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Review Article

Comparative Efficacy of Topical Anti-inflammatory Corticosteroids

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THE ANTI-INFLAMMATORY corticosteroids represented by hydrocortisone and related synthetic analogs have gained an unchallenged position of paramount utility in dermatologic therapy during a span of little more than a decade. Having unique effectiveness in selected and even fatal dermatoses when employed systemically, the corticosteroids have been utilized even more frequently by topical administration in the successful management of a variety of inflammatous skin lesions.

Despite the documented efficacy and widespread use of these agents, the mechanism by which they exert their often profound anti-inflammatory activity remains largely unknown. It is nevertheless well established that the corticosteroids are only palliative rather than curative in action. They must be utilized continually until the disease undergoes spontaneous remission or cure or until the noxious pro-inflammatory stimuli are eliminated by other means. Consequently, the tremendous topical utility of the corticosteroids is based not only on their marked clinical effects but also because, with few exceptions, they may be used with impunity over long periods when applied to the skin.

The major objective of this review is to analyze the experimental and clinical studies that have contributed to our understanding of the process or manner in which the various topical corticosteroids produce their therapeutic responses. Another purpose of the report is to unite in summary fashion the results from a group of dispersed publications, each dealing almost exclusively with individual anti-inflammatory compounds.

Considerable emphasis is placed on various comparisons among the corticosteroids in an effort to focus attention on a variety of important variables that may be responsible for producing appreciable differences in clinical effectiveness. While all of the parameters that influence topical activity could not be included in one review, it is hoped that the factors selected for attention will help make future investigations on topical corticosteroids even more fruitful than those reported in the past decade.

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HISTORY

The advent of the profoundly successful anti-inflammatory corticosteroid era was heralded by the auspicious discovery by Hench and collaborators in 1949 (1, 2) that cortisone and cortisone 21-acetate were dramatically effective in the treatment of the complicated and frequently crippling rheumatic arthritis. The initial high cost and limited supply of cortisone led these and other investigators to search for steroidal substitutes which might possess similar or superior anti-inflammatory properties. Of several adrenal-corticosteroids evaluated, only hydrocortisone was found to have clinical efficacy comparable to that of cortisone (2).

The primary discovery by Hench stimulated

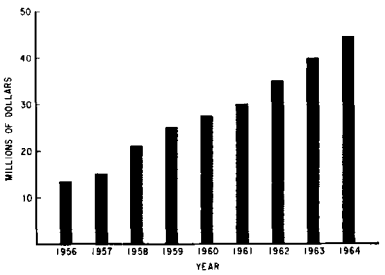

Fig. 1.—Estimated annual domestic sales of topical corticosteroid products at manufacturer's level.

numerous investigators to evaluate cortisone in other diseases. Quickly, several ophthalmologists began to extol the virtues of topical cortisone 21-acetate for therapy of various inflammatory eye conditions (3–6). The dermatologists, however, were less fortunate during this early period. While cortisone 21-acetate was effective systemically in the treatment of certain dermatoses, it was disappointingly devoid of anti-inflammatory activity when applied topically and essentially ineffective or erratically effective when administered as a suspension or solution by intradermal injection (7). Fortunately, this dilemma was only temporary, for it was discovered subsequently that hydrocortisone and hydrocortisone 21-acetate produced pronounced anti-inflammatory responses when applied topically in a variety of inflammatous dermatitides (8–10). Hydrocortisone 21-acetate, but not the free alcohol, also was effective intradermally in improving local lesions of spontaneous disease or those induced experimentally by application of primary irritants, especially certain sensitizing agents (8–11).

The experimental and clinical proof that hydrocortisone and its 21-acetate derivative possessed appreciable topical anti-inflammatory activity marked the beginning of a new epoch in dermatology. Being generally safe and free from the development of drug tolerance and lacking odor, staining, or irritating or sensitizing properties, these compounds employed in various vehicles and in combination with antibiotics soon facilitated eloquently the successful management of a large number of dermatoses.

Today there are available for topical therapy a dozen or more newer synthetic derivatives of hydrocortisone. The majority of these compounds were produced by various groups of active chemists and biologists (12–18), whose primary objectives were to discover and develop derivatives which upon systemic administration would have lessened propensities for eliciting side effects, such as suppression of the pituitary-adrenal axis, disturbance of electrolyte metabolism, and proclivity to peptic ulcers and osteoporosis. While many of the disadvantages of systemic steroidal therapy remain to be circumvented, there accrued from these expanded efforts several new and more effective compounds for topical use. Subsequent research culminated in the discovery of several synthetic analogs having various degrees of preferential topical activity (19–23). The selective topical efficiency is based on improved therapeutic indices, manifested by increased topical potencies with less systemic effects from percutaneous absorption when lower drug concentrations are applied.

Perhaps the most dramatic way to demonstrate the enormous therapeutic utility of the topical anti-inflammatory corticosteroids is to examine the growth of this market in the United States, as shown in Fig. 1. This graph, depicting estimated sales at the manufacturer's level, reflects the ever-increasing use of topical corticosteroids. It was estimated that by the end of 1964 the topical market would be essentially equivalent to the market for oral anti-inflammatory corticosteroids.[1]

A compilation containing the trade names of over 100 available topical corticosteroidal products has been published (24). This number swells to a prodigious several hundred product items when the various drug concentrations are included.

TOPICAL CORTICOSTEROID STRUCTURES

The chemical configurations of the major topical corticosteroids employed clinically along with the topically ineffective cortisone structure are displayed in Fig. 2. One or more portions of several of the structures have been circled to emphasize the positions where the hydrocortisone molecule has been modified chemically to produce synthetic analogs possessing different physicochemical properties and altered topical potencies. Some of the compounds are employed topically both as the free alcohol and as the 21-acetate ester.

Structure-Activity Relationships.—The basic structural features required for topical anti-inflammatory activity are those possessed by hydrocortisone, the naturally occurring and principal anti-inflammatory corticosteroid produced by the human adrenals (25, 26). The most critical structural configuration is the perhydrocyclopentanophenanthrene ring system containing the 11β-hydroxy group. Other chemical groups necessary for activity of the parent hormone are the C-4, C-5 double bond together

[1] Data from Marketing Research Unit, The Upjohn Co.

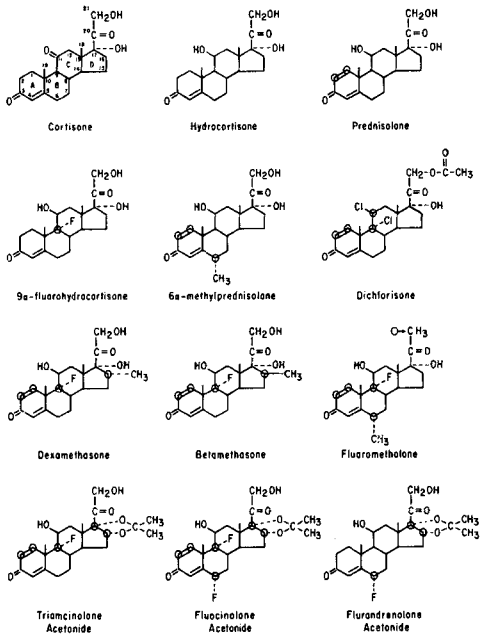

Fig. 2.—Chemical structures of the major topical corticosteroids employed clinically.

with the 3-ketone of the A-ring and the 17,21-dihydroxyacetone side chain.

The reason hydrocortisone is active by every route of administration, while cortisone is ineffective when applied to the skin or injected intradermally or intra-articularly, has puzzled investigators for more than a decade. Lack of absorption is not a primary factor, for absorption is obviated by the injection technique, and Malkinson has shown that hydrocortisone and cortisone 21-acetate are absorbed to about the same extent following application to normal skin (27, 28). Although Malkinson also has shown that hydrocortisone is absorbed to a greater extent than cortisone 21-acetate when applied after the corneum has been removed by stripping (29), this difference most conceivably could be due to the lower solubility, slower dissolution rate, and the concomitantly reduced availability of the cortisone ester when applied to the more hydrophilic barrierless epidermis.

Other evidence suggests strongly that the answer to the problem resides in the basic lack of appreciable extrinsic anti-inflammatory activity by the cortisone molecule. Several of the clues are: (*a*) hydrocortisone is the principal anti-inflammatory corticosteroid of the human adrenal (25); (*b*) both endogenous and exogenous hydrocortisone and cortisone are interconverted systemically to account for the systemic activity of cortisone (30); (*c*) 2α-methylcortisone is without systemic activity because conversion to the hydrocortisone configuration is blocked by the 2-methyl substituent (26, 31); and (*d*) *in vitro* studies by Berliner (32) also indicate that conversion from cortisone to hydrocortisone is necessary for activity.

While these data are reasonably convincing regarding the lack of intrinsic activity for cortisone, the concept is fortified by the observation of Malkinson (33) which indicates that human skin *in vitro* is capable of converting some cortisone to hydrocortisone. Yet topical cortisone is ineffective, despite the possibility of this conversion to an active form. Perhaps any transformed hydrocortisone is removed from the skin by the vascular and lymphatic channels too readily for attainment of an effective level in the tissues. Or the amount converted could be too little for activity to become evident.

Hydrocortisone and Synthetic Analogs.—All of the chemical groups which appear essential for the anti-inflammatory activity of hydrocortisone are not critical for the topical activity of several of the synthetic corticosteroids. This phenomenon is manifested by virtue of the marked increase in anti-inflammatory potency which may ensue when the hydrocortisone structure is modified by the addition of certain activity-potentiating groups to key positions in the parent molecule. Such enhancement of activity may be so profound that it compensates more than adequately for any reduction in activity engendered otherwise by the concomitant loss of a basic functional group.

One example of this principle is represented by the unique properties of fluorometholone. Examination of the structures in Fig. 2 will reveal that in fluorometholone the essential side chain of hydrocortisone has been altered by deoxygenation at C-21, a change which characteristically lowers anti-inflammatory potency (26, 34). However, three additional modifications have been made to form fluorometholone: dehydrogenation at C-1 and C-2, 6α-methylation, and 9α-fluorination. Together, these changes increase markedly the topical anti-inflammatory efficacy. The preferential action of fluorometholone is based on an oral potency in man of only one to two times that of hydrocortisone (35, 36) and a topical potency of 40 times hydrocortisone on inflammatous skin lesions (20, 37–40). Thus, undesirable effects from percutaneous absorption of topically applied fluorometholone should be remote relative to such effects from equivalently effective concentrations of hydrocortisone.

An explanation for the disparity between the oral and topical activity of fluorometholone has

not been formulated with absolute certainty. However, the presence in the molecule of three activity-potentiating substituents (26) together with a probable low order of inactivating enzymatic action in the skin (33) doubtlessly accounts for much of the increase in topical potency. The low oral and, by inference, low systemic potency likely results from the extreme vulnerability of the 21-deoxy side chain to inactivation by hepatic enzymes. This hypothesis is based on the fact that both fluorometholone and progesterone possess the 21-deoxyconfiguration. Progesterone has a very low order of oral activity. It is absorbed well (41) but is inactivated readily by the liver (42, 43).

Another interesting structure-function relationship is that displayed by prednisolone, which differs from hydrocortisone only by the presence in the A-ring of an additional double bond between C-1 and C-2. This rather subtle yet significant alteration increases the oral potency to about four times that of hydrocortisone and reduces the electrolyte disturbing properties of the natural hormone (26). In contrast, however, the topical activity of prednisolone is only one or two times that of hydrocortisone (20, 44–46). Since the increased oral potency of prednisolone is due to a slower rate of metabolic inactivation (47), the lower topical potency suggests again that enzymatic destruction plays a less significant role in modifying the activity of topically applied anti-inflammatory corticosteroids. This conclusion is strengthened by the skin incubation studies of Malkinson (33), showing a low order of conversion of hydrocortisone to the inactive cortisone, and by similar studies of Goldman (48), showing little if any metabolism of prednisone or prednisolone over 9 hr. of incubation.

Triamcinolone represents another unique example of a discrepancy between oral and topical effectiveness determined by structural features. This 16α-hydroxylated corticosteroid, like 6α-methylprednisolone, displays five times the oral potency of hydrocortisone (26). But, unlike the latter compound, which is equally potent orally and topically (20, 26, 34, 49, 50), triamcinolone is only equivalent to hydrocortisone topically, as determined by this author in an experimental procedure to be described later in this review (20). The mechanism for this oral-topical discrepancy has not been established, but the disparity most likely could be due to the presence in the molecule of the 16-hydroxy substituent, which doubtlessly alters the solubility, polarity, and other physicochemical properties in a manner unfavorable for high topical absorption or especially for retention in the skin subsequent to penetration through the horny layer. Evidence for this postulate is obtained from the marked increase in potency of triamcinolone when both the C-16 and C-17 hydroxyls are masked, as in the highly active derivative, triamcinolone-16,17-acetonide (51, 52). Furthermore, McKenzie (53) attributes the low topical activity of triamcinolone to poor cutaneous penetration relative to the markedly increased penetrability of the 16,17-acetonide derivative.

TABLE I.—RELATIVE R_f VALUES OF CORTICOSTEROIDS

Compd.	Bush B-5 System	Mattox-1 System
Triamcinolone	0.04	0.12
Prednisolone	0.27	0.25
Hydrocortisone	0.37	0.29
6α-Methylprednisolone	0.46	0.37
Hydrocortisone 21-acetate	0.90	0.67
Triamcinolone-16,17-acetonide	0.91	0.71

That the physicochemical properties of the corticosteroids may affect penetrability and retention in the skin as well as other factors bearing on topical anti-inflammatory activity may be appreciated from a perusal of Table I, which lists the relative R_f values for several of the compounds under discussion. These paper chromotographic values were determined recently[2] using the same paper for all compounds to obtain more meaningful relative figures. The Bush B-5 system (54) and a modified Mattox-1 system (55) were employed.

From Table I and from the data discussed previously, it can be seen that, except for hydrocortisone 21-acetate, the more polar and less mobile compounds have the lesser topical potencies. The difference in polarity between triamcinolone and triamcinolone-16,17-acetonide is especially noteworthy in view of the higher topical potency of the acetonide derivative. An exception to the trend is the large difference in polarity of hydrocortisone and hydrocortisone 21-acetate which disagrees with the available data indicating equivalent clinical efficacy for these compounds. One explanation for this paradox is that the less polar hydrocortisone 21-acetate may penetrate the skin with greater ease and may be better retained in the corium than hydrocortisone. But the latter compound, having greater solubility (56) and a probable faster dissolution rate when applied to the skin, would present more molecules in solution for absorption. The net result, when the compounds are applied in crystalline form in the usual topical vehicles, could be similar clinical efficacy.

[2] Determinations by Mr. L. M. Reineke, Biochemical Research Unit, The Upjohn Co.

A different explanation is needed to account for the equivalent topical potencies of hydrocortisone and triamcinolone. Perhaps in this case the expected lower activity of triamcinolone, due to decreased penetration or reduced tissue retention of this more polar compound, is compensated for in part by the higher intrinsic activity of the 9α-fluorinated structure. Of course, many other factors, such as vehicle effects, organic/aqueous partition coefficients, hydrolysis of esters, and tissue binding and metabolism, must be considered when one attempts to explain how the various corticosteroids behave after topical administration.

Two other marketed corticosteroidlike compounds which have rather unusual structural features are dichlorisone and δ-5-hemisuccinoxypregnenolone. Both compounds lack the 11β-hydroxyl which is the most necessary functional group for the anti-inflammatory activity of hydrocortisone (57). The basis for the topical action of these somewhat bizarre noncorticoids remains to be explored in more detail. It has been reported that pregnenolone is devoid of topical anti-inflammatory activity in inflammatous dermatoses (58).

This section on structure-activity relationships will be concluded with a discussion of two additional corticosteroids which, like prednisolone and triamcinolone, have lower topical than oral anti-inflammatory potencies. These compounds are dexamethasone and betamethasone, the 16α-methyl and 16β-methyl analogs of 9α-fluoroprednisolone, respectively.

Both of these corticosteroids have marked oral potency in the range of about 30 times that of hydrocortisone (59–62). However, their topical potencies are only about tenfold that of hydrocortisone, as judged from the therapeutic activity of the marketed concentrations (63, 64). Since the high oral potency is attributable to protection from metabolic inactivation of the 17,21-dihydroxyacetone side chain by steric hindrance from the 16-methyl substituents (65–67), the lower topical potency once again affords support to the concept that resistance to metabolism in the skin must not be a major determinant of topical corticosteroid potency. Moreover, that changes in the physicochemical properties of the corticosteroids may affect topical activity greatly is exemplified once more by the great boost in the topical potency of betamethasone by esterification to form the less polar but highly effective topical derivative, betamethasone 17-valerate (68). The latter compound looks very promising on the basis of preliminary clinical reports.

LABORATORY ANIMAL STUDIES

The traditional animal experimentation, necessary before evaluation of new compounds in humans, poses several perplexing problems that are specifically peculiar to the topical area. The difficulties are due not only to the inherent anatomic, physiologic, and biochemical differences between man and laboratory animals (69) but also because, even in man, the corticosteroids often display large differences in relative potency and efficacy when employed by different routes of administration.

A serious and often ignored problem of testing corticosteroids topically on animals is the instinctive and obsessive licking and rubbing by the animal to remove or displace the foreign topical medications. The licking may result in large oral doses of corticosteroid, thereby magnifying systemic manifestations. Topical effects may be reduced or contaminated by combined topical and systemic responses. Procedures involving covering and binding of treated areas with occlusive dressings may alter absorption and the pharmacological and toxicological responses to the test agents profoundly (70). Collars and other restraints to prevent licking produce stress with elaboration of endogenous corticosteroids (71); this confounds the purity of the test procedures involving anti-inflammatory agents.

The skin of the pig often has been purported as ideal for testing topical preparations. While this may be true in some respects, recent studies have shown that pigskin is distinctly different from that of the human (72).

Despite these difficulties with topical testing, preliminary animal studies by other routes of administration may be helpful when the results are used intelligently. Oral and parenteral studies in animals are useful for screening for anti-inflammatory activity in a variety of chemical configurations. Profiles regarding absorption, distribution, and metabolism are useful, while toxicity profiles are mandatory.

Some of the animal procedures which have been used with some success need only to be summarized for this review. Anti-inflammatory activity has been determined by the implanted cotton-wool pellet procedure (73, 74), the granuloma pouch technique (75, 76), the eosinophil assay (77), hepatic glycogen deposition (78), and the alleviation of experimentally induced arthritis in rats (79). Mineralocorticoid activities have been appraised by electrolyte excretion patterns (80) and pituitary-adrenal inhibition by adrenal weight loss (67).

Various topical methods have been used for

defining irritation and sensitizing potentials. Although the marketed corticosteroids are devoid of these undesirable properties, the methods are nevertheless necessary, particularly for the adjuvants in topical formulations. Several methods for measuring irritation and sensitization have been published for animal (69, 81, 82) and human studies (83–86).

Although animal testing may eliminate many corticosteroids from further consideration as potential topical candidates because of inactivity or undesirable pharmacologic and toxicologic profiles, the insurmountable problems in conducting mass clinical trials on the large number of potentially useful compounds spurred the development of several preclinical procedures in humans to bridge more effectively the large gap between animal studies and final clinical evaluations. A review of this human screening methodology will be presented following a discussion of some of the earlier probing experiments, which formed a foundation for the more standardized human assays.

EXPLORATORY EXPERIMENTAL STUDIES

The initial probing studies were conducted early in the history of the corticosteroids when there were few guidelines concerning the manner in which these compounds exerted their local anti-inflammatory action. Indeed, the types of dermatoses which were benefited by topical corticosteroids had yet to be classified. This area was explored initially by Goldman (7, 8, 11), who studied the ability of topical and intradermal cortisone and hydrocortisone to improve clinical diseases and to suppress inflammation induced experimentally by application of primary irritants and sensitizing agents. These studies revealed that cortisone 21-acetate and an aqueous-soluble ester of cortisone were practically devoid of local anti-inflammatory activity. Hydrocortisone was effective by both routes of local administration. An exception among Goldman's observations was that even hydrocortisone 21-acetate was not effective universally. It did not suppress inflammation induced by turpentine, cantharidin, poison ivy antigen, and histamine, nor did it benefit clinical urticaria.

Additional experiments by Goldman provided the following important facts regarding local corticosteroidal behavior: (*a*) the highly aqueous-soluble hemisuccinate and phosphate esters of prednisolone, like those of cortisone, lacked anti-inflammatory activity when employed intradermally (87); (*b*) histological, histochemical, electron microscopic, and X-ray diffraction studies of skin sections following intradermally injected corticosteroids revealed characteristic masses containing the injected crystals and showed that the least aqueous-soluble hydrocortisone 21-acetate crystals persisted *in situ* for several months. The more aqueous-soluble crystals of hydrocortisone remained in the skin for somewhat shorter periods, and the highly aqueous-soluble corticosteroidal ester salts were absorbed so rapidly that they could not be detected in the biopsy specimens (48, 87); and (*c*) paper chromatographic assays of corticosteroid levels in biopsied skin confirmed the above findings and also showed smaller, less persistent, and more variable corticosteroid levels after topical inunction, compared to intradermal administration of the same compounds (88).

The results obtained by Goldman were verified subsequently by the clinical studies of Sulzberger (9, 10) and by experimental explorations by Atkinson (89) and Dostrovsky (90). The latter author found that hydrocortisone 21-acetate had an inhibitory effect when injected intradermally with leishmania vaccine; but the inhibition was considerably reduced when hyaluronidase also was present in the injected formulation.

An obvious conclusion from these exploratory studies is that an appreciable level of an active corticosteroid must be present at the site of action if a detectable degree of anti-inflammatory activity is to be observed. Thus, the low and rapidly diminishing corticosteroid levels in the skin following intradermal injection of the highly aqueous-soluble esters explains the lack of significant activity with this species (87). This simple explanation is less plausible for the less effective and more erratic activity of intradermal hydrocortisone, compared to hydrocortisone 21-acetate, for the aqueous solubility of 0.28 mg./ml. for hydrocortisone (56, 91) is appreciably low compared to that of the highly aqueous-soluble esters. A more probable explanation is that the slow rate of dissolution of the hydrocortisone crystals, as shown by their persistence in the skin, when coupled with a likely rapid clearance of the molecules as they become dissolved, prevents accumulation of an adequate concentration of hormone at the site of action. In contradiction, the higher organic/aqueous partition coefficient and lower polarity of hydrocortisone 21-acetate doubtlessly favors preferential tissue uptake rather than clearance of the latter compound. The distribution coefficients between water and benzene are 0.35 or less than unity for hydrocortisone and 15.3 for hydrocortisone 21-acetate (92).

The inhibitory action of intradermally injected hydrocortisone 21-acetate on several types of

patch test reactions and the lack of such activity against urticarial and histamine-induced lesions (8, 11) led Brodthagen (93) to investigate whether prior inunction with a 2% ointment of hydrocortisone 21-acetate could block the reactions produced in normal skin by the tuberculin test, by patch tests in sensitive individuals, and by scratch tests with histamine. He obtained negative results when the tests were tried following daily applications of the corticosteroid for a period of 3 days. However, when cantharidin was used to induce blisters and the blister tops were removed to eliminate the stratum corneum before application of the ointment, definite inhibitions of the reactions to tuberculin, histamine, and the specific allergens were obtained. These studies emphasize the importance of the epidermis as an effective and major barrier to the percutaneous absorption of the cortiscosteroids.

Haxthausen (94), apparently unaware of the above results, attempted to prevent the development of electrophoretically induced eczematous reactions by applying preparations containing various corticosteroids, immediately following electrophoresis of the antigens. Hydrocortisone 21-acetate, at 2.5% concentrations in petrolatum, in creams, and in a Carbowax vehicle, exerted only a weak to moderate inhibition in three of 12 test sites. Other corticosteroids showing similar or slightly better inhibition were hydrocortisone, 9α-chlorohydrocortisone, and a Xantogen derivative of hydrocortisone. Although Haxthausen was not happy with the inconsistency and lack of complete suppression of the eczematous reactions, the low order of activity may have been due to insufficient corticosteroid in the tissues during the early stages of development of the eczema. For, contrary to his belief, the allergic process probably commenced in the skin, immediatedly following introduction of the antigen by electrophoresis. The corticosteroids would not have had sufficient time to penetrate during the early phase of the developing reaction.

The early report by Jarvinen (95) that 14 patients treated with massive oral doses of cortisone had decreases in the reaction of their skin to ultraviolet light led several investigators to attempt to reproduce these results using topical corticosteroids. For example, Everall (96) observed no reduction in ultraviolet erythema when 2.5% concentrations of cortisone or hydrocortisone as the 21-acetates were applied immediately before irradiation or once daily for 3 successive days prior to exposure to irradiation on the fourth day. The reasons for his failure to observe any inhibition of erythema seem obvious now. Twenty-two of the volunteers were treated only with the topically ineffective cortisone ester. The negative results in the remaining three subjects treated with hydrocortisone 21-acetate require two different explanations. In the cases treated immediately before irradiation, there was doubtlessly inadequate time for the steroid to be absorbed to block the ensuing reaction. When the last of three daily treatments was applied 24 hr. prior to irradiation, the tissue levels of corticosteroid likely faded to subthreshold concentrations by the time of induction of the erythematous stimuli. The significance of the relation between the time of drug administration and the inducement of experimental inflammation will be covered later in this review, when Scott's (97) studies on percutaneous absorption of hydrocortisone will be discussed.

Despite the above objections regarding experimental details, both Kanof (98) and Langlo (99) also were unable to prevent the occurrence of ultraviolet erythema by the prior application of hydrocortisone ointments. There is no apparent explanation for these failures, which are in disagreement with the successful results of Scott (100), who conducted extensive studies in this area.

Scott's organized and laborious approach to the delineation of some of the factors important for the evaluation of topical corticosteroid action began with the investigation of the percutaneous absorption of hydrocortisone-4-C^{14}, using the autoradiographic technique (97). He demonstrated that the corticosteroid entered the skin both through the transepidermal and follicular routes. Radioactive material reached the basal cells 2 hr. after application and diffused throughout the corium during the next 4 hr. Sixteen hours after a single application, almost all of the absorbed hydrocortisone presumably had passed into the systemic circulation.

These results suggested the need to evaluate the time of application of the hormones in relation to the inducement of experimental inflammation. Accordingly, Scott (100) treated small areas of normal skin with different corticosteroid preparations at various times before and after irradiation with ultraviolet light or application of dilutions of mustard oil or nitric acid as primary irritants. His results indicated effective inhibition of inflammation when the hormones were applied from 2 to 8 hr. prior to the stimulus. The maximum inhibition was achieved with 6 hr. of pretreatment, which corresponded to the time when the absorption studies indicated the highest tissue levels of corticosteroid would prevail. Applications of drug 16 hr. before, immediately before, or at various times after introduction of the

pro-inflammatory stimuli were essentially ineffective.

All of the subjects did not display inhibitory responses, nor was the inhibition absolute in many of the cases. Scott concluded that the degree of the anti-inflammatory response produced by 1% hydrocortisone, 0.25% 9α-fluorohydrocortisone, or 5% ACTH varied inversely with the intensity of the inflammatous stimuli.

It must be assumed that the more consistent inhibitory responses observed in these experiments, compared to those discussed previously, are attributable to certain important but indefinite factors in addition to the critical time sequence for the topical drug applications. These indefinite factors may involve the specific emission characteristics of the ultraviolet instruments employed, differences in the mechanisms by which the primary irritants produce inflammation, seasonal variations in the response of the skin to irritants and to drugs, and other unknown variables. However, none of these parameters is helpful for describing why ACTH was topically effective in Scott's studies. It is difficult to rationalize how this protein can exert a direct anti-inflammatory action, or how it can penetrate the skin to any significant degree.

Before proceeding to a review of topical assay methodology, it appears worthwhile to relate a general concept on corticosteroid action which may facilitate an understanding and appreciation of why these anti-inflammatory agents are sometimes dramatically effective, while at other times they behave variably or are even ineffective. This concept does not pertain to the unknown mechanism of corticosteroid action. Instead, it is simply this author's opinion of the manner in which the corticosteroids exert their anti-inflammatory effect, both in experimental situations and in therapeutic applications.

According to this philosophy, the anti-inflammatory corticosteroids act only preventively or prophylactically and exert an inhibitory influence only on the developmental aspect of the inflammatory process. They primarily do not reverse established inflammation. Rather, in progressing or stabilized systemic or local inflammatory diseases, the corticosteroids act effectively by checking further reaction of the affected tissues to the continuing influx of pro-inflammatory stimuli. The degree of benefit characterized by reduced inflammation is determined by a new equilibrium based upon the strength and persistence of the noxious stimuli and the tissue concentration and intrinsic activity of the corticosteroid administered. The onset of objective and subjective relief of symptoms varies as the time required to establish the new equilibrium. The inflammation established prior to institution of therapy subsides to a lower level as the new equilibrium develops. As the corticosteroids are palliative rather than curative, flare-ups occur upon withdrawing therapy unless the noxious stimuli have been reduced or eliminated.

When the corticosteroids are evaluated in experimentally induced inflammations, it must be appreciated that complete suppression of the reaction requires an adequate tissue concentration of an effective corticosteroid from the time of the appearance through the duration of action of the phlogistic stimuli. If there is inadequate corticosteroid coverage during any phase of the inflammatory sequence, the inflammation propagated during this interval will assume its natural unabated course. If erythema is characteristic of the experimental reaction, for instance, some reddening of the lesion will occur, and the activity of the corticosteroid will be interpreted as being only partially effective.

In concluding this section, some consideration also should be given to the fact that most of the experiments were conducted with hydrocortisone, which is not an extremely potent topical agent. Some of the recently developed and more meaningful assays were conducted with highly potent compounds. These newer synthetic analogs made the older methods more reproducible and useful and allowed development of new techniques based upon more definite and consistent anti-inflammatory responses.

TOPICAL CORTICOSTEROID ASSAYS

Intradermal Screen.—The failure to produce an experimental inflammation which could be moderated consistently by topical corticosteroids led Witkowski (101) to devise a screening procedure based on the ability of intradermally injected compounds to suppress pustule formation induced above the injected sites by the 24 hr. application of croton oil. Treatment responses were graded visually to the nearest 25% inhibition of pustules using 1.0 mg. of cholesterol in the suspending vehicle as the control. Corticosteroid activity was rated according to the milligrams of unknown required to produce a level of suppression equivalent to a standard, such as hydrocortisone.

This test provided a valuable tool for determining rapidly whether a variety of different corticosteroids possessed anti-inflammatory activity in man. A major advantage was the use of inflammation as the criterion for activity

rather than some proximate action, such as glucocorticoid or eosinopenic activity. Nonetheless, the procedure suffers immeasurably as a topical screen, for it ignores, as the author acknowledged, several critical topical parameters, such as drug-vehicle relationships and percutaneous absorption. This defect is most prominent in the results (101) showing triamcinolone to be approximately 40 times as potent and 6α-methylprednisolone to be 20 times as effective as hydrocortisone. The actual topical potencies of these compounds relative to hydrocortisone are more nearly 1:1 for triamcinolone (20) and 5:1 for 6α-methylprednisolone (20, 49, 50).

The weak anti-inflammatory activity of several highly aqueous-soluble corticosteroid esters in the intradermal procedure confirms the results reported earlier by Goldman (87). Likewise, the higher potencies of the 21-acetate esters of several compounds are in agreement with previous reports on intradermal studies (8, 11). This acetate factor should never be ignored when interpreting comparative topical and intradermal data. Rather, the higher potencies of the acetates should tend to cancel when the standard for comparison is the corresponding ester derivative.

Tetrahydrofurfurylalcohol (THFA) Assay.—This method has been employed successfully by Schlagel (20) in more than 150 separate assays involving over 4,000 volunteer subjects. A similar but not identical procedure also has been utilized extensively by Brunner (102) and to a lesser degree by other investigators (103).

The technique consists of the production of erythema under a patch by an 18-hr. application of THFA, an irritant alcohol. Anti-inflammatory activity of the corticosteroids is quantitated on the basis of their ability to reduce or inhibit the erythema when they are dissolved in THFA and applied simultaneously with the irritant.

Ten treatment patches usually are applied. One serves as a control, three represent different concentrations of a standard, and the remaining six treatments involve multiple doses of two different test compounds.

Thirty subjects are required for a reliable dose-response assay, and three such assays are necessary to establish a precise figure on potency. Criticism of the need for this many subjects for quantitative results is not justified. The literature is replete with erroneous conclusions based on too few tests or subjects for obtaining reliable and quantitative answers.

Some of the advantages of the THFA procedure are: (*a*) the test compounds are applied topically which conforms to the route employed clinically; (*b*) the assay end point is erythema, a major characteristic of inflammation; (*c*) the technique is performed easily and rapidly; and (*d*) the assay was designed in a manner to account for the known variations in topical responses and to permit quantitative evaluations of the results by recognized statistical procedures.

TABLE II.—COMPARISON OF TOPICAL ANTI-INFLAMMATORY CORTICOSTEROID POTENCIES BY AN EXPERIMENTAL AND CLINICAL METHOD

Compd.	Potency In THFA Procedure with S. E.	Potency By Clinical Evaluations
Hydrocortisone	1.0	1
Cholesterol	0	0
Cortisone	0.04	Not detectable
Triamcinolone	1.0 ± 0.2	Not reported
Prednisolone	1.8 ± 0.5	2
6α-Methylprednisolone	4.7 ± 1.3	5
9α-Fluorohydrocortisone	9.0 ± 2.4	10
Dexamethasone	19.0 ± 5.1	10
Fluorometholone	42.0 ± 11.0	40
Triamcinolone-16,17-acetonide	33.0 ± 10.0	40

The major disadvantages of the method are that the compounds are tested in solution rather than in their respective vehicles, and the subjects can be used safely and reliably only once, due to more pronounced and variable responses to the irritant on repeat applications.

A number of the corticosteroids evaluated in the THFA assay by the author are shown in Table II. This table presents the potencies determined by the experimental procedure and gives the corresponding clinical potencies in so far as the latter figures can be ascertained from various clinical reports which will be discussed later in this review.

As can be deduced from the data in Table II, generally good agreement exists between the experimentally and clinically determined values. Absolute agreement is not necessary, for the objective of the experimental method primarily is to focus on a reliable order of potency, so that drug concentrations selected for clinical evaluation will more likely produce the desired therapeutic activity. Otherwise, expensive and cumbersome clinical probing studies are necessary.

It should be emphasized that the topical anti-inflammatory potencies of the corticosteroid 21-esters, determined in the experimental assay, differ from the potencies of the corresponding corticosteroid free alcohols. The highly aqueous-soluble esters display only 0.1 or less of the activity of their free alcohols, while the less soluble 21-acetates are three to five times as potent as their parent compounds. While this problem can be avoided experimentally simply by using an

identical ester for the standard corticosteroid, the question of which forms of the corticosteroids provide the greatest therapeutic utility remains to be determined by additional and more definitive clinical evaluation.

The fact that the experimentally determined potency differences of the esters are not caused by disparities in cutaneous corticosteroid penetration was shown by recovery experiments wherein the quantity of corticosteroid remaining at treatment sites was determined following application of the compounds according to the standard assay procedure (20). The highly soluble and least potent hydrocortisone hemisuccinate and the least soluble but most potent hydrocortisone 21-acetate were absorbed to the same extent as hydrocortisone free alcohol. The relatively high absorption, amounting to approximately 35% for each compound, was likely due to the use of an organic solvent as the irritant-vehicle and to the use of occlusive dressings over the treated areas.

Perhaps the general apathy regarding the potential limitations and virtues of the corticosteroid esters in clinical situations results from earlier studies showing approximate therapeutic equivalency for hydrocortisone and hydrocortisone 21-acetate (104). This somewhat negative attitude may be abandoned soon as the result of recent reports showing markedly altered topical potencies of betamethasone by esterification at the 17 and 21 positions in the molecule (68, 105).

Topical Vasoconstrictor Procedures.—The cuanetous vasoconstrictive property of topically applied anti-inflammatory corticosteroids has been utilized by several researchers for the evaluation of these agents. Apparently, only the newer more potent corticosteroids are capable of producing consistent vasoconstriction in normal unbroken skin, while all of the commercially available compounds are active when applied to abraded skin or when applied to healthy skin under occlussive dressings (53). The increased activity on abraded skin results from removal of the major barrier to percutaneous absorption (106). Occlusive dressings increase drug penetration as the result of increased temperature and increased hydration of the skin (107).

Plastic Tape Stripping Method.—The standardized trauma and subsequent inflammation induced in the skin by removal of the horny layer with repeated application and removal of Scotch tape strips (108, 109) was utilized first by Wells (110) to evaluate the topical anti-inflammatory action of hydrocortisone. He found hydrocortisone effective in allaying the erythema surrounding the stripped areas, beginning after about 4 hr. and becoming maximal after 12 hr. under the conditions of the experiment.

This technique was expanded by Heseltine (111) into an assay for comparing the activity of various concentrations of triamcinolone-16,17-acetonide with hydrocortisone. Lotions containing the corticosteroids were applied to segments of an elongated stripped area on the forearm. The inhibitory effects of the corticosteroids on the vascular effects of the epidermal stripping (112) were determined at various intervals after the drugs were applied.

The results indicated that 0.025% and 0.1% concentrations of triamcinolone-16,17-acetonide were equivalent in activity and more effective at 2 hr. and 6 hr. posttreatment than a 1.0% concentration of hydrocortisone and a 0.01% concentration of the acetonide derivative. Hydrocortisone at 1.0% had activity similar to that of 0.01% triamcinolone-16,17-acetonide. The inhibitory effects from the triamcinolone-16,17-acetonide preparations also appeared to persist longer than those produced by hydrocortisone. Responses to the latter compound began to fade 2 or 3 hr. after a single application.

Though these experimentally determined comparisons agree with certain clinical data on these agents (113), the validity of the procedure with other corticosteroids remains to be determined. Some skepticism of the method is justified on the basis that the major barrier to absorption has been removed. The results also may have been different if hydrocortisone 21-acetate had been used as the standard. While both hydrocortisone and the acetate ester are available as marketed products, the latter appears to have greater activity in several experimental procedures, including vasoconstrictive methods (20, 53, 101).

Several important advantages of Heseltine's procedure as a screening tool should not be overlooked. It is simple and rapid. Most importantly, the corticosteroids may be compared by application in their respective vehicles.

Occlusive Dressing Techniques.—The clinical observation that treatment of dermatoses with corticosteroids under Saran Wrap produced pallor of the lesion and of the surrounding normal skin suggested to McKenzie (70) that vasoconstriction might be used as an index of percutaneous corticosteroid absorption. This stimulus led to several fruitful absorption studies by McKenzie (53, 70) and to a comparative study of the activities of numerous betamethasone esters by the same investigator (114).

In the absorption studies, various tenfold dilutions of the corticosteroids as solutions or suspensions in 95% ethanol were applied for 16

hr. to different sites on the forearms of volunteers. After solvent evaporation, the areas were protected by covering with perforated metal guards (open method) or occlusive dressings. Vasoconstrictive end points were evaluated 1 hr. after removing the protectors or dressings.

McKenzie reported that the 21-acetates were more effective vasoconstrictors than the parent alcohols, while the highly aqueous-soluble phosphate and hemisuccinate ester salts usually were less effective (53). Compounds with less than tenfold differences in potency could not be distinguished easily because these were the minimum dilutions employed. Comparative vasoconstriction produced under the metal guards and under occlusion with Saran Wrap indicated that occlusive dressings increased the absorption of hydrocortisone 21-acetate, prednisolone 21-acetate, 6α-methylprednisolone 21-acetate, dexamethasone, 9α-fluorohydrocortisone 21-acetate, and flurandrenolone, triamcinolone, and fluocinolone 16,17-acetonides one hundredfold and prednisolone alcohol only tenfold.

These initial studies culminated in the development by McKenzie (114) of a more sophisticated and well-controlled assay for the comparative evaluation of a large number of esters of betamethasone. The results indicated that betamethasone 17-valerate, on the basis of vasoconstrictive activity under occlusive dressings, was 4.5 times as potent as betamethasone alcohol and 3.6 times as potent as fluocinolone 16,17-acetonide. Early clinical trials with betamethasone 17-valerate confirmed a high potency for this new derivative (68). However, the 0.1% concentration at which it was evaluated clinically against standard products must be considered appreciably high on the basis of the high comparative potency determined in the experimental assay. Comparative studies with additional concentrations are needed for more precise delineation of the clinical efficacy of this new ester.

Perhaps the most exciting and thought-provoking results derived from the vasoconstrictive procedures were the discovery and proof by Vickers (115) that a reservoir exists in the stratum corneum for corticosteroids after they have been applied under occlusive dressings. This phenomenon would appear to account, secondarily to increased penetration, for the increased efficacy of the topical corticosteroids when the occlusive regimen is employed (70).

The existence of such a reservoir was postulated earlier by Malkinson (27) to account for the prolonged urinary excretion of hydrocortisone-4-C^{14} following a single topical application. The phenomenon was revealed serendipitously to Vickers when skin sites blanched by corticosteroids applied previously under Saran Wrap became constricted again when different sites were treated with corticosteroid, and the whole area, including the original patch sites, was occluded for a second experiment. With the two corticosteroids studied, the acetonides of triamcinolone and fluocinolone, the reservoir, depicted by vasoconstriction after repeated occlusion, persisted for from 3 to 15 days in the subjects tested.

Proof that the corticosteroid reservoir was confined essentially to the stratum corneum was provided ably by results showing lack of a reservoir following intradermal corticosteroid injections or after applications to stripped skin. The established reservoir could be abolished also by subsequent stripping of the area of treatment. The results from present studies by Vickers dealing with several factors, which may explain the wide variation in the duration of the reservoir, are awaited with much interest.

The fascinating data from the vasoconstrictive studies and assays conducted thus far focus attention anew on the important relationships between the physicochemical properties of the corticosteroids and their topical potencies and activities. The older, more polar, more aqueous-soluble corticosteroids, particularly the 21-alcohols and the highly aqueous-soluble ester salts, are less potent and less effective than the newer, highly substituted, less polar, and less soluble corticosteroids, as determined both in the vasoconstrictive procedures and clinical trials. Furthermore, simple esterification with various monobasic acids at C-21 and at C-17 tends to increase topical anti-inflammatory activity over that possessed by the parent hormones, apparently as the result of physicochemical changes in the direction of decreased polarity and aqueous-solubility and increased organic/aqueous partition coefficients (68, 114).

Careful scrutiny of the data also suggests that considerable care must be taken with any attempt to extrapolate experimentally determined corticosteroid potencies for predicting clinical efficacy. For example, certain physicochemical factors, such as high aqueous solubility, which depress experimentally determined potency may tend to enhance clinical efficacy by rendering more corticosteroid molecules available for absorption when the compounds are applied in microcrystalline form in a variety of vehicles. Or this efficiency-promoting solubility or dissolution rate factor may in turn be opposed to a lesser or

greater extent by the greater penetration and tissue retention of the less polar derivatives.

A final comment worthy of note involves McKenzie's (53) interpretation of the role of percutaneous absorption as it applies to the increased activity of the corticosteroids when applied under occlusive dressings. He concluded correctly in studies reported as absorption experiments (53, 70) that absorption for a number of compounds under occlusive bandages was increased one hundredfold over absorption after standard procedures of application. However, his conclusion, based on vasoconstriction rather than direct absorption measurements, that the corticosteroid acetates are better absorbed and the aqueous-soluble ester salts more poorly absorbed than their parent alcohols may be incorrect or an oversight in data interpretation. It seems just as feasible that the observed differences in vasoconstrictive activity may be due to different degrees of tissue retention subsequent to penetration of the horny layer. Accordingly, the most effective 21-acetates may present the tissues with the highest corticosteroid levels subsequent to passing the horny barrier. The least potent but most soluble ester salts doubtlessly are cleared from the tissues more rapidly, while the corticosteroid alcohols likely possess both intermediate clearance and retentive values. Thus, the question of the relative importance of absorption and retention for activity of topical corticosteroids will not be settled until absorptive-time data are available for a number and variety of the compounds. The recent absorption studies by Malkinson are a step in this direction (116).

CLINICAL EFFICACY STUDIES

A comparative analysis of the numerous publications dealing with the therapeutic efficacy of the topical anti-inflammatory corticosteroids is extremely difficult. Many of the reports deal with studies designed simply to demonstrate efficacy in a variety of inflammatous dermatoses. The majority of other publications involve the desirable direct comparison of the newer corticosteroids with the popular standard, hydrocortisone. But there are only a few studies comparing efficacy directly among the newer compounds.

Another recognized problem common to many topical clinical studies has been the general poor quality of design, execution, and analysis of data. Only recently have statistical procedures been utilized (117, 118). However, even these often complex analyses cannot compensate for the serendipitous collection of data from less than stringent experimental formats.

Aside from the problems attributable to the human element, there are numerous study variables that can be controlled only with extreme difficulty, if at all, by individual investigators. Since the majority of dermatologic subjects are out-patients, variables such as time, duration, and frequency of applications as well as drug quantities applied can be controlled only to an uncertain degree. Environmental factors, such as temperature and humidity, may confound the issue further by independently altering drug absorption and subsequent relief of symptoms. Variations among different patients in their degree of continued contact with the eczematous-inducing irritants and allergens during therapy often may make highly effective formulations appear less potent or inactive. Frequent evaluations of topical therapy are always desirable; yet these are extremely troublesome to the busy physician and are even more impracticable and revolting to the ambulatory patient. Thus, it appears that a happy medium must be met between the requirements for a rigidly designed and executed study protocol and the less desirable but practical and feasible procedures utilized in the past.

Despite many complications, in addition to those enumerated, the conclusions from several independent clinical evaluations with a given corticosteroid generally establish a trend in therapeutic efficacy which becomes reasonably well corroborated by extended studies and wide therapeutic use. It is on this somewhat broader view that the clinical papers on the topical corticosteroids will be scrutinized for this review. With this rather submissive approach, it must be emphasized that some conclusions have had to be based on isolated data and on general impressions obtained from considering the topical corticosteroid field as a whole.

Hydrocortisone Preparations.—The original and skillfully conducted explorations by Sulzberger and Witten (9, 10, 119, 120), on the therapeutic utility of various hydrocortisone preparations, established several cardinal concepts and guidelines for topical anti-inflammatory therapy that remain fixed and unchallenged even today. These illustrious clinicians utilized the method of symmetrical paired comparison (121) for the evaluation of various concentrations of hydrocortisone and hydrocortisone 21-acetate in different vehicles and in a variety of disease entities. Some of the conclusions from these investigations are summarized here. (*a*) Hydrocortisone and hydrocortisone 21-acetate formulations were essentially equivalent in therapeutic efficacy. (*b*) With few

exceptions, the 5% preparations were only a little more effective than the 2.5% ointments; the 2.5% formulations were more effective than the 1.0% preparations; and 0.5% and 0.25% concentrations were sometimes effective, although generally less so than the 1.0% ointment. (*c*) Objective and subjective relief of symptoms were usually obvious within 24 hr. after initiation of therapy. (*d*) The hydrocortisone preparations were highly effective in a variety of inflammatous skin diseases, especially the eczematous dermatoses. (*e*) Lesions involving certain areas with relatively thin skin, such as eyelids, in and around ears, on the scrotum and penis, on the perianal and perivulvar areas, and on the nipple and areolar responded more favorably than lesions of the same dermatoses involving other areas. (*f*) The hydrocortisone ointments did not irritate, produce allergic sensitization, stain the skin, lose effectiveness with continued application, or produce evidence of systemic side effects, even on long-term administration. (*g*) The corticosteroid formulations were highly acceptable to the patients, for they were not uncomfortable or unsightly when rubbed in well.

The results and conclusions published by Sulzberger and Witten were verified in most every respect by several other early and fairly extensive clinical trials by Alexander (122), Friedlaender (123), Robinson (124), and Frank (125). These documentary studies also indicated the advantage of the addition of antibiotics, particularly neomycin, to help control secondary pyogenic infections (123, 124). Of some significance was Frank's conclusion (125) that, although hydrocortisone and hydrocortisone 21-acetate were both effective topical anti-inflammatory agents, hydrocortisone as the free alcohol appeared somewhat more effective in his series of cases. Sulzberger and Witten (121) observed the same trend in one of their studies; however, they apparently judged correctly that the slightly larger number of treatment preferences favoring the free alcohol were not significant in view of the preponderance of cases where the efficacy of the two compounds was indistinguishable. It is unfortunate that the question of the relative quantitative efficacy of hydrocortisone and hydrocortisone 21-acetate remain incompletely answered, for, in essence, these compounds have been used interchangably as standards in the clinical evaluation of the newer synthetic corticosteroids.

Hydrocortisone Versus Synthetic Analogs.—An attempt will be made in this section to review the clinical studies involving comparisons of the topical anti-inflammatory efficacy of the major corticosteroids with the usual standards, 1.0% concentrations of hydrocortisone or hydrocortisone 21-acetate. Publications dealing with direct comparisons among some of the newer analogs also will be discussed in the latter portion of this review.

Prednisolone.—An evaluation of the therapeutic utility of prednisolone is appreciably complicated by the fact that topical preparations are available containing prednisolone as the free alcohol, as the 21-acetate, and as the highly water-soluble 21-sodium phosphate form. A prednisolone aerosol product also is marketed. Nevertheless, the results of the majority of the clinical trials comparing the standard creams and ointments suggest rather convincingly, but without statistical proof, that the fourfold greater potency of oral prednisolone over hydrocortisone does not hold for topical therapy. Rather, most of the studies indicate that prednisolone possesses at best only a twofold increase in topical efficacy over hydrocortisone. Thus, Sloane (126) reported that prednisolone was slightly more effective than prednisolone 21-acetate, but that both preparations at concentrations of 0.5% were only equivalent to 0.5% hydrocortisone alcohol. Smith (127) likewise found 1.0% hydrocortisone somewhat more efficacious than 0.5% prednisolone, while Frolow (128) concluded that prednisolone was no more effective than double its concentration of hydrocortisone. The results from similar comparative studies by Zimmerman (129) appear to agree with those above, although this investigator concluded that 0.5% prednisolone may be slightly superior to 1.0% hydrocortisone.

In contrast to the above data, both Borrie (130) and Inman (131) reported that 0.25% prednisolone was equivalent in clinical utility to 1.0% hydrocortisone. However, Borrie treated alternate patients rather than using the symmetrical paired comparison procedure, while Inman employed hydrocortisone 21-acetate rather than hydrocortisone as the standard. He also used different vehicles for the two corticosteroids under comparison and applied the compounds under occlusive dressings over extensive periods of time. Such prolonged periods of maintenance therapy tend to mask therapeutic differences which often may be detected only in the early stages of therapy (132).

While these data on prednisolone are only pseudoquantitative at best, the trend seems definitely in favor of the general conclusion that 0.5% prednisolone preparations are approximately equivalent in clinical efficacy to 1.0% hydrocortisone products.

9α-Fluorohydrocortisone.—The substitution of fluorine for hydrogen in the 9α-position of hydrocortisone increased the oral potency eight- to tenfold with respect to such parameters as eosinopenic and hyperglycemic potencies and ASTH-suppressing, nitrogen-wasting, and anti-inflammatory activities (36, 133, 134). The topical anti-inflammatory action was increased also by a factor of 10 on the basis of studies by Witten (135), Robinson (136), and Lubowe (137), showing that 0.1% and 0.25% concentrations of 9α-fluorohydrocortisone 21-acetate were, in the majority of paired comparisons, equivalent in activity to 1.0% and 2.5% concentrations of hydrocortisone or hydrocortisone 21-acetate, respectively. None of these investigators observed any untoward systemic symptoms when the topical preparations were applied from two to four times daily for periods ranging from a few weeks to several months. However, both Livingood (138) and Fitzpatrick (139) demonstrated sufficient percutaneous absorption, particularly from the above concentrations of 9α-fluorohydrocortisone 21-acetate in lotion form, to induce obvious states of clinical edema. More definitive studies by these investigators indicated that appreciable sodium retention occurred, as judged from weight gain and urinary excretion values. They concluded that this newer corticosteroid should be employed with care on inflammatous dermatoses and that it should be avoided in patients with hypertension, toxemia of pregnancy, congestive heart failure, and nephritis.

Although there are no direct comparisons of the relative percutaneous absorption of hydrocortisone and 9α-fluorohydrocortisone, the marked electrolyte-regulating potency of the latter compound in dogs and man of about 125 times that of hydrocortisone (26) accounts for the sodium retention and edema following topical therapy with this agent. Products containing 9α-fluorohydrocortisone are used little today because of the availability of other effective and safer corticosteroids.

Fluorometholone.—Like many of the synthetic analogs of hydrocortisone shown in Fig. 2, fluorometholone contains several chemical substituents which increase the intrinsic anti-inflammatory activity greatly (26). However, it differs from all other topical corticosteroids by possessing the C-21-deoxy configuration which, when the compound is administered systemically, apparently renders the side chain highly labile to metabolic inactivation by hepatic enzymes (42, 43). Such vulnerability to biologic inactivation is probably much reduced in the skin, as shown by incubation studies (33, 48). Futhermore, compounds like prednisolone and dexamethasone owe much of their increased systemic activity to protection against enzymatic reduction (59–62), while their topical potencies are increased to a lesser degree, most likely as the result of the less significant role of inactivating enzymes in the skin.

The combination of high intrinsic potency and low systemic activity imparts in fluorometholone a preferential topical utility whereby a low concentration of applied hormone can produce effective topical therapy with reduced risks of systemic side effects following percutaneous absorption. The preferential action of fluorometholone is of the order of 20 to 1, on the basis of a topical potency of 40 and an oral potency of 1 to 2 relative to hydrocortisone (20, 35–40). Consequently, when the clinically equivalent concentrations of 0.025% fluorometholone and 1.0% hydrocortisone 21-acetate are utilized in the treatment of inflammatous dermatoses, much less fluorometholone is available for absorption and subsequent systemic effects. Furthermore, the percentage of applied corticosteroid absorbed is similar for fluorometholone and hydrocortisone 21-acetate, as determined in studies under preparation for publication by the author.

The clinical efficacy of 0.025% fluorometholone preparations has been demonstrated in several therapeutic studies (38, 39, 140). Williams (37) and Wyman (40) also have shown 0.025% fluorometholone to be equivalent in clinical activity to 1.0% hydrocortisone 21-acetate preparations.

Dexamethasone and Betamethasone.—These highly substituted corticosteroids differ in structure only with respect to the orientation of the 16-methyl group, which is in the α-position in dexamethasone and the β-position in betamethasone. Both compounds are highly potent and equally effective oral anti-inflammatory agents (26, 62). Only meager data are available on their relative topical activities.

Several investigators have conducted non-comparative studies, the results of which indicate that 0.1% dexamethasone (141, 142) and 0.2% betamethasone (143) possess moderate degrees of topical anti-inflammatory efficacy. Vickers (64) determined in a well-controlled double-blind evaluation that 0.1% betamethasone 21-phosphate and 1.0% hydrocortisone were equally effective, while betamethasone 21-phosphate and betamethasone were also equally efficacious. These results differ from those obtained in a smaller study by Inman (144), who reported no differences in the topical effectiveness of 0.04% dexamethasone and 1.0% hydrocortisone lotions. Accordingly, additional clinical comparisons are

necessary before any concrete conclusions can be made regarding the topical merits of these two 16-methylated corticosteroids relative to hydrocortisone.

6α-Methylprednisolone 21-Acetate.—This was one of the first compounds among a newer group of synthetic corticosteroids to be evaluated and marketed at topical concentrations which frequently produced more dramatic and superior therapeutic responses than 1.0% hydrocortisone formulations. Both Kile (145) and Goldberg (49) reported, for example, that problem cases that had failed to respond to previously applied corticosteroids often responded with gratifying results to topical 6α-methylprednisolone 21-acetate. Several other investigators (146–148) also have obtained excellent therapeutic results using 0.25% 6α-methylprednisolone 21-acetate in a unique cream base which approximates the composition of normal human skin liquids (148, 149).

Perhaps the most definitive demonstration of the therapeutic superiority of 0.25% 6α-methylprednisolone 21-acetate over 1.0% hydrocortisone 21-acetate was the extensive double-blind study conducted by Kaufman (50). From his direct comparisons in 477 patients with corticosteroid responsive dermatoses, 69% of the cases responded better to the 6α-methylated corticosteroid, 20% responded equally to the two preparations, while only 11% had better results with the standard product.

The more pronounced therapeutic efficacy of the 6α-methylated compound was confirmed also in a double-blind evaluation by Olansky (150), wherein a 1.0% 6α-methylprednisolone 21-acetate formulation was compared to a preparation containing 2.5% hydrocortisone 21-acetate. Of the 31 patients treated, several had severe and resistant dermatoses. The 6α-methylprednisolone 21-acetate preparation was consistently more effective in 28 of these patients. These data suggest that the maximum effective concentration of hydrocortisone 21-acetate is greater than 2.5%, or the plateau for this corticosteroid is lower than that for 6α-methylprednisolone 21-acetate.

Like the 1.0% and 2.5% concentrations of hydrocortisone, the 0.25% concentration of 6α-methylprednisolone 21-acetate is available as a general-purpose product, while a 1.0% concentration of the latter corticosteroid is marketed for initiating therapy, especially in the severe or resistant inflammatous dermatitides.

Triamcinolone-16,17-acetonide.—This interesting corticosteroid was the first of three different acetonide derivatives to be evaluated topically and shown to exhibit anti-inflammatory qualities superior to topical hydrocortisone. The higher order of topical activity must be attributable to the more ideal physicochemical properties imparted by the 16,17-acetonide configuration, for the parent compound, triamcinolone, is only equal to hydrocortisone topically (20). Futhermore, the acetonide derivative and triamcinolone have equivalent oral anti-inflammatory potency (151).

The majority of clinical reports with this agent involve double-blind comparisons of the topical efficacy of 0.1% triamcinolone-16,17-acetonide and 1.0% hydrocortisone in ointment, cream, and lotion vehicles. All of these publications indicate some degree of superiority for the acetonide preparation in the majority of cases evaluated (21, 52, 152, 153). The superiority of the 0.1% triamcinolone-16,17-acetonide generally entails a more rapid onset of relief of symptoms and an earlier or more complete clearing of lesions, especially in the more severe dermatoses. The results of clinical evaluations with other concentrations of triamcinolone-16,17-acetonide will be discussed later in this review.

Fluocinolone-16,17-acetonide.—A perusal of the corticosteroid structures shown in Fig. 2 will indicate that this compound differs from the acetonide of triamcinolone only by the addition of a fluoro substituent at the C-6 position of the B-ring. Although the 6α-fluoro modification increases intrinsic anti-inflammatory potency so that fluocinolone-16,17-acetonide is more potent milligram per milligram than triamcinolone-16,17-acetonide, the topical therapeutic effectiveness of these two acetonides appears to be quite similar when they are compared at their usual marketed concentrations. Thus, triamcinolone-16,17-acetonide at a concentration of 0.1% is apparently as efficacious as the more potent fluocinolone-16,17-acetonide, the latter at the lower standard concentration of 0.025% (154).

There also are available several reports describing the excellent therapeutic responses produced by 0.025% fluocinolone-16,17-acetonide in the treatment of a variety of inflammatous dermatoses (155–162). The results of these efficacy evaluations have been confirmed by more quantitative direct comparisons which attest to the therapeutic advantages of 0.025% fluocinolone-16,17-acetonide over 1.0% hydrocortisone in a high proportion of the patients under evaluation (163–165).

Perhaps some additional support for the excellent topical activity of 0.025% fluocinolone-16,17-acetonide is provided by the results of two studies showing appreciable effectiveness in dermatoses resistant or refractory to prior therapy with other

topical corticosteroids (166, 167). Although the authenticity of these observations cannot be questioned, the results showing effectiveness in cases previously treated unsuccessfully with 0.1% triamcinolone-16,17-acetonide places a great deal of suspicion on the relevancy of this type of evaluation. This suspicion is indicated clearly on the basis of other direct comparisons showing 0.1% (154) and even 0.025% triamcinolone-16,17-acetonide (168) to be equivalent therapeutically to 0.025% fluocinolone-16,17-acetonide.

Flurandrenolone-16,17-acetonide.—The results of topical efficacy studies with the standard 0.05% concentrations of flurandrenolone-16,17-acetonide in ointment, cream, and lotion vehicles support the conclusion that this corticosteroid is quite effective in the alleviation of inflammation of the skin (103, 169, 170). Double-blind, symmetrical, paired comparisons have indicated that 0.05% flurandrenolone-16,17-acetonide, like the two acetonides discussed previously, is somewhat superior in therapeutic efficacy to preparations containing 1.0% hydrocortisone (171–173). This superiority usually appeared in the form of more rapid and complete relief of symptoms. With continued treatment, the tendency was for the flurandrenolone-16,17-acetonide and hydrocortisone-treated areas to show the same degree of improvement (172). This phenomenon, whereby higher concentrations of a given corticosteroid or the more potent of two different compounds tend to produce faster or more dramatic initial benefit with subsequent leveling-off of therapeutic differences as the lesions subside in severity, is observed commonly with all the corticosteroids employed topically. It is a major variable in comparing the efficacy of different corticosteroids or in evaluating different concentrations of the same compound.

This portion of the review dealing with the clinical evaluations of the individual corticosteroids may be concluded with a summary which places all of the major topical corticosteroids into two categories. One group contains those products which may be considered similar in clinical utility to 1.0% concentrations of hydrocortisone or hydrocortisone 21-acetate. Such products are those containing 0.5% prednisolone, 0.1% 9α-fluorohydrocortisone, 0.1% dexamethasone and betamethasone, 0.025% fluorometholone, 0.25% dichlorisone, and 0.5% hydrocortisone diethylamino-acetate hydrochloride (174–177). The second category includes those products which, in a reasonable proportion of the patients treated, produce anti-inflammatory responses somewhat superior to those engendered by 1.0% hydrocortisone. These products are 0.25% 6α-methylprednisolone 21-acetate, 0.05% flurandrenolone-16,17-acetonide, 0.1% triamcinolone-16,17-acetonide, and 0.025% fluocinolone-16,17-acetonide.

Many products also contain less than 1.0% hydrocortisone. These are utilized mostly in mild conditions and for maintenance therapy or are designed as special purpose products containing tars, antiseptics, or other therapeutic agents. For severe inflammatous dermatoses, preparations are available containing 1.0% 6α-methylprednisolone 21-acetate or 0.5% triamcinolone-16,17-acetonide. The 6α-methylprednisolone 21-acetate product has been shown to be superior to 2.5% hydrocortisone 21-acetate (150). The superiority of 0.5% triamcinolone-16,17-acetonide over 0.1% concentrations of the same compound also has been demonstrated to occur in about one-third of the cases compared (178, 179).

CLINICAL COMPARISONS AMONG THE NEWER CORTICOSTEROIDS

With the advent of several corticosteroids more effective than hydrocortisone, some recent emphasis has been placed on direct comparisons among the newer agents as opposed to indirect evaluations using 1.0% hydrocortisone as the reference standard. Many of these studies have been better controlled and analyzed by established statistical procedures.

Grekin (180) has shown by a double blind well-controlled investigation that 0.25% 6α-methylprednisolone 21-acetate and 0.05% flurandrenolone-16,17-acetonide possess equivalent therapeutic efficacy. The same strength of the latter compound has been shown to be equal in effectiveness to 0.1% triamcinolone-16,17-acetonide by Bluefarb (181).

In other studies, the results of which pertain to the 16,17-acetonides of the corticosteroids to be discussed, Readett (182) has determined that the 0.025% and 0.1% concentrations of the triamcinolone derivative could not be distinguished clinically. The controlled studies by Peterkin, indicating equivalent effectiveness for the 0.1% and 0.025% concentrations of the derivatives of triamcinolone and fluocinolone, respectively, (154) and the studies by Orentreich (168), showing no therapeutic differences between 0.025% strengths of the triamcinolone and fluocinolone acetonides, are in agreement with Readett's conclusion.

Since the above studies were reasonably standardized and controlled evaluations, their lack of ability to discriminate between even fourfold differences in the concentrations of the same

compound emphasizes the extremely poor precision and quality of clinical trials with topical corticosteroids. The general lack of quantitative data stresses the need for even more stringent study protocols for controlling the numerous variables and for making the results of studies more reliable and useful clinically.

OCCLUSIVE DRESSING THERAPY

The most recent significant advance in dermatologic therapy has been the increased therapeutic benefits derived from applying the anti-inflammatory corticosteroids under occlusive pliable plastic dressings like Saran Wrap or Handi-Wrap. With this procedure, topical corticosteroid preparations, which may be ineffective by the conventional method of application, often produce dramatic improvement or even complete clearing of otherwise recalcitrant dermatoses.

The plastic film technique was reported originally by Garb (183), who employed it as a means of keeping podophyllin ointment in place. Subsequently, it was adopted for highly successful topical corticosteroid therapy by Sulzberger (184) and Scholtz (185) and since has been utilized extensively by numerous dermatologists. Gratifying results have been observed in the treatment of resistant or stubborn lesions of diseases, such as chronic eczemas, lichen simplex chronicus, hypertrophic lichen planus, generalized exfoliative psoriasis, and especially torpid plaques of psoriasis.

The markedly potentiated anti-inflammatory responses obtained with occlusive corticosteroid procedures occur primarily as the result of increased penetration of the corticosteroids. For example, McKenzie's observations indicate that the use of Saran Wrap occlusive dressings increases the penetration of various corticosteroids by a factor of one hundredfold (53, 70). This enhanced penetration not only supplies higher concentrations of drug to the lower epidermis and corium but also produces a reservoir of corticosteroid in the stratum corneum (115). Thus, occlusive dressings provide a form of surface depot therapy.

The increased penetrability of the corticosteroids when applied under plastic bandages appears to be due to the elevated temperature and humidity which, with the dammed-up sweat and sebum, macerates the epidermal surface and the material in the follicles (70, 184, 186, 187). The integrity of the epidermal barrier, thus disrupted, becomes more permeable. Other probable factors are (*a*) confinement of the corticosteroids to the site of application, (*b*) facilitated diffusion of the drug in the vehicle, and (*c*) greater solubility and faster dissolution rate of the microcrystalline corticosteroids in the warm humid environment under the occlusive dressings.

The epidermal reservoir of corticosteroid, once established by increased drug penetration, most likely is maintained after removal of the plastic film (115) as the result of dehydration of the stratum corneum and subsequent precipitation of the corticosteroid in the desiccated keratin.

Occlusive therapy with the corticosteroids has several disadvantages (188). Frequent complications developing under the dressings are folliculitis, miliaria rubra, pyodema, atrophic striae, absorption-id reactions, foul odor, and reactions to the tape and plastic films (189–191). A major disadvantage of considerable concern is systemic corticosteroid effects resulting from enhanced percutaneous absorption. While this is scarcely a problem with the customary mode of corticosteroid application (192–195), with the exception of 9α-fluorohydrocortisone (138, 139, 196), very definite depression of the pituitary-adrenal axis following occlusive corticosteroid therapy has been demonstrated by several investigators (197–199). This impairment of adrenal cortical function could be quite hazardous in subjects exposed to stress as in physical injury, infections, and surgery.

Although these local and systemic complications following occlusive corticosteroid therapy should never be ignored or underestimated, the frequently dramatic responses obtained in torturesome and recalcitrant dermatoses by many investigators using this mode of therapy indicate that the procedure is necessary and justified when employed with the utmost judiciousness. However, more studies under a variety of experimental conditions are necessary for establishing permissive limits of percutaneous absorption and the ultimate safety and usefulness of occlusive therapy.

References (200–213) reflect the recent flurry of interest in the use of the surface depot type of topical corticosteroid administration. These reports may be consulted for details regarding the nature of the dermatologic entities treated successfully, the variations in the techniques of application of the occlusive dressings, and the comparative clinical effectiveness of various corticosteroids or different corticosteroid concentrations, especially the 16,17-acetonide compounds.

PERCUTANEOUS CORTICOSTEROID ABSORPTION

There have been several reviews of this subject in recent years (53, 70, 116, 214). Barr (106) also discussed the corticosteroids in his review of the general topic of percutaneous absorption.

There are at least two parameters that could alter appreciably corticosteroid absorption that have not been emphasized in the above publications. One of these factors is the important relationship which exists between the concentration of the applied drug and the rate at which it is released from the suspension-type vehicles. Such vehicles are typical of the topical preparations in which the corticosteroids are employed.

As Higuchi (215) has shown, the rate of release of the active agent from the base is proportional to the square root of the concentration, the solubility and diffusion remaining constant for a given corticosteroid. Accordingly, in order to double the rate of drug release, the concentration of the corticosteroid would have to be squared rather than merely doubled. If it is acknowledged that the rate of release of medicament is related closely to availability of drug to the skin, then the concentration-release factor could play a significant role in determining the amount of corticosteroid absorbed and the ultimate therapeutic activity of the preparation under evaluation. This suggests that greater care should be exercised in the selection of the concentration at which the corticosteroids are compared. Otherwise, anticipated therape tic differences may not be realized.

The other variable which complicates studies on percutaneous corticosteroid absorption is the cutaneous vasoconstriction that may be produced, especially by the more potent topically applied compounds. Once vasoconstriction is induced by the corticosteroid molecules absorbed initially, the rate and degree of subsequent absorption may be retarded effectively. This phenomenon has been studied and thoroughly discussed by Malkinson (116).

One of the most interesting and recent contributions to the scanty knowledge of absorption parameters is the report by Stoughton (214) on the influence of dimethylsulfoxide (DMSO) on percutaneous absorption in humans. His studies showed the absorption of fluocinolone-16,17-acetonide to be enhanced about fivefold when incorporated in 10% or 25% DMSO. The increased penetration of the corticosteroid induced by occlusive dressings was not increased further by the use of DMSO in the vehicle.

While it is conceivable that this or similarly acting organic solvents could be utilized in special situations for increasing corticosteroid penetration and enhancing therapeutic responses, it is doubtful whether they would be well tolerated when used routinely on inflammatous lesions. The majority of dermatoses for which the anti-inflammatory corticosteroids are indicated are highly susceptible to the presence in vehicles of even mildly irritating substances. Even with the rather bland vehicles commonly employed, there is a fairly high incidence of stinging and burning, especially upon initial contact of the tender lesions with the corticosteroid preparations. Futhermore, topical corticosteroid therapy must be discontinued because of worsening of the lesions in approximately 3% of the cases tested (216). This is due in most instances to sensitivity of the inflamed lesions to irritating substances in the vehicles employed (217–219).

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Research Articles

Deuterium Isotope Effects in Nonenzymatic Transamination of L-Glutamic Acid

By SONG-LING LIN, MARTIN I. BLAKE, and FREDERICK P. SIEGEL

L-Deuterio-glutamic acid was isolated in pure form from algae grown in nutrient solution containing better than 99 per cent D_2O. The pH profile for the transamination of glutamic acid with pyridoxal shows a peak at pH 4. The presence of acetate buffer increases the reaction rate up to about seven times when compared to the unbuffered system. Protio-glutamic acid reacts about 2.1 times faster than deuterio-glutamic acid. The reaction appears to be general acid and general base catalyzed.

IN BIOLOGICAL systems transamination involves the transfer of an amino group between certain amino and keto acids. The discovery of the occurrence in nature of the aldehyde and amine forms of vitamin B_6 by Snell (1) led to the suggestion that pyridoxal and pyridoxamine may be interconvertible in transamination reactions, and that this vitamin may function as a coenzyme in enzymatic transamination. It was later demonstrated (2) that reversible interconversion between pyridoxal and pyridoxamine may take place by nonenzymatic transamination. The mechanism of the enzymatic and nonenzymatic reactions has been the subject of a review by Snell and Jenkins (3). Pyridoxal catalysis received a thorough treatment in a recent text edited by Snell *et al.* (4).

Blake *et al.* (5) studied the deuterium isotope effect in the transamination reaction of alanine and deuterio-alanine with pyridoxal. The effect of pH, metal ion, and nature and concentration

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