Chapter 6

METHODS TO ACHIEVE SUSTAINED DRUG DELIVERY

The Chemical Approach

Anthony A. Sinkula

The Upjohn Company
Kalamazoo, Michigan

I. INTRODUCTION

The basic premise for chemical sustained drug release is the localization of drug in some biological depot or site within the organism, with subsequent slow release from that site to provide the active form of the drug in therapeutically efficacious quantities over a given span of time. The medicinal chemist has two options in this regard. (1) In conjunction with the biologist, he can prepare analogs of a lead compound in an attempt to enhance duration of effect. Preparation of such analogs can be considered chemically and biologically independent of other congeners in the series. The lead compound is not regenerated in vivo and structure-activity relationship comparisons between members in the series have limited value from a predictive standpoint. (2) Preparation of prodrug derivatives can be undertaken also to increase sustained release properties of drug molecules. In this instance, however, the parent molecule is regenerated in vivo by enzymatic or nonenzymatic hydrolytic mechanisms. The effect of chemical modification on sustained release effect can thus be evaluated because the parent molecule can always be used as a standard reference whether assessment is based upon physicochemical or pharmacological property changes in the derivative series.

The chemical approach to sustained release drug delivery represents a unique and imaginative departure from conventional approaches. When using this approach, however, the chemist is severely limited in the application of sound theoretical principles on which to base his rationale. While the physical approach is relatively well-grounded in theory, the chemical approach is highly intuitive in nature and its success is based primarily upon the experience, expertise, and creativity of these scientists engaged in such an endeavor. One is effectively prevented from resorting to standard methods in which a given drug or class of drugs can be made to conform to modern concepts of sustained release. Each individual drug represents a new challenge that must include considerations of chemical synthesis, physicochemical properties and their relationship to the biopharmaceutics and pharmacokinetics of the derivative, toxicity, and the resultant bioactivity of the modified drug.

When a drug is chemically modified, the resulting changes in physicochemical properties of the analog or derivative are likely to produce pharmacological and biochemical changes different from those found in the parent or reference drug molecule. The predictability of these changes is difficult to assess. Obviously the ultimate clinical changes produced as some function of chemical alteration vary from drug to drug. Frequently it is not possible to alter only one property of a drug (such as duration of effect) by the chemical approach. This is perhaps the primary reason why definitive structure-property correlations are lacking and why an overall

rationale for the chemical approach to sustained release for many classes of drugs has not been forthcoming. However, an awareness of the effect of chemical modification on solubility, partitioning behavior, distribution, metabolism, etc. may lend meaningful insights into the ultimate effect in vivo.

Thus, altering solubility (hydrophilic-lipophilic balance) may alter absorption, distribution, metabolism, and excretion to different degrees in a given series of analogs or derivatives. Some ways that the chemical approach has been used to enhance sustained release properties are by altering the following:

1. Degree and rate of protein or tissue binding
2. Degree of tissue or organ localization and distribution and subsequent release from such sites
3. Degree and rate of resorption
4. Degree and rate of metabolism; in the case of prodrugs, the degree and rate of conversion to the bioactive species plus subsequent metabolism
5. Rate of excretion

Examples of each case or combination thereof will be discussed with each class of drug where appropriate.

Based on the preceding considerations, the medicinal chemist must then be aware of the interdisciplinary input necessary in planning a successful chemical approach to sustained release drug delivery. It no longer becomes strictly a chemical approach but one that relies on the expertise and contributions of the formulator, the biologist, the pharmacokineticist, and the clinician. Feedback from all of these scientists ultimately determines the success of the chemical approach. The most clinically useful chemical sustained release drug delivery systems are those that have been "fine-tuned" by the effective use of such a multidisciplinary approach.

The criteria for gauging the so-called sustained release phenomenon are twofold: (1) the sampling of relevant compartments (blood, tissue, bile, urine) and determination of rate of appearance or disappearance of drug as some function of time, and (2) the observation of some objective or subjective pharmacological, biochemical, or clinical response produced by varying concentrations of drug (dose-dependent kinetics) versus time. Method (1) has been widely employed when appropriate sampling and assay methodology are available. In this regard, the revolution in assay techniques and enhanced sensitivity holds great promise for accurate determination of extremely minute quantities of highly potent drugs and their metabolites. This method is limited in utility, however, by the paucity of sampling sites within the organism. Thus, therapeutic levels of antibiotics

might best be determined in tissue rather than blood. Analogously, therapeutic levels of neuroleptics are difficult to determine in brain tissue and, unless blood and tissue levels of these agents can be correlated, sampling from such a central compartment is hardly meaningful. In such cases, then, determination of drug concentrations in easily accessible compartments such as blood or urine may give clues to bioavailability but not necessarily to bioactivity.

Method (2) has historically been the route whereby chemical sustained drug release has been evaluated. This has especially been true in lower mammalian species, such as the mouse and rat, where low circulating or tissue levels of drug preclude instrumental analytical methodology. Early investigators made wide use of pharmacological and biochemical endpoints to assess duration of drug effects. Particularly relevant are the long-acting steroids, e.g., weight gain in sex organs. Clinical evaluative techniques have included a variety of rating scales, for example, in the evaluation of depot neuroleptics. Perhaps the greatest value in assessing sustained drug release would represent a marriage of the best of both methods. The implications of methodology and assessment will be discussed in greater detail in following sections.

The literature concerning the chemical approach to sustained release drug delivery is seldom identified as such. Rather, a chemist and biologist, in synthesizing and evaluating a series of chemically related drugs, seek initially to enhance potency or spectrum of activity while minimizing toxic side effects. If it so happens that during the biological testing of these compounds a duration effect is noted, and it is decided that such an effect may be advantageous, further synthesis and evaluation are undertaken. On the other hand, there are a variety of studies and experiments designed ultimately to achieve a sustained duration effect. Some exhibit a high degree of sophistication in the design, application, and evaluation of such effects and eventually produce successful drug candidates. Others fail for a variety of reasons. None are based on an entirely rational approach. This is not to say that the chemical approach has not been successful. Hopefully the examples of drug classes as well as individual drugs discussed in the following sections will illustrate this premise.

This review is purposely selective, due to the constraints of space as well as the desire to avoid redundancy. It is designed to impart the flavor of the chemical approach to a variety of widely differing series of drug classes. The steroids have been discussed in detail because of the large volume of literature available. Further, the types of derivatives and analogs used to modify steroids are applicable to virtually every other class of drugs. The methods of evaluation and assessment of sustained release effects discussed for steroids again have been used, with appropriate modification, with other drugs.

In this chapter, the terms sustained drug release, sustained drug delivery, depot, long-acting, extended duration, etc., are used interchangeably and reflect the particular choice of terminology by investigators whose work is being discussed. Tables and figures are used freely throughout the text to provide a convenient condensation of much relevant information that supplements the more detailed discussion of specific drugs.

A selected list of additional reading is included for those interested in further pursuing the literature on drugs that are not covered in this review (see Appendix).

II. CLASSES OF DRUGS AMENABLE TO CHEMICAL MODIFICATION

A. Steroids

The use of the chemical approach in the design of sustained release drug derivatives has perhaps achieved its greatest success with the steroids. The early use of these potent (and many times, toxic) agents was characterized by inefficient utility based on an inadequate understanding of steroid biochemistry and pharmacology. Poor absorption and rapid metabolism and excretion necessitated frequent oral administration. Esterification of steroids bearing a hydroxyl group was found to improve their duration of activity. Early workers wondered how such steroid esters exerted a sustained bioactivity effect and what the altered properties of these esters were that could be responsible for this extended duration of effect. In general, the differences in the duration of effect between a variety of derivatives (esters, ethers, etc.) of several types of steroids (androgens, estrogens, adrenocorticoids, norsteroids) reside in the alteration of certain physicochemical properties (solubility and its influence on lipophilic-hydrophilic character) and pharmacokinetic properties. The preparation of long-acting steroids requires derivatives with extended biological half-lives. The judicious chemical alteration of the parent steroid molecule presumably modifies the time course of the drug such that transport, distribution, site or tissue localization, metabolism, and excretion are favorable toward sustained bioavailability [1]. Since the vast majority of sustained release steroids are prodrugs, in vivo hydrolysis of the ester or ether becomes necessary for bioactivity. Methods of slowing the hydrolysis rate of derivatives include preparation of long chain fatty acid esters and derivatives sterically hindered at or near the site of hydrolysis. Since most long-acting steroids are administered as insoluble suspensions or oil-soluble solutions, other considerations include (1) the solubility of the derivative in the vehicle (e.g., oleaginous) and the bulk aqueous phase

(extracellular water at the site of administration) where the release rate is solubility-controlled, and (2) the diffusion of drug derivative from the vehicle where release is diffusion-controlled.

Once the derivative is released (solubilized) at the site of administration, two factors become important: (1) regeneration of the parent molecule at or near the site by slow, nonenzymatic hydrolysis on contact with body or tissue fluids, e.g., testosterone ethers, and (2) regeneration of the parent bioactive substance by enzymatic hydrolysis either at the site or in a central compartment (e.g., blood) [2]. Although most steroid esters and ethers can be treated as prodrugs, there appear to be exceptions to this generalization and these will be treated in subsequent parts of this section.

1. Metabolism

The question of whether steroids exert their effects in a derivatized form (intrinsic bioactivity) or require metabolism (e.g., hydrolysis to parent steroid-extrinsic bioactivity) remains largely unresolved. Part of the problem resides in the complexity of assay methodology and technique. Early testing of long-acting steroid efficacy was concerned with the measurement of growth of tissue intimately involved with steroid physiology. Thus, weight gain in seminal vesicle, prostate, capon comb, or levator ani muscle over controls for various periods was taken as an indication of the potency and duration of the steroid derivative under investigation. Analogously, such steroid derivatives can be evaluated clinically by attempting to quantify similar endpoints in humans, i.e., growth of pubic hair, sexual libido, quantity of semen, frequency of erections, etc. A second type of assay frequently employed is the measurement of changes in urinary excretion of 17-ketosteroids (17-KS) and historically this has been used in a manner similar to weight gain assays. An increase in urinary excretion of 17-KS is seen after both oral and intramuscular administration of testosterone derivatives, for example, but is not seen with other androgens [3]. The use of 17-KS as an assay procedure is unreliable because urinary excretion is influenced by the basal status as well as the stress conditions under which an individual exists [4]. Examples include age, level of development, sex, disease, emotional stress, and exercise. Ideally, the effects of a sustained anabolic effect could be monitored and correlated with blood levels of either parent steroid or derivative and urinary excretion of 17-KS should reflect this trend. Unfortunately, steroidal physiological activity can occur at levels below detection of changes of 17-KS excretion levels thus further testifying to the unreliability of the method. Weight gain studies in lower species and clinical evaluation of secondary sex effects in man, however, appear to be reasonable methods of evaluating steroid potency and duration.

Scant information exists concerning the metabolism of steroid analogs and prodrugs, as such metabolism affects duration of activity. Two distinct processes are important: (1) hydrolysis and/or metabolism of the steroid "reversible derivative" (ester, ether, etc.) to the parent steroid, and (2) further metabolic degradation of the parent to excretory products, e.g., 17-KS. Since most steroids possessing sustained activity properties are of the "reversible derivative" or prodrug type, consideration will primarily be limited to this chemical class of steroid. Gould et al. [5] suggested that the enhanced potency and duration of certain halo- and alkylphenoxyacetate esters of testosterone was due to the protection afforded testosterone until it reaches the site of action. Once there, hydrolysis occurs and the supply of hormone is at least as adequate and perhaps more so than that indigenous to the intact testis. This intuitive notion implicitly resides in most reported studies as the basis for the rationale for the design of long-acting steroid derivatives. Of the early workers in this area, Kochakian [6] was one of the first to acknowledge that differences in duration for a series of testosterone esters might be due to their different rates of hydrolysis in vivo. It was also thought that the relative ease of base-catalyzed hydrolysis of ester groups at the 3 and 17 positions could be correlated with in vivo hydrolysis rates of acetate and benzoate esters of androsterone, estrone, and α-estradiol. A study of the solubilities of testosterone, progesterone, and estradiol in mammalian blood and the observation that, on equilibration, testosterone propionate and estradiol benzoate were as soluble as the parent steroids led Bischoff et al. [7] to suspect the existence of steroid serum esterase. These esters are less soluble in serum initially than are the parent steroids. Others experiments with estrone and estradiol esters using human and rabbit serum demonstrated that such steroid esters are indeed hydrolyzed, and rate of hydrolysis was acetate > propionate > benzoate > palmitate [8]. Esterase activity was also found in liver and kidney homogenates of the mouse [9]. Testosterone propionate, cortisone acetate, and deoxycorticosterone acetate were found to be substrates amenable to hydrolysis in these homogenates. Demonstration of enzyme substrate steroid esterase activity was extended to a variety of tissue homogenates from the human, horse, ox, rabbit, and rat [10]. Junkmann [11], in an excellent review of long-acting estrogens and androgens, commented that perhaps these types of steroid esters were exhibiting their effects as the intact derivative. He based this reasoning on the fact that the in vitro enzymatic rate of hydrolysis of such esters does not parallel in vivo activity. During the discussion of this paper, several participants argued for and against the merits of steroid esters being intrinsically active. Segaloff cited experiments which involved local application of steroid esters in castrated mice. These esters were shown to be the most effective and this was used as evidence for

ester intrinsic activity at the end organ. Mumson similarly commented concerning local application of testosterone propionate to the chicks' comb. Both arguments, however, fail to account for the possibility of hydrolysis at the target organ site as suggested by Gould et al. [5]. Further, Lozinski, studying the efficacy of testosterone and estradiol-17-monosulfate esters, found no androgenic or estrogenic effects when administered in doses 10 times greater than those effective for the parent steroid. This was cited as evidence that the free 17-hydroxyl group is critical for bioactivity. A study by Myers et al. [12] of the aliphatic esterases and their inhibition by tri-o-cresyl phosphate revealed some interesting results. If testosterone propionate was administered parenterally to castrated rats pretreated with such esterase inhibitors, their prostate and seminal vesicles showed no weight gain. Administration of testosterone elicited the usual androgenic response. Animals not pretreated with esterase inhibitor, and injected with testosterone propionate, demonstrated a response equal to testosterone implying that hydrolysis probably occurs at the site of bioactivity. Alibrandi and co-workers [13] similarly speculated on the site of hydrolysis for orally administered short and medium chain androgen esters.

Van der Vies [14], in a comparative investigation of the anabolic and androgenic activities of nandrolone (19-nortestosterone) decanoate and phenylpropionate, found these esters to be rapidly hydrolyzed in rat blood. The duration of activity of these esters was determined by the rate of absorption from the injection site. No free nandrolone was found at the injection site but hydrolysis was shown to occur in vitro in a variety of different tissue (Table 1). Nandrolone esters are not hydrolyzed by enzymes present in muscle (as evidenced by the presence of only intact nandrolone ester at site of injection) but are extensively metabolized in liver and plasma of rats. Comparison of percent hydrolysis of nandrolone phenylpropionate in other mammalian plasma revealed interesting species esterase specificity and activity. The esterase activity in rats is extremely high while a lower activity is seen with canine and human plasma (Table 2).

Oral activity of testosterone undecanoate was demonstrated in the rat with most of the ester being hydrolyzed in the intestinal wall [15]. A tissue distribution study of the radioactive steroid [4-^{14}C]hydroxyprogesterone-17α-caproate indicated that hydrolysis did not occur at the 17-ester position [16]. Failure to recover any pregnane-3α,17α,20-triol, the major metabolite of 17α-hydroxyprogesterone, was cited as proof. It was thought that the enhanced activity of 17α-substituted analogs was due to a greatly reduced rate of enzymatic reduction of the C-20 ketone due to steric inhibition by the caproate ester [17]. The increased lipophilicity of the ester may also enhance resorption and transport of this agent to the target site. Cortisone diacetate, however, is converted to cortisone chemically as well as by microflora of the gut and is active as the parent steroid [17].

TABLE 1. Degradation of Nandrolone Esters by Tissues In Vitro

Substances incubated[a]	Recovery of substance (%)
Homogenate of rat M. L. A.[b] (50 mg/ml)	
Nandrolone phenylpropionate	104
Homogenate of rat gastrocnemius muscle (250 mg/ml)	
Nandrolone phenylpropionate	95
Homogenate of rat liver (250 mg/ml)	
Nandrolone phenylpropionate	0
Nandrolone decanoate	62
Nandrolone oleate	101
Hog pancreas extract[c]	
Nandrolong phenylpropionate	86
Nandrolone oleate	94
Rat plasma	
Nandrolone phenylpropionate	0.1

[a]Incubations with homogenates at 37°C for 4 hr, with plasma for 17 hr. Concentration of ester: 1 mg per ml. It was shown by means of thin-layer chromatography that liver and plasma hydrolyzed the esters to nandrolone.
[b]M. L. A. = levator ani muscle.
[c]Containing 60 mU of lipase per ml.
Source: Reproduced with permission from Ref. 14.

Rapala et al. [18], in a systematic investigation of the influence of the adamantyl group on drug molecules, found a sharp separation of myotropic and androgenic effects with 19-nortestosterone-17β-adamantoate. This dichotomy was not seen with either 19-nortestosterone or its 17β-decanoate ester. The phenomenon was attributed to the fact that the adamantoate ester was not hydrolyzed but was efficacious per se (Figure 1). An unusual antiinflammatory steroid ester, dexamethasone-21-isonicotinate [19] was

TABLE 2. Degradation of Nandrolone Phenylpropionate in Plasma

Substance incubated with nandrolone phenylpropionate[a]	Recovery of nandrolone phenylpropionate (%)
Water	98
Rat plasma	0.1
Rat plasma, sterile	0
Rat plasma, previously heated to 50°C during 30 min	68
Human plasma	77
Dog plasma	63

[a]Incubation at 37°C for 17 hr, concentration 1 mg per ml. It was demonstrated by means of thin-layer chromatography that nandrolone phenylpropionate was hydrolyzed to nandrolone.
Source: Reproduced with permission from Ref. 14.

studied with particular reference to species differences in hydrolysis rate [20,21]. This ester was rapidly hydrolyzed in rat and rabbit sera (90 and 99% respectively, within 10 min). The ester half-life in human serum, however, was 90-100 min (Figure 2). Such studies as this dramatically illustrate the danger in relying too heavily on data generated in lower mammalian species and extrapolating such information to use in humans. Useful data on which one might base meaningful extrapolations on interspecies drug metabolism are lacking. An understanding of the variety and importance of esterases involved in steroid ester metabolism, especially in humans, is rudimentary [22].

An investigation in humans of the pharmacokinetics and metabolism of 6-chloro-1,2α-methylene-4,6-pregnadiene-17α-acetoxy-3,20-dione (cyproterone acetate) indicated no deesterified steroid in circulating plasma [23]. The compound was assayed as the 2-^{3}H-labeled derivative. The absence of any 20α-hydroxyl derivative, the main metabolite of free cyproterone, accounted for the relatively higher blood levels of the metabolically stable acetate ester. As far as can be ascertained, this derivative represents another example of a steroid ester that is possibly an intrinsically bioactive form of this useful therapeutic class of drugs.

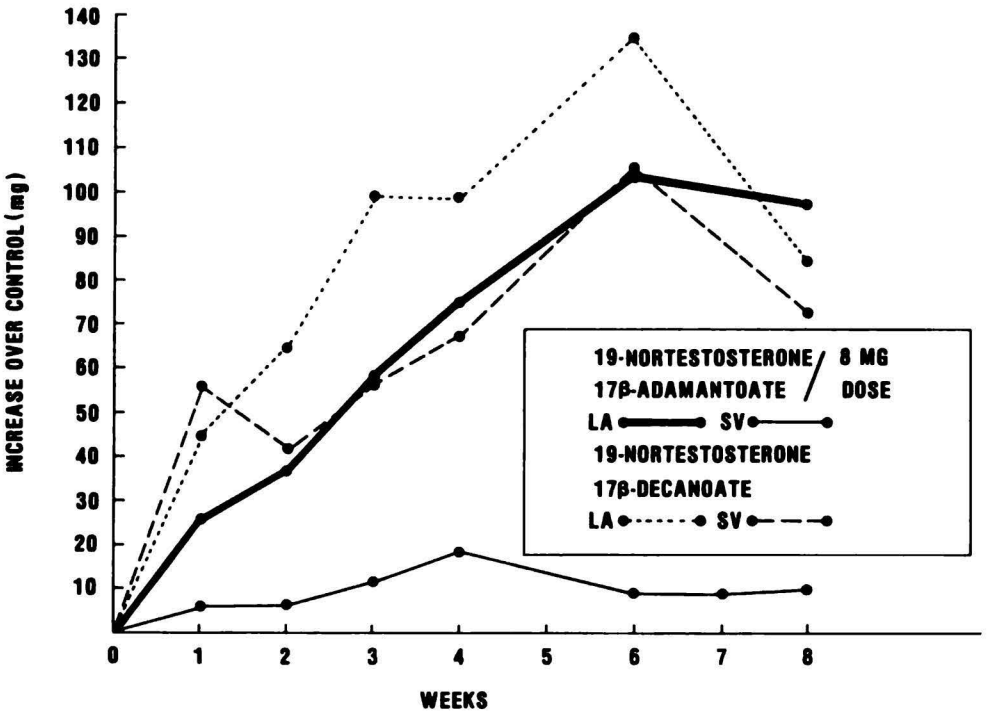

Fig. 1. Levator ani and seminal vesicle response to a single subcutaneous administration of steroid esters. (Reprinted with permission from Ref. 18; copyright by the American Chemical Society.)

Concern over the lack of use of purified reasonably well-defined enzymes in most steroid metabolism studies led Schöttler and Krisch [24] to study 22 steroid esters using carboxylesterase (EC 3.1.1.1) isolated from pig liver microsomes. Their purpose was to determine whether steroid ester hydrolysis was catalyzed by steroid-specific esterases or by widely distributed unspecific esterases such as carboxylesterase. Of those esters studied, which included androgens, corticosteroids, and estrogens, no correlation was found between chemical structure and hydrolysis rate. The best substrates were found to be estrone acetate and estradiol benzoate. Several esters were not hydrolyzed, including the 21-hemisuccinate esters of prednisolone and cortisol, probably due to the anionic character of the terminal portion of the acid ester [25,26]. Others not hydrolyzed were 17α-hydroxyprogesterone-17-caproate and dexamethasone-21-isonicotinate. The majority of esters followed Michaelis-Menten kinetics

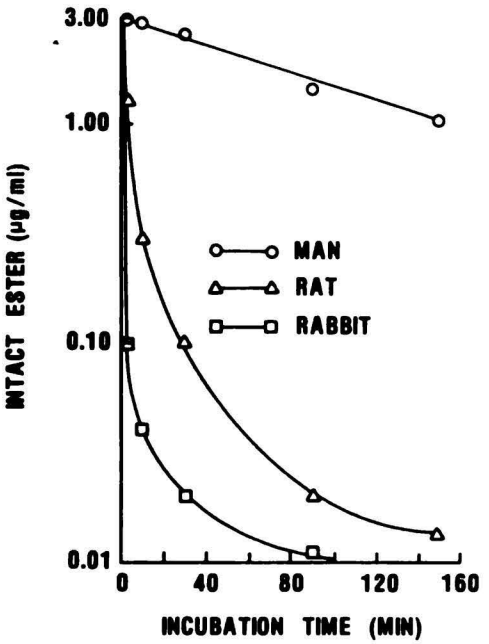

Fig. 2. Time dependence of the hydrolysis of dexamethasone-21-isonicotinate in human, rat, and rabbit serum. The ordinate is divided logarithmically in order to represent the low concentrations in rat and rabbit serum. (Reproduced with permission from Ref. 21.)

with the Michaelis constants falling in the range of 10^{-5} to 10^{-6} M. Table 3 features the relevant constants determined in the course of this investigation.

Evidence for the hydrolysis of estradiol valerate and estradiol benzoate given intramuscularly was dramatically demonstrated in a study of estrogen-withdrawal migraine in premenstrual women [27]. Administration of an injection of estradiol valerate following migraine attack resulted in rapid and sustained plasma levels of free estradiol lasting from 7 to 12 days. Four subjects were used and a similar pattern was seen in each subject (Figure 3). Estradiol benzoate was hydrolyzed and excreted more rapidly (Figure 4). Sustained plasma levels of free estradiol were not seen after oral administration of estradiol valerate thus emphasizing the importance of route of administration for therapeutically significant duration of effect. The method utilized for determining free estradiol was based on competitive protein binding and made use of estrogen-binding macromolecules of sheep uterine cytosol [28].

TABLE 3. Kinetic Parameters of the Hydrolysis of Steroid Hormone Esters by Pig Liver Esterase

Substrate	Parameter[a] K_m (M)	k_{cat} (min^{-1})	k_{cat}/K_m (liters $mole^{-1}$ min^{-1})
Androgens			
Testosterone cyclopentyl propionate	2.0×10^{-6}	1250	6.25×10^{8}
Testosterone propionate	1.4×10^{-5}	1385	9.89×10^{7}
Testosterone isobutyrate	4.6×10^{-6}	935	2.03×10^{8}
Testosterone oenanthate	4.3×10^{-6}	3740	8.70×10^{8}
Anabolics			
Nortestosterone phenylpropionate	4.6×10^{-6}	3675	8.17×10^{8}
Estrogens			
Estradiol-17β-cyclopentylpropionate	3.5×10^{-6}	735	2.10×10^{8}
Estradiol-17β-valerate	5.4×10^{-6}	1765	3.27×10^{8}
Estradiol-3-benzoate	3.0×10^{-6}	5020	1.67×10^{9}
Estradiol-17β-propionate	1.0×10^{-5}	5020	5.02×10^{8}
Estrone acetate	4.2×10^{-5}	8350	1.99×10^{8}
Corticosteroids and derivatives			
16-Methyl-prednisolone-21-diethylaminoacetate	8.3×10^{-5}	885	1.07×10^{7}
Corticosterone acetate	1.3×10^{-5}	1250	9.62×10^{7}
11-Deoxycorticosterone acetate	1.3×10^{-5}	5020	3.85×10^{8}

[a] K_m = Michaelis constant; k_{cat} = catalytic rate constant; k_{cat}/K_m = specificity constant.
Source: Reproduced with permission from Ref. 24.

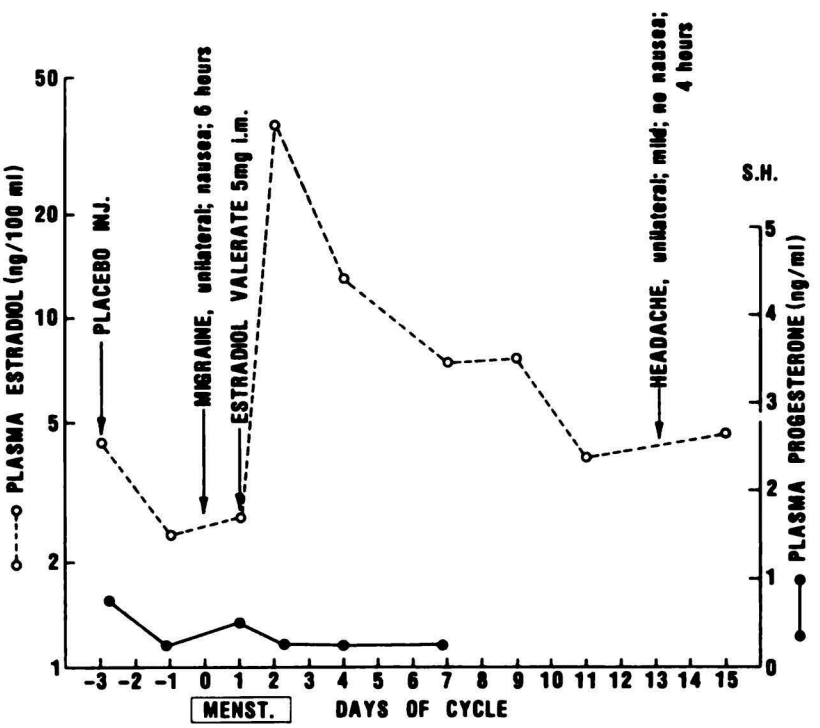

Fig. 3. In one patient (S. H.), injection of estradiol valerate caused a second--but atypical--migraine attack. (Reprinted with permission of the author from Ref. 27; copyright 1975, The New York Times Media Company, Inc.)

Not all sustained release steroid derivatives were designed to be hydrolyzed enzymatically in vivo. Thus, Cross et al. [29] synthesized several acid labile 17β-tetrahydropyranyl ethers of androstane and 19-norandrostane. Orally, such derivatives demonstrated good bioactivity probably due to hydrolysis in the acidic pH of the stomach. Subcutaneous injection of these ethers showed low androgenic-anabolic activity when compared to testosterone. Alkyl steroid-17β-yl mixed acetals of aliphatic and cycloaliphatic ketones behaved similarly [30]. A series of benziloyl hydrazones was thought to also hydrolyze under acidic conditions [31].

In summary, it is not readily apparent what factors are critical for classification of steroid derivatives as being intrinsically or extrinsically active. While most esters, acetals, ketals, and ethers act as prodrugs, exceptions to this rule exist. Obviously more research is needed in the

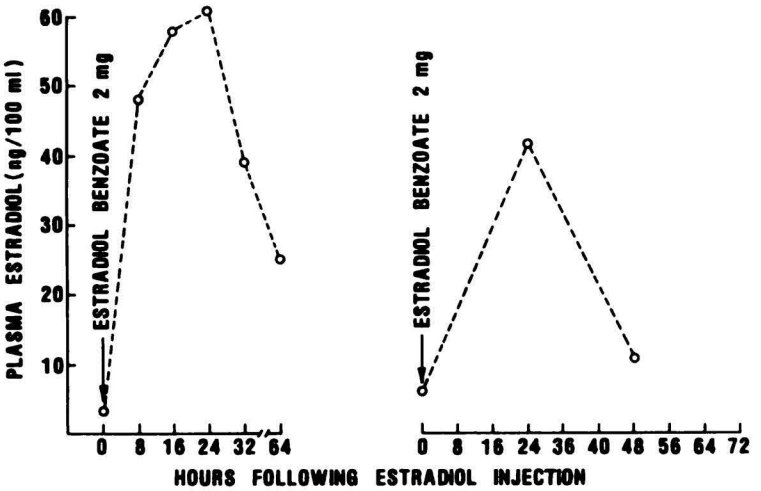

Fig. 4. The effect of short-acting estradiol benzoate injected during the early follicular phase. The peak in plasma estradiol was short-lived and was not followed by a second migraine attack in either patient. (Reprinted with permission of the author from Ref. 27; copyright 1975, The New York Times Media Company, Inc.)

area of steroid metabolism, specifically in the knowledge of possible enzyme systems that catalyze the hydrolysis of such derivatives in vivo, especially in humans. Further, clinical pharmacokinetic studies would be valuable in obtaining answers to many of these questions. Development of more specific and sensitive assays, such as radioimmunoassays, would definitely constitute a great step forward in solving such problems.

2. Quantitated Structure-Activity Relationships

Solubility

In an effort to correlate solubility with the duration and intensity of activity of testosterone esters, James and Roberts [32] determined the aqueous solubilities of several esters varying in length from formate (C_1) to valerate (C_5). The solubilities of this homologous series decreased logarithmically with the addition of each successive methylene unit. Mole fraction solubility (H_2O) ranged from 2.50×10^{-7} (formate ester) to 1.40×10^{-8} (valerate ester). The logarithms of their distribution coefficients (ethyl oleate-water) increased as a function of chain length ($\sim 10^4$ for

formate ester to $>10^5$ for valerate ester) [33]. The differences in duration of activity between esters was thought to be related to their distribution coefficients since diffusion from the injected oil solution to the surrounding aqueous tissue phase was the rate-determining process. All studies were performed in rats with radiolabeled [4-^{14}C]testosterone and its esters. Biological half-lives were determined in rat gluteus muscle and whole body from first-order rate constants obtained by least-squares analysis. The results are shown in Figure 5. It was not possible to obtain evidence regarding the metabolic fate of the esters since all derivatives were labeled on the testosterone parent (C_4) molecule rather than the ester. The poor correlation of rate of elimination of drug from muscle with distribution coefficient was due to the fact that ethyl oleate is absorbed at rates similar to those of the steroids themselves [34].

Measurement of the solubilities and R_m values [$R_m = \log (1/R_f - 1)$, where R_f is the retardation factor] of several steroid esters was undertaken to evaluate the utility of the solubility ratios as an approximation of their distribution coefficients and as a comparison of biological activity [35]. Unfortunately, enough exceptions existed to warrant caution in their application.

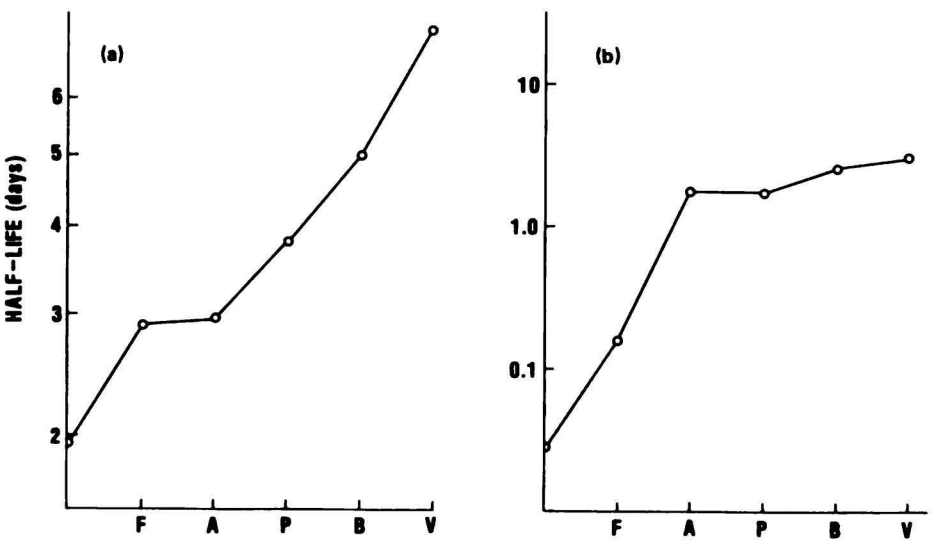

Fig. 5. Biological half-lives in (a) rat and (b) muscle. F, formate; A, acetate; P, propionate; B, butyrate; and V, valerate. (Reproduced with permission from Ref. 33.)

A significant correlation between R_m values (determined by a reversed-phase thin-layer chromatography [TLC] method [36]) and testosterone ester bioactivity was reported by Biagi and co-workers [37]. A linear relationship was found between R_m and the logarithm of biological response (BR) as follows:

$$\log BR = 0.295 + 0.416R_m\left(\frac{n}{7}\right)\left(\frac{s}{0.085}\right)\left(\frac{r}{0.934}\right) \quad (1)$$

where n is number of derivatives tested, s is the standard deviation, and r is the multiple correlation coefficient. Table 4 lists the testosterone esters tested along with the relevant constants generated using Eq. (1).

James [38] demonstrated that the previous results of Biagi correlated well with biological response only for the range of esters tested. Deviation was seen with the formate as well as the decanoate esters and correlation was no longer linear. The use of other chromatographic phases was suggested to enhance predictive ability for such deviant esters.

TABLE 4. Lipophilic Character and Biological Activity of Androgenic Compounds

Compound	R_m values	Time of maximum effect[a]	
		Observed log BR	Calculated log BR
Testosterone	0.60	0.00	0.04
17-acetate	0.22	0.12	0.20
17-propionate	0.05	0.25	0.27
17-butyrate	0.09	0.39	0.33
17-isobutyrate	0.09	0.43	0.33
17-valerate	0.25	0.48	0.40
17-caprinate	0.96	0.61	0.69

[a]Data are expressed on a molar basis and calculated on a relative scale with testosterone.

Source: Reproduced with permission from Ref. 37.

TABLE 5. Anabolic Activities and Times of Maximum Anabolic Effect of Nandrolone Esters

Ester	Log distribution coefficient	Log time of maximum effect[a] 1st maximum Obs.	1st maximum Calc.	2nd maximum Obs.	2nd maximum Calc.	Anabolic activity[b] (mg days) at dose (μg) of: 250	500	750	1000	Log anabolic activity for 1 mM dose Obs.	Predicted
Butyrate	4.838	0.778	0.820	1.041	1.074	547 (162)	669 (224)	876 (133)	1488 (234)	2.783	2.776
Hexanoate	5.284	1.041	0.941	1.176	1.172	968 (413)	2142 (583)	2557 (519)	3731 (651)	3.163	3.174
Heptanoate	5.429	0.954	0.977	1.230	1.204	1079 (267)	3193 (583)	3463 (519)	6559 (449)	3.287	3.249
Octanoate	5.786	1.114	1.076	1.279	1.282	1063 (363)	2701 (348)	3856 (399)	5557 (395)	3.281	3.320
Nonanoate	5.658	0.954	1.042	1.279	1.254	1047 (576)	2509 (573)	2960 (684)	5080 (742)	3.264	3.313
Decanoate	5.904	1.146	1.108	1.322	1.308	1410 (541)	3192 (502)	4055 (509)	7735 (668)	3.409	3.307
Undecanoate	6.166	1.146	1.179	1.322	1.366	729 (271)	2470 (473)	3393 (365)	6576 (416)	3.192	3.216

[a]Obs., observed value; Calc., calculated value.

[b]Figures in parentheses represent the 95% confidence limits of the results.

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TABLE 6. Effect of Unsaturation and Branching of the Side Chain Upon Incorporation of Testosterone Esters into Liposomes

Testosterone ester	R_m value	Incorporation[a] (moles per mole of lecithin ± range)
Crotonoate	0.30	0.14 ± 0.01
Benzoate	0.59	0.04 ± 0.01
2-Octenoate	0.98	0.56 ± 0.03
3-Octenoate	0.98	0.55 ± 0.04
Undecylenate	1.53	0.56 ± 0.08
2-Methylpropionate	0.45	0.11 ± 0.01
4-Methylpentanoate	0.79	0.25 ± 0.02

[a]Average of 4 or 5 determinations.
Source: Reprinted with permission from Ref. 42.

A subsequent study by James et al. [39] correlated R_m values, hydrolysis rates, and androgenic activities of several testosterone esters in rats. Enzyme-ester substrate hydrolysis rates were determined using rat liver homogenate. Initial hydrolysis rates were generated from the results by computer. Catalytic constants were calculated from hydrolysis rate-enzyme concentration plots. Weight gain in sex organ was dependent on ester hydrolysis rate, and steroid ester lipophilicity determined the duration of activity [40].

Nandrolone (19-nortestosterone) esters have also been the subject of a quantitated structure-activity relationship study [41]. The relationship between log anabolic activity and log distribution coefficient (ethyl oleate-water) was binomial, i.e., two bioactivity peaks were observed at all dose levels for all esters. This response was rationalized as being due to (1) absorption of steroid ester from the injection site and its hydrolysis, and (2) storage and release of ester from body fat with subsequent ester hydrolysis. Good agreement was found between the observed and calculated distribution coefficients and anabolic activities for all esters tested (Table 5).

The effect of chain length and lipophilic character (R_m) of testosterone esters and their incorporation into liposomes has been investigated [42]. Maximum incorporation was achieved with testosterone octanoate. Interestingly, the natural membrane sterol, cholesterol, also contains a C_8

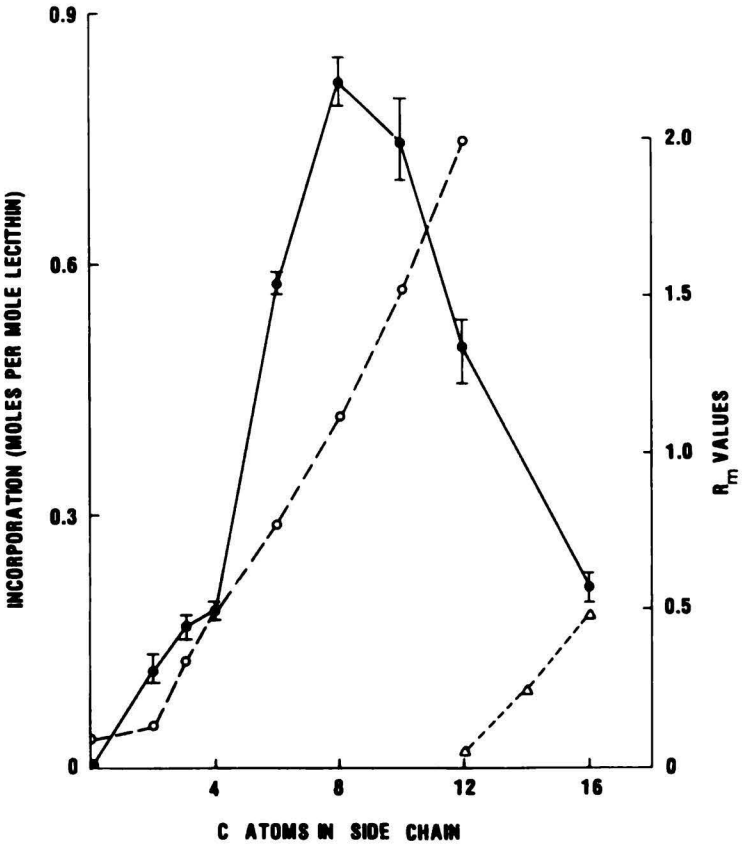

Fig. 6. Relationship between the incorporation into liposomes, the length of the side chain, and the lipophilic character (R_m) of testosterone esters: •— •, incorporation into liposomes; o— o, R_m values in methanol-water (65:35, v/v); and Δ— Δ, R_m values in methanol-water (85:15, v/v). Incorporation into liposomes is given as the mean of four determinations ± range. (Reproduced with permission from Ref. 42.)

side chain. Apparently testosterone esters of C_9 or longer would not be more efficiently incorporated into liposomes than the octanoate ester (Figure 6). Lipophilicity of saturated, straight-chain testosterone esters varying from 2 to 16 carbon atoms increased in a linear fashion. Branched side chains decreased incorporation by about 50% suggesting that R_m values (lipophilicity) are useful only in restricted cases, i.e., straight-chain esters to a maximum of eight carbon atoms (Table 6).

Other quantitated structure-activity relationship studies have been concerned with R_m chromatographic values of testosterone derivatives and their erythrocyte membrane binding properties [43], R_f and R_m values

of testosterone derivatives and bioactivity correlations [44], and distribution coefficient-anabolic activity comparisons with a series of nandrolone esters [45]. Improved correlation between lipophilic character and bioactivity for a number of testosterone esters was found by the use of hydrophobic fragmental constants (f values) [46].

The use of techniques for the evaluation of hydrophobic characteristics of drugs and structure-activity relationships based on such characteristics has recently been reviewed [47].

What follows is a discussion of several steroid prodrugs and analogs designed explicitly as sustained release preparations with particular attention focused on the variety and diversity of modifying groups, their routes of administration, and the methodology utilized in assessment of sustained bioavailability.

3. Androgens

a. Testosterone

Interest in long-acting forms of testosterone dates back to 1936 when Miescher and co-workers [48] synthesized and studied a large number of testosterone aliphatic esters. This interesting study illustrated differences in duration and intensity as a function of chain length for 11 testosterone esters. Two methods of determining the intensity and duration of activity of these esters were utilized: (1) capon comb technique, which involved injecting ester into healthy capons and observing the percent growth of the comb as a function of time, and (2) castrated rat technique, in which rats were castrated at a weight of 60-80 g and not used until 16-21 days after castration. Three procedures were then employed to evaluate duration and potency. (1) Ester was injected for a number of consecutive days and the animals were sacrificed on the day following the final injection. Sex organs were weighed to determine the weight increase [49]. This weight increase was taken as an indication of steroid efficacy. (2) Alternatively, the animals were administered drug twice in a 5 day period, sacrificed, and the organs examined. (3) The final procedure was evaluation of effect after a single injection. This method more clearly enables one to determine the duration of effect of a given amount of steroid ester without resorting to the more involved methodology and interpretation of a multidose study. Figure 7 illustrates the effect of altering ester chain length and dose on the duration and intensity of testosterone as measured by capon comb growth. It can be seen that onset of action and intensity is greatest with the short chain esters such as formate, acetate, and propionate. This is probably due to the fact that solubility at the injection site is sufficient to allow rapid absorption to occur. It may also be due to facile hydrolysis of the

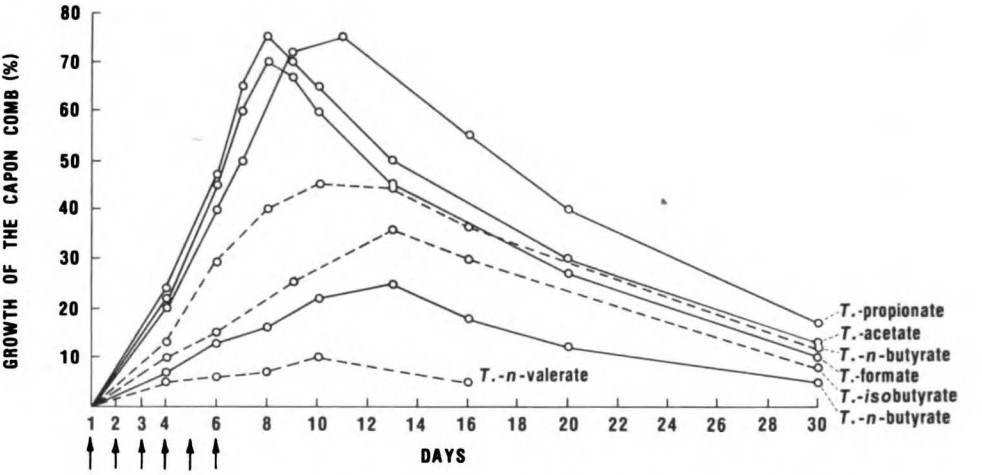

Fig. 7. Effect of testosterone esters on the capon comb (daily injections during six days) with daily dose of 50 γ (solid line) and 100 γ (broken line). T = testosterone. (Reproduced with permission from Ref. 48.)

esters to testosterone. The medium chain esters (butyrate, isobutyrate and valerate) show decreased duration and intensity, probably due to a combination of decreased solubility and greater resistance to enzymatic hydrolysis. Figure 8 is a fine graphic example showing the differences in onset of action, intensity, and duration of action of several testosterone esters of equivalent activity. Again, the differences are striking between the short-chain unhindered esters and the intermediate and sterically hindered esters. The benzoate, stearate, and palmitate esters (not shown on Figure 8) are virtually without effect. Under similar conditions, all esters were compared to the activity of one international unit (15 γ) of testosterone. The figures in parentheses after each ester in Figure 8 correspond directly to this baseline value.

Using the rat as the biological model for probing duration of steroid effect, it was found that this evaluative technique revealed similar correlations to that of the capon growth assay. Using either the sequential injection (day 1 and day 6) or the single injection techniques, the duration, onset of action, and intensity correlated roughly with length of ester and degree of steric hindrance. Further, similar trends were noted whether weight gain of the seminal vesicle or the prostate was utilized as the basis of comparison (Figures 9 and 10). Miescher et al. [50] further studied duration effects with a series of aralkyl, haloalkyl, and aryl esters and amides of testosterone and androsterone.

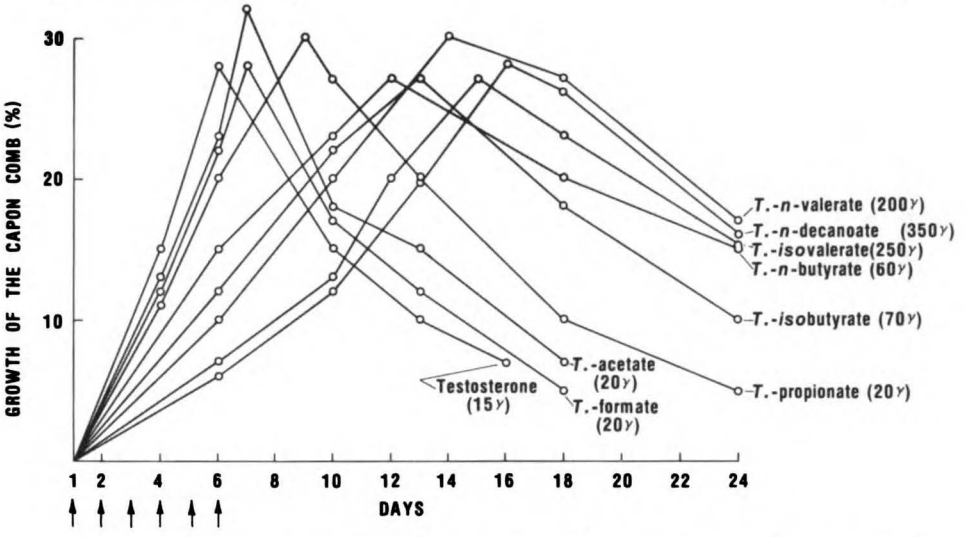

Fig. 8. Time course of effect of approximately 1 international capon unit of different testosterone esters on the capon comb. T = testosterone. (Reproduced with permission from Ref. 48.)

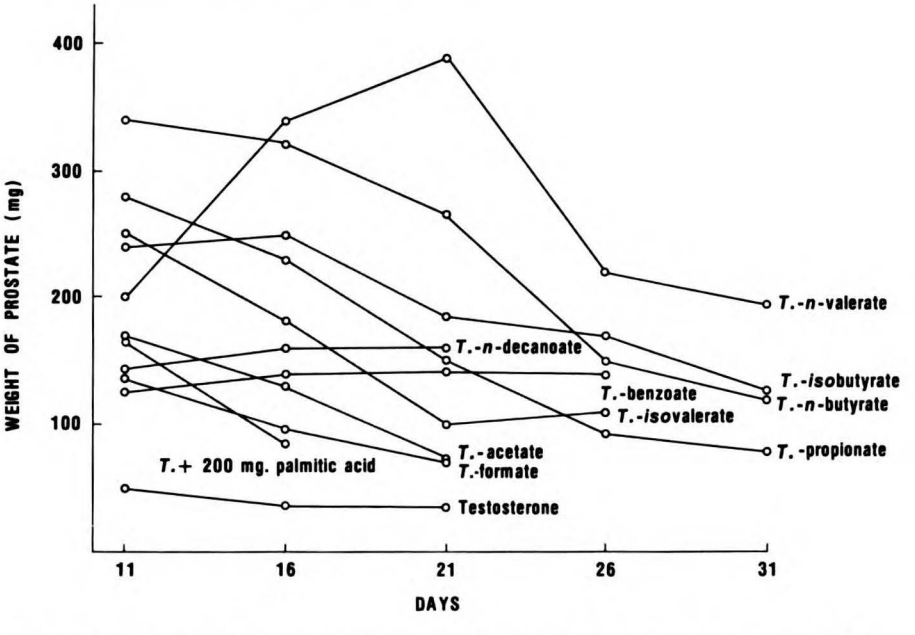

Fig. 9. Effect of testosterone esters on the weight of prostate (sequential injection method, i.e., injections on the first and sixth day). T = testosterone. (Reproduced with permission from Ref. 48.)

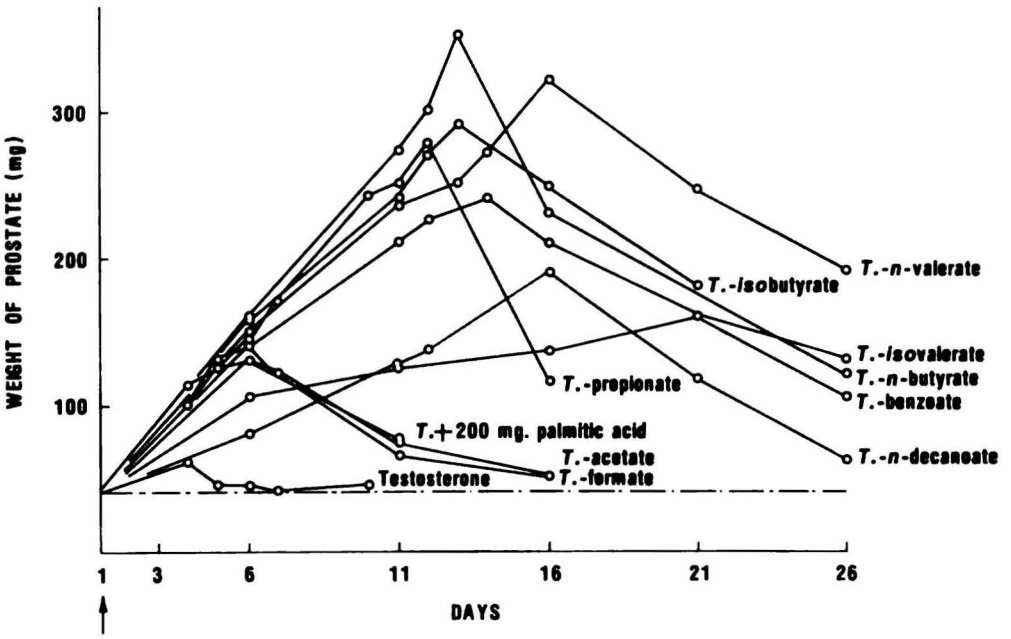

Fig. 10. Effect of testosterone esters on the weight of prostate (single injection method). T = testosterone. (Reproduced with permission from Ref. 48.)

Kochakian, in the evaluation of testosterone oxime, benzoate, propionate, and diacetate, as well as androstanediol benzoate, attempted to standardize his assay methodology so that meaningful comparisons might provide clinically useful data [6]. The assay involved castrated rats and its purpose was to determine the effect of testosterone esters on the regeneration of the prostate and seminal vesicles of these animals. Particular attention was directed to (1) the time of castration and (2) the physiology involved in defining the ancillary tissue that might be considered an integral part of either the seminal vesicle or prostate and whose growth might be affected by these androgenic derivatives. These results corroborated the findings of Miescher et al. [50].

Since testosterone and its derivatives possess anabolic as well as androgenic activity, assays have been developed which are able to differentiate the two effects. Fortunately, the rat can again be used for this purpose. The impact of androgenicity can be determined by weight gain in seminal vesicles or prostate, and anabolic activity can be evaluated by

weight gain of levator ani muscle [51]. Testosterone β-cyclopentylpropionate is an extremely potent anabolic and androgenic agent of several weeks duration [52]. This ester of testosterone, when compared with testosterone and testosterone propionate, was found to be more potent and longer lasting [53]. These studies were carried out in patients exhibiting prepuberal hypogonadism and the effects of these derivatives were evaluated by assaying urinary 17-KS (metabolic and excretion products of the administered steroids) for the amounts and duration of excretion. Semen volume, libido, and potentia were also determined.

A study by Ott and co-workers [54] evaluated the effect of various cycloalkyl esters of testosterone on the androgenic response (seminal vesicle and prostate weight gain) in the rat. Of the esters tested, testosterone β-cyclopentylpropionate again provided the greatest duration of activity. Comparisons with the propionate ester and an aqueous suspension of microcrystalline testosterone dramatize the differences in potency and duration effects of this ester (Figure 11). This study provides an excellent example of the superiority of a chemical sustained release form of a drug over the physical formulation.

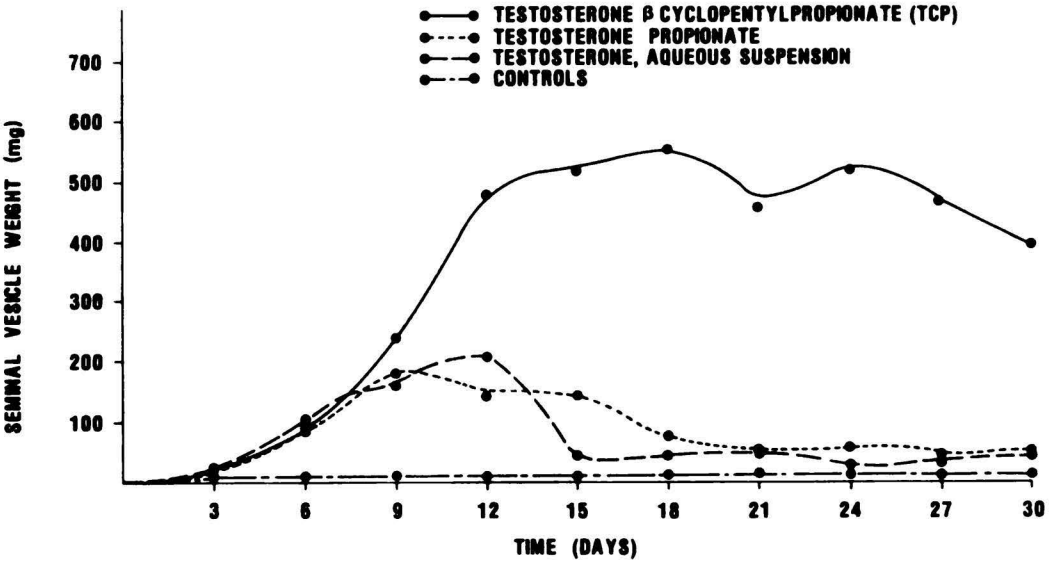

Fig. 11. Testosterone β-cyclopentylpropionate--comparison with other androgen preparations administered by injection. (Vehicle: cottonseed oil, each ml of which, for a single-dose injection, contained the testosterone ester in an amount equivalent to 5 mg of testosterone). Microcrystalline testosterone, 5 mg in 0.2 ml of saline, was given in a single-dose injection. (Reproduced with permission from Ref. 54.)

The use of a physicochemical approach for extending testosterone bioavailability [55] has been investigated in humans. The duration of the increased 17-KS excretion was found to depend on the chemical nature of the testosterone derivative as well as the type of formulation used. Three esters were used (propionate, isobutyrate, and n-valerate) in several different formulations (oil solution, emulsion, pellet implant, and aqueous suspension containing crystals of varying size) and the results are summarized in Table 7. As is readily apparent, the duration of effect is determined by the testosterone derivative, its physical state, and the formulation. For testosterone propionate, the oil solution and emulsion exhibit no difference in duration of effect. This appears reasonable since, upon injection, the emulsion breaks, the oil particles coalesce, and absorption of derivative is similar to the oil solution where release rate from the vehicle becomes a diffusion-controlled process. The interesting difference in duration (7 days versus 9 days) of testosterone-n-valerate aqueous suspension and oil solution, while not significantly different, can nevertheless be explained by mechanisms that contribute to the bioavailability of each dosage form. The solubility of testosterone-n-valerate aqueous suspension at the injection site is dependent on particle size. Bioavailability of this ester initially will primarily be a solubility-limited phenomenon. The oil solution solubility is dependent on the rate of diffusion of the derivative from the oil vehicle as well as its solubility in physiological water. Thus, the extended duration of effect from oil is primarily diffusion-controlled. The difference in amounts of testosterone (as 17-KS) recovered from each formulation of testosterone-n-valerate (12% versus 38%) has been attributed to incomplete absorption of the suspension formulation [55]. Moreover, greater and more complete absorption from the injection site is achieved with an oil solution rather than a crystalline suspension [56]. The effect of particle size on duration is readily apparent with testosterone propionate (small crystals versus "commercial" crystals). Testosterone isobutyrate represents a case of steric hindrance and its effect on duration. Again, recovery of 17-KS is low due to incomplete absorption. The pellet implant technique using testosterone propionate further represents the effect on duration by a gross reduction in surface area available for solubilization of the derivative. It should be noted that one further mechanism is important in determining duration and that is the rate of enzymatic or nonenzymatic hydrolysis of derivative to provide therapeutically significant amounts of testosterone. This is readily apparent in the differences of duration of testosterone propionate, valerate, and isobutyrate esters.

In brief, the following considerations are important in determining duration of androgenic effect when injectable steroid prodrugs or analogs are formulated in a variety of ways:

TABLE 7. Effect of Physical and Chemical Variables on the Duration of Effect of Testosterone Esters

Ester/formulation[a]	Route of administration	Duration of effect	Amount of testosterone recovered as 17-KS (%)
T propionate in oily solution	Intramuscular	3-4 days	44
T propionate emulsion	Intramuscular	4 days	47
T-n-valerate crystals (<0.1 mm)	Intramuscular	7 days	12
T propionate small crystals (0.04-0.1 mm)	Intramuscular	8 days	36
T-n-valerate in oily solution	Intramuscular	9 days	38
T propionate commercial crystals (0.05-0.2 mm)	Intramuscular	12 days	45
T-isobutyrate crystals (0.05-0.2 mm)	Intramuscular	20 days	17
T propionate pellet implantation	Subcutaneous implant	4-5 weeks	14

[a]T = Testosterone.
Source: Data from Refs. 55 and 57.

1. Diffusion rate of derivative from vehicle
2. Solubilization in biological water at and around the injection site
3. Absorption of derivative from injection site
4. Distribution and subsequent hydrolysis of derivative to parent bioactive steroid species
5. Metabolism of both derivative and parent steroid
6. Excretion of both derivative and parent steroid

Processes in steps 4, 5, and 6 may occur sequentially or simultaneously.

Kupperman et al. [3] reviewed the factors involved in evaluation of depot activity of steroid esters as a variability of formulation and also commented on the validity of methods of determining duration of testo-

sterone androgenic effect. Other studies have investigated the variables involved in duration of activity (formulation and chemical modifications) of orally administered testosterone derivatives [13].

Gould et al. [5] studied the effect of chain branching of testosterone esters and its role in reducing activity and duration through inhibition of ester hydrolysis. All esters with dialkyl substituted chains, e.g., diethylacetate, and with 2-alkyl substitution, exhibited poor activity. The exception was testosterone isobutyrate. A rough correlation was found between the rate of base-catalyzed hydrolysis in vitro with the bioactivity and duration of effect of these esters in vivo. This finding implies that such esters must be hydrolyzed in vivo to exert their androgenic activity and further that such activity and its duration are dependent on the rate at which hydrolysis occurs. These esters then qualify as prodrugs of testosterone. An analysis of the structure-activity relationships of several substituted cyclohexanecarboxylic acid esters of testosterone revealed interesting correlations [5]. These esters can be likened to conformationally restricted disubstituted acetic acid derivatives since the alkyl groups are held in place by the cycloalkyl ring. Exceptions are the 2-methyl and the trimethyl derivatives. The cyclohexanecarboxylate derivatives containing 3- and 4-alkyl groups were equal in intensity to the heptanoate and nonanoate straight-chain acid esters [48,50,58,59] but peak activity and duration were longer (Table 8). Chain extension to include methylcyclohexylacetates decreased both potency and duration. If the chain was extended to phenyl or cycloalkyl propionates and butyrates, intensity and duration were similar to testosterone heptanoate (Table 9). Testosterone (4-cyclohexyl) hexanoate was unique however, because its onset of action was delayed but maintained at virtual peak levels for 14-18 weeks in the rat.

A series of phenoxyalkanoate esters of testosterone showed rather high intensity and duration of activity (Table 10) [5,60]. The 4-t-butylphenoxyacetate and 4-chlorophenoxyacetate derivatives were effective for 16 weeks (in mice) and the latter had peak activity more than two times greater than testosterone propionate. Aqueous suspensions (in rats) of the phenoxyacetate and 4-t-butylphenoxyacetate esters revealed peak levels greater than three times those of testosterone propionate. Since phenoxyalkanoic acids are known to be plant growth regulators, and since this type of testosterone ester exhibits similar activity in mammals, it was suggested that the biochemical mechanisms of plant and animal growth control might be similar in origin [61].

Other steroid structure-activity relationship studies indicated that while esterification dramatically increased duration, potency was almost entirely dependent on the presence of an oxygen function in the molecule [62]. Attempts to replace such oxygen with other atoms, e.g., nitrogen,

TABLE 8. Substituted Cyclohexanecarboxylates of Testosterone

Ester	Duration[a]	Method[b]	Intensity[c]
Cyclohexanecarboxylate	3,3,3	A, B, C	1,2,1
2-Methylcyclohexanecarboxylate	3,0,2	A, B, C	1/2,0,1
3-Methylcyclohexanecarboxylate	3,3,3	A, B, C	3,2,2
4-Methylcyclohexanecarboxylate	4,3,3	A, B, C	3,3,3
4-Ethylcyclohexanecarboxylate	3	A	3
4-Propylcyclohexanecarboxylate	3	A	2
4-Isopropylcyclohexanecarboxylate	3	A	2
3,3,5-Trimethylcyclohexanecarboxylate	2,0	A, C	1/2,0

[a]Duration of effect is rated as follows: 0 = no effect (seminal vesicle weighs less than one-half of the TP [testosterone propionate] maximum); 1 = ca that of TP (2-2.5 weeks in mice, 3-4 weeks in rats); 2 = 1-2 x TP; 3 = 2-4 x TP; 4 = 4-6 x TP.

[b]The different procedures were as follows: A, 2.5 mg of ester in oil in mice; B, 7.5 mg of ester in oil in rats; C, 7.5 mg of ester in aqueous suspension in rats.

[c]Intensity is rated on seminal vesicle weights as follows: 0 = < 1/2 TP maximum; 1/2 = 1/2-1 x TP; 1 = TP (55-75 mg in mice, 200-250 mg in rats); 2 = 1 to 1-1/2 x TP; 3 = 1-1/2 to 2 x TP; 4 =>2 x TP.

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result in loss of activity. Testosterone-3-oxime-17-propionate is about one-twentieth as potent as testosterone-17-propionate. Testosterone is also 40 times as potent as its 3-thiosemicarbazone derivative [63]. Gleason [64] and Gleason and Parker [31] investigated the differences in both potency and duration of action in testosterone derivatives substituted variously at the 3, 17, and the 3-17 positions. Thus, a comparison of testosterone and its 3-benziloyl hydrazone (Structure [1]), 17-heptanoate [2], and 3-benziloyl-17-heptanoate [3] derivatives revealed no potency and duration effects for [1], reflecting loss of activity by replacement of oxygen. Evidently metabolic hydrolysis to testosterone does not occur in vivo. The similarities in potency for [2] and [3] are striking since regeneration of

TABLE 9. Miscellaneous Esters of Testosterone

Ester	Duration[c]	Method[c]	Intensity[c]
3-Ethoxypropionate[a]	1	A	1
3-(n-Butoxy)-butyrate	3,2	A, B	2,1
2-Furoate[a,b]	3[d]	A	2
2-Chlorobenzoate	0	A	0
4-Chlorobenzoate	0	A	0
4-Ethoxybenzoate	3,2	A, C	2,2
3,4-Dimethoxybenzoate	1	A	1/2
p-Toluate	2	A	1
Phenylacetate[a]	3,3	A, C	2,3
Cyclohexylacetate[a,b]	3	A	1
3-Methylcyclohexylacetate	2[d]	A	1
4-Methylcyclohexylacetate	2[d]	A	1
3-Cyclopentylpropionate[a]	3,3,1	A, B, C	3,3,1
3-Cyclohexylpropionate[a,b]	3	A	3
3-Phenylpropionate[a]	3[d],3	A, B	3,3
trans-cinnamate	2	A	2
2-Phenylbutyrate	0	A	0
4-Phenylbutyrate	3,2	A, B	3,2
4-Cyclohexylhexanoate	3,4	A, B	3,4
1-Naphthylacetate	1	A	1
Diphenylacetate[a,b]	0	A	0

[a]Included for comparison.
[b]Ref. 54.
[c]See Table 8, footnotes a, b, and c.
[d]Extrapolated value.
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TABLE 10. 17-Aryloxyalkanoates of Testosterone

Ester	Duration[a]	Method[a]	Intensity[a]
Phenoxyacetic	3,3,3	A, B, C	3,3,4
2-Chlorophenoxyacetic	3[c],4	A, C	2,3
4-Chlorophenoxyacetic	4,3	A, C	4,2
4-Bromophenoxyacetic	3,4	A, C	3,3
2,4-Dichlorophenoxyacetic	3,3,3	A, B, C	3,3,4
2,4,5-Trichlorophenoxyacetic	4,3,3	A, B, C	3,3,3
2,4,6-Triiodophenoxyacetic	--	--	--
4-Methoxyphenoxyacetic	3,3	A, C	3,4
4-Methylphenoxyacetic	3	A	3
4-t-Butylphenoxyacetic	4,4,4	A, B, C	3,3,4
(-)-2-Phenoxypropionic[b]	3[c],2[c]	A, C	2,2
(+)-2-Phenoxypropionic[b]	3[c],3	A, C	2,3

[a]See Table 8, footnotes a, b, and c.
[b]Resolved by crystallization of the mixed esters of the racemic acid.
[c]Extrapolated value.
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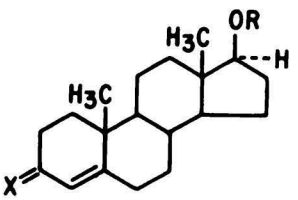
testosterone in vivo probably occurred. Duration of [3] is significantly longer (16 weeks) than that for [2] and probably represents a delayed hydrolysis of the 3-benziloyl hydrazone moiety (Figure 12). The growth response curve using rat seminal vesicle weight is qualitatively similar to the prostate weight gain.

An elegant chemical rationale, based on relative hydrolysis rates, provided the basis for the synthesis of several unusual prodrug derivatives of testosterone [65]. It was intended that such derivatives would be efficiently absorbed orally and metabolized slowly, either enzymatically or nonenzymatically, to exert a sustained duration of androgenic effect.



X	R	
O	H	Testosterone
C_6H_5 C_6H_5 OH C - C - NH - N - O	H	[1]
O	O ‖ - C$(CH_2)_5CH_3$	[2]
C_6H_5 C_6H_5 OH C - C - NH - N - O	O ‖ - C$(CH_2)_5CH_3$	[3]

The derivatives included hemiesters (testosterone acid succinate), mixed amide-esters (testosterone amidosuccinate and N,N-dimethylamidosuccinate), esters (pyruvate, ethyl succinate) and ethers (testosterone ethylene ketal methoxymethyl ether and tetrahydropyranyl ether). No derivative possessed potency or duration greater than the parent steroids testosterone or methyltestosterone. The results of this endeavor illustrate quite vividly the necessity for a biological as well as a chemical basis for the design of long-acting testosterone preparations.

The use of unique testosterone derivatives such as ethers and acetals is at least partially vindicated by the work of Cross et al. [29] in which a variety of 2'-tetrahydropyranyl ethers of methyl- and nortestosterone were synthesized. Several of these acid-labile derivatives exhibited increased potency and a corresponding duration of activity over the parent molecules in rats. An unusual delay in attainment of maximum response was seen with the 17-trimethylsilyl ether of testosterone [66]. The maximum androgenic response to a single subcutaneous dose of testosterone propionate in the rat occurred at 7-10 days. The same response with testosterone-17-trimethylsilyl ether was observed at 20-30 days. It appears that this ether derivative was somewhat more potent than the propionate ester as the weights of the accessory organs (seminal vesicle and ventral prostate)

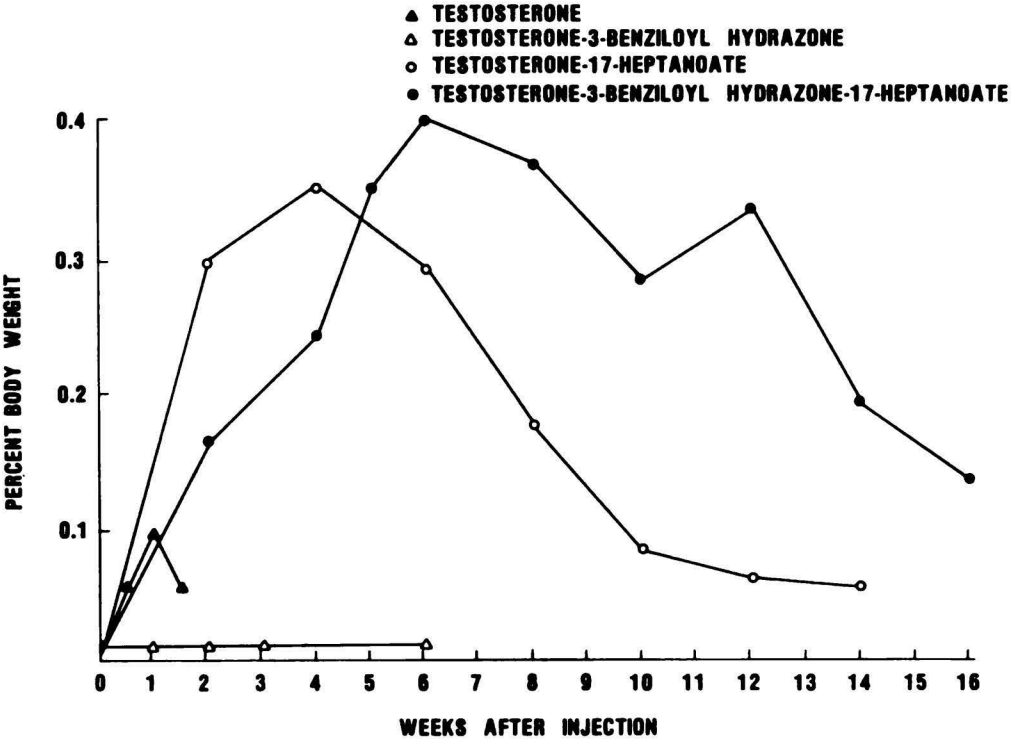
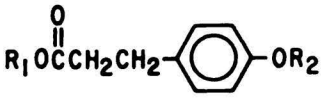


Fig. 12. Growth response of prostate expressed as percent of body weight. (Reproduced with permission from Ref. 31.)

exhibited a significantly greater gain. This same trend was also seen for the myotrophic effect (levator ani muscle weight gain) but was less dramatic. Studies with 17β-yl mixed acetals of testosterone and methyltestosterone disclosed similar effects [30].

A new chemical type of long-acting steroid ester was described in 1960 by Diczfalusy [67]. These were steroid esters of p-alkoxyphenyl propionic acid (p-alkoxyhydrocinnamic acid esters).



R_1 represents the steroid portion of the derivative and included testosterone, cortisone, 17β-estradiol, and 17α-hydroxyprogesterone, while R_2 was varied with alkyl groups of chain lengths from 1 to 12 carbon atoms. This type of ester derivative provides an ideal model in which to study the

effect of carbon chain length on duration of activity. Interesting differences in duration were found when various substituted and unsubstituted testosterone propionate esters of testosterone were tested in the rat ventral prostate assay (Figure 13) [68]. Introduction of a phenyl group in propionate ester significantly increased potency. The p-hydroxyphenyl addition likewise increased potency as well as duration. Optimal potency and duration was seen with testosterone-p-hexoxyhydrocinnamate. This ester was more potent and of greater duration than several commercially available testosterone esters [67]. Similar correlations were noted with 19-nortestosterone p-alkoxyhydrocinnamate esters [67]. It is not known if these esters possess anabolic activity per se or if they require hydrolysis to exert their effects.

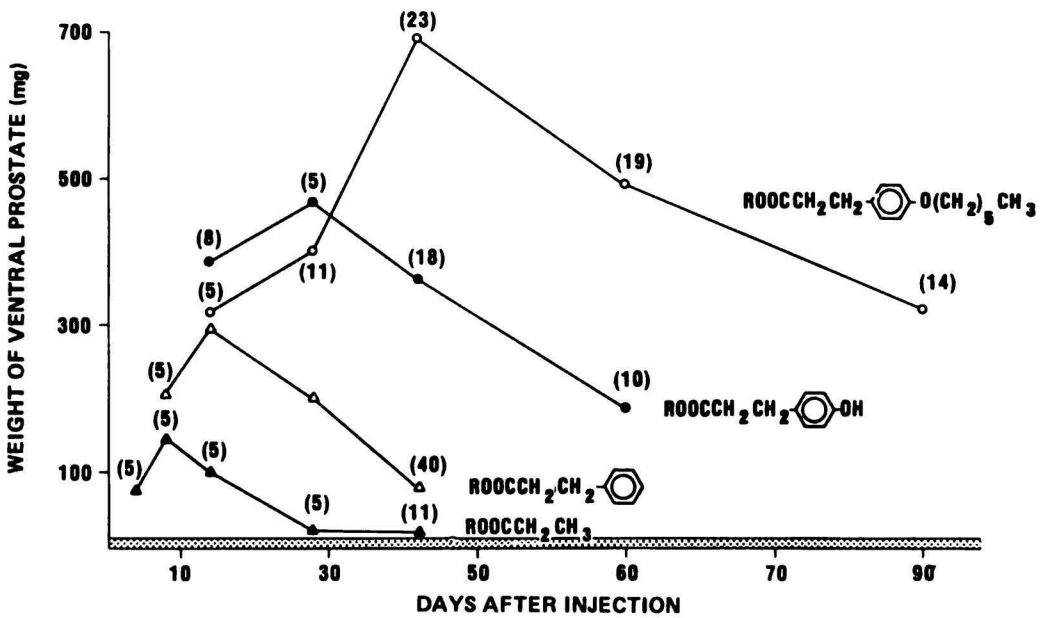


Fig. 13. Weight of the ventral prostate in castrated adult rats treated with various p-alkoxyhydrocinnamates of testosterone, as a function of the number of carbon atoms in the p-alkoxy part of the molecule. (Zero indicates a p-hydroxy group.) The animals were killed 42 days after a single intramuscular injection of the equivalent weight of 4.35 mg of testosterone in oil. Figures in parentheses indicate the number of animals used. Shaded area corresponds to the 95% fiducial range of the ventral prostate weight in castrated rats. (Reproduced with permission from Ref. 68.)

b. Nortestosterone

Nortestosterone (19-nortestosterone, nandrolone, 17β-hydroxyestr-4-en-3-one) is the 19-demethyl analog of testosterone. Like testosterone, it possesses both androgenic and anabolic properties. The discovery that certain 19-nortestosterone derivatives produced significant anabolic (myotropic) effects to the exclusion of androgenic effects at certain dose levels led many investigators to search for other 19-nortestosterone derivatives that enhanced this split in activity. In this regard, several long-acting 19-nortestosterone esters have been evaluated. Esters such as phenylpropionate, decanoate, docosanoate (C_{22}), and oleate (C_{18}) exhibited good duration of activity as well as a high anabolic/androgenic ratio [69, 70]. Scribner et al. [71] postulated best anabolic activity with esters that exhibited optimal rather than maximal lipophilicity and enzymatic hydrolysis. To this end, several bicyclo[2.2.2]octane-and-oct-2-ene-1-carboxylic acid esters were prepared and evaluated. The unsubstituted and 4'-methylbicyclo ester derivatives produced unusually long-acting anabolic effects in the rat ventral prostate, seminal vesicle, and levator ani assays.

A number of 19-nortestosterone terpenoate esters were evaluated for anabolic activity [72] using the myotropic-androgenic methodology of Hershberger et al. [49]. Several acyclic terpenoates produced significant response over a period of 21 days. The cyclic terpenoate esters were inactive due probably to the degree of steric hindrance and lack of ester hydrolysis in vivo.

Treatment of metastatic breast cancer was attempted with the potent androgen, 7α-methyl-19-nortestosterone acetate at dose levels of 10 mg, 33 mg, and 100 mg weekly [73]. Its duration of regression, duration of therapy, and survival time in 10 subjects was compared with those of dromostanolone propionate (17β-hydroxy-2α-methylandrostan-3-one propionate). The summary of duration of effect at various doses for survival of responders and nonresponders [73] is as follows:

Dromostanolone propionate	
100 mg	responders 18+ months; failures 7-9 months
200 mg	responders 18+ months; failures 6-7 months
7α-Methyl-19-nortestosterone acetate	
10 mg	responders 21+ months; failures 8-9 months
33 mg	responders 24-25 months; failures 8 months
100 mg	responders 22 months; failures 5-6 months

It thus appears that 7α-methyl-19-nortestosterone acetate significantly increases survival at lower doses than does dromostanolone propionate.

Other studies involving nortestosterone and its derivatives are discussed under Testosterone (Section II.A.3.a).

4. Estrogens and Progestogens

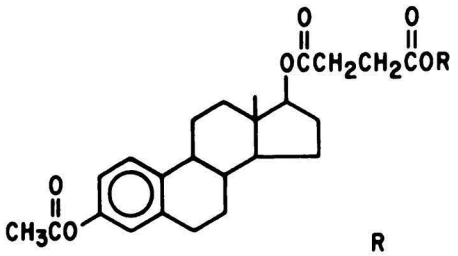
As with androgenic steroids, the primary chemical approach utilized to produce long-acting estrogens has been via the prodrug derivative. Early work in this area was concerned with mono- and bis-esters of estradiol and estrone [74,75]. These studies of Miescher et al. stand as classics in the field, and subsequent investigations have merely refined the underlying rationale and methodology. The bioassays used in the evaluation of duration of estrogenic activity are twofold: (1) Uterus growth in immature rats. Increased weight of the uterus is noted as a function of estrogen potency. The time that the uterus weight remains significantly greater than that of controls is taken as an indication of the estrogen derivatives' duration of effect. (2) Estrus test. The day of onset as well as the duration of estrus in castrated rats are used as indicators of potency and duration. The error inherent in the tests is about ±10% for the uterus growth assay and ±15% in the estrus test. Recent studies by Rubin et al. [76] have confirmed a linear relationship between log dose and uterine weight response in mice. Assay design established according to Bliss [77] reduced the standard error to ±13%. Methods of determining duration of estrogenic response in human subjects have included (1) duration of time interval between last injection and onset of withdrawal bleeding in castrated women with intact uteri, (2) changes in vaginal smears and character of the cervical mucous, and (3) symptomatic patient response [78].

Estrone esters displayed a typical structure-activity relationship that varied according to the length of the ester chain (Figure 14). Esters larger than the octanoate showed a rapid decrease in duration (the C_{16} ester was inactive) reflecting perhaps a high degree of aqueous insolubility, thus limiting absorption and bioavailability.

Similar relationships can be seen for estradiol-3-benzoate and several estradiol-3,17-bisesters (Figure 15).

Junkmann has reviewed the biology of a large variety of estrone and estradiol long-acting ester derivatives [79].

A novel approach to the prolongation of estrogenic activity involved the use of oligomeric estradiol esters [80]. Such oligomers contain from two to four steroid molecules and are covalently linked via succinate esters. The preparation of these steroid esters was achieved by use of some unique chemistry. Thus, reaction of 3-acetoxy-estradiol-17β-hemisuccinate with N,N'-carbonyldiimidazole afforded an intermediate that was condensed with estradiol or its acetate ester to form a variety of oligomers, several of which greatly enhanced duration of estrogenic activity. Bioassay involved daily analysis of the vaginal smears of oophorectomized rats receiving subcutaneous injections of test compound. Increasing the chain length was found to enhance duration of vaginal estrus to about 120 days (Compound [7] versus 80 days for estradiol-17β-undecylate (Figure 16). The dimeric derivatives (as isomeric esters, [4], [5], [6]) also exhibited extensive duration of activity.



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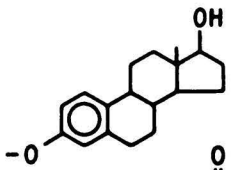

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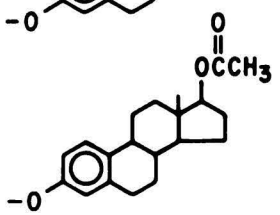

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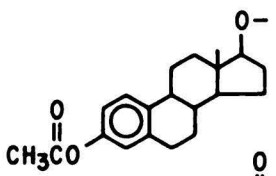

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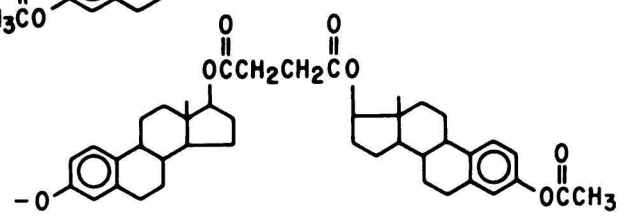
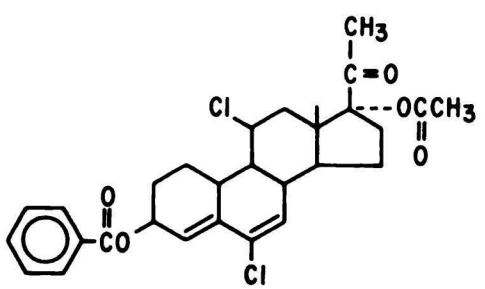


[7]

The benzoate ester of 6, 11β-dichloro-3β, 17-dihydroxy-19-nor-4, 6-pregnadien-20-one-17-acetate [8] was found to possess prolonged o-progestational and antiestrogenic activity compared to its Δ^4-3-ketone analog [81].



[8]

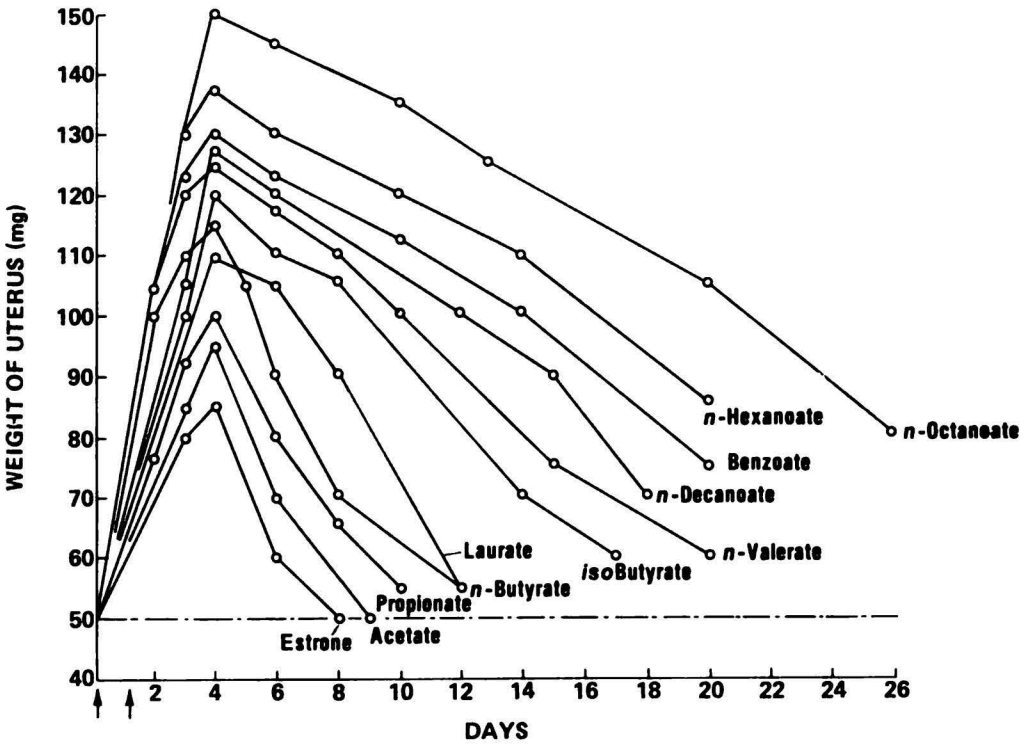


Fig. 14. The effect of estrone and its esters on the weight of the uterus of young rats after administration of a total dose of 50 γ of hormone given in equal parts on two consecutive days. (Reproduced with permission from Ref. 74.)

Duration of anestrus of [8] was twofold greater than that of the Δ^4-3-ketone analog (15 versus 8 days) as determined by uterus weight differences in ovarectomized rats as well as changes in uteri carbonic anhydrase activity in immature rabbits (modified McPhail test). The presence of the 3-benzoate ester was thought to inhibit metabolic breakdown and possibly increase storage of [8] in body fat tissue.

A series of 17α-alkynyl estradiol-3-cyclopentyl ether 17-benzoate esters were shown to exhibit an interesting and prolonged antigonadotrophic activity approximately twice (16 versus 32 days) that of the parent ovarian inhibitor quinestrol (17α-ethynyl estradiol-3-cyclopentyl ether) [82]. The use of substituted and unsubstituted 17-benzoate esters appears to be an efficient and uncomplicated method of varying antigonadotrophic activity

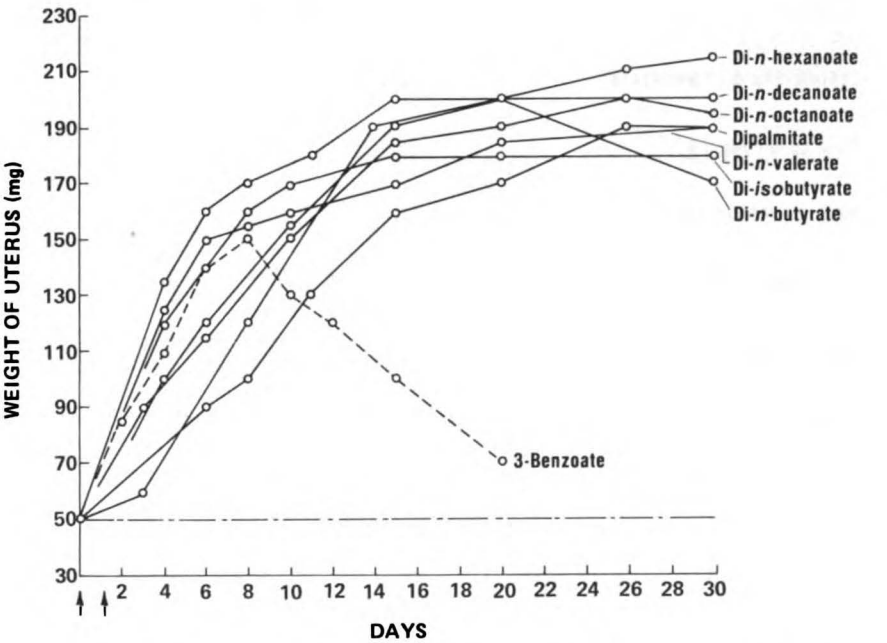


Fig. 15. The effects of some higher aliphatic diesters of estradiol and estradiol-3-benzoate on the weight of the uterus of young rats after administration of a total dose of 50 γ of hormone given in equal parts on two consecutive days. (Reproduced with permission from Ref. 75.)

as well as inducing a high and prolonged inhibition of testes growth in male animals. Such compounds find utility as sterilizing agents in noxious animal species such as mice and rats.

Several estratriene-3-alkyl, -cycloalkyl, and -heterosulfonyloxy derivatives were tested and found to demonstrate sustained androgenic, anabolic, gestational, and estrogenic activity [83]. Estratriene formate esters were claimed to display prolonged hypocholesteremic bioactivity [84].

A rather successful chemical approach to the fabrication of long-acting steroids has been via steroidal ethers. Meli and Steinetz, in an excellent review [85] of steroidal ether biology and metabolism, delineated those factors deemed important to the bioactivity of these unique drug derivatives:

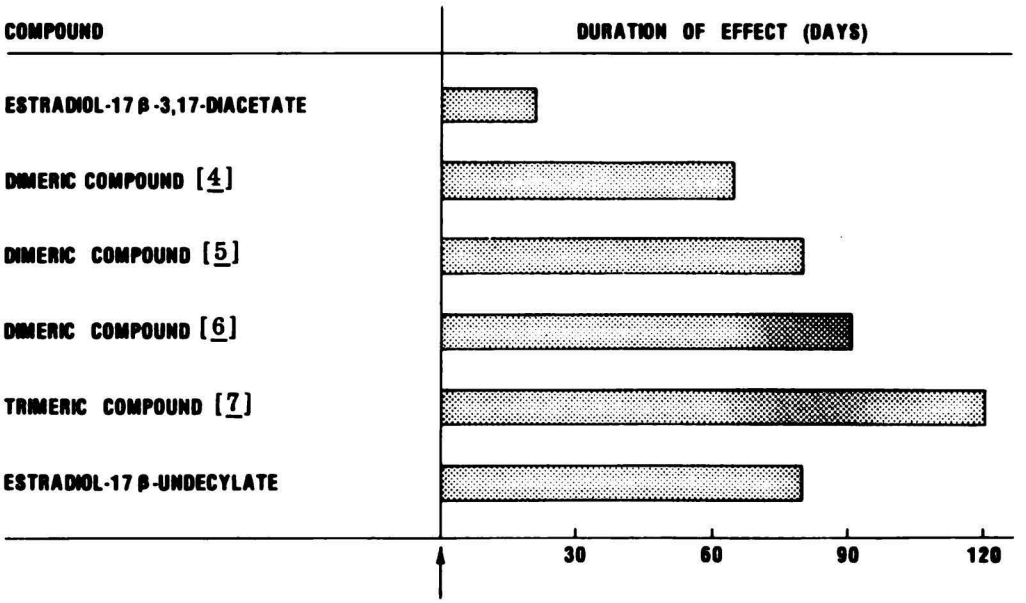


Fig. 16. Mean duration of action of depot-estrogens after a single subcutaneous injection of 40 μg per rat. (From Ref. 80.)

1. Steroidal 3-ethers and 3-enol ethers may be bioactive per se.
2. Sustained bioactivity is due to slow hydrolysis of the ether linkage.
3. Ether linkage hydrolysis occurs, but metabolic products are different from those of the parent steroid.
4. The ether linkage influences the pharmacokinetics (absorption, distribution and storage, excretion patterns and rate, and metabolism) of the steroid.

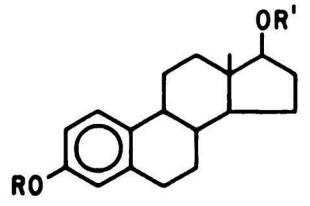
The route of administration plays an important role in the ultimate duration of effect of such ethers. Since the ether linkage is acid-labile, hydrolysis would seem assured when these substances are administered orally (acid hydrolysis in the stomach). This is true of the 3-enol ethers of methyltestosterone and 17α-acetoxyprogesterone [86-88]. The lowered activity seen when enol ethers are administered subcutaneously is probably due to lack of absorption (high lipophilicity) and to storage in fat depots rather than to lack of hydrolysis. This appears reasonable since the findings are based on rat studies and subcutaneous fat accounts for approximately 50% of rat total body fat. Highly lipophilic steroids would be slowly released from such compartments. This same effect appears to be operative with estradiol and testosterone esters [11]. Conversely, bioactivity may be

decreased due to lack of metabolic inhibition. Thus, the lowered bioactivity seen after oral administration of 19-norsteroid enol ethers is perhaps due to lack of a 19-methyl group that would inhibit its rate of metabolism [89].

Overall, it appears that most ethers of this type again act as prodrugs and require hydrolysis to the parent steroid prior to expression of bioactivity. An important consideration concerning activity and duration of steroidal ethers is that the parent molecule is as important as the ether moiety itself in the determination of the ultimate pharmacokinetic and bioavailability parameters, and spectrum of activity of these drugs.

In the great majority of cases, estrogen-3-ethers are less active subcutaneously than orally [90,91]. Ethynyl estradiol-3-cyclopentyl ether, on oral administration, was stored in body fat and slowly released over a long period of time [92,93]. Partitioning and storage of this ether derivative into brain tissue may also account for its effective inhibition of pituitary gonadotrophic hypersecretion. Estrogenic effects of this derivative lasted for several months after termination of administration.

The presence of C-17 alkyl moieties in both natural and synthetic estrogens has been implicated in heightened hepatotoxicity of these steroids [94]. Several estradiol-3-ester-17-ether derivatives were synthesized in an effort to circumvent this toxicity of the parent steroid. Estradiol-17-cyclohexenyl ether-3-propionate (orestrate [9]) and estradiol-17-cyclooctenyl ether-3-benzoate ([10]) demonstrated a separation of estrogenic and hepatotoxic effects in the rat (decrease of sulfobromphthalein serum levels over quinestrol) [95,95]. The delay of hepatic metabolism of ester-ether derivatives with subsequent distribution to target organ was thought to be responsible for lack of toxicity.



R	R'	
$C_2H_5C(=O)-$		[9]
$C_6H_5C(=O)-$		[10]

Disteroidal ethers [97] and estratriene-17β-yl enol ethers and acetals [98] have also been utilized as oral and parenteral long-acting estrogens, androgens, and corticoids.

5. Adrenocortical Hormones

The antiinflammatory agent, hydrocortisone, has been investigated with regard to sustained bioactivity. The effect of esterification of hydrocortisone on liver glycogen and antiinflammatory activity has been studied [99]. Of the esters tested, hydrocortisone-21-caproate prolonged liver glycogen activity to the greatest extent. Liver glycogen deposition was taken as an objective evaluation of the true bioactivity of a glycogenic steroid [100]. For determination of antiinflammatory activity, the degree of decrease of foreign body granulomas (induced by subcutaneous implantation of compressed cotton pellets) was determined after steroid ester administration [101]. The trimethylacetate derivative was more readily absorbed but was not efficacious to any greater extent. Cortisone-21-trimethylacetate, however, was found to have a longer duration of activity than cortisone [102]. Duration of activity was thought to be due to a combination of reduced rates of absorption and ester hydrolysis. The liver glycogen activity for a series of hydrocortisone esters is shown in Table 11.

Significant correlations between potency and lipophilicity were found for a series of corticosteroid esters of various types. These topically active steroids were evaluated by the skin blanch test which is characterized by subepidermal capillary blood vessel vasoconstriction [103,104]. This effect is important in appraising the value of topical corticosteroids since the swelling and redness of inflammation is due directly to vasodilation of these vessels. Thus, the structure-activity relationships of several hydrocortisone-17-esters were determined using this technique [105]. A high correlation was found between biological response and lipophilicity of steroid ester, hydrocortisone-17-butyrate and 17-valerate esters being most potent. That such esters act as prodrugs has not been definitely settled. It has been speculated that the activity of betamethasone-17α-20-orthoesters are hydrolyzed by acidic components present in sweat [104]. This type of ester readily hydrolyzes under acidic conditions in vitro [106].

Difluorocorticosteroid 17,21-methylorthoesters, 17-monoesters, and 17,21-diesters have also been studied for topical antiinflammatory activity [107]. Interestingly, none of the above-mentioned topical steroids have been evaluated for depot activity. Although most esters evaluated would probably exhibit prolonged activity per se, the use of ointment bases that release drug over an extended period would make such evaluation most difficult.

TABLE 11. Liver Glycogen Activity of Some Hydrocortisone Esters

Repletion test: Dose, molecular equivalent of 1.5 mg hydrocortisone acetate/100 g rat per day, s.c.[a] for 6 days			Prolongation test: single s.c.[a] doses of the molecular equivalent of 8.25 mg hydrocortisone acetate/100 g rat		
Ester	Response[b]	Activity[c]	Prolongation[d] activity (7 days)	Absorbed in 7 days (mg)	Activity[e] (wt. absorbed)
Free alcohol	285	=	--	--	--
Acetate	370	--	347	1.3	267
Propionate	455	=	--	--	--
Trimethylacetate	351	=	362	3.6	100
Diethylacetate	504	=	--	--	--
t-Butyl acetate[f]	90	<	101	1.8	57
Caprylate	601	>	--	--	--
Caproate[g]	643	>	737	5.5	135
Stearate	75	<	59	3.1	19
Benzoate	88	<	144	2.6	55
Phenylacetate	383	=	--	--	--
Cortisone acetate	389	=	--	--	--
Cortisone t-butyl acetate	136	<	--	--	--

[a]s.c. = subcutaneously. [b]Milligrams liver-reducing substances, per 100 g body weight, above control values. [c]Activity compared with hydrocortisone acetate by Student's t test. [d]Area under curves relating liver-reducing substances to days on test. [e]Calculated as hydrocortisone acetate. [f]Hydrocortisone t-butyl acetate was about one-half as active as hydrocortisone alcohol in a one-day mouse liver glycogen test when administered orally (Method III). [g]Hydrocortisone caproate was about as active as hydrocortisone alcohol in the mouse test (Method III). Source: Reproduced with permission from Ref. 99.

TABLE 12. Duration of Activity in Various Corticosteroid Derivatives

Parent steroid	Modification	Route of administration	Ref.
Prednisolone, hydrocortisone	Pyrophosphate-21-esters and salts	Intramuscular	109
Prednisone, prednisolone	Alkyl carbonate ester	Oral, subcutaneous	110
Aldosterone	Aryl ester	Oral, subcutaneous	111
Corticosteroid general structure	21-Sulfate and 21-phosphate esters and salts thereof	Intramuscular	112
Pregnatrienepyrazol	21-m-Sulfobenzoate ester	Oral, parenteral, topical	113
Betamethasone, dexamethasone	21-Adamantyl-1-carboxylic acid ester	Intramuscular	114

Certain fluocinolone acetonide* 21-esters exhibited prolonged activity over unesterified fluocinolone acetonide, as seen in the liver glycogen and edema reduction assays [108]. Thus, greatest duration was found with fluocinolone acetonide 21-benzofuranate while relatively shorter durations were seen with fluocinolone acetonide 21-acetate and fluocinolone acetonide.

Other corticosteroids exhibiting depot activity are summarized in Table 12.

B. Neuroleptics

Neuroleptics are drugs used in the treatment of mental disorders, especially psychoses. Included in this frame of clinical reference are the tranquilizers which are employed in the treatment of common psychoneuroses and in somatic disorders. A depot neuroleptic has been defined as one

*6α,9α-difluoro-16α-hydroxyprednisolone 16,17-acetonide.

which can be administered in such a form and manner that a single dose affords therapeutic tissue levels for at least 1 week [115]. In many ways, the depot neuroleptics are strikingly similar to the depot steroids in the following ways. (1) Both utilize the prodrug derivative as the primary chemical form. (2) The intramuscular route of administration (as aqueous suspensions or drug esters in oil solution [116]) is the primary method of treatment. (3) Both diffuse slowly from the injection site and both experience dilatory metabolism and excretion. (4) Due to their high potency, both are ideally suited to depot therapy. Therapeutic efficacy is adequate at the microgram level.

The primary chemical methods of enhancing neuroleptic duration are by ester and salt formation. Neuroleptic esters have extremely high oil/water partition coefficients thus enabling slow diffusion of the drug from the oil vehicle into the tissue fluid at the injection site. Hydrolysis of the ester occurs by esterases and the parent molecule is usually transported from the injection site in the unesterified form. Esterases available from other sources such as the blood and brain are also responsible for hydrolysis of that small amount of drug ester that leaves the injection site intact. Very little ester has been detected outside of the original site of administration [117].

Generally, as with depot steroids, the longer the fatty acid ester chain, the greater the duration of effect. By the same token, the lower the aqueous solubility of a salt derivative, the longer the duration. The duration of action of neuroleptic derivatives can be determined in two ways. (1) After administration of a single dose, the plasma concentration of active drug and/or metabolite is measured as a function of time. Meaningful correlations, however, should previously be established between plasma level and therapeutic effect. (2) Measurement and correlation of drug effect (dose: response) on specific function can also be utilized. An example is the inhibition of conditioned avoidance reaction (CAR) for neuroleptic assessment in animal models. In human subjects, assessment might rely on degree of clinical improvement based on the Brief Psychiatric Rating Scale or Wing Nurse Behavior Rating Scale [118].

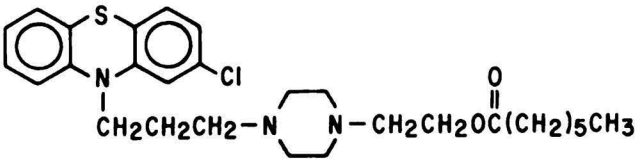
Historically, the extrapolation of drug duration effects from lower mammalian species to humans has been difficult at best; this situation is no different for the neuroleptics. As is common, the duration of effect varied with dose, making comparisons of various neuroleptic derivatives difficult. Further, equidose duration effects vary as a function of the animal species being utilized [119].

Long-acting neuroleptics are derived from a variety of chemical classes including phenothiazine, thioxanthene, benzazepine, and diphenylbutyl piperidine.

1. Phenothiazines

a. Perphenazine

The use of perphenazine enanthate (heptanoic acid 2-(4-[3-(2-chlorophenothiazin-10-yl)propyl]-1-piperazinyl)ethyl ester) [11] as an injectable long-acting neuroleptic was studied by van Kempen [120] in a group of 11 patients. Administration of a 100 mg intramuscular dose resulted in significant urinary levels of perphenazine for greater than 4 weeks. Of the dose given, 6% was recovered as free perphenazine and 22% as the sulfoxide metabolite. Increasing the dose also produced a relative percentage increase in amounts of drug and metabolite excreted.



[11]

b. Pipothiazine

Several esters of pipothiazine (2-dimethylsulfamoyl-10-[3-(4-hydroxyethylpiperidino)propyl]phenothiazine) have been evaluated as long-acting neuroleptics, including the undecylenate [12] and palmitate [13]. Evaluation of these esters along with fluphenazine heptanoate (see [15]) revealed that duration of effect increased as a function of the increase of the chain length of the esters [121]. An unusual method was utilized to demonstrate duration differences between the esters. The dog was used as the biological model and the test was antagonism against apomorphine-induced vomiting, a sensitive technique used to evaluate the efficacy of neuroleptic agents. As can be seen in Figure 17, efficacy (ED_{50}) is plotted against duration (days). A clear difference is evident between the esters. Note that the peak of effect for fluphenazine enanthate (heptanoate) is about 6 hr whereas it is of longer onset for the undecylenate ester (~2 days) and palmitate ester (4-7 days). These varied onsets of action are probably a reflection of the solubility and rates of hydrolysis, transport, and excretion of the various esters. Log ED_{50} varies linearly with time over a portion of the curve and from this can be determined a rough half-life for each derivative. This is also illustrated in Figure 17.

Plasma level studies in the rat with radiolabeled pipothiazine palmitate (intramuscular injection) revealed that a reasonable duration of effect correlation could be obtained from the ED_{50} versus time plot. The half-life calculated from the plasma concentration-time curve was about 15 days. Based on in vitro enzyme hydrolysis studies with rat plasma and liver and brain homogenates demonstrating ester hydrolysis [122], and augmented with chromatographic evidence, it was concluded that the free pipothiazine was the bioactive species.

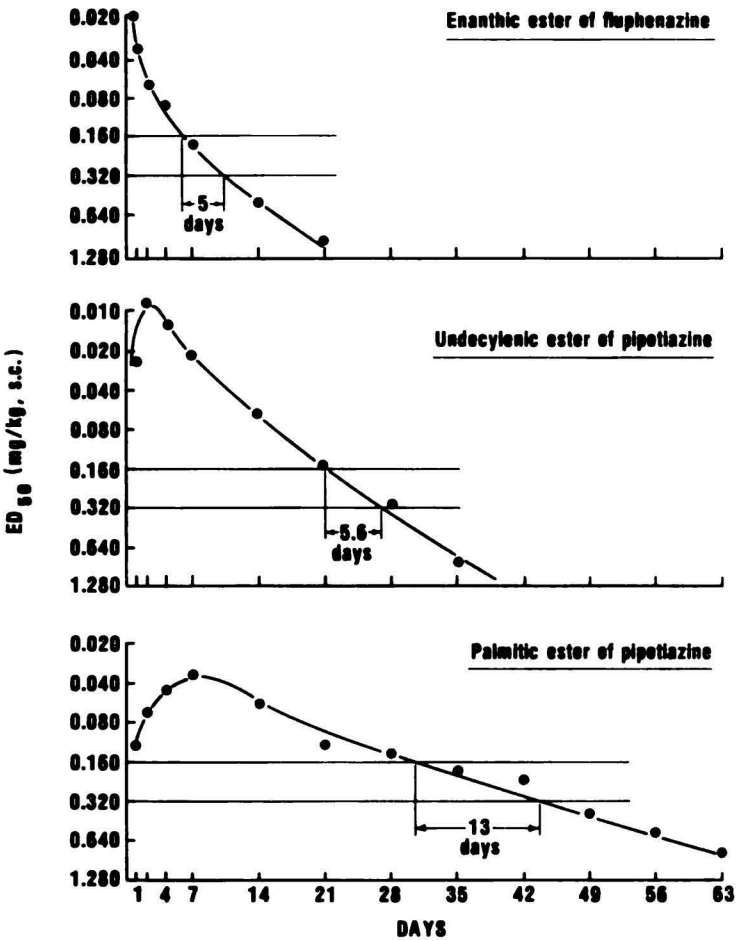
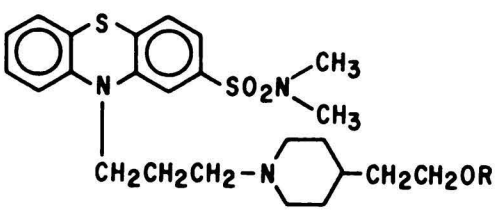


Fig. 17. Antiapomorphine activity (vomitings) in the dog. Variations of ED_{50} versus time. (Reproduced with permission from Ref. 121.)



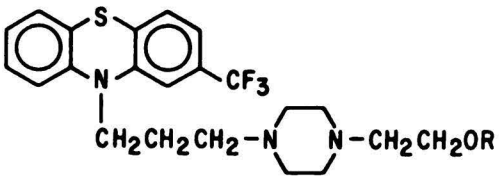
$$R = -\overset{O}{\overset{\|}{C}}(CH_2)_9CH_3 \qquad [12]$$

$$-\overset{O}{\overset{\|}{C}}(CH_2)_{14}CH_3 \qquad [13]$$

Pipothiazine undecylenate was studied in a group of chronic schizophrenics and found to relieve such symptoms as depressive mood, anergia, and psychomotor inhibition [123]. Mean duration of action was 2 weeks using an average dose of 60-80 mg [124]. Pipothiazine palmitate, moreover, was found to satisfactorily maintain schizophrenic patients with an average single intramuscular injection of 152 mg for 4 weeks [125]. Depending on the patient, best results, with a minimum of extrapyramidal side effects, were obtained with a once-monthly dose of 100-150 mg. Other clinical studies on these esters have dealt with the advantages of long-acting neuroleptics in minimizing patient compliance problems [126,127] and reduction of toxic side effects [128].

c. Fluphenazine

Another potent tranquilizer derived chemically from phenothiazine is fluphenazine (4-(3-[2-(trifluoromethyl)phenothiazin-10-yl]propyl)-1-piperazine ethanol) [14]. Like most other agents of its class, its onset (~1 hr) and duration of action (6-8 hr) are relatively short. By chemically extending the duration it was hoped to reduce frequency of administration, eliminate uncertainties associated with oral administration (bioavailability, toxicity) and reduce or eliminate problems encountered with patient compliance [129]. Toward this end, the heptanoate (enanthate) [15] and decanoate [16] esters were prepared and evaluated.



R	
H	[14]
$-C(CH_2)_5CH_3$	[15]
$-C(CH_2)_8CH_3$	[16]

Early animal studies established [15] as a powerful tranquilizer of long duration. One dose (32 mg/kg subcutaneously) in an oil vehicle blocked CAR in rats for about 2 weeks [130,131]. Drug effects lasted for almost 1 month in blockage of apomorphine-induced emesis in dogs. Ebert and Hess [117] examined the distribution and fate of [15] in the rat. Using ^{14}C-radiolabeled [15], it was found that distribution of injected drug was widespread with a preponderance remaining in the carcass (60% of dose at day 1 and 8% at day 21), smaller amounts being detectable in viscera, brain, lungs, and liver. The metabolites identified were free fluphenazine and its sulfoxide as well as [15]. The brain contained only free fluphenazine [14]. Interestingly, the percentage inhibition of CAR decreased as the brain levels of [14] decreased. Over half of the dose was excreted in the feces suggesting that resorption is an important route of elimination. Recovery of urinary metabolites amounted to 22%. Pharmacological half-life correlated well with the presence of radioactivity in the animal. The half-life was determined to be 10-11 days. Observation of the injection site after administration of [^{14}C]fluphenazine enanthate in the dog revealed radioactivity still present at the site after 21 days [132]. Since the excretion half-life of [15] was shorter for unformulated versus formulated drug given intravenously, it was decided that biological disposition was partially rate-limited due to release of highly lipophilic [15] from the oily vehicle rather than to excretion. Intramuscular injection of [15] (which exists as a viscous liquid) without any vehicle was also found to possess slow-release characteristics. The ester was hydrolyzed to free fluphenazine when incubated in dog plasma. Like the rat, a majority of the radioactivity was excreted via the bile.

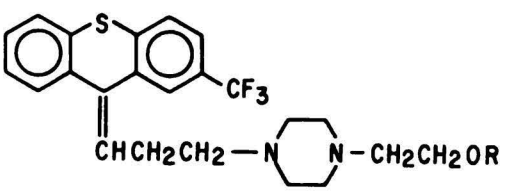
Clinical observations with chronic schizophrenics in remission indicated that adequate control was possible with doses of [15] ranging from 25-100 mg per month [133].

Fluphenazine decanoate [16] has received attention, clinically, similar to that of [15]. The decanoate ester was found to extend duration of activity to about 4 weeks with significantly fewer extrapyrimidal side effects than seen with [15] [134]. Further, resocialization of patients treated with [16] was such that their family, professional, and social adaptation significantly improved. Chronic inpatient schizophrenics known to deactivate chlorpromazine via oral administration showed marked clinical improvement when given [16] intramuscularly [118]. Based on the Brief Psychiatric Rating Scale, improvement was significant at $p < 0.05$. In another study with 212 chronic schizophrenics, the use of this depot neuroleptic reduced hospital readmissions from 191 to 50 and hospital stays from 8713 to 1335 days [135].

2. Thioxanthenes

Flupenthixol

Flupenthixol (2-trifluoromethyl-(3-[1-(2-hydroxyethyl)-4-piperazinyl] propylidene)-9-thioxanthene) [17] is an orally effective thioxanthene neuroleptic that has found wide acceptance in psychiatry. As is true for many orally administered neuroleptic agents, the frequency of dosing to maintain therapeutic levels is often accompanied by side effects, primarily extrapyramidal in nature. Sedation is also common. Again these shortcomings can be circumvented by changing the route of administration and the chemical form administered. The flupenthixol derivative used is the decanoate ester [18], and the route is intramuscular and this combination has met with wide acceptance in the psychiatric community. Nymark et al. [136] compared duration of action and incidence of side effects of the dihydrochloride salt, the free base, and the decanoate ester of [17] in mice, rats, and dogs. Flupenthixol, because of its asymmetry in the ring carbon, can exist in two stereoisomeric forms. The α-isomer is more active than the β-form [137] and this distinction should be noted when interpreting results from various studies. Inhibition of CAR in the rat using various chemical forms of [17] intramuscularly and orally at several dose levels is summarized in Table 13. Prolongation of neuroleptic effect was equally well demonstrated in the dog antiapomorphine model where activity was apparent for 3 weeks. Observation of duration of the same effect of [18] in the rat, however, was considerably shorter, lasting only 4 days. Again, such results point out the danger of relying on one species for evaluation of drug activity in animal models [138]. Administered in an oil vehicle, [18] provides therapeutic neuroleptic concentrations with a delayed onset of action having prolonged duration that is devoid of toxic side effects. The metabolism and excretion of [18] has been investigated in rats and dogs



R	
H	[17]
$-C(CH_2)_8CH_3$ (O)	[18]

TABLE 13. Duration of Inhibition of Conditioned Avoidance Response in Rats after Administration of Various Preparations of α-Flupenthixol

Preparation	Dose (mg/kg)	Route[a]	Duration (days)
[17], 2HCl, aqueous	2.5	p.o.	1-2
	5.0		1-2
[17] base in oil	3.75	i.m.	7-8
	7.5		11-12
[18] in oil	2.5	i.m.	7-8
	5.0		15-16
	10		22-23
	20		29

[a]p.o., peroral; i.m., intramuscular.
Source: Reproduced with permission from Ref. 136.

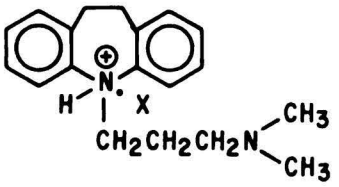
[139]. The ester was released intact from the depot and subsequently hydrolyzed affording bioactive [17]. It was postulated that [18] penetrated the blood-brain barrier and then hydrolyzed to [17], unlike fluphenazine enanthate which appears to enter the brain as fluphenazine [117]. Detection of maximum blood level concentrations of ^{3}H-labeled [18] were seen in dogs 7 days postadministration. In rats, the maximum was reached in 24 hr, the difference being attributed to more rapid rate of release and more efficient ester hydrolysis. Metabolites included traces of unchanged [18] and its sulfoxide as well as [17] and its sulfoxide. The presence of both the α and β isomers was demonstrated. Clinical pharmacokinetic studies [140] were undertaken in 10 patients using ^{3}H-labeled [17] (oral) and [18] (intramuscular). Oral [17] peak concentrations were evident in 3-8 hr after dosing. Maximum intramuscular values were observed 11-17 days postinjection with plateau values averaging 2-3 weeks in duration. Cerebral spinal fluid levels were 29-55% of the corresponding serum values. Steady-state serum levels after injection were more than twice the oral peak levels after single dose administration. The fact that metabolism occurs in the intestinal wall during absorption probably contributes to lower oral bioavailability. A single injection of [18] given every 2 weeks is equivalent to an oral 1 mg dose of [17] administered three times daily. A comparative pharmacokinetic study in animals and man further substantiated the

depot effect of [18] [141]. Peak blood levels were seen after injection at 6 hr in rats, 7 days in dogs, and 11-17 days in humans. Plateau levels were found only in humans, reflecting the more rapid metabolism and excretion in lower mammalian species. Good correlation between pharmacological and clinical response was demonstrated.

3. Dibenzazepines

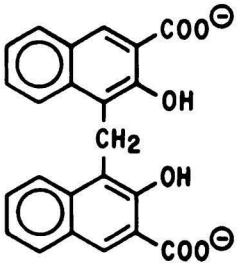
Imipramine

Imipramine (5-[3-dimethylaminopropyl]-10,11-dihydro-5H-dibenz[b,f] azepine) [19] is the original tricyclic antidepressant of the dibenzazepine family useful in treating endogenous and reactive depression [142]. The depot form of [19] is the pamoate salt [20] which has been successfully used once a day by the oral route. Wilson et al. [143] successfully used [20] in the treatment of depression in 40 patients and side effects were greatly diminished over those of [19]. Depressive symptomatology was improved significantly in male versus female subjects. A double-blind study in 43 patients with primary depression indicated that one 75 mg daily dose of [20] was equivalent to three 25 mg doses of [19] [144]. While not dramatic in terms of duration, administration of [20] was viewed as important in reducing patient compliance dilemmas. Other investigators have confirmed the relevance of once-a-day dosing of [20] and its positive consequences in patient well-being [145].



X

Cl [19]

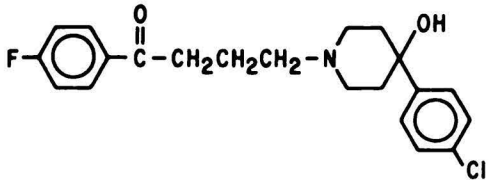


[20]

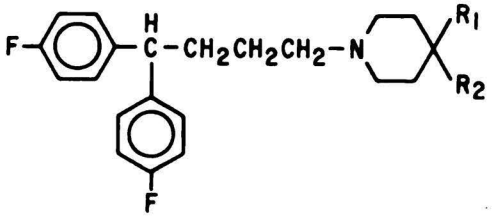
4. Diphenylbutylpyridines and Phenylbutylpyridines

Haloperidol and pimozide

Haloperidol (4-[4-(p-chlorophenyl)-4-hydroxypiperidino]-4'-fluorobutyrophenone) [21] is a potent, orally active central nervous system depressant, sedative, and tranquilizer. It is well absorbed and peak plasma levels are seen within 2-6 hr after administration [146]. Excretion is slow, with trace amounts being detectable in plasma for several weeks. Comparative pharmacology studies of [21] and pimozide (1-(1-[4,4-bis(p-fluorophenyl)butyl]-4-piperidyl)-2-benzimidazolinone) [22] in several species indicated that the latter was much less toxic and of longer duration than the former [147]. It is almost equipotent when given orally or by injection and its oral effective duration is about 24 hr, outlasting the duration of chlorpromazine by several hours.



[21]



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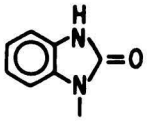
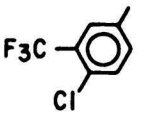
H	
OH	

[22]

[23]

A structural analog of [22], penfluridol (4-[4-chloro-α,α,α-trifluoro-m-tolyl]-1-[4,4-bis(p-fluorophenyl)butyl]-4-piperidinol) [23] has been found to possess duration of activity approaching 1 week [148]. Its onset of action is gradual and smooth, peak activity being reached in 24-48 hr. This lack of "burst effect" reduces toxic side effects seen with many other neuroleptic agents. Comparative durations of activity (antiapomorphine) are shown for [21], [22], and [23] in Figure 18. The effective dose of drug in mg/kg that prevents vomiting in 50% of the dogs injected subcutaneously with a standard dose of apomorphine HCl is the ED_{50} value; ED_{50}^{t} is the ED_{50} t hours after oral administration, and ED_{50}^{min} is the lowest effective value in mg/kg [148]. Table 14 summarizes relevant activity and duration parameters for a wide variety of chemically related neuroleptic agents.

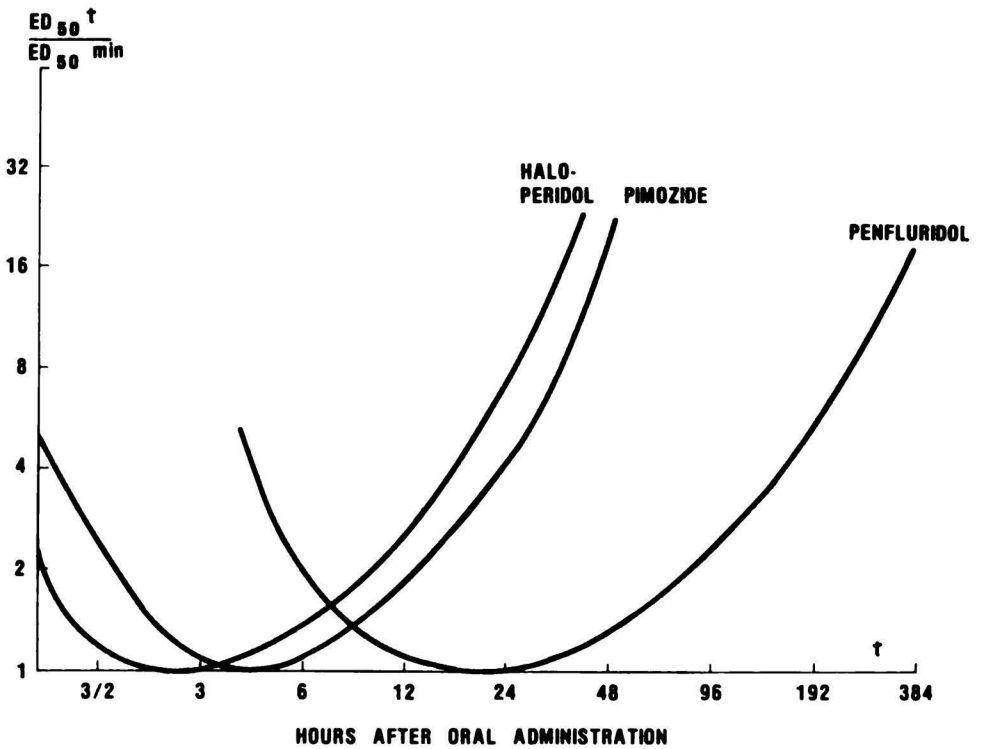


Fig. 18. Antiapomorphine test in dogs. Ratio (ED_{50} at t hours after administration)/(lowest ED_{50}) versus t for haloperidol, pimozide and penfluridol. (Reproduced with permission from Ref. 148.)

TABLE 14. Antiapomorphine Test in Dogs.[a]

Generic names	ED_{50}^{min} (mg/kg)	Peak effect (hr)	Onset (hr)	Duration (hr)
Droperidol	0.0036	1.2	0.4	3.3
Benperidol	0.0013	2.8	0.8	8.1
Trifluperazine	0.47	1.4	0.4	8.6
Pipamperone	1.48	2.1	<0.3	8.6
Thioperazine	0.11	3.1	0.6	8.7
Trifluperidol	0.067	1.8	<0.3	9.1
Spiperone	0.0014	4.1	1.4	9.2
Perphenazine	0.35	2.4	0.4	9.5
Fluphenazine	0.10	4.1	1.2	11
Prochlorperazine	2.03	1.8	0.3	13
Haloperidol	0.027	3.4	0.7	19
Pimozide	0.018	4.2	1.1	24
Penfluridol	0.023	24	4.2	165

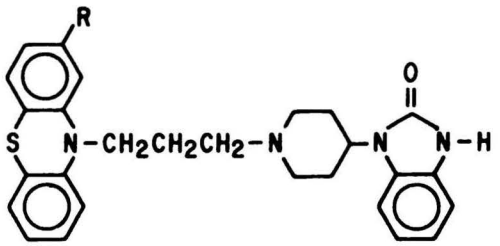
[a]Lowest ED_{50} values in mg/kg (ED_{50}^{min}) and time in hours after oral administration at which these ED_{50}^{min} occur (peak effect) derive from the minimum of a fitted polynominal function. Onset and duration in hours are measured at four times the ED_{50}^{min} (intersection with the polynominal function when ED_{50}^{min} is multiplied by a factor of four).
Source: Reproduced with permission from Ref. 148.

Chemically combining the phenothiazine and benzimidazolinone nuclei into one molecule produced several nontoxic neuroleptic agents of relatively long duration [149]. Efficacy and duration results of selected compounds in this series are listed in Table 15.

TABLE 15. Efficacy and Duration of Selected Neuroleptics

Compound of example	Antiapomorphine test in dogs (oral)		Amphetamine antagonism in rats (oral)[a]
	ED_{50} (mg/kg)	Duration (hr)	ED_{50} (mg/kg)
[24]	0.07	50	0.63
[25]	0.06	80	2.5
[26]	0.06	55	0.16

Source: Ref. 149.
[a]Duration does not appear in table source.



R	
H	[24]
CF_3	[25]
Cl	[26]

C. β-Lactam Antibiotics

1. General

Sustained antibacterial activity has long been recognized as a valuable and necessary consideration in the treatment of many acute disease states caused by a variety of pathogenic and nonpathogenic bacteria. The basis for rational therapy in such disease states has been disputed. The use of plasma or serum drug concentrations has been widely employed as an objective means for the evaluation of safe and efficacious drug therapy. In many cases, however, the locus of infection resides in tissues, and eradication is based on bactericidal or bacteristatic drug concentration at this

site. Since sampling of drug at such sites within the body can often be done only with great difficulty, attempts have been made to correlate drug concentration in blood with antibacterial response. Such efforts are based on the premise that a steady-state equilibrium exists between free drug concentration in blood and tissue fluid at the site of activity. Mattila [150] has discussed several factors that may be important in the verification of this premise.

1. Analytical methodology must be sufficient to accurately determine drug levels in various body fluids.
2. The effects of the drug should be reversible.
3. The nature of the bioactive species should be known, i.e., parent drug, metabolite, etc.
4. Drug pharmacokinetics should be evaluated.
5. Quantitation of the drugs' effect is necessary.
6. An appreciation of idiosyncratic behavior with the drug is desirable.

Ercoli et al. [151], in a study of the duration of activity of penicillin G in the rat, found that duration varied with the absolute weight of the animal, i.e., larger rats produced longer durations of drug in the blood. In 30 rats infected 2-6 hr after disappearance of drug from the blood, 10 survived the observation period of 21 days. It was thought that this "chemotherapeutic effectiveness" was present 4-8 hr after disappearance of drug from blood and that an additive effect after repeated dosing was operative. Other reasons put forth to explain this phenomenon were (1) induction of the immune response in the host, (2) influence of the treatment schedule [152], (3) the presence of penicillin in the microorganism after excretion by the host, and (4) the persistence of therapeutic penicillin levels in tissue, although undetectable in blood. Eagle et al. [153] also suggested that the penicillin-damaged organisms were disposed of by host defense mechanisms after the drug itself had been excreted. Further, Weinstein and co-workers [154] found penicillin present in fibrin clots for significant intervals after it was unassayable in plasma. This finding may be significant because of the known abundance of cavities, abscesses, and densely fibrotic areas that are a usual consequence of such infectious diseases. Slow diffusion of penicillin from such areas may account for prolonged activity. Eagle aptly summarized in Figure 19 the dilemma of the clinical investigator in attempting to determine the factors that hinder or enhance the rational use of sustained action antibacterial chemotherapy [155].

Thus, from many of these early speculations evolved the idea of the use of chemically modified forms of antibiotic to provide long-acting preparations of these important drugs.

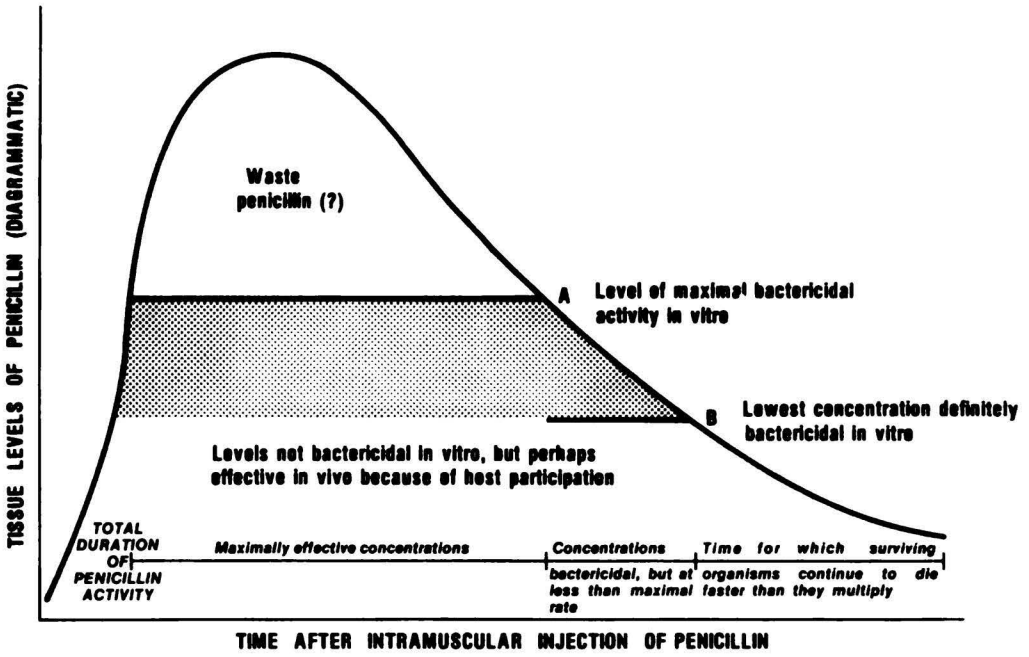


Fig. 19. A diagrammatic representation of the tissue concentrations of penicillin after its intramuscular injection in aqueous solution, considered in relation to the bactericidal activity of these concentrations. Penicillin levels A and B correspond to the following: A is that which is maximally effective in vitro, and B is the lowest concentration which effects a net reduction in vitro in the number of viable organisms. (Reproduced with permission from Ref. 155.)

2. Penicillins

a. Salts

One of the earliest salts tested in human subjects was procaine penicillin G [156]. Administration of a 1 ml suspension of salt in cottonseed oil afforded therapeutic blood levels of penicillin G for up to 24 hr. The duration of activity was dose-related as seen with subjects receiving a 3 ml dose who had significant blood levels of drug for at least 36 hr. A 4 ml dose, however, afforded significant 48 hr blood levels. This procaine penicillin salt has been widely used as a repository form of penicillin via the parenteral route of administration.

The early 1950s saw the introduction of a long-acting, nearly tasteless, and stable salt of penicillin G (N,N'-dibenzylethylenediamine

dipenicillin G; DBED penicillin) useful by both the oral and intramuscular routes of administration [157-159]. It was felt that the low aqueous solubility of this salt (0.15 mg/ml at 23°C) would enhance its formulation stability as well as reduce the objectionable bitterness that is characteristic of penicillin G and procaine penicillin G. Further, rate-limited dissolution at the injection site would substantially enhance the depot effect. Stability studies revealed negligible loss of potency of an aqueous suspension (287,000 units/ml) stored at 25°C for 1 year.

Initial trials in rabbits indicated adequate blood levels of penicillin for durations of 15 days. Blood level studies in humans given a single 300,000 unit intramuscular dose of DBED penicillin afforded average blood levels of 0.018 units/ml for up to 144 hr. One subject exhibited penicillin blood levels at 264 hr [158]. A single oral dose (2,500,000 units in 4 ml) in aqueous suspension yielded average serum levels of 0.67 units/ml at 240 hr [158]. The exceptionally prolonged serum levels of this salt proved useful in the eradication of penicillin-sensitive organisms responsible for syphilis, gonorrhea, and streptococcal infections [160-162].

The effect of dose of DBED penicillin on duration of activity was studied by Welch et al. [159]. A majority of subjects receiving a 300,000 unit intramuscular dose maintained blood levels for 10 days, compared to 17 days for a 600,000 unit dose. A combination dose of 300,000 units of DBED penicillin and 300,000 units of procaine penicillin G produced penicillin blood levels seven times greater than DBED penicillin alone at the 1 hr sampling time, about four times greater at 4 hr, and equivalent levels after 24 hr. Blood levels persisted in most subjects for 11 days. Procaine penicillin G in this study acted as a readily available short-duration form of penicillin while DBED penicillin maintained longer-term penicillin levels. Orally, 200,000 unit tablets of DBED penicillin G produced significant blood levels for 8 hr in ambulatory adult male subjects. Approximately 25% of the subjects exhibited penicillin blood levels ranging from 0.018 to 0.068 units/ml at 12 hr postadministration. Oral administration of an aqueous suspension (300,000 units/ml) produced results similar to that of crystalline potassium penicillin and procaine penicillin.

Concern about the possible toxicity of N,N'-dibenzylethylenediamine (DBED) and DBED penicillin led Seifter and co-workers [163] to study these entities in a variety of mammalian species including rats, mice, rabbits, and dogs. It was found that DBED dihydrochloride was about 1.5 times more toxic than procaine hydrochloride (intragastric dosing in the mouse), and DBED penicillin was of equivalent toxicity (low) to procaine penicillin, due perhaps to slow absorption from the site of administration, which allows for adequate metabolism and excretion [163]. Oral administration of the DBED and procaine salts to fasted dogs caused vomiting and salivation at doses of 50 mg/kg.

Since both procaine hydrochloride and DBED dihydrochloride are local anesthetics, it would appear reasonable to assume that such salts of penicillin might circumvent or reduce local irritation or pain at the injection site. Injection of DBED penicillin aqueous suspension into the hind limb muscle of the rabbit disclosed minor inflammatory response but left large crystalline deposits that resulted in histiocytic granulation tissue in 1-2 weeks. Such large deposits may account for the slow absorption of drug from the injection site which contributes to the long duration of DBED penicillin. Procaine penicillin produced a similar inflammatory response, focal tissue necrosis, and scar tissue. An investigation further elaborating on the disposition of DBED and DBED penicillin G from the intramuscular injection site was undertaken by Walkenstein et al. [164]. They found that (^{14}C-methylene)-labeled DBED was transported from the site almost quantitatively after 8 hr while some penicillin G salt was still evident after 3 days. A subsequent study [165], using ^{14}C-labeled DBED penicillin G and [^{14}C]-potassium penicillin G, indicated the potassium salt to be rapidly transported from the site within 12 hr whereas about 15% of the DBED salt remained after 3 days (Figure 20).

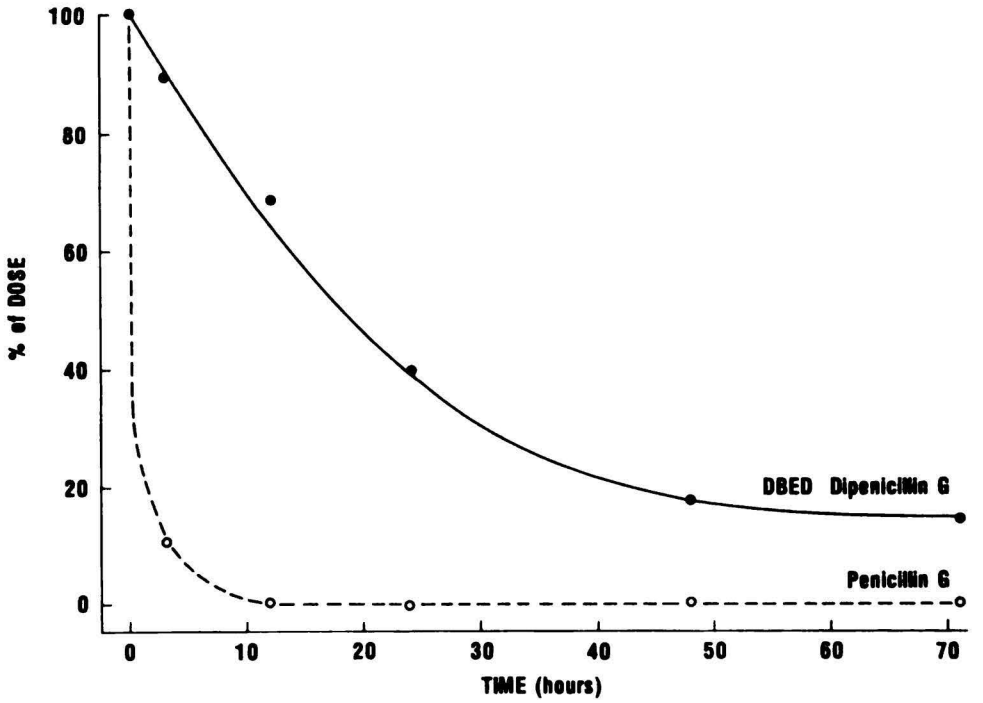
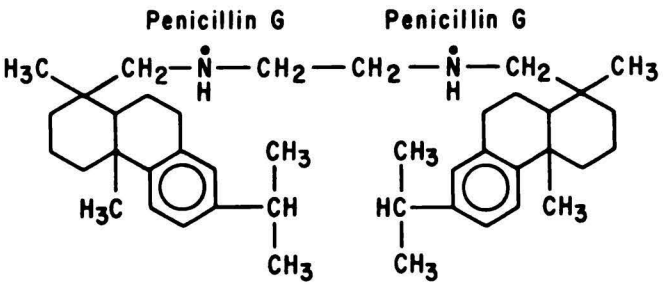


Fig. 20. Rate of disappearance of labeled carbon of DBED [^{14}C]dipenicillin G and [^{14}C]penicillin G from intramuscular injection site. (Reproduced with permission from Ref. 164; copyright by S. Karger AG, Basel.)

Long-acting DBED salts of ampicillin (α-aminobenzylpenicillin) and methylene-ampicillin have also been prepared [166].

An unusual diamine salt of penicillin G, N,N'-bis-(dehydroabietyl)-ethylenediamine dipenicillin G (hydrabamine penicillin G) [27], was found to possess properties similar to DBED penicillin G [167,168].

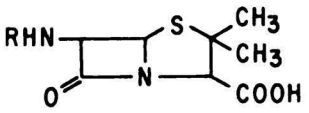


[27]

b. Analogs and derivatives

The design and synthesis of semisynthetic penicillins, i.e., analogs and prodrug amide and ester derivatives, provides an excellent means of generating new long-acting forms of these antibiotics. They differ from salts in that such chemical modifications may result in altering properties of the antibiotic other than duration of activity. For example, analog formation at the 6-amino group of 6-aminopenicillanic acid may result in compounds having differences in potency, spectrum of activity, and toxicity, as well as duration. Comparisons are difficult since one can never compare a large series of analogs with a standard reference compound. Prodrugs, however, normally revert to a parent molecule by virtue of enzymatic and/or pH effects in vivo and provide a means for comparison of a given property with a standard or parent compound. It is still possible, however, to synthesize analogs and make comparisons to a standard compound that is chemically similar to the analog series. Differences in potency or toxicity could possibly be minimized by adjustment of the dose administered. It would then be reasonable to make rough comparisons in, for example, duration of activity.

One such study approximating the above situation was undertaken with a series of α-phenoxyalkylpenicillin analogs in which certain characteristics of the different analogs were found to vary rather consistently as a function of the length and configuration of the alkyl side chain [169]. It was found that the serum penicillin concentration increased with an increase in alkyl chain length. Significant differences were seen at the 4 hr and 6 hr sampling times with all analogs ([29]-[34]) compared to phenoxymethylpenicillin (Penicillin V, [28], Table 16).



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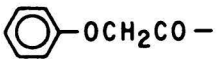
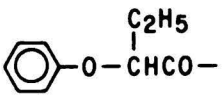
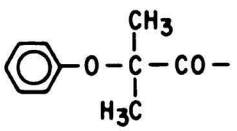
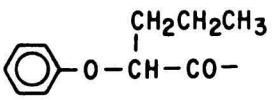
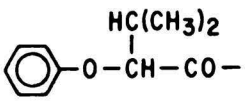
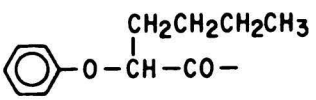
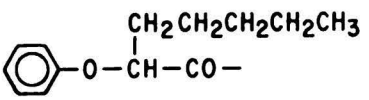
	phenoxymethyl	[28]
	phenoxypropyl	[29]
	phenoxyisopropyl	[30]
	phenoxybutyl	[31]
	phenoxyisobutyl	[32]
	phenoxyamyl	[33]
	phenoxyhexyl	[34]

The synthesis and evaluation of C_3 amide derivatives of penicillins G and V prompted Yurchenko et al. [170] to speculate on the depot effect observed with these derivatives in mice. Comparisons of both duration and potency of DBED penicillin G with the 1,2-benzisothiazol-3(2H)-one 1,1-dioxide amide derivatives of penicillin G and penicillin V [35] and [36], respectively, revealed significant differences between DBED penicillin G and [35] and [36]. With high doses, all three depot forms protected mice for 16 days prior to infection with Streptococcus pyogenes C203. The ED_{50} values (effective dose required to cure 50% of the infected animals) for [35] and [36] were lower than those for DBED penicillin G, indicating a greater potency of the two amide derivatives on a weight-for-weight basis. A

TABLE 16. Serum Concentrations after Oral Administration of 0.3 g of Seven Phenoxyalkylpenicillins

Hours after oral dose	Serum concentration (μg/ml) ± mean error of mean[a]						
	Phenoxy-methyl	Phenoxy-propyl	Phenoxy-isopropyl	Phenoxy-butyl	Phenoxy-isobutyl	Phenoxy-amyl	Phenoxy-hexyl
1	2.09 + 0.22 - 0.20	3.02 + 0.51 - 0.44	5.40 + 0.59 - 0.53	6.69 + 1.10 - 0.95	7.07 + 0.73 - 0.66	8.32 + 1.08 - 0.96	10.13 + 1.34 - 1.18
2	0.46 + 0.08 - 0.07	1.19 + 0.32 - 0.25	1.52 + 0.37 - 0.30	2.86 + 0.42 - 0.36	2.23 + 0.51 - 0.42	2.01 + 0.21 - 0.19	3.41 + 0.62 - 0.53
4	0.08 + 0.02 - 0.01	0.35 + 0.09 - 0.07	0.70 + 0.10 - 0.09	0.97 + 0.21 - 0.17	0.66 + 0.17 - 0.14	0.37 + 0.07 - 0.06	1.10 + 0.12 - 0.11
6	0.02 + 0.001 - 0.002	0.08 + 0.01 - 0.01	0.17 + 0.04 - 0.03	0.23 + 0.06 - 0.05	0.11 + 0.03 - 0.02	0.07 + 0.02 - 0.02	0.31 + 0.05 - 0.04

[a]Means calculated after logarithmic treatment of the single values.

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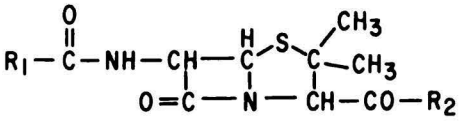
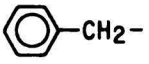
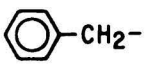
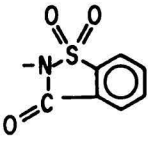
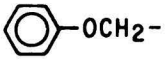
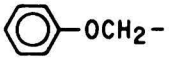
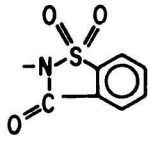
reflection of the depot activity of [35] and [36] is apparent at 8, 12, and 16 days post-treatment. Smaller ED_{50} values were necessary for protection of the infected mice. These findings are summarized in Table 17. Similar results were noted when the infecting organism was Staphylococcus aureus Smith.



R_1	R_2	Penicillin
	OH	Penicillin G
		[35]
	OH	Penicillin V
		[36]

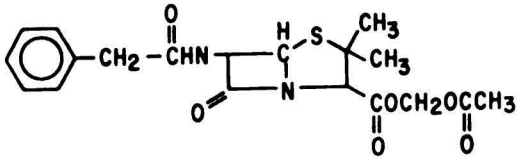
While DBED penicillin G, being a salt, merely dissociates to release bioactive penicillin G, the amides constitute a more complicated situation. It was thought by Yurchenco et al. [170] that these derivatives might be ingested by wandering macrophages and subsequently taken up by sessile macrophages of the reticuloendothelial system. Eventually, enzymatic hydrolysis of the amide releases the active species. This example is illustrative of some of the complexities involved in attempting to rationalize mechanisms for depot bioavailability. In reality, perhaps several interdependent mechanisms (chemical, physical, physiological, enzymatic) are responsible for the ultimate sustained action properties seen with any given drug molecule.

TABLE 17. Comparative Duration of Activity and Antibacterial Effectiveness of DBED Penicillin G, [35], and [36] in CD-1 Mice Infected with Streptococcus pyogenes

Post-treatment infection time (days)	ED_{50}, mean ± SD[a] (mg/kg)		
	DBED penicillin G	[35]	[36]
0	7.4 ± 0.9	2.1 ± 0.5	2.0 ± 0.3
2	135.6 ± 6.1	25.2 ± 1.6	19.3 ± 0.0
4	390.0 ± 108.3	39.8 ± 4.2	30.5 ± 4.5
8	629.4 ± 162.2	94.4 ± 27.2	47.1 ± 12.2
12	750.0 ± b	286.1 ± 75.6	190.6 ± 9.4
16	1,004.4 ± 265.5	540.0 ± 115.5	266.1 ± 6.1
20	1,451.9 ± 33.3	1,600.0 ± 0.0	>800.0
Controls, % survivors mean ± SD	3.6 ± 1.5	2.5 ± 1.6	2.4 ± 1.5

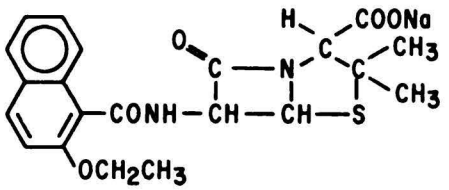
[a]SD = standard deviation.
[b]ED_{50} range: 533.0 to 800.0.
Source: Reproduced with permission from Ref. 170; copyright by S. Karger AG, Basel.

The longer duration of blood levels of the 3-acetoxymethyl ester of benzylpenicillin (penamecillin) [37] in the dog was attributed to slow enzymatic hydrolysis by nonspecific esterases [171]. While potassium benzylpenicillin and potassium phenoxymethylpenicillin exhibited therapeutic blood levels within 30 min, peak levels (0.1 μg/ml) of [37] were reached in an average of 60 min and maintained for 8 hr. The release of benzylpenicillin from [37] provides an excellent rationale for use of the prodrug approach in the design of long-acting penicillin derivatives. Similar studies have confirmed the validity of this approach with other penicillins [172,173].



[37]

An effort to enhance duration of activity by the synthesis of a metabolically resistant penicillin analog has been attempted. Walkenstein et al. [174] determined the distribution, metabolism, excretion, and duration of activity of nafcillin (6-[2-ethoxy-1-naphthamido] penicillanic acid) [38] in dogs. This penicillinase-resistant penicillin analog distributed widely in all major organs and tissue within 30 min of intramuscular administration. Peak plasma levels (8.2 μg/ml) were seen within 15 min, and after 8 days levels of drug or metabolite amounted to about 1 μg/ml. Orally, peak levels were 1.4 μg/ml at 2 hr and 0.25 μg/ml on day 8. Resistance to hydrolysis is probably due to the steric bulk of the substituted naphthyl group on the side chain of the molecule and may account for its rather long residence time in vivo. Repeated doses of 500 mg every 6 hr, however, must be administered to maintain therapeutic efficacy [175].

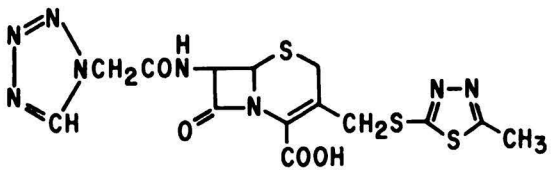


[38]

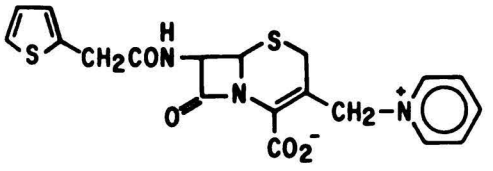
3. Cephalosporins

The cephalosporins, a class of antibiotics related chemically to the penicillins, have virtually been ignored in relation to structure-activity studies designed to improve sustained bioactivity. A comparative serum level study by Cahn et al. [176] revealed significant differences between three of the most frequently prescribed cephalosporins. Cefazolin [39] produced significantly higher serum concentrations than either cephaloridine [40] or cephalothin [41] in normal volunteers. Cefazolin levels were about two to three times those of cephaloridine at all dose (intramuscular) levels (Figure 21). The differences were significant. The levels of [39] were of longer duration due probably to more efficient absorption from the injection site. With the limited number of compounds evaluated, however, structure-activity relationships are not apparent.

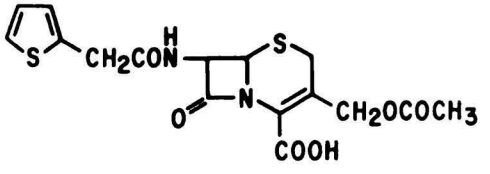
Examination of other cephalosporin analogs [177] and prodrug esters [178, 179] have yielded candidates possessing improved depot bioavailability characteristics when compared to standard compounds.



[39]



[40]



[41]

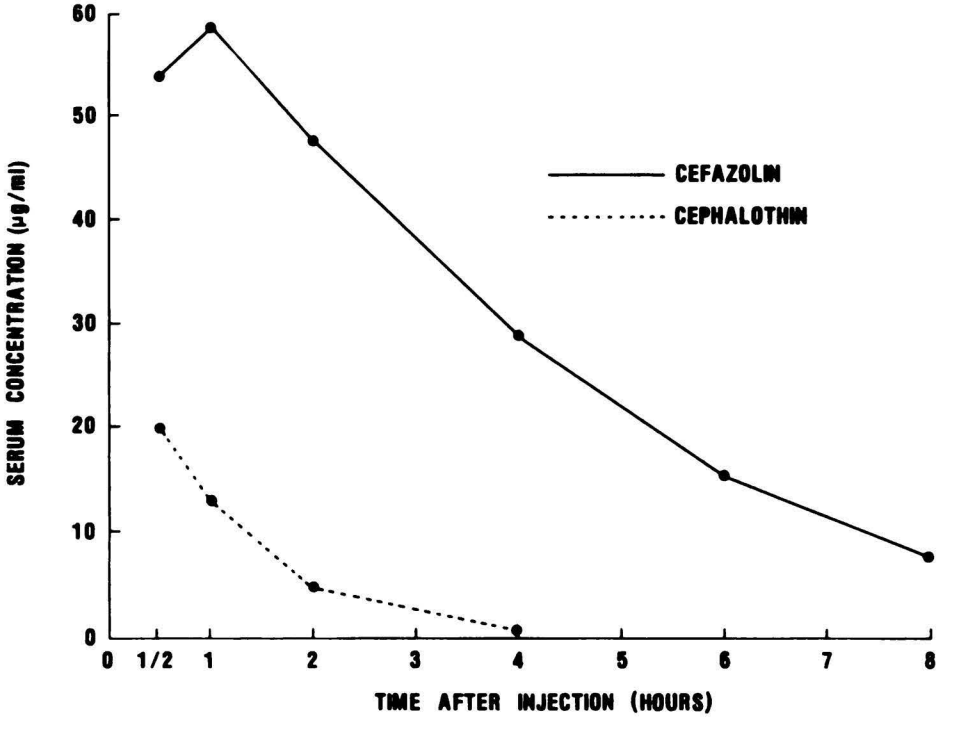


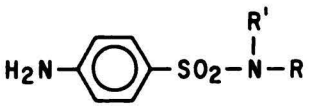
Fig. 21. Average serum concentration of cefazolin and cephalothin following a single 1 g intramuscular injection. (Reproduced with permission from Ref. 176.)

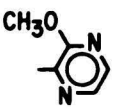
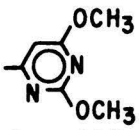
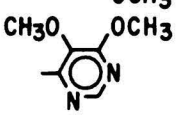
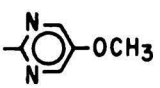
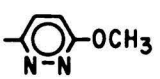
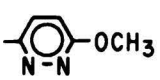
D. Sulfanilamides

The sulfanilamides are bacteriostatic agents that competitively inhibit bacterial synthesis of folic acid from p-aminobenzoic acid. Their use has been unquestioned as relatively safe agents in a variety of situations including short- and long-term gram-positive and gram-negative bacterial infections as well as parasitic infections such as malaria and tuberculosis. Although the benefits of sulfanilamide and its derivatives have long been known, the use of long-acting forms is fairly new [180]. Relatively minor chemical modifications within the molecule can result in profound biological changes and thus it is difficult to predict structure-activity relationships. However, some physicochemical and pharmacokinetic parameters that have been studied to elucidate both potency and duration of action include solubility (ionized and un-ionized species), pK_a, protein binding, volume of distribution, half-life, and metabolism and excretion phenomena [180-184]. The long-acting sulfanilamides appear to be so due to low renal clearance (extensive tubular reabsorption) which in turn is related to the lipophilic character of the molecule. This high lipid solubility enhances absorption through the cell membranes of the tubular epithelia [185]. Therapeutically useful sulfanilamides are thought to be antibacterial in the range of 5-15 mg % [185]. The long-acting sulfas are usually administered once daily while ultralong-acting sulfas are efficacious at doses of 1-1.5 g per week [180]. The remainder of this discussion will be limited to a treatment of selected examples of long- and ultralong-acting sulfanilamides. The choice of structures is intentional, highlighting the isomeric similarities among several important drugs, some in current use. The relationship between physicochemical properties and duration of activity will be examined where appropriate.

1. Sulfamethoxypyrazine

This interesting sulfa drug (2-sulfanilamido-3-methoxypyrazine) [42] was thoroughly studied by Wiegand et al. [183]. The plasma half-life was about 67 hr with a volume of distribution (unbound drug) of approximately 0.73 liter/kg. Very little metabolism (acetylation) occurs, 95% being unchanged drug. This lack of polar metabolite formation probably accounts for its long duration of activity. The drug is not highly bound to plasma protein and absorption and elimination follow first-order kinetics. It was felt that once-a-day maintenance therapy would be sufficient after administration of an initial loading dose of four to five times the maintenance dose. The question of the relevance of drug plasma levels correlative with clinical efficacy was explored by assessing these levels against in vitro plasma antibacterial activity [184]. Plasma samples were tested by a broth-dilution procedure using a Proteus vulgaris strain as indicator organism. Chemical assays were also run. The results are graphically illustrated in Figure 22.



R	R'		
	H	Sulfamethoxypyrazine	[42]
	H	Sulfadimethoxine	[43]
	H	N'-(5,6-dimethoxy-4-pyrimidinyl)-sulfanilamide	[44]
	H	Sulfamethoxydiazine	[45]
	H	Sulfamethoxypyridazine	[46]
		N'-Acetylsulfamethoxy-pyridazine	[47]

Antibacterial titers paralleled serum concentration of [42] over a span of 96 hr at the high dose. Lower doses indicated less correlation at times to 48 hr but with less variance at 72-96 hr. The plasma concentrations exhibited were considered to be adequate to exert antibacterial activity yet low enough to be safe. Other studies have confirmed the use of [42] as an effective long-acting sulfanilamide in dermatovenerology [186] and urology [187].

2. Sulfadimethoxine

Sulfadimethoxine (N'-[2,6-dimethoxy-4-pyrimidinyl] sulfanilamide) [43], in early studies, fulfilled the criteria for a potentially successful long-acting sulfa drug, namely, antibacterial bioactivity and low toxicity in animal test systems, rapid and efficient absorption upon oral administration,

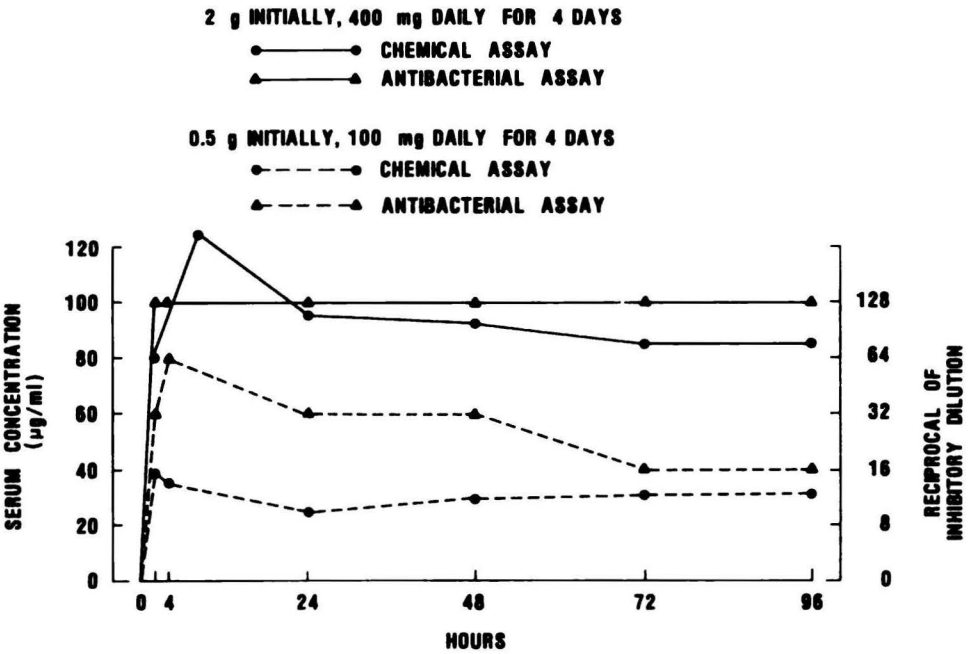


Fig. 22. Concentration of [42] in plasma and plasma antibacterial titer. (Reproduced with permission from Ref. 184.)

and slow metabolism and excretion in humans. In a six-patient crossover study, Boger [188] compared the duration of free (unconjugated) sulfanilamide plasma levels of sulfadimethoxine with another long-acting sulfa, sulfamethoxypyridazine [46]. Also included in the study was the medium-duration sulfaethidole (sulfaethylthiadiazole). Both [43] and [46] produced plasma sulfa concentrations above 10 mg % within 4-6 hr and maintained them at this level for over 24 hr. One dissimilarity between these two drugs is their metabolic and excretion patterns; [43] is excreted as conjugate in 15-25% of the dose whereas 40-50% of [46] is excreted in conjugated form. Their plasma level profiles, however, are very similar. The variance noted in excretion patterns is perhaps due to minor resorption differences in each molecule.

An interesting clinical study [189] in pediatric subjects with [43], [46], and [47] (a palatable pediatric form) revealed significant differences in the concentration versus time curves between [43] and [46] on one hand and [47] on the other (Figure 23). Blood levels of [47] from 0 to 24 hr are approximately half of those of [43] and [46], indicating poor absorption and/or rapid

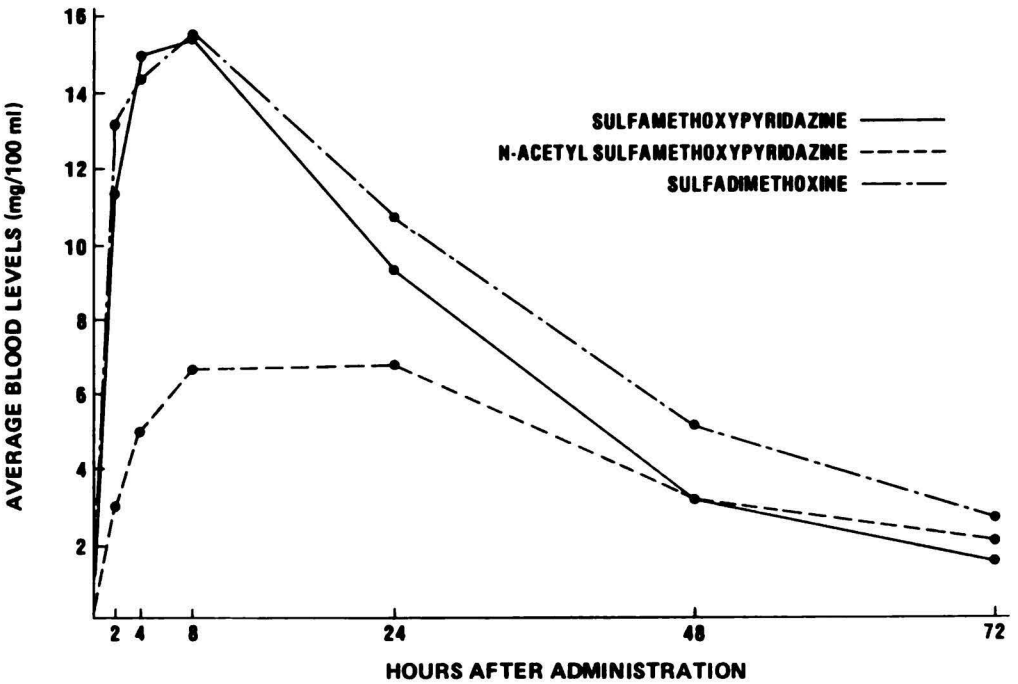


Fig. 23. Comparison of sulfonamide blood levels of three sulfonamides after oral administration of a single dose, 50 mg/kg. (Reproduced with permission from Ref. 189.)

excretion. Differences from 48 to 72 hr are not significant. The acetyl derivative could be viewed as a conjugated (acetylated) form of [46] and this might account for more rapid excretion. Daily dosing, as is customary even for long-acting sulfanilamides in clinical practice, affords adequate blood levels of [47] (Figure 24). Virtually identical results are also obtained with [43] and [46] on a daily dosage regimen.

Follow-up studies on larger patient populations verified the use of [43] as an effective long-acting sulfanilamide [190, 191].

3. Sulfamethoxydiazine

The absorption, excretion and renal clearance of sulfamethoxydiazine (N'-[5-methoxy-2-pyrimidinyl] sulfanilamide) [45] was investigated in a study of 12 subjects [192]. Results obtained paralleled those of previously discussed sulfas. Single doses of 1-2 g produced peak serum levels of 8-13.4 mg % in 6-8 hours. Half of this amount was still present after 48 hr. Only

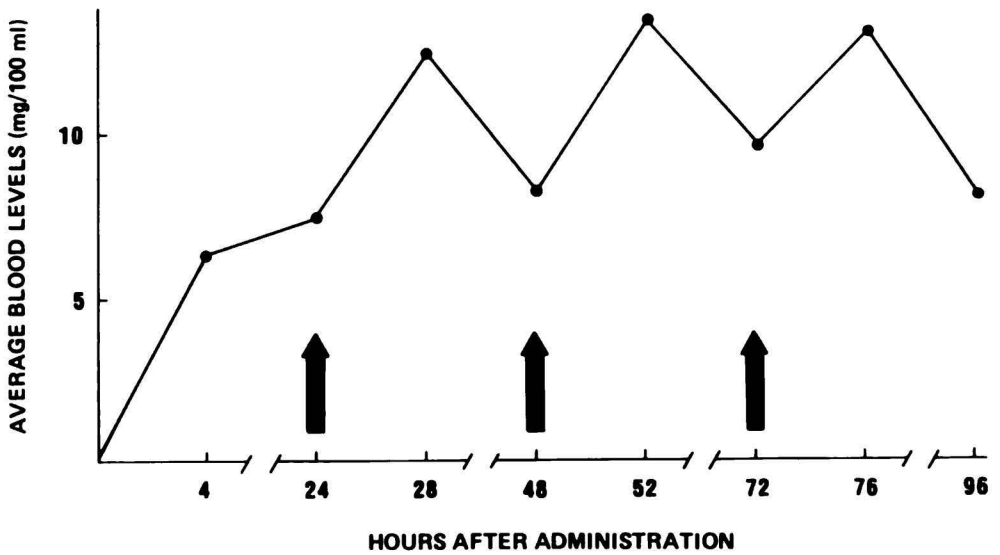


Fig. 24. N'-acetyl sulfamethoxypyridazine blood levels obtained 4 and 24 hr after a daily dose, 25 mg/kg, given for four consecutive days. (Reproduced with permission from Ref. 189.)

5% of the drug was in the N^4-acetylated form, which is important since this acetylated conjugate does not inhibit bacterial growth. Higher plasma levels were produced with higher loading doses. Therapeutic levels of 6-14 mg % were produced with a loading dose of 1-2 g followed by daily maintenance doses of 500 mg. Renal clearance of the drug was low, reflecting the long duration of activity of [45]. About 25-50% is bound to protein and obviously is not cleared in the bound form, thus the duration is further enhanced [193].

Crystalluria (presence of drug crystals in the urine due to precipitation in the kidney tubules) is an undesirable consequence of sulfa drugs that are poorly soluble, and this phenomenon becomes an important consideration in the design of long-acting sulfanilamides. Nelson has written extensively on the subject [194-197] and found that crystalluria can be prevented if such factors as urine minimum flow rate, drug solubility, and excretion rate are considered. These factors are related according to Eq. (2):

$$\text{Minimum flow rate} = \frac{\text{excretion rate}}{\text{solubility}} \tag{2}$$

The solubility of N^4-acetylated [45] (the species most likely to precipitate) in urine at pH 5.7 and 7 was found to be 10.5 and 78.4 mg %, respectively. Compound [45] itself has twice the solubility, in pH 5.7 urine, of the acetylated conjugate and would not be expected to precipitate. The N^4-glucuronide of [45] also posed no solubility problem. Based on urine flow rates of 60 ml/hr and excretion rates (acetylated drug) of 0.8-6.2 mg/hr, a 1.5 g oral dose of [45] should cause no crystalluria problems.

4. Sulfamethoxypyridazine

Sulfamethoxypyridazine (N'-[6-methoxy-3-pyridazinyl] sulfanilamide) [46] was designed as a long-acting sulfanilamide while its N^1-acetyl prodrug derivative [47] serves as a tasteless palatable form for the same use. The prodrug is hydrolyzed in the gastrointestinal tract and absorbed as [46]. The fact that blood levels of [47] are low (see Figure 23) may be a reflection of the incomplete gut hydrolysis and subsequent absorption of this chemical species. The criteria that make [46] a good drug are good gastrointestinal absorption, low N^4-acetylation, wide tissue distribution including penetration into the brain, very slow urinary excretion, high urine solubility, and adequate potency. A single dose of 4 g of [46] gave peak blood levels of 15 mg % and persisted in appreciable concentrations for 105 hr [198]. Conjugate formation averaged less than 15%. Little or no drug diffused into red blood cells. Free drug was cleared from plasma at about 1.8 ml per minute. The N^4-acetylated conjugate cleared at a rate of 19.8 ml of plasma per minute or about 10 times as fast as [46]. Other workers have found average peak levels greater than 20 mg % upon administration of a single 2 g dose [199]. Drug was measurable in plasma for as long as 168 hr. This excellent long-acting antibacterial sulfanilamide has been successfully used in pyelonephritis [200], pediatric meningitis [201], and a variety of persistent chronic infections [202].

An ultralong-acting sulfanilamide, [44], was studied in babies and older children and found to have a half-life of 93.3 hr [205]. This long duration was attributed to high tubular reabsorption and high protein binding. The possibility also exists that the excretory systems in such young subjects are not fully developed and persistent levels of drug remain for inordinately long periods of time.

Several reviews have dealt, in part, with long-acting antibacterial sulfanilamides [180, 181, 202-204].

E. Local Anesthetics

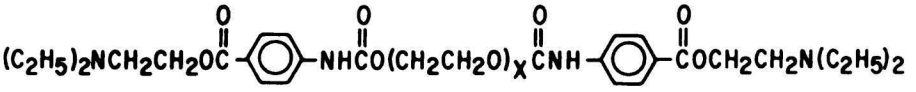
The value of this class of drugs is attributable to their ability to produce localized insensitivity to pain by the blocking of impulse transmission in pain-conveying nerves. Most attempts to enhance the duration of activity

of local anesthetics have been incidental. Primary emphasis has centered around the improvement of potency. In many instances, however, an increase in potency is accompanied by a disproportionate increase in toxicity. While structure-potency relationships are quite well defined, structure-duration correlations are only vaguely understood.

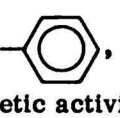
The relationship between chemical structure and physicochemical properties of local anesthetics is important because these properties affect absorption, tissue distribution, and metabolism, and ultimately will influence onset and duration of action, potency, and toxicity. The majority of these agents are esters or amides and their duration of effect is basically a function of the enzymatic stability of these covalent linkages. Hydrolysis results in loss of local anesthetic activity. A classic study of the in vitro human serum hydrolysis rates of a wide variety of esters and amides was undertaken by Levine and Clark [206]. In general, they found amide bonds to be extremely stable and not hydrolyzed by serum. Ester-containing local anesthetics are enzymatically labile but can be stabilized by (1) steric hindrance, i.e., substitution at the α position of the alcohol or carboxylic acid, (2) increasing the chain length of the alcoholic group, and (3) increasing the steric bulk on the terminal nitrogen portion of the alcohol group. Kalow [207] examined the relationship between chemical structure and relative serum hydrolysis rates for several local anesthetics. The unhindered ester-type anesthetics were the most rapidly hydrolyzed while the more hindered esters and the amide derivatives were slowly hydrolyzed. Kalow postulated the existence of an esterase that was responsible for the hydrolysis of procaine and he presented evidence for the identity of "procainesterase." The duration of local anesthetic activity in rabbits was found to be roughly correlated with serum levels of procainesterase activity [208].

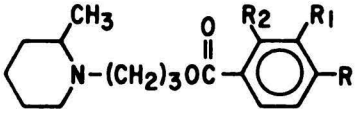
1. Ester-type Local Anesthetics

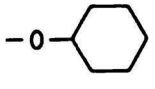
Polyethylene glycol (PEG) carbamate derivatives of procaine were synthesized and shown to possess duration of effect that was related to the type of PEG used [209]. Thus, in the Bulbring and Wajda anesthesia test, procaine endures for 30-45 min while procaine-PEG 400 [48] and procaine-PEG [49] last for 60-100 min and 180 min, respectively. Molecular weight may not be the most important factor in determining duration of procaine effect as the lower polymer exhibited longer effect. The authors suggested that other factors such as lipophilicity and permeability behavior may also play an important role. Macromolecular dextran-procaine derivatives also exhibit long durations of anesthesia [210].



X	
10	[48]
4	[49]

Experiments by Rose et al. [211] were designed to elucidate the factors responsible for duration of anesthetic effects in a series of substituted piperidine derivatives. Duration of anesthesia was measured by the activity on the rabbit sciatic nerve. For a series of substituted benzoates of 3-(2-methylpiperidino) propanol [50], extension of alkyl chain length, especially R, prolongs activity with no concomitant increase in toxicity or irritation. When R =  , toxicity is very pronounced; when R and R_1 are -OH, local anesthetic activity is lost. Substitution of the 3-(2-methylpiperidino)propyl moiety with esters other than benzoate or with carbamates, ethers, or ketones produced mostly inactive derivatives. Those that showed activity were toxic. Two of the most promising derivatives [51] and [52] were further tested in six human subjects by intracutaneous injection on the volar surface of the forearm. Duration of anesthesia was evaluated by stroking the area of injection with a wisp of cotton. Complete anesthesia lasted for 31 min with [51] and 58 min with [52].



R	R_1	R_2	
H	H	H	[50]
$-O(CH_2)_3CH_3$	H	H	[51]
	H	H	[52]

The relationship of duration of anesthesia with certain physicochemical properties in a series of isoanesthesine derivatives was examined by Büchi and Perlia [212]. In a group of variously substituted ethyl and propyl

p-aminobenzoates, the degree of local anesthetic activity increased with increasing substitution (Table 18) and could reasonably be correlated with aqueous solubility and lipophilicity.

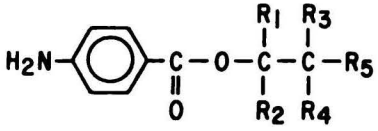
2. Amide-type Local Anesthetics

The more successful modifications of the procaine molecule have been the amides and the sterically-hindered amides, commonly referred to as the xylidides. The amide-type local anesthetic duration of activity is due to the resistance of the amide bond to enzymatic hydrolysis. Further, substitution at positions ortho to the amide linkage greatly enhance resistance to hydrolysis. The ortho-substituent used in virtually all local anesthetics of this type is the methyl group. Table 19 lists some of the common amide-type local anesthetics that have been used in past and current clinical practice.

Åkerman et al. [213] studied the rates of hydrolysis of several amino-acylanilides similar in structure to xylocaine and related this parameter to toxicity and nerve-blocking effect. With local anesthetics, toxicity as well as duration was diminished or eliminated by rapid metabolism (hydrolysis). Mouse liver amidases were utilized as the enzyme source and toxicity LD_{50} was evaluated in male albino mice. Nerve-blocking effect (anesthesia) was determined on isolated sciatic frog nerve and was expressed in relation to xylocaine [54]. Table 20 summarizes the findings in this study. Hydrolysis rate decreased with an increase in number of substituents near the amide bond, particularly on the carbon α to the carboxyl group. In general, the degree of amide hydrolysis correlated well with toxicity. Cumulative toxicity was lower for the more rapidly hydrolyzed derivatives.

Several local anesthetics have sufficiently long durations of activity but, because of their duration, high concentrations accumulate and toxic symptoms and tissue irritation result. The evaluation of etidocaine [55] showed promise as a relatively nontoxic, long-acting anesthetic. Pharmacokinetic studies in five volunteers indicated a favorable therapeutic ratio [214]. The mean blood clearance of [55] was 1.24 ± 0.2 liter min^{-1} and, coupled with a slow net biphasic absorption pattern, toxicity was minimal and duration relatively sustained. Sensory and motor onset for [55] were dose-independent while duration of anesthesia was dose-related [215]. Duration of activity varied from 190 to 319 min in the 100 to 300 mg range (sensory), respectively, and 148 to 261 min in the same dose range for motor anesthesia. Systemic and local side effects were minimal or absent. Other investigators have found that onset of action is somewhat dose-related with amide-type local anesthetics [216]. Thus, a threefold increase in dose of prilocaine [58] reduced onset of anesthesia by greater than 50% (Figure 25). A less dramatic difference was seen with [56]. This study also confirmed the dose-duration of effect relationship and illustrated the role of the chemical nature of the derivative in demonstrating individual variabilities within this relationship.

TABLE 18. Physicochemical Properties of the Isoanesthesine Series

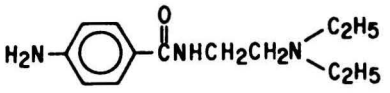
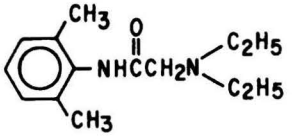
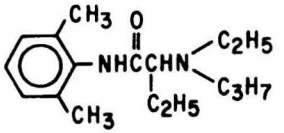
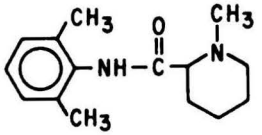
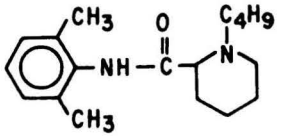


R_1	R_2	R_3	R_4	R_5	pK_a	Water solubility, pH = 7.4 (mM/liter)	Distribution coefficient	Surface activity[a] at: 0.25 mM	0.125 mM	0.03 mM	Average duration of total anesthesia (min)
H	H	H	H	H	2.65	4.20	38	1.2	--	--	12.3
H	H	CH_3	H	H	2.68	1.67	147	2.1	1.6	--	14
H	H	CH_3	CH_3	H	2.64	0.98	482	7.9	3.3	--	14.8
H	H	CH_3	CH_3	CH_3	2.64	0.27	1499	15.0	10.4	--	16.2
CH_3	H	H	H	H	2.66	2.06	111	1.6	0.7	--	21
CH_3	H	CH_3	H	H	2.65	1.28	388	5.9	2.0	--	23
CH_3	H	CH_3	CH_3	H	2.67	0.34	1270	13.1	7.6	0.8	18
CH_3	H	CH_3	CH_3	CH_3	2.63	0.24	3258	--	16.1	5.0	25.2
CH_3	CH_3	H	H	H	2.74	0.51	345	4.1	1.5	0.4	27
CH_3	CH_3	CH_3	H	H	2.70	0.53	1111	11.9	7.2	1.7	30.8
CH_3	CH_3	CH_3	CH_3	H	2.72	0.34	2780	--	15.1	4.9	35.5
CH_3	CH_3	CH_3	CH_3	CH_3	2.73	0.03	7780	--	--	10.8	60.2

Source: Ref. 212.

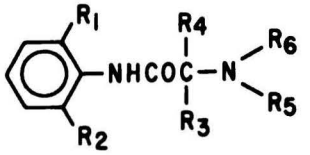
[a]Units do not appear in table source.

TABLE 19. Common Amide-type Local Anesthetics with Long Duration

Chemical structure	Common name	Chemical name	Structure number
	Procaine amide	p-Amino-N-(2-diethylaminoethyl)benzamide	[53]
	Xylocaine	2-Diethylamino-2',6'-acetoxylidide	[54]
	Etidocaine	N-Ethylpropylamino-2-butyroxy-2',6'-xylidide	[55]
	Mepivacaine	1-Methyl-2',6'-pipecoloxylidide	[56]
	Bupivacaine	dℓ-1-Butylpipecoloxylidide	[57]

	Prilocaine	2-(Propylamino)-O-propionotoluidide	[58]
	Dibucaine	2-Butoxy-N-(2-diethylaminoethyl)cinchoninamide	[59]

TABLE 20. Enzymatic Hydrolysis, Acute Toxicity, and Nerve-blocking Action of a Series of Aminoacylanilide Derivatives



R_1	R_2	R_3	R_4	R_5	R_6	Rate of hydrolysis by mouse liver homogenate (μmol g^{-1} min^{-1})[a]	Acute toxicity in mice, LD_{50} (mmol/kg)		Blocking action on nervous conduction in isolated frog sciatic nerve[b] (Lidocaine = 1.0)
							Intravenous	Subcutaneous	
CH_3	CH_3	H	H	H	CH_3	0.04	0.19	2.80	0.1
CH_3	CH_3	H	H	H	C_2H_5	0.12	0.22	1.44	0.2
CH_3	CH_3	H	H	H	C_3H_7(-n)	0.53	0.11	0.95	0.9
CH_3	CH_3	H	H	H	C_3H_7(-iso)	0.39	0.18	1.87	0.5
CH_3	CH_3	CH_3	H	H	C_2H_5	<0.01	0.13	1.71	0.7
CH_3	CH_3	CH_3	H	H	C_3H_7(-n)	0.02	0.06	1.00	1.0
CH_3	CH_3	H	H	C_2H_5	C_2H_5	<0.01	0.09	1.00	1.0
CH_3	CH_3	CH_3	H	CH_3	CH_3	<0.01	0.09	0.92	0.9
CH_3	CH_3	CH_3	H	C_2H_5	C_2H_5	< 0.01	0.06	0.81	1.1
CH_3	CH_3	H	$-(CH_2)_4-$		CH_3	< 0.01	0.10	0.95	0.6
CH_3	H	H	H	H	CH_3	0.24	0.35	4.71	0.1
CH_3	H	CH_3	H	H	C_2H_5	0.06	0.20	2.97	0.2
CH_3	H	CH_3	H	H	C_3H_7(-n)	0.07	0.16	2.67	0.6

CH_3	H	CH_3	H	H	C_3H_7(-iso)	0.01	0.17	2.84	0.5
CH_3	H	CH_3	H	H	C_4H_9(-n)	0.21	0.07	1.55	1.0
CH_3	H	CH_3	H	H	C_4H_9(-iso)	0.39	0.07	2.03	1.0
CH_3	H	CH_3	CH_3	H	C_3H_7(-n)	<0.01	0.09	2.88	0.9
CH_3	H	CH_3	CH_3	H	C_6H_{13}	<0.01	0.06	2.16	1.0
CH_3	H	H	H	C_2H_5	C_2H_5	0.08	0.14	5.84	0.2
CH_3	H	CH_3	H	C_2H_5	C_2H_5	0.05	0.15	4.17	0.7
H	H	CH_3	H	H	C_3H_7	0.20	0.17	1.90	0.6

[a]Incubation mixture: 0.5 ml of mouse liver homogenate (1:10 in 0.1 N tris buffer, pH 8.5) and 0.4 μmol of the tritiated compound. Final volume, 1.0 ml. The incubation was performed for 10 to 30 min at 37°C.
[b]Decrease in amplitude of action potential on incubation for 5 min with 5.0 mM solutions (Tasaki-Ringer, pH 7.4).
Source: Reproduced with permission from Ref. 213.

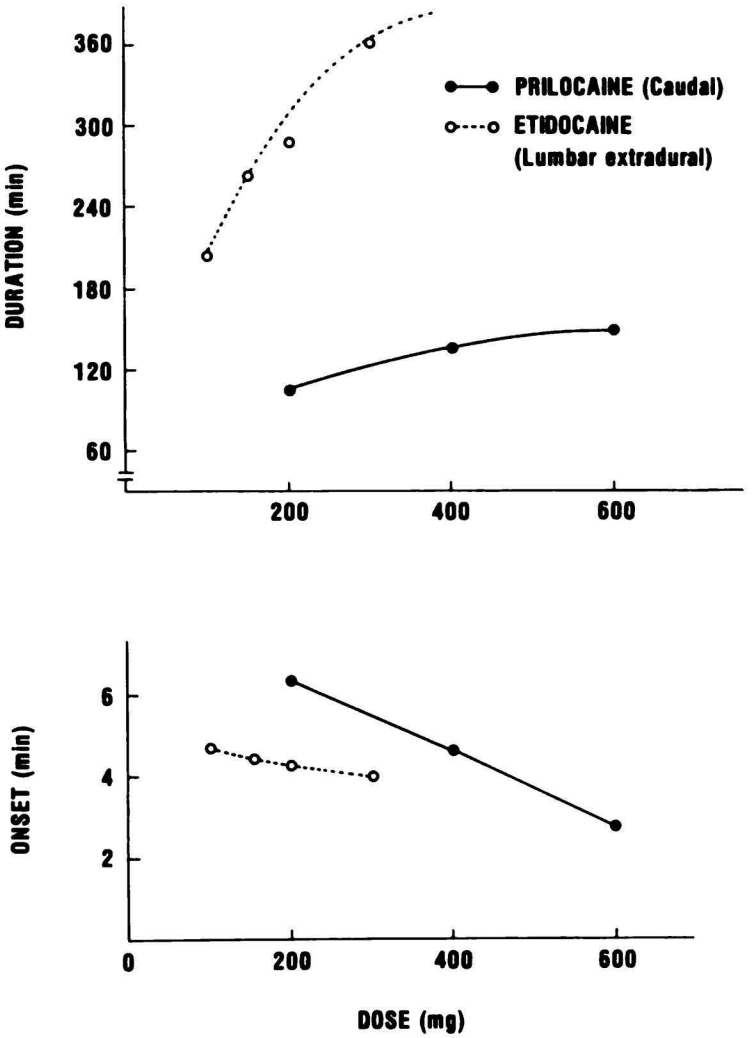


Fig. 25. Relationship between dose of prilocaine and etidocaine and the duration of sensory analgesia (above) and initial onset of sensory analgesia (below) following caudal or lumbar extradural administration. (Reproduced with permission from Ref. 216.)

Other variables are equally important in evaluating the duration and onset of different local anesthetic derivatives. Table 21 dramatically illustrates the effect of the site of administration of different derivatives as a function of onset and duration of action. These differences are ultimately a consequence of the chemical and physical properties of the individual local anesthetics.

TABLE 21. Variations in Onset and Duration of Anesthesia as a Function of Anesthetic Technique

Parameter	1% Xylocaine ulnar nerve block		0.5% Etidocaine brachial plexus block	
	Intraneural injection	Extraneural injection	Axillary	Supraclavicular
Sensory onset (min)	4.0 ± 1.2	3 ± 0.7	8–9	8.77
Sensory duration (min)	54.9 ± 9.0	165 ± 21.8	453 ± 66	572 ± 203

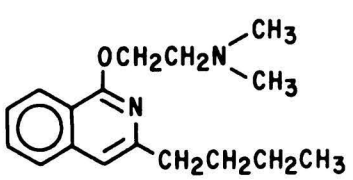
Source: Reproduced with permission from Ref. 216.

The metabolism [217], pharmacokinetics [218], and clinical evaluation [216] of the amide-type long-acting local anesthetics have been reviewed.

Various physicochemical properties and their relationship to potency and duration of anesthetic activity has been comprehensively treated by Büchi and Perlia [212].

3. Miscellaneous

A local anesthetic chemically resembling both procaine and dibucaine was found to combine the best features of both when tested topically in the rabbit [219]. This hybrid derivative was 3-butyl-1-(2-dimethylamino-ethoxy)isoquinoline [60] and was 10 times more potent and one-half as toxic as dibucaine [59]. Its duration of anesthesia was extremely long and varied with concentration (Table 22). The idea of combining what appear to be the essential pharmacophores of two or more drug molecules into a series of hybrid derivatives represents another successful chemical variation for the improvement of certain desirable drug characteristics.



[60]

TABLE 22. Local Anesthetic Activity after Topical Application to Rabbit Eyes

Compound	Number of tests	Concentration (%)	Duration of anesthesia (min)
Cocaine	10	1.0	18
Procaine	20	1.0	±2
Dibucaine	20	0.01	69
	20	0.001	None
[60]	20	0.1	514
	20	0.01	199
	20	0.001	75

Source: Reproduced with permission from Ref. 219; copyright 1951, The Williams and Wilkins Co., Baltimore.

F. Phenethylamines

The phenethylamine nucleus provides the chemical backbone for a variety of physiologically active amines such as amphetamine, ephedrine, dopamine, and their isomers and congeners. Many of these agents are administered orally but suffer from extremely low plasma and brain tissue levels and consequently short duration of activity. Metabolizing enzymes such as monoamine oxidase (MAO) present in the gastrointestinal tract wall deactivate amines before they reach their site of action. Further, such amines ionize and it is well known that, within limits, the degree of absorption is related to the concentration of undissociated molecules available at the absorption site. The lipophilicity of the resulting undissociated molecules obviously is important and also affects absorption and transport. Any attempts to enhance the duration of effect of phenethylamines should thus have a twofold purpose: (1) to enhance absorption and transport to the brain by the use of lipophilic derivatives and/or analogs and (2) to decrease premature metabolism by chemically protecting the molecule at metabolically vulnerable sites such as phenolic hydroxyl groups and amino groups. Early investigators in this area were Verbiscar and Abood who synthesized carbamate esters of phenethylamine and various isomers of amphetamine, ephedrine, and hydroxyamphetamine [220]. Radiotracer studies in mice with several carbamate esters of d-amphetamine revealed a delayed onset

and greater duration of effect. Transport of d-amphetamine across the blood-brain barrier was facilitated via these derivatives with subsequent hydrolysis in brain tissue. By selectively modifying the alcoholic portion of such carbamate esters, rate-controlled in vivo release of bioactive amine could be achieved to extend duration [221,222]. Several phenethylamines covalently linked to polymers were tested for extended duration [223]. The polymer backbones used were starch and polymethacrylic acid. The onset of action of N-methacryloylphenethylamine monomer after injection was rapid (2 min) while effects lasted longer than 6 hr. N-methacryloylephedrine showed activity for about 4 hr whereas its copolymer activity was evident up to 48 hr. The phenethylamine-starch polymer presented sedation in mice lasting 5-24 hr. Toxicity of the polymer adducts was generally lower than that of the parent compounds.

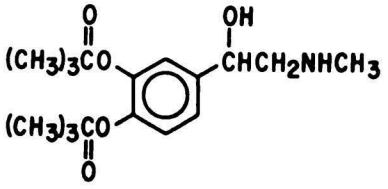
A series of isoproterenol [224] and epinephrine [225] esters exhibited significantly different half-lives of hydrolysis when incubated in serum and plasma of the horse, rabbit, and human (Table 23). Apparently, hydrolysis rate is not only dependent on the nature of the ester but on the species of mammal as well. While the half-lives of the derivatives vary only over a matter of seconds, they can still be considered to exhibit a significant prolonged duration over the parent drug molecule.

The bispivalate ester of epinephrine ([61]) has been examined in the treatment of glaucoma [226]. Glaucoma is the consequence of increased intraocular pressure (IOP) in the eye and epinephrine has been the traditional drug of choice. One of the problems inherent with the use of epinephrine is its short duration. The evaluation of [61] versus epinephrine in the rabbit produced the results shown in Figure 26. It is readily apparent that [61] affords a more consistent and sustained drop in IOP at a

TABLE 23. Hydrolysis Rates of Isoproterenol and Epinephrine Esters in Serums

Ester	Half-life (sec)		
	Horse	Rabbit	Human
3,4 Bisacetate	20	45	180
3,4 Bispropionate	50	90	240
3,4 Bisbutyrate	100	160	360
3,4 Bispivalate	600	720	1800

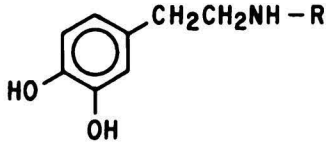
Source: Refs. 224 and 225.



[61]

lower and possibly safer dose than does epinephrine. The reasons for this consistency appear to be due to slower metabolism of [61] by catechol-o-methyl transferase (COMT), an enzyme that methylates the meta hydroxyl group in epinephrine. This metabolic step is dependent on the rate of hydrolysis of the 3-pivalate ester. Moreover, the increased lipophilicity of [61] may contribute to greater absorption and thus greater bioavailability allowing the use of smaller doses.

Dopamine ([62]) is another phenethylamine analog receiving increased attention in human disorders such as renal hypertension, renal blockade, congestive heart failure, and possibly other dysfunctions of the kidney. Its duration of effect is short, due to metabolic breakdown by MAO in the gut and to poor gastrointestinal absorption. Biel et al. [227] and Jones and coworkers [228] synthesized a large number of amino and hydroxy derivatives of [62] designed to be cleaved by enzymes indigenous to the body at such a rate as to produce therapeutic levels of drug for extended periods of time. Prolongation of vasodilator effect for 4-6 hr was accepted as a reasonably long duration. An amino acid derivative, N-L-isoleucyldopamine [63] was selected for further study. In vitro enzyme hydrolysis studies with amino-acylarylamidase (also present in renal tissue) demonstrated slow cleavage of the amide. Doses of 12 and 24 mg/kg of [63] administered intraduodenally to anesthetised dogs produced increases in renal blood flow for 155 and 172 min, respectively. Plasma contained both [62] and [63]. A minimum of systemic hemodynamic side effects were noted at the 12 mg/kg dose.



R

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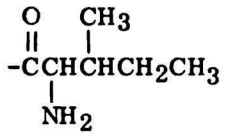
[62]



[63]

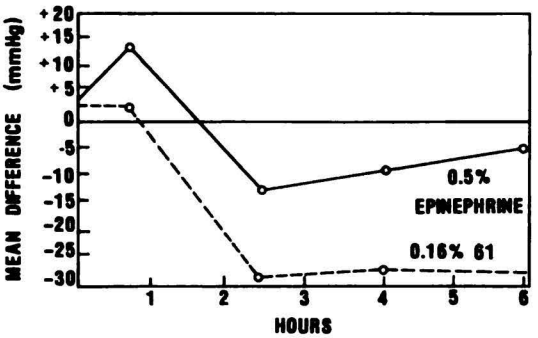


Fig. 26. Comparison of intraocular pressure effects in rabbits. (Reproduced with permission from Ref. 226.)

The species specificity of several dopamine amides was investigated by Minard and co-workers [229]. Since these derivatives were expected to be prolonged renal dilators, the presence of amidase enzymes in the kidney was considered critical for rate-limited hydrolysis. Table 24 outlines some interspecies differences and similarities between hydrolysis rates for several of these amides. There appear to be no obvious structure-hydrolysis rate correlations in this series although stereochemical (substrate:enzyme receptor fit) factors may be operative. Whatever subtle chemical differences are applicable, they seem to be consistent from species to species. As mentioned previously, of those dopamine amides tested in the dog, [63] showed the greatest promise as a potential renal vasodilator in human medicine. Studies of a similar nature and employing a similar approach to the production of long-acting vasodilators have been conducted with ^{14}C-labeled dopamine and N-alanyldopamine [230]. Casagrande and Ferrari synthesized several 3,4-diacyl dopamine derivatives that appeared to have good potential but they did not test them for potency or duration [231]. Alkyl esters of DL-dopa were reported in a similar manner [232].

G. Antimicobacterials

1. Antileprotic Agents

The toll taken in terms of human misery and suffering by leprosy and tuberculosis is inestimable. Both diseases are of long gestation and duration and are extremely refractory to complete eradication and clinical cure of the offending organisms. It is well known that the acid-fast bacilli *Mycobacterium tuberculosis* and *M. leprae* are the causative agents of tuberculosis and leprosy respectively.

Sulfones

The current drug of choice for leprosy is dapsone (diaminodiphenyl sulfone, DDS, 4,4'-sulfonyldianiline), [64]. Its duration of activity is relatively short (0.3 g orally twice weekly in humans) and, due to the chronic nature of this disease, repository forms of this drug have been sought [233].

A series of prodrug derivatives (Table 25) of dapsone was prepared by Elslager et al. [234] designed to hydrolyze in vivo by either enzymatic or nonenzymatic means. Several 4'-(N-acylsulfanilyl) anilides, in which the acyl group varied from hydrogen to palmityl, were found to exhibit a broad range of antileprotic depot bioactivity. The variety of half-lives of the prodrug derivatives was due to a combination of aqueous solubility at the injection site and relative degree of hydrolysis of the prodrug. Optimum depot activity was seen with acedapsone [66] at a solubility of 0.003 mg/ml. This derivative was slowly hydrolyzed by deacetylases [235]. Comparative data on several similar antileprotic prodrugs are listed in Table 26.

Several polymeric sulfones and sulfone Schiff bases were prepared to determine their repository antileprotic and antimalarial effect on the respective test organisms. Several exhibited extended bioactivity in mice inoculated with Plasmodium berghei (malaria) and M. leprae (leprosy). All compounds tested were active for longer than 8 weeks in mice and had half-lives ranging from 10 to 39 days in rats. The majority afforded protection intermediate between dapsone and acedapsone. Several of these including 4'-(N-benzylidenesulfanilyl) acetanilide [69], 4'-[N-(p-acetamidobenzylidene) sulfanilyl] acetamide [70], 4'[N-(3,5-dichlorosalicylidene) sulfanilyl] acetanilide [71], and several polymeric forms of the sulfones [72]-[74] are summarized in Table 27.

Several excellent reviews on the antileprotic sulfones have appeared [233,236-238].

2. Antitubercular Agents

Isoniazid

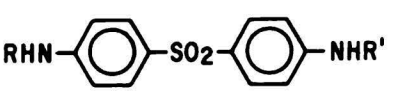
The use of isoniazid [75] as an antitubercular agent is well known [239, 240]. This drug, however, exhibits toxicity on repeated dosing and is characterized by peripheral and optic neuritis, central nervous system stimulation, and hepatitis. Several interesting derivatives of this agent have been made, and one showing both favorable lack of toxicity and acceptable level of therapeutic activity is 1,1'-methylenebis(2-isonicotinylhydrazine) [76]. Acute toxicity in mice was found to give an LD_{50} of 3900 mg/kg.

TABLE 24. Species Specificity of Renal Amidases

Amino acid moiety	Initial hydrolysis rates of amides of dopamine				
	Rat[a]	Dog[a]	Monkey[a]	Human[a]	Pig[b]
L-alanine[c]	1.000	1.000	1.000	1.000	1.000
L-phenylalanine	0.50	0.64	1.13	0.89	0.76
Glycine	0.37	0.18	0.22	0.26	0.56
L-serine	0.10	0.10	0.12	0.07	0.09
L-isoleucine	0.04	0.009	0.06	0.08	0.05

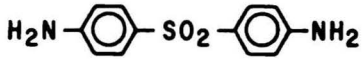
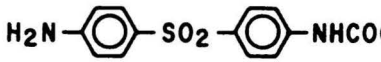
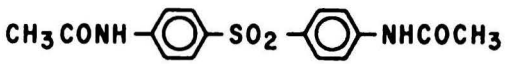
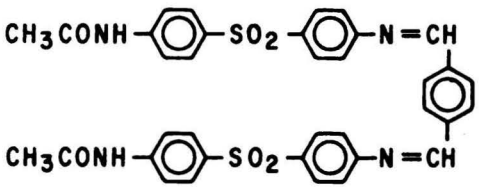
[a]Tissues were homogenized in 0.9% saline at a proportion of 1 g per 25 ml, centrifuged for 5 min at 26 g, and the amidase activity of the supernatant was measured at pH 6.9 with L-alanyldopamine as substrate.
[b]Crystalline porcine arylamidase.
[c]The initial hydrolysis rate with L-alanyldopamine was arbitrarily assigned a value of unity for each of the four species.
Source: Reproduced with permission from Ref. 229; copyright by Academic Press, Inc.

TABLE 25. Prodrug Derivatives of Dapsone



Name	R	R'	Other names	Structure number
Diaminodiphenyl sulfone	H	H	Dapsone, DDS	[64]
4'-Sulfanilylacetamide	H	$-COCH_3$	MADDS	[65]
4',4'''-Sulfonylbisacetanilide	$-COCH_3$	$-COCH_3$	Acedapsone, DADDS	[66]
4,4'-Diformamidodiphenylsulfone	-CHO	-CHO	Diformyldapsone, DFD	[67]

TABLE 26. Comparative Antimalarial, Antileprotic, and Metabolic Data on DDS, MADDS, DADDS, and PSBA[a]

Drug	Structure	Estimated weeks mice protected		Rats			
				Urinary Excretion			
		P. berghei	M. leprae	% Excreted in 30 days	Half-life (days)	Peak blood level (μg/ml)	Peak methemoglobin levels (g/100 ml)
DDS [64]		<1	2	57	9	13.8	3.9
MADDS [65]		3.5	-	50	32	1.3	1.2
DADDS [66]		12	>8	7	>200	0.2	0
PSBA[b] [68]		5-7	>8	40	55	0.4	0.2

[a]Drugs given in a single 400 mg/kg subcutaneous dose in benzyl benzoate castor oil (BBCO) or H_2O.
[b]4',4'''-[p-phenylenebis(methylidyneimino-p-phenylenesulfonyl)]bisacetanilide.
Source: Reproduced with permission from Ref. 236.

TABLE 27. Comparative Antimalarial, Antileprotic, and Metabolic Data on Other Sulfone Schiff Bases and Bioploymers[a]

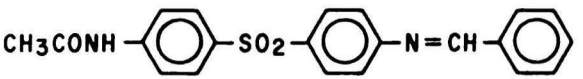
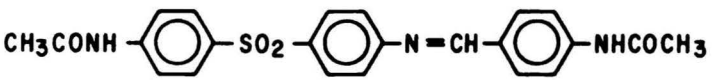
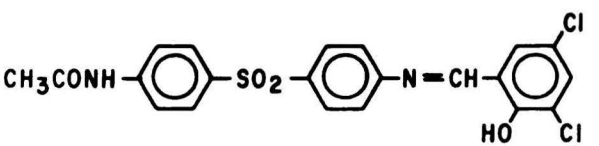
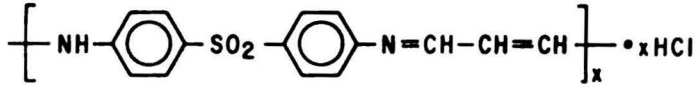
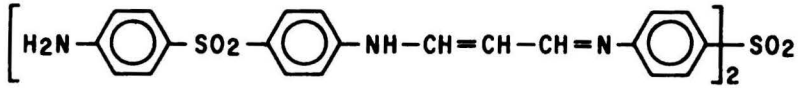
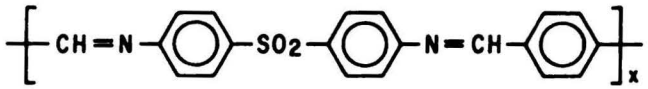
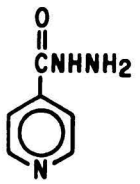
Drug	Structure	Estimated weeks mice protected		Rats			
				Urinary Excretion		Peak blood level (μg/ml)	Peak methemoglobin levels (g/100 ml)
		P. berghei	M. leprae	% Excreted in 30 days	Half-life (days)		
[69]		6	>8	44	27	--	--
[70]		9	>8	85	10	--	--
[71]		9	>8	31	35	0.7	1.0

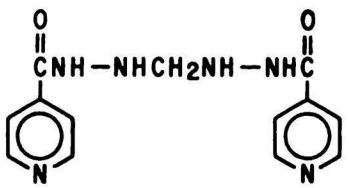
TABLE 27 (continued)

Drug	Structure	Estimated weeks mice protected: P. berghei	Estimated weeks mice protected: M. leprae	Rats: Urinary Excretion: % Excreted in 30 days	Rats: Urinary Excretion: Half-life (days)	Rats: Peak blood level (μg/ml)	Rats: Peak methemoglobin levels (g/100 ml)
[72]		8.5	>8	32	25	--	--
[73]		3.5	>8	34	39	--	--
[74]		4	>8	50	30	--	--

[a]Drugs given in a single 400 mg/kg subcutaneous dose.
Source: Reproduced with permission from Ref. 236.



[75]



[76]

The duration of protection of this relatively insoluble drug implanted in mice infected with strain H37Rv Mycobacterium tuberculosis was approximately 25 days. Table 28 shows the protective effect of [76] in relation to time of insertion of pellets prior to infection. Studies in human tuberculosis patients following oral administration of [75] and [76] indicated a lower peak concentration of [76] (probably due to slower absorption and/or hydrolysis while exhibiting a slower rate of excretion (Figure 27). Administration of [76] as a single dose of 10 mg/kg per day was adequate to maintain therapeutic concentrations of isoniazid (assayed as isonicotinic acid [243]) for at least 24 hr after the last dose and yet remain free of toxic symptoms. This appears to be a good example of maintaining therapeutic concentrations of a drug while preventing toxic symptoms as shown on Figure 27.

Another example illustrating the chemical depot approach to the prevention of toxic symptoms in antimicobacterial chemotherapy is the use of hinconstarch in pulmonary tuberculosis. Hinconstarch is the condensation product of equimolar amounts of isoniazid and p-aminobenzalthiocarbazone (p-ABT) with the aldehyde groups on periodate-oxidized starch [244]. Although p-ABT has been shown to be the most suitable adjuvant to isoniazid from a bacteriological standpoint [245], its toxicity prevents its widespread use for this purpose. It was hoped that covalently bonding both this adjuvant and the antitubercular agent to a carrier (starch) for slow release in vivo would overcome this toxicity problem. It was found that administration of 40-45 mg of hinconstarch per kilogram of body weight daily allowed therapeutic levels (>1.5 γ per ml) of bioactive agent to appear and yet remain free of gastrointestinal or renal toxicity [246]. A further study of the in vivo metabolic breakdown of hinconstarch produced the following urinary metabolites: acetyl isoniazid, isoniazid, isonicotinic acid, isonicotinyl glycine, diisonicotinyl hydrazine, and 5-p-acetamidophenyl-2-amino-1,3,4-thiadiazole [247, 248]. It thus appears that hinconstarch is an effective prodrug of isoniazid, slowly releasing nontoxic amounts of isoniazid after administration.

TABLE 28. Protective Effect of Pellets of [76] in Intravenous Infection[a] of Mice with Strain H37 Rv.

Insertion of pellets (days before infection)	Total observation (days)	Protection rate[b]	No. neg. cultures / No. cultures taken
0	25	9/9	5/9
3	24	8/9	5/8
7	28	5/10	1/10
14	35	3/10	1/10
21	42	0/10	1/9
42	63	0/9	0/8
Controls	24-25	0/20	1/20

[a]Infection: 0.5 ml of a 10^{-1} dilution of a 7-10 day-old culture in Dubos' medium. Treatment: 25 mg pellet implanted subcutaneously.
[b]Number of mice free of lesions/number of treated mice.
Source: Reproduced with permission from Ref. 241.

An excellent and comprehensive review on Antimycobacterial drugs has been written by Lewis and Shepherd [249].

H. Antimalarials

Malaria is a malady having the potential for debilitating and disabling vast segments of the world population. The disease is transmitted by several parasitic Plasmodium species including P. vivax, P. malariae, and P. falciparum. Treatment of the disease is long-term and resistance has been known to develop. Several classes of drugs are utilized in malarial chemotherapy and are broadly categorized by the following chemical types: 4- and 8-aminoquinolines, diaminoquinazolines, diaminopyrimidines, dihydrotriazines, biguanides, quinolinemethanols, and a variety of sulfonamides and sulfones. Since malaria requires treatment for extensive periods of time, many concerted efforts have been undertaken to develop depot or repository forms of antimalarial agents. Elslager [236] has set

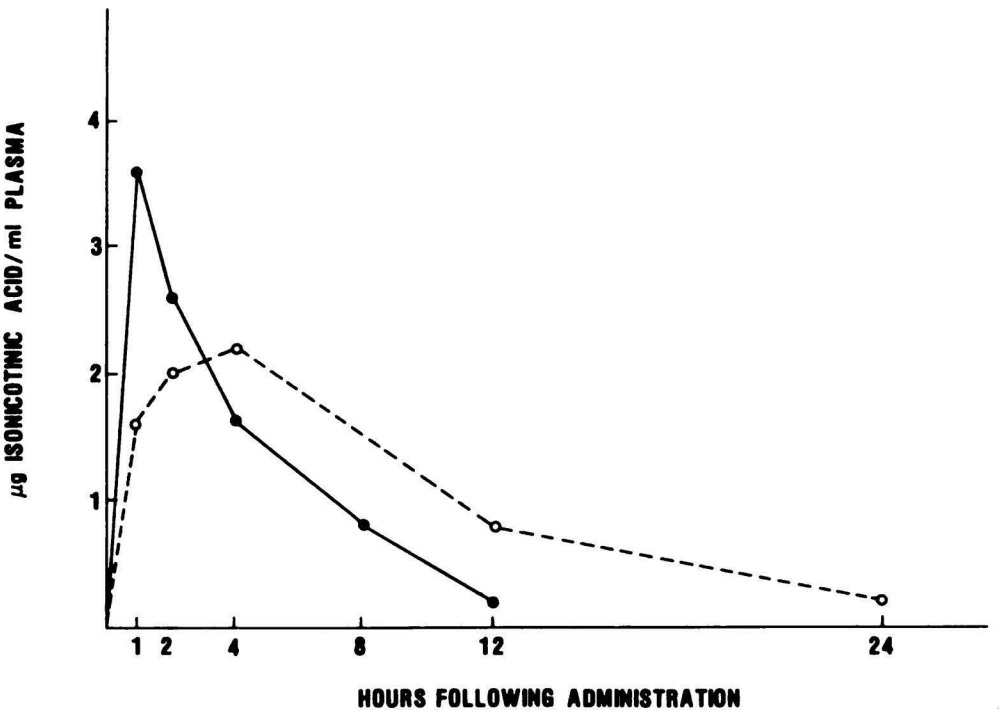


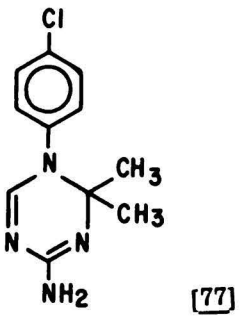
Fig. 27. Plasma concentrations in the same subject following oral administration of 200 mg each of [75] (solid line) and [76] (broken line). (Reproduced with permission from Ref. 242.)

down some features necessary for the design of effective depot antimalarial drugs: (1) Administration should be limited to a single dose with release of therapeutic drug concentrations over a prolonged period of time. (2) The drug should exhibit toxicity specific to the offending parasite. (3) Absorption and release from the site of administration should ideally follow zero-order kinetics. (4) Accumulation of drug should not occur after absorption; metabolism and excretion rates should roughly parallel absorption rate. (5) Irritation at the site of administration should be negligible. (6) Both normal and resistant strains of the parasite should be drug-susceptible.

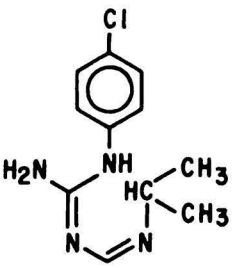
The variety and number of depot antimalarials is extensive and an effort will be made to only review some of the more prominent drugs.

1. Dihydrotriazines

The most prominent and extensively studied drug among this chemical class of substances is cycloguanil (4,6-diamino-1-[p-chlorophenyl]-1,2-dihydro-2,2-dimethyl-S-triazine) [77]. Interest in this drug derived from the fact that it is the deaminated cyclized metabolite of cycloguanide HCl [78]. Drug [77] is rapidly excreted and thus is less bioactive than [78]. A series of substituted benzoic and naphthoic acid salts of [77] provided the basis for evaluation of depot activity in mice inoculated with P. berghei [250]. The solubility of the salts in pH 7 phosphate buffer varied from 0.03 mg/ml (pamoate) to 7 mg/ml (2,4-dihydroxybenzoate). A rough correlation was seen between solubility and duration of activity (as measured by protection of 50% or more of the mice from P. berghei challenge). Cycloguanil pamoate protected for more than 8 weeks while other salts varying in solubility from 0.04 to 2.44 mg/ml afforded protection of 1-2 weeks. Substituted dibenzofuranote and binaphthoate salts proved to be toxic and of little therapeutic value.



[77]



[78]

The administration of 5 mg/kg of cycloguanil pamoate intramuscularly in human prisoner volunteers furnished protection for 8-12 months against subsequent challenges of P. vivax and P. falciparum [251-253]. Contacos and co-workers [254] found protection was adequate when urinary levels were 0.25 mg or greater. Duration of protection, utilizing such urinary excretion criteria, lasted for 8-9 months.

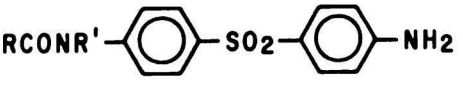
Studies with pyrimethamine pamoate salts in mice, similar to those described above, have been undertaken with similar results [250,255].

2. Sulfones

A number of sulfone derivatives, as well as possessing significant antileprotic activity, are antimalarial agents. The fact that such antimalarial drugs as diaminodiphenyl sulfone (DDS, [64]) are toxic and require frequent administration has probably precluded their use on a large scale. Drug

[64] has virtually no antimalarial depot bioactivity, protecting mice infected with P. berghei for less than 7 days. Its therapeutic response, however, is elicited by a mechanism unrelated to the dihydrotriazines such as cycloguanil pamoate. If depot forms of [64] could be produced, the combination of sulfone and dihydrotriazine antimalarials might provide a counterforce to strains resistant to either drug. Elslager [236] has designed several promising drug leads by modification of the free amino groups on [64]. Of the monosubstituted DDSs (4'-sulfanilylanilides) synthesized, a general trend was found between aqueous solubility (pH 7 phosphate buffer) and depot activity. In general, derivatives more soluble than 0.03 mg/ml showed little depot activity. Compounds of solubility 0.001 mg/ml and below exhibited sustained activity of from 4 to 10 weeks. Several of the drugs are listed in Table 29.

TABLE 29. Repository Antimalarial Effects of p-Sulfanilylanilides against P. berghei in Mice Following a Single Subcutaneous Dose of 400 mg/kg



RCONR'	Solubility in pH 7 buffer (mg/ml)	Repository activity PMW[a]
HCONH	0.27	-
CH_3CONH	0.04	+
CH_3CH_2CONH	0.027	+
$CH_3CONCH_2CH_2CH_3$	0.12	-
$CH_3(CH_2)_5CONH$	0.001	++
$CH_3(CH_2)_{10}CONH$	<0.0005	+++

[a]Activity is based on the number of weeks 50% of the mice were protected (PMW), and activity ratings were assigned within the following ranges (weeks protected): +++, 7-10; ++, 4-7; +, 1-4; -, <1.

Source: Reproduced with permission from Ref. 236.

Disubstituted derivatives exhibited a parabolic relationship in the solubility range of 0.013 to 0.0035 mg/ml with maximum depot activity occurring with the disubstituted acetamido sulfone (acedapsone). Below a solubility of 0.0001 mg/ml, depot bioactivity was low and inconsistent. The disubstituted derivatives are shown in Table 30. From these studies acedapsone was selected for further investigation as a depot antimalarial drug. Table 31 outlines some relevant data with regard to the depot activity of acedapsone in a variety of mammalian species. An interesting difference is evident between the duration of depot activity (weeks protected, 12 versus <1) of this drug in mice and rats. This apparent contradiction is reconciled by a species-specific metabolic hydrolysis of [66] to [64]. Rat enzyme systems are unable to hydrolyze acetamido groups at a sufficient rate to generate the bioactive [64], and the lower the bioactivity, the lower the protection. Deacetylation occurs readily in the mouse and thus affords protection. Good protection was also demonstrated in humans. The frequency of dosing acedapsone is once every 10 weeks. Acedapsone, when admixed with cycloguanil pamoate, is a potent, long-lasting antimalarial prophylactic suppressive, and the combination is also useful against strains of P. berghei that are resistant to either drug administered individually. A summary of the clinical results are listed below and in Table 32 [237, 256].

P. vivax:
1. Strains susceptible to chlorguanide: 5 months
2. Strains resistant to chlorguanide: no protection

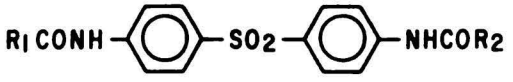
P. falciparum:
1. Strains susceptible to chlorguanide and pyrimethamine: 4 months
2. Strains resistant to chlorguanide and pyrimethamine: 3 months
3. Strains from Southeast Asia resistant to chlorguanide, pyrimethamine, and the 4-aminoquinolines: variable protection, approximately 2 months
4. Strains from Brazil possibly resistant to chloroquine, 3-4 months

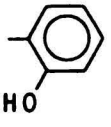
Several excellent reviews and monographs have been written treating various aspects of depot forms of antimalarial agents [236,237,257-259].

I. Antihypertensives

Several chemical classes of drugs have been evaluated as antihypertensives (hypotensives). The majority of such agents have been used clinically in primary or essential hypertension, i.e., elevated blood pressure of unknown

TABLE 30. Repository Antimalarial Effects of 4'-(N-Acylsulfanilyl)anilides Against P. berghei in Mice Following a Single Subcutaneous Dose of 400 mg/kg

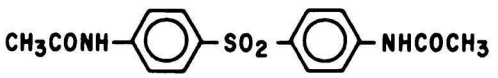


R_1	R_2	Solubility in pH 7 buffer (mg/ml)	Repository activity PMW[a]
H	H	0.013	++
H	CH_3	0.012	++
CH_3	CH_3	0.0030	++++
CH_3	C_2H_5	0.0090	+++
C_2H_5	C_2H_5	0.0035	+
CH_3	$(CH_2)_4CH_3$	<0.0001	+++
CH_3		<0.001	-
$(CH_2)_4CH_3$	$(CH_2)_4CH_3$	<0.0001	+
C_6H_5	C_6H_5	<0.0001	-
CH_3	$(CH_2)_{10}CH_3$	<0.0001	++
$(CH_2)_{10}CH_3$	$(CH_2)_{10}CH_3$	<0.0002	-
$(CH_2)_{14}CH_3$	$(CH_2)_{14}CH_3$	<0.0002	-

[a]Activity is based on the number of weeks 50% of the mice were protected (PMW), and activity ratings were assigned within the following ranges (weeks protected): ++++, >10; +++, 7-10; ++, 4-7; +, 1-4; -, <1.
Source: Reproduced with permission from Ref. 236.

etiology. Gray et al. [260] prepared a series of unsymmetric bis(ammonium) pyridine (Table 33) and piperidine (Table 34) derivatives possessing significant "split" between hypotensive and ganglionic blocking activites. Structural features that are favorable to optimal and prolonged hypotensive

TABLE 31. Repository Antimalarial and Antileprotic Effects of Acedapsone (Hansolar, DADDS, CI-556)

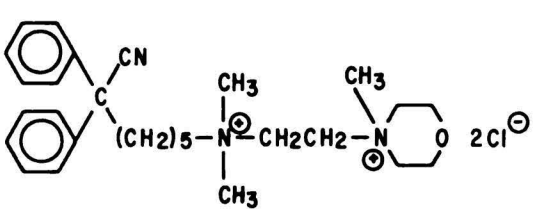


Species	Strain	Host	Single subcutaneous or intramuscular dose (mg base/kg)	Weeks protected
P. berghei	S	Mouse	400	12
P. berghei	Cy-R	Mouse	200	>5
P. berghei	S	Rat	400	<1
P. cynomolgi	S	Monkey	50	9-38
P. falciparum	Multi-R	Human	3.25	6
P. falciparum	Py-R	Human	5-8	4-21

Source: Reproduced with permission from Ref. 237.

activity include the size, planarity, polarity, and flexibility of the head (substituted pyridine or piperidine portion of the molecule), as well as the spatial relationships between the polar portions of the molecule. One factor of critical importance for duration of action is the rate of metabolism of the derivative. Thus, planar, completely conjugated derivatives ([81] and [85]) are thought to be rapidly metabolized whereas their nonconjugated counterparts ([80] and [84]) exhibit a greatly enhanced duration of action. Several derivatives displayed hypotensive effects for more than 4 hr.

A study of a series of ganglion-blocking agents revealed 356C54 [90] to be a potent hypotensive, lowering blood pressure significantly for 7-12 hr with minimal side effects [261].



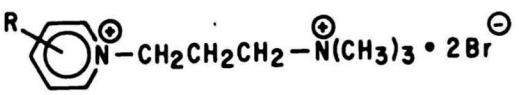
[90]

TABLE 32. Repository Effects of Dapolar[a] Against Susceptible and Drug-resistant Plasmodia

Plasmodium	Strain	Host	Single subcutaneous or intramuscular dose (mg base/kg)	Weeks protected
Berghei	S	Mouse	200	>5
Berghei	Cy-R	Mouse	200	5
Berghei	DDS-R	Mouse	200	5
Cynomolgi	S	Monkey	50	16-33
Vivax	S	Human	3-10	21
Vivax	CG-R	Human	3-10	Nil
Falciparum	S	Human	3-10	17
Falciparum	CG, Py-R	Human	3-10	13
Falciparum	Multi-R	Human	3-10	<8

[a]A 1:1 mixture of acedapsone and cycloguanil pamoate.
Source: Reproduced with permission from Ref. 237.

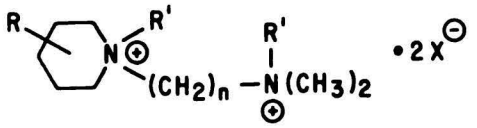
TABLE 33. Antihypertensive Pyridine Salts



Drug	R	Relative hypotensive activity[a]
[80]	2-(1-Naphthylethyl)	100
[81]	4-(1-Naphthylethenyl)	5
[82]	2-(3-Indenylethyl)	40
[83]	2-(3-Indolylethyl)	50
[84]	4-(1-Indolylethyl)	150
[85]	4-(3-Indolylethenyl)	4
[86]	4-(1-Methyl-3-indolylethyl)	80
[87]	2-(1-Methyl-3-indolylethyl)-5-ethyl	120

[a]Intravenously in anesthesized dogs; relative activities are primarily weighted on the basis of duration at about the same percent maximum fall in blood pressure and are compared to a potency of 2 mg/kg of hexamethonium bromide = 1.
Source: Reprinted from Ref. 260; copyright by the American Chemical Society.

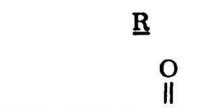
TABLE 34. Antihypertensive Piperidine Salts



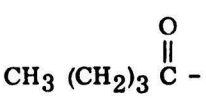
Drug	R	R'	n	X	Relative hypotensive activity[a]
[88]	4-Benzyl	CH_3	3	Br	5
[89]	4-Benzyl	CH_3	2	I	5

[a]See Table 33, footnote a.
Source: Reprinted from Ref. 260; copyright by the American Chemical Society.

A hexapeptide similar in activity to eledoisin was chemically modified with a view toward providing longer-lasting hypotensive and vasodilating activity [262]. Of several acyl derivatives synthesized, the butyryl [91] and valeryl [92] peptides significantly increased the duration of hypotension over the parent peptides.



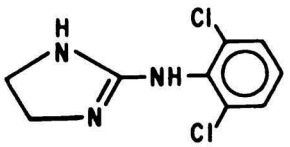
[91]



[92]

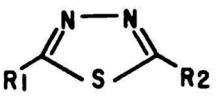
Administration of either derivative in diacetin resulted in a greater than twofold increase in duration. The use of this hexapeptide provides an excellent model for improvement of depot bioavailability via adjustment of hydrophobic-hydrophilic relationships within the molecule. Introduction of suitable hydrophobic fragments at the ω-amino group of lysine in the molecule could account for alteration of distribution to arrive at proper solubility between water and tissue membrane. Hydrophilicity was maintained by the contribution of the free α-amino group on lysine.

The observation of an antihypertensive side effect in a drug being tested for nasal decongestion led to the examination of 2-[(2,6-dichlorophenyl)amino]-2-imidazoline hydrochloride (Catapresan) [93] for blood pressure lowering activity. An intravenous dose of less than 1 mg provided a maximum blood pressure lowering within 30-120 min for a duration of 6-8 hr [263-266]. Oral administration resulted in a delayed onset but the effect and duration were similar. Bock et al. [263] found an intravenous dose of [93] could last up to 4 hr whereas a large oral dose caused lowered blood pressure for as long as 36 hr. The initial hypotensive effect, however, was too intense. A smaller dose of [93] administered three times daily eliminated this initial "burst effect" [267].

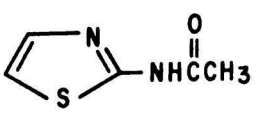


[93]

A series of analogs related to 2-amino-1,3,4-thiadiazole [94] were found to possess antihypertensive activity [268]. When R_1 is H or lower alkyl and R_2 is varied from -NHCHO to $-N(CH_3)COCH_3$, hypotensive activity of short duration was noted. A thiazole analog, however, caused a significant reduction in blood pressure for 24 hr. Administration of 100 mg/kg of 2-acetamido-1,3-thiazole [95] to spontaneously hypertensive rats produced blood pressure lowering effects of long duration. Repeated administration, however, resulted in tolerance development within 48 hr. No meaningful structure-duration of activity relationships were apparent with these series of compounds.



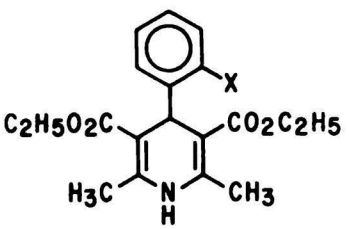
[94]



[95]

A study of a large variety of substituted 1,4-dihydropyridines revealed that aromatic substitution at the 4 position greatly enhanced duration of hypotension on oral administration to anesthesized dogs [269]. While 3,5-dicarbethoxy-2,6-dimethyl-4-phenyl-1,4-dihydropyridine [96] produced

marked hypotension of long duration intravenously, no effect was noted on oral administration. It was found that the chemical nature of the 4-substituent was critical for oral activity. Of the substituents tested, optimum activity was seen with heteroaryl and substituted phenyl groups. The heteroaryl derivatives were found to be toxic and were excluded from further study. The ortho substituted phenyl derivatives, particularly 3,5-dicarboethoxy-2,6-dimethyl-4-(2-trifluoromethylphenyl)-1,4-dihydropyridine [97] lowered blood pressure in anesthesized dogs at doses of 1 mg/kg lasting up to 8 hr. Hypotensive activity was minimal when [97] was converted to the pyridine analog or when the diesters were converted to the free carboxylic acid.



X	
H	[96]
CF_3	[97]

J. Hypoglycemics

1. Insulin Salts and Complexes

The need for long-acting hypoglycemic agents is real since diabetes is a disease treated by prophylaxis rather than cure. The use of insulin as replacement therapy in the hormone-deficient diabetic harks back to the eventful discoveries of Banting and Best [270]. Insulin, however, has a rather short duration of action of approximately 6-8 hr. Hagedorn and co-workers [271] formed an insoluble zinc protamine complex of insulin which, on subcutaneous injection, afforded therapeutic levels of insulin for up to 36 hr. Other insulin complexes possessing depot activity include zinc globin insulin [272], zinc insulin [273], and protamine insulin [274]. Table 35 reviews several long-acting insulin complexes, listed in the British Pharmacopoeia (BP), that have been utilized in diabetes [275].

TABLE 35. Pharmaceutical Injections of Insulin BP

Preparation	Year introduced	pH	Buffer	Description	Duration of effect (hr)
Injection	1923	3.0-3.5	--	Solution	6-8
Neutral injection	1964	6.6-7.7	Acetate	Solution	8-9
Protamine zinc	1936	6.9-7.4	Phosphate	Amorphous particles; rod-shaped crystals	<36
Globin zinc	1939	3.0-3.5	--	Solution	18-24
Isophane	1946	7.1-7.4	Phosphate	Rod-shaped crystals (about 20 μm long)	<28
Zinc suspension (amorphous) "Semilente"	1951	7.0-7.5	Acetate	Amorphous particles (<2 μm diameter)	12-16
Zinc suspension (crystalline) "Ultralente"	1951	7.0-7.5	Acetate	Rhombohedral crystals (about 20 μm across)	<36
Zinc suspension "Lente"	1951	7.0-7.5	Acetate	Amorphous particles (30%), rhombohedral crystals (70%)	<24
Biphasic	1964	6.6-7.2	Acetate	Insulin in solution (25%), rhombohedral crystals (75%)	18-22

Source: Reproduced with permission from Ref. 275.

Complexes of various insulin salts with protamine have been formed with the claimed advantages of rapid onset of action, prolonged duration of action, and extended storage stability [276]. Of the complexes formed (ammonium, potassium, lithium, and sodium) the sodium insulin-protamine complex compared favorably with NPH insulin in duration of activity (Figure 28). A dextran complex of insulin had a hypoglycemic effect 20-30% longer than insulin itself [277]. Alkali metal or ammonium salts of insulin having a dual activity (rapid onset and prolonged duration), especially for use in pediatric diabetes, have been claimed [278].

2. Insulin N- and O-acylates

The traditional chemical approaches to depot insulin preparations have been by the use of combinations of insoluble amorphous and crystalline salts and complexes. Recent interest has centered around the use of insulin prodrug esters and amides to produce sustained blood sugar lowering effects. This concept was utilized in an effort to provide better and more consistent control of blood sugar levels. Further, in certain cases, the diabetics' immune response produces antibodies to insulin with subsequent

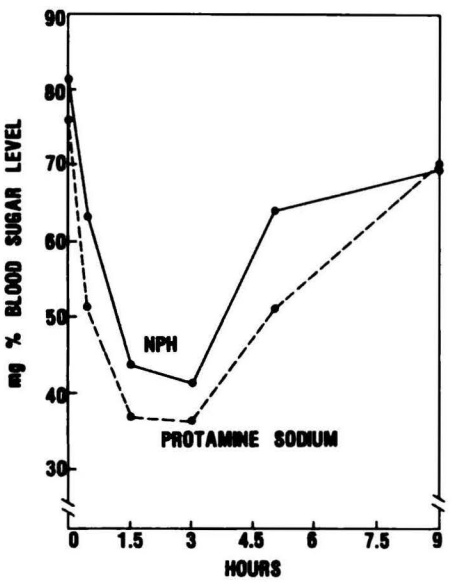


Fig. 28. The effect of sodium insulin-protamine complex (protamine sodium) versus NPH insulin in the degree and duration of blood sugar lowering. (From Ref. 276.)

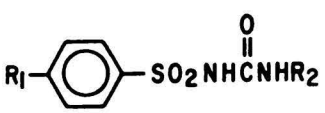
resistance to its action [279]. Acyl derivatives hopefully could be produced with acceptable immunological properties. Thus, blocking insulin amino groups via anion-forming substituents (e.g., succinyl and glutaryl) accounted for prolonged residence of insulin in vivo; such blocking appears to delay enzymatic or acidic hydrolysis of these substituents to insulin. Acylating the tyrosine hydroxyl groups afforded inactive insulin prodrugs which slowly reverted to insulin in vivo and also produced a depot effect. It was also postulated that the presence of anionic groups (enhanced negative charge) might contribute to minimize aggregation of insulin molecules and explain the observed reduction in antigenicity of these insulin prodrug derivatives.

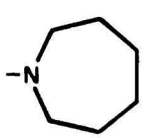
Acylation of the free N-terminal amino groups of glycine (position A-1), phenylalanine (position B-1) and lysine (B-29) resulted in insulin derivatives possessing rapid onset and long duration of activity as well as possible lowered antigenicity [280]. A series of alkanedioic acid prodrug derivatives of insulin are also claimed to possess depot bioactivity [281].

A brief review has recently appeared updating progress in prolonged action insulin derivatives [282].

3. Synthetic Hypoglycemic Agents

The orally effective synthetic hypoglycemic agents are useful in the management of the stable type (maturity-onset, mild adult, or nonketotic) of diabetes mellitus. The majority of these agents are sulfonylureas and act by stimulating the secretion of insulin from the beta cells of the pancreatic islets. Those in clinical use include acetohexamide [98], chlorpropamide [99], tolazamide [100], and tolbutamide [101].



R_1	R_2	Names
CH_3CO-		Acetohexamide, 1-[(p-acetylphenyl) sulfonyl]-3-cyclohexylurea [98]
Cl	$-CH_2CH_2CH_3$	Chlorpropamide, 1-(p-chlorophenylsulfonyl)-3-propylurea [99]
CH_3		Tolazamide, 1-(hexahydro-1H-azepin-1-yl)-3-(p-tolylsulfonyl)urea [100]
CH_3	$-CH_2(CH_2)_2CH_3$	Tolbutamide, 1-butyl-3-(p-tolylsulfonyl)urea [101]
H_2N	$-CH_2(CH_2)_2CH_3$	Carbutamide, 1-butyl-3-sulfanilylurea [102]

It is apparent that the sulfonylureas bear a striking resemblance to the sulfanilamides, particularly the sulfanilylthiadiazoles. In contrast to their structural similarities, however, the sulfonylureas are devoid of any antibacterial activity. Further, the presence of a p-amino group is not necessary for hypoglycemic activity [283,284] (see carbutamide, [102]). The duration of activity of several of the sulfonylureas is variable and most probably is dependent on a variety of factors, such as degree and rate of absorption, volume of distribution, extent of metabolism, and excretion rate. Perhaps the most important parameter affecting duration is metabolism. Tolbutamide is rapidly oxidized to the inactive p-carboxy metabolite [285] with a metabolic half-life of approximately 5 hr [286]. A single dose of tolbutamide which produces an effective hypoglycemic response lasts about 7 hr in humans. The half-life of chlorpropamide, on the other hand, is about 35 hr and that of carbutamide 40 hr. There is little correlation of serum half-life with volume of distribution (Table 36). Excretion of chlorpropamide is relatively slow (96 hr for 80-90% of a single dose to be excreted in urine) with total elimination taking 3-7 days. Its peak hypoglycemic effect is retarded (3-6 hr) and persists for about 24 hr.

The duration of effect of carbutamide is dependent on its rate of acetylation and excretion. In diabetics a satisfactory reduction in blood sugar lasts for up to 24 hr [288]. Acetohexamide has a half-life of about 1 hr and a duration of effect of 12-24 hr [289,290]. However, the metabolically reduced form of acetohexamide, p-α-hydroxyethylhexamide, has a half-life of 4-6 hr and possesses equal or greater hypoglycemic activity than acetohexamide and may be responsible for its longer duration of activity [291].

TABLE 36. Mean Half-life and Percentage of Body Weight in which Three Sulfonylureas were Distributed

Name	Half-life[a] (hours)	Volume of distribution as percentage of body weight (approx. values)
Carbutamide	40 (8)	45
Tolbutamide	3.5 (6)	20
Chlorpropamide	34.5 (8)	18

[a]Numbers of individuals in parentheses.
Source: Reproduced with permission from Ref. 287; copyright by the New York Academy of Sciences.

Smith et al. [292], in a study of the metabolism of sulfonylureas in humans, found a good correlation of duration of hypoglycemic effect with total sulfonylurea in the serum. The compounds studied included tolbutamide, 1-(p-acetylbenzenesulfonyl)-3-(hexahydro-1H-azepin-1-yl) urea, (U-18,536), acetohexamide, tolazamide, and chlorpropamide, and their metabolites. The metabolites of U-18,536 and acetohexamide had longer half-lifes than the parent compounds, lending support to the belief that duration of effect was due, at least in part, to such metabolites. Table 37 lists the half-lives of several sulfonylureas and metabolites. The short duration of action of tolbutamide is probably due to its rapid metabolism and excretion. Tolazamide, which surprisingly is not metabolized, exhibits a relatively long half-life compared to that of the other sulfonylureas studied. Chlorpropamide is metabolized only to a very minor extent to p-chlorobenzenesulfonamide [293] and its long half-life is probably due to a combination of resistance to metabolism and high lipophilicity.

McLamore et al. [285] have studied the effect of arylsulfonylurea structure as a function of activity and duration. Table 38 lists several combinations that have been tested. Optimum activity and duration was seen with unsubstituted as well as p-chloro, fluoro, and methyl substituents, while a low order of activity was observed with o-methyl, naphthyl, and heteroaryl substituents.

Gandhi and Jindal [294], in an extensive study of sulfonylurea structure-activity relationships, essentially confirmed the findings of McLamore et al.

Modification of the alkylurea portion of the molecule virtually destroyed hypoglycemic activity and duration [285], as shown below:

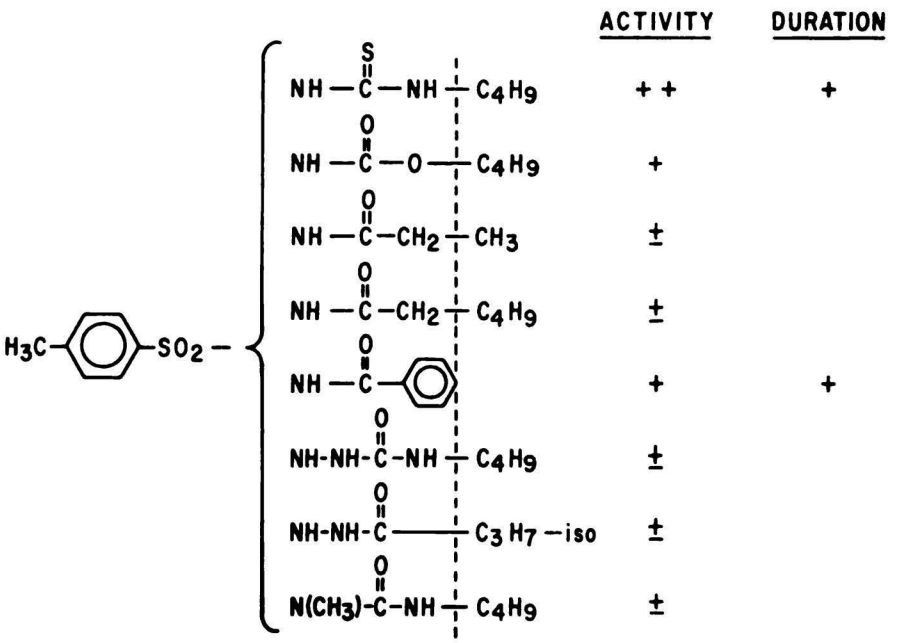
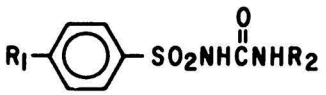
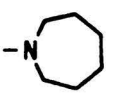
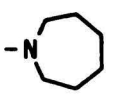
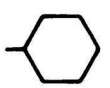
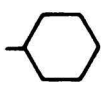
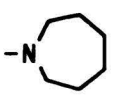


TABLE 37. Half-lives of Sulfonylureas of the General Structure



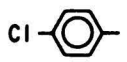
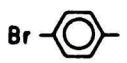
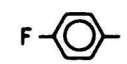
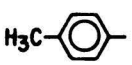
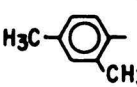
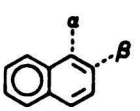
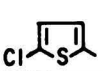
Name	R_1	R2	Half-life (hours)	Number of subjects	Ref.
Tolbutamide	CH_3	$-CH_2(CH_2)_2CH_3$	5.6	59	286,300-302
Tolbutamide metabolite	HOOC-	$-CH_2(CH_2)_2CH_3$	0.5	4	300,303
U-18,536	CH_3CO-		2.1 ± 0.5	8	292
U-18,536 metabolite			3.0 ± 0.6	8	292
Acetohexamide	CH_3CO-		1.3 ± 0.1	10	292
Acetohexamide metabolite			4.6 ± 0.9	10	292
Tolazamide	CH_3		7.0 ± 0.4	9	292
Chlorpropamide	Cl	$-CH_2CH_2CH_3$	35	20	286,287,301,303,304

Source: Reproduced with permission from Ref. 292; copyright by Grune and Stratton, Inc.

4. Miscellaneous

A series of adamantylamine salts of various alkylbiguanides have been claimed as sustained release hypoglycemics [295]. Butylbiguanide adamantoate showed marked improvement over the hydrochloride salt. Similar claims have been made for a series of N_1-(1-adamantyl) biguanides [296].

TABLE 38. Effect of Aryl Substitution on Hypoglycemic Potency and Duration

Ar—$SO_2NHCONHR$			
Ar	R	ACTIVITY	DURATION
	C_3H_7	++++[a]	+++
(CHLORPROPAMIDE)			
	C_3H_7	++++	+
	C_3H_7	++	++
	C_4H_9	+++	++
(TOLBUTAMIDE)			
	C_4H_9	±	
	C_3H_7	++	++
	C_3H_7	+++	++
	α C_4H_9	±	++
	β C_4H_9	+	++
	C_4H_9	++	+
	C_4H_9	++	+

Source: Reproduced with permission from Ref. 285; copyright by the New York Academy of Sciences.

[a]Quantitative significance of + and - does not appear in table source.

Several reviews have appeared on the effect of structure and hypoglycemic activity for several chemical classes of agents [297-299].

III. SUMMARY: FUTURE TRENDS

The future portends many attractive developments in the increasingly more efficient and rational use of the chemical alternative to sustained drug release systems. Major areas where research can be concentrated are analytical methodology, chemistry, and multidisciplinary interaction.

A. Analytical Methodology

As the sophistication and sensitivity in assay techniques increases, the routine determination of drugs at the lower than picogram levels becomes a reality. Especially relevant will be the techniques that might enable the analyst to determine quantitatively the levels of drug and metabolites directly from biological material, e.g., radioimmunoassay. Advances in instrumentation, such as that in mass spectroscopy (MS), high pressure liquid chromatography (HPLC), and specialized nuclear magnetic resonance techniques lend support to these contentions. Moreover, combinations of analytical techniques such as HPLC-MS and gas chromatography-MS allow simultaneous separation and characterization of complex mixtures of metabolic products. Examples of such combinations useful in this regard include mass fragmentography [305], isotope dilution mass spectrometry [306], electron capture detection-mass fragmentography [307] and field desorption mass spectrometry [308]. The use of radiolabeled drugs for distribution and metabolism studies is already finding wide use. Advances in technology that permit radiolabeling in various parts of the drug molecule allow analysts to determine specific metabolic sites within the molecule and to establish the importance of biochemical intervention in such metabolism. Organ and whole-body autoradiography, emerging as noninvasive and nondestructive techniques, will enable the biologist and clinician to ascertain the extent of distribution or localization for drugs variously administered and should provide alternatives to sampling of drugs at inaccessible sites within the organism. Feedback from these various sources might provide the chemist with additional input on which to base further synthetic efforts.

B. Chemistry

New and unusual modifying groups could be systematically evaluated for their effect on physicochemical properties (solubility, partitioning behavior, etc.) as well as on pharmacological, pharmacokinetic, and toxicological properties. Perhaps structure-activity relationships could be more carefully designated as structure-potency, structure-duration, or structure-toxicity relationships.

Controlled release rates might be studied with variously hindered substituents. Prodrugs, for example, could be designed with substituents possessing various degrees of lability. Mixtures of such derivatives could be administered simultaneously, eliminating the need for loading doses now considered so necessary in sustained drug therapy.

The use of polymers as carriers or backbones for slow release of covalently bound drugs should gain wider acceptance. Discerning application of techniques of oligomerization, mesomerization, and cross-linking might improve sustained release properties to suit a given situation. Such polymeric drug combinations might also act as their own vehicles.

More attention will be given to the design of chemical synthetic programs that are geared toward correlative studies. Planning should include considerations of solubility, crystal growth studies (polymorphism), and other physicochemical properties amenable to control by chemical modification.

C. Multidisciplinary Interaction

The journal and patent literature involving chemical sustained release studies is exceedingly fragmental. The chemist initially synthesizes a variety of drug analogs or derivatives that appear interesting from a chemical point of view. The biologist then evaluates these compounds in a screen-type assay and notes those substances that provide further interest biologically. Toxicology studies then eliminate those drugs deemed unacceptable for preclinical and clinical testing. The formulation specialist prepares delivery systems for the candidate or candidates that remain. If clinical testing proves unsuccessful, the entire process is repeated in approximately the same sequence. Retrospective reviews of those relatively few drug candidates that eventually reach the market place generally give the impression that the effort from the beginning was multidisciplinary in scope. In reality it is usually multidisciplinary after the fact. A true program that spans the relevant disciplines is one that is designed as such prior to any laboratory effort on the part of the chemist. Since he is the wellspring, via his derivatives and analogs, of all subsequent effort in this program sequence, his drug candidates should represent the best thinking of all involved. Granted, the chemist may have to learn some biology and the biologist some chemistry, but such a priori effort can only enhance the success of the ultimate outcome.

An excellent example of this approach is cited by Wingard [309] who recently discussed the use of enzyme devices for sustained release drug delivery. He envisions at least two cases where a combined effort might be used. Case (a) is drug release from an impregnated membrane

that is enzymatically controlled. The immobilized enzyme is used to control the transport rate of drug to surrounding biological milieu. Ideally, release would be at a constant zero-order rate. This type of delivery system might suitably lend itself to correlative studies involving various series of drug analogs. Case (b) appears to be an intriguing method of regulating the rate of release of prodrugs in vivo. As can be seen in Figure 29 the delivery system represents a creative combination of chemistry, enzymology, and membrane technology. A, the substrate or prodrug, is hydrolyzed by an appropriate enzyme E, to afford P, the bioactive parent molecule. Molecule P then diffuses through the membrane at a controlled rate and is the chemical form exclusively available to surrounding interstitial fluid. This method would ensure hydrolysis of the prodrug to the bioactive species without reliance on enzymes indigenous to the mammalian species under investigation. Further, since the derivative portion of the molecule remains within the microcapsule, toxicity of this chemical entity may not be as paramount an issue in the design of such prodrug derivatives. Such a system is perfectly suited for the study of a multitude of prodrug derivatives with any number of commercially available enzymes. Wingard further derived mathematical expressions for rates of conversion of A to P as well as overall rate of drug delivery under steady state conditions.

Gregoriadis has considered a similar rationale for drug delivery with the use of liposomes [310].

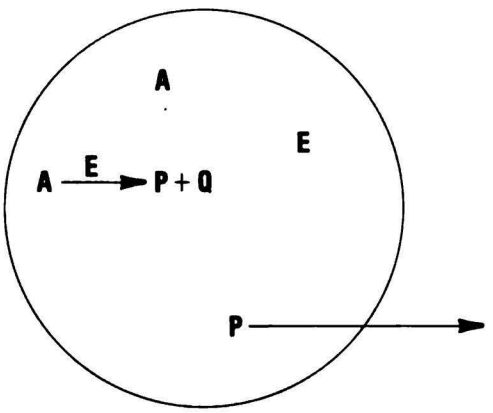


Fig. 29. Drug delivery capsule for case (b). A, substrate or prodrug; E, enzyme; P, active drug (the product of the reaction of A catalyzed by E); and Q, inert nondiffusable portion of the prodrug molecule. Only P can move through surrounding membrane. (Adapted with permission from Ref. 309.)

IV. APPENDIX: ADDITIONAL READING

This appendix contains lists of references to drugs that are not covered in this chapter. It is included for readers who are interested in perusing the literature on a particular drug or class of drugs.

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