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THE PSORIASIS BIO-ASSAY FOR TOPICAL CORTICOSTEROID ACTIVITY

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Abstract. A semi-quantitative bio-assay method for topical corticoid activity in human skin disease was reported by us in 1967, and a dose–response curve for fluocinolone acetonide was described. The procedure has been refined and shortened, and dose response curves are described for additional compounds: fluocinonide, betamethasone 17-valerate, flurandrenolide, and hydrocortisone. Multiple sites are tested simultaneously utilizing microgram doses, thus precluding the possibility of systemic therapeutic effect or other adverse effects. Results closely parallel known clinical activities, and the method is considered of value to detect active new therapeutic agents.

METHODS AND MATERIALS

In an earlier report (10) we described a dose–response curve obtained with a single compound, fluocinolone acetonide. As then, our test subject is the chronic stabilized psoriatic. In many patients with psoriasis, there will be periods of months or even years when the cutaneous eruption is in a "stabilized" phase. The lesions remain static in size even though there may be some daily fluctuation in the amount of scaling, and there may be minor variations in erythema. No new lesions are appearing and the isomorphic (Koebner) phenomenon cannot be elicited. A pretreatment period of about 2 weeks of observation is desirable to establish that the patient is in this stable phase.

We have limited our subjects to adult males and non-pregnant females, and have accepted no one on either systemic corticoid or methotrexate within the preceding 3 months.

Since our original report, the procedure has been modified, as follows:

1. Test sites are outlined with pre-cut adhesive squares, each 1.1 cm^2. Dermicel® Tape is best for this purpose. It adheres well, and irritation and sensitization are extremely rare.

2. All materials are used on a double-blind coded basis. Positive and negative controls are included in each assay.

3. Multiple sites (12–18) are treated simultaneously in the same patient.

4. Formulation is applied to each test site in a volume of 0.5 minim delivered from an 0.5 cc glass syringe.

5. A square of plastic film (Saran® Wrap) slightly larger than the test site is applied over the area. The medication is spread by light pressure on the film, and the plastic is then sealed to the underlying border. To further secure the dressings we often cover the area with elastic netting.

6. Dressings are applied daily and remain in place 24 hours. The area is cleansed gently with water, followed by acetone. After the skin is dry, the area is photographed and a "reading" made.

7. Results are recorded as follows: 0 = unchanged or less than full involution. + = complete return of epidermis to "normal".

A "normal" epidermis is one in which no scaling is present, no thickening is noted by palpation, texture and skin markings appear normal, and there is no elevation above normal skin level. There may be a residual erythema. This end point is one on which different observers can agree. No attempt is made to estimate degrees of partial improvement. With threshold concentrations there is difficulty in interpretation, i.e., the difference between total clearing and almost total clearing. As it is here that bias may enter and objectivity be impaired, all readings are best made by the same observer.

8. The final reading is made on the fifth day, when the fourth dressing is removed. At each new dressing the entire procedure is repeated, with new adhesive squares applied each time.

9. At the time of each dressing it is essential that sites be carefully marked so that precisely the same area will be treated.

The five-day schedule has evolved from the original schedule (reported in our first publication) of dressings twice weekly, with final reading on day 14–15. Subsequently, a schedule of dressings on days 1, 3, 5, and 8, with final reading on day 10 gave equally consistent results. We have used the 10-day schedule sufficiently to be confident that it is reliable. Experience with the five-day schedule is more limited, but appears to give comparable results.

Wherever possible we utilized commercially available formulated creams. This applied to fluocinolone acetonide 0.01% and 0.025%, flurandrenolide 0.05%, hydrocortisone 0.5% and 1.0%, and betamethasone valerate 0.1%. All concentrations of fluocinonide were in the cream base expected to be available commercially. Other concentrations of other compounds were prepared in an aqueous cream base of stearic acid, propylene glycol, sorbitan monostearate, monooleate, and polyoxyethylene sorbitan monostearate.

Table I. *Results of psoriasis assay*

Number of positive responses/number of sites tested

Concentration (%)	Fluocinolone acetonide	Fluocinonide	Betamethasone valerate	Flurandrenolide	Hydrocortisone
0.00025	1/15	1/15			
0.001	2/15	8/15	3/15	0/15	
0.0025	6/15	10/15	8/15	3/15	
0.005	13/15				
0.01	14/15	14/15	9/14	7/15	0/15
0.025	14/15[a]				
0.05				14/15	
0.1			15/15		
0.5					1/15
1.0					0/15

[a] Not included in potency computations.

RESULTS

Responses are recorded in Table I. The response data were analysed by Finney's (2) probit technique. Each percentage response was transformed to a "probit" unit, and a set of parallel, straight-line dose–response relations was simultaneously fitted to the data by the method of weighted least squares. One dose response line is established for each compound. Assumptions of linearity and parallelism were checked and satisfied. The dose–response lines are depicted in Fig. 1.

Estimated potencies and associated 95% confidence intervals were computed. These are given in Table II. The 95% confidence interval is the interval which with 95% probability brackets the true potency.

DISCUSSION

Animal assay procedures long have been available to quantitate the activities of corticosteroids. Traditional methods (1, 6) have utilized oral or parenteral administration to measure the involutional effect on a corticoid-sensitive organ, such as the thymus gland, or to measure the prevention of an artificial subcutaneous inflammation, as in the case of the granuloma pouch. More recently, animal assays have been developed (11, 13) which employ topical application of the test agent to assess the ameliorative action in an artifically-induced localized inflammation.

From the viewpoint of the clinician, animal assays suffer from two obvious deficiencies. They involve end points in systems other than naturally-

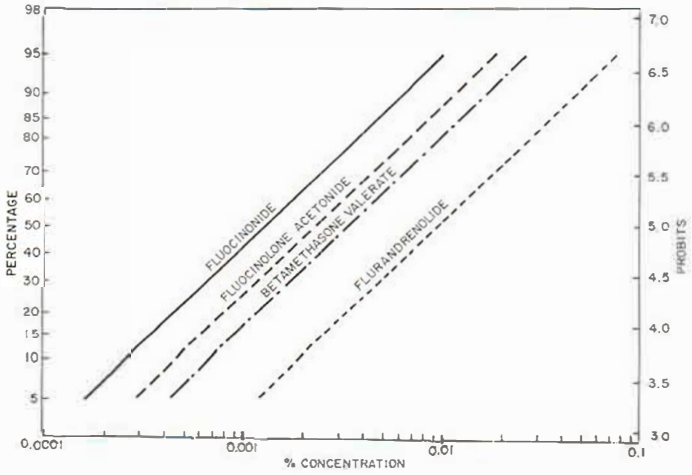

Fig. 1. Dose–response curves for the four steroids tested.

occurring dermatoses. Secondly, they do not involve human skin or anything even closely resembling it.

To overcome the latter objection, efforts have been made by numerous workers over many years to devise topical assays in man. Those assays have concentrated upon the effects of locally-applied corticosteroids to normal skin to produce a physiological effect, such as vasoconstriction, or to influence the course of experimentally-produced pathology. In recent years, the simplicity and reproducibility of the method has led to rather wide use of the vasoconstrictor assay of McKenzie & Stoughton (7). Recently Place and others (8) have modified that assay to permit more precise quantitation and simultaneous screening of large numbers of compounds.

Earlier workers attempted to prevent or ameliorate an induced erythema. Kalz & Scott (5) reported successful use of Grenz ray, and the same workers in another paper (12) discussed experiences with inflammatory responses induced by ultraviolet, mustard oil, or nitric acid. Wells (14) employed the erythema subsequent to skin-stripping to assess corticoid effects. Other forms of inflammation reported successful were the tetrahydrofurfuryl alcohol irritation method of Schlagel & Northam (9) and the croton oil method of Witkowski & Kligman (15), in which anti-inflammatory agents are studied to determine their activity in preventing pustule formation.

Some of the assay systems mentioned above have fallen into disuse because of difficulties in technique or in standardization. Some methods use atypical vehicle systems, such as alcohol solutions, which minimize the prospect of extrapolating test results to clinical expectations. With some methods, the corticosteroid action is preventive rather than therapeutic, materials being applied to normal skin before inflammation is produced, again raising some question about applicability to clinical practice. All of the above bio-assay methods, of course, fail to deal with spontaneously-occurring skin disease.

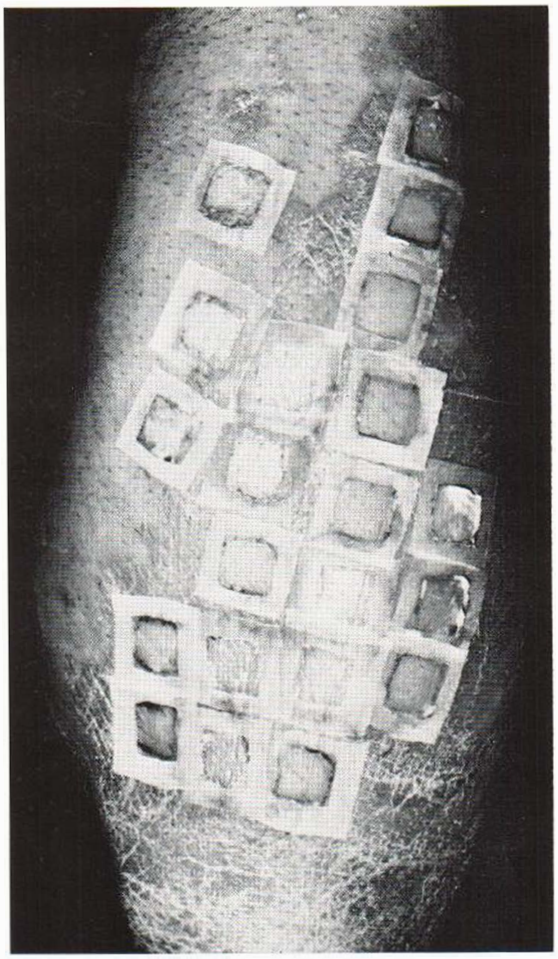

Fig. 2. After 24 hours of treatment, prior to cleansing of sites. Illustrates multiple sites tested simultaneously, and excellent adherence of Dermicel® squares.

Table II. *Relative potencies*

Hydrocortisone was not calculable because of lack of response in this system.

Compound	Potency	95% confidence intervals Lower	Upper
Fluocinolone acetonide	1[a]	—	—
Fluocinonide	1.85	0.98	3.49
Betamethasone valerate	0.69	0.36	1.34
Flurandrenolide	0.24	0.13	0.47

[a] Calculations are based on the arbitrary assignment of 1 to fluocinolone acetonide.

One effort made to anticipate this latter objection was the work of Haxthausen (4), who attempted to block the development of allergic eczematous lesions induced in sensitive patients. Difficulties in the technical procedure may account for the little follow-up given to this work. No attempts were made to quantitate that procedure.

The usual therapeutic assessment of topical corticosteroids is somewhat less than ideal. The use

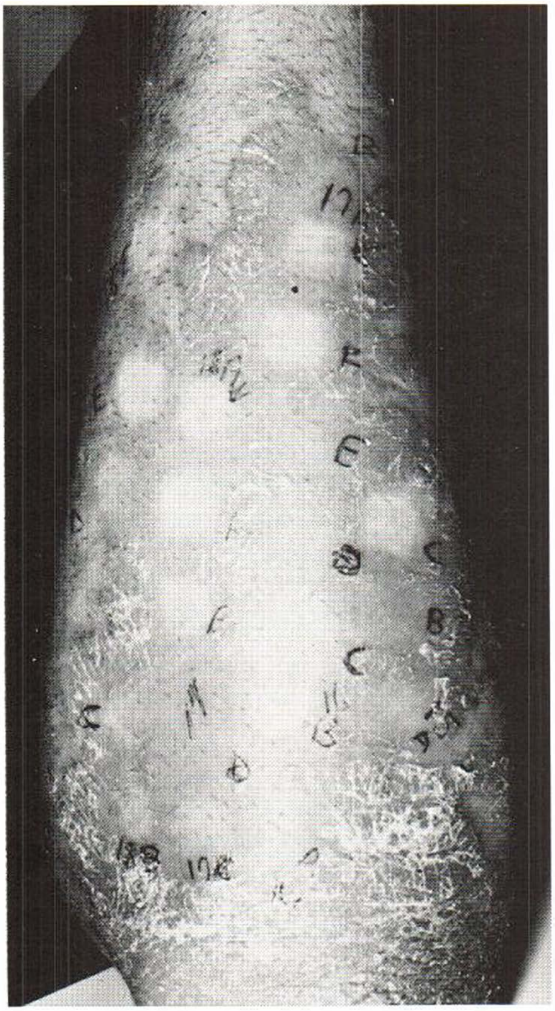

Fig. 3. Appearance of test sites at time of final reading, one-half hour after removal of fourth dressing. "Positive" results show return of epidermis to apparent normality. Blanching of skin evidently the result of persistent vasoconstriction. Negative sites show no significant suppression of epidermis, and any vasoconstriction which may be present is not apparent through the unchanged epidermis.

of double-blind methodology, comparing test agent against placebo or against an active reference compound, has become relatively standard in recent years. Far from standard from study to study are such factors as: indications treated, status of lesions, frequency and methods of application, duration of therapy, and criteria for therapeutic end points. Because of these weaknesses, there is considerable difficulty in comparing results of different studies, either with the same or with differing compounds.

What has concerned us particularly are the paradoxical results sometimes seen within a single study. Prominent among the difficulties is the frequent inability to distinguish clinically the effects of corticosteroids which in animal tests or in non-therapeutic human assays (such as vasoconstrictor tests) have very wide differences in activity. In other words, compounds sometimes a hundredfold apart in biologic activity often turn out to be comparable in the conventional double-blind clinical trial. A prime reason may be the frequent use of concentrations well in excess of minimum effective doses. In the common study using commercial preparations which have empirically selected concentrations, often equal or nearly equal for the two active agents, compound A may be present at 50 times its minimum effective dose and compound B may be used at 5 times its minimum effective level. With such overages it is hardly surprising that these two preparations are essentially indistinguishable, although one is ten times more active than another.

To fully define relative potency, what is clearly needed is a therapeutic system wherein we determine dose response curves for each new compound.

For all the foregoing reasons, we devised a human bio-assay system using topical application of varying concentrations of corticosteroid, applied to a corticosteroid-responsive, but difficult, and reasonably standardized dermatologic lesion. With respect to the lesion, the chronic stabilized psoriatic has been considered by us to be closest to the ideal.

It approaches a "standardized" clinical lesion. Classical chronic psoriasis lesions are more nearly comparable from patient to patient than those of any other cutaneous disease in the "inflammation" group.

Psoriasis is an especially good subject for corticoid assay because of the acanthotic, hyperplastic epidermal component. Suppression of the epithelium is a prominent and readily observable effect of topical corticoid activity. For example (Figs. 3 and 4), the area of epidermal suppression is sharply limited and coincides precisely with the shape of the area to which the medication was applied, providing a tissue change accessible to examination without special procedure or instrumentation.

Our experience is in agreement with the ob-

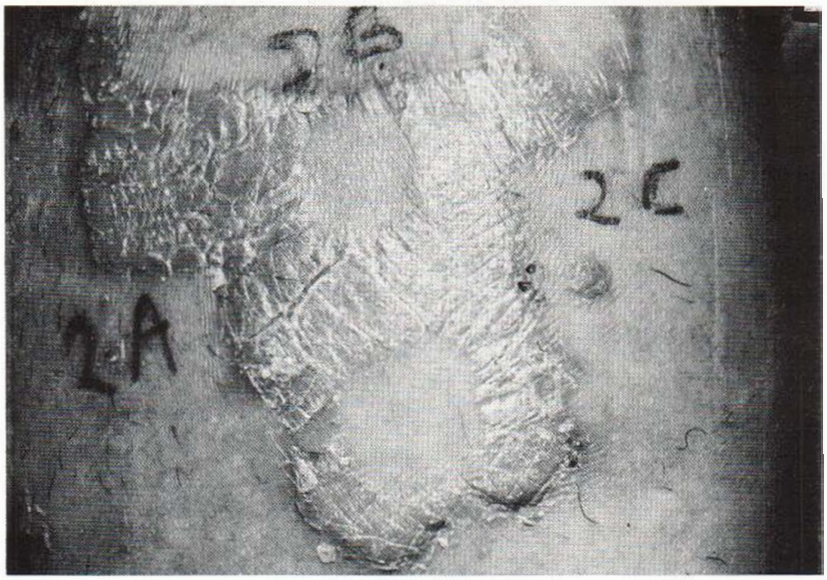

Fig. 4. Site above left: complete failure of response, rated "Negative". Center site above: partial response, rated "Negative". Center site lower: epidermis appears normal, rated "Positive".

servations of Goldman et al. (3), who stated that testing in chronic psoriasis is easiest to interpret and most consistent in response.

For assay purposes a plaque can be divided into many small test sites for comparative, simultaneous testing (Fig. 2). This represents the ideal "control" situation, since it eliminates the problem of finding matching patients, comparable lesions, and other variables. At any given time all of a patient's lesions generally are in the same state of activity, and all lesions are affected equally by any systemic influence. Spontaneous, abrupt fluctuations in activity do not occur (by definition) in chronic stabilized psoriasis, thus presenting a "steady state" for testing.

The stabilized state is requisite to reliability of the assay. If spontaneous involution is beginning, then false positive results will be obtained, since lesions will respond to agents of very low potency, and if exacerbation and increasing activity are occurring, false negative results are the result.

The assay described here has many benefits and some limitations. It is a semi-quantitative assay with good precision, having a lambda value of 0.11. It is possible to use regular formulated materials, making it a relevant and practical test. A number of compounds and/or concentrations can be tested simultaneously in the same subject, thus permitting the use of relatively small numbers of test subjects and the inclusion of positive and negative controls in each testing. The all-or-none end point is easy to read. The use of square treatment sites eliminates any problem relative to natural involution of lesions or correspondence with anatomical or vascular patterns. Systemic drug effects through percutaneous absorption are obviated by the use of small test sites and minute amounts of drugs.

For those compounds which have been available commercially, there is remarkable consistency between the results of this assay and the findings of experienced clinicians. Hydrocortisone is ineffective in psoriasis, even in concentrations up to 1.0%. Better than 90% effectiveness is achieved by fluocinolone acetonide at 0.01%, the low concentration which generally is preferred for occlusive use. Better than 90% effectiveness is reached by flurandrenolide at its standard concentration, 0.05%. At its standard commercial concentration, 0.1%, betamethasone valerate reached greater than 90% effectiveness.

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