasing concentrations of the drug over a threshold value of about 0.5 mg./ml. Atropine and several other anticholinergic drugs when mixed with sucrose solutions decreased the clearance rate of sucrose. The self-depression of atropine absorption and the inhibited absorption of sucrose are probably both due to the local pharmacological action of atropine. Sund and Schou (21) conclude that although the exact mechanism is not known, atropine probably interferes with local blood flow at the injection site. Atropine also has interesting local effects on the inflammatory process. Houck (22) administered intradermal injections of 50% croton oil in peanut oil to rats. Croton oil, a water-immiscible vesicant, produces wounds that are reproducible. He found that the microcirculatory insufficiency characteristic of injuries produced by the diluted croton oil was eliminated by the subcutaneous administration of atropine. He proposed that one of the primary effects of atropine was to accelerate wound healing by restoring the biological continuity of the wound with the circulation and surrounding tissue.

Molecular Size—As a first approximation, the diffusion coefficient of a spherical or nearly spherical drug molecule that is larger than the solvent molecules is inversely proportional to its molecular radius (or weight). Thus, in a diffusion-controlled process, large molecules would be expected to have slower penetration rates than smaller ones.

However, none of the equations so far presented in this paper give the investigator any indication as to whether an injected molecule would be absorbed primarily *via* capillary vessels, lymphatic vessels, or both. Table I summarizes some of the literature relating molecular or formula weights of injected substances and their probable primary absorption routes. From the subcutaneous site it appears that molecules or ions having low molecular weights are absorbed primarily *via* the capillaries, while molecules having high molecular weights appear to be absorbed primarily *via* lymph vessels. Sund and Schou (20) followed the clearance rate of labeled carbohydrates from rat muscle. Table II lists the substances used, their molecular weights, aqueous diffusion coefficients, and the fraction of drug cleared 5 min. after the injection. As expected, it can be seen from this table that with an increase in molecular weight, there is a decrease in clearance rate. The fact that the fraction cleared at the end of 5 min. correlates with the diffusion coefficient, they state, is evidence that diffusion is the main driving force for the absorption. These investigators did not determine experimentally which of the molecular species was cleared primar-

Table I—Relationship Between Molecular or Formula Weight of Chemical Species and Probable Route of Absorption Following an Intramuscular or Subcutaneous Injection

Mol. Species	Mol. or Formula Wt.	Route of Administration	Probable Primary Absorption Route	Ref.
$^{24}NaCl$	58	i.m.	Capillary	(145)
Strychnine (salt?)	>334	s.c.	Capillary	(38)
$^{59}FeCl_3$	270	s.c.	Capillary	(146)
^{59}Fe-labeled plasma	?	s.c.	Lymphatic	(146)
Black tiger snake venom	>20,000	s.c.	Lymphatic	(38)
India cobra venom	2500–4000	s.c.	Capillary	(38)
Russell viper venom	~30,000	s.c.	Lymphatic	(38)
Diphtheria toxin	~70,000	s.c.	Lymphatic	(38)
Tetanus toxin	?	s.c.	Lymphatic	(38)
Iron polysaccharide complexes	10,000–20,000	i.m.	Lymphatic	(37)
Neolymphins[a]	High	i.m.	Lymphatic	(24)
Iron–sorbitol–citrate complexes	<5000	i.m.	~16% lymphatic ~50–60% capillary	(147)

[a] The neolymphins are neomycinpolymetacrillate, neomycindextransulfate, and neomycincarboxymethyl starch.

Table II—Carbohydrate Absorption from Rat Muscle (20)

Substance	Mol. Wt.	Aqueous Diffusion Coefficient × 10^6	Fraction of Drug Cleared 5 min. After i.m. Injection × 10
D-Mannitol-1-^{14}C	182	8.7	~7
Sucrose-^{14}C	342	7.5	~6
Inulin-methoxy-^{3}H	3000–4000	2.1	~2
Inulin-carboxyl-^{14}C	3000–4000	2.1	~2
Dextran-carboxyl-^{14}C	60,000–90,000	~0.5	~0.7

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ily by the capillary or by lymphatic routes, although they pointed out that the drainage through lymph channels could be of relatively greater importance for the absorption of large molecules compared to smaller ones.

Lewis (23) attempted to explain why sudden anaphylactic deaths occasionally occurred in humans after subcutaneous injections of macromolecules like diphtheria antitoxin when absorption of this substance from the subcutaneous site was known to be slow. The test substance he used was horse serum. Table III shows that the serum was absorbed slowly from the subcutaneous region of dogs, probably because the absorption took place largely *via* the lymphatics. Massage of the wheal produced by the injection and the use of high injection pressures resulted in a more rapid appearance of the serum in the thoracic duct lymph. Lewis concluded that the serum's absorption rate was too slow to account for cases of anaphylactic death in humans, and that the probable reason for this phenomenon was an accidental intravenous injection of the serum.

The effect that molecular size or weight can have on the route of drug clearance from an injection site has been demonstrated by Málek and co-workers (24). They prepared salts of an antibiotic base like streptomycin or neomycin with high molecular weight anionic substances like polyacrylic acids, sulfonic or phosphorylated polysaccharides, and natural polycarboxyl acids. After an intramuscular injection of neomycin sulfate into a dog, a peak blood level of about 20 mcg./ml. was obtained at 2 hr., which fell to zero at 12 hr. After an intramuscular injection of neomycindextransulfate (neolymphin II), a peak neomycin blood level of about 3 mcg./ml. was obtained at 2 hr., and its concentration in the blood remained nearly constant at 1 mcg./ml. from 8 to 24 hr. They termed these macromolecular salts "antibiolymphins." According to the authors, the antibiolymphins were absorbed from the injection site primarily *via* the lymphatic system in contrast to the corresponding sulfate salt, which presumably was absorbed *via* the capillaries, although the authors did not state this.

TABLE III—APPEARANCE OF SUBCUTANEOUSLY ADMINISTERED HORSE SERUM IN LYMPH AND BLOOD OF DOGS UNDER VARIOUS CONDITIONS (23)

Conditions	Time to Detect —Presence of Horse Serum in— Thoracic Duct Lymph	Blood
No massage at site	40 min.	3.5 hr.
	...[a]	1.5 hr.[a]
Massage at site	15–20 min.	2 hr.
High-pressure injection	<5 min.	40 min.

[a] Thoracic duct not cannulated.

pH—Madison and Christian (25) studied the influence of pH on the absorption rate of $^{22}NaCl$ and $^{23}NaCl$ administered subcutaneously in rats. The magnitude of the absorption rate was evaluated on the basis of the amount of radioactivity present in a sample of heart blood removed 45 min. after the injection. From pH 2.5 to 10 inclusive there was little effect on the normal absorption rate of sodium ion. At pH values of 1.0 and 2.0, a decrease in sodium ion absorption rate was observed, while at pH values of 11.0 and 12.0 an increase in rate occurred.

In the case of organic bases, the pH of the injected solution can often have a profound effect on absorption rate. Cutts and Walker (26) repeated the work of White and Claflin (27). They confirmed that in mice intraperitoneal injections of the nitrogen mustard HN_2, methyl-bis(β-chloroethyl)-amine hydrochloride, at two different pH values resulted in different LD_{50} values. At pH 2 the LD_{50} was about 5 mg./Kg., while at pH 8 it was about 2 mg./Kg. White and Claflin (27) showed under their injection conditions that little HN_2 would have undergone cyclization. Since HN_2 has a pK′ of 6.45 at 15° (28), the molecule exists almost entirely as the water-soluble protonated form at pH 2. But at pH 8 a significant fraction of the amine exists in the undissociated form. On the basis of pH-partition considerations (29), the drug's absorption and distribution at the higher pH value should be facilitated resulting in the lower value found for the LD_{50}.

PHAGOCYTOSIS

Although passive diffusion is an important mechanism for drug absorption, the process of phagocytosis may also be involved in drug absorption from subcutaneous and intramuscular sites. Rees and co-workers (30) studied the systemic distribution of dimethylpolysiloxane,[2] following its subcutaneous administration, in mice. They stated that while the mechanism of absorption and systemic distribution of the silicone fluid in mice is at present unknown, it may be distributed to the viscera by gaining entrance to the general circulation (*via* capillaries?) or lymphatic channels. However, the authors suggest that the most likely mechanism is by the process of phagocytosis by histocytes.

When sodium urate crystals are injected subcutaneously into animals and man, an acute inflammatory response occurs accompanied by phagocytosis of the crystals by mononuclear and

[2] Dow Corning MDX 4-4011.

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