vere and fatal periarteritis in 4 to 8 weeks. Similar lesions were seen after injection of one-half as much of this hydrocarbon, but they developed more slowly. Equivalent levels of 10-methyl-1,2-benzanthracene or the solvent alone were ineffective. 2. The most severe lesions were observed in pulmonary arteries; this finding is in accord with the cyanotic conditions of some rats. Hyaline necrotic lesions were observed in renal arterioles and glomerular capillaries. 3. A possible immunological basis for these lesions is discussed.

1. Kussmaul, A., Maier, R., *Dtsch. Arch. Klin. Med.*, 1866, v1, 484.

2. Gruber, G. B., *Virch. Arch. Path. Anat. u. Physiol.*, 1925, v258, 447.

3. Rackeman, F. M., Greene, J. D., *Tr. Assn. Am. Phys.*, 1939, v54, 112.

4. Rich, A. R., *Bull. Johns Hopkins Hosp.*, 1942, v71, 123.

5. Vaubel, E., *Beit. z. Pathol. Anat.*, 1932, v89, 375.

6. Masugi, M., Sato, Y., *Virch. Arch. f. Path. Anat. u. Physiol.*, 1934, v293, 615.

7. Rich, A. R., *Bull. Johns Hopkins Hosp.*, 1943, v72, 65.

8. ———, *ibid.*, 1952, v91, 109.

9. Germuth, F. G., Jr., *J. Exp. Med.*, 1953, v97, 257.

10. Ehrich, W. E., Seifter, J., Forman, C., *ibid.*, 1949, v89, 23.

11. Hopps, H. S., Wissler, R. W., *J. Lab. Clin. Med.*, 1946, v31, 939.

17. Stanton, M. F., Stoufer, R., *Am. J. Path.*, 1957, v71, 375.

13. ———, *ibid.*, 1945, v77, 43.

14. Selye, H., Bois, P., *Can. Med. Assn. J.*, 1956, v75, 720.

15. ———, *Endocrinology*, 1956, v59, 646.

16. ———, *Brit. Med. J.*, 1957, p183.

17. Stanton, M. F., Stoufer, R., *Am. J. Path.*, 1957, v33, 1099.

18. Lillie, R. D., *Histopathologic Technic*, p188, Blakiston Co., Philadelphia, 1948.

19. Kingsley, D. M., *Stain Tech.*, 1935, v10, 127.

20. Davidson, J., Ball, J., Platt, R., *Quart. J. Med.*, 1948, v17(N.S.), 175.

21. Hawn, C. V., Janeway, C. A., *J. Exp. Med.*, 1947, v85, 571.

22. Williams, M. G., *J. Invest. Dermatol.*, 1958, v30, 13.

23. Miller, E. C., *Cancer Research*, 1951, v11, 100.

24. Wiest, W. G., Heidelberger, C., *ibid.*, 1953, v13, 250.

25. Heidelberger, C., Moldenhauer, M. G., *ibid.*, 1956, v16, 442.

26. Miller, E. C., Miller, J. A., *ibid.*, 1952, v12, 547.

27. Green, H. N., in Raven, R., *Cancer*, v3, 1, Butterworth & Co., Ltd., London, 1958.

Received April 14, 1959. P.S.E.B.M., 1959, v101.

Comparative Anti-Inflammatory Efficacy of Topically Applied Steroids on Human Skin. (25041)

CARL A. SCHLAGEL AND J. I. NORTHAM (Introduced by R. W. Heinle)
Research and Development Divisions, The Upjohn Co., Kalamazoo, Mich.

Hundreds of hydrocortisone analogs have been synthesized in the search for more ideal anti-inflammatory agents. It is desirable to evaluate these newer compounds in animals and humans as quickly as possible. This paper describes results obtained using a modification of an unpublished assay method recommended by Brunner and Finkelstein (Research Laboratories, The Toni Co., Chicago). The original method will be reported by these authors.

Method. Immediately prior to testing, 7 accurately weighed steroid samples are separately dissolved in 15 ml tetrahydrofurfuryl alcohol (THFA).* This irritant alcohol serves both as solvent for test steroids and as agent for producing experimental erythema. Potencies of the steroids are determined from their ability, when applied simultaneously with the irritant, to inhibit THFA-induced contact dermatitis. THFA alone is used as control. Twenty-four white male volunteers at least 21 years of age are used for each test. One by 3″ Elastoplast† bandages are used to

* Quaker Oats Co., Chicago, Ill.

† Duke Labs., South Norwalk, Conn.

TABLE I. Comparison of Anti-Inflammatory Potency of Steroids Determined by Experimental Method with Potencies Determined by Clinical Trial or Wide Dermatological Use.

Test steroid	Estimated potency (× 1% hydrocortisone) with S.E.	Clinical potency (× 1% hydrocortisone)
Cholesterol	0	0
Cortisone	.04*	Not detectable†
Hydrocortisone	1.0	1†
Prednisolone	1.8 ± .5	2†
6α-methylprednisolone	4.7 ± 1.3	4‡
9α-fluorohydrocortisone	9*	10†
6α-methyl-9α-fluoro-21-deoxyprednisolone (fluorometholone‖)	42.0 ± 11.0	40§

* Not tested sufficiently for reliable estimation of stand. error.
† See ref. 1.
‡ " " 2.
§ Unpublished data from Dept. of Clinical Investigation, Upjohn Co.
‖ Oxylone: Registered trademark, Upjohn Co.

apply 0.25 ml of each solution. These treatments are assigned to subjects and to positions on forearms so that effects associated with subjects and positions can be eliminated from treatment comparisons. The actual design constitutes a set of three 8 x 8 Latin Squares. With this design each steroid sample and the control is tested at one site on each volunteer. For a quantitative assay, 3 or 4 concentrations of the unknown are compared with 3 concentrations of hydrocortisone. the reference steroid. Test solutions are in contact with the skin from late afternoon until next a.m. Assay sites are scored visually according to degrees of developed erythema using an erythema scale of zero to 4. A zero score indicates no erythema or a completely inhibited reaction. Scores of 1, 2, and 3 represent minimal, mild, and moderate degrees of erythema. A score of 4 denotes a pronounced erythema or no inhibition of the THFA induced reaction. With certain exceptions, one should refrain from retesting the same individuals to avoid more severe and aberrant responses. The mean erythema score for each steroid concentration subtracted from mean control value yields a numerical improvement value (response) used in evaluating relative potencies of test steroids.

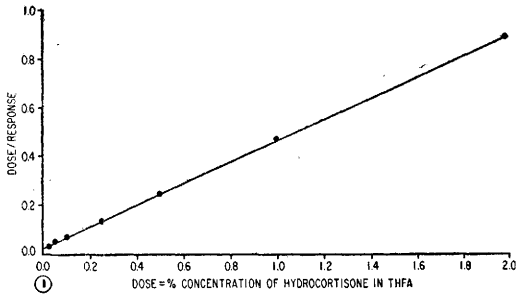
Results. During the test an uninhibited erythema develops at control sites treated with THFA alone. At sites previously occupied by the steroids dissolved in THFA, the erythema is checked completely or held at various degrees of abeyance depending upon potency and concentrations of the steroids being studied.

Several steroids have been tested sufficiently in multiple-dose assays to permit statistical analysis and more precise quantitation of potencies (Table I). This study reveals the very excellent agreement between potencies of steroids as estimated in this assay and potencies determined in topical treatment of steroid responsive dermatoses. These clinical values are based on what may be considered the average effective topical steroid concentrations. Estimated potency values were determined by quantitative technic described below.

Intensive study of assay data indicated that the theory of Stetten(3) concerning relationship of hormone dosage to physiological response applied to steroids of Table I. This finding has permitted comparison of steroids with respect to: (a) capacity of target organ to bind hormone, (b) affinity of hormone for binding sites, and has also determined, in part, the nature of statistical analysis and method of estimating potency.

According to Stetten's(3) theory, dosage divided by response should be a linear function of dosage for a given steroid and target organ. Thus: (1) $D/R = A + B \cdot D$, where D = dose, R = response, A is the intercept on the D/R axis, and B is the slope of line. The value of 1/B is a relative measure of capacity (total number of sites for physiological responsive attachment of hormone to target organ). The value of B/A is a relative measure of affinity of hormone for binding sites.

Evidence for applicability of Stetten's(3) approach is shown in Fig. 1 where ordinates, D/R, were calculated in one assay by taking R as mean improvement over control at given concentration of hydrocortisone for 24 volunteers. Such plots of D/R against D for the



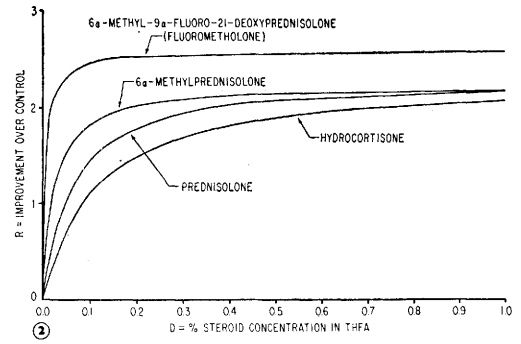


FIG. 1. Relationship of dose/response to dose for hydrocortisone in THFA.

FIG. 2. Estimated response/dose curves for four steroids according to theory of Stetten; $R = D/(A + B \cdot D)$.

anti-inflammatory steroids usually give linearity and also homogeneous variation in dose/response.

Estimates of A and B for each steroid have been obtained by the method of least squares applied to the plot of D/R against D. Table II summarizes these estimates for 4 of the steroids studied.

Within the framework of Stetten's theory, certain conclusions may be obtained from Table II. Values of B are statistically fairly similar for hydrocortisone, prednisolone, and 6α-methylprednisolone; this indicates that the target organ exhibits equal capacity to bind these steroids. However, the B value for fluorometholone is statistically significantly lower (beyond the 5% level) than those obtained for other steroids. Hence, the target organ has greater capacity to bind fluorometholone than it has to bind the other 3 steroids. The capacity to bind fluorometholone relative to hydrocortisone is .431/.385 or 1.12 ± .03.

This 12% increase in capacity for fluorometholone indicates that a greater anti-inflammatory response should be obtained with maximum effective concentration of fluorometholone than with maximum effective concentration of hydrocortisone. The increased capacity has great practical significance, since in treatment of severe or resistant inflammatory dermatoses, fluorometholone should be capable of producing a greater anti-inflammatory response than that possible with even very high concentrations of hydrocortisone. Of even greater significance is the fact that, while fluorometholone is 42 times as effective as hydrocortisone topically, it is only about equal to hydrocortisone by oral or intravenous administration(4,5). This suggests that fluorometholone should be an ideal steroid for highly effective topical anti-inflammatory treatment without the danger of any deleterious hormonal side-effects due to systemic activity.

Among those compounds which have a common B value, the ratio of A values gives an estimate of relative steroid potency (Table III).

However, the relative potency of steroids with different capacities (different values of B) cannot be determined on the basis of the ratio of the A (intercept) values. Therefore, the potency of fluorometholone was calculated from equations in Table II for fluorometholone and hydrocortisone, or determined graphically from smooth graphical plots of R against D [from $R = D/(A + B \cdot D)$] for these 2 steroids (Fig. 2). From these plots it was readily determined that 0.024% fluorometholone gave the same response as 1% hy-

TABLE II. Estimates of Parameters in Stetten's Theory for 4 Steroids.

Steroid	D/R = A + B·D	Stand. error of A	Stand. error of B
Hydrocortisone	D/R = .0480 + .431D	.0058	.005
Prednisolone	" = .0271 + .425D	.0074	.014
6α-methylprednisolone	" = .0103 + .443D	.0026	.014
Fluorometholone	" = .00225 + .385D	.0004	.009

TABLE III. Estimated Potency of Prednisolone and 6α-Methylprednisolone Relative to Hydrocortisone.

Steroid	Value of A	Potency relative to hydrocortisone	Stand. error of relative potency
Hydrocortisone	.0480	1	
Prednisolone	.0271	1.8	.5
6α-methylprednisolone	.0103	4.7	1.3

drocortisone. Hence, at the clinically useful 1% hydrocortisone level, fluorometholone was 1/.024 or 42 times as potent as hydrocortisone. Since these original observations were made, this high potency for fluorometholone has been demonstrated repeatedly in numerous additional assays where it has been compared both with hydrocortisone and with other steroids.

Summary and conclusions. 1. A reliable assay procedure has been presented for rapidly quantitating the anti-inflammatory potency of steroids on human skin. 2. Estimated steroid potency values can be used to predict reliably the proper steroid concentration to be studied in topical clinical trials. 3. Data have been adapted to the Stetten theory which concerns relationship of hormone dosage to physiological response. This permitted comparison of steroids with respect to capacity of target organ to bind hormone and affinity of hormone for binding sites. 4. The target organ has increased capacity to bind fluorometholone relative to hydrocortisone. This means that some optimum concentration of fluorometholone is capable of producing a greater anti-inflammatory response than that possible even with very high doses of hydrocortisone. 5. That fluorometholone is approximately 40 times as potent as hydrocortisone topically yet only about equal to hydrocortisone in systemic activity suggests that fluorometholone is a unique compound for topical anti-inflammatory therapy, since even if absorption from the skin occurred, the systemic effect would be negligible.

1. Robinson, R. C. V., Robinson, H. M., Jr., *J.A.M.A.*, 1956, v49, 260.

2. Goldberg, L. C., *Antibiot. Med. and Clin. Ther.*, 1958, v5, 372.

3. Stetten, D., Jr., *Science*, 1956, v124, 365.

4. West, K. M., *Metabolism*, 1958, v7, 441.

5. Kleeman, C. R., Koplowitz, J., Maxwell, M. H., *Clin. Res.*, 1958, v6, 54.

Received April 20, 1959. P.S.E.B.M., 1959, v101.

Evolution of Herpes Simplex Cellular Lesions Observed *in vitro* by Phase Contrast Microcinematography. (25042)

G. BARSKI AND R. ROBINEAUX (Introduced by A. B. Sabin)

Institut Gustave Roussy, Villejuif, (Seine) and Centre d'Immunopathologie, Hôpital St. Antoine, Paris

Since first attempts of *in vitro* cultivation of herpes simplex virus by Rivers *et al.*(1), Andrewes *et al.*(2), and others, many authors have described specific lesions produced by this virus in cells maintained *in vitro*. However, despite considerable amount of information accumulated so far, some points concerning phenomena occurring in herpes virus infected cells have remained controversial. Our purpose was to record by continuous observations with phase contrast microcinematography the herpes infectious process in living cells, and to compare these data with those obtained by routine histological techniques. We were thus able to follow step by step the interrelationships between different cell constituents and organelles during development of the herpetic cell lesion and, on an intercellular level, to study stage by stage the formation of giant cells characteristic of this infectious process.

Material and methods. Cultures of trypsinized rabbit kidney cells were prepared in