THE EFFECT OF THE TOPICAL APPLICATION OF CORTICOTROPHIN, HYDROCORTISONE, AND FLUOROCORTISONE ON THE PROCESS OF CUTANEOUS INFLAMMATION*

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Since the introduction of the steroid† hormones and ACTH into clinical therapy in the past decade, there have been innumerable therapeutic demonstrations of the efficacy of corticotrophin, cortisone, hydrocortisone, and more recently, fluorohydrocortisone, in the alteration of the response mechanism of the body to inflammation (1). The systemic administration of these hormones has yielded fairly uniform results in the experimental study of their effects on inflammation, but only conflicting observations have been reported with tests involving their local application. In contrast stands the well-corroborated evidence of their topical therapeutic effectiveness (2).

In 1949, Ragan commenced a series of investigations which led to the experimental demonstration of an alteration in the inflammatory response in animals maintained on systemic steroid therapy (ACTH and cortisone) (3). Subsequent investigators have employed these hormones, and hydrocortisone locally by injection, and have observed an inhibitory effect both on inflammation and the healing process in animals (4, 5). These last points have been most ably shown in Selye's work with the “granuloma-pouch” technic (6).

Jarvinen, in 1951, transferred the experimental field to man. Still using the systemic administration of the hormones, he studied the alteration in the cutaneous response to ultra-violet radiation, noting in some cases a diminution in the degree of erythema produced (7). Long and Favour (8), the following year, and Dougherty and Appel (9) in 1954, demonstrated an alteration in the tuberculin-like forms of inflammation in patients who received the hormones systematically. Lovell (10) in 1953, confirmed this finding, but was unable to obtain any change in immediate wheal reactions.

Goldman et al. (11), in a series of experiments, failed to demonstrate any alteration in local inflammatory response following the injection of cortisone at the test site, but could obtain an inhibition of patch test responses after the local injection of Compound F. Attention was then directed to the latter material after the suggestion was made by Rothman that hydrocortisone would be more likely to have a demonstrable peripheral effect than would cortisone. Dougherty (9) confirmed the accuracy of this remark in a number of experiments using the local injection of hydrocortisone to alter the reaction to a primary irritant. However, Everall (12) in 1952 was unable to obtain any parallel results with the

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† The steroid hormones, for the purposes of this article, will refer to cortisone, hydrocortisone, or fluorohydrocortisone.
topical application of the same hormone. In 1954, using also the local application of hydrocortisone ointment, Sidi and Bourgeois-Gavardin did effect an alteration of the cutaneous reaction to croton oil, but were unable to produce any predictable results, concluding then that the effects were undependable (13). Thus, despite the repeated success obtained therapeutically with local hydrocortisone ointments, experiments designed to measure this effect have so far either failed to show any change or have produced only equivocal results.

Preliminary random tests carried out convinced us that there was an anti-inflammatory effect that could be predictably shown and we therefore set about to construct a series of experiments to demonstrate these alterations.

A. The influence of the following factors was studied:
1. The relationship between the time of application of the hormone and the application of the inflammatory stimulus.
2. The duration of the contact of the hormones with the skin.
3. The concentration of the hormones used.
4. The intensity of the inflammatory stimulus used.
5. The characteristics of the skin tested. (Pigmentation and thickness of epidermis)

B. A larger number of tests was then carried out under set experimental conditions taking into consideration the effect of the above mentioned modifying factors.

MATERIALS AND METHODS

1. All tests were performed on the normal skin of human volunteers. A total of 149 persons was tested, both males and females, ranging in age from 7 to 86 years. Included among these were 12 patients with no adrenal function (6 patients with total bilateral adrenalectomy, and 6 patients with long-standing Addison's disease.)
2. The test site in all cases was an area of 1 square cm. on the upper part of the back, below the level of the dorsal spine of the scapula. In no case was there any pretreatment of the area before testing.
3. Corticotrophin, in the form of the commercially available powder and ground hydrocortone tablets were used. These hormones were incorporated into a watersoluble ointment base, consisting of glycerin, cetyl alcohol, sodium lauryl sulfate and water. The fluorohydrocortisone was used in the form of the commercially available ointment since none of the powder was at hand. Varying concentrations of the hormones were used in some of the experiments as outlined below but for part B of the study 5% corticotrophin, 1% hydrocortisone and 0.25% fluoro hydrocortisone were employed.
4. 50 mg quantities of the test ointment were applied to an area of 1 sq. cm., and massaged into the skin with 6 circular movements of the third digit. The area was then covered with a transparent cellophane disc.
5. The application was effected at time intervals ranging from 24 hours before to 24 hours after the introduction of the inflammatory stimulus, and contact of the hormone ointment with the skin was maintained for time periods ranging from $\frac{1}{2}$ to 8 hours.
6. The hormone-containing ointment was removed with water, alcohol and ether at the desired time.
7. The inflammatory stimuli consisted of:
   a. An erythema dose of ultraviolet light delivered by a Bircher mercury contact lamp. The time of exposure necessary to produce an erythema was determined beforehand for each case. The usual time ranged from 3 to 7 seconds.
   b. Mustard oil in varying concentrations, prepared by dilution with liquid petrolatum,
were used in some of the experiments, but the major part of the study was carried out with an 80% concentration.

c. Nitric acid in aqueous solution; 4%, 10% and 15% concentrations were used in a small group for comparison, but the last named strength was employed for the main test group. Both the primary irritants were kept in contact with the skin site for 24 hours by means of a cellophane patch, and removed at the end of that time with a suitable solvent.

8. In all cases observations were made immediately after application of the inflammatory stimulus, and at approximately 2 hour intervals until the maximum development of the inflammatory reaction was achieved. The time of appearance of the reaction, its rate of progress and the final degree of response were noted. In the case of the ultraviolet light reaction the fully developed response was graded in terms of the erythema of the control site. For the primary irritants the inflammatory reaction was graded as follows:
   a. Erythema.
   b. Erythema plus obvious edema.
   c. Additional formation of papules or vesicles.
   d. Necrosis.

9. Controls were used for all tests, and comprised:
   a. Normal skin untreated prior to induction of inflammation.
   b. Normal skin pretreated with the empty ointment base.
   c. Skin pretreated with ointment containing inert protein.
   d. In some cases epidermal stripping (Pinkus) was carried out immediately before the application of the inflammatory stimulus, in order to remove the superficial layers of the epidermis and any of the hormones contained therein.

RESULTS AND DISCUSSION

The influence of the modifying factors

1. The time of application of the hormones in relation to the induction of inflammation. In order to demonstrate this relation a series of tests were carried out upon a group of 20 patients. Each of the hormones was applied locally at various time intervals within a range of 48 hours, (from 24 hours before to 24 hours after the use of the ultraviolet light or the irritant). As illustrated in Figure 1,* application of the hormone before the inflammatory stimulus resulted in an inhibition of the inflammatory response. The maximal inhibitory effect was observed at a time interval of from 6 to 8 hours, but even with an interval as short as 2 hours, complete inhibition was noted in 50% of the tests. Prolongation of the time interval beyond 8 hours led to a rapid diminution of the effect with no alteration of the inflammatory response if there was a lapse of 16 hours.

   If inunctions were carried out less than one hour before, or at any time up to 8 hours after the use of the stimulus, 60% of the cases failed to show an alteration in inflammatory response. Only 10% showed any degree of inhibition of the reaction, while about 30% showed an exacerbation beyond the normal response. Parallel results were obtained with all the hormones tested.

* This figure was constructed from values obtained using ultraviolet light as the inflammatory stimulus. However, the same results hold true with the primary irritants, although modifications of our usual procedure were necessary in testing the effect of hormone application after the stimulus; the primary irritants remained in contact with the skin for shorter time periods of about 10 minutes.
2. The duration of contact of the hormone with the skin. The results demonstrated in Table 1, illustrate the observation that a total duration of hormone contact with the skin of only 1 hour was sufficient for the manifestation of an inhibition of inflammation. The maximal inhibition was obtained with 2 hours’ contact, whether the hormones were removed at this point or left on the skin for the full 6 hours before application of the irritant. This failure to increase the effect with increase in contact time beyond 2 hours suggests that the skin can either absorb or utilize only limited quantities of hydrocortisone. The existence

<table>
<thead>
<tr>
<th>Duration of Contact o Hormone on Skin</th>
<th>Effect of Hydrocortisone on Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>hr.</td>
<td>Complete inhibition</td>
</tr>
<tr>
<td>1/2</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

16 patients tested, using 60% mustard oil, applied 6 hours after the 1% hydrocortisone ointment, in all cases except the last, in which the interval was 8 hours.
Fig. 2. Autoradiograph demonstrates the presence of C\textsuperscript{14}-hydrocortisone in the basal layer of the epidermis after a total of 1 hour's contact of the hormone-containing ointment with skin surface. There are in addition a few collections of the material about the superficial blood vessels in the corium.‡

of such a saturation level is also indicated by the failure to augment the effect by reapplications of the hormones during the ensuing 6 hours.

A few studies using autoradiography demonstrated the presence of the C\textsuperscript{(14)}-labelled hydrocortisone in the basal layer of the epidermis one hour after its external application, while either the hormone itself or at least the C\textsuperscript{(14)} had entered the cutis after 2 hours (Figures 2 and 3).

3. The concentration of the hormones. A direct relation was established between the concentration of the hormone used and its inhibiting ability. (Table 2.) It is of interest to note that an optimum effect was obtained with 1% hydrocortisone, 0.25% of fluorohydrocortisone and 5% corticotrophine and that higher concentrations did not increase their effectiveness. This observation again suggests a saturation factor, related either to the ability of the skin to absorb the hormone, or to its ability to react to the hormone. One might assume that the latter possibility is more likely in view of the similar molecular weight of hydrocortisone and fluorohydrocortisone; it would be difficult to understand the failure of the skin to absorb any more than 0.25% of fluorohydrocortisone, whilst accepting up to 1% of hydrocortisone.

‡ The Autoradiographic work was supported by a grant of Merck Co. Ltd., Canada.
FIG. 3. Autoradiograph illustrates the dispersion of C\textsuperscript{14} hydrocortisone through the skin after 6 hours' time lapse from the point of hormone application.

TABLE 2
The concentration of hormone; its influence on the ability of the hormone to inhibit inflammation

<table>
<thead>
<tr>
<th>Hormone Application</th>
<th>Concentration</th>
<th>Complete Inhibition</th>
<th>Partial Inhibition</th>
<th>No Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticotrophin</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>0.25</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>0.50</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Fluorocortisone</td>
<td>0.05</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>0.1</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

10 patients were tested, using mustard oil. Ointments were applied 6 hours before the stimulus was given.
4. The intensity of the stimulus used. An inverse relation was observed between the intensity of the inflammatory stimulus employed, and the efficacy of a given hormone concentration in influencing the resultant tissue reaction (Table 3.)

5. Characteristics of the skin tested. No correlation was found between the degree of pigmentation of the skin and the degree of inhibition of the inflammatory reaction. Several areas of vitiligo were tested and found to react like their normally pigmented counterparts (Table 4).

The histological study of skin biopsies of the test site in a small group of cases suggested an inverse relationship between the thickness of the epidermis (in terms of cell layers) and the degree of modification of the inflammatory response by hydrocortisone. Several patients with scars showed complete inhibition of inflammation in these areas and less alteration in the adjacent skin. Amongst those patients in the main group who manifested no inhibition of inflammatory response were a number with skin thicker than average.

The possible influence of skin characteristics was noted, but these features were not used as criteria in the selection of cases or test sites.

### TABLE 3

*The intensity of the stimulus; its influence on the ability of the hormones to inhibit inflammation*

<table>
<thead>
<tr>
<th>Concentration of Irritant (Mustard oil)</th>
<th>Complete Inhibition</th>
<th>Partial Inhibition</th>
<th>No Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACTH</td>
<td>Hydrocortone</td>
<td>ACTH</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>9</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>40</td>
<td>9</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>60</td>
<td>7</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>80</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

10 patients used. Hydrocortisone 1% and ACTH 5% in ointment form were applied 6 hours before the irritant and remained on the skin the entire 6 hours.

### TABLE 4

*The thickness of the epidermis; its influence on the ability of the hormones to inhibit inflammation*

<table>
<thead>
<tr>
<th>Thickness of Epidermis (cell layers)</th>
<th>No. of Cases</th>
<th>Effect on Inflammation in Test Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Complete inhibition</td>
</tr>
<tr>
<td>5–6 (scar)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>8–9</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>10–11</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>12–14</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>
The application of the steroid hormones and corticotrophin under controlled conditions: their effect on the process of inflammation

On the basis of the results obtained in the first series of experiments, we were able to investigate a larger group of 121 patients, under conditions controlled with respect to the first four variables considered above. For this purpose, ointments were employed using the hormones in strengths of 1% hydrocortisone, 0.25% fluorohydrocortisone, and 5% corticotrophin. The ointment applications were all effected 6 hours prior to the induction of inflammation, and allowed to remain in contact with the skin surface for the entire 6 hour period. The inflammatory stimulants used included in erythema dose of ultraviolet radiation, mustard oil in 80% concentration, and a 15% aqueous solution of nitric acid. These irritants were applied about 3 hours after removal of the hormone-containing ointment.

The three forms of inhibition which were observed are depicted in Figure 4. "A" illustrates by means of a curve, the development of the inflammatory reaction as seen in the control sites, and in those few instances in which hormone application failed to alter the response. "B" depicts a delay in the onset of the reaction, but with the subsequent full development of the inflammatory response. "A" and "B" were regarded as showing no inhibition of inflammation. Curve "C" demonstrates the same phenomenon of "delay", but in addition there is an inhibition in the progress of the reaction so that it fails to reach the intensity seen in the control sites. This was considered as partial inhibition of inflammation. Curve "D" represents the complete suppression of the inflammatory response as seen in more than 75% of the tests. This was classified as complete inhibition.
of inflammation. It may be noted here that in no case was there alteration of the very early faint erythema which occurs within a few minutes of the application of a primary irritant.

The delay phenomenon was most readily observed in the development of the erythema resulting from ultraviolet light. In the control site, the erythema made its appearance 2 to 3 hours after exposure to the radiation, increasing to its maximum in about 12 hours, and persisting essentially unchanged for 2 to 3 days before becoming pigmented. In the test sites this sequence is altered so that the erythema appeared only 6 to 12 hours after exposure, reached a maximum in 18 hours, and faded by 24 hours. In those cases which manifested a "total inhibition", there was complete absence of any visible reaction except for a mild and transitory erythema which appeared in test and control sites alike, immediately after contact with the inflammatory stimulants.

The results are presented in detail in Table 5. Hydrocortisone completely inhibited the normal inflammatory response to the application of primary irritants in 75% of the tests; while 50% of the areas exposed to ultraviolet irradiation failed to develop any erythema in contrast to the control sites. 13% of the sites tested with the primary irritants and 34% of those tested with ultraviolet rays showed a partial inhibition of the process of inflammation as manifested by a failure to reach the degree of the response seen in the control areas, either
Fig. 6. Photograph illustrates the inflammatory reaction elicited by mustard oil, 60% (upper row) and 80% (lower row). The pair on the far left are the control sites; the pairs proceeding from left to right have been pretreated with ACTH, hydrocortisone, and fluorocortisone. Note the necrosis which has appeared in the control test with 80% mustard oil.

<table>
<thead>
<tr>
<th>Inflammatory Stimulus</th>
<th>No. of Cases</th>
<th>Complete Inhibition</th>
<th>Partial Inhibition</th>
<th>No Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Corticotrophin</td>
<td>Hydrocortone</td>
<td>Fluorocortone</td>
</tr>
<tr>
<td>Ultraviolet erythem...</td>
<td>96</td>
<td>38</td>
<td>50</td>
<td>66.7</td>
</tr>
<tr>
<td>Mustard oil, 80%</td>
<td>92</td>
<td>66</td>
<td>77</td>
<td>79</td>
</tr>
<tr>
<td>Nitric acid, 15%</td>
<td>88</td>
<td>59</td>
<td>76.5</td>
<td>67.9</td>
</tr>
</tbody>
</table>

N.B. The hormone-containing ointment was applied 6 hours prior to application of the stimulus, and remained on the skin for the entire 6 hours. Results are expressed as percentage of the total number of cases tested.

Hormone concentrations—corticotrophin, 5%; hydrocortisone, 1%; fluorocortisone, 0.25%.

with or without a delay in the appearance of the reaction. The remainder failed to show any alteration in the response beyond a delay phenomenon noted in a few cases. Tests with ACTH gave similar results—60% of the tests showed complete inhibition of the normal reaction to primary irritants, while 40% failed to develop an erythema after ultraviolet exposure. Partial inhibition of these
reactions was noted in approximately 30% of tests with primary irritants and in 45% of the tests with ultraviolet light (Table 5).

Discussion of the possible mechanism of the inhibitory phenomena observed

Our experiments clearly demonstrate an anti-inflammatory effect of the topically applied steroid hormones and ACTH, provided that adequate tissue concentrations are present at the time that the inflammatory stimulus is applied.

The possibility that the observed effects are non-specific in nature has been eliminated by the use of adequate controls—the ointment base and inert proteins. A light screening effect of these hormones could not account for the results obtained in our experiments because:

1. Inhibition was not obtained in those tests in which the time of contact between hormone and skin was too short.
2. A light screening effect could not explain the delay phenomenon.
3. The inhibitory effect persisted even after epidermal stripping.
4. Closely parallel results were obtained with ultraviolet irradiation and the primary irritants.

Absorption of the hormone and the presence of a critical tissue concentration appear to be essential for the inhibition of inflammation. This concept is supported by 3 observations:

a. The necessity of a minimum time of contact of the hormone with the skin before introduction of the irritant.
b. The necessity for a minimum time lapse between hormone application and induction of inflammation.
c. The apparent influence of the thickness of the epidermis. The loss of the inhibitory effect if the time lapse between application of the hormone and the inflammatory stimulus exceeds 8 hours suggests diffusion from the test site and/or destruction of the hormone in tissue.

One may therefore conclude that the hormones studied are capable of inhibiting the process of experimental inflammation if they are present in the skin in sufficient concentration at the time when the inflammatory agent is first introduced on the surface. The application of the hormones to the site after the process has been initiated either produces no effect whatsoever—in most instances—or an exacerbation of the inflammatory process. On the basis of these observations one might suggest that the hormones exert a protective act on the skin concerned, but cannot halt the development of inflammation once the process has been initiated.

As yet, our knowledge of the fundamental mechanisms of inflammation is limited. Neither the factors involved in its initiation, or those responsible for its evolution and propagation are fully appreciated. This restriction in our understanding of the pathophysiology of inflammation does not permit us to attempt any definitive explanation of the role of the steroid hormones in its alteration. At the present time there are three main theories as to the means by which these hormones alter the course of inflammation.

1. An effect on the vascular apparatus. Nilzen who proposed this theory
based his thesis on the finding that under his experimental conditions, the steroids protected the skin more effectively against croton oil, a vascular irritant, than against cantharidin, an epidermal irritant. His deductions appear to be derived from insufficient evidence (14).

2. Inhibition of the mesenchymal response. This theory, put forth by Selye (15), is based upon the proposition that necrosis and inflammation are two entirely separate processes. By means of his "granuloma-pouch" technic, he demonstrated that the presence of hydrocortisone prevented the inflammatory reaction in the wall, but did not inhibit the necrosis of tissue resulting from the introduction of a primary irritant—croton oil—into the lumen. On the basis of these results he postulated that the hormone acted by preventing the formation of a connective tissue barrier, thereby actually enhancing the necrosing action of the irritant. This theory presupposes that the process of inflammation occurs only in mesenchymal tissue, and that teleologically inflammation protects against tissue necrosis. Selye denies the protective action of the hormones on cell reactions, as originally suggested by Hench (16).

As illustrated in Figure 6, under our experimental conditions, we were able to prevent tissue necrosis in test sites exposed to a primary irritant (mustard oil or croton oil), after pretreatment with one of the steroids.

3. Alteration of intracellular enzyme activity. Menkin (17) has stressed the ability of these hormones to alter the cellular ability to produce such materials as leucotaxin. His experiments with intrapleural inflammation failed to reveal any interaction of the hormone hydrocortisone with the leucotaxin directly. However, some of his technics have been questioned by such investigators as Harris.

On the basis of our own experiments we may make several deductions as to the mode of action of these hormones:

1. Systemic action. This can be excluded since the same tests carried out in patients who had longstanding, clinically evident Addison's disease, or who had been previously subjected to a total bilateral adrenalectomy gave results identical to those obtained in euadrenal individuals. Secondly, the sites used for control purposes failed to show the effects observed in the test sites. Finally, topical application of hydrocortisone ointments to normal individuals, those with denuded skin surfaces, and patients with exfoliative dermatitis was not followed by any alteration in eosinophil levels (blood) or in urinary 17-keto-steroid excretion (18 & 19).

2. Prevention of entrance of the irritant into skin. This explanation does not appear likely since the very early erythema and the early burning sensation subjectively noted are observed with equal intensity in control and test sites alike, within a few minutes of the application of the irritant. Secondly, if a material such as fluorescein is placed on the skin with the irritant, the dye is found to be under the stratum corneum within a few minutes, indicating the entrance of the irritant into the skin.

3. Inhibition of the primary reaction of tissue to the inflammatory stimulus. The initial erythema associated with a primary irritant and UVL is visualized
equally in control and test sites. If this effect is an indication of the first phase of inflammation in the responding tissue, then the steroid does not act at this point.

4. Inhibition of the production of secondary substances which, it has been suggested, are both the products and the propagators of inflammation. The fact that despite removal of the inflammatory stimulus the hormones are unable to inhibit the inflammatory process if they are applied after its initiation, permits the conclusion that the steroids do not act at this stage.

5. Inhibition of tissue reactivity to any secondarily released chemicals. An alteration in the tissue response within the treated area is suggested by three observations referable to the delay phenomenon:
   a. There was a number of instances in which we noted a delay in the appearance of the inflammatory process. This would suggest that the hormone had held the process in check only until its activity was lost either by destruction or dispersion, whereupon the process resumed its evolution.
   b. A similar phenomenon has been demonstrated by Favour (20), in connection with tuberculin testing in patients receiving steroid therapy. If these hormones are administered in dosage sufficient to prevent the appearance of a positive tuberculin reaction, this suppression will persist as long as the hormone therapy is continued. However, if therapy ceases at any time within a period of about three weeks from the time of the test, the positive reaction will appear within 36 hours.
   c. Similar observations have been made by ourselves using patch tests with known contactants in patients with predetermined hypersensitivity (e.g., penicillin). The patch tests were kept in place for 24 hours, and upon removal inhibition was noted in the areas pretreated with hydrocortone. However, this inhibition lasted only as long as hormone application was continued, but if this were stopped within 3 days, the reaction appeared immediately, and developed to the full, while the control test was already fading.

The extra adrenal effect of corticotrophin

Throughout the entire experimental study it was found that the activity of the commercially available corticotrophin paralleled that of hydrocortisone, even in those cases where no functioning adrenal tissue was present. Approximately five times the concentration of ACTH was necessary to achieve results within the range of those obtained with hydrocortisone. The optimum duration time of contact of ACTH on skin appeared to be slightly longer. About 66% of the areas pretreated with corticotrophin showed inhibition of the inflammatory response initiated by mustard oil, while 38% showed inhibition of the ultraviolet induced erythema. No such effect was noted with thyrotrophic hormone—the only other pituitary hormone which we had available. In the control sites, heat-inactivated ACTH failed to effect any inhibition.

This effect does not appear likely to have been achieved through systemic absorption and subsequent influence on endogenous adrenocortical hormone production, since, firstly, identical results were obtained in patients with no
functioning adrenocortical tissue, secondly, the quantities used were relatively
minute compared with those ordinarily needed to stimulate normal adrenal
function, and finally, the ten cases in whom steroid excretion studies and eosin-
ophil levels were available at the time of testing failed to show any alteration in
these gauges of adrenal function.

This local alteration of tissue reaction could be explained through one of two
modes of action—1) the local production of an hydrocortisone-like hormone; 2)
the direct action of ACTH on tissues.

The extra-endocrine production of a hormone is not without parallel in the
case of the thyroid hormone; there have as yet been no reports of an absolute
“0” reading for a protein-bound iodine level even in the most flagrant case of
myxedema, in whom the thyroid has been removed in toto at operation. There
has in addition been one experimental report in which radiothyroxine was
identified in the peripheral circulation of a case of myxedema to whom I$^{131}$ had
been administered (21). However, in the case of extra-adrenal hydrocortisone
production, several attempts have so far failed to identify it in the skin subjected
to treatment with corticotrophin in vitro.*

As far as the direct tissue activity of corticotrophin is concerned, we may
again cite the examples seen with other trophic hormones, such as TSH and
STH (somatotrophic), both of which have been revealed to have an action on
cellular metabolism apart from that on their target glands. (22) Menkin, in his
studies of the products of inflammation, noted that at least one of these latter
was inhibited by ACTH and not by cortisone. While it is still not clear that these
materials occur naturally as separate substances one can at least remark that
certain areas of inflammation are affected more by ACTH than by cortisone.

We have tried to corroborate the local action of ACTH by its use in almost
50 cases of various dermatoses, using hydrocortone and control ointment bases
as controls. We have been able to demonstrate an improvement in about 60%
of these trial cases.

Clinically, an extra-adrenal effect of corticotrophin has been suggested by the
therapeutic response of cases of pemphigus vulgaris to whom ACTH has been
administered alone. Many of these patients had previously been on massive
doses of cortisone, sufficient to depress adrenocortical function over long periods
of time but not enough to control the disease. Despite this, the patients showed
a healing of their lesions, when placed on ACTH, although laboratory estima-
tion of endogenous adrenocortical stimulation failed to reveal any change in the
target gland.

SUMMARY AND CONCLUSIONS

1. A study was made of the alterations in the process of cutaneous inflamma-
tion effected by the topical application of hydrocortisone, fluorocortisone, and
corticotrophin.

2. The primary irritants—mustard oil and nitric acid—were used, together
with erythema-inducing doses of ultraviolet rays.

* These experiments were carried out through the courtesy of the Canada Packers’
Company in their laboratories.
3. Five major factors influenced the results obtained in the test situations:

a. *Relation between time of hormone application and induction of inflammation.* Effective inhibition of inflammation resulted from the application of the hormone 2 to 8 hours prior to the stimulus. The maximum effect was achieved in about 6 hours. A time interval over 16 hours failed to produce any effect. If applied immediately before or after the stimulus, the hormone produced no alteration whatsoever in the majority of cases, an exacerbation of inflammation in about 1/2 of the tests, and inhibition in a few cases only.

b. *Duration of contact of hormone with skin.* The minimal effective contact time was one hour. Prolongation of contact time over 2 hours gave no increase in effect.

c. *Concentration of hormone applied.* Optimal and about equal effects were seen with concentrations of 1% hydrocortisone, 0.25% fluorocortisone, and 5% corticotrophin.

d. *Intensity of inflammatory stimulus applied.* There was an inverse relationship between the intensity of the stimulus and the degree of inhibition observed in the inflammatory response.

e. *Thickness of epidermis.* An inverse relation was suggested between the thickness of epidermis in terms of cell layers and the hormonal effect.

4. If the hormones were applied under optimal conditions in our experiments, complete inhibition of inflammation induced by primary irritants was observed in 75% of cases using hydrocortone and fluorocortone, and in 60% of cases using ACTH. 50% of the cases exposed to erythema-inducing doses of ultraviolet radiation failed to develop a reaction if pretreated with these same hormones. Partial inhibition was observed in the inflammatory response in a smaller number of tests.

5. The established minimal time period of contact of hormone with skin was thought to be related to the time necessary for penetration of the hormone through skin. This explanation was supported by autoradiography. The time limits for demonstration of the effects of the hormones was thought to suggest a later destruction or loss of the hormones from the tissues tested.

6. On the basis of these experiments, it was concluded that these hormones were capable of the inhibition of inflammation, provided that they were present in adequate concentration in the tissues at the time of application of the inflammatory stimulus.

7. The mechanism of this action is discussed and the conclusion reached that the hormones inhibit the reaction of the cells to the primary products of inflammation.

8. The comparable results noted with corticotrophin are described and the mechanism of this reaction discussed. This phenomenon appears to be due to an extra-adrenal effect of the trophic hormone, and the possibility is suggested that it acts directly on the target cells in its primary biochemical role.

**ADDENDUM**

REFERENCES

ANTI-INFLAMMATORY EFFECT OF STEROID HORMONES

DISCUSSION

DR. ALLAN L. LORINCZ (Silver Spring, Md.): In this study, the direct local anti-inflammatory effect ascribed to topically applied corticotrophin is, of course, contrary to the generally accepted concept that the therapeutic effects of corticotrophin are indirectly brought about through increased production of adrenal corticosteroids. I wonder whether the direct effect described here, instead of being a corticotrophin effect, is not rather the result of the local vasoconstrictor action of pitressin as there is considerable pitressin activity in the usual corticotrophin preparations.

DR. JAMES W. BURKS (New Orleans, La.): At the risk of adding a clinical flavor to this highly scientific presentation I would like to offer myself as a singular example where the steroids failed to temper the effect of the sun. Forgetting my sun screen preparation and losing my hat in the excitement of fishing for six hours in the Nassau sun are my excuses for this severe actinodermatitis. Within one hour after this trip I began applying hydrocortisone lotion and ointment and have continued using it for the past four days.

This personal experience tends to confirm the results of studies recently completed by Dr. William George and myself at Charity Hospital in New Orleans. In an attempt to prevent or minimize the actinodermatitis which occurs so frequently in the treatment of vitiligo with psoralens and sun, innunctions of hydrocortisone ointment were made for periods up to seven days before exposure. Also a number of these patients used the ointment immediately after exposure to the sun. In our opinion no prophylaxis or reduction in intensity of the reaction resulted. On the other hand when actinodermatitis did develop in these patients hydrocortisone ointment was therapeutically effective.

DR. JOHN D. KRAFCHUK (New Orleans, La.): Treatment of patients without adrenal glands was mentioned. It must be assumed that these individuals were given some sort of steroid therapy, or else they would not have remained alive. Thus this group would represent patients with perhaps more than adequate circulating steroid levels, and not devoid of steroids, as was implied. The demonstration of local effects due to ACTH is, I believe, unique. Heretofore, experimental and clinical studies have indicated that the only effect due to ACTH was on the adrenal gland. In adrenalectomized animals, the administration of ACTH has no effect. If, as demonstrated here, ACTH does have local tissue effects, it represents a new phase of activity of this compound for further consideration.

DR. STEPHEN ROTHMAN (Chicago, Ill.): Drs. Malkinson and Ferguson in our department have studied the percutaneous absorption of C\(^{14}\) labelled hydrocortisone by analyzing the urine. In this way they find a very rapid and considerable absorption. The beautiful radioautograph of the presenters seems to indicate that the absorption is via hair follicles.

DR. ALLENE SCOTT (in closing): Thank you very much.

As far as the action of corticotrophin, we have no means of answering whether it is ACTH, pitressin or some other pituitary hormone. We used both the commercially prepared ACTH and also ACTH which has been thought to be rela-
tively purified, but certainly more experiments will have to be done to rule out
the actions of other hormones, trophic in nature, contained in the material.

In the adrenalectomized cases it is quite true that those patients with Addi-
son's disease were maintained on cortisone at the time the tests were done but
in our patients who served as controls we were giving systemic cortisone in that
dosage, and no alteration of the control test area was seen. In the adrenal-
ectomized patients, they were all tested prior to their introduction to the hor-
mone therapy. Essentially the same effects were noted, as with adrenalectomized
patients—a partial or total suppression of reaction in the pretreated areas, and
no alteration of the response in the control sites.