Dermal Spreading of Hyaluronidase as Influenced by Prolonged Local Treatment with Adrenal Cortical Extract.* (18198)

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The inhibition of the spreading activity of hyaluronidase by adrenal cortical extract and various adrenal cortical steroids has been reported by several investigators(1-4). Opsahl (1) and Seifter *et al.*(4) believed that adrenal hormones may inhibit hyaluronidase indirectly by increasing the resistance of the ground substance to enzymatic depolymerization. These investigators did not rule out the possibility that adrenal steroids inhibit the enzyme by direct action. Since Castor and Baker(5) have demonstrated that prolonged local treatment of the skin with adrenal cortical extract induces marked structural changes in the dermis which appear to involve the ground substance, this report is concerned with the study of the effect of pretreatment of the skin with adrenal extract on the spreading action of hyaluronidase.

Materials and methods. Eight adult male rats of the Long-Evans strain were used, 5 being treated and 3 serving as controls. For 4 months 0.1 cc of an adrenal extract (ACE) in 25% alcohol† was applied to the dorsal cervical region just caudal to the right ear. The controls were treated on the right side in a comparable area with 25% alcohol. In both groups, the left side received no treatment. The indicator employed was a hemoglobin solution. The final injection quantity was 0.05 cc containing 25 units of hyaluronidase† in an isotonic solution of the indicator. The time for the injection was 25 seconds. The animals were quieted by the subcutaneous injection of 0.2 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 hair was clipped closely from the proposed injection sites and the animals were secured loosely for constancy of position.

The manipulations of the injection procedure were controlled carefully as suggested previously(6) and the intradermal injections were made in comparable sites on each side of the animal. The measurements made were the length and width of the ellipsoidal area of spread. The first measurement was made 60 seconds after the beginning of the injection and then every minute for 15 minutes; every 5 minutes through 30 minutes; every 10 minutes through one hour and every half hour through 2 hours. A total of 736 observations was made on the 8 test animals. The increase in area for each time interval over that of the initial injection area was then calculated. The arithmetic means of all increases were determined and plotted graphically, the ordinate being the logarithm of time in seconds and the abscissa the increase in area of spread over the initial area in square millimeters. Eighty-seven per cent of all single observations fell within a range of 6 sq. mm from the plotted arithmetical means.

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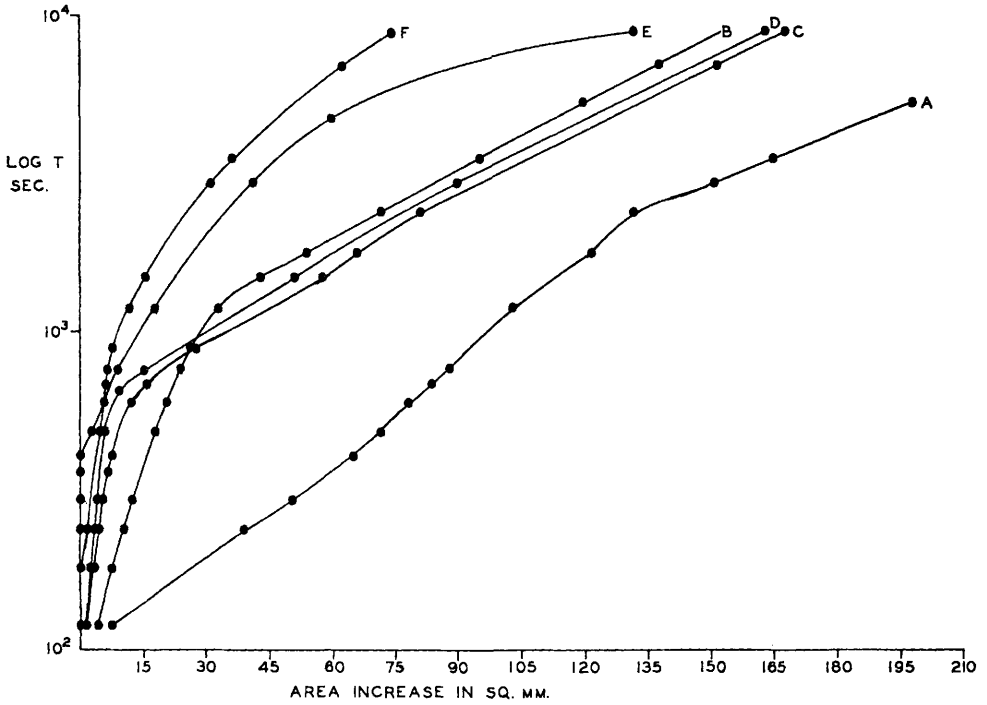

FIG. 1.

Graphic presentation of results expressed as arithmetic means: Curve A, enzyme plus indicator on treated side of ACE animals; Curve B, control side of ACE animals; Curve C, treated side of alcohol animals; Curve D, control side of alcohol animals. Curves E and F represent the observations made on a single ACE animal using indicator alone. Curve "E" is the treated side. Curve "F" is the control side.

After a period of 10 days, the experiment was repeated on a single adrenal cortical extract treated animal using indicator solution without hyaluronidase to determine the effect of local pretreatment on the spread of the indicator alone.

Results. The results are presented graphically in Fig. 1. Curve A represents the spread of hemoglobin indicator plus enzyme as influenced by the previous application of adrenal cortical extract. The initial slow phase of spreading which has been described(6) is much less distinct, resembling more a straight line function.

Curve B presents the results from the untreated side of the group of animals receiving ACE locally on the opposite side. After an initial slow phase which is intermediate between the determinations from both sides of the alcohol control animals and the right side of the ACE animals, the rapid phase of spread conforms closely to the spread of the alcohol-treated group. Curves C and D, respectively, represent the right (alcohol-treated) and left (no treatment) sides of the alcohol control animals. No significant difference is seen between the two.

Curve E is a single experiment employing an isotonic saline preparation of the indicator without enzyme on the ACE side of one animal; Curve F is the control (no treatment) side of the same animal. While no quantitative conclusions can be made from this single examination, it is readily apparent that the pattern of spreading action is distinctly different from the experiment in which the enzyme was injected (Curve A).

Discussion. The observation that pretreatment of skin with adrenal extract increases the rate and extent of spread by hyaluronidase is contrary to the reports of others(1-4) who demonstrated an opposite effect when adrenal hormones and hyaluronidase were administered concurrently. Our previous work(6) suggested that the substrate concentration serves as a limiting factor in the slow phase

of spread. However, it appears that adrenal extract so modifies the connective tissue ground substance as to accentuate the spreading. From the shape of Curve A, which demonstrates a definite alteration in the initial slow phase of diffusion, one may infer that the substrate (hyaluronic acid) has been so reduced in amount and/or modified in character that the substrate is no longer as sharply a limiting factor in the enzymatic activity as on the untreated side.

It has been shown previously(6) that irrespective of the enzyme concentration and/or the volume injected, all "normal" spreading activity is characterized by an initial slow phase followed by a more rapid phase of spreading. The observation in this study that indicator alone (Curve E) does not spread as rapidly in the ACE treated area as does the enzyme plus indicator emphasizes the fact that the result is not due entirely to the extreme thinning of the dermis(5), but that there has been some structural alteration in the ground substance which renders it more susceptible to hydrolysis by the enzyme.

The significant alteration in the slow phase of spreading in Curve B suggests that there is a concurrent systemic effect on the ground substance even though the treatment always had been localized. This alteration lends further support to the contention that the action of ACE is primarily on the slow phase since no significant difference from the alcohol controls (Curves D and C) is apparent in the rapid phase.

From the foregoing conclusions, one may assume that the difference in the slow phase of spreading in the ACE-treated skin may be characteristic of the alteration in the ground substance; the exact nature of this change is as yet unknown. These observations raise the possibility that prolonged action of adrenal cortical compounds on connective tissue ground substance might be a factor in the etiology of the collagen diseases.

Summary. An accentuation of hyaluronidase activity was observed in skin which had been treated locally wtih adrenal cortical extract for 4 months. This effect was the result of a probable alteration in the connective tissue ground substance.

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Effect of Antibiotics on Mortality from Internal Radiation.* (18199)

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Rats dead of P^{32} poisoning commonly show bacterial lesions at autopsy. Because of the possibility that bacterial toxemia might play a rôle in radiation death, an investigation was made of the protective effect of antibiotics.

Methods. A total of 248 white male rats of the C. F. Wistar strain weighing about 175 g each were evenly divided into 124 treated rats and 124 untreated controls. The animals were kept in individual cages in an air-conditioned unit permitting constant temperature and humidity. Radioactive phosphorus was injected intraperitoneally in amounts ranging from 3 to 6 μc/g body weight. Streptomycin and penicillin were employed in combination, since the tissue lesions showed both gram negative bacilli and gram positive cocci. The doses were 12 mg of streptomycin twice daily and 20,000 units of penicillin every other day and these were given intramuscularly in the hind extremities. Of the 124 rats which received antibiotics, 49 were pretreated for periods ranging from 3 to 10 days, while in 75 treatment was begun immediately after injection of the P^{32}. In all instances the antibiotics were discontinued

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