AMERICAN PHARMACEUTICAL ASSOCIATION / ACADEMY OF PHARMACEUTICAL SCIENCES

Design of Biopharmaceutical Properties through Prodrugs and Analogs

Edward B. Roche, *Editor*

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Published by
AMERICAN PHARMACEUTICAL ASSOCIATION
ACADEMY OF PHARMACEUTICAL SCIENCES
2215 Constitution Avenue, N.W.
Washington, DC 20037

A Symposium

Sponsored by
Medicinal Chemistry Section
APhA ACADEMY OF PHARMACEUTICAL SCIENCES
November 1976 National Meeting
Orlando, Florida

International Standard Book Number: 0-917330-16-1
Library of Congress Catalog Card Number: 77-81663

AMERICAN PHARMACEUTICAL ASSOCIATION
2215 Constitution Avenue, N.W.
Washington, DC 20037

Printed in the United States of America

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PREFACE

This volume contains the papers presented during the symposium on the Design of Biopharmaceutical Properties Through Prodrugs and Analogs held in Orlando, Florida, November 16 and 17, 1976. This symposium was sponsored by the Medicinal Chemistry Section of the APhA Academy of Pharmaceutical Sciences, and was held as part of the 21st National Meeting of the APhA/APS. The program was intended to provide a rather broad coverage of the subject. As the title indicates, authors were allowed the latitude to describe the development of both prodrugs and analogs having specific effects on absorption, distribution, metabolism, and excretion.

The first chapter provides a suitable overview of the general symposium topic. This is followed by a chapter considering drug-receptor interactions. This current state-of-the-art approach provides the necessary awareness of the fact that during the design of biopharmaceutical properties, those structural features required for eliciting the response in the target tissue must be maintained.

The third chapter provides a review and a functional classification of groups or substituents which provide varying degrees of selective distribution to target tissues. The intention was to provide a background in physicochemical and biochemical properties important to selectivity. The next two chapters describe quantitative approaches to analyzing the effects of substituents on the distribution properties and actions of drugs.

The sixth chapter discusses the effects of structure on pharmacokinetics. The emphasis is on prodrugs and problems associated with measuring active and inactive forms of a drug. The next two chapters describe approaches to the control of drug transport across membranes. The first of these describes a novel approach to the development of polar character in amine drugs, while the latter discusses the improvement of intestinal absorption using a physical model.

The topics of *in vitro* and *in vivo* stability are presented in the next four chapters. Chapter 9 discusses the use of substituent constants as a means of predicting chemical stability of drug systems. Chapter 10 describes the application of structural specificities for various enzyme systems to the design of prodrugs. The design of structural features affecting drug metabolism is approached through the ideas presented in Chapter 11. Examples of the use of physical organic principles in the design of prodrugs are discussed in Chapter 12.

Aspects of drug solubility, topical absorption, and the design of taste properties round out the symposium, and comprise the last three chapters.

This volume presents the basic principles of organic, physical, and biochemistry which are important to the design of biopharmaceutical properties, and illustrates these principles with many examples. The authors have presented the reader with sufficient information to understand the concepts, and to initiate research in this important area of drug design.

I would like to take this opportunity to thank Dr. Walt Morozowich for his extremely valuable discussions and assistance in organizing this symposium. My thanks also goes to the participants for their efforts during the symposium and their diligence in submitting manuscripts for this publication. A special note of thanks is given to Mrs. Sue McQuade for her secretarial assistance in preparing this volume. I would also like to acknowledge and thank Mr. Walid Al-Turk and my wife, Vickie, for their assistance in preparing the index.

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May 13, 1977

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Chapter 1

PERSPECTIVE ON PRODRUGS AND ANALOGS IN DRUG DESIGN

Anthony A. Sinkula

The alteration of bio-pharmaceutical properties by the use of prodrugs and analogs for improvement of drug delivery is of extreme interest to a growing number of pharmaceutical scientists. Eloquent testimony for this premise is evident by the number and diversity of subjects that will be discussed during the course of this symposium. The term bio-pharmaceutical property is somewhat general in meaning and can perhaps be separated into pharmaceutical (physicochemical) and biological components. The pharmaceutical aspects of the definition include considerations such as solubility, stability and related physicochemical phenomena responsible for pharmaceutical elegance (taste, odor, patient acceptance). Alteration in biological properties by the use of prodrugs and analogs result in significant clinical improvement of the drug.

The Analog

In the case of the analog, biological alteration can be manifested by enhancement of potency, spectrum of activity and increase of the therapeutic ratio. In certain situations, however, analogs have been utilized to enhance bio-pharmaceutical properties other than those mentioned above. A comparison of selected antidiabetic sulfonylurea analogs, for example, exhibited significantly different duration of effect characteristics. The duration of activity of several of these 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 (1) with a metabolic half-life of approximately five hours (2). A single dose of tolbutamide which produces an effective hypoglycemic response lasts about seven hours in man. The half-life of chlorpropamide, on the other hand, is about 35 hours and that of carbutamide 40 hours. There is little correlation of serum half-life with volume of distribution (3). Excretion of chlorpropamide is relatively slow (96 hours 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 hours) and persists for ~24 hours.

The duration of effect of carbutamide is dependent on its rate of acetylation and excretion. In diabetics a satisfactory reduction in blood-sugar levels lasts for up to 24 hours (4). Acetohexamide has a half-life of about one hour and duration of effect of 12-24 hours (5). The metabolically reduced form of acetohexamide, p-α-hydroxyethylhexamide however, has a half-life of 4-6 hours and possesses equal or greater hypoglycemic activity than acetohexamide and may be responsible for its longer duration of activity (6). Similarly, the longer duration of effect seen with the 15-methyl prostaglandins *vs.* the nor methyl prostaglandins is probably also due to inhibition of metabolism (7). Another bio-pharmaceutical property that has been modified via the analog approach is absorption. Clindamycin [7(S)-chloro-7-deoxylincomycin] hydrochloride is a semisynthetic antibiotic derived from lincomycin. Its activity against gram-positive aerobes and gram-positive and gram-negative anaerobic pathogens is greater than that of lincomycin. Clindamycin is well absorbed from the GI tract and produces

serum levels considerably greater than lincomycin (8,9). Increased absorption is probably due to the high degree of lipophilicity of clindamycin compared to lincomycin. While analogs can be utilized in the improvement of certain bio-pharmaceutical properties of drugs, the likelihood of decreasing and possibly destroying potency and intrinsic activity is high and thus the analog approach must be used with great care for this purpose.

The Prodrug

Prodrugs can be utilized to modify a variety of both pharmaceutical and biological properties and include (a) modification of the pharmacokinetics of the drug *in vivo* to improve absorption, distribution, metabolism and excretion; (b) improve bioavailability by increasing aqueous solubility; (c) increase drug product stability and (d) enhance patient acceptance and compliance by minimizing taste and odor problems, eliminating pain on injection and decreasing gastrointestinal irritation. The use of the prodrug approach to modify bio-pharmaceutical properties has been extensively reviewed (10,11).

Screening and Synthetic Approaches to Drug Discovery and Design

Historically, the rational approaches to the discovery of new drugs evolved from the idea of the lead compound. The earliest approach involved a random screening procedure designed to identify substances with interesting pharmacological activity. The most bioactive substances were further analyzed for specific bioactivity and utility. When such chemically identifiable compounds were thoroughly characterized, the second phase of discovery involved a synthetic program aimed at increasing potency and spectrum of activity as well as eliminating undesirable properties of the lead compound. This approach is currently being exploited in various degrees by pharmaceutical academia and industry and has come to be known as the analog and prodrug approach to new drug discovery.

The preparation of 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 (SAR) comparisons between members in the series traditionally have had limited value from a predictive standpoint. In the case of prodrugs, however, the parent molecule (lead compound) is regenerated *in vivo* by either enzymatic or non-enzymatic hydrolytic mechanisms. The effect of prodrug chemical modification on bio-pharmaceutical properties can thus be evaluated because the parent molecule is utilized as the standard reference whether assessment is based upon physicochemical or pharmacological property changes in the derivative series.

The Analog-Prodrug Hybrid

Another type of drug, presently classified as neither analog nor prodrug, appears with increasing frequency in the world journal and patent literature. Based on present-day drug design rationale and evaluative methodology, these relatively biostable derivatives possess elements of both analog and prodrug (analog-prodrug hybrid). Several examples of this phenomenon appear in the literature and the following discussion will highlight this problem using steroids as the specific class of drugs and duration of activity (sustained release) as the bio-pharmaceutical property to be modified.

Steroids and the Analog-Prodrug Hybrid

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 man, 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 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 (12). 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 (13). 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 physiologic 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 bio-pharmaceutical properties. Two distinct processes are important: (a) hydrolysis and/or metabolism of the steroid "reversible derivative" (ester, ether, etc.) to the parent steroid and (b) further metabolic degradation of the parent to excretory products, e.g., 17-ketosteroids. Gould et al. (14) 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 (15) 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. (16) to suspect the existence of steroid serum esterase. These esters are less soluble in serum initially than are

the parent steroids. Other 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 (17). Esterase activity was also found in liver and kidney homogenates of the mouse (18). 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 (19). Junkmann (20), 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 pro and con on 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. (14). Further, Lozinski, studying the efficacy of testosterone and estradiol-17-monosulfate esters, found no androgenic or estrogenic effects when administered in doses 10X 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 (21) 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 (22) similarly speculated on the site of hydrolysis for orally administered short and medium chain androgen esters.

Van der Vies (23), 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 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 tissues.

Nandrolone esters are not hydrolyzed by enzymes present in muscle (as evidenced by presence of only intact nandrolone ester at site of injection) but is extensively metabolized in liver and plasma of rats. Comparison of per cent 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.

Oral activity of testosterone undecanoate was demonstrated in the rat with most of the ester being hydrolyzed in the intestinal wall (24). A

tissue distribution study of the radioactive steroid 4-C^{14}-hydroxyprogesterone-17α-caproate indicated that hydrolysis did not occur at the 17-ester position (25). 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 (26). 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 (26).

Rapala et al. (27), 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. This phenomenon was attributed to the fact that the adamantoate ester was not hydrolyzed but was efficacious per se.

An unusual antiinflammatory steroid ester, dexamethasone-21-isonicotinate (28) was studied with particular reference to species differences in hydrolysis rate (29,30). This ester was rapidly hydrolyzed in rat and rabbit sera (90 and 99% respectively, within ten minutes). The ester half-life in human serum, however, was 90-100 minutes.

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 man. Useful data on which one might base meaningful extrapolations on interspecie drug metabolism are lacking. An understanding of the variety and importance of esterases involved in steroid ester metabolism, especially in man, is rudimentary (31).

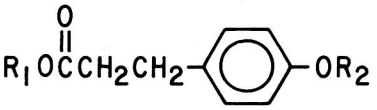
An investigation in man of the pharmacokinetics and metabolism of 1,2α-methylene-6-chloro-pregna-4,6-diene-17α-acetoxy-3,20-dione (cyproterone acetate) indicated no de-esterified steroid in circulating plasma (32). The compound was assayed as the 2-^{3}H labeled derivative. The absence of any 20α-hydroxyl 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.

Concern over the lack of use of purified, reasonably well-defined enzymes in most steroid metabolism studies led Schottler and Krisch (33) 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 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 and included the 21-hemisuccinate esters of prednisolone and cortisol and was probably due to the anionic character of the terminal portion of the acid ester (34,35). Others not hydrolyzed were 17α-hydroxyprogesterone-17-caproate and dexamethasome-21-isonicotinate. The majority of esters followed Michaelis-Menten kinetics with the Michaelis constants falling in the range of 10^{-5} to 10^{-6}M.

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 (36). Administration of an injection of estradiol valerate following migraine attack resulted in rapid and sustained plasma levels of free estradiol lasting from seven to twelve days. Four subjects were used and a similar pattern was seen in each subject. Estradiol benzoate was hydrolyzed and excreted more rapidly. Sustained plasma levels of free estradiol were not seen after oral 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 (37).

Not all sustained release steroid derivatives were designed to be hydrolyzed enzymatically in vivo. Thus, Cross et al. (38) 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 (39). A series of benziloyl hydrazones was thought to also hydrolyze under acidic conditions (40).

A new chemical type of long-acting steroid ester was described in 1960 by Diczfalusy (41). 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. 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 were tested in the rat ventral prostate assay (42). Introduction of a phenyl group in the propionate ester significantly increased potency. The p-hydroxylphenyl 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 (41). Similar correlations were noted with 19-nortestosterone p-alkoxyhydrocinnamate esters (41). It is not known if these esters possess anabolic activity per se or require hydrolysis to exert their effects.

A rather successful chemical approach to the fabrication of long-acting steroids has been via steroidal ethers. Meli and Steinetz, in an excellent review of steroidal ether biology and metabolism, delineated those factors deemed important to the bioactivity of these unique drug derivatives (43):

(a) Steroidal 3-ethers and 3-enol ethers may be bioactive per se.

(b) Sustained bioactivity is due to slow hydrolysis of the ether linkage.

(c) Ether linkage hydrolysis occurs, but metabolic products are different than those of parent steroid.

(d) 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 (44-46). 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 (20). 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 (47).

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 (48,49). Ethynylestradiol-3-cyclopentyl ether, on oral administration, was stored in body fat and slowly released over a long period of time (50,51). Partitioning and storage of this ether derivative into brain tissue may also account for its effective inhibition of pituitary gonadotrophin 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 (52). 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) and estradiol-17-cyclooctenyl ether-3-benzoate demonstrated a separation of estrogenic and hepatotoxic effects in the rat (decrease of sulfobromphthalein serum levels over quinestrol) (53,54). The delay of hepatic metabolism of ester-ether derivatives with subsequent distribution to target organ was thought to be responsible for lack of toxicity.

The use of unique testosterone derivatives such as ethers and acetals is at least partially vindicated by the work of Cross et al. (38) 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 (55). The maximum androgenic response to a single subcutaneous dose of testosterone propionate in the rat occurred at 7-10 days. The same reponse 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) 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 (39). Whether these chemically modified forms of testosterone act as analogs or prodrugs or as a hybridized form is largely unresolved.

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 (56,57). 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 (58). 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 (57). This type of ester readily hydrolyzes under acidic conditions in vitro (59).

Difluorocorticosteroid 17,21-methylorthoesters, 17-monoesters and 17,21-diesters have also been studied for topical antiinflammatory activity (60). 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.

Certain fluocinolone-acetonide*-21-esters exhibited prolonged activity over unesterified fluocinolone-acetonide as seen in the liver glycogen and edema reduction assays (61). Thus, greatest duration was found with fluocinolone-acetonide-21-benzofuranate while relatively shorter durations were seen with fluocinolone-acetonide-21-acetate and fluocinolone-acetonide.

In sum, it is not readily apparent what factors are critical for classification of steroid derivatives as being intrinsically or extrinsically active. While many esters, acetals, ketals and ethers act as prodrugs, exceptions to this rule exist. More research is needed in the area of steroid metabolism, specifically in the knowledge of possible enzyme systems that catalyze the hydrolysis of such derivatives in vivo, especially in man. 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.

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