Influence of ACTH and Cortisone upon Alteration in Capillary Permeability Induced by Hyaluronidase in Rats.* (18341)

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The scope and nature of the physiological processes which come under the influence of the adrenal cortex is only beginning to be appreciated. Alterations induced in the animal body by the administration of adrenal cortical steroids are widespread and are obtained in many tissues(1). It has been suggested(2-4) that the plasticity of the ground substance of connective tissue may be, in part, under the control of these steroids. The permeability of connective tissues and membranes to dyes, particulates and fluids is increased following local application of hyaluronidase preparations(5,6,7). Intravenous administration of the enzyme produces an increase in capillary permeability(8,9). Following adrenal stimulation (produced by ACTH, epinephrine or stress conditions) or the administration of Compound E or adrenal cortical extract, the alterations in connective tissue and membrane permeability resulting from hyaluronidase are reported to be suppressed(3,5,10).

The present investigation demonstrates the inhibitory effect of ACTH and cortisone acetate on the increase in capillary permeability induced by the intravenous administration of a hyaluronidase preparation.

Materials and Methods. Female Sprague-Dawley rats weighing approximately 184 g were fed Purina chow and water *ad lib.* Weights were noted at the start of the experimental period and at the time of the hyaluronidase injection. Two mg of Evans blue in 0.2 or 0.5 ml of saline were administered intravenously by tail vein. One-half or one ml of a saline solution of bull testis hyaluronidase, assaying 184 units† per mg, and containing 2000 units, was given by tail vein immediately following the dye. Control animals received an equal volume of saline. The concentration of Evans blue in the blood, total serum protein, and hematocrit were de-

TABLE I. Influence of ACTH and Cortisone Upon Serum Dye Concentration, Serum Protein Concentration, and Hematocrit Measured 30 Min. After Inj. of a Hyaluronidase Preparation.

	No treatment*	ACTH	Cortisone
Evans blue conc. corrected for body wt/ml serum			
Saline	205 ± 4.2	194 ± 4.9	199 ± 4.1
Hyaluronidase	155 ± 3.3	177 ± 3.5	168 ± 5.3
% inhibition	—	67 ($p<.001$)†	40 ($p<.05$)
Serum protein concentration g %			
Saline	6.40 ± .11	6.20 ± .2	6.78 ± .1
Hyaluronidase	5.32 ± .19	5.84 ± .1	6.12 ± .2
% inhibition	—	67 ($p<.05$)	40 ($p>.1$)
Hematocrit—%			
Saline	48.6 ± 1.3	52.2 ± 1.8	51.8 ± 1.5
Hyaluronidase	57.2 ± 0.9	51.3 ± 2.3	52.4 ± 2.1
% inhibition	—	110 ($p<.01$)	93 ($p<.01$)

* The values are the means and standard errors for each treatment group. Each group consisted of 10 rats. The experiment was done in 2 parts, 4 animals per group in the first part and 6 animals per group in the second part.

† P values are for the order of significance of the difference in hyaluronidase effect between treated and untreated animals.

† The unit is arbitrary as previously described (11).

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TABLE II. Influence of Duration of Treatment with Cortisone Acetate Upon Disappearance of Evans Blue from the Blood of Rats Injected with Hyaluronidase. Evans blue concentration corrected for body weight per ml of blood.

	12 hr		24 hr		48 hr		96 hr	
	Control	Cortisone acetate†	Control	Cortisone acetate	Control	Cortisone acetate	Control	Cortisone acetate
Saline	126 ± 1.4	127 ± 2.7*	126 ± 2.2	122 ± 0.5	132 ± 4.4	131 ± 1.9	121 ± 3.1	122 ± 0.8
Hyaluronidase	90 ± 7.0	92 ± 5.4	75 ± 3.7	85 ± 7.6	82 ± 3.5	101 ± 5.0	97 ± 2.4	113 ± 3.4
% inhibition		3(p>.9)		26(p>.1)		40(p<.05)		64(p<.01)

* This group contained 4 animals; all other groups consisted of 5 animals.
† 5 mg per animal per day.

termined 30 minutes following the administration of the enzyme by methods previously described(12). In the experiments shown in Table I, the dye concentration was measured in the serum; whole blood was used in subsequent experiments. The ACTH‡ was administered subcutaneously in 2 daily doses of 2.5 mg per 0.5 ml of an 0.85% saline solution. Cortisone acetate was given subcutaneously as a suspension diluted with saline so that each animal received 2.5 mg twice a day except as indicated in Table II. Evans blue concentration following the injection of a constant quantity of dye varies inversely as the plasma or blood volume and blood volume varies as the body weight(13). In order to compare the serum or blood dye concentrations in animals of differing weights a correction was introduced. Dye concentration in μg per ml of serum or blood was multiplied by the body weight of the animal taken at the time of the test. The values were all divided by 200 g to yield concentration of dye per ml of serum or blood for a 200 g rat. These values are recorded in the tables.

Results. The experiment in Table I was designed to test whether ACTH or cortisone acetate would inhibit the increase in capillary permeability induced by testicular extract. One group of animals was pretreated for 5 days with ACTH, another with cortisone acetate, and a third received no treatment. Each group was then divided in half. One half of the animals received saline and Evans blue intravenously, the other half received hyaluronidase and Evans blue. Table I demonstrates that both ACTH and cortisone acetate inhibited the effects of the hyaluronidase. The inhibition of the change in the 3 indices of enzyme action studied (dye concentration, serum protein, and hematocrit) was statistically significant in all but the effect of cortisone upon the serum protein change.

The experiment shown in Table II was evolved to determine how soon the inhibitory action of cortisone acetate becomes manifest. Groups of 5 animals each were injected subcutaneously twice daily with the steroid suspension while control animals were treated with saline. Twelve, 24, 48, and 96 hours following initiation of therapy, rates of Evans blue disappearance were tested in groups of animals after injection of enzyme solution or saline. Although the inhibition of the effect of testicular extract appeared 24 hours following initiation of therapy, a statistically significant reduction did not occur until 48 hours had elapsed.

A single large dose of 20 mg of cortisone acetate, as obtained, in 0.8 ml of the commercial diluent§ was administered subcutaneously to a group of 10 animals. Controls received an equal volume of the diluent subcutaneously. Twelve hours later they were tested as in the previous experiment. No inhibition was demonstrable.

Discussion. The exact nature of the action

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‡ ACTH was obtained through the courtesy of Dr. Edwin E. Hays of the Armour Laboratories. The dosage refers to LA-1-A standard.

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§ Cortisone acetate suspension was purchased from Merck and Co.

of intravenously administered hyaluronidase in the rat is unknown. The increased rate of disappearance of Evans blue from the circulation, the decrease in serum protein concentration, and the increased hematocrit following injection of the enzyme indicate an increase in capillary permeability. Histologic examination of the grossly edematous areas in these animals(14,15) does not show rupture of the capillary wall as seen by Zweifach and Chambers(16).

Present knowledge(17,18) of the *in vitro* action of partially purified testicular extracts makes it reasonable to assume that the enzyme acts upon such polysaccharides as hyaluronic acid and chondroitin sulfate. If these polysaccharides are components of the intercellular substance of the capillary, the present work suggests that the intercellular substance becomes resistant to the action of testicular hyaluronidase following administration of ACTH and cortisone. To the extent that there is increased resistance of the capillary to the action of testicular enzyme our data agree with the reports of others who found that local and distant administration of adrenal cortical extract inhibited the spreading and permeability increasing effect of testicular extract in skin(3,19) and synovial membranes(4).

An immediate effect due to local application of adrenal substances and the rapid emergence of an effect following distant administration(3,4,19) suggest a direct action of the compounds on connective tissue. Inhibition *in vitro* of the action of hyaluronidase on membrane permeability(5) is also consistent with the hypothesis of a direct action. In contrast, our data show no significant inhibition until 48 hours of treatment had been given. From the results of this investigation one may postulate an intermediate step between the administration of cortisone and its action on the tissue. The following possibilities exist: 1) cortisone acetate is not the active substance and must be modified by some cellular process to another compound which acts directly on the tissue or the enzyme, or 2) cortisone affects the cells of the mesenchyme which in turn alter the intercellular substance. Winter and Flataker present evidence indicating that cortisone acetate is not the locally active compound. Such activity, however, was found to be present in adrenal cortical extract(20). That cortisone acetate has an effect on mesenchymal cells has been demonstrated(21,22).

Summary. Intravenous administration of 2000 units of partially purified testicular hyaluronidase in rats induced an increased rate of disappearance of Evans blue, a decreased concentration of serum protein, and an increased hematocrit. These manifestations of an increased capillary permeability due to the action of the enzyme preparation were largely inhibited by 5 days pretreatment with subcutaneous administration (5 mg per day) of either ACTH or cortisone acetate. A minimum of 24 to 48 hours of pretreatment with cortisone acetate was required for a discernible inhibition of the hyaluronidase effect. The evidence suggests an intermediate step between cortisone acetate and its action on connective tissue.

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