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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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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.

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-

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

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.

polymorphonuclear leucocytes (31). A similar acute inflammatory response occurs after subcutaneous injections of microcrystalline ammonium and potassium urates, uric acid, xanthine, hypoxanthine, calcium carbonate, and creatinine. Seegmiller and co-workers (32) have shown that when microcrystalline sodium urate and sodium orotate suspended in a 50% glycerol–water vehicle were injected subcutaneously in man, 24 hr. later the site in most subjects showed warmth, swelling, erythema, induration, and tenderness. There was essentially no reaction to control subcutaneous injections of the vehicle after the immediate pain and wheal had subsided 6 to 8 hr. after the injection. Malawista and Seegmiller (33) have shown, when humans were pretreated with colchicine, the inflammatory response to subcutaneously administered monosodium urate monohydrate and sodium orotate microcrystals was substantially reduced over controls not so pretreated. Malawista (34) has shown that colchicine interferes with the amoeboid motility of individual human polymorphonuclear leucocytes. Other substances like actinomycin D, 6-mercaptopurine, and puromycin have been found to block lymphocyte emigration into a region of inflammation (35). An interesting but as yet unexplained finding is that the inflammatory response to subcutaneous injections of amorphous sodium urate particles was much less than that found with microcrystalline preparations of the same salt (32).

Gómez-Luz (36) showed that when microcrystals of insoluble oxytetracycline were administered subcutaneously into man, crystals could be found in the peripheral blood either intracellularly or free and in human bone marrow 6 to 14 days following the injection. Patients suffering from nonspecific urethritis received the insoluble oxytetracycline subcutaneously, and microcrystals were found in their urethral discharges. Gómez-Luz explained these observations by suggesting that polymorphonuclear leucocytes and monocytes engulfed the microcrystals by the process of phagocytosis, and these crystals then were transported to the general circulation and finally to the site of infection.

Beresford and co-workers (37) showed that most of the absorption of iron polysaccharide complexes injected into rabbit muscle tissue occurred during the initial 72 hr. The absorption during this time was mediated in part by the inflammatory reaction evoked, and in part by lymphatic transport of the iron complexes. The iron complexes remaining after 72 hr. were fixed by tissue macrophages that seemed to impede further absorption.

BIOLOGICAL FACTORS

There are several biological factors, in addition to those mentioned, which can influence the rates of drug absorption. Some of these factors include body movement, pre-existing disease conditions, the age of the animal, the anatomical region into which the injection is made, and the condition of the tissue at the site.

Body Movement—If a molecule is absorbed primarily *via* the lymphatic route, body movement or exercise can often have a profound effect on how rapidly it reaches the general circulation. Muscular movement has long been known to increase the flow of lymph fluid along the lymphatic vessels. When an animal is at rest, little or no lymph flows (5). The effect that body movement has on the absorption rate of a substance in solution can be seen from the work by Barnes and Trueta (38). They injected black tiger snake venom subcutaneously into rabbits. Since this venom has a relatively large molecular weight (>20,000), it is transported from the injection site almost exclusively by the lymphatic drainage. Control animals lived no longer than 150 min. following the injection, while animals whose injected limb was immobilized by a plaster cast lived more than 8 hr.

Controlled physical activity has also been shown to have an effect on the rate at which subcutaneously implanted procaine penicillin G implants were absorbed in rats. Ballard (39) found that the mean absorption rate per mean pellet area[3] in animals rotated in a rodent activity cage at 3.83 r.p.m. was 2.57×10^{-3} Gm. hr.$^{-1}$ cm.$^{-2}$, while that of nonrotated control animals was 1.93×10^{-3} Gm. hr.$^{-1}$ cm.$^{-2}$. These differences were shown to be statistically significant.

Disease Conditions—Bauer and co-workers (40) noted that patients with cardiac failure and patients with myxedema had prolonged absorption half-lives following a jet injection of ^{131}I solutions into their thighs. When the myxedemic condition was reversed by appropriate thyroid medication, the absorption half-life of the drug returned to the normal range. De Groot and co-workers (41) noted delayed intramuscular absorption of a triiodothyronine formulation in one hypothyroid subject studied. Using the same jet injection technique, Bauer *et al.* (42) studied the absorption half-life of ^{131}I from the thighs of patients suffering from myocardial infarctions. None of the patients had congestive heart failure. In one patient the half-lives were

[3] An error in the caption at the top of column five of Table I (39) indicated that all the rates per unit area were tenfold slower than they should be.

60, 29, 14, and 8 min., respectively, on the first, eighth, fourteenth, and thirty-first days after the infarction. In healthy controls the mean absorption half-life for ^{131}I was 9 (range: 6–14) min. Irons (43) followed the blood levels of penicillin in patients suffering from a variety of bacterial infections after injections of an aqueous suspension of procaine penicillin G. He commented that a depression of penicillin blood levels tended to occur in a patient with severe cardiac failure because there was poor absorption of the drug from the injection site.

Animal Age—Lee (44) studied the effect of age on the acute toxicity of subcutaneously administered chlorpheniramine maleate and diphenhydramine hydrochloride in rats. He found that the LD_{50} of chlorpheniramine during the first 16 days of life varied between 175 and 230 mg./Kg., but after 25 days of life the LD_{50} was 360–365 mg./Kg. A similar trend was found for diphenhydramine. There was no apparent difference in the activity of enzymes concerned with the metabolism of both drugs in the livers of 15- and 40-day-old rats. Lee concluded that the development of greater resistance to both drugs with increasing age could be explained by a decrease in the amount of drug absorption from the subcutaneous region. The reason why age should influence drug absorption from the subcutaneous region is not known at present. However, if one may speculate, there are at least two possible reasons for this phenomenon.

Ballard and Menczel (19) studied the subcutaneous absorption half-life of aqueous benzyl alcohol solutions in rats. The mean absorption half-life for four animals was 1.6 hr., but the range was from 0.96 to 2.58 hr. under conditions where the tissue area exposed to the drug solution was constant. They suggested that one probable reason for the large interanimal variation in $t_{0.5}$ could be attributed to differences in the membrane thickness exposed to the drug solution in the abdominal region for the several animals. It is well known that the thickness of the subcutaneous tissue among humans is not constant (45). In Eqs. 4 and 6 it can be seen that the penetration coefficient is inversely proportional to δ, while $t_{0.5}$ is directly proportional to δ. It is reasonable to suppose that as young animals age the thickness of their subcutaneous tissue may increase as well, resulting in increases in $t_{0.5}$ and increases in LD_{50} values for drugs whose acute toxicity can be correlated with drug blood-level.

Another hypothesis among several others deals with the question: what influence does animal age have on the chemical composition of subcutaneous fat tissue? The penetration rate of a lipid-soluble drug should be directly proportional to the lipid/water partition coefficient of the drug. (See Eq. 4.) If the chemical composition of this tissue changes with age, and the apparent partition coefficient of the drug differs in younger and older tissue, then variations in absorption rate would be expected. Using gas chromatographic analysis Bondrup *et al.* (46) studied the triglyceride composition of subcutaneous fat obtained from premature stillborn and full-term stillborn children, and normal, lean, and obese adult patients. Between the children and adult groups they found that there was a pronounced difference in the relative percentages of C_{12} to C_{18} triglycerides in the two groups. The testing of these two hypotheses must await more data on the relative thicknesses of the subcutaneous tissue surrounding injected solutions in young and older animals, and apparent partition coefficient data for drugs in these triglyceride systems.

Anatomical Region and Tissue Condition—The absorption rate of subcutaneously or intramuscularly administered drug can often be profoundly affected by the anatomical region into which the drug is injected and the condition of the tissue found at the site. Nora and co-workers (47) studied the effect of the route of insulin administration in the management of diabetes mellitus in children. They found no significant difference in absorption rate of ^{131}I-labeled lente insulin from subcutaneous and intramuscular tissues at a given anatomical region, but they did find significant differences at different sites. For example, the mean absorption half-life for the drug following intramuscular arm injections was 224 min., and that for the subcutaneous arm injections was 232 min. By comparison, the mean absorption half-life of the drug for intramuscular thigh injections was 314 min., and that for the subcutaneous thigh injections was 310 min. They concluded that variability in diabetic control potentially could be encountered when alternating the anatomical region of the injection between arm and thigh, but such a problem would not be anticipated between the subcutaneous and intramuscular sites at a given anatomical region.

Burchell and Swasdio (48) compared the absorption rates of atropine injected by various routes. The absorption rate of drug administered by various routes could be compared by recording the time interval between drug injection and the advent of tachycardia. Maximum pulse-rate elevation was noted at about 7 min. after intravenous administration of atropine, 28 min. after broad ligament administration, and

40 min. for both subcutaneous and intramuscular drug administration. They did not discuss the reasons for the unexpected rapid absorption of drug from the broad ligament.

Braid and Scott (49) studied the systemic absorption of the local anesthetic drugs, lidocaine and prilocaine, in humans after using two types of regional nerve block, namely, lumbar epidural block and intercostal regional block. Their work showed that much higher serum levels of both drugs follow intercostal block compared to epidural block. The authors ascribed the higher serum levels found with the intercostal block to differences in the relative vascularity of the tissues at the two injection sites. Although the epidural space contains numerous blood vessels, there are comparatively few capillaries available for drug absorption.

The condition of the tissue at the injection site can also affect drug absorption rate. Root and co-workers (50) studied the subcutaneous absorption of insulin labeled with radioactive iodine in diabetics and volunteers. In the controls and patients with uncomplicated diabetes, the absorption rates of tagged insulin were nearly identical. Drug absorption rate was retarded in diabetics when insulin was injected into regions where induration or an insulin pad had formed. The pads disappeared when injections in these regions were discontinued. After a recovery period, the tissue regained its ability to absorb insulin rapidly once again. Their results agreed with the clinical observation that insulin tends to lose its effectiveness when injected in insulin pads.

Chavarría *et al.* (51) administered cycloguanil pamoate intramuscularly to patients with dermal leishmaniasis. One of the treatment failures occurred in a child with a suppurating abscess at the injection site. When the abscess was drained of pus, particles of the yellow drug were found indicating that it had not been completely absorbed. The authors suggested that this complication might explain the treatment failure in this patient.

ENZYME EFFECTS

It has been known for years that when the enzyme hyaluronidase is added to a drug solution intended for intramuscular use, the onset of drug action is more rapid than when the drug alone is used (52–54). Hyaluronidase hydrolyzes hyaluronic acid, a component of tissue ground substance, which limits the spread of fluids at the injection site. Thus, after hydrolysis, the area of drug distribution in the tissue is increased with the result that absorption rate is also increased (55). The enzyme chymotrypsin, on the other hand, influences drug absorption by a mechanism not yet understood. Seneca and Peer (56) administered orally enteric-coated tablets of this enzyme to volunteers. Four hours later the subjects again received chymotrypsin orally, and 100 mg. of tetracycline hydrochloride intramuscularly. A control group was treated in the same manner except that its members received placebo tablets instead of the enzyme. At the end of 6 hr. the placebo groups had excreted about 13% of the injected dose of tetracycline in the urine, while the enzyme groups excreted about 37% of the dose over the same time. Serum levels of drug at various times following its injection were not reported for these subjects. However, when tetracycline hydrochloride and chymotrypsin were given orally, the mean level of antibiotic in the serum and the cumulative amount of drug excreted up to 12 hr. were significantly higher than when tetracycline hydrochloride was given orally alone. The authors state that chymotrypsin either interferes with the excretion of tetracycline or it increases drug absorption rate. On the basis of the evidence provided in the above experiment and the literature (57, 58), the authors concluded that interference with drug excretion was probably not the mechanism for the enhanced blood levels.

FORMULATION—PHYSICAL ASPECTS

The preceding section discussed some of the mechanisms and factors affecting drug absorption In this section some aspects of parenteral drug formulation will be considered. Nash (59) pointed out that most reviews written about pharmaceutical suspensions devote little space to a discussion of the formulation of parenteral suspensions. There are formulation problems associated with these dispersed systems which other suspension systems do not share. Some of these factors are product sterility, syringeability, ease of resuspension, and drainage.

The manufacture of sterile parenteral suspensions requires careful control at the following stages: (*a*) final drug recrystallization, (*b*) drug micronization, (*c*) drug sterilization, (*d*) vehicle sterilization, (*e*) aseptic wetting of the sterile powder with a portion of the sterile vehicle, (*f*) aseptic dispersion and milling of the bulk suspension, and (*g*) aseptic filling of the sterile containers with the finished suspension. The use of sound parenteral formulation techniques and sterility controls in the first place results in reductions in the foreign matter content of such powders and the chances of contaminating the formulation with pyrogenic materials.

One of the most important physical properties of a good parenteral suspension is its syringeability, or the ability of a solution or suspension to pass easily through a hypodermic needle. Properties that tend to reduce the formulation's syringeability can be traced to increases in the vehicle's viscosity and density, increases in the particle size of solids in the suspension, and increases in the percentage of drug suspended in the formulation. Nash states that probably the most important of the above factors contributing to a decrease in syringeability is the viscosity of the formulation. He believes one practical standard for syringeability is that the entire contents of a syringe should pass easily through a 25-gauge hypodermic needle.

The resuspendability of a suspension may be defined in terms of the minimum amount of shaking that is required to suspend particles that have settled in the container. A practical consideration is how much effort will a busy nurse or physician exert to resuspend settled particles? Ideally, the best answer to this problem is to formulate initially a "permanent suspension," or a physically stable, flocculated suspension.

A parenteral suspension should exhibit good drainage. Good drainage can be seen when the suspension separates cleanly from the inner walls of the container. The current practice of coating containers and plugs with silicone preparations makes good suspensions drain better and helps minimize the tendency toward bad drainage of over-flocculated systems.

Rheology—The pharmaceutical industry in recent years has come to rely heavily on sophisticated rheological measurements in order to help solve some of the difficult stability problems encountered in pharmaceutical systems. However, Boylan (60) points out that pharmaceutical literature is nearly devoid of rheological studies involving parenteral products. In his review, he gives examples of the effect that the formula and vehicle can have on the rheological properties of some commercially available procaine penicillin G suspensions.

Polymorphism—In recent years drug product formulators, government agencies, and others have become increasingly aware of the importance of identifying and characterizing the physical and biological properties of drug polymorphs. For example, both chloramphenicol palmitate and stearate have two polymorphic forms, one form of the ester in each case is therapeutically active and the other is not (61). In 1965 the Food and Drug Administration (FDA) amended its regulations to include a limit of not more than 10% of polymorph A, the therapeutically inactive form, in chloramphenicol palmitate suspension (62).

Hayden and co-workers (63) studied the absorption spectra of 175 reference standard compounds under controlled conditions. Except in resolution and slight relative intensity reversals, they observed no significant infrared spectral changes when potassium halide disks containing 150 of the standards were remeasured after periods of 2 weeks to 6 months. However, 25 compounds[4] displayed significant spectral variations after standing. They attributed the changes exhibited by these compounds primarily to crystalline form transitions. The conditions of crystallization, force of grinding, or the disk storage period influenced the crystalline forms and their infrared spectra. Lees (64) cites other examples where drugs were converted from one polymorphic form to another by apparently innocuous grinding procedures.

Mesley and Johnson (65) studied the infrared spectra of pharmaceutically important steroids with particular reference to the occurrence of polymorphism. Of 35 steroids examined 19 had two or more polymorphic forms.[5]

The articles by Macek (3) and Collard (66) consider some of the physical problems associated with the formulation of drugs intended for parenteral use. Polymorphic changes and particle size control in suspensions are among the topics discussed. Identification of the various polymorphic or solvate forms of drugs is also important because it has been shown that the subcutaneous absorption rate of one form may differ from that of another (2, 67).

Scheindlin (68) has briefly reviewed the use of stabilizers in parenteral drug formulations. He discusses the following classes of stabilizers: buffers, antioxidants, chelating agents, protective colloids, and miscellaneous stabilizers such as nonaqueous solvents. Anschel (69) reviewed the literature dealing with some of the methods of solubilizing some steroid hormones and antibiotics.

FORMULATION—BIOLOGICAL ASPECTS

Apart from the physical aspects of parenteral

[4] Betazole hydrochloride, calcium panthothenate, carbinoxamine maleate, dihydrostreptomycin sulfate, epinephrine bitartrate, ergonovine maleate, estradiol, estradiol benzoate, hydroxystilbamidine isethionate, menadione, mercaptopurine, nifuroxime, nitrofurantoin, oxytetracycline, phenindamine tartrate, phentolamine methanesulfonate, probenecid, pyrilamine maleate, sodium diatrizoate, sulfamethazine, sulfamethoxypyridazine, tetracaine hydrochloride, thiamine hydrochloride, trimethaphan camphorsulfonate, and zoxazolamine.

[5] Compound (number of solid forms identified): betamethasone (2), cortisone acetate (7), dexamethasone acetate (4), dimethisterone (2), ethinyl estradiol (3), fludrocortisone acetate (4), fluocinolone acetonide (2), hydrocortisone (2), methylprednisolone (2), norethandrolone (2), norethynodrel (2), prednisolone (2), prednisolone trimethylacetate (3), prednisone (2), prednisone acetate (2), progesterone (2), spironolactone (3), testosterone (3), and triamcinolone (2).

formulation, the test of whether a given formulation will be acceptable also depends upon performance *in vivo*. It has been known for some time that formulation variables can affect the animal's response to the product under study, sometimes to a large degree.

Particle Size—The effect of particle size on the availability of a suspension has been demonstrated by Foglia and co-workers (70). They administered the estrogenic substance *p*-hydroxypropiophenone (PHP) in saline subcutaneously to spayed female rats. When the size of the suspended particles was about 10,000 μ^3, and a high dose was maintained for a long time, they observed no estrus. When samples of the same powder were recrystallized to yield a particle size of about 2,000 μ^3 or less, they observed estrogenic activity. After samples of these active microcrystals were recrystallized to yield particles having the original size of about 10,000 μ^3, they became inactive again. The authors concluded that PHP appears to be a weak estrogenic substance, and its activity depends upon the crystal size injected.

Solubilizers—Kopáčová and Vrba (71) studied the local anesthetic activity of 1.5, 3, and 6% concentrated solutions of benzocaine in highly concentrated aqueous solutions of the solubilizing agents antipyrine, dimethylformamide, diacetone alcohol, and butane-1,2,4-triol. They assumed that the depot activity of the benzocaine was due to prolonged absorption following the drug's precipitation at the injection site. All benzocaine solutions caused undesirable local reactions. Reactions were greatest with the solubilizers butane-1,2,4-triol and antipyrine, and least with dimethylformamide. As to the degree of anesthesia, benzocaine in dimethylformamide was somewhat less effective than benzocaine in the solubilizers antipyrine and diacetone alcohol.

Viscosity—Coles *et al.* (72) studied the effect of aluminum monostearate–paraffin gels on the antigen response to *Clostridium welchii* type D toxoid. When these formulations were injected subcutaneously into rabbits and guinea pigs, the level and duration of the antitoxin titer in the blood was directly related to the viscosity of the vaccine formulations studied. In attempting to explain why an iron–polysaccharide complex (IPH) marketed for human use was poorly absorbed from muscle tissue of rats, Beresford and co-workers (37) surmised that it had a higher viscosity than the other iron–polysaccharide complexes tested. Also, its molecular weight might lie beyond the range capable of penetrating the lymphatics.

Base and Salt Forms—Monash and Gibbs (73) studied the acute toxicities of a number of local anesthetics administered intraperitoneally and subcutaneously to mice. They dissolved the salts in normal saline, and prepared a suspension of the base using polysorbate 80 and normal saline. The LD_{50} data are shown in Table IV. Intraperitoneal injections of both bases and salts resulted in approximately equal toxicity. This suggests that these forms of the drugs were absorbed from the peritoneal region into the circulation at nearly similar rates. In general, absorption from the subcutaneous tissue was not as rapid as it was from the intraperitoneal region. The result was that the acute toxicity of the salt and base were both reduced in most cases. After subcutaneous injections of the two forms of the drug, the acute toxicity of the base was always less than that for the salt form of the drug. The authors stated that this occurred because the base was less water soluble and hence was absorbed more slowly. However, their results raise some unanswered questions. Why should the salt and base forms of the drug have substantially the same degree of toxicity when administered by the intraperitoneal route, yet have different degrees of toxicity when administered subcutaneously? Should not the argument concerning the lesser solubility of the base compared to its salt and the differences in their absorption rates apply equally well to both injection sites? Also, what possible influence does the polysorbate 80 and the particle size distribution of the suspension have on drug absorption rate from either absorption site?

Solvent Effects—Foldes and co-workers (53) studied the influence of solvent on the myoneural effects of succinylcholine administered intramuscularly to humans. The chloride salt of the drug was made into a 10% solution by dissolving it in water and in 0.9% saline. When succinylcholine was dissolved in water, the onset of action was more rapid and its intensity greater than when the solvent was normal saline. In the water group apnea developed in 155 (S.E. ± 15.6) sec. and lasted

TABLE IV—ACUTE TOXICITY OF SALTS AND BASES OF SOME LOCAL ANESTHETICS IN MICE (73)

	Median Lethal Dose, mg./Kg.			
	i.p. Injection		s.c. Injection	
Anesthetic	Base	Salt	Base	Salt
Tetracaine	60	60	85	50
Benoxinate	37	30	75	37
Dibucaine	50	35	60	35
Lidocaine	200	160	275	225
Procaine	225	225	1000	500
Pramoxine	300	300	900	750

881 (S.E. ± 93.4) sec. in 9 out of 10 subjects. In the saline group apnea developed in 253 sec. and lasted 993 sec. in only 3 out of 10 subjects. The authors suggested that the delayed onset and lesser intensity of action of the intramuscularly injected succinylcholine dissolved in normal saline were due to the retardation of its rate of absorption that prevented the development of a sufficiently high plasma concentration. There are at least two possible reasons for this phenomenon. First, both succinylcholine chloride solutions are hypertonic with respect to the body fluids. However, of the two, the saline solvent is more hypertonic. Thus, there should be a greater net flow of water toward the injection site in the case of the saline vehicle with the result that drug absorption rate is decreased due to the greater solvent drag effect. Second, in water the diffusing electrolyte is moving under the influence of the chemical potential gradient for that ionic species, and the electrical field produced by the motion of the oppositely charged ion or ions. The more mobile Cl^- will tend to diffuse more rapidly than the succinylcholine^{2+} species. In so doing, a microscopic charge separation will tend to occur. Since it is an experimental fact (10) that macroscopic charge separation does not occur, the diffusion velocity of the slower ion will increase, while that of the Cl^- will decrease. When succinylcholine chloride is diffusing in an aqueous medium having an appreciable sodium chloride concentration, the microscopic conditions prevailing in the solution are different from what they were before. Now there is no symmetry of the "ionic atmosphere" which was found where the drug was diffusing in water. The succinylcholine^{2+} (or tracer ion) is moving relative to a background of nondiffusing Cl^-, while in the former case all ions are diffusing with the same velocity (10). Regardless of the mechanisms involved, on the basis of the biological data provided by Foldes *et al.* (53) it would appear that the diffusion rate of the succinylcholine ion is retarded in the saline vehicle resulting in a decreased absorption rate.

Potency Testing—There exists a danger that a parenteral product will be evaluated in terms of the "active" ingredient contained in the formulation without due consideration to other pharmaceutically important substances present in the preparation, such as solubilizers, preservatives, buffering agents, and the like. Bueno-Montano *et al.* (74) studied what effect chlorobutanol, ethanol, phenol, sodium acetate, and propylene glycol might have on the *in vitro* spontaneous contractile patterns of isolated human uterus tissues. They found all of these agents to be depressant to isotonically contracting uterine smooth muscle. The authors point out that the presence of agents commonly used in commercial preparations may complicate the *in vitro* appraisal of a given product.

Foreign Particulate Matter—The parenteral product formulator must determine the origin and nature of foreign particulate matter which might be found in a product and eliminate the sources of this contamination. This problem is so important that the FDA sponsored a symposium in July 1966 entitled "The Safety of Large Volume Parenteral Solutions." Abstracts of the papers presented at this meeting and other pertinent work are included in *References 75–80.*

Scherr (81) and Zolov (82) have suggested that a possible cause of abscess formation found in some patients receiving subcutaneous emulsion therapy is due to metal particles introduced into the formulation during emulsification. In addition, Caplin (83) proposed that other foreign bodies such as cotton gauze and rubber stopper particles might give rise to untoward local reactions during emulsion therapy, although he does not cite experimental evidence for his statement.

Another source of foreign matter may arise as a result of coring when the needle is passed through the rubber closure of a vial or the skin. Magath and McClellan (84) noted when needles were inserted through stoppered vials, that rubber fragments might be cut out. They injected these particles into the gluteal muscle of guinea pigs and found that they migrated quickly from the injection site into the popliteal space. Magath and McClellan concluded that it was unlikely that the fragments would produce much damage unless they were accidently injected into a blood vessel or if they gave rise to an infection. Gibson and Norris (85) directed their research toward finding the answers to these questions: How often does a hollow needle piercing the skin core out a fragment of tissue? What is likely to cause this to occur? And what is the possible fate of the fragments? Using needles having bores in the range commonly used for intravenous solutions, they recovered skin fragments 69% of the time. They noted no real difference between new and resharpened needles. This needle coring is probably due to a very sharp or ragged back bevel. However, attempts to blunt the back bevel did not greatly reduce the number of skin fragments. Some of the fragments may remain in the needle bore, partially or completely blocking it. Some may become sus-

pended in the injection fluid or lodged in the barrel causing the piston to stick. The remainder will be ejected along with the fluid. Gibson and Norris (85) state that it is difficult to assess the clinical significance of their findings, since many thousands of injections are given daily and no harmful effects have been attributed to skin emboli. This fact suggests that little is to be feared from the injected skin fragments *per se*. However, the authors suggest that unless the skin is carefully prepared prior to the injection, there is a risk that an infection may occur at the subcutaneous, intramuscular, or pulmonary sites. Parenthetically, Dann (86) recently has provided evidence that the routine procedures for skin preparation before injections are unnecessary, unless the skin is obviously dirty. Also, pretreatment of the skin with an antiseptic was judged to be unnecessary when measle vaccine was injected intradermally and subcutaneously by means of a dermojet instrument (87). Some investigators have attempted to avoid this potential problem. Thompson *et al.* (88) used 18- to 20-gauge Huber point hypodermic needles to facilitate the injection of suspensions and to minimize the likelihood of introducing skin fragments into animals. Charlebois (89) says the problem of coring skin or closures by needles is of particular importance now with the growing popularity of epidural injections. To eliminate the possibility of injecting a core of skin into the epidural space, the physician should make the actual injection with a needle other, and smaller, than the one used to pierce the skin. To eliminate the possibility of injecting a core of the closure into the epidural space, the local anesthetic drug should *not* be obtained by piercing a pliable membrane. The container closure should be removed before withdrawing the solution. The author further concludes that no drug that has been obtained by piercing a pliable membrane should be injected into a patient, and multiple-dose vials should not be used by the medical profession.

Carcinogenesis—The product formulator should always be alert to the potential problem of carcinogenesis. Roe (90) has reviewed the evidence for carcinogenic activity of various pharmaceutical agents and preparations. Table V shows his categorization of these substances into high, medium, and low carcinogenic risk groups. Several substances listed are of interest to the parenteral product formulator. Roe comments on the significance of tumor induction in subcutaneous tissues of rats and mice. The induction of cancers in these rodents at a subcutaneous site may well be a result of physical rather than chemical carcinogenesis similar to the induction of neoplasms following the implantation of chemically inert materials. Also, rats and mice are especially susceptible to the induction of local tumors following the subcutaneous or intramuscular injection of various substances. For these reasons Roe states that it is generally agreed that not much weight can be given to positive results obtained from injections in rats and mice unless they are supported by positive results from other experiments employing other methods of exposure.

Nevertheless, the subcutaneous route is frequently used to screen substances for their possible carcinogenic activity. For example, the colors, blue VRS, green S, and brilliant blue FCF

TABLE V—ATTEMPT TO CATEGORIZE SPECIFIC PHARMACEUTICAL AGENTS AND PREPARATIONS INTO HIGH, MEDIUM, AND LOW CARCINOGENIC RISK GROUPS (90)

HIGH RISK
All biological alkylating agents, especially β-naphthyl-di-2-chloroethylamine
Urethan
Isonicotinic acid hydrazide
Arsenic
Phenylbutazone
Creosote and coal tar preparations
Estrogens
Materials such as plastic sponges used in surgery
Combination of ^{131}I and goitrogens
3-Phenyl-5β-diethylaminoethyl-1,2,4-oxadiazole
MEDIUM RISK
Penicillins given by s.c. or i.m. injection
Actinomycins given by s.c. or i.m. injection
Iron dextran, saccharated iron oxide, ferric sodium gluconate complex, and ferrous glutamate given by s.c. or i.m. injection
Tannic acid and tannins
Medicinal paraffin
Goitrogens
Cortisone
Pronetalol[a]
8-*o*-Hydroxyquinoline
Vaccine preparations from which the presence of oncogenic viruses has not been excluded
Griseofulvin
LOW RISK
Methotrexate
Sulfonamide
Chloramphenicol
Chlorpromazine
Reserpine
Thalidomide
Carbon tetrachloride and chloroform (in doses which do not cause obvious liver damage)
Injections of colloidal gold or silver
Spermicidal contraceptive preparations, except possibly those containing 8-*o*-hydroxyquinoline
Carrageenin
Carboxymethylcellulose
Progesterone and oral contraceptives
Cocarcinogenic agents including phenols, essential oils, and surface active agents

[a] Alderlin.

were tested subcutaneously in rats to determine whether they might be safe for use as food colors (91). Poirier and co-workers (92) studied the carcinogenic activities of *N*-hydroxy-2-acetylaminofluorene (*N*-hydroxy-AAF) and some of its metal chelates after subcutaneous administration into rats. They noted an approximate correlation between the retention times of the potassium salt of *N*-hydroxy-AAF and its various metal chelates and the incidence of sarcomas found at the injection site. Certain chelates (*e.g.*, Ni^{2+}, Co^{2+}, Fe^{3+}, and Cu^{2+}) had moderate to high incidences of sarcomas following one to four injections. For these chelates the half retention time, or the time when only one-half of the administered *N*-hydroxy-AAF species remained at the site, ran from 4 to 50 days. The potassium salt and the Mn^{2+} and Zn^{2+} chelates showed less activity at the injection site. Their half retention times ranged from 2.5 hr. to 2.5 days.

Hueper (93) investigated the carcinogenic potential of hypertonic (25%) solutions of the sugars: *l*-arabinose, anhydrous *d*-dextrose, *d*-galactose, lactose, *d*-levulose, *d*-maltose, *l*-sorbose, and *d*-sucrose. These solutions were administered subcutaneously to rats and mice twice a week for periods up to 2 years. Only 2 rats out of 60 animals given the sorbose solution developed sarcomas at the injection site. No tumors were found at this site in rats and mice injected with the other sugar solutions, injected with water, or traumatized by needle puncture. One conclusion of interest was that nonspecific chronic irritation (*e.g.*, repeated water injections and needle trauma) did not represent a mechanism for carcinogenesis in this case.

It is of interest that Roe (90) catagorized mineral oil as being in the medium carcinogenic risk group as shown in Table V, since it is used in the food-processing industry of this country (94). Boitnott and Margolis (95) studied lymph nodes taken from the porta hepatis at autopsy. They found in 38 cases (78%) that the nodes contained large oil droplets that were deposits of liquid saturated hydrocarbons. They state that in all likelihood these hydrocarbons were derived in large part from absorbed mineral oil from the intestinal tract. The high incidence of oil droplets found in these lymph nodes seemed out of proportion to laxative and other medicinal uses of mineral oil, although quantitative data on this point are not available. They cite evidence that the use of mineral oil in the food-processing industry is currently estimated to result in the ingestion of an average of 47.5 Gm. of mineral oil per capita per year in this country. Although there are no data on the absorption of mineral oil at dosage levels comparable to those present in foods, they felt that this yearly intake seemed adequate to explain the amounts and incidence of the oil found in their autopsy specimens.

The FDA recently terminated many of the investigational new drug (IND) applications for clinical investigations of a mineral oil-emulsifier product. Allergists had been adding antigens to the product in order to treat specific allergies of patients. The mineral oil and emulsifier, generally mannide monooleate, tended to act as a sustained-release medium for the antigens, permitting less frequent injection of the patient. Tests used for measuring the biological safety of mannide monooleate have been detailed by Berlin (96). Fiero (97) has reviewed the industrial procedures used to purify the grade of mineral oil used in medicine and pharmacy. The position of the FDA and the Committee on Mineral Oil Adjuvant Preparations concerning the hazards of mineral oil adjuvants has been detailed in a letter sent to an allergist (98). Allergists (99–102) have responded to the action taken by the FDA in letters and editorials. It appears that considerably more testing will be required before a safe, generally accepted repository material will be available for routine clinical use.

Injection Pain—The intensity of pain resulting from some intramuscular or subcutaneous injections may range from a mild or transient unpleasant sensation to a severe reaction which might necessitate the termination of the therapy. Since pain may arise from parenteral injections to these regions, the product formulator should be aware of some of the causes of such pain. Travell (103) has classified the different causes of the immediate pain following injections. Immediate pain may arise from: (*a*) local irritation due either to the antiseptic used on the skin or the properties of the parenteral formulation (*e.g.*, pH, tonicity, or chemical action on the tissues), (*b*) mechanical trauma due to the introduction of the needle to the injection site, or to the sudden distension of the tissues resulting from a too rapid discharge of the syringe contents, and (*c*) abnormal sensitivity of the tissue at the injection site. She states that some of the chief causes of delayed pain of injection include infection, aseptic irritation and necrosis, antigenic reactions, reactions to pyrogens, and painful muscle spasm.

The pain may be classified according to its origin as cutaneous, subcutaneous, or intramuscular pain. Cutaneous pain originates from the

stimulation of sensory nerve receptors found in the skin. To eliminate skin pain, Travell recommends that a sharp needle, free of barbs, having the smallest diameter compatible with the viscosity of the solution be used. A cooling spray (*e.g.*, ethyl chloride) should be applied to the site for 2 or 3 sec. before the injection, and the instant the liquid disappears, the needle should be quickly inserted through the skin. Weeks and Travell (104) found that at a skin temperature of 15° one-third of the subjects had anesthesia to a needle prick, while at 10° or below all had anesthesia to the same stimulus. Travell (103) states that the subcutaneous tissues are relatively insensitive to the movement of the needle unless the fascia of the underlying muscle tissue is entered and pulled. Subcutaneous pain can be induced by the injection of an irritant material or a hypotonic solution such as distilled water. The addition of a local anesthetic to the vehicle injected may reduce, but not entirely eliminate, the immediate pain response from the subcutaneous tissues. In contrast to skin, normal voluntary muscle tissue is practically insensitive to the insertion of a needle. However, hypersensitive or "trigger" areas may exist in the muscle tissue of some patients. When these areas are stimulated by pressure, palpation, or the insertion of a needle, pain may result which can be referred to adjacent regions. It can be seen from this brief account that pain derived from subcutaneous or intramuscular injections may arise not only from the formulation injected, but from the techniques used in making the injection, as well as certain predisposing conditions found in the target tissue.

A classic method used by the parenteral product formulator to minimize injection pain has been to add a local anesthetic such as benzyl alcohol, procaine, lidocaine, and the like to the preparation (103, 105). Miller (106) has been critical of the use of such local anesthetics in parenteral products on the grounds that the pain reflects tissue damage, and this is not corrected merely by blocking of the sensory nerves in the region. Perhaps it would be better if the offending substance could be identified and the product modified in its formulation so that the amount of irritation caused could be reduced.

Untoward Reactions—Fortunately, over the years there have been remarkably few serious untoward reactions reported in the literature directly resulting from the techniques used in injecting parenteral formulations. Such injuries may provide the basis for malpractice suits. In a recent case over a quarter of a million dollars was awarded to a patient for damages incurred after an intragluteal injection of penicillin (107). Table VI lists some of the drugs associated with severe complications following intra-arterial or intramuscular injections. The injuries include nerve damage (*e.g.*, sciatic), gangrene of extremities, progressive fibrosis of muscle (*e.g.*, quadriceps), and others. Lachman (45) states there is complete agreement among all authors that damage to the sciatic nerve after intragluteal injection is essentially independent of the drug used. While the damage has in large measure been attributed to the techniques used for making the injection, physical and possibly chemical properties of the injected substances must also have contributed to the injuries reported. A number of authors have discussed injection procedures that should be followed to minimize complications (18, 45, 108–116).

Tables VII and VIII list some of the reported milder local reactions after the administration of selected drug solutions and suspensions. The preparation of Tables VI–VIII was facilitated by recent advances in the abstracting of adverse drug reaction reports (117–119).

At present the causes of many of the local tissue reactions following drug administration are not well understood. Part of the difficulty stems from the fact that the mechanisms by which body

TABLE VI—SUBSTANCES ADMINISTERED IN REPORTED COMPLICATIONS FOLLOWING INTRA-ARTERIAL AND INTRAMUSCULAR INJECTIONS

Drug	Ref.
Ascorbic acid	(150)
Barbital and aprobarbital mixture	(151)
Bismuth tartrate potassium	(152)
Caffeine sodium benzoate	(153)
Chloramphenicol and the succinate ester	(153, 154)
Chlorpromazine	(18)
Digitoxin	(153)
Epinephrine	(155)
Erythromycin	(154)
Gamma globulin	(153)
Iron dextran	(153)
Meperidine HCl and promethazine HCl mixture	(149)
Paraldehyde	(157)
Penicillin and its salts in various vehicles	(107, 109, 150, 151, 153, 154, 158–166)
Promazine and salts	(149, 153)
Quinine and salts	(167, 168)
Sodium amobarbital	(148, 149)
Sodium thiopental	(121, 149, 151, 172)
Streptomycin and salts	(150, 153, 158, 161, 166)
Sulfonamides	(169–171)
Tetracycline and salts	(108, 153, 154, 166)
Thialbarbital[a]	(156)
Vitamin K	(150, 153, 154)

[a] Trademarked as Kemithal by Imperial Chemical Industries, Ltd., Wilmslow, Cheshire, England.

TABLE VII—LOCAL REACTIONS REPORTED FOR SELECTED DRUG SOLUTIONS[a]

Active Ingredient	Vehicle	Route of Administration and Untoward Local Reactions in One or More Humans or Animals	Species	Ref.
Ampicillin (as sodium salt)	.....	i.m.: severe pain at injection site	Human	(173)
Bis-(4-hydroxyiminomethyl-pyridine-1-methyl) ether dichloride[b]	.....	i.m.: dull, burning pain following injection using plastic self-injection ampul, which is manually emptied, but painless when automatic type is used. Pain assumed to be caused by needle movement	Human	(135)
Butyl-*p*-aminobenzoate 5%, procaine 1%, procaine HCl 0.25%[c]	Propylene glycol 78%, polyethylene glycol 300 2%, in distilled water	Intercostal nerve block: neuritis, inflammatory reactions, and lasting absence of nerve function resulted	Human	(174, 175)
Capreomycin	.....	i.m.?: bleeding at injection site	Human	(176)
Capreomycin	.....	i.m.: s.c. "lumps" at injection site from first week on	Human	(177)
Capreomycin	.....	i.m.: mild pain or stinging initially, palpable nodules, diffuse induration at injection site	Human	(178)
Capreomycin	.....	i.m.: sterile abscess of hip (due to injection technique?)	Human	(179)
Cephaloridine	Sterile water or isotonic saline	i.m.: moderately severe pain at site	Human	(180)
Chloramphenicol sodium succinate	Aqueous or saline	i.m.: local pain at injection site, sometimes severe	Human	(181)
Dihydrotriazine hydrochloride[d]	40% benzyl benzoate in castor oil	s.c.: local induration and frequently open lesions at injection site	Mice	(88)
Diphtheria–tetanus vaccine	No lidocaine	s.c.: initial pain occurring immediately after the injection and reaching a peak within a few minutes. Pain partly due to mechanical distension caused by the fluid, but mainly due to some unknown factor in the vaccine	Human	(105)
	Lidocaine added	s.c.: 12 of 13 volunteers felt less pain with the added lidocaine; one noted no difference		
Emulsion therapy with various pollens or molds	Adjuvant: 35% mannide monooleate, 65% mineral oil	s.c.: abscess at injection site	Human	(82)
Iron–dextran complex[e]	Aqueous	i.m.: injection abscess (may be due to faults in aseptic technique). Injections very painful and produced staining of skin	Human	(183)
Iron–sorbitol–citrate complexes[f]	Aqueous	i.m.: pain and skin discoloration at injection site	Human	(184, 185)
Nafcillin[g]	.....	i.m.: local pain and induration when given frequently in the same area	Human	(186)
Oxytetracycline ethanol–ammonium magnesium salt	80% propylene glycol in water, 2% lidocaine	i.m.: single injections almost invariably well tolerated, with only occasional complaints of mild pain lasting 15–30 min. Multiple injections usually well tolerated when given at 24-hr. intervals. More frequent injections occasionally associated with discomfort and more lasting pain	Human	(187)
N-(Pyrrolidino-methyl) tetracycline	Water, lidocaine	i.m.: local pain or discomfort at injection site	Human	(189)
N-(Pyrrolidino-methyl) tetracycline[h]	Water, lidocaine, ascorbic acid	i.m.: little discomfort at time of injection, but 15–20 min. later subjects complained of moderately severe pain at injection site lasting 1.5–2.5 hr. Without lidocaine same formulation caused moderate to severe pain from 1 min. to 2–3 hr. after	Human	(190)
Sodium fusidate[i]	Aqueous	s.c., i.p.: local tissue injury with necrosis at injection site, probably this irritating effect connected with the surface-active properties of the drug	Mice	(182)

(*Continued on next page*)

TABLE VII (*Continued.*)

Active Ingredient	Vehicle	Route of Administration and Untoward Local Reactions in One or More Humans or Animals	Species	Ref.
Sodium pentobarbital	2% benzyl alcohol in 60% propylene glycol in water, pH = 10.5	s.c.: pain usually present, firm nodule at injection site; i.m.: usually no pain	Human	(188)
Thiamine hydrochloride	Aqueous	i.m.: intense pain lasting on av. 122 sec. Addition of 0.5% procaine HCl reduced duration of pain to av. of 20 sec.	Human	(103)
7-(Thiophene-2-acetamido) cephalosporanate sodium[j]	Normal saline	i.m.: slight to moderately severe pain at injection site	Human	(191)
Trypsin	Gelatin in double-blind (DB) study Saline in alternating (A) study	i.m.: in "DB" study *ca.* 30% of subjects developed local tenderness at injection site with nodule formation. In "A" study local reaction was 10%, no nodule formation occurred	Human	(201)
Vitamin D_3 hydrosol	Water, ethanol polyoxyethylene ricinoleic acid	i.m.: hemorrhagic focal area 1 week after initial injection. After 3 weeks calcification occurred on muscle surface and dermis of skin in contact with musculature	Rat	(192)

[a] Consult references cited for pharmacologic or possible toxic effects in other tissues. [b] Toxigonin. [c] Efocaine. [d] 4,6 Diamino-1-(*p*-chlorophenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine hydrochloride (DHT). [e] Imferon. [f] Jectofer. [g] Unipen. [h] Syntetrin. [i] Fucidin. [j] Keflin.

TABLE VIII—LOCAL REACTIONS REPORTED FOR SELECTED DRUG SUSPENSIONS[a]

Active Ingredient	Vehicle	Route of Administration and Untoward Local Reactions in One or More Humans or Animals	Species	Ref.
Betamethasone sodium phosphate + betamethasone acetate[b]	Aqueous	i.m.: stinging, burning, or acute pain at injection site	Human	(193)
Cycloguanil pamoate	Lipid: 40% benzyl benzoate in castor oil	s.c.: transient palpable masses, and open lesions	Mice	(88)
	Aqueous: 1.5% pectin + 0.1% polysorbate 60	s.c., i.m.: palpable masses occurred only with very large injections	Monkey	(88)
Cycloguanil pamoate	40% benzyl benzoate in castor oil	i.m.: suppurating abscess developed at injection site	Human	(51)
Hydrocortisone acetate	0.9% NaCl, 0.5% Na carboxymethylcellulose, 0.1% methylcellulose, 0.19% polysorbate 80, 0.24% methylparaben, 0.026% propylparaben, water *q.s.*	i.t.[c]: pain at injection site or referred pain to face or behind eye	Human	(194)
Insulin	Aqueous	s.c.: local reactions may occur in the form of red painful indurated areas at injection site and may persist for 2–3 weeks, then gradually disappear. Insulin atrophy i.c.[d]: induration, necrosis, ulceration and scarring	Human	(195)
Monosodium urate	5% dextrose in water	s.c., i.d.[e]: erythema, induration, local temperature elevation	Human	(196)
Monosodium urate monohydrate	50% glycerin in water	s.c.: erythema, induration, warmth, tenderness	Human	(33)
Sodium orotate	50% glycerin in water	s.c.: erythema, induration, warmth, tenderness	Human	(33)
Tetracycline phosphate	Lidocaine, monoacetin	i.m.: local discomfort or pain	Human	(189)
Triamcinolone acetonide	Aqueous	i.m.: subcutaneous fat atrophy	Human	(197)
Triamcinolone acetonide	0.9% benzyl alcohol, 0.75% Na carboxymethylcellulose, 0.04% polysorbate 80 in water, NaCl *q.s.* for tonicity	i.m.: atrophy in area of injection, s.c. fat necrosis at injection site	Human	(198)
Triamcinolone acetonide	Aqueous	i.m.: atrophy of s.c. fat, local abscess	Human	(199)
Triamcinolone acetonide	Aqueous	s.c.: atrophy of keloids	Human	(200)

[a] Consult references cited for pharmacologic or possible toxic effects in other tissues. [b] Celestone Soluspan. [c] Intraturbinate. [d] Intracutaneous. [e] Intradermal.

cells recognize injected foreign materials are complex. An explanation of these local tissue reactions is not generally possible at the molecular level. Some of the research concerned with foreign body reactions and the mechanisms of phagocytic discrimination has been reviewed by Boyden (120). On a macroscopic level some of the causes for these local tissue reactions include the following. After injections of sodium thiopental, Davies (121) believed that necrosis of tissue at the site was caused in part by direct toxic action of the solution, and in part by infection. Subcutaneous nodule formation following "intramuscular" injections has been attributed to erroneous deposition of the formulation into the subcutaneous region (45). After an intramuscular injection, painful subcutaneous nodules (15) sometimes form because leakage of the solution occurs back along the needle tract. In other cases the subcutaneous nodule formation is caused by the deposition of material into the subcutaneous region because needles of insufficient length were used (18). McLean *et al.* (122) discusses some of the causes of local untoward reactions to repository therapy; they conclude that a sterile abscess forms because a foreign body type of irritative process occurs. Goldman (123) discusses the local reactions following intra- and sublesional injections of corticosteroid suspensions. While local reactions are uncommon, they include (*a*) pain and discomfort, (*b*) hemorrhage, (*c*) atrophy, (*d*) secondary infection (local and diffuse), (*e*) pigmentation changes, (*f*) hypersensitivity reactions, and (*g*) panniculitis. If a relatively large amount of corticosteroid crystals is injected superficially, a slough may develop which appears to be due to a blockage of local circulation as well as changes in the local ground substance. If sloughing does not occur, the depot may remain visible in the skin for long periods of time.

Hanson (124) investigated some of the local toxic effects of the antibiotics: chloramphenicol, chloramphenicol sodium succinate, oxytetracycline, tetracycline, and their vehicles after subcutaneous and intramuscular injections into rabbits. Cioc and von Schilling (125) describe histological changes in rabbit and pig muscle tissue produced by injections of a number of drugs.[6]

Newer Techniques—Up to this point, the traditional syringe-and-needle method of introducing drugs subcutaneously and intramuscularly has been discussed almost exclusively. The history of the development of injection therapy has been thoroughly discussed by several authors (1, 126–128) and will not be reviewed again at this time. However, newer devices are appearing on the market. Under certain circumstances they may have advantages over the more conventional devices. Some of these instruments involve the principle of jet injection whereby the drug solution is forced through the epidermis and dermis as a fine dispersion under high pressure (40, 42, 87, 129–133). Some reports in the literature indicate that dosing errors might result from the use of some jet-injection instruments due to calibration problems (130, 132). (See Table IX.)

TABLE IX—SUMMARY OF RECORDINGS COMPARING THE DOSAGE-DELIVERANCE RELIABILITY OF TWO JET-INJECTION INSTRUMENTS AND A PIPET TECHNIQUE (132)

Anticipated Vol. Delivered, ml.	Mean % of Anticipated Vol. Actually Delivered		
	Jet No. 1	Jet No. 2	Pipet
1.0	82	113	100
0.7	94	119	101
0.5	97	128	102
0.2	39	170	98

Another instrument using the syringe-and-needle principle is the Hypule, which is designed for unit dosage systems. It is a prefilled, disposable device which delivers an exact volume of solution subcutaneously (134). Erdmann *et al.* (135) studied the absorption and excretion of an alkyl-phosphate antidote,[7] following its injection muscularly into humans, using two types of sterile, self-injection ampules: a plastic type,[8] which the user empties manually after puncture, and an automatic type,[9] which is self-emptying.

Prolonged-Action Formulations—One of the problems of evaluating the absorption rates of solids administered as suspensions *via* the subcutaneous or intramuscular routes is that no convenient method exists today for estimating the surface area of the solid exposed to the absorbing membranes while *in situ*. The effective area of the suspension exposed to the absorbing tissues varies according to the injection techniques used and the physical properties of the suspension. Since the effective area of solid exposed to tissues is not known, precise estimates of the drug absorption rate

[6] Dipyrone (50%), thiamine, ferro dextran complex, liver extract, vitamin B_6, dihydroergocornine-cryptine-cristine methanesulfonate (ergot fluid extract), trimethyl 3-oxyphenyl ammonium bromide, liver extract with vitamins B_1 and B_{12}, α-(*N*-β-diethylaminoethyl)aminophenylacetic acid, calcium bromide, and eucalyptol, guaiacol, and sage compound.

[7] Toxogonin, E. Merck AG, Darmstadt, Germany.

[8] Gotole, Penicillin-Gesellschaft Dauelsberg, Göttingen, Germany.

[9] Autule, Penicillin-Gesellschaft Dauelsberg, Göttingen, Germany.

cannot be made, since the absorption rate is directly proportional to this area at any time. Where applicable one quantitative method of evaluating the absorption rate of solids is the use of the pellet implantation technique of drug administration. For example, suppose the parenteral product formulator intends to formulate a suspension and has several salt, polymorphic, or solvate forms of a given drug. He should be able to establish in a given species and a given absorption site the rank order for the mean absorption rates per mean pellet areas. From this and other information at his disposal, the investigator could make a preliminary estimate as to which of the drug forms should be studied in detail for possible use in the suspension formulation.

For a single implanted sphere of a drug the weight of solid at any time can be calculated (136) from:

$$W = \frac{\pi\rho}{6}(D^0 - kt)^3 \qquad \text{(Eq. 7)}$$

and for an implanted disk:

$$W = \frac{\pi\rho}{4}(D^0 - kt)^2(h^0 - kt) \qquad \text{(Eq. 8)}$$

where W is the weight of the solid at any time, ρ is the apparent density of the solid, D^0 is the initial diameter of the disk or sphere, h^0 is the initial height of the disk, k is the mean absorption constant having dimensions of length $\times$ time^{-1}, and t is the duration of pellet implantation. It is assumed that D^0 or $h^0 \geqslant kt$, and that the shape of the solid is not distorted during the implantation period. Once the value of k has been determined for one geometric shape of a particular drug in a given animal species at a given absorption site, it can be used to estimate the probable absorption rate characteristics for other geometric shapes of the same drug.

Recently, Ballard and Goyan (137) made use of an analog computer to study the kinetics of drug absorption, distribution, metabolism, and elimination of a model drug, sulfadiazine, in all forms following its administration as subcutaneous disk-shaped implants in rats. Since the partially absorbed solid was recovered from the implantation site, the mean absorption constant, k, could be determined by Eq. 8. The cumulative amount of free and acetylated drug was followed as a function of time. The kinetic model used is shown in Fig. 1. The term Si is the amount of drug in the disk at the implantation site, S is the amount of free drug in the fluids of distribution in the animal, Se is the cumulative amount of free drug excreted unchanged in the urine, Sa is the amount of acetylated drug in the fluids of distribution in the animal, and Sae is the cumulative amount of acetylated drug excreted into the urine up to any time. For purposes of achieving material balance between the amount of drug absorbed and that actually recovered in the urine as free and acetylated drug, it was necessary to postulate an amount of drug or metabolite, Su, in some unknown compartment. If Su represents free drug, then it is the cumulative amount excreted to any time by a route other than *via* the urine. In this case k_5 would be ignored. If Su is the amount of undetected metabolite to any time, then Sue is the cumulative amount of that metabolite excreted by some route to any time. While the absorption constant, k, has units of length $\times$ time^{-1}, the constants k_1, k_2, k_3, k_4, and k_5 are assumed to be first-order rate constants for the respective processes in units of time^{-1}. In Fig. 2, curve 1 (Se) shows the cumulative amount of free drug, and curve 2 (Sae) shows the cumulative amount of acetylated drug excreted *via* the urine as a function of time. Curve 3 (Su or Sue) is defined by material balance considerations and is the cumulative amount of free drug (Su), or the cumulative amount of an unspecified metabolite (Sue) excreted *via* an unknown route to any time. In the model the constant k_2 is assumed to be very large compared to k_1, and k_5 is very large compared to k_4. Curve 4 (S) represents the amount of free drug in the fluids

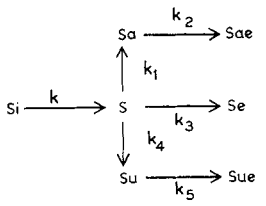

Fig. 1—Kinetic model showing disposition of sulfadiazine in all forms following its administration as a subcutaneously implanted disk-shaped pellet in a rat (137). Consult text for details. [Reprinted with permission from Med. Biol. Eng., *4, 483 (1966).]*

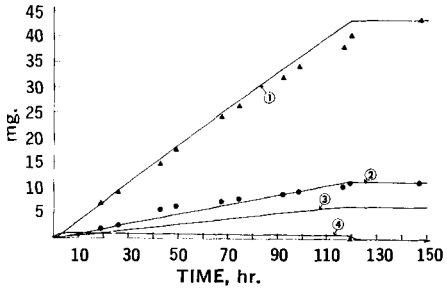

Fig. 2—Analog computer plots for rat B. Consult text for details concerning curves 1–4. Unpublished data (137). Key: ▲, free sulfadiazine; ●, acetylated sulfadiazine.

of distribution in the animal at any time. With curve 4 (S) one notes that after the peak amount of free drug is reached in the fluids of distribution about 10 hr. after implantation, there is a very gradual decline in amount until the pellet is removed (vertical arrow at 118.3 hr.), whereupon an exponential drop occurs. This gradual decline in amount would be expected because the pellet in losing mass with time, is also changing area very slowly.

There are several advantages in using the analog computer in *in vivo* drug kinetic studies such as the one illustrated here. First, it is generally simple to write out the differential equations for the proposed steps in the kinetic model shown in Fig. 1, and it is often possible to integrate these equations (137). However, their direct solution becomes extremely difficult by ordinary digital methods, particularly when one or more rate constants are not known exactly. Second, it is possible with an analog program to plot the probable amount-time course of drug or metabolites in compartments *not* analyzed directly in the investigation (*e.g.*, curves 3 and 4, Fig. 2). Third, once the pharmacokinetic constants have been determined for a particular shape of a drug implant in a given species and site of implantation, it should be possible to simulate with the computer the distribution and elimination time courses of the drug and its metabolites with other implant shapes and implantation times, even though the experiment was not actually undertaken *in vivo*.

A striking example of how the usefulness of a drug can be enhanced by preparing a repository formulation can be seen with the antimalarial drug cycloguanil pamoate. Shortly after chlorguanide was introduced, *in vitro* evidence indicated that the intact drug was essentially inactive, and that an active metabolite, 4,6-diamino-1-(*p*-chlorophenyl) - 1,2 - dihydro - 2,2 - dimethyl - *s* - triazine (DHT), was responsible for the activity of the drug. The hydrochloride salt of DHT is less active than chlorguanide against *P. cynomolgi* in monkeys when given orally or parenterally. The decreased activity of DHT compared to chlorguanide in monkeys is believed to be due to the rapid excretion of the soluble metabolite (88).

The pamoate salt of DHT, cycloguanil pamoate, has a solubility of 0.03 mg./ml. in pH 7, 0.1 *M* phosphate buffer. The drug has been formulated as parenteral suspensions in a lipid vehicle consisting of 40% benzyl benzoate and 60% castor oil, and in an aqueous vehicle consisting of 1.5% pectin and 0.1% polysorbate 60[10] in water. The drug was injected intramuscularly or subcutaneously as a 10 to 25% suspension in the vehicles described above. As a result of their experiments, the authors (88) made the following tentative generalizations regarding the repository effects of cycloguanil pamoate against *P. cynomolgi* in monkeys: the type of vehicle had little influence, the period of protection was related to the amount of drug per injection site, injection of a given amount at a single site was as effective as similar injections at multiple sites, and either intramuscular or subcutaneous routes were used without markedly different results.

From a pharmacokinetic standpoint, the rate-limiting step in the disposition of cycloguanil pamoate in the body is the dissolution rate of the salt in the fluids present at the injection site. As a consequence of the slow release of the drug from this site and the rapid excretion of the active metabolite *via* the urine, low plasma levels have been found. The plasma level of DHT following the injection of cycloguanil pamoate would mimic in many ways an extremely slow intravenous infusion of a soluble salt of DHT. If cycloguanil pamoate were administered as an implanted disk instead of as a suspension having an ill-defined area in contact with the tissues, the amount of free DHT in the fluids of distribution in the animal at any time would be represented qualitatively by the shape of curve 4 in Fig. 2. Since certain strains of malaria parasites have become increasingly resistant to some of the antimalarial drugs, attempts have been made to prepare parenteral repository preparations containing mixtures of drugs. This work has been reviewed by Powell (138).

The use of computers in pharmacokinetic studies has been discussed by Wagner (139). Analog, digital, and hybrid computers have been used principally for the rapid numerical analysis of data and for pharmacokinetic simulation. Wagner (139) points out that the equations and models used are always oversimplifications of the real system and their appropriateness in a given experiment can be judged only by their ability to describe the observed data and the accuracy with which they make predictions of future observed data. Some of the examples described in Wagner's paper include a pharmacokinetic analysis of the serum concentrations of linocomycin following an intramuscular injection of the drug hydrochloride, an analog computer prediction of the multiple-dose serum levels of lincmycin observed after a series of constant-rate intravenous infusions, and the digital computer simulation of serum levels produced by depot intramuscular preparations of lincomycin.

[10] Tween 60, Atlas Chemical Industries, Inc., Wilmington, Del.

In the past few years a number of other prolonged-action formulations have been reported on in the literature. Rizkallah and Taymor (140) gave, to normal females, intramuscular injections of various combinations of dihydroxyprogesterone aceophenide and estradiol enanthate in an attempt to discover which formulation would give the most satisfactory cyclic uterine bleeding response, yet produce minimum side effects. Kowarski and Bar-Natan (141) reported details of the biological responses to a number of prolonged-action diethylstilbestrol formulations intended for subcutaneous administration. Laffan and co-workers (142) studied the prolonged-action effects of fluphenazine enanthate dissolved in sesame oil after subcutaneous administration to animals. One preliminary report (143) casts doubt on the effectiveness of this formulation given intramuscularly in treating some schizophrenic patients. Linna *et al.* (144) managed to produce experimental hypertension in rats by prolonged subcutaneous administration of norepinephrine bitartrate in an oily vehicle.

In summary, this review has attempted to consider some of the biopharmaceutical aspects of drug absorption from subcutaneous and intramuscular routes of administration. A portion of the literature dealing with the formulation and administration of such parenteral formulations also has been discussed. From the foregoing it is clear that there are many areas where more basic research is needed. If this review stimulates others to conduct such research, one of the several goals of this paper will have been achieved.

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Keyphrases

Biopharmaceutics, drugs–parenteral
Drug administration—subcutaneous, intramuscular
Endothelial tissue—drug absorption
Diffusion—drug absorption
Phagocytosis—drug absorption
Biological factors—drug absorption
Drug formulations—absorption, biological factors

Research Articles

Procaine Interaction with the Corneal Surface and its Relation to Anesthesia

By VICTOR F. SMOLEN and FREDERICK P. SIEGEL*

The effects of pH and procaine on the apparent density of charged groups fixed to the corneal surface of guinea pigs were studied. The net density of fixed charge was found to be negative over the pH range of 5 to 9 and decreased in the presence of procaine. These results are interpreted in terms of procaine interaction with the surface and its implications with regard to the factors that may influence the characteristics of the anesthetic response. The electrometric method employed allowed the study to be performed under physiological conditions using live animals. The method is general and can be equally applied to study other drug–tissue interactions.

THE CORNEA possesses a moist surface primarily composed of amphoteric colloids; pH and the interaction of solutes determine the extent to which attached groups are ionized and the resulting net density of fixed charge on the surface. The present study is an effort to elucidate the role of these factors in the mechanism by which the effectiveness of procaine in the eye is increased in alkaline solution (1–4).

An electrometric method modified from Joseph *et al.* (5, 6) was used to determine the apparent fixed charge density of the corneal surface of the guinea pig in response to variations in the pH of applied buffer solutions in the presence and absence of procaine. The cornea is particularly suited to this approach due to its relative simplicity and accessibility. It has also been the subject of many investigations with local anesthetics. Reliable pharmacological data particularly with procaine were found to be readily available (1). The method is generally applicable to the study of other tissue surfaces and other drugs as well. It allows the results to be obtained under nearly physiological conditions without damage to the tissues involved. Thus, in the present study, the apparent density of fixed charge of the corneal surface was determined under the same conditions that are known to influence its anesthetic response to procaine.

THEORETICAL

The dissociation of counterions from ionogenic groups covalently bonded to a tissue surface or the absorption of ions onto neutral surface sites gives

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