the strip of guinea pig intestine. When extracted for histamine by the method of Barsoum and Gaddum, there is no evidence that exudin contains any histamine. Its behavior toward cortisone and its indiffusibility indicate that leukotaxine and histamine are dissimilar in nature to exudin. Finally, the intravenous injection of ACTH suppresses the local increase in capillary permeability referable to exudin. The possibility of these findings in regard to a further understanding of the mechanism of ACTH in various infectious conditions are discussed.

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Dermal Spreading of Hyaluronidase as Influenced by Prolonged Local Treatment with Certain Steroid Hormones.* (18860)

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Previous reports from this laboratory have demonstrated the increase in rate and alteration in pattern of dermal spreading by hyaluronidase as influenced by prolonged local (1) and parenteral (2) treatment with adrenocortical extract. As a result of these studies, it was desirable to examine the effects on enzymatic spreading of prolonged local pretreatment with some individual steroids.

Materials and methods. The steroids chosen for investigation were desoxycorticosterone,† 11-dehydro-17-hydroxycorticosterone (cortisone)† and progesterone.† The first 2 were supplied in the free alcohol form and were dissolved in 25% alcohol at a concentration of 1 mg/cc. The progesterone was dissolved in 50% alcohol at the same concentration. Twenty-one litter mate adult male rats of the Long-Evans strain in the weight range of 295 ± 75 g were used. For 5 months 0.1 cc of the desoxycorticosterone solution in 25% alcohol was applied daily to the dorsal cervical region just caudal to the right ear of 6 animals covering an area of approximately 10 sq cm; identical applications of the cortisone solution were made on 6 other animals. Four control animals were treated in a comparable site with 25% alcohol. Three animals were treated in the same manner and dosage with the progesterone preparation and their 2 controls received applications of 50% alcohol. In all 5 groups the left side of each animal was left untreated. Body weights were determined and regrown hair on the neck was clipped at weekly intervals after hair growth patterns had been recorded (3). The technic employed for study of the spreading action of hyaluronidase has been described in detail by Hayes and Reed (4). The hair was clipped closely from the proposed injection sites on each side of the neck and the animals were secured loosely for constancy of position. The animals were quieted by the subcutaneous injection of 0.3 cc of a veterinary solution of sodium pentobarbital (6.5% solution) and were anesthetized lightly with ether only for the duration of the initial injection. The indicator employed was an isotonic solution of hemoglobin. The final injection quantity was 0.05 cc containing 25 units of hyaluronidase.†

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1. Hayes, M. A., Reed, T. G., and Baker, B. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 361.

2. Hayes, M. A., and Baker, B. L., in press.

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3. Butcher, E. O., *Anat. Rec.*, 1934, v61, 5.

4. Hayes, M. A. and Reed, T. G., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 357.

The rate of injection was 0.01 cc/5 sec. The manipulation of the injection procedure was controlled carefully and intradermal injections were made in comparable sites on each side of the dorsal midline of the neck. One study was made on each side of each animal.

Measurements were made of the length and width of the ellipsoidal area of spread. The first measurement which was made at the termination of the injection was taken as a baseline. Then, observations were made every minute for 15 minutes; every 5 minutes through 30 minutes; every 10 minutes through one hour, and every 15 minutes through 2 hours. The increase in area for each time interval over that of the initial injection area was calculated. The arithmetic means were plotted, using the ordinate as the increase in area of spread over the initial area in sq mm and the abscissa as the logarithm of the time in seconds.

Results. There was no significant difference in the rate of body weight gain in any of the animals. Eighty-four per cent of the individual measurements in this entire study fall within the range of 4 sq mm of the plotted arithmetical means.

Complete inhibition of hair growth was produced by desoxycorticosterone and cortisone for 3 months prior to the period of testing. Progesterone, on the other hand, disturbed the symmetry of regrowth on the 2 sides but did not show evidence of inhibition. In the alcohol control animals no alteration in hair regrowth was detectable.

Fig. 1 graphically presents the results of the hyaluronidase spreading activity as influenced by prolonged prior treatment with desoxycorticosterone and with cortisone. Curve B represents spreading action on the treated side of the desoxycorticosterone animals and curve F the treated side of the cortisone animals; curve A and curve E are their respective controls showing rate of spreading on the untreated sides of the same animals. Curves C and D illustrate the enzyme activity on the two sides of the alcohol control animals, respectively. It is apparent that there is no significant difference between right and left sides of alcohol control animals and the untreated sides of the experimental animals. The chief difference appears to be restricted to the area of steroid treatment resulting in a marked accentuation of enzyme activity and a significant alteration in the pattern of spreading as is apparent in the slow phase of spreading, as defined in other investigations reported from this laboratory(1,2,4).

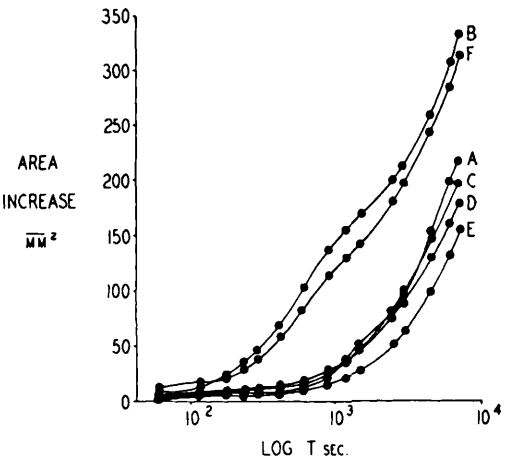

FIG. 1. Alterations in rate and pattern of spreading resulting from prolonged local pretreatment: Curve A, untreated side of desoxy animals; Curve B, treated side of desoxy animals; Curve C, untreated side of alcohol animals; Curve D, treated side of alcohol animals; Curve E, untreated side of cortisone animals; Curve F, treated side of cortisone animals.

In Fig. 2, on the other hand, progesterone pretreatment shows a different effect. In the slow phase of enzymatic spreading (to 10^3 sec) there is no significant difference between the treated and untreated sides of the experimental animals and either side of the alcohol control animals. The principal effect of progesterone has been a marked increase in enzymatic spreading in the rapid phase of spreading. The effect on enzymatic spreading was not influenced by the state of hair growth activity.

Discussion. Hayes and Baker(2) have suggested that adrenocortical hormones may affect the hyaluronidase enzyme-substrate complex in 2 antagonistic ways as measured by the dermal spreading of an indicator. Prolonged pretreatment locally(1) or parenterally(2) accentuates enzymatic spreading by altera-

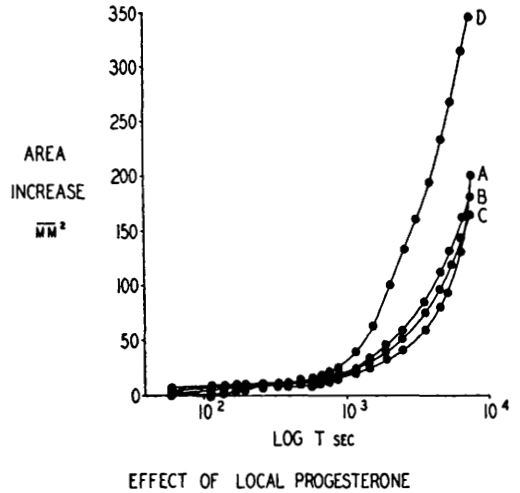

FIG. 2. Alterations in rate of spreading resulting from prolonged pretreatment: Curve A, untreated side of alcohol animals; Curve B, treated side of alcohol animals; Curve C, untreated side of progesterone animals; Curve D, treated side of progesterone animals.

tions in the connective tissue ground substance; acutely elevated levels of circulating adrenocortical hormones restrict enzymatic spreading by direct inhibition of the enzyme (2). Since these results may explain the contradiction concerning inhibition of enzyme activity by adrenocortical extract(5-7), it may also explain the contrary reports of inhibition by cortisone(5,6,8,9,10). The results of the present study demonstrate a marked *accentuation* in area of hyaluronidase spreading with all 3 steroids, desoxycorticosterone, cortisone and progesterone, as determined by measurements at 10^4 seconds. There is one great dissimilarity between the action of the steroids with an adrenal type of structure and the sex hormone; with the adrenal steroids, the slow phase of spread is modified so that it nearly has been abolished; while, with progesterone there is no difference in the slow phase of spread from any of the control situations.

It is interesting that prolonged pretreatment with desoxycorticosterone, the "salt active" steroid, or 11-dehydro-17-hydroxycorticosterone, the "carbohydrate active" steroid has an identical effect on hyaluronidase spreading of an indicator substance, particularly since Seifter, Baeder, and Begany(7) in an acute experiment, found synovial permeability to be increased to a maximum by either the enzyme or desoxycorticosterone. The individual effects were not additive with simultaneous administration of both enzyme and steroid.

The alteration in the slow phase of spreading as produced by the adrenal steroids and not by progesterone further strengthens the suggestion made by Hayes, Reed, and Baker (1) that the characteristic effect of prolonged treatment with adrenocortical steroids is on the slow phase of spreading activity.

Summary. An accentuation of hyaluronidase activity has been observed in skin which had been treated locally for 5 months with desoxycorticosterone, 11-dehydro-17-hydroxycorticosterone and progesterone. Only the adrenocortical steroids produced a characteristic alteration in the slow phase of spread. These alterations are interpreted as representing possible alterations in connective tissue ground substance, specifically related to steroids of adrenocortical origin.

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