Long-Acting Parenteral Drug Formulations

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Introduction

Unlike transdermal drug administration, by which percutaneous absorption of drugs is always limited by the impermeable stratum corneum, and oral drug administration, by which bioavailability of drugs is often subjected to variation in gastrointestinal absorption and the biotransformation by hepatic "first-pass" metabolism, parenteral drug administration, especially intravenous injection, can gain easy access to systemic circulation with complete drug absorption and therefore reach the site of drug action rapidly.

Parenteral drug administration via intramuscular or subcutaneous injection, while not so fast-acting as an intravenous injection, still achieves therapeutically effective drug levels fairly rapidly if the drugs are administered in aqueous solution formulations. This rapid drug absorption, unfortunately, is also accompanied by a rapid decline in blood drug levels. The usual outcome is production of a fairly rapid onset, but a relatively short-acting therapeutic response. For the sake of effective treatment, it is desirable to maintain the blood drug levels within the therapeutic effective concentration range for the length of a treatment.

Continuous intravenous infusion is recognized as a superior mode of drug administration which can be tailored to maintain a constant and sustained drug level within a therapeutic concentration range for as long as required for effective treatment. Additionally, it also provides a means of direct entry into systemic circulation for drugs which are labile to hepatic "first-pass" metabolism and/or suspect of producing gastrointestinal incompatibility. Unfortunately, such a mode of drug administration entails certain risks and, therefore, necessitates continuous hospitalization during treatment and requires close medical supervision.

To duplicate the benefits of continuous intravenous drug infusion without its potential hazards, much effort has been invested in the development of depot-type parenteral controlled drug release formulations. These development efforts have generated a number of injectable depot formulations, e.g., penicillin G procaine suspensions (Duracillin®, Lilly; Crysticillin®, Squibb; Wycillin®, Wyeth), cyanocobalamin-Zn-tannate suspensions (Depinar®, Armour), medroxyprogesterone acetate suspensions (Depo-Provera®, Upjohn), fluphenazine enanthate and decanoate in oil solutions (Prolixin Enanthate® and Prolixin Decanoate®, Squibb), ACTH-Zn-tannate/gelatin preparations (H.P. Acthar®, Armour), microcrystalline desoxycorticosterone pivalate in oleaginous suspension (Percorten® Pivalate, Ciba), testosterone enanthate (Delatestryl®, Squibb), testosterone enanthate/estradiol valerate in ethyl oleate BP repository vehicle (Ditate-DS®, Savage), nandrolone decanoate injection (Deca-Durabolin®, Organon), and Insulin-Zinc suspensions (Ultralente®, Lente®, and Semilente®, Novo), to name just a few.

Parenteral administration of a drug in depot formulation of aqueous or oleaginous suspension or oleaginous solution into a subcutaneous or muscular tissue results in the formation of a depot at the site of injection. This depot acts as a drug reservoir which releases drug molecules continuously at a rate determined to a large extent by the characteristics of the formulation, leading to the sustained absorption of drug molecules from the formulation. The nature of the vehicle used, either aqueous or oleaginous, and the physico-chemical characteristics of the drug or its derivatives as well as the interactions of drug with vehicle and tissue fluid will determine the rate of drug absorption and, hence, the duration of therapeutic activities.

Sustained release of parenteral drugs from depot formulations, in many cases, reduces the inherent disadvantages of conventional parenteral drug administration. Benefits to be derived from parenteral controlled drug release formulations may be said to relate primarily to achievement of a constant and sustained therapeutic drug level with a reduced frequency of injection. In other words, the injectable depot formulation is developed with the primary objective of simulating the continuous parenteral drug administration of intravenous infusion on a more practical basis. It often results in such additional benefits as reduced drug dose, decreased side effects, and/or improved drug utilization.

Approaches to Parenteral Controlled Drug Administration

Several pharmaceutical formulation approaches may be applied to the development of parenteral controlled drug

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release formulations. The most commonly used techniques are:

a. Use of viscous, water-miscible vehicles, such as aqueous solutions of gelatin or polyvinylpyrrolidone.
b. Use of water-immiscible vehicles, such as vegetable oils, plus water-repellent agents, such as aluminum monostearate.
c. Formation of thixotropic suspensions.
d. Preparation of water-insoluble drug derivatives, such as salts, complexes, and esters.
e. Dispersion in polymer beads or microcapsules, such as lactide/glycolide homopolymers or copolymers.
f. Co-administration of vasoconstrictors.

These techniques may be used alone, such as aqueous insulin-zinc suspensions, or in combination, such as penicillin G procaine suspension in vegetable oil gelled with aluminum monostearate.

Application of these techniques has produced a variety of depot formulations, which may be classified on the basis of the processes of controlled drug release. They are:

Dissolution-Controlled Depot Formulations

In this type of depot formulations, the rate of drug absorption is controlled by slow dissolution of the drug particles in the formulation or in the tissue fluid surrounding the formulation. Rate of dissolution (1), $(Q/t)_d$, under sink conditions, is defined by:

$$\left(\frac{Q}{t}\right)_d = \frac{S_a D_s C_s}{\delta_d} \qquad \text{(Eq. 1)}$$

where S_a is the surface area of drug particles in contact with the medium, D_s is the diffusion coefficient of drug molecules in the medium, C_s is the saturation solubility of the drug in the medium, and δ_d is the thickness of the hydrodynamic diffusion layer surrounding the drug solid.

Basically, there are two approaches that can be utilized to control the dissolution of solid drug to prolong absorption and, hence, therapeutic activity of the drug.

Formation of Salt/Complexes with Low Aqueous Solubility. Aqueous soluble basic or acidic drugs can be rendered depot effective by transformation into salts with extremely low aqueous solubility. Typical examples are the preparation of penicillin G procaine (C_s = 4 mg/ml) and penicillin G benzathine (C_s = 0.2 mg/ml) from aqueous soluble alkali salts of acidic penicillin G, and the preparations of naloxone pamoate (2) and of naltrexone-Zn-tannate (3, 4) from the aqueous soluble hydrochloride of basic naloxone and naltrexone, respectively.

Aqueous suspensions of penicillin G benzathine (Bicillin®, L-A, Wyeth), of penicillin G procaine (Crysticillin A.S., Squibb), and of penicillin G benzathine-penicillin G procaine combination (Bicillin C-R, Wyeth) and oleaginous suspensions of penicillin G procaine, of naloxone pamoate, and of naltrexone-Zn-tannate in vegetable oils gelled with aluminum monostearate all produce prolonged therapeutic activities.

Suspension of Macrocrystals. Large crystals are known to dissolve more slowly than small crystals. This is known as the "macrocrystals principle" and can be applied to control the rate of drug dissolution. Typical examples are the aqueous suspension of testosterone isobutyrate for intramuscular administration (Fig. 1) and of diethyl stilbestrol monocrystals for subcutaneous injection.

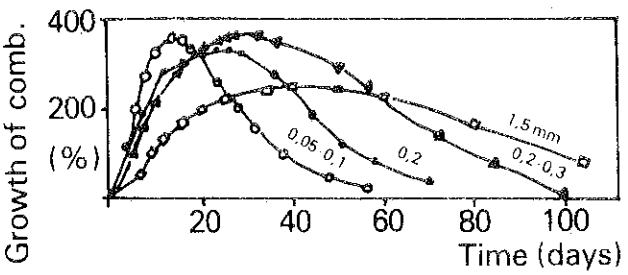


Figure 1—Effect of particle size of testosterone isobutyrate on the growth of the comb of capons (in %) [reproduced, with permission, from Lippold, *Pharm. Int.*, **1**, 60 (1980)].

One exception to this macrocrystals principle was observed with penicillin G procaine suspension in gelled peanut oil for intramuscular injection (5, 6). With large particles (>150 μm), the blood levels of penicillin rise rapidly, reach a peak level, and then fall relatively fast. However, with micronized particles (≤5 μm) the peak serum concentration of penicillin is reduced, while the therapeutic effective dose level is prolonged substantially. On the contrary, in the plain peanut oil suspension, i.e., without the addition of aluminum monostearate, or in aqueous suspension this macrocrystals principle was followed fairly well. The observed exception was rationalized as due to the possibility that the micronized particles of penicillin G procaine may reach the interface of tissue fluid/gelled suspension at a rate which is significantly slower than the large particles; therefore, a smaller rate of interfacial dissolution is achieved for micronized particles than for the large ones (1).

The major drawback of these two types of injectable depot formulations is that the release of drug molecules is not of zero-order kinetics as expected from the theoretical model defined by Eq. 1. Two reasons account for this deviation: (*a*) the surface area, S_a, of the drug solids diminishes with time, because of increased drug release, and (*b*) the saturation solubility, C_s, of the drug in the medium cannot be easily maintained because of rapid absorption.

Adsorption-Type Depot Preparations

This type of depot preparation is produced by binding of drug molecules to adsorbents. In this case, only the unbound, free species of the drug is available for absorption. As soon as the unbound drug molecules are absorbed, a fraction of the bound drug molecules will be released to reach a new equilibrium, at which the equilibrium concentration of free, unbound drug species, $(C)_f$, will be determined by a Langmuir relationship as defined by:

$$\frac{(C)_f}{(C)_b} = \frac{1}{b(C)_{b,m}} + \frac{(C)_f}{(C)_{b,m}} \qquad \text{(Eq. 2)}$$

where $(C)_b$ is the amount of drug (in mg) adsorbed by 1 g of adsorbent; $(C)_{b,m}$ is the maximum amount of drug (in mg) adsorbed by 1 g of adsorbent and can be estimated

from the linear plots of $(C)_f/(C)_b$ vs. $(C)_f$; and b is a constant and can be determined from the intercept and $(C)_{b,m}$.

This type of depot preparation is exemplified by vaccine preparations, in which the antigens are bound to highly dispersed aluminum hydroxide gel to sustain their release and, hence, prolong the duration of the stimulation of antibody formation.

Encapsulation-Type Depot Preparations

This type of depot preparation is formed by encapsulating drug solids within a diffusion barrier or dispersing drug particles in a diffusion matrix. Both diffusion barrier and diffusion matrix are fabricated from either biodegradable or bioabsorbable macromolecules, such as gelatin, dextran, polylactide, lactide/glycolide copolymers, phospholipids, and long-chain fatty acids and glycerides. Typical examples are naltrexone pamoate-releasing biodegradable microcapsules (7), liposomes (8), and norethindrone-releasing biodegradable lactide/glycolide copolymer beads. Release of drug molecules is controlled by the rate of permeation across the diffusion barrier and the rate of biodegradation of the barrier macromolecules.

Esterification-Type Depot Preparations

This type of depot preparation is formed by synthesizing the bioerodible esters of a drug and then formulating it in an injectable formulation which forms a drug reservoir at the site of injection. The rate of drug absorption is controlled by interfacial partitioning of drug esters from the reservoir to tissue fluid and the rate of bioerosion of drug esters to regenerate the active drug molecules. It is exemplified by the fluphenazine enanthate (Prolixin Enanthate, Squibb), nandrolone decanoate (Deca-Durabolin, Organon), and testosterone cypionate (Depo-testosterone cypionate®, Upjohn) in oleaginous solution.

Development of Parenteral Controlled Drug Release Formulations

Long-Acting Penicillin Preparations

Penicillin in the form of aqueous soluble sodium or potassium salt is rapidly absorbed from subcutaneous and intramuscular sites of parenteral administration. The intramuscular route is preferred.

Following intramuscular administration of aqueous solutions of the sodium (or potassium) salt of penicillin G, rapid absorption is achieved with high peak serum levels of penicillin. These high serum penicillin concentrations then decline rapidly in a matter of few hours due to the rapid urinary excretion of penicillin after its absorption from the site of injection.

It is known that 80% of urinary excretion is by tubular secretion and the remaining 20% is by glomerular filtration. The renal tubular secretion of penicillin can be interferred with and blocked by co-administration of some drugs, such as phenylbutazone, aspirin, and indomethacin, leading to enhancement and prolongation of the effective blood levels of penicillin (9).

A number of pharmaceutical techniques were utilized to extend the therapeutic activity of penicillin by preparing long-acting formulations of penicillin for intramuscular administration. The earliest approach was to reduce the aqueous solubility of penicillin by converting the aqueous soluble sodium (or potassium) salt into extremely low aqueous soluble salts, such as penicillin G procaine (with aqueous solubility of 4 mg/ml). Intramuscular administration of penicillin G procaine in vegetable oil produces a depot effect which sustains therapeutic blood level of penicillin for 24 to 48 hr. Gelation of this oil suspension with 2% aluminum monostearate further prolongs the therapeutic blood level of penicillin to 96 hr.

Another approach was to prepare aqueous suspensions of the relatively aqueous insoluble penicillin salts. This type of depot preparations was found most acceptable to medical professionals. However, this formulation has achieved only a limited success in sustaining the therapeutic blood levels of penicillin during the initial stage of development. Penicillin G procaine in aqueous suspension, for example, was able to maintain the therapeutic blood level of penicillin for only 12 to 24 hr. It was later discovered that the absorption and, hence, the therapeutic blood levels of penicillin can be significantly prolonged by maintaining a high solid/water ratio in the aqueous suspensions of penicillin G procaine (10). Further development has resulted in the preparation of long-acting thixotropic suspensions of penicillin G procaine (11). It is exemplified by Duracillin (Lilly), Crysticillin (Squibb), and Wycillin (Wyeth).

Depo-Penicillin in Oleaginous Suspensions. An injectable depot formulation of penicillin was developed by dispersion of the micronized crystals of penicillin G procaine in vegetable oil, such as peanut or sesame oil, which was gelled with aluminum monostearate (5). Intramuscular injection of this formulation was reported to produce a sustained therapeutic blood levels of penicillin in both animals and humans (5, 6, 12).

The duration of the depot effect of this parenteral controlled drug release formulation is dependent on the following formulation variables:

Type and Size of Penicillin G Procaine Crystals. The

TABLE I. Effect of Crystal Types of Penicillin G Procaine on the Intramuscular Bioavailability of Penicillin in Rabbits[a]

Crystal Types[b]	Serum Penicillin Concentration	
	Peak Level[c], units/ml	Duration[d], hr
Crystallized from water	1.18	162
Crystallized from acetone/water	0.59	146
Crystallized from propanol:		
Large crystals	2.19	93
Small crystals	0.76	152

[a] Compiled from the data by Buckwalter and Dickison, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 661 (1958).

[b] Suspensions were prepared to contain 300,000 units/ml of micronized penicillin G procaine of one crystal type in peanut oil gelled with 2% aluminum monostearate.

[c] Peak serum level of penicillin was reached within 1 hr.

[d] Duration in hours with serum penicillin concentration higher than the minimum therapeutic concentration of 0.03 units/ml.

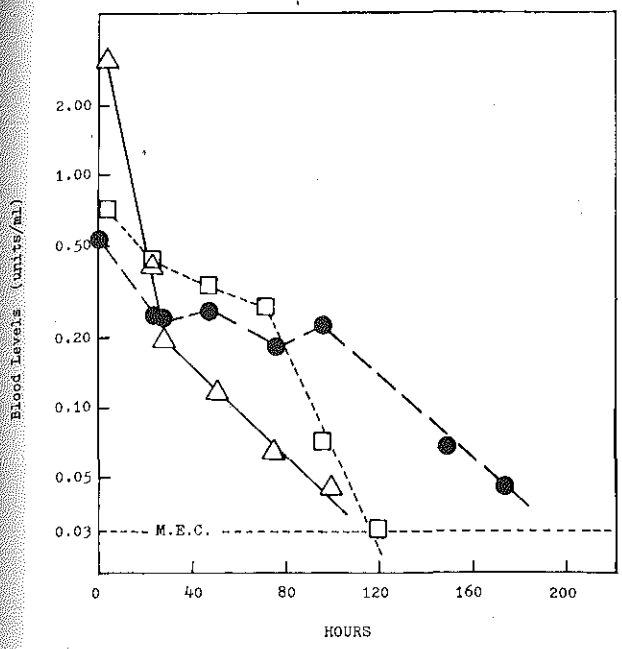


Figure 2—Effect of particle size of penicillin G procaine on the intramuscular bioavailability of penicillin in rabbits [plotted from the data by Buckwalter, U.S. patent 2,507,193 (May 9, 1950)]. Each suspension contains 300,000 units/ml of penicillin G procaine with particle size of △ 150–175 μm, □ 45–60 μm, or ● <5 μm in peanut oil gelled with 2% of aluminum monostearate. A dose of 50,000 units/kg was administered intramuscularly into each rabbit.

crystal type of penicillin G procaine, even after micronization, was found to affect the magnitude of peak serum concentration of penicillin and also the duration of therapeutic penicillin levels (Table I). The differences in bioavailability observed among crystals precipitated from different solvent systems may be due to polymorphic or solvate formation changes in the penicillin crystals.

The duration of therapeutically effective serum levels of penicillin was sustained remarkably, while the peak serum penicillin concentration was suppressed, by using a small particle size of penicillin G procaine (Fig. 2). The result is an exception to the macrocrystal principle demonstrated earlier by testosterone isobutyrate (Fig. 1).

In fact, the macrocrystal principle is also followed by penicillin G procaine crystals when formulated in aqueous suspensions (Table II) or in vegetable oil suspensions without the addition of aluminum monostearate.

Type and Amount of Aluminum Stearates. Without the addition of aluminum monostearate, oleaginous suspensions of micronized penicillin G procaine crystals in either peanut oil or sesame oil show no advantageous over aqueous suspensions in terms of maintenance of sustained therapeutic blood levels of penicillin (Table III). In either aqueous or oleaginous suspensions, therapeutically effective levels could be maintained for, at most, 24 hr.

TABLE III. Intramuscular Bioavailability of Penicillin from Aqueous and Oleaginous Suspensions of Penicillin G Procaine in Rabbits[a]

Suspensions[b]	Vehicles	Serum Concentration (units/ml), hr 1	4	24	28
Aqueous	Water	2.14	2.22	0.06	—[c]
Oleaginous	Peanut oil	3.70	2.50	—[c]	—[c]
	Sesame oil	5.53	2.27	0.03	—[c]

[a] Compiled from the data by Buckwalter and Dickison, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 661 (1958).
[b] Each suspension contains 300,000 units/ml of micronized penicillin G procaine particles.
[c] Concentration is lower than the therapeutic effective levels of penicillin.

With the addition of aluminum monostearate into the vegetable oils to form a gel, the intramuscular bioavailability of penicillin from the penicillin G procaine suspension is significantly prolonged in duration, while the peak serum level of penicillin is suppressed (Fig. 3). The effect appears to be dependent of the concentration of aluminum monostearate. The therapeutic effective concentration of penicillin is prolonged by approximately five times and the peak plasma level is suppressed sevenfold when the suspension is gelled with 1% aluminum monostearate. Serum penicillin levels are maintained over the minimum therapeutic concentration for longer than 168 hr when the aluminum monostearate content is increased from 1 to 2%. However, incorporation of more than 2% aluminum monostearate into the vegetable oils appears to have only limited benefit for prolongation of the duration of effective penicillin levels. On the contrary, the suspension, which contain more than 2% aluminum monostearate, is too viscous for practical use. Aluminum monostearate produces a relatively similar effect in sesame oil and peanut oil.

Replacement of aluminum monostearate with either di- or tri-stearate appears to have very little effect on the intramuscular bioavailability of penicillin (Fig. 4). On the

TABLE II. Effect of Particle Size of Penicillin G Procaine on the Serum Penicillin Levels in Rabbits from Aqueous Suspensions[a]

Particle Size[b], μm	Average Serum Levels[c], hr 1	4	24	28	48	72
150–250	1.37	1.29	0.82	0.86	0.31	0.12
105–150	1.24	1.50	0.76	0.28	0.16	—[d]
58–105	1.54	1.44	0.47	0.25	0.12	—[d]
35–38	1.64	1.51	0.62	0.33	0.15	—[d]
<35	2.40	2.36	0.33	0.16	0.07	—[d]
1–2	2.14	2.22	0.06	—[d]	—[d]	—[d]

[a] Compiled from the data by Buckwalter and Dickison, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 661 (1958).
[b] Each aqueous suspension contains 300,000 units/ml of penicillin G procaine with a specific particle size range.
[c] Average serum levels of penicillin in units/ml.
[d] Concentration is lower than the therapeutic effective levels of penicillin.

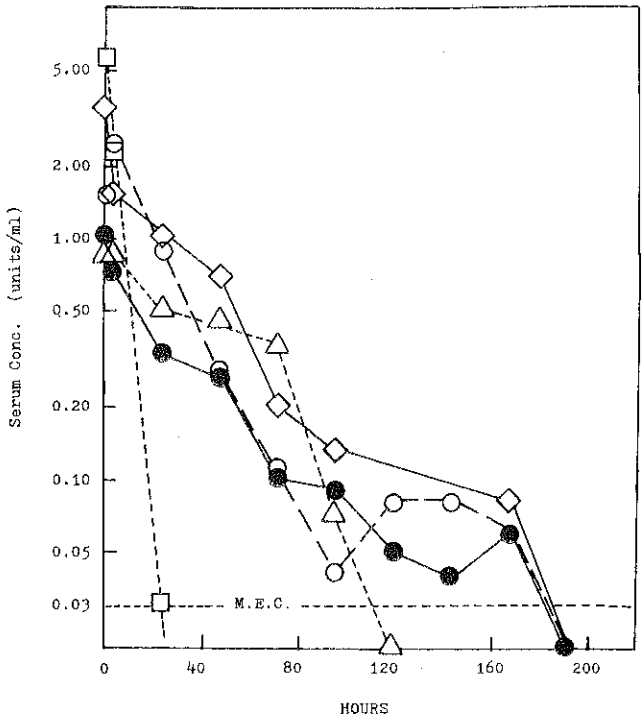


Figure 3—Effect of aluminum monostearate content in penicillin G procaine suspension on the intramuscular bioavailability of penicillin in rabbits [plotted from the data by Buckwalter and Dickison, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 661 (1958)]. Each suspension contains 300,000 units/ml of micronized penicillin G procaine in sesame oil gelled with aluminum monostearate: □ 0%, △ 1%, ● 2%, ◇ 3%, and ○ 4%.

other hand, the prolongation of intramuscular bioavailability of penicillin by aluminum stearate can not be duplicated by beeswax. Addition of beeswax (up to 5%) has produced only a slight effect, if any, on the duration of therapeutic penicillin levels (6).

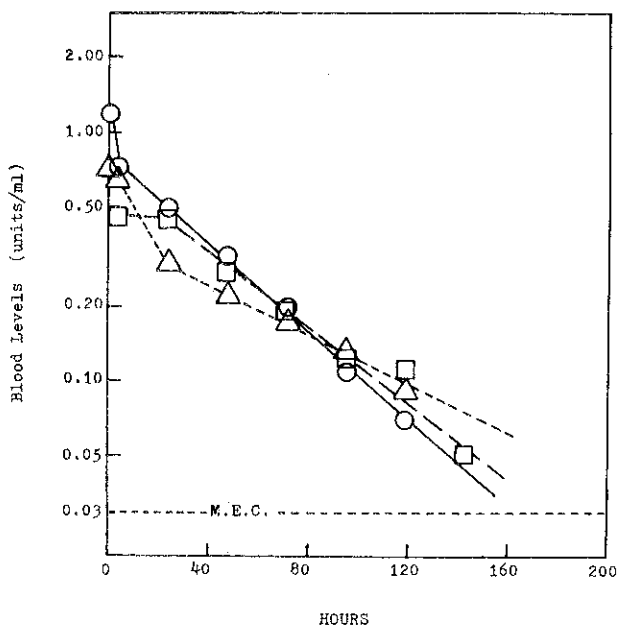


Figure 4—Effect of aluminum stearate on the intramuscular bioavailability of penicillin in rabbits [plotted from the data by Buckwalter and Dickison, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 661 (1958)]. Each suspension contains 300,000 units/ml of micronized penicillin G procaine in peanut oil gelled with 2% of ○ monostearate, □ distearate, and △ tristearate.

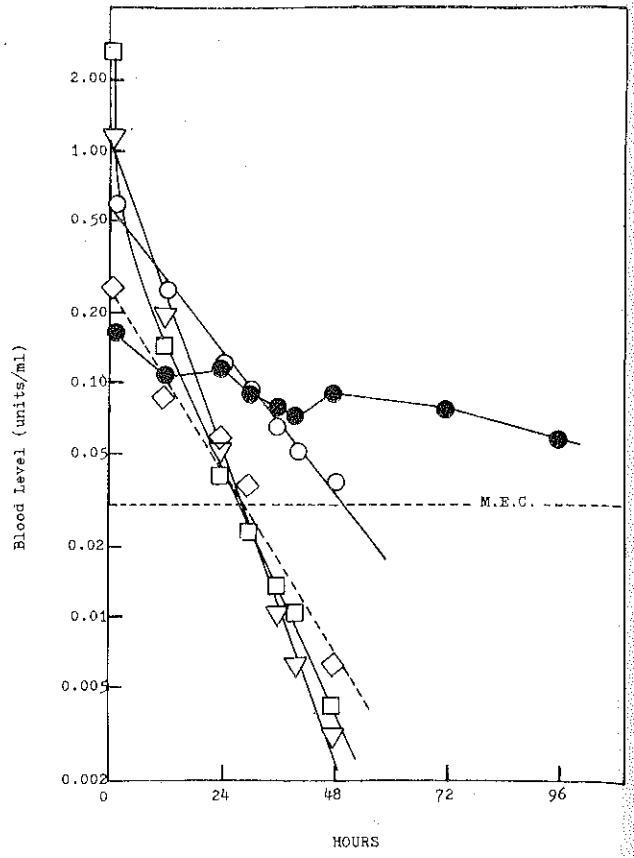


Figure 5 —Comparative clinical bioavailability of penicillin from intramuscular administration of various penicillin G salts in peanut oil suspension gelled with 2% of aluminum monostearate [plotted from the data by Thomas et al., *J. Am. Med. Assoc.*, **135**, 1517 (1948)]. ● penicillin G procaine (95 %, ≤5 μm), ○ penicillin G procaine (≥50 % over 50 μm), □ penicillin G sodium (≥50% over 50 μm), and ◇ penicillin G aluminum (≥50% over 50 μm).

The depot effect of penicillin G procaine suspension in gelled vegetable oils appears to be related to the combined effects of: (*a*) reduction in aqueous solubility of penicillin G by the formation of low aqueous solubility procaine salt, and (*b*) retardation in intramuscular drug absorption by the formation of compact cohesive depots within the muscular tissue as soon as the suspension is injected.

Clinical Performance of Depo-Penicillin Suspensions. The clinical bioavailability of penicillin G procaine suspension in gelled peanut oil was compared with other formulations as shown in Figure 5. Results indicated that penicillin G procaine (with particle size 50% or more over 50 μm) in peanut oil gelled with 2% aluminum monostearate produces a longer depot action than in ungelled peanut oil. On the other hand, the aqueous soluble sodium salt and aqueous insoluble aluminum salt of penicillin G in gelled peanut oil produced the same duration as penicillin G procaine salt in ungelled oil. Completely unexpected from the macrocrystal principle, the micronized penicillin G procaine (with particle size 95% less than 5 μm) in gelled peanut oil yielded a much longer repository action than the large penicillin G procaine crystals in the same formulation.

Further evaluation in 1008 patients demonstrated that the depo-penicillin formulations with micronized penicillin G procaine crystals suspended in gelled peanut oil is capable of maintaining a sustained therapeutically effective con-

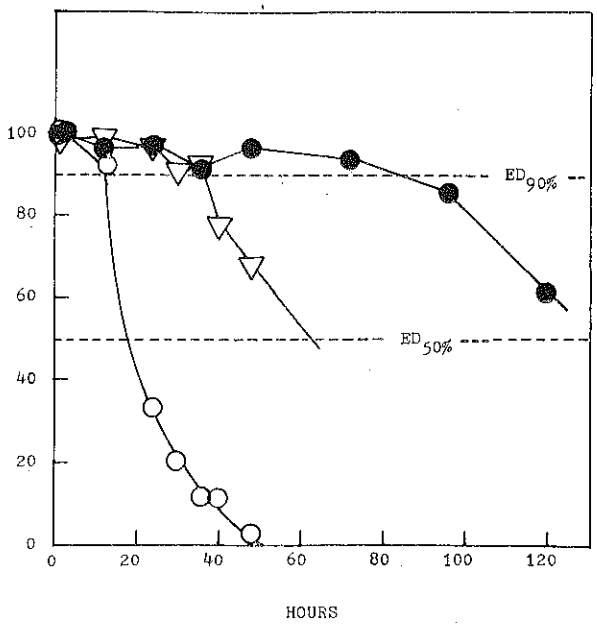


Figure 6—Comparative clinical performance of penicillin G procaine suspension [plotted from the data by Buckwalter and Dickison, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 661 (1958)]. The percent of patients treated has serum penicillin concentration greater than the minimum therapeutic level of 0.03 units/ml is used as the indicator for clinical effectiveness. Key: O penicillin G procaine suspension in peanut oil, ∇ large particles of penicillin G procaine in peanut oil suspension gelled with 2% aluminum monostearate, ● micronized penicillin G procaine particles in peanut oil suspension gelled with 2% aluminum monostearate.

centration (≥0.03 units/ml) in 90% of the patients for longer than 80 hr and in 50% of the patients for 132 hr (Fig. 6). In comparison, the same formulation with non-micronized penicillin G procaine crystals was able to achieve only 30 and 62 hr, respectively. Without the gelation with 2% aluminum monostearate, the micronized penicillin G procaine suspension in plain peanut oil maintained efficacy in 50% of the patients for only 17 hr.

The concept and methodology of depo-penicillin oil suspension has also been satisfactorily utilized in the development of parenteral controlled drug release formulation for relaxin (13), naloxone and naltrexone pamoate (2), naltrexone-Al-tannate and naltrexone-Zn-tannate (3), and aurothioglucose (Solganal®, Schering).

Depo-Penicillin Aqueous Suspensions. Several years after development of depo-penicillin oleaginous suspensions, Abbott Laboratories discovered that the therapeutic serum concentration of penicillin can also be substantially prolonged by formulating penicillin G procaine in an aqueous thixotropic suspension (10, 11). It was accomplished by maintaining a high solid/vehicle ratio (40–70% w/v of milled and micronized penicillin G procaine particles). Its prolonged action is due in part to the fact that these thixotropic suspensions tend to form compact and cohesive depots at the site of intramuscular injection, leading to slow release of penicillin G procaine and, in part, to the low aqueous solubility of the procaine salt of penicillin G, which renders intramuscular absorption of penicillin under the control of dissolution of penicillin G procaine in the tissue fluid.

Thixotropy is a desirable rheological behavior which gives a stable suspension during shelf-life and also has the necessary flow properties required for manufacturing, processing, and injection. Soon after intramuscular injection, it regains its original structure and forms a compact depot at the site of injection.

TABLE IV. Effect of Structure Breakdown Point and Other Physical Properties of Aqueous Penicillin G Procaine Suspensions on Depot Formation[a]

Suspensions[b]	Particle Size, μm	Specific Surface, cm^2/g	Structure Breakdown Point, dyne-cm	Depot Shape[c]
A	12–105	>5,000	0	Flat and fanned
B	8.5–89	7,620	102,000	Oval
C	2.5–90	20,200	411,000	Spherical
D	3.2–95	25,000	414,000	Spherical
E	2.3–79	24,000	578,000	Spherical

[a] Compiled from the data by Ober et al., *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 667 (1958).
[b] Each suspension contains 55% of penicillin G procaine solids.
[c] It was determined by injecting, using a 20-gauge needle, the trial suspensions into a 2% gelatin gel. The formation of depot and its shape can be observed and photographed.

The most important feature of the rheogram for thixotropic suspensions is the existence of a structural breakdown point. It is a measure of the structure of the suspension prior to any application of a shear force.

The capability of a suspension to form a depot structure can be evaluated by either in vivo or in vitro methods (11). It was concluded that to form a spherical depot, the aqueous suspensions of penicillin G procaine required a structural breakdown point of at least 10^5 dyne-cm (Table IV). Suspensions that possessed no structural breakdown point at all, even though they were paste-like and contained high penicillin units, produced no depot at all.

The structural breakdown point of aqueous penicillin G procaine suspension was reported to depend upon the content of solids in the suspension and their specific surface. The structural breakdown point was observed to increase in proportion to the increase in specific surface and solids content. A series of combinations of specific surface and solids content for powders with broad particle size distribution can be selected to give a suspension of any desired structural breakdown point.

Clinical bioavailability of several aqueous suspensions of penicillin G procaine was compared as shown in Figure 7. Results indicated that those suspensions, which have a structural breakdown point greater than 10^5 dyne-cm and which are capable of forming a spherical depot structure, tended to give a longer duration of therapeutic penicillin serum levels.

The question of possible plugging of hypodermic needles by the suspension is also important from a practical standpoint. It was found that suspensions which are made up of penicillin G procaine crystals with specific surface in excess of 10^4 cm^2/g and structural breakdown point values below 10^6 dyne-cm will have good injectability through a 20-gauge hypodermic needle.

Long-Acting Insulin Preparations

There are two million insulin-dependent diabetics in the

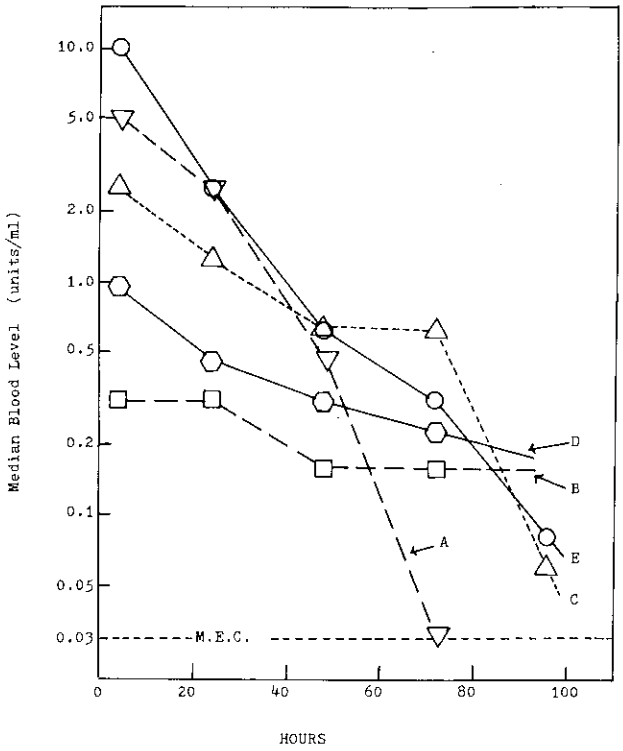


Figure 7—Comparative clinical bioavailability of penicillin from the intramuscular administration of various aqueous penicillin G procaine suspensions listed in Table IV.

United States alone. Clinically, they need regular administration of exogenous insulin for the treatment of their diabetes mellitus.

Insulin is a polypeptide hormone. The possibility of delivering insulin orally has been attractive but there has been little success in achieving acceptable bioavailability by the oral route due to inactivation of insulin by gastrointestinal enzymes, like pancreatic enzymes (14, 15). Several attempts were made to facilitate the oral absorption of intact insulin by co-administration of Trypan Red to inhibit gastric digestion, of Malachite Green to prevent tryptic digestion, and of saponin to aid insulin absorption (16). One of the most recent attempts has involved the incorporation of insulin in liposomes. All these efforts have produced a net normoglycemic activity incomparable with parenteral insulin administration, due to extensive hepatic "first-pass" metabolism following oral absorption.

Other routes of insulin administration that have been investigated include nasal, sublingual, rectal, and topical administrations which aim to bypass gastrointestinal and hepatic metabolism. All methods have yielded incomplete and/or uncertain absorption of this polypeptide hormone.

Because of the aforementioned failures, insulin has been given to diabetic patients exclusively by parenteral administration via the subcutaneous route. The therapeutic activity of insulin is evident within about 1 hr following subcutaneous injection. The duration of action is, however, relatively short, with plasma half-life being approximately 40 min for I^{131}-labeled insulin and less than 9 min for unlabeled insulin. It was found that the duration of action is not linearly proportional to the size of the insulin dose injected, but is a simple function of the logarithm of the dose, i.e., insulin is inactivated by the liver at a rate which is proportional to its concentration in the blood. The usual duration of regular insulin injection USP is between 4 and 8 hr, it, therefore, requires two to four injections daily for proper control of severe diabetes. For practical purposes, diabetic patients are trained to inject themselves (18).

Improper injection technique has been found to be responsible for the poor parenteral bioavailability of insulin. A diabetic patient could easily inject a correct dosage too deeply into the muscular tissues or not deep enough (intradermally or into the subcutaneous fat tissues). Insulin not properly injected in the subcutaneous region, where normal absorption of insulin takes place, may become temporarily trapped, resulting in areas of lumpiness or swelling. This lumpiness tends to occur when injections are given to the same region for several days. However, if the patient should traumatize or exercise the lumpy area, insulin could be suddenly taken into the the blood, resulting in adverse insulin reactions.

Consideration of the aforementioned problems associated with insulin administration, development of a long-acting parenteral insulin preparation becomes a critical need. It is important for good control of a patient's blood glucose and for obviating the need for multiple daily injections.

An important advance in the prolongation of insulin's activity was made by complexing insulin with protamine, an aqueous soluble, strongly basic simple protein isolated from the sperm or the mature testes of fish (19). This protamine insulinate has an isoelectric point at pH 7.3 and is, therefore, relatively insoluble in tissue fluids at physiological pH. When injected subcutaneously, it slowly releases insulin for a prolonged period of time (up to 24 hr). However, protamine insulinate was found unstable and its stability was later improved by adding zinc chloride to the preparation to form protamine-Zn-insulin complex (20). When injected into the loose subcutaneous tissue, protamine-zinc-insulin suspension provides a prolonged normoglycemic activity (over 36 hr). Unfortunately, protamine-Zn-insulin preparation has a slow onset and the normoglycemic action of insulin is not usually evident until 6–8 hr after administration and takes 14–20 hr to reach its peak level. Isophane insulin suspension, USP is a similar preparation as prota-

TABLE V. Normoglycemic Activity and Duration of Various Commercial Insulin Products[a]

Insulin Preparations	Normoglycemic Activity[b] Onset, hr	Peak, hr	Duration, hr
Insulin Injection, USP	0.5–1.0	2–3	6
Semilente Insulin[c]	0.5–1.0	5–7	12–16
Lente Insulin[c]	1.0–1.5	8–12	24
Ultralente Insulin[c]	4–8	16–18	>36
Globin-Zn-insulin injection, USP	~2	8–16	~24
Isophane insulin suspension, USP	1–1.5	8–12	~24
Protamine-Zn-insulin suspension, USP	4–8	14–20	~36

[a] Compiled from *Physicians' Desk Reference*, 33th ed., Medical Economics, Oradell, NJ, 1979.
[b] The time at which activity is evident.
[c] Novo Terapeutisk Laboratorium A/S.

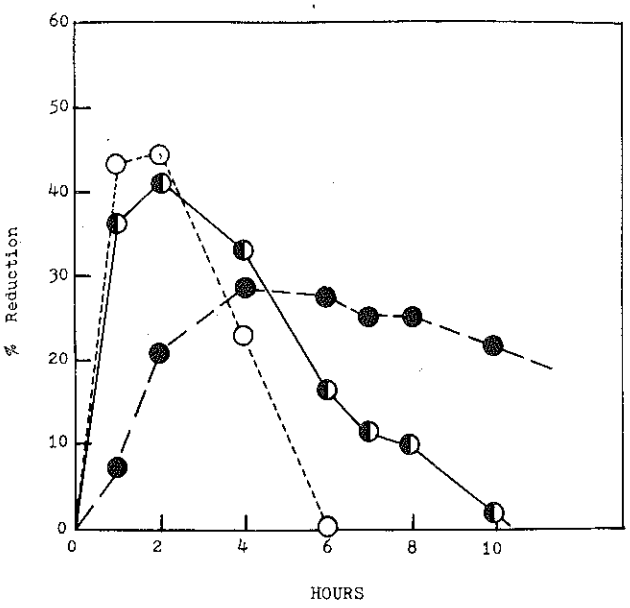


Figure 8—Comparative normoglycemic activity of Ultralente, Semilente, and regular insulin preparations in 26 rabbits by crossover test [plotted from the data by Hallas-Moller, *Diabetes*, **5**, 7 (1956)]. Each rabbit received 3 units of insulin. Key: O regular insulin, ◐ Semilente insulin, and ● Ultralente insulin.

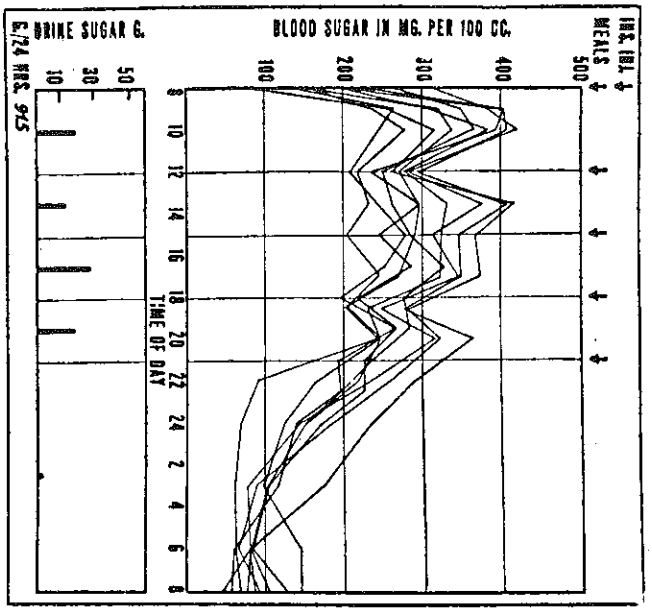


Figure 9—24-hr blood sugar profiles in nine diabetics after one subcutaneous injection of Ultralente insulin suspension (average dose is 55 units) at 8 a.m. [reproduced, with permission, from Hallas-Moller et al., *Science*, **16**, 394 (1952)].

mine-Zn-insulin but with fairly rapid onset (1–1.5 hr) and moderate duration of activity (24 hr). Globin-Zn-insulin injection is another typical example of protein-insulin complex prepared from zinc chloride and globin, a conjugated protein isolated from beef blood. It provides a relatively similar insulin release pattern, in terms of onset and duration of action, as Isophane insulin suspension (Table V).

It was later discovered that the duration of normoglycemic activity of insulin can be prolonged substantially without the need of adding proteins, like protamine and globin, into the preparations. It was achieved by "controlling crystallinity" of insulin in the presence of zinc chloride (21).

Insulin reacts with zinc chloride and precipitates as an aqueous insoluble Zn-insulin complex. Depending upon the pH, it may precipitate either as an amorphous or crystalline solid. Insulin crystals (10–40 μm) with high zinc content could be precipitated from acetate buffer at pH 5–6. This crystalline insulin zinc complex is absorbed very slowly and has a prolonged duration of normoglycemic activity. Subcutaneous injection of these insulin crystals by suspension in buffer solution at pH 7.3, the insulin is slowly released and absorbed (4–8 hr to the onset of action) and maintains activity for more than 36 hr (22, 23),. This preparation is named Ultralente insulin (Novo Terapeutisk Laboratorium A/S, Copenhagen, Denmark).

On the other hand, the amorphous insulin precipitated at higher pH (from pH 6 to 8) with low zinc content is absorbed more readily and has a duration of action which is shorter than Ultralente. When administered subcutaneously the insulin in the amorphous insulin-zinc (particle size <2 μm) suspension is quickly released and absorbed (with onset of action within 1 hr) and has a shorter duration of normoglycemic activity (12–16 hr). This preparation is called Semilente insulin (Novo). Both Ultralente and Semilente insulin produce longer duration of normoglycemic activity than the regular insulin injection USP (Fig. 8). The clinical efficacy of the long-acting ultralente insulin is illustrated in Figure 9.

One injection daily, which is desired by both diabetic

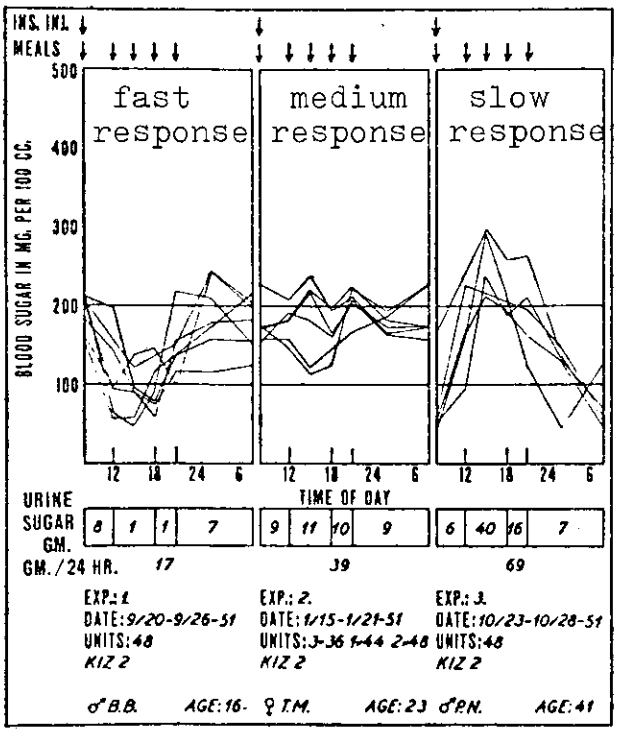


Figure 10—Different types of blood sugar responses in three diabetic patients following one daily injection of Ultralente insulin suspension at 8 a.m. for 6 days [reproduced, with permission from Hallas-Moller et al., *J. Am. Med. Assoc.*, **150**, 1667 (1952)].

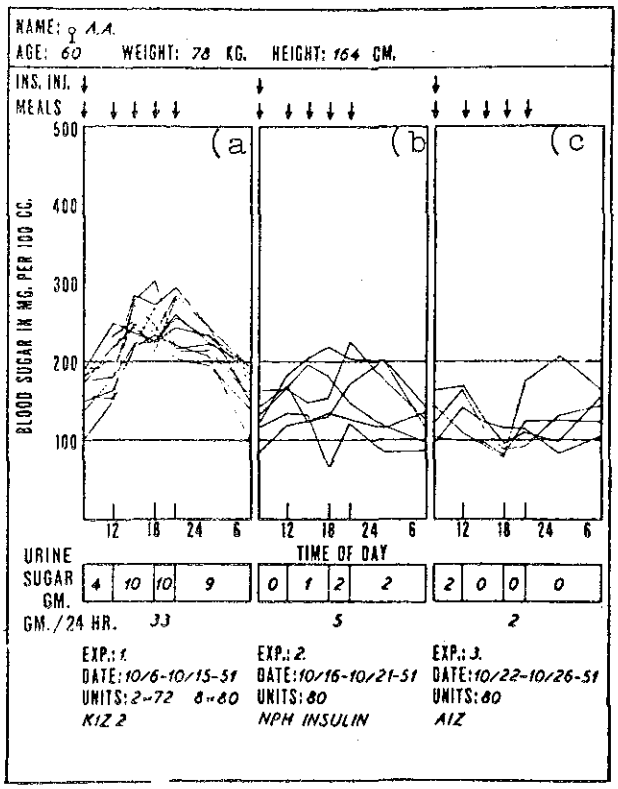


Figure 11—Comparison of blood sugar responses of patient A.A. to daily injection of a) Ultralente insulin, b) Isophane insulin, and c) Semilente insulin suspensions at 8 a.m. for 4–9 days [reproduced, with permission, from Hallas-Moller et al., *J. Am. Med. Assoc.*, **150**, 1667 (1952)]. See Table V for the onset, peak, and duration of normoglycemic activity of each preparation.

patients and physicians, requires adequate timing of the insulin preparation in use. There is no simple relationship between the blood sugar response of a diabetic and the activity range of the insulin preparation.

Patients can be classified, according to the promptness of their response to an insulin injection, into fast, medium, and slow reaction types. These three types of diabetic patients respond very differently to even the same kind of insulin preparation (Fig. 10). The blood sugar response of a diabetic patient also depends on the activity range of the insulin preparation (Fig. 11). The clinical studies concluded that the fast reaction-type patients need a relatively slow-acting insulin preparation in order to maintain the blood sugar level within the range of 100–200 mg/100 ml, while the slow reaction-type patients require a relatively fast-acting preparation (24). The aim is to obtain the type of blood sugar response closely simulating the one in Figure 11 (c).

One of the advantages is that the Ultralente and Semilente insulin preparations are mutually miscible and, because of this, the range of activity can be formulated. A typical combination is Lente insulin (Novo), which consists of seven parts of crystalline and three parts of amorphous insulin-zinc complexes. It gives an intermediate acting form of insulin. Its effect is evident in approximately 1–1.5 hr, reaches its peak level within 8–12 hr, and has a duration of action for 24 hr.

These three long-acting insulin preparations—Ultralente, Lente, and Semilente—with activity ranges from 12 to 36 hr (Table V) provide the three reaction types of diabetic patients a good selection of insulin preparations for single daily injection, depending upon their blood sugar responses. Clinical testing of these insulin-zinc suspensions in 65 patients with severe cases of diabetes indicated that satisfactory blood sugar control can be obtained in such a patient with just a single daily injection of the appropriate form. (24). These long-acting insulin preparations have been commercialized in the United States by both Lilly and Squibb under the license of Novo Terapeutisk Laboratorium A/S.

Other novel approaches for maintaining long-term normoglycemia in diabetes have also been recently explored. These include mechanical insulin delivery systems that control blood glucose concentrations by means of feedback-controlled or pre-programmed insulin delivery (25) and insulin-releasing subdermal polymeric devices (26).

Long-Acting Vitamin B_{12} Preparations

Administration of vitamin B_{12} by the oral route is limited and variable due to the fact that its absorption from the gastrointestinal tract is mediated by two separate and distinct mechanisms. The more important of these is mediated by the gastric intrinsic factor of castle, a glycoprotein secreted by the gastric parietal cells. This gastric intrinsic factor is easily saturated by vitamin B_{12} at amounts as small as only 1.5 to 3 μg and, additionally, the attachment of the vitamin B_{12}-intrinsic factor complex to receptors on the ileal surface requires calcium and a pH above approximately 6. After a delay of several hours at and within the ileal mucosa, the vitamin is transported in the blood stream to the liver and other organs. It was reported that interference with this mechanism is responsible for the overwhelming majority of the megaloblastic anemias seen in the United States. The other mechanism of vitamin B_{12} absorption, which is independent of intrinsic factor, appears to be a mass-action effect, possibly diffusion, and accounts for the absorption of approximately 1% of any dose of free vitamin B_{12} along the entire length of the small intestine. These two mechanisms for oral absorption of vitamin B_{12} overlap to a variable degree, depending on daily dietary vitamin B_{12} intake and the quantity of the vitamin released from its bound state.

On ther other hand, vitamin B_{12} is quantitatively and rapidly absorbed from intramuscular and subcutaneous sites of injection. The plasma level of cyanocobalamin reaches its peak within 1 hr after intramuscular injection. Therefore, parenteral administration of vitamin B_{12}, given by intramuscular or deep subcutaneous injection, is the medication of choice for treatment of pernicious anemia and other vitamin B_{12} deficiency states. The usual maintenance treatment of patients with pernicious anemia is with monthly injections of 30 to 1000 μg of cyanocobalamin. Unfortunately, much of the injected dose is lost in the urine. For these reasons, plus the convenience of injections at intervals greater than a month (because it must continue for life), it becomes very desirable to develop a parenteral controlled drug release formulation for vitamin B_{12}.

The very first approach to develop a parenteral controlled vitamin B_{12} release formulation was to formulate the vita-

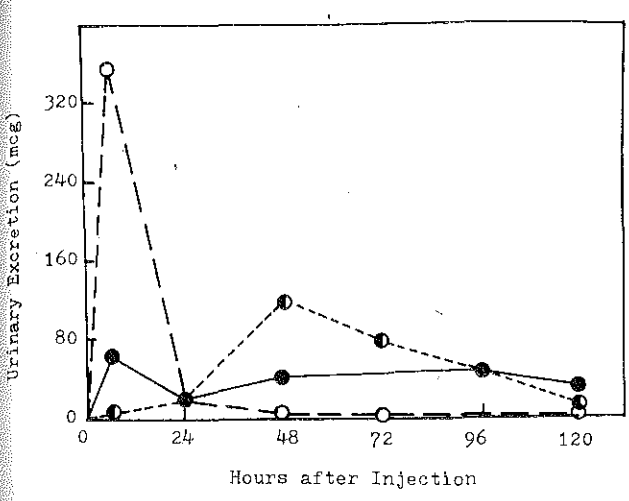


Figure 12—Comparative subcutaneous bioavailability of vitamin B_{12} in rats from various vitamin B_{12} preparations [plotted from the data by Thompson, *Bull. Parenteral Drug Assoc.*, **14**, 6 (1960)]. Key: ○ B_{12} in saline, ◐ B_{12}-Zn-tannate complex in gelled sesame oil, and ● Depinar (mean of three tests on three commercial lots).

min B_{12} in a concentrated (32%) partially-hydrolyzed gelatin solution. However, no sustained release behavior was detected in human testing (27).

In the second approach, crystalline vitamin B_{12} was suspended in sesame oil gelled with 2% aluminum monostearate. This approach did achieve a significant prolongation in vitamin B_{12} absorption as compared to aqueous vitamin B_{12} injection. Unfortunately, urinary excretion of this vitamin was still unacceptably excessive.

The third approach is to synthesize an insoluble derivative of vitamin B_{12}, called cyanocobalamin-Zn-tannate complex, and then suspend it in sesame oil gelled with 2% aluminum monostearate. This preparation did achieve a significant prolongation in the absorption of vitamin B_{12}. Urinary loss is significantly reduced (Fig. 12). On the other hand, the simple salts of vitamin B_{12}-Zinc and vitamin B_{12}-tannate did not produce any prolongation of vitamin B_{12} absorption. The results are in strong contrast with the sustained release accomplished by aqueous insulin-zinc suspensions discussed earlier and vasopressin tannate (Pitressin® Tannate in oil, Parke-Davis) for antidiuretics. The prolongation of biological activity for both preparations are achieved by simple salt formation.

In clinical testing, the cyanocobalamin-Zn-tannate preparations also produce a more steady and prolonged serum level of vitamin B_{12}. This development effort resulted in the commercialization of a parenteral long-acting vitamin B_{12} product, Depinar (Armour), which consists of a combination of a readily absorbable vitamin B_{12} and a sustained release vitamin B_{12}-Zn-tannate. This preparation produces a very small urinary loss (Fig. 12) and much of the vitamin B_{12} dose is gradually released from the injection site and deposited in the liver. It provides adequate maintenance therapy when given at intervals of 8 to 12 weeks (1 mg of cyanocobalamin in 1 ml of injection).

Long-Acting Adrenocorticotropic Hormone Preparations

Adrenocorticotropic Hormone (ACTH) is a polypeptide hormone which stimulates and regulates the adrenal contex to secrete adrenal steroids, mainly the corticosteroids.

The adrenocorticotropic activity of ACTH is destroyed easily by proteolytic enzymes in the gastrointestinal tract; therefore, exogenous ACTH is ineffective when given orally.

On the other hand, ACTH is readily absorbed from parenteral sites, and is usually administered by intramuscular injection and occasionally by intravenous infusion. Following intravenous administration ACTH rapidly disappears from the circulation with plasma half-life of only 15 min in man. In the rat, it was observed that only 0.2% of the hormonal activity is recovered in the target organ—the adrenal at 5–15 min after a rapid intravenous injection; 10 to 20% of the activity can be recovered from the non-target organ, the kidneys. A proteolytic system, probably fibrinolysin, has been detected in the blood that may inactivate ACTH (28).

The maximum effects of ACTH on the adrenal can be achieved when optimal amounts of the hormone are acting continuously. In studies with continuous infusion of a fixed dose of ACTH for periods varying from 30 sec to 48 hr, it was observed that ketosteroid excretion increases with the duration of infusion. It means that when the ACTH is given by slow intravenous infusion over a prolonged period of time, much of the injected dose is able to act on the adrenal cortex. This also occurs, but to a lesser extent, when ACTH is administered intramuscularly in aqueous solution (28). Development of a slowly released ACTH injectable formulation thus becomes indispensable.

ACTH is commercially prepared from bovine, porcine, ovine, and cetacean pituitaries. This hormone shows a strong affinity to tissue proteins. Adsorption onto the tissue proteins was reportedly responsible for the low effectiveness of ACTH after a single subcutaneous or intramuscular

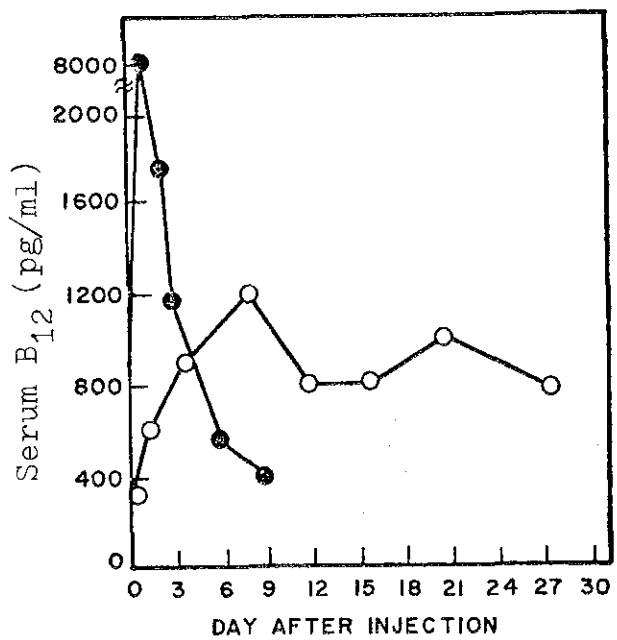


Figure 13—Comparative serum levels of vitamin B_{12} in humans after intramuscular injection of 500 μg of vitamin B_{12} (●) and a molar equivalent amount of vitamin B_{12}-Zn-tannate complex (○) [plotted from the data by Thompson and Hecht, *Am. J. Clin. Nutr.*, **7**, 311 (1959)].

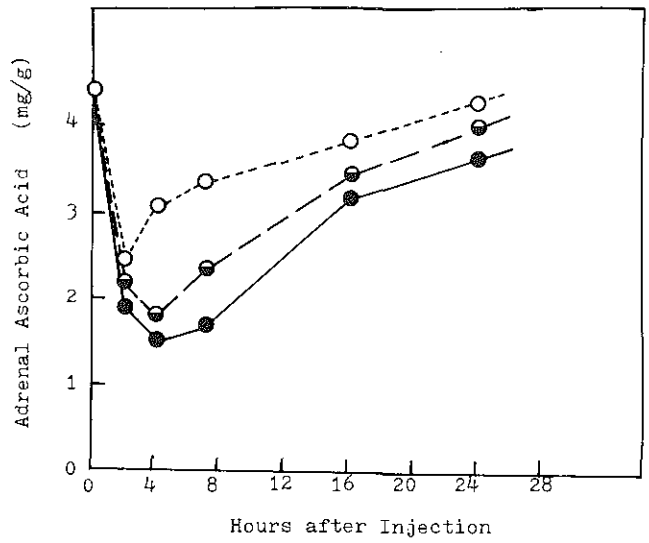


Figure 14—Effect of gelatin on adrenal ascorbic acid response in hypophysectomized rats to ACTH injections [plotted from the data by Thompson, *Bull. Parenteral Drug Assoc.*, **14**, 6 (1960)]. Key: O ACTH in saline, ⊖ addition of 16% of gelatin, and ● addition of 32% of gelatin.

injection of ACTH in aqueous solution. Only a small fraction of the administered dose is absorbed into the blood stream (29). Gelatin was found to inhibit the protein binding of ACTH. Addition of partially hydrolyzed gelatin into the injectable ACTH solution was noted to enhance the adrenal ascorbic acid responses in hypophysectomized rats (Fig. 14). This activity was observed to increase with increased gelatin concentration in the ACTH injection. Results of this investigation provided the foundation for development of a repository corticotropin injection (H.P. Acthar gel, Armour), a long-acting ACTH injectable formulation which contains a highly purified preparation of ACTH in 16% gelatin solution. It gives a rapid onset and prolonged action to stimulate the functioning adrenal cortex

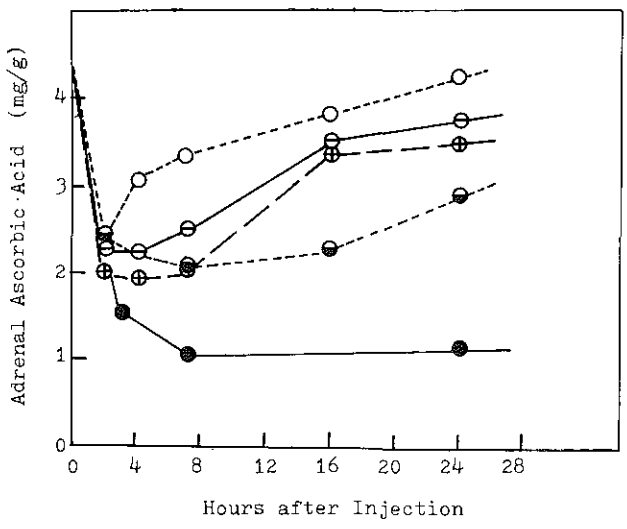


Figure 15—Comparative adrenal ascorbic acid responses in hypophysectomized rats to various ACTH preparations [plotted from the data by Thompson, *Bull. Parenteral Drug Assoc.*, **14**, 6 (1960)]. Key: O ACTH in saline, ⊖ ACTH with aluminum phosphate, ⊕ ACTH with aluminum phosphate and 16% gelatin, ◒ ACTH suspension in sesame oil gelled with 2% aluminum monostearate, and ● ACTH-Zn-tannate complex with gelatin.

to produce and secrete the adrenal steroids. This preparation is active for a duration of 24 hr—a significant improvement over the regular corticotropin injection (Acthar, Armour), which has a duration of action of only 8 hr.

The subcutaneous absorption and, hence, biological activities of ACTH can also be sustained to various degrees by adsorption onto aluminum phosphate, suspension in sesame oil gelled with aluminum monostearate, or formation of ACTH-Zn-tannate complex (Fig. 15). Addition of hydrolyzed gelatin was found to further enhance the activity of various ACTH preparations. The ACTH-Zn-tannate complex in gelatin solution was found to produce the most efficient and long-acting adrenocorticotropic effect.

With prolonged absorption, ACTH-Zn-tannate/gelatin preparation achieved a more intense response on adrenal ascorbic acid depletion, adrenal weight, and thymus weight in hypophysectomized rats than ACTH in saline or ACTH-gelatin preparation at the same dose from a single injection (Fig. 16). A significantly longer duration of activity was produced by ACTH-Zn-tannate/gelatin preparation.

The activity-time profile of ACTH-Zn-tannate/gelatin preparation was further compared in humans, using the cumulative urinary 17-hydroxy-steroid excretion as the indicator, with ACTH-gelatin preparation (H. P. Acthar gel) and ACTH-Zn-tannate complex (Fig. 17). Apparently, the ACTH-Zn-tannate/gelatin preparation produced a vastly greater effect than the complex alone and ACTH-gelatin preparation.

Long-Acting Steroid Preparations

So far, the discussion has been focussed on the use of insoluble salts, such as penicillin G procaine, or insoluble complexes, such as cyanocobalamin-Zn-tannate, to achieve prolongation of drug action. Alternatively, the objective of sustained drug activity can also be accomplished by esterification of the drugs to form water-insoluble, but oil-soluble pro-drugs. Probably, the area in which the pro-drug approach has been utilized extensively has been in the development of long-acting injectable steroid preparations.

Androgenic Steroids. Testosterone given by mouth is readily absorbed, but such administration is almost completely ineffectual inasmuch as the hormone is metabolized by the liver before reaching systemic circulation. Testosterone injected as a solution in oil is also quickly metabolized and excreted and the androgenic effect is small.

The androgenic activity of testosterone was reportedly enhanced and prolonged by esterification (30). The biological half-life and the times of maximum effect for a series of testosterone esters in an oleaginous intramuscular injectable formulation was found to be closely related to the oil/water partition coefficient of these fatty acid esters (31, 32). Further investigation revealed that the difference in the oil/water partition coefficients of the homologous series of formyl through valeryl esters of testosterone is largely determined by the variation in their aqueous solubilities, since their solubilities in oil vehicle, like ethyl oleate, are approximately equal.

It was further demonstrated by several investigators that longer durations of androgenic activity of testosterone from

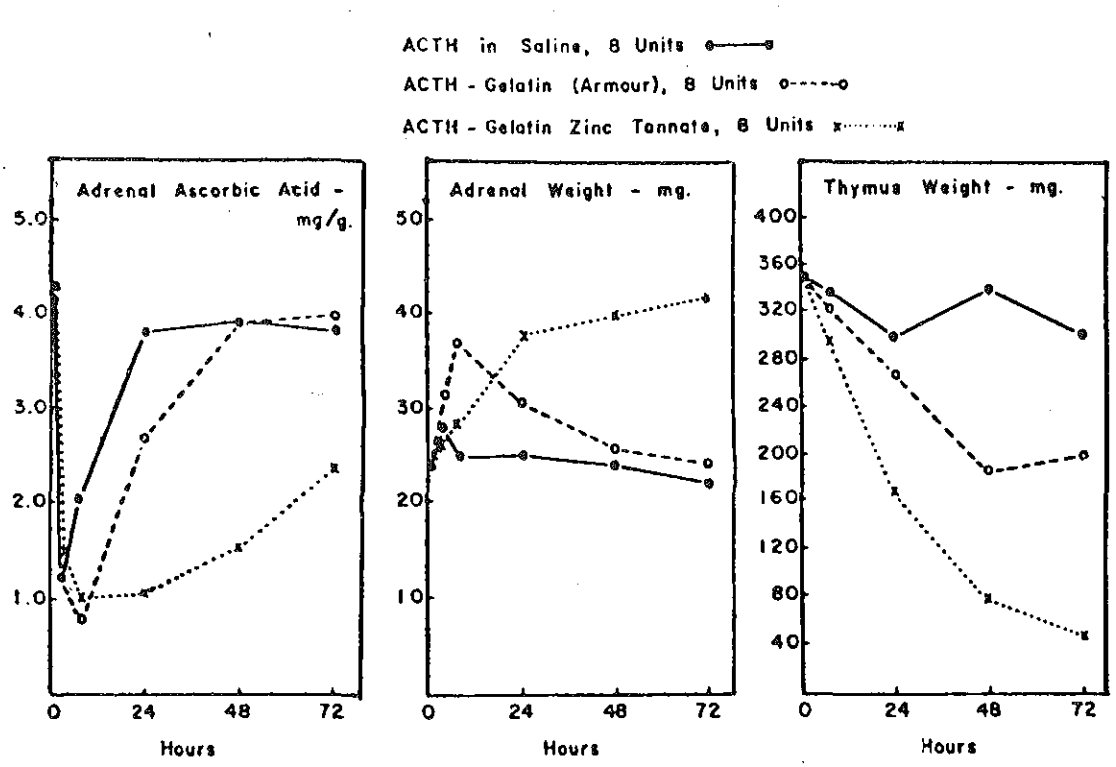


Figure 16—Biological activities and duration of ACTH-Zn-tannate/gelatin preparation in hypophysectomized rats as compared to ACTH in saline and ACTH-gelatin preparation [reproduced, with permission, from Thompson, *Bull. Parenteral Drug Assoc.*, **14**, 6 (1960)].

an intramuscular injection can be achieved by the acylation of the 17β-hydroxy group in testosterone molecule (33–42). The duration of testosterone action was effectively prolonged as the chain length of the acyl group increased. This observation was rationalized as that the longer the chain length of acyl group, the slower the release of testosterone esters from the oleaginous vehicle due to the reduced water/oil partition coefficient, leading to a decrease in the rate of regeneration of the active testosterone moiety in the body to exhibit its androgenic activity. More analyses on the effect of chain length and partition coefficient on the biological activity will be done later in the discussion on 19-nortestosterone. Miescher and his associates (30) were the first to promote the use of the long-chained esters of testosterone as injectable depot form of testosterone.

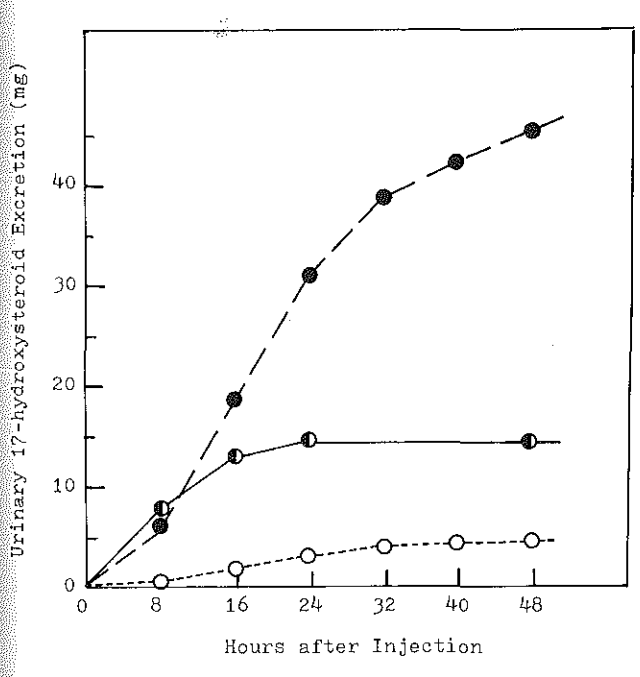


Figure 17—Time course for the cumulative urinary 17-hydroxysteroid excretion in humans in response to the subcutaneous administration of various ACTH preparations [plotted from the data by Thompson, *Bull. Parenteral Drug Assoc.*, **14**, 6 (1960)]. Keys: ● ACTH-Zn-tannate/gelatin; ◐ ACTH-gelatin, and ○ ACTH-Zn-tannate (each contains 80 units of ACTH).

Studies of a series of saturated and non-saturated esters of testosterone later revealed that the β-cyclopentyl propionate ester of testosterone injected intramuscularly in cottonseed oil is a superior depot formulation of testosterone. It was concluded from the results of the relative growth of seminal vesicles in castrated rats. These investigations provide the foundation for the commercialization of testosterone cypionate injection, USP (Depo-testosterone cypionate, Upjohn). The enanthate ester of testosterone (Delatestryl, Squibb) is another testosterone ester that provides excellent prolonged androgenic activity. Following a single intramuscular injection of 1-ml oily solution, both formulations produce a sustained androgenic activity for a period of about 4 weeks. The continuous release of testosterone from the esters is thought to resemble closely the endogenous production of testosterone.

Testosterone enanthate is also formulated with a long-acting estrogen, estradiol valerate, in ethyl oleate BP repository vehicle (Ditate-DS, Savage) for the prevention of postpartum breast engorgement.

The esterification approach was also applied to 19-nortestosterone (nandrolone) to prolong its anabolic activity after intramuscular or subcutaneous injection (43–47). The effect of esterification on the anabolic activity of nandrolone is illustrated in Figure 18. The data indicate that following

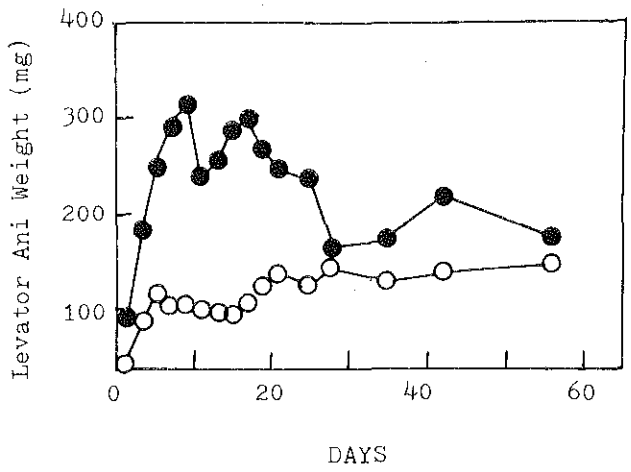
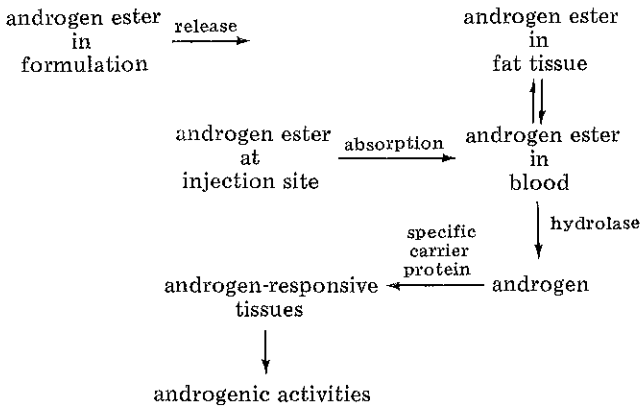


Figure 18—Effect of nandrolone nonanoate on the growth of levator ani in castrated rats [plotted from the data by Chaudry and James, *J. Med. Chem.*, **17**, 157 (1974)]. Key: ● injection of 1 mg dose of nandrolone nonanoate, O control rats.

injection, nandrolone nonanoate produces a substantial increase in the weight of levator ani in castrated rats, as compared to control rats, for a prolonged period of time.

It was interesting to observe that there are two peaks of activity with all the nandrolone esters and at all dose levels. Multiple regression analysis of these maxima suggested that the times for the second peak activity can be correlated with the ethyl oleate/water partition coefficient (in a semilogarithmic relation), but not the first maxima. Similar results were also observed with testosterone esters (31). The biological half-lives of the testosterone esters in rats were found to be linearly related to the ethyl oleate/water partition coefficient, but the half-lives for the disappearance of drug at the injection site were not. These observations were rationalized as the result of distribution/buildup of esters in the body fat tissues and its subsequent release at a later time (Scheme I).



Scheme I

Based on Scheme I, the first peak activity noted in the levator ani weight–time profiles (Fig. 18) is the result of the androgen released from the androgen ester dose absorbed into the systemic circulation; the second maxima is produced by the androgen released from the androgen ester built up earlier in the body fat tissues. The anabolic activity (in mg-days) for each nandrolone ester can be calculated from the area under the levator ani weight—time curves (Fig. 18).

TABLE VI. Anabolic Activities and Times to Maximum Anabolic Effect of Nandrolone Esters[a]

Esters	Time to Maximum Effect: 1st max., days	Time to Maximum Effect: 2nd max., days	Anabolic Activity, g-day[b]: 1-mg Dose	Anabolic Activity, g-day[b]: 1 m*M* Dose[c]
Butyrate	6	11	1.49	0.61
Hexanoate	11	15	3.73	1.46
Heptanoate	9	17	6.56	1.94
Octanoate	13	19	5.56	1.91
Nonanoate	9	19	5.08	1.84
Decanoate	14	21	7.74	2.56
Undecanoate	14	21	6.58	1.56

[a] Compiled from the data by Chaudry and James, *J. Med. Chem.*, **17**, 157 (1974).
[b] Correction has been made for control weights. Each data point was computed from at least 32 determinations.
[c] Molar concentration of nandrolone base.

The results in Table VI show that on a weight basis the butyrate has the lowest anabolic activity and the decanoate the highest in the homologous series of nandrolone esters. A similar pattern also develops when anabolic activities of nandrolone esters are compared on the basis of nandrolone content alone (in m*M* dose).

The data in Table VI were found to be related to the ethyl oleate/water partition coefficient in the following ways:

(*a*) The time to the second peak of anabolic activity ($t_{p,2}$) is highly correlated to the partition coefficient (*p.c.*):

$$\log (t_{p,2}) = 0.219\ (0.028) \log (p.c.) - 0.015\ (0.157) \quad \text{(Eq. 3)}$$

(*b*) The anabolic activity (*AA*) shows a binomial relationship with the partition coefficient (*p.c.*):

$$\log (AA) = 7.33\ (1.51) \log (p.c.) - 0.636\ (0.138) \log (p.c.)^2 - 17.8\ (4.1) \quad \text{(Eq. 4)}$$

The parabolic relationship demonstrated by the anabolic activity of nandrolone esters has been seen frequently with many other drugs and systems (48). The maximum observed in the parabola represents an optimum combination of hydrophilicity and lipophilicity in a drug molecule. The good correlation (with correlation coefficients of 0.96 and 0.97, respectively) shown in Eqs. 3 and 4 suggests that the anabolic activity and duration of nandrolone esters are highly dependent upon the lipophilic nature of the esters. This analysis concludes that nandrolone decanoate produces a maximum anabolic activity in this series of nandrolone esters (43).

These investigations provide a fundamental understanding of the mechanisms for the prolonged anabolic activity of nandrolone decanoate (Deca-Durabolin, Organon). The decanoate ester is longer acting than another commercially available long-acting anabolic product—nandrolone phenylpropionate (Durabolin, Organon). The nandrolone decanoate can be administered into the gluteal muscle monthly whereas the nandrolone phenylpropionate needs to be injected weekly.

Estrogenic Steroids. By oral administration, the natural estrogens, such as estradiol, are subject to extensive hepatic

"first-pass" metabolism. They are mostly degraded by conversion to less active products, such as estriol, by oxidation to unknown non-estrogenic substances, and by conjugation with sulfuric and glucuronic acids (49). By parenteral administration in vegetable oil, estradiol is rapidly absorbed and quickly metabolized with a plasma half-life of about 1 hr.

Long-acting estrogenic steroids useful in the treatment of estrogen deficiency in women, such as menopause, postovariectomy, postmenopause, thus became desirable and have been of interest to a number of scientists. Pro-drug approach by esterification of 3- and/or 17-hydroxyl groups of estradiol has shown to produce prolonged estrogenic action by intramuscular administration (50–55). It was found that the aryl and alkyl esters of estradiol become less and less polar as the size of the substituents increase; correspondingly, the rate of parenteral absorption is progressively slowed down and the duration of estrogenic activity prolonged. Several long-acting estrogenic steroids have been made commercially available. They are outlined as follows:

Estradiol Benzoate (Progynon Benzoate®, Schering). Prepared by benzoylation of the 3-hydroxyl of estradiol. It is an oil-soluble ester from which the active β-estradiol is slowly released from the oleaginous formulation at the site of intramuscular injection. It provides sustained therapeutic level of estrogen for several days.

Estradiol benzoate has intrinsic biological activity about half that of natural estradiol, but owing to its sustained action, it is more efficacious than estradiol. It is used in the treatment of the same conditions as estrone but is several times more active.

Estradiol Valerate (Delestrogen, Squibb; Duratrad, Ascher). Prepared by esterifying estradiol at both the 3- and 17-positions by treatment with valeryl chloride in pyridine and then removing the 3-valerate by treatment with potassium carbonate in aqueous methanol. It is very slowly released and absorbed from an oil suspension injected intramuscularly and provides a therapeutic level of estradiol for a duration of about 3 weeks.

It has been formulated in combination with the long-acting testosterone enanthate in ethyl oleate BP repository vehicle (Ditate-DS, Savage) for the prevention of postpartum breast engorgement and for the menopausal syndrome in those patients not improved by estrogen alone.

Estradiol valerate may also be administered along with a progestational drug in the management of primary or secondary amenorrhea and functional uterine bleeding.

Estradiol Dipropionate (Ovocylin Dipropionate, Ciba). Prepared by esterifying estradiol at both the 3- and 17-positions by treatment with propionyl chloride in pyridine. It is half as potent as estradiol benzoate, but because of a more sustained depot-action the dipropionate becomes more potent than benzoate with respect to the cumulative maintenance dosage.

It is longer acting than estradiol valerate when injected as a solution in vegetable oil.

Estradiol Cypionate (Depo-Estradiol, Upjohn). Prepared by the same method described earlier for the valerate, using cyclopentyl propionyl chloride as the esterificant. As demonstrated earlier by testosterone cypionate (Depotestosterone cypionate, Upjohn), the 17-cyclopentylpropionate ester of estradiol also produces a sustained hormonal activity. Intramuscular injection in vegetable oil solution, estradiol cypionate has a more prolonged estrogenic activity than those of the benzoate, valerate, or dipropionate. Its average duration of action is 3–8 weeks.

Progestational Steroids. The rate of turnover for progesterone is extremely rapid with a plasma half-life of only about 5 min. Inactivation takes place largely in the liver; the characteristic metabolite is pregnane-3α, 20α-diol, which is excreted in the urine in conjugated form with glucuronic acid (49).

Progesterone injected in oil solution is readily absorbed but it is degraded at a rate that is too rapid for optimal therapeutic efficiency. In fact, it is extremely difficult to achieve effective blood levels with any convenient dosing schedules. In animal tests several doses per day were found more effective than the same dose given once daily, and less frequent dosage is quite inefficacious (49, 56).

The first parenterally active, long-acting progestational formulation is the development of medroxyprogesterone acetate. Aqueous suspensions of micronized medroxyprogesterone acetate crystals (DepoProvera, Upjohn) produce a sustained intramuscular progestational activity for a duration of 2–4 weeks.

The pro-drug concept has also been applied in development of long-acting injectable progestational preparations, but the primary interest has been centered on the development of injectable depot formulation for contraception, such as norgestrel 17β-fatty acid esters. This development will be discussed under *Long-Acting Contraceptive Preparations.*

Adrenal Steroids. As discussed earlier, under regulation of ACTH the adrenal cortex synthesizes, from cholesterol, two classes of adrenal steroids—the corticosteroids and the adrenal androgens (28).

The corticosteroids have numerous and diversified physiological functions and pharmacological actions. They affect the metabolism of carbohydrate, protein, fat, and purine; electrolyte and water balance; and the functional capacities of the cardiovascular system, the kidney, skeletal muscle, the nerve system, and other organs and tissues. Furthermore, the corticosteroids endow the human with the capacity to resist all types of noxious stimuli and environmental changes (28). Traditionally, they have been classified into mineralocorticoids and glucocorticoids. Desoxycorticosterone, the prototype of the mineralocorticoids, is highly potent in regard to sodium retention but practically inactive in liver glycogen deposition. On the other hand, cortisol, the prototype of the glucocorticoids, is highly potent in regard to liver glycogen deposition but weak in sodium retention activity.

The coricosteroids are required for replacement therapy in adrenal insufficiency, such as Addison's disease. Both mineralocorticoids and glucocorticoids may be needed to approximate the equivalent of their physiological body concentrations. Glucocorticoids are additionally used to treat rheumatic, inflammatory, allergic, neoplastic, and other disorders (56).

Treatment of Addison's disease has been greatly advanced by the use of desoxycorticosterone. Although the defects in carbohydrate and protein metabolism are not corrected by this mineralocorticoid alone, life can be maintained by its intelligent administration.

All the natural corticosteroids are poorly effective by oral administration, because of rapid, extensive hepatic "first-pass" metabolism. Hence, they must be given parenterally for systemic effects. Desoxycorticosterone has a serum half-life of about 70 min, so daily intramuscular injection becomes necessary for adequate maintenance treatment of Addison's disease. Because Addison's disease is a permanent disorder and medication is needed for life, development of a parenteral long-acting desoxycorticosterone preparation is most desirable.

The corticosteroid activity of desoxycorticosterone was remarkably prolonged by converting its acetate to pivalate. It was prepared by condensation of desoxycorticosterone with trimethylacetyl chloride in pyridine solution. Intramuscular injection of microcrystalline desoxycorticosterone pivalate in oleaginous suspension (Percorten Pivalate, Ciba) produced a very long duration of action. It is administered once every 4 weeks as compared to daily injections required for desoxycorticosterone acetate (Percorten Acetate in oil, Ciba).

Other commercially available long-acting injectable corticosteroid preparations with activity prolonged by esterification are betamethasone acetate (Celestone® Soluspan® suspension, Schering), methylprednisolone acetate (Depo-medrol®, Upjohn), and triamcinolone hexacetonide suspension (Aristospan®, Lederle), to name a few.

Long-Acting Anti-Psychotic Preparations

The use of drugs for the treatment of psychotic disorders has become widespread only since the mid-1950s. Today, the phenothiazines, the most prescribed anti-psychotic agent, as a class are among the most widely used drugs in the practice of medicine. They are used on a grand scale to modify attitudes and emotions of psychiatric patients—a tranquilizing effect.

Fluphenazine, the trifluoromethyl phenothiazine derivative, is the most potent phenothiazine available for the management of manifestations of psychotic disorders. Both laboratory and clinical studies demonstrate that this drug exhibits several important different features from other phenothiazines. It is more potent, exhibits a more prolonged duration of action, is less likely to induce hypotension, is less sedative, and does not potentiate CNS depressants, and anesthetics to the same degree as other phenothazines.

In the management of psychotic disorders, patient compliance has always presented a major difficulty that challenges the medical profession. Development of a long-acting injectable anti-psychotic preparation will certainly provide a practical solution to minimize this non-compliance problem.

The prolongation of anti-psychotic activity of fluphenazine can be achieved by esterification (57–60). It is exemplified by the development of fluphenazine enanthate (Prolixin Enanthate, Squibb) and fluphenazine decanoate (Prolixin Decanoate, Squibb). They were prepared by esterifying fluphenazine with either enanthoyl chloride or decanoyl chloride in the presence of pyridine. The esterification of fluphenazine with the enanthate moiety markedly prolongs the duration of anti-psychotic action without unduly attenuating its beneficial effects (61).

Intramuscular or subcutaneous administration of either ester in sesame oil solution produces the anti-psychotic action for an average duration of 2 weeks. The onset of action generally appears between 24 to 72 hr after injection, and the effects of the drug on psychotic symptoms become significant within 48 to 96 hr. Amelioration of symptoms continues for 1 to 3 weeks or longer. They are used for management of schizophrenia and other psychotic disorders and are particularly effective in modifying psychotic behavior patterns and ameliorating such symptoms as agitation, delusions, and hallucinations.

The esterification approach has also been utilized to achieve longer duration of action for other neuroleptic drugs (62–67). A typical example is the development of α-fluphenthixol. It has been substantially prolonged by converting its dihydrochloride salt (with an oral dose of 5 mg/kg/day) to the decanoate ester (which requires an intramuscular injection of 10 mg/kg for a 10-day period). A single intramuscular injection of α-fluphenthixol decanoate in oil produces a fairly steady neuroleptic activity, in terms of the inhibition of a conditional avoidance response, for a duration much longer than that achieved by multiple oral daily intakes of the dihydrochloride salt (Fig. 19). The neuroleptic activity from oral administration of α-fluphenthixol dihydrochloride fluctuates greatly. The metabolic pattern of the esters was found to be identical to those of the parent compound (62).

Long-Acting Anti-Malarial Preparations

Anti-malarial drugs may be roughly classified into two quite distinct groups. The first group is rapid in shizontoc-

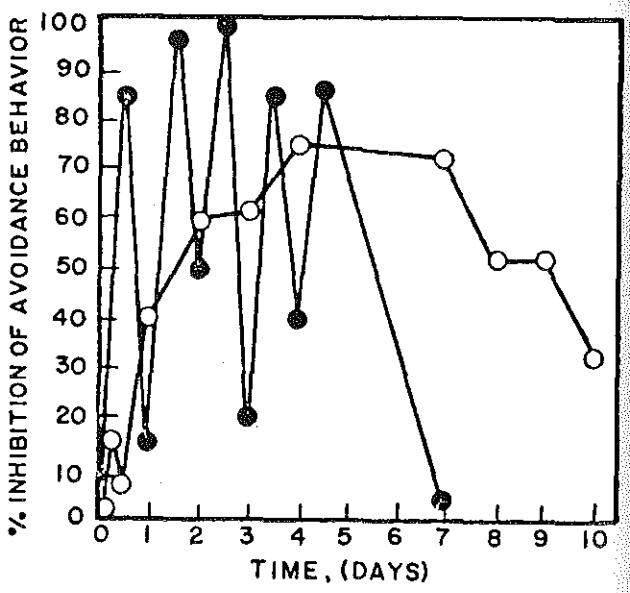


Figure 19—Comparison in the neuroleptic activity and duration, in terms of the inhibition of a conditional avoidance behavior, between single intramuscular injection of α-fluphenthixol decanoate in oil (O) and multiple oral administration of α-fluphenthixol dihydrochloride (●) [plotted from the data by Nymark et al., *Acta Pharmacol. Toxicol.*, **33**, 363 (1973)].

idal action, difficult for originally sensitive strains to develop resistance, and nonspecific in mechanism of action. They interact with, and alter the properties of, both microbial and host DNA without discrimination. Their selective toxicity depends upon selective accumulation at the intracellular milieu of the parasite. Drugs in the second group are characterized by a schizontocidal effect that is slow in onset and dependent upon the stage of multiplication of the parasite. Their mechanism of action appears to be much more specific than that of the first group. Drugs in the second group either interfere with the incorporation of *p*-aminobenzoic acid into folic acid, a process that does not occur in mammals, or bind to plasmodial dihydrofolate reductases (68). These specific mechanisms of action are exemplified by 4,4′-diaminodiphenyl sulfone and chloroguanide, respectively.

Elslager has reviewed the chemotherapy of malaria and discussed at great lengths various means by which the duration of anti-malarial action could be extended (69). The acylation of 4,4′-diaminodiphenyl sulfone and the formation of sparingly water-soluble cycloguanil pamoate are the two most interesting examples:

Cycloguanil Pamoate. Cycloguanil is the active metabolite of chloroguanide, whose anti-malarial action is related to the binding and inhibition of plasmodial dihydrofolate reductase. It has a short duration of action because of rapid excretion. This fact stimulated Thompson and his coworkers to synthesize compounds that would act for much longer periods of time (70). Their objective was to develop a single-dose, parenteral, depot preparation that would, in a well-tolerated dose, protect humans from malarial infection for prolonged periods, and preferably have immediate action as well.

Various water less soluble salts of cycloguanil were prepared with objective of sustaining the anti-malarial activity of cycloguanil. It became apparent that the ability of the drug to be released from an intramuscular depot formulation is highly dependent upon the solubility of the drug in the tissue fluid at the injection site relative to drug solubility in the formulation vehicle. If the drug has low solubility in the physiological fluid, following an intramuscular injection, the depot formulation will form a drug reservoir in the muscular tissues and gradually release the active drug moiety for absorption into systemic circulation. It is known that the aqueous solubility of any amine salt is dependent upon the type of counter anion with resultant solubility equal to the square root of the solubility product of the amine and its counter anion and is highly pH dependent. The investigations revealed that the duration of anti-malarial activity of various cycloguanil salts was linearly proportional to their aqueous solubilities (Fig. 20). This linear relationship can be expressed mathematically by:

$$\log (\text{PMW}) = -0.71\ (\pm 0.11) \log C_s + 0.077 \quad \text{(Eq. 5)}$$

Equation 5 suggests that the lower the aqueous solubility of the cycloguanil salts, the longer the duration of anti-malarial action. Results indicate that the pamoate salt of cycloguanil, with aqueous solubility of 0.03 mg/ml at pH 7, possesses the desired properties.

Laboratory tests with *P. berghei*-infected mice and *P.*

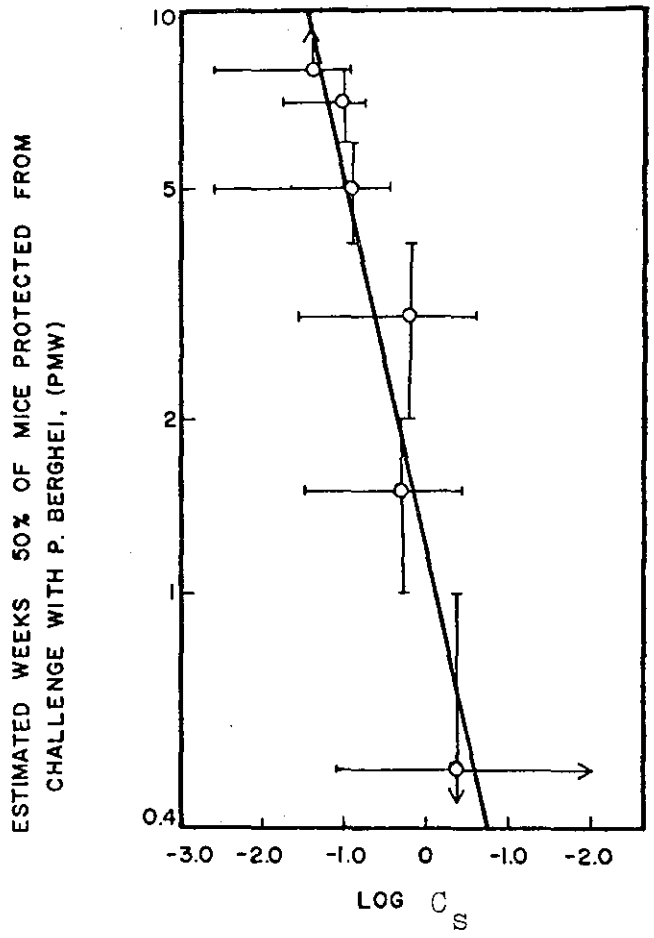


Figure 20—Linear relationship between log PMW, the estimated duration (in weeks) with 50% of mice protected from *P. berghei* challenge, and log C_s, the aqueous solubility of cycloguanil salts [reproduced, with permission, from Higuchi and Stella, *ACS Symp. Ser. No. 14*, 51 (1975)].

cynomolgi-infected monkeys showed that well-tolerated doses give protection in mice up to 8.5 weeks when given subcutaneously (70, 71). The prolonged anti-malarial activity was confirmed to be due to slow release of the active moiety from a depot formed at the site of injection and not due to the formation of a systemic reservoir (72).

Subsequent studies in man showed that a single intramuscular injection of cycloguanil pamoate at a dose of 5 mg/kg provides a protection against *P. falciparum* and *P. vivax* for several months. It functioned as a prolonged infusion of a therapeutic effective concentration of soluble dihydrotriazine in the blood, which appears to prevent the growth or even the survival of both erythrocytic and preerythrocytic parasites. Up to 80% of the injected dose was found to remain at the site of injection 2 weeks after administration and small amounts could still be detected for as long as 56 weeks later (70). The rate of absorption of the drug from intramuscular sites was determined to follow a first-order process, which was influenced greatly by the particle size of the cycloguanil pamoate (73), as expected from dissolution kinetics.

Clinically, it was observed that the use of cycloguanil pamoate with 4,4′-diacetoamidodiphenyl sulfone in 1:1 combination (Dapolar®) further extended the duration of anti-malarial protection, delayed the emergence of resistant

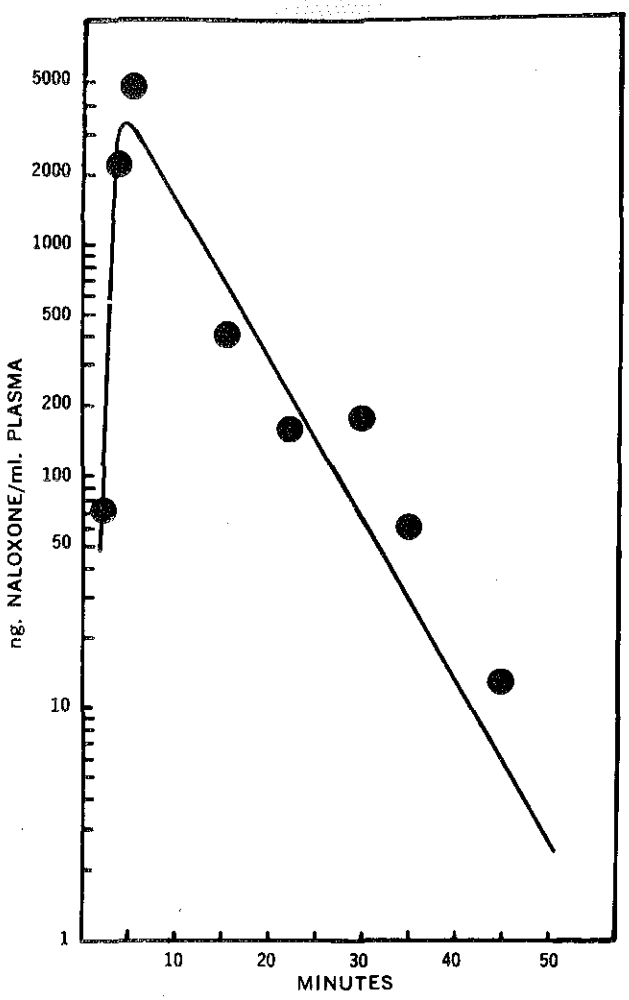


Figure 21—Plasma profile of naloxone in rats following the oral administration of 100 mg/kg of naloxone [reproduced with permission from Weinstein et al., *J. Pharm. Sci.*, **62**, 1416 (1973)].

strains, and even provided some protection against strains already less sensitive to either drug alone (68).

4,4′-Diacetoamidodiphenyl Sulfone. Diaminodiphenyl sulfone attributes its anti-malarial activity to the inhibition of folic acid synthesis in the parasites. The acylation of 4,4′-diaminodiphenyl sulfone produces 4,4′-diacetoamidodiphenyl sulfone (Acedapsone®, Hansolar), which was reported to give a prolonged anti-malarial action following intramuscular injection.

It was found to be useful in the treatment of *P. falciparum* in humans for 42 days after a single 3.25 mg/kg intramuscular injection. It is slowly hydrolyzed in the body to regenerate the active anti-malarial diaminodiphenyl sulfone (74).

Long-Acting Anti-Narcotic Preparations

Narcotic addiction affects the lives of hundreds of thousands of individuals and creates a great deal of socio-economic hardship to the community. These narcotic addicts become psychologically dependent on the drug and believe that the effects produced by self-administration of the narcotics are necessary for maintaining an optimal state of well-being. In extreme forms, the abuser exhibits the characteristics of a chronic relapsing disease (75).

Recently, attention has focused in the use of narcotic antagonists for the treatment of narcotic addiction (76–80). The rationale for the use of narcotic antagonists in the treatment of narcotic addiction is that the antagonists block the euphoric effects of a narcotic drug taken during an addict's rehabilitation and make it pleasureless, thus extinguishing the drug seeking behavior and removing the addict's incentive for continued use.

For the treatment of narcotic addiction and dependence, methadone was found useful and used extensively. Unfortunately, it introduced some complications of its own since methadone is, basically, a substitute for heroin and is itself also an addicting drug subject to abuse (81, 82).

An ideal narcotic antagonist should be free of agonistic (morphinelike) properties and effective for at least 1 week. Naloxone is known to be a pure narcotic antagonist which does not possess any agonistic properties, such as respiratory depression (83, 84). Long-term administration of naloxone is not associated with dysphoria nor is there an abstinence syndrome on abrupt withdrawal. Unfortunately, its duration of action is short and oral bioavailability is poor due to extensive hepatic "first-pass" metabolism. Therefore, large oral doses, to 3 g daily, are required for a 24-hr effective blockade to heroin challenge (82, 85). Additionally, naloxone, unlike methadone, provides no incentive to the addicts to return for frequent maintenance therapy. Patient compliance thus becomes a critical issue. Development of long-acting anti-narcotic preparations using naloxone or other narcotic antagonists thus becomes urgent for maximum patient compliance and successful rehabilitation.

Oral Bioavailability and Hepatic First-Pass Metabolism. Oral therapeutic effectiveness of both opiate-type narcotic agonists, such as morphine, and antagonists, such as naloxone, was reported to be extremely poor (75). The poor therapeutic effectiveness by the oral route was found not a result of low gastrointestinal absorption but was attributed to an extensive hepatic "first-pass" metabolism (86).

Naloxone, for example, is rapidly absorbed with a peak plasma level achieved in 5 min after oral administration (Fig. 21) and after 90 min, absorption is virtually complete (Table VII). However, the maximum amount of intact naloxone detected in the rat plasma is only 0.19% of the naloxone dose administered orally. This extensive metabolism was attributed to enzymatic metabolism of naloxone by the liver (Table VIII). The major pathway for hepatic "first-pass" metabolism in most species appears to be the conjugation of the 3-phenolic OH group with glucuronic

TABLE VII. Oral Absorption of Naloxone from In Vivo Intestinal Loop in Rats[a]

Time, min,[b]	% of Naloxone Dose	
	Remaining[c]	Absorbed
30	55.5 ± 17.9	44.5
60	18.8 ± 12.5	81.2
90	4.7 ± 3.6	95.3

[a] Compiled from the data by Weinstein et al., *J. Pharm. Sci.*, **62**, 1416 (1973).
[b] Time following the administration of 500 mg naloxone into ligated intestinal loop.
[c] Mean (± *SD*) of GLC readings.

TABLE VIII. Hepatic Metabolism of Naloxone in Rats[a]

Time, min[b]	% of Naloxone Dose	
	Intact[c]	Metabolized
30	61.0 ± 17.7	39.0
60	32.9 ± 13.2	67.1
120	13.5 ± 0.1	86.5

[a] Compiled from the data by Weinstein et al., *J. Pharm. Sci.*, **62**, 1416 (1973).
[b] Time after incubation of naloxone with rat liver slices.
[c] Mean (± *SD*) of four determinations.

acid to form naloxone-3-monoglucuronide, the major metabolite. Several minor metabolites, such as 3-sulfate and free and conjugated naloxol can also be detected in urine (87, 88). These results lead to the conclusion that the lower oral therapeutic effectiveness of naloxone is therefore the result of rapid hepatic "first-pass" metabolism (86). The *N*-dealkylation and reduction of the 6-keto group of naloxone were also reported to occur in man (89, 90).

Pharmacokinetic analysis indicated that naloxone is rapidly absorbed with an absorption half-life of 0.72 min and rapidly eliminated with an elimination half-life of 16 min (86). This value of elimination half-life agrees closely with the 20 min determined in rats (91) and the 19 min obtained in guinea pigs (92).

The increasing knowledge of the pharmacokinetic behavior of narcotic agonists and antagonists has opened new possibilities of a more rational approach to anti-narcotic treatments. Together with studies on other drug-induced biochemical changes, pharmacokinetic investigations can provide deeper insight into the mechanisms of narcotic antagonism.

Comparative Pharmacokinetics of Narcotic Antagonism. The concentration profiles of naloxone and of morphine in the serum and brain tissues of rats after subcutaneous administration of an equivalent dose are compared in Figure 22. It appears that the peak serum levels (~1 μg/ml), time to peak serum levels (<30 min), and serum half-life (40 min) for naloxone and morphine are comparable (93, 94).

However, the brain entry and egress of these two compounds differ markedly. Peak brain level of naloxone occurs within 30 min and declines by 50% within 1 hr, whereas the peak brain level of morphine is reached within 1 hr and sustained for up to 2 hr. At peak concentration, the brain/serum ratio is only 0.1 for morphine whereas for naloxone it is 15 times greater. As serum levels decline, the ratio becomes greater for morphine (approaches 0.5), while for naloxone it remains in a narrow range of 1.5–2.0. Therefore, at equivalent serum levels, one will find 3–4 times more naloxone in the brain than morphine. These observations suggest that naloxone gets into the brain more easily than does morphine. The maximum levels of naloxone in brain tissues achieved by subcutaneous administration were found to be approximately 11-fold those of morphine with an equivalent dose (88). The rapid uptake by brain tissues and high ratio of brain/serum concentrations for naloxone may well explain the potent anti-narcotic activity of naloxone. Unfortunately, the rate of disappearance of naloxone from the CNS is much faster than morphine (88, 93). Rapid

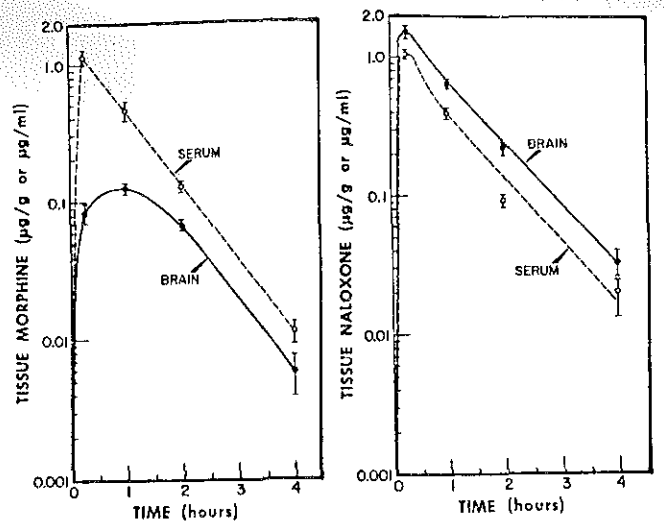


Figure 22—Serum and brain concentrations of morphine and of naloxone in rats after subcutaneous administration of 5 mg/kg dose. Each data point represents the mean for 3–5 animals. Vertical bars are standard errors [reproduced, with permission, from Berkowitz et al., *J. Pharmacol. Exp. Ther.*, **195**, 499 (1975)].

hepatic metabolism and high elimination rate appear to account for the short duration of action for naloxone.

The effects of long-term subcutaneous morphine administration on the ratio of brain/plasma morphine concentrations were studied, and it was found that this ratio was maintained in a narrow range of 0.45 to 0.54 as the subcutaneous morphine dose was increased fivefold and the duration of treatment was prolonged from 3 days to 10 days (95).

If both narcotic agonist and antagonist are removed from the receptor site compartment by a first-order rate process, the time course of the narcotic action can be expressed by:

$$\log\left(\frac{D'_N - D_N}{D_N}\right) = (\log f_{NA} D_{NA} + \log K_{NA}) - 0.43\, k_{eNA} t \quad \text{(Eq. 6)}$$

where D'_N and D_N are the doses of a narcotic agonist administered in the presence and absence of a given dose of antagonist (D_{NA}) to produce an equivalent pharmacological response, k_{eNA} is the first-order rate constant for elimination of narcotic antagonist from the receptor site compartment, f_{NA} is the fraction of narcotic antagonist dose absorbed into the receptor site compartment, and K_{NA} is the affinity constant of the antagonist-receptor complex (91).

As expected from Eq. 6, plots of $\log [(D'_N - D_N)/D_N]$ against time should yield a series of parallel straight lines (Fig. 23) with

$$\text{slope} = -0.43\, k_{eNA} \quad \text{(Eq. 7)}$$

and

$$\text{intercept} = \log f_{NA} D_{NA} + \log K_{NA} \quad \text{(Eq. 8)}$$

The relationship between the narcotic antagonistic activity, P_{NA}, and the doses of narcotic antagonist can be established from Eq. 8:

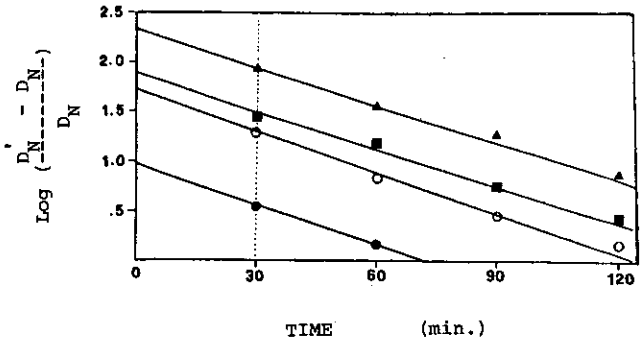


Figure 23—Linear relationship of log $[(D'_N - D_N)/D_N]$ against time after morphine at 4 doses of naloxone using the tail compression test in the rats. Key: ● 0.01, ○ 0.05, ■ 0.10, and ▲ 0.25 mg/kg (plotted from the data by Tallarida, in *Factors Affecting the Action of Narcotics*, Adler et al., Eds., Raven Press, New York, NY, 1978).

$$P_{NA} = \log K_{NA} + \log f_{NA} \qquad \text{(Eq. 9}a\text{)}$$

$$= \text{intercept} - \log D_{NA} \qquad \text{(Eq. 9}b\text{)}$$

An P_{NA} value of 2.57 ± 0.03 was obtained for the narcotic antagonism between naloxone and morphine (91). This P_{NA} value determined from *in vivo* animal testings is a useful and reproducible pharmacological constant in assessing the selectivity of antagonists and also in characterizing pharmacological receptors in different isolated tissues.

The competitive antagonism of naloxone was demonstrated in an *in vitro* comparative binding study to rat brain tissues with an opiate agonist, dihydromorphine (96).

The binding of dihydromorphine to rat brain tissues may be separated into two components: one is a saturable component and the other non-saturable. A marked regional difference was observed in the distribution of the saturable dihydromorphine binding in the brain as due to the difference in the concentration of the saturable binding sites within various brain regions. The saturable binding sites from various brain regions appeared to have similar affinities for dihydromorphine except for the binding sites from cerebral cortex which had a higher affinity. In contrast, the saturable binding sites for naloxone in various brain regions showed different degrees of affinities for naloxone.

Naloxone was found to contain at least two types of saturable binding sites; one of which is not available to the binding of dihydromorphine. The saturable binding sites in cerebellum are predominantly naloxone-specific, whereas those in striatum are capable of binding both naloxone and dihydromorphine.

Development of Long-Acting Anti-Narcotic Formulations. As discussed earlier, a most desirable component of addiction treatment would be that the anti-narcotic activity of a narcotic antagonist formulation should be long enough that frequent dosing would not be necessary to aid the addict in becoming dissociated from his opiate-taking desire.

There seems to be some differences of opinion as to how long-acting an ideal anti-narcotic formulation should be. Some investigators believe that a day or two is long enough whereas others feel that a duration of 20 months or longer would be better (97). The current consensus is that durations of at least 1 to 3 months are desirable. In order to achieve an anti-narcotic activity with a desired duration of one to three months, a logical approach is to develop long-acting drug delivery systems for the controlled administration of an acceptable but short-acting antagonist.

Efforts to achieve the development of long-acting drug delivery systems were launched in the early 1970's by the city of New York Public Health Department and by the National Institute on Drug Abuse (NIDA). Presently, the program supported by NIDA includes 6 contracts that are concerned with the development of new drug delivery systems and 3 contacts that are involved in the clinical evaluation of the drug delivery systems developed (98).

Two approaches have been successfully utilized for the development of parenteral controlled release anti-narcotic formulations:

Insoluble Salts/Complexes of Narcotic Antagonists in Oil Suspensions. One approach to achieving a long-acting anti-narcotic activity is the development of low solubility salts and ionic complexes of potential narcotic antagonists. This approach has already been successfully applied in other therapeutic areas discussed earlier, such as penicillin G procaine, vitamin B_{12}-Zn-tannate, and cycloguanil pamoate.

For example, the intramuscular bioavailability of penicillin G was markedly prolonged by the use of depot preparations consisting of relatively insoluble salts of penicillin G in a suitable vehicle. It was reported that the therapeutic blood levels of penicillin G persist 12 to 24 hr if penicillin G was administered as procaine salt, an ion pairing salt, in aqueous suspension. Therapeutic blood levels were prolonged to 24–48 hr if procaine penicillin G was administered in oil suspension and to 48–96 hr if the oil suspension was gelled with aluminum stearate. By changing to a different type of ionic pair-type salt, such as benzathine penicillin G, which has an aqueous solubility 20 times lower than the procaine salt, the therapeutic blood levels of penicillin G was further sustained to one week or longer (99).

Approximately, 100 mono- and poly-basic organic acids, such as salicylic acid and tannic acid, were evaluated for their ability to form water-insoluble salts with narcotic antagonists, such as naloxone and naltrexone, and to form water-insoluble complexes with polyvalent metallic ions, such as Zn^{2+}, Al^{3+}, Mg^{2+}, and Ca^{2+} (100).

Using the mouse tail-flick test, the duration of anti-narcotic activity of representative salts and Zinc complexes of naloxone was evaluated (3, 101, 102). Results indicated that the intramuscular anti-narcotic activity of naloxone was significantly prolonged by forming insoluble salts and Zn-complexes (Table IX). Naloxone HCl, a water-soluble salt itself, showed significant antagonistic activity only to 4 hr even at the very high dose of 4 mg/kg (naloxone base equivalent). The activity was sustained to 8 hr with the formation of pamoate and tannate salts and to 16 hr with 5-*t*-octyl salicylate. The narcotic antagonistic activity was further prolonged to 24 hr with the formation of Zn-tannate complex. On the other hand, incorporation of zinc had no further effect on the prolongation of antagonistic activity of pamoate and 5-*t*-octyl salicylate. It appeared that the

TABLE IX. Duration of Antinarcotic Activity of Naloxone Salts and Zinc Complexes in Mice[a]

Naloxone Derivatives[b]	Percent Antagonism[c]					
	40 min	4 hr	8 hr	16 hr	24 hr	48 hr
Salts						
Hydrochloride	99	79	16	3	10	—
Pamoate	100	100	94	11	1	—
Tannate	99	87	66	6	3	—
5-*t*-Octyl salicylate	100	78	58	43	18	17
Complexes						
Zn-pamoate	100	100	86	9	0	—
Zn-tannate	95	99	90	41	42	23
Zn-5-*t*-octyl salicylate	99	96	29	57	25	14

[a] Compiled from the data by Gray and Robinson, in *Narcotic Antagonists*, Braude et al., Eds., Raven New York, NY, 1974, p. 555.
[b] Intramuscular injection in peanut oil (4 mg/kg of naloxone base equivalent).
[c] Percent antagonism to a standard morphine sulfate dose of 20 mg/kg administered intraperitoneally 30 min before mouse tail-flick tests (12 mice each).

incorporation of zinc had the most significant sustaining effect on the duration of antagonistic activity of the naloxone tannate preparations. Similar results were also observed with other narcotic antagonists, such as cyclazocine and naltrexone.

The duration of anti-narcotic activity of the salts and Zn-complexes of naloxone was found to be roughly correlated with their percent dissociation: hydrochloride (100%) > pamoate (74.1%) > 5-*t*-octyl salicylate (62.5%) > tannate (53.3%). The incorporation of zinc substantially reduced the percent dissociation of tannate from 53.3 to 13.6–14.3%, but not pamoate (3).

Simultaneously, a long-acting naloxone pamoate injectable formulation was developed and patented (2). It consists of naloxone pamoate suspension in peanut oil or sesame oil gelled with 2% aluminum monostearate. The duration of narcotic antagonistic activity of this long-acting formulation has been evaluated in mice by Straub tail tests and in rats by narcosis tests (Table X). Results demonstrated that with naloxone HCl (50 mg/kg) the Straub tail reaction of oxymorphine HCl (2 mg/kg) was blocked at 15 min, but not at 24 hr. With naloxone pamoate suspension, complete protection (100%) was achieved for a duration up to 24 hr, and 77.8% of the mice were still protected at 48 hr.

TABLE X. Duration of Antinarcotic Activity of Naloxone Pamoate Suspension[a]

Time, hr	% Protection against Oxymorphone HCl[d,e]	
	Straub Tail[b]	Narcosis[c]
0.25	100	100
24.0	100	75
48.0	77.8	62.5
72.0	0.0	37.5

[a] Suspension in peanut oil gelled with 2% aluminum monostearate.
[b] Each of the nine CF#1-S male albino mice was administered subcutaneously with a dose of naloxone pamoate suspension (50 mg/kg) and then challenged with a subcutaneous daily dose of 2 mg/kg oxymorphone HCl in solution.
[c] Each of the eight CFN male albino rats was administered subcutaneously with a dose of 10 mg/kg naloxone pamoate suspension and then challenged with a subcutaneous daily dose of 1 mg/kg oxymorphone HCl in solution.
[d] At the same dose level, naloxone HCl in aqueous solution produced 100% protection at 0.25 hr, but no protection at all at 24 hr.
[e] Compiled from the data by Lachman et al., U.S. patent 3,676,557 (July 11, 1972).

The comparative anti-narcotic activity in rats also illustrated that the aqueous naloxone HCl solution produced a complete narcotic blockade in rats only at 15 min but none at 24 hr. On the other hand, naloxone pamoate suspension showed a significantly longer narcotic blockade; at 15 min, all of the treated rats were protected from oxymorphone-produced narcosis; at 24 hr, 75% were still protected; at 48 hr, 62.5%; and at 72 hr, 37.5%.

The duration of narcotic antagonistic activity of this naloxone pamoate suspension was also evaluated in 12 dogs. Intramuscular injection of 10 mg/kg of naloxone pamoate suspension was found to protect the dogs from any serious narcotic depression for at least 7 days in all of the treated dogs, for at least 14 days in most of the animals, and for 21 days in a few of them. By comparison, the same dosage of naloxone HCl in aqueous solution was not able to give adequate protection for even one day. Additional investigations in non-narcotized animals, the intramuscular administration of naloxone pamoate suspension at a dosage equivalent to 10 mg/kg of naloxone HCl produced no significant effect on behavior, respiration, blood pressure, heart rate, ECG, or body temperature as did the naloxone HCl. At higher doses, naloxone pamoate showed substantially less convulsant action and toxicity than naloxone HCl, presumably due to the sustained release of the naloxone from the relatively insoluble pamoate salt suspension in oil (naloxone pamoate has an oil solubility that is 12 times lower than naloxone itself).

The effects of the formation of insoluble salts with poly-basic organic acids and of insoluble complexes with polyvalent metallic ions on the duration of anti-narcotic activity of a narcotic antagonist can be illustrated by naltrexone, a close analog of naloxone.

It was reported that naltrexone is essentially as pure an antagonist as is naloxone and 2–3 times as potent as naloxone. When administered orally for narcotic blockade in rats, naltrexone HCl is about eight-fold as active and about three times as long-acting as naloxone HCl on a mg dose basis (103). The difference between naloxone and naltrexone in their duration of oral activity may be related to the difference in the pharmacokinetic rate processes in their permeation from blood circulation to target tissue compartment, which may contain the receptor sites.

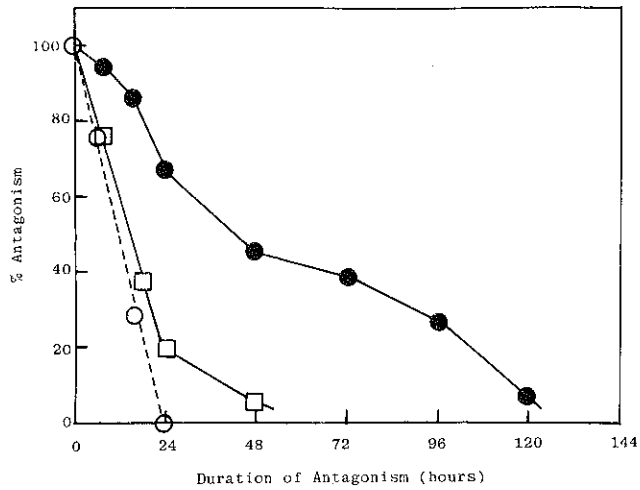


Figure 24—Comparative antagonistic activity of naltrexone preparations in peanut oil against the analgesic effect of morphine sulfate in mice. Key: O naltrexone HCl, □ naltrexone tannate, ● naltrexone-Zn-tannate (4 mg/kg naltrexone base, each) [plotted from the data by Gray and Robinson, *J. Pharm. Sci.*, **63**, 159 (1974)].

The intramuscular anti-narcotic activity of naltrexone was slightly prolonged by the conversion of its hydrochloride to tannate salt. With the formation of Zn-tannate complex, the activity of naltrexone showed at least a threefold increase in its duration (Fig. 24). A further roughly threefold prolongation of anti-narcotic activity was achieved when the peanut oil suspension of naltrexone-Zn-tannate was gelled with aluminum monostearate (Fig. 25). Nineteen days after intramuscular administration, this naltrexone-Zn-tannate gelled suspension still produced 70% antagonism against the analgesic effect of morphine sulfate in mice (4). Effect of aluminum monostearate on the intramuscular activity of naltrexone-Zn-tannate may be explained by its water repellency, resulting in the reduction of interfacial dissolution rate of naltrexone-Zn-tannate from the oil suspension (99).

TABLE XI. Extent of Dissociation of Naltrexone Salts and Zn-Complex[a]

Naltrexone Derivatives	Percent Dissociation[b]
Naltrexone HCl	100
Naltrexone tannate	38.2
Naltrexone-Zn-tannate	11.5

[a] Compiled from the data by Gray and Robinson, *J. Pharm. Sci.*, **63**, 159 (1974).
[b] In an isotonic phosphate buffer at pH 7.3 and 37 °C.

As illustrated by naloxone, the duration of anti-narcotic activity of naltrexone salt and Zn-tannate complex was also related to the extent of in vitro dissociation (Table XI), though all produced the same ED_{80} values for narcotic antagonism as does naltrexone base at a dose of 0.02 mg/kg.

Biodegradable Narcotic Antagonist-Containing Beads and Microcapsules in Aqueous Suspensions. Recently, a biodegradable drug delivery system was developed from the homopolymer or copolymer of lactic acid and glycolic acid (104). In a biological environment, these biodegradable lactide/glycolide polymers are broken down by enzymes into innocuous metabolites, such as carbon dioxide and water, and the drug impregnated within the polymer matrix or encapsulated in the microcapsules is thus released into the tissue fluid at the site of administration. The biodegradable polymer has been extensively applied to the development of long-acting delivery systems for narcotic antagonists (105–109).

A considerable advantage in the long-term administration of narcotic antagonists would be achieved, if the drug-containing polymeric drug delivery system could be delivered into the body in the form of very fine particles by hypodermic injection. The long-term bioavailability of narcotic antagonists, such as naltrexone, from the lactide/glycolide copolymer after parenteral administration

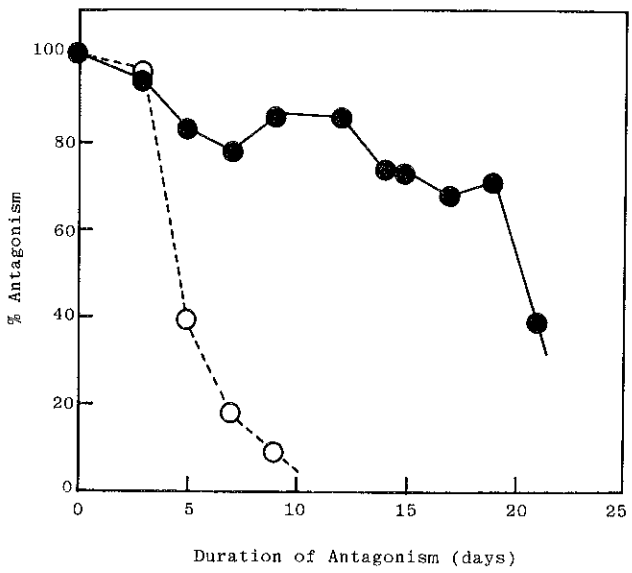


Figure 25

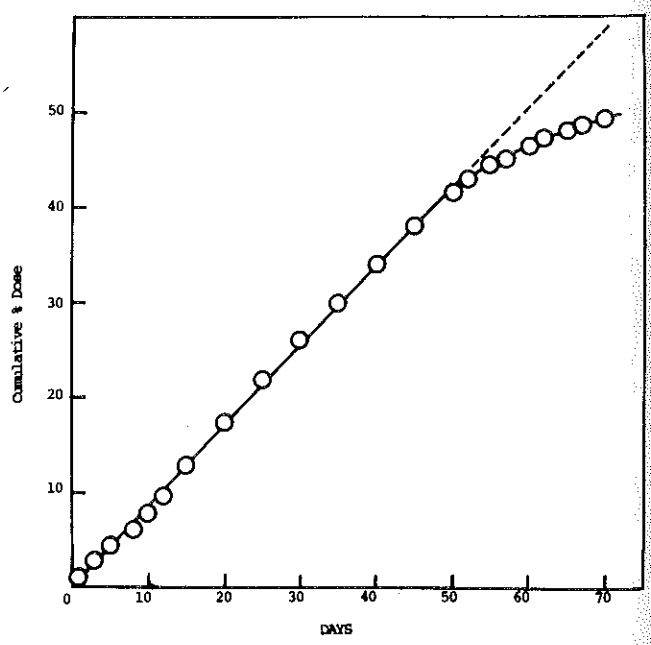


Figure 26

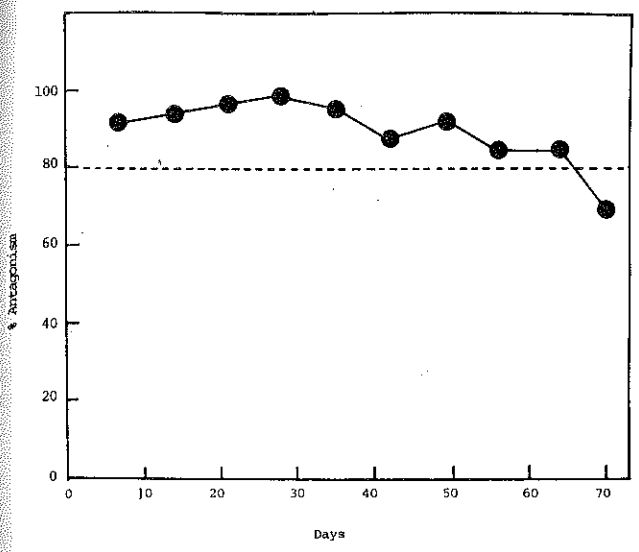


Figure 27

in mice is illustrated in Figure 26. The cumulative urinary excretion profile indicates that the in vivo release of naltrexone from lactide/glycolide copolymer beads is fairly constant for a duration up to 53 days. 0.83% of the dose was released daily. The anti-narcotic activity of this long-acting naltrexone formulation was evaluated and found to achieve a significant anti-narcotic activity against morphine challenge for two months (Fig. 27). With poly(lactic acid) beads as the delivery system, naltrexone was able to achieve narcotic antagonism for a duration of 3–4 weeks in several animal species (Table XII).

Under the sponsorship of NIDA, an injectable microcapsule formulation has also been developed using the biodegradable poly(lactic acid) polymer as the long-acting delivery system for naltrexone and naltrexone pamoate (110).

Long-Acting Contraceptive Preparations

It is known that progestational steroids, such as the natural progesterone, in high doses suppress the pituitary release of luteinizing hormone (LH) and the hypothalmic release of the LH-releasing factor (LRF), thus preventing ovulation (56).

TABLE XII. Duration of Antinarcotic Activity of Naltrexone-Dispersed Poly(lactic Acid) Beads in Animals[a]

Animals Tested	Dose [b], mg/kg	Duration [c], days
Dogs	17	29
Monkeys	30	20
Mice	39	21
Rats	240	24

[a] Compiled from the data by Yolles et al., *J. Pharm. Sci.*, **64**, 348 (1975).

[b] Injectable suspension of 35% naltrexone/poly(lactic acid) beads (500–710 μm) in 7% CMC gel.

[c] Duration of antinarcotic activity was determined in rats and mice by the tail pinch test; in dogs by measuring the flexor reflex, the skin twitch reflex, the pulse rate, and the pupillary diameter; and in monkeys by determining the changes in morphine-induced prolongation of interblinking time.

In addition to the development of progestational steroid-releasing vaginal rings for monthly intravaginal contraception and progesterone-releasing IUDs for yearly intrauterine contraception, several successful attempts have also been made to develop injectable, long-acting contraceptive formulations for long-term, continuous fertility control.

The first attempt was to inject the natural progesterone in oleaginous solution; it was found that progesterone is readily absorbed from the sites of injection, but it is also degraded at a rate that is too rapid for optimal therapeutic effectiveness. In fact, results suggested that it is extremely difficult to achieve effective blood levels for progesterone with any convenient dosing schedules. In animal tests, administration of several doses per day is more efficacious than the same dose administered once daily, and less frequent dosing is always inefficacious (49, 56).

Development of an injectable, long-acting contraceptive formulation has been made possible via the use of long-acting derivatives of progesterone. Relatively successful results have been obtained in preclinical and/or clinical studies with the following preparations:

(*a*) Medroxyprogesterone acetate in aqueous suspensions (Depo-Provera, Upjohn)
(*b*) Dihydroxyprogesterone acetophenide/estradiol enanthate in oleaginous solutions (Deladroxate, Squibb)
(*c*) Norethindrone in biodegradable polymer beads
(*d*) Norethindrone enanthate in oleaginous solutions (Norigest, Schering AG)
(*e*) Norgestrel 17 β-fatty acid esters in oleaginous solutions

These long-acting, injectable contraceptive preparations contain either progestin alone or in combination with an estrogen (111–115). Their development will be individually discussed.

Depo-Provera C-150. Depo-Provera C-150 (Upjohn) is an aqueous suspension of 150 mg of microcrystalline medoxyprogesterone acetate. It is recommended for intramuscular injection deep into the gluteal muscle, one dose every 3 months.

Depo-Provera has been made commercially available as a long-acting injectable preparation for various gynecologic and obstetric indications since 1960 and for treatment of inoperative, advanced endometrial carcinoma since 1972. However, its potentials as an injectable long-acting contraceptive formulation was not officially accepted until late 1974 (IND was filed in the July of 1963, but the NDA was not approved by the Food and Drug Administration until September of 1974). It has been used as an injectable contraceptive preparation in the British Commonwealth.

The long-acting contraceptive activity of parenterally administered medroxyprogesterone acetate in aqueous suspension formulation is believed to be the result of one or more of the following actions (116, 118).

(*a*) Suppressing ovulation by the inhibition of the preovulatory surge of luteinizing hormone (LH) and follicle stimulating hormone (FSH).

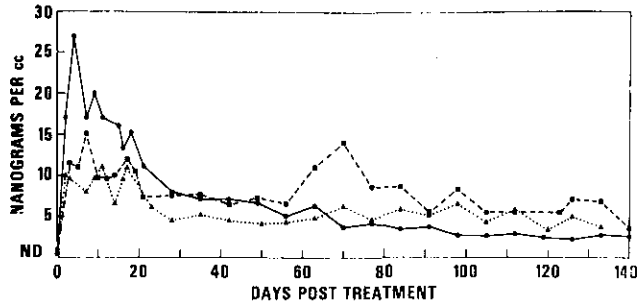


Figure 28

(*b*) Impeding nourishment of the blastocyst within the endometrial cavity by alteration of the secretory transformation of endometrium.

(*c*) Reducing the penetration of spermatozoa into the uterus by an increase in the viscosity of cervical mucus.

The first action was substantiated clinically by gonadotropins bioassay in patients receiving a single injection of Depo-Provera C-150 (117) and by double antibody radioimmunoassay in 84 patients who had been on Depo-Provera for 3 to 24 months (118). Both measurements demonstrated that the LH and FSH levels in treated patients do not differ significantly from control values except for the observation that the pre-ovulatory peaks of both gonadotropin hormones are eliminated from the treated patients.

Between July of 1963 through March of 1973 a total of 11,500 women were treated with various dosage regimens of Depo-Provera for a total contraceptive experience of 208,894 woman-months (119). Ovarian biopsies on subjects receiving Depo-Provera revealed the existence of follicles in all stages of development with small follicular cysts, and absence of corpora lutea. Histomorphological and histochemical studies of ovaries from Depo-Provera-treated patients are comparable with those of a normal ovary except for the absence of a corpus luteum (120).

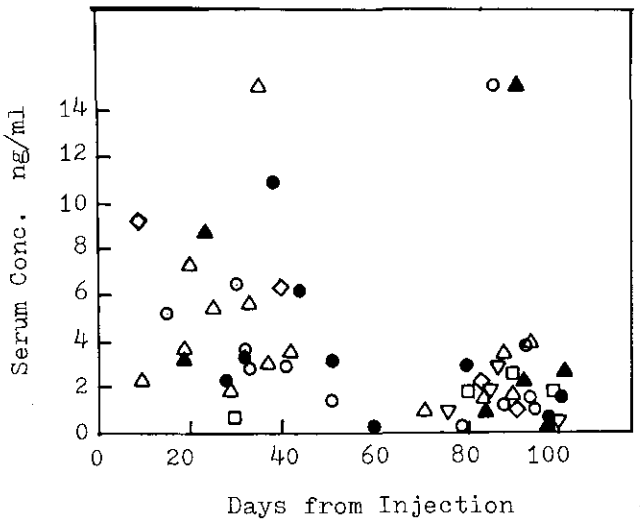


Figure 29

TABLE XIII. Life Table Analysis of the Clinical Effectiveness of Depo-Provera[a]

Events	Clinical Effectiveness[b,c] C-150	C-300
Pregnancies	0.3	2.3
Termination		
Medical	19.1	10.3
Personal	22.5	20.7
Continuation rate	58.1	66.7

[a] Compiled from Schwallie and Assenzo, *Fertil. Steril.*, **24,** 331 (1973), and Scutchfield et al., *Contraception*, **3,** 21 (1971).

[b] The statistic is based on more than 25,000 woman-months of use with Depo-Provera C-150, 150-mg dose every 3 months and more than 10,000 woman-months of use with C-300, 300-mg dose every 6 months.

[c] Expressed as events per 100 women through 12 months of use.

The serum concentration profile of medroxyprogesterone acetate after intramuscular administration of a single 150-mg dose of Depo-Provera was monitored with radioimmunoassay for much longer than the prescribed treatment period of 90 days (117, 119, 121). Concentration of medroxyprogesterone acetate in the systemic circulation was found to be in the 10–25 ng/ml range during the first 20 days after the injection, then dropped to a steady-state level of 4–8 ng/ml for up to 140 days (Fig. 28) and to <3 ng/ml during the remaining 150 to 200 days.

The serum levels of medroxyprogesterone acetate in patients receiving multiple doses of Depo-Provera, one injection every 90 days, were also monitored at various time intervals (Fig. 29). A median serum level of 3.5 ng/ml was obtained. A comparison of the medians from various injections revealed that the serum medroxyprogesterone acetate levels at the end of the 7th injection period is no different from the serum concentrations detected at the end of the 1st injection period. This observation suggests that there is no accumulation of medroxyprogesterone acetate in the body over the treatment period.

There is wide variation in the continuation rates at the end of one-year use reported from various clinical studies. Life table analysis indicates that approximately 58–67% of the patients who have chosen this method of contraception still continue using it after one year of medication (Table XIII) (122, 123).

Clinical effectiveness of Depo-Provera C-150 was evaluated in 1,123 subjects for a total of over 14,000 months (124). Results showed that the intramuscular administration of Depo-Provera C-150 at 3-month intervals is a suitable, effective means of contraception. Only one method-related pregnancy and three patient-failure pregnancies occurred in over 14,000 months of use. This translates to a pregnancy rate of 0.3 per 100 woman-year. This result is comparatively better than other methods of fertility control (Table XIV). In contrast to the metabolic effects commonly noted with the uses of progestin–estrogen combination oral contraceptives, no apparent change in liver function, lipid metabolism, or blood pressure has been detected during Depo-Provera treatment (125).

After discontinuing the Depo-Provera treatment, conception regained in 12 months in over 60% of the users (124). In another investigation, results also indicated that

TABLE XIV. Comparison in Pregnancy Rate between Depo-Provera and Other Methods of Birth Control[a]

Methods	Rate of Pregnancy[b] Method Failure	Combined Failure
Depo-Provera C-150	0.085	0.3
Oral contraceptives		
Combined regimen	0.1	0.7
Sequential regimen	0.5	1.4
Intrauterine devices[c]		
Large Lippes loop	1.9	2.7
Saf-T-Coil	1.9	2.8
Condom/diaphragm	2.6	—
United States Clinics		
Diaphragm & spermicidal jelly or cream	—	17.9
Vaginal foam	—	28.3
Vaginal jelly or cream alone	—	36.8

[a] Compiled from the data by Powell and Seymour, *Am. J. Obstet. Gynecol.*, **110**, 36 (1971).
[b] Expressed as the number of pregnancy per 100 woman-year.
[c] Non-medicated, conventional IUDs.

resumption of ovulation and fertility occurred in the majority of women within 1 year after stopping treatment (122); however, a delayed resumption of regular menses and ovulation was observed in 20–25% of the users for longer than 1 year.

Elevated progesterone concentrations, which are inconsistent with the levels found at post-ovulation, were detected at 200–245 days after treatment. The elevation in progesterone levels signaled the return of the ovaries to normal ovulatory cycles at the end of medication.

Vaginal cytology and endogenous estrogen excretion were also determined in a group of women who had been on continuous Depo-Provera treatment for 21–41 months. Results showed that the mean estrogen values in the treatment group were suppressed by approximately 50% below the mean values of normal menstruating women on Day 7 to Day 8. However, the mean estradiol values were still higher than the levels measured in post-menopausal women. No atrophy pattern and no absence of superficial cells were noted.

Urinary estrogen levels measured in 20 patients were found moderately depressed after a single injection of Depo-Provera, but returned to pretreatment levels within 7–20 weeks after treatment. However, results of another study revealed no statistically significant difference from controls in urinary estrogen levels in 12 women treated with Depo-Provera over a period of one year.

Serum estradiol levels were also analyzed in 121 women who had received Depo-Provera for 1–5 years. Mean serum estradiol levels were in the range normally found in the early follicular phase of women with ovulatory cycles. Nearly all the serum estradiol measurements revealed levels which are higher than those found in post-menopausal women (126). Separate investigation in 20 women, who have been treated with Depo-Provera for at least 18 months, indicated that the ratio between estradiol and estrone in plasma was 2:1 as in untreated women (127). They also observed a variation in estrogen levels in the 12-week treatment period with a significant rise at the end of treatment, indicating no possible accumulation of medroxyprogesterone acetate in the body at the end of medication.

IND on Depo-Provera for injectable contraceptive uses was filed in July of 1963. An NDA on Depo-Provera C-150 was approved by the Food and Drug Administration in September 1974 as an injectable, long-acting contraceptive for limited use in patients who are unwilling or unable to use other contraceptives. The dose recommended for contraception is one injection of Depo-Provera® C-150 every 3 months.

Several undesirable side effects, primarily the frequent amenorrhoea and the erratic, unpredictable interval between the injection and the occurrence of menstruation, were reported. These side effects probably accounted for the high patient "drop-out" rate, which reached an average of 42% at the end of 9 months. They were reportedly mitigated later by co-administration of long-acting estradiol cypionate with the Depo-Provera treatment (128). Results of intramuscular injection of this combination—25 mg of medroxyprogesterone acetate and 5 mg of estradiol cypionate in 104 patients, one dose every 28 to 32 days, for a period of 4 to 15 months—indicated that 100% fertility control has been achieved. Aside from the minor change in the menstrual patterns, no significant side effects were noted. This monthly injectable contraceptive formulation caused no discomfort to the users and the dropout rate was found to be less than 5% at the end of 9 months as compared to the 42% reported with Depo-Provera C-150.

Endometrial biopsies performed in the first month of treatment revealed distinct phases of proliferation and secretion with varying degrees of hypoplasia, which became the dominating feature after 6 to 7 months of medication. No apparent change in breast morphology and function, and neither mastalgia nor breast discomfort was reported.

Deladroxate. Deladroxate (Squibb) is a once-a-month intramuscular contraceptive preparation. It is composed of 150 mg of dihydroxyprogesterone acetophenide and 10 mg of estradiol enanthate and provides, when administered parenterally, simultaneous prolonged progestational and estrogenic activities for approximately 3 weeks. It was reported that it gives a steroid ratio that is adequate for the control of ovulation (129). This combination produced a menstrual cycle which simulates most closely the normal menstrual pattern in humans in terms of length and quantity of menstrual flow. When given on Day 8 of the cycle, it gave a mean cycle length of 27.8 days.

Extensive clinical testing was conducted in 60 centers worldwide with over 70,000 cycles in 8,000 women. Only one occasional pregnancy was reported to occur during the first treatment cycle. No pregnancies have ever occurred beyond the second treatment cycle (130). Of the 22 patients who had completed 24 consecutive monthly injection of Deladroxate, 16 had returned to spontaneous ovulatory cycles within 12–42 weeks after termination of the treatment. Normal ovulatory cycles can be stimulated to return more rapidly, if necessary, by the use of clomiphene, an anti-estrogenic drug that blocks the negative feedback action of estrogens on the hypothalamus.

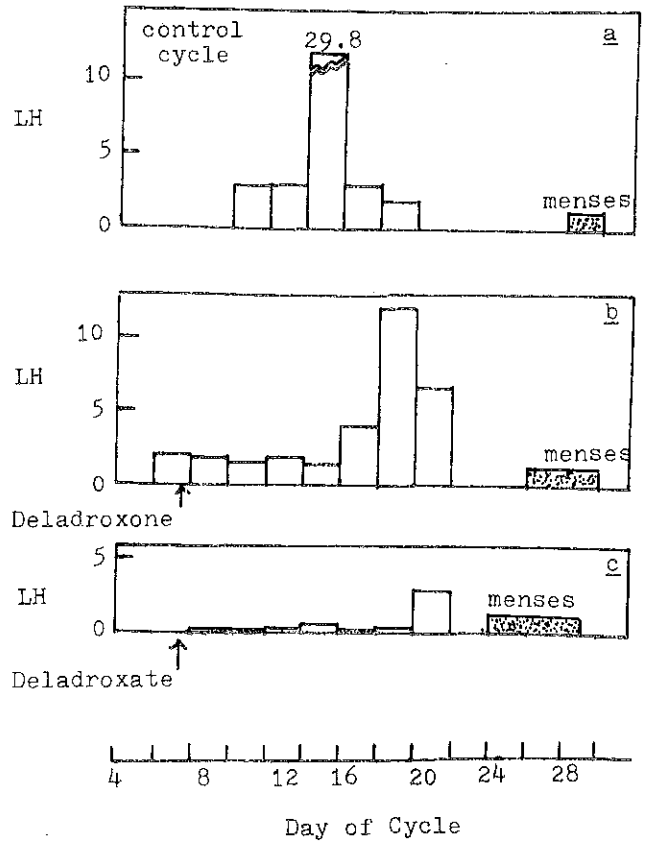


Figure 30

Additional clinical evaluations in 189 women, who were administered one dose of Deladroxate every month on the 7th, 8th, or 9th day following the onset of menstruation, for 1,255 cycles also reported no pregnancies during a 24-month period (131).

When used alone, dihydroxyprogesterone acetophenide (Deladroxone, Squibb) was observed to cause a delay in the appearance of the LH peak, which shows up approximately 4 days after the expected mid-cycle surge, and inhibition of ovulation. The use of dihydroxyprogesterone acetophenide and estradiol enanthate in combination (Deladroxate), on the other hand, markedly depressed the level of LH in addition to the delay in its appearance (Fig. 30). Results of both immunoassay of LH and radioimmunoassay of serum gonadotropins levels further confirmed that the suppression of midcycle LH peak is the mechanism of contraceptive action for Deladroxate. Both levels of LH and FSH are depressed and ovulation is inhibited during its medication.

Norethindrone-Releasing Biodegradable Polymer Bead Suspension. A long-acting, injectable contraceptive norethindrone formulation was developed by first preparing biodegradable norethindrone-dispersing polymeric beads (90–180 μm in particle size) of 90% lactide/10% glycolide copolymer and then suspending them in 1% w/v aqueous methyl-cellulose solution (132).

Results of intramuscular administration of this formulation in four female baboons indicated that norethindrone is released at a rate fairly constant with a daily dose of approximately 1.075 mg (or 1.92% of the administered dose) for the first 3 weeks. Burst effect is minimal (Fig. 31). The release rate from week 3 through week 7 is also fairly constant, but it decreases by threefolds to a daily dose of 364 μg. Beyond week 7, the amount of norethindrone released daily becomes negligibly small. Totally, 58.4% of the administered dose were excreted during a 7-week observation.

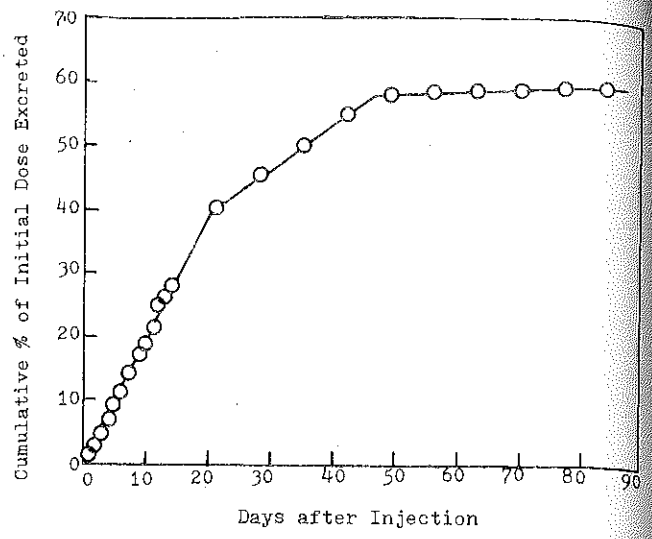


Figure 31—Cumulative excretion of radioactivity (expressed as percent of initial dose of norethindrone) from four baboons injected intramuscularly with biodegradable polymer beads of 90% lactide/10% glycolide copolymer containing 20% w/w of norethindrone [plotted from the data by Gresser et al., *Contraception*, **17**, 253 (1978)].

Intramuscular bioavailability of norethindrone from this injectable contraceptive preparation was also evaluated in small animals, e.g., rats, and larger animals, e.g., dogs. It was found that the in vivo release rate of norethindrone, represented by the total excretion rate in both urine and feces, is linearly proportional to the initial dose of norethindrone impregnated in the polymer matrix (Fig. 32). It appears that the intramuscular release rate of norethindrone

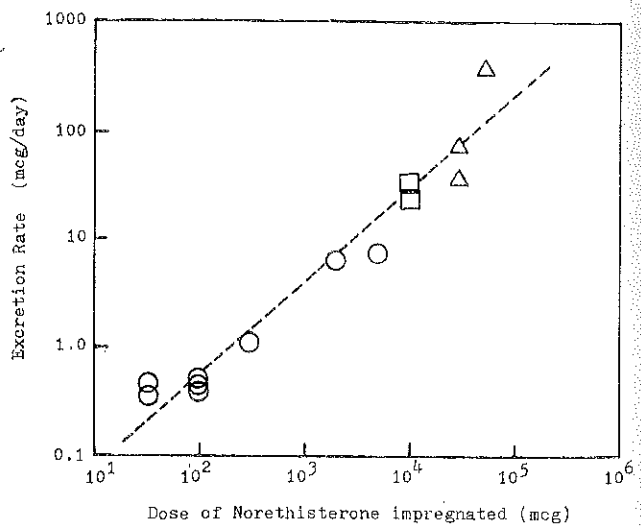


Figure 32—Dependency of the excretion rate of norethindrone in three animal species on the initial dose of norethindrone impregnated in the polymer beads [plotted from the data by Gresser et al., *Contraception*, **17**, 253 (1978)].

from the biodegradable polymer beads is independent of the animal species. The linear relationship between the rate of excretion of noretindrone and its dosage in the polymer beads is defined by:

$$\log\left(\frac{Q}{t}\right)_e = 0.82 \log (\text{dose}) - 1.85 \qquad \text{(Eq. 10)}$$

where $(Q/t)_e$ is the total excretion rate of norethindrone measured in urine and feces.

Equation 10 suggests that the intramuscular release rate of norethindrone can be controlled to give a therapeutic dose of norethindrone by impregnating adequate amount of norethindrone into the biodegradable polymer beads.

Norethindrone Enanthate in Oleaginous Solution. Another injectable, long-acting norethindrone formulation was also developed by synthesizing the aqueous-insoluble, but oil-soluble enanthate ester of norethindrone.

Intramuscular administration of 200 mg of norethindrone enanthate in oleaginous solution (Norigest, Schering AG) to 130 young fertile women, one dose every 3 months, for a total of 2,300 months of observation produced only 3 pregnancies, which occurred during the second or third month after the last injection (133). This result translates into a method-related failure of 2.3%. A total of 25.3% of the patients dropped out of the treatment due to side effects, such as the disturbance of menstrual cycles, or for other reasons. They became pregnant between the 4th and 7th months after the last medication.

Additional clinical trials of this contraceptive formulation in 160 patients, with a dosing schedule of one injection every 10 weeks, suggested that this preparation causes fewer complaints of bleeding and weight gain than Depo-Provera discussed earlier. It also yielded a more rapid return of ovulation following the termination of medication. More clinical evaluations are currently underway in several countries.

The mechanism of action for this long-acting contraceptive preparation appears to be attributed to the changes norethindrone has made in the cervical mucus after its release, leading to the interference of sperm survival and ascent. The modifications observed in the endometrium and endosalpinx may also contribute to some extent to contraception. Gynecologic laparotomy of 6 women revealed no signs of ovulation or the formation of corpus luteum during the medication (133).

The clinical trials in the United Stated of this injectable contraceptive formulation were recently recommended by the Fertility & Maternal Health Drug Advisory Committee of the FDA (F-D-C reports, Oct. 1, 1979).

Long-Acting Norgestrel 17 β-Fatty Acid Esters. Daily oral administration of *d*-norgestrel at low dose (30 μg) has been recognized as a useful method of hormonal contraception (134, 135). Recently, several efforts have been devoted to the development of long-acting *d*-norgestrel formulations for sustained, continuous fertility control in humans. The bioavailability and contraceptive activity of *d*-norgestrel have been effectively prolonged by impregnating the steroid in matrix-type, ring-shaped vaginal silicone devices, by encapsulating the drug in subdermal implants, or by esterifying the *d*-norgestrel with long-chained

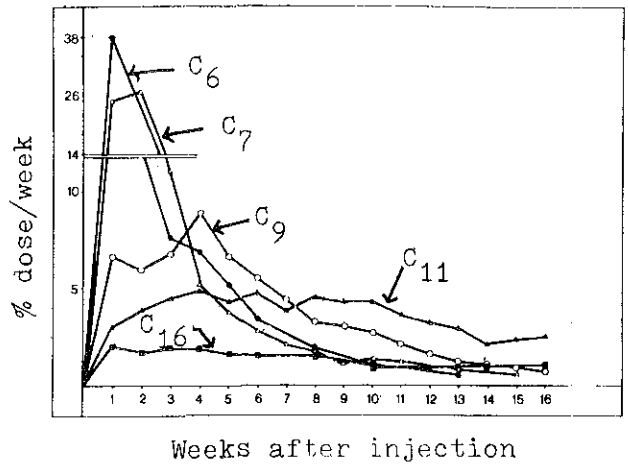


Figure 33—Mean weekly excretion of ^3H activity after intramuscular injection of 50 mg of *d*-norgestrel esters (800 μCi^{3}H) in 1 ml of castor oil/benzyl benzoate (6:4, v/v) into each of two beagles for up to 8 weeks after administration and, for one animal, up to 16 weeks after administration [replotted from the data by Humpel et al., *Contraception*, **15**, 401 (1977)]. Key: ● hexanoate, △ heptanoate, O nonanoate, ▲ undecylate, and ■ hexadecanoate.

fatty acids to yield an injectable long-acting contraceptive formulation (136, 137).

Five *d*-norgestrel 17 β-fatty acid esters with alkyl chain length ranging from 6 to 16 carbon atoms (n = 4–14) were synthesized and intramuscularly administered to dogs in a castor oil/benzyl benzoate formulation (136). The bioavailability of *d*-norgestrel from these esters was found to be dependent upon the chain length of these fatty acids; that is, the longer the chain length, the slower the release rate of *d*-norgestrel from the depot formulation and hence the longer the duration of contraceptive activity (Fig. 33). The total percent dose excreted 8 weeks after intramuscular injection was found to decrease exponentially as increasing the alkyl chain length of fatty acid esters (Fig. 34).

Clinical effectiveness of this injectable *d*-norgestrel depot

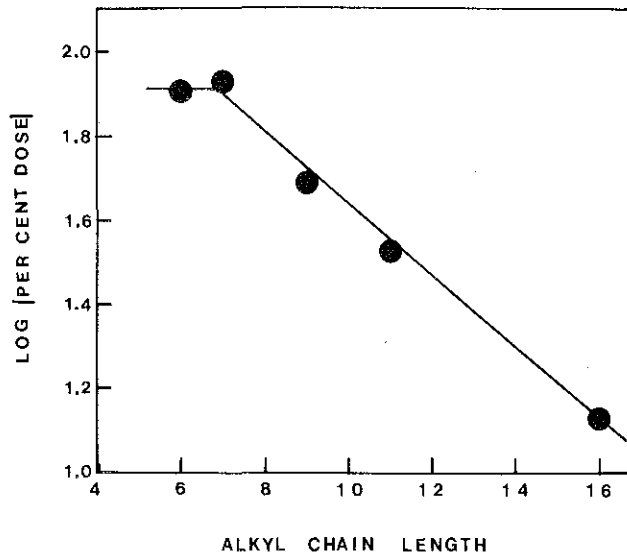


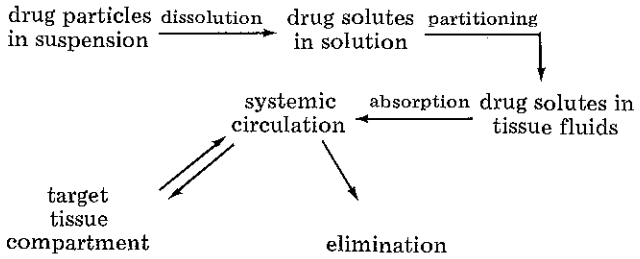
Figure 34—Effect of alkyl chain length of fatty acids on the 8-week urinary excretion of d-norgestrel from intramuscular d-norgestrel esters depot formulations (50 mg) in beagles. [plotted from the data by Humpel et al., *Contraception*, **15**, 401 (1977)].

formulation for long-acting fertility control was evaluated in 8 women using a single intramuscular injection of 100 mg *d*-norgestrel 17 β-undecylate (137). A peak serum level of *d*-norgestrel ranging from 400 to 700 pg/ml was achieved in 6 subjects within 1–2 days after administration. This serum *d*-norgestrel concentration dropped, thereafter, to a level varying between 50 and 150 pg/ml. This drug level was maintained for a period of at least 130 days. A higher serum *d*-norgestrel concentration was seen in two other patients. Two of the 8 subjects had normal first treatment cycles followed by anovulatory cycles. All other subjects had anovulatory first treatment cycles, followed by ovulatory cycles. Poor bleeding control was registered in all patients. The observed variation of *d*-norgestrel bioavailability among patients may be related to the variation in the extent of cleavage of *d*-norgestrel from the fatty acid esters after release from the depot formulation.

Biopharmaceutics of Parenteral Controlled Drug Administrations

In order for a drug, which is administered intramuscularly or subcutaneously, to reach the site of action to execute its therapeutic activity, it must first be released from its formulation and absorbed into the systemic circulation from the site of injection and then transported to the target tissues.

For a drug administered intramuscularly or subcutaneously in the form of a suspension, in either aqueous or oleaginous vehicle, or an oleaginous solution the extent and the rate of availability of the drug to the site of drug action is frequently found to be controlled by the slowest (rate-limiting) step in the following pharmacokinetic sequence (Scheme II).



Scheme II

Effects of Physico-Chemical Properties

Parenteral absorption is greatly dependent upon the composite effect of the following physico-chemical parameters of the formulation:

(*a*) Rate of dissolution of drug solids in the formulation vehicle.
(*b*) The particle size and crystal habit of drug solids.
(*c*) The pK_a value of the drug molecule.
(*d*) The pH value of the formulation.
(*e*) The (tissue fluid/vehicle) partition coefficient of the drug.
(*f*) The solubility of the drug in the biological fluids at the site of injection.
(*g*) The lipophilicity of the drug molecule.
(*h*) The presence of other ingredients in the formulation and their interaction with the drug molecule.

All of these parameters may play important roles in determining the time of onset, the magnitude and the duration of a therapeutic response of a parenteral controlled drug release formulation. In addition, physiological conditions such as blood flow around the site of injection can also be quite important in affecting the extent of drug activity.

In many instances the slowest step, i.e., the rate-determining step, in Scheme II, is the dissolution of drug solids in the formulation vehicle and/or the interfacial partitioning of drug molecules from the vehicle to the surrounding tissue fluid. Any factor which influences the rate of dissolution will ultimately affect the parenteral absorption of drug. For example, decreasing the particle size of the drug solids in the suspension will lead to the increase in the total surface area of the drug particles in the formulation, which generally result in an enhancement in the rate of drug dissolution. The effect of particle size was demonstrated by the dependency of intramuscular bioavailability of phenobarbital suspension on particle size (138). Blood levels of phenobarbital were reported to increase by reducing the particle size of phenobarbital (Fig. 35). The intramuscular absorption of penicilline G procaine suspension was also reportedly affected by the particle size (139). The average peak levels were found to increase two folds from 1.24 to 2.40 units/ml when the particle size of procaine penicillin G was reduced from 105–150 μm to smaller than 35 μm (Table II).

Alternatively, the effect of particle size on drug dissolution can also be applied to sustain the release of a drug over a prolonged period of time. It can be achieved by increasing the particle size of the drug solids in the formulation (macrocrystal principle). It was illustrated earlier by the effect of particle size of testosterone isobutyrate on the growth of comb (Fig. 1) and of penicillin G procaine in aqueous suspension on the serum levels of penicillin (Table II). Results suggested the larger the particle size, the longer the duration of biological activity and serum level of the drugs. One exception to the macrocrystal principle was

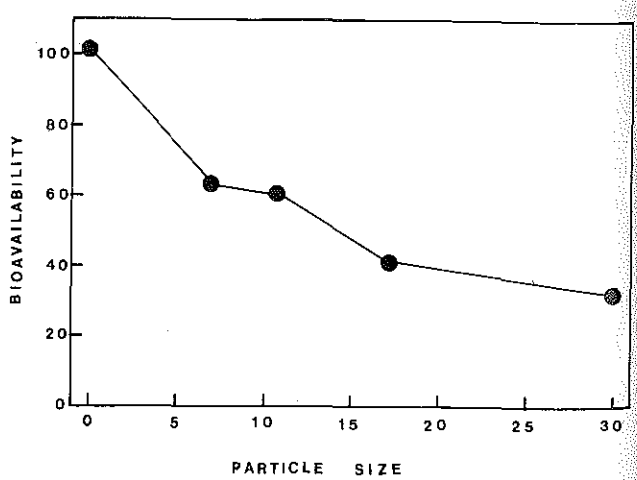


Figure 35—Dependency of intramuscular bioavailability (%) of phenobarbital in beagle dogs on the particle sizes (microns) of phenobarbital in suspensions [plotted from the data by Miller and Fincher, *J. Pharm. Sci.*, **60**, 1733 (1971)].

demonstrated by suspensions of micronized penicillin G procaine in vegetable oil gelled with aluminum monostearate (Fig. 2).

By altering the solubility of the drug in the formulation one can either increase or decrease the rate of dissolution. A number of parameters can alter drug solubility. For example, polymorphism was reported to be responsible for a number of clinically significant differences in drug activity. It is known that approximately one in every three organic compounds exhibits polymorphic behavior. In general, there is only one stable crystalline form. Other polymorphic forms are less stable and usually have greater solubility. The polymorph with greater solubility will give a faster rate of dissolution and therefore a more rapid absorption of the drug. It was demonstrated for chloramphenicol and novobiocin, in which only the amorphous form exhibits biological activity (139).

For weakly acidic or weakly basic drugs, the drug molecules can exist in either the unionized or the ionized state, and the degree of ionization depends on the dissociation constant (K_a) of the drug and the pH of the medium as defined by Henderson-Hasselbalch equation:

(*a*) *For a weakly acidic drug*

$$\log \frac{(A^-)}{(HA)} = pH - pK_a \qquad \text{(Eq. 11)}$$

(*b*) *For a weakly basic drug*

$$\log \frac{(B)}{(BH^+)} = pH - pK_a \qquad \text{(Eq. 12)}$$

For acidic drugs at pH values below their pK_a the drug molecule would exist predominately in the unionized form (HA). On the other hand, the basic drug molecule would exist mainly in the protonated form (BH^+). The unionized, (HA) or (B), and ionized, (A^-) or (BH^+), species exhibit different solubility behavior and lipophilicity. The solubility of these drugs can be altered by alteration of the pH of the vehicle in which the drug particles are suspended, leading to an increase (or decrease) in the rate of dissolution in the hydrodynamic diffusion layer and the magnitude of partitioning of drug molecules from the formulation to the tissue fluids at the site of administration.

The viscosity of the suspension can also affect the rate of dissolution by altering the solution diffusivity of the drug molecule in the vehicle. It was demonstrated by the observation that use of 35 v/v% aqueous glycerin as the vehicle decreases the acute subcutaneous toxicity of isoniazid and streptomycin sulfate.

Certain suspensions show thixotropic behavior, which means that when stirred very gently or let stand for some time, they show a nearly infinite viscosity and when shaken or stirred vigorously they become more fluid in their consistency and flow more readily. A thixotropic suspension has three advantages: (*a*) under storage the suspension is stabilized by its structure and high viscosity; (*b*) when the suspension is shaken prior to injection, it becomes fluid enough to pass through a hypodermic needle; and (*c*) once the suspension reaches the site of injection in the muscle tissue the suspension structure regenerates and a compact depot results. This thixotropic behavior was demonstrated in the development of penicillin G procaine suspension (11). It was found that those suspensions having structural break-down point values in excess of 10^5 dyne-cm give spherical depots (Table IV). The structural breakdown point is the point at which the viscosity of the suspension suddenly becomes reduced or the structure of the suspension begins to break in response to an increase in stirring rate. It was also noted that penicillin G procaine suspensions having a structural breakdown point values greater than 10^6 dyne-cm tend to give rise to excessive needle plugging due to high specific surface or high solid content or both. It was concluded that an acceptable intramuscular suspension formulation should contain drug solids with a specific surface of 10^4 to 3×10^4 cm^2/g and a structural breakdown point between 10^5 to 10^6 dyne-cm.

TABLE XV. Effects of Vehicles on the Biological Activities of Androgens

Androgens	Vehicles	Biological Activities [a] Prostate Glands, mg	Seminal Vesicles, mg
Testosterone[b]	Control	41	14
	Glycerol (50%)[c]	36	12
	Sesame oil	70	41
	Wool fat	128	98
	Mineral oil	44	15
	Mineral oil + palmitic acid[d]	145	126
Androsterone[e,f]	Olive oil	78	18
	Arachis oil	133	21
	Castor oil	149	19
	Propylene glycol	199	37

[a] The weight of organs at one day after the last injection.
[b] Compiled from the data by Ballard, in *Sustained and Controlled Release Drug Delivery Systems*, Robinson, Ed., Marcel Dekker, New York, 1978, chap 1.
[c] 50% glycerol in water.
[d] 50 mg/day.
[e] Compiled from the data by Ballard and Nelson, in *Remington's Pharmaceutical Sciences*, Osol and Hoover, Eds., 15th ed., Mack, Easton, PA, 1975, chap. 91.
[f] Subcutaneous injection of 10 mg androsterone in 2 ml vehicle.

As early as in 1936 the influence of vehicle used in the formulation on the biological activity of drugs was already recognized. It was reported that the biological activity of androgens greatly depended on the types of vehicle used (Table XV). It is interesting to note that the androsterone was more than twice as effective in stimulating organ growth when administered in the water-miscible propylene glycol vehicle than in the water-immiscible olive oil vehicle. It may be due to the precipitation of steroid from propylene glycol vehicle after injection as the result of dilution of vehicle by tissue fluid. It gives a crystalline mush at the site of injection (140). However, the use of 50% glycerol for testosterone did not achieve the same effect (Table XV).

The data in Table XV suggest that the androgenic activity of testosterone and androsterone in oleaginous solutions is very much dependent upon the type of oil vehicles used. For example, maximum activity is achieved for testosterone when it is administered in wool fat, but no increase in activity is observed as compared to control when it is administered in mineral oil. However, the addition of pal-

TABLE XVI. Effect of the Volume of Arachis Oil on the Biological Activity of Testosterone in Rats[a]

Volume[b], ml	Biological Activities[c]	
	Prostate Glands, mg	Seminal Vesicles, mg
2	61	27
5	183	118
10	219	163

[a] Compiled from the data by Ballard and Nelson, in *Remington's Pharmaceutical Sciences*, Osol and Hoover, Eds., 15th ed., Mack, Easton, P A, 1975, chap. 91.
[b] Subcutaneous administration of 2 mg of testosterone in either 2, 5, or 10 ml of arachis oil to 2 groups of rats in 10 equal daily injections.
[c] The weight of organs at one day after the last injection.

TABLE XVII. Comparative Androgenic Activities of Testosterone and Its Propionate and Effect of Solution Volume[a]

Androgens[b]	Seminal Vesicle Weight[c]	
	0.2 ml	0.8 ml
Testosterone	28	10
Testosterone propionate	58	140

[a] Compiled from the data by Honrath et al., *Steroids*, **2**, 425 (1963).
[b] 5 mg of testosterone or its propionate dissolved in either 0.2 or 0.8 ml of sesame oil and injected as a single dose subcutaneously into each of castrated male rats.
[c] The weight changes (in mg/100 g rat) of the seminal vesicles at the end of 10 days.

mitic acid has significantly improved the biological activity of testosterone in mineral oil. Furthermore, the subcutaneous activity of testosterone was also found to depend on the volume of oil vehicle injected (Table XVI). The androgenic activity of testosterone in arachis oil solution increases as increasing the volume of vehicle used to dissolve the same amount of testosterone. However, a reverse trend is observed when testosterone is administered in sesame oil (Table XVII). On the other hand, administration of testosterone propionate in 0.8 ml sesame oil give a substantially greater androgenic activity than in 0.2 ml oil. In both cases, testosterone propionate produces a greater biological activity than testosterone itself at the same dose level. The results suggest that the androgenic activity of testosterone can be greatly enhanced by the formation of a long-acting ester, such as propionate; this improvement in activity is almost three times greater by increasing the volume of vehicle from 0.2 to 0.8 ml.

The studies discussed above illustrate how important the formulation can be in affecting the biological response of a parenteral controlled drug release formulation. The ideal oil utilized as a vehicle for a long-acting, injectable formulation, either as solution or suspension, should meet the following requirements (141):

(*a*) Chemically, it should be a stable oil and neutral in reaction, i.e., containing no excess free fatty acids. It should not react with the drug to form toxic degradation products.
(*b*) Physically, it should not be too viscid to pass readily through a hypodermic needle. It should have good thermal stability at both high, i.e., sterilization, and low temperature, i.e., freezing.
(*c*) Biologically, it should be inert and non-irritating. The oil should be essentially free of antigenic properties and be rapidly absorbed from the site of injection soon after the end of medication and leave no residue.

Before a drug molecule can reach the systemic circulation for distribution to its target tissue it must first penetrate a series of biological (cell) membranes. Biological membranes are highly complex in structure but can be described as composed of lipids and proteins. The lipid layer, which is made of phospholipids and cholesterol sandwiched in-between two protein layers, is the backbone of the biological membrane. The phospholipid molecules are arranged in a bilayer structure with the lipophilic portion of each molecule directing inward and the hydrophilic portion faces toward the outer protein layers, which lend mechanical strength to the membrane. From the structure and composition, it is logical to assume that the biological membrane presents a lipoidal barrier to penetration of drug molecules. That means that drugs require a proper lipophilicity to penetrate through the membrane. This lipophilicity can be correlated with the partitioning of drug molecule across an oil/water interface. Permeation of drugs through the cell membranes of the oral cavity, the gastrointestinal epithelium, the skin tissues, into the bile, central nervous system, tissue cells, and kidney has been reportedly related to the oil/water partition coefficient of the drug molecules. Even with intravenous administration, where absorption of drug into the blood circulation is not required, distribution of the drug from the circulation to the site of action, i.e., target tissues, still depends greatly on the lipophilic characteristics of the drug molecules.

Intramuscular or subcutaneous drug administration requires an absorption step before drug molecule can gain access to the blood circulation for tissue distribution. Thus, any factors that influence this absorption step will also affect the rate at which active drug enters systemic circulation. In addition to the physico-chemical properties of the drug molecule itself as analyzed earlier, the physiological conditions such as blood flow from the site of injection can also be quite important in determining the onset and magnitude of drug activity. It was demonstrated that epinephrine delayed the subcutaneous absorption of a number of drugs due to its constricting action on the vascular bed in the zone of absorption by reducing blood flow. On the contrary, drug absorption was enhanced by the incorporation of hyaluronidase as due to its spreading effect on the injected drug solution over a larger area of connective tissue, leading to the exposure of the drug molecules to a larger surface area of absorption (139).

Effect of Physiological Conditions

Increase in muscular activity, which produces an increased blood flow to the muscles, may also result in an enhancement of drug absorption from the site of injection. The degree of body movement, for instance, was reported to affect the duration of effective penicillin blood levels following the intramuscular administration of penicillin G procaine suspension in oil. The mean duration with plasma penicillin concentrations above 0.039 units/ml was main-

tained for 33 hr in pneumonia patients as compared to only 12 hours in ambulatory patients.

Effect of body movement on intramuscular bioavailability was also observed in the patients administered with aqueous suspension of penicillin G procaine into the gluteal region. The mean peak serum levels attained at 1 to 2 hours in outpatients (0.72 ± 0.28 units/ml) was more than twice as high as that for hospital patients (0.29 ± 0.05 units/ml). Particularly high serum concentrations were detected in those outpatients who played active sports during medication. The observed higher plasma drug levels in outpatients can be attributed to muscular movement in walking and running, which promote the intramuscular absorption of penicillin from the gluteal region by reducing the hydrodynamic diffusion layer of tissue fluid which surrounds the depot formulation and by increasing regional blood flow during and after exercise.

Effect of body movement on intramuscular drug bioavailability is also dependent upon the site of injection. It was found that intramuscular absorption of depot penicillin G benzathine produced a longer penicillinemia in the active training group of Navy recruits than in the less active, hospitalized group. The difference was observed only when the traditional upper, outer quadrant of the gluteal region is used for drug administration. No difference was noticed when injection was made on the anterior gluteal region on the lateral thigh.

The criteria used to determine the route and the site for parenteral drug administrations are (142):

(*a*) The desired rate and extent of systemic absorption.
(*b*) The extent of local tissue irritation, nerve damage, and inadvertent blood vessel entry.
(*c*) The inherent irritation of the drug or its formulation, acidity, basicity, or concentration.
(*d*) The total volume of the formulation to be injected.
(*e*) The dosing frequency of injections.
(*f*) The age and physical condition of the patient.

The two major routes of administration for injectable, long-acting depot formulations are:

Subcutaneous Route of Drug Administration. It is generally limited to non-irritating, water-soluble drugs that are well absorbed from adipose and connective tissue sites which, in comparison to muscle, are poorly perfused with blood (143, 144). Subcutaneous administration of insulin preparations is a well-known example.

It is extremely important that sites for repeated subcutaneous injections, such as insulin self-administration in diabetics, be rotated frequently to prevent local tissue damage and the accumulation of a depot of unabsorbed drug. The volume for a single subcutaneous injection is usually small, which could range from 0.5 to 1.5 ml (Table XVIII).

Intramuscular Route of Drug Administration. The ideal site for intramuscular injection is deep into the muscle and away from major nerves and arteries. The best sites are the gluteal, the deltoid, and the vastus lateralis (145).

The gluteal muscle has become the most common site for intramuscular injection, since it has a greater muscle mass, permitting the injection of larger volumes of fluid (Table XVIII). The best suited area is the upper and outer quadrant, which has a minimum danger of the needle piercing the sciatic nerve or the superior gluteal artery. Careful localization of the injection site is of primary importance. Injections should be made lateral and superior to a line drawn from the posterior superior iliac spine to the greater trochanter. In infants, the lateral or anterior thigh is recommended (146).

The deltoid muscle is thick and also has a superior blood supply, which provides the fastest absorption and systemic effects of all the intramuscular sites (147, 148). Owing to the non-yielding tendinous septa in the upper and lower regions of this muscle, only a small area in the center provides a satisfactory site for drug administration and the volume of a single injection is also smaller than for the gluteal muscle (Table XVIII). This site is found to locate at 2 cm below the acromion.

The subcutaneous and intramuscular bioavailabilities of drugs were compared in dogs using butorphanol tartrate (149). Results suggested that there is basically no significant difference between these two routes of drug administrations in terms of absorption lag times, peak serum drug levels, pharmacokinetics, and the areas under the serum concentration vs. time curves. These routes of drug administrations are considered bioequivalent. Both routes are

TABLE XVIII. Common Routes, Sites, and Volumes for Parenteral Controlled Drug Administrations[a]

Injection Routes	Injection Sites		Single Volume Injected	
	Tissue	Location	Usual, ml	Range, ml
Subcutaneous	Fatty layer underlying skin	Abdomen at naval level, buttocks, lateral upper hips, thighs, back middle of upper arm	0.5	0.5–1.5
Intramuscular	Gluteus medius	Upper outer quadrant of buttocks	2–4	1–6
	Ventrogluteal	Central upper hip	1–4	1–6
	Quadriceps femoris	Central mid-thigh	1–4	1–6
	Vastrus lateralis	Outer mid-thigh	1–4	1–6
	Deltoid	Outer triangular muscle of extreme upper arm at shoulder	0.5	0.5–2

[a] Compiled from the data by Newton and Newton, *J. Am. Pharm. Assoc.*, NS 17, 685 (1977).

very sensitive to the extent of regional blood and lymph flows.

Pharmacokinetic Basis of Parenteral Controlled Drug Administrations

To maintain an effective therapeutic activity over the prescribed period of treatment, it is important that the amount of a drug released from a parenteral drug delivery system should be sufficiently large to makeup the quantity of drug eliminated out of the body during the same period of time. Two pharmacokinetic models should be discussed:

Model A: If the rate of release of a drug from a parenteral long-acting drug delivery system follows pseudo-zero-order kinetics, then

$$(D)_D \xrightarrow{k_0} (D)_B \xrightarrow{k_e} (D)_E$$

Scheme III

where $(D)_D$, $(D)_B$, and $(D)_E$ are the total amounts of drug in the drug delivery system injected into the body intramuscularly or subcutaneously, absorbed into the body other than at the site of injection, and eliminated out of the body, respectively; k_0 is the pseudo-zero-order rate constant of drug delivery; and k_e is the first-order rate constant of elimination.

The rate of change in the total amount of drug in the body, $(D)_B$, in relation with time is thus defined by:

$$\frac{d(D)_B}{dt} = k_0 - k_e(D)_B \qquad \text{(Eq. 13)}$$

or

$$\frac{d(D)_B}{dt} = k_0 - k_e C_B V_D \qquad \text{(Eq. 14)}$$

where C_B is the concentration of drug in the sampling compartment, such as blood circulation; and V_D is the volume of distribution for that drug.

Integration of Eq. 14 gives:

$$C_B = \frac{k_0}{k_e V_D}(1 - e^{-k_e t}) \qquad \text{(Eq. 15)}$$

Equations 13 and 14 do not contain the $(D)_D$ term. It indicates that the concentration of drug in the sampling compartment, C_B, and, thus, $(D)_B$, the total amount of drug in the body, are independent of the total amount of drug exists in the parenteral drug delivery system, $(D)_D$, when the drug molecules are programmed to release at a pseudo-zero-order rate profile.

k_e, the first-order rate constant for the elimination of drug from the body, appears in both the denominator and the exponential term of Eq. 15. It suggests that if the biological half-life of the drug is short, i.e., a short-acting drug, such as naloxone and ACTH, then the k_e term is large and the magnitude of k_0 value has to be large in order to maintain an effective therapeutic blood level, C_B. In other words, the rate of release of a short-acting drug from the parenteral controlled release drug delivery system has to be sufficiently high to maintain a constant, therapeutically effective level of the drug in the body.

At some time after the drug administration when the $e^{-k_e t}$ term is approaching zero, a steady-state drug concentration as defined by:

$$(C_B)_{ss} = \frac{k_0}{k_e V_D} \qquad \text{(Eq. 16)}$$

is then established and maintained for as long as the drug is being released at the rate constant of k_0 from the parenteral drug delivery system. Equation 16 indicates that the steady-state blood level of a drug, $(C_B)_{ss}$, can be controlled by controlling the rate of release, k_0, of the drug from the long-acting drug delivery system injected.

Model B: If the rate of release of a drug from a parenteral long-acting drug delivery system follows a first-order kinetics with a first-order rate constant of drug release, k_1, then

$$(D)_D \xrightarrow{k_1} (D)_B \xrightarrow{k_e} (D)_E$$

Scheme IV

and the rate of change of the total amount of drug in the body is defined by:

$$\frac{d(D)_B}{dt} = k_1(D)_D - k_e(D)_B \qquad \text{(Eq. 17)}$$

The concentration of the drug in the sampling compartment can be described by:

$$C_B = \frac{k_1(D)_D(e^{-k_e t} - e^{-k_1 t})}{V_D(k_1 - k_e)} \qquad \text{(Eq. 18)}$$

Equation 18 suggests that C_B is dependent upon the total concentration of the drug in the parenteral drug delivery system, $(D)_D$. That means that as $(D)_D$ is getting smaller as due to the continuous release of the drug, C_B becomes smaller in proportion as defined by Eq. 18. In addition, both $e^{-k_e t}$ and $e^{-k_1 t}$ terms are decreasing with time. In other words, this pharmacokinetic model dictates that if the release of drug from the long-acting drug delivery system is of first-order kinetics in nature, the concentrations of drug in the blood circulation, C_B, and in the body, $(D)_B$, will be a direct function of the total amount of the drug incorporated in the drug delivery system, $(D)_D$, and are thus decreasing with time.

The pharmacokinetic analyses discussed above conclude that a parenteral drug delivery system which contains a drug with a long-biological half-life and releases it at a zero-order rate process is preferable to a system which does not have these features (97).

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