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Effects of Hyaluronidase and Cortisone on Connective Tissue Studied Electrometrically.* (23074)

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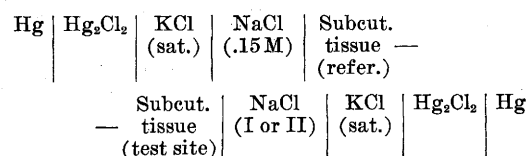
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The ground substance of connective tissue behaves in many respects as heterogeneous colloid consisting of negatively charged aggregates. Of these, mucoproteins form a prominent component(1). The physico-chemical state of the ground substance is modulated by variations in internal environment and through reversible action of certain hormones and enzymes(2-5). An example of reversible behavior, reported in this paper, is the resynthesis of connective tissue colloids following disaggregation induced by hyaluronidase.

Many of the properties of the ground substance have been related to colloidal charge density, which can be determined electrometrically by measurement of liquid junction potentials(1-3). The electrometric method was used in this investigation to study disaggregation and reformation of connective tissue colloids in normal and cortisone treated rabbits after injection of hyaluronidase. The rate at which this occurs is believed to reflect a synthetic activity of cells. The results suggest that this procedure may be useful as a test of biologic resiliency of connective tissue.

Material and methods. Liquid junction potentials were measured serially on 22 albino rabbits, approximately 3 months of age. Eight of these animals received two 5 mg intramuscular injections of cortisone acetate (Merck) 48 and 24 hours prior to time of analysis. In 4 of these rabbits, electrometric

determinations were also made before cortisone was administered. The animals were anesthetized by an intraperitoneal injection of 3% sodium pentobarbital. A 20-gauge one-half inch hypodermic needle was then inserted into the dermal layer of the shaved anterior abdominal wall. A reference junction was formed by placing a similar needle under the skin of the left hind leg.[†] After the desired salt solutions were instilled, the needles were connected to small syringe barrels filled with saturated KCl in agar which, in turn, were joined by saturated KCl bridges to calomel half cells. A Leeds-Northrop type K-2 potentiometer and galvanometer completed the circuit, which may be represented as follows:



A baseline potential (E) was measured with 0.15 M NaCl (solution I) at both junctions. In each instance final value was based on the average of 3 consecutive readings taken at approximately 30-second intervals, and agreeing within one millivolt. The isotonic saline was then withdrawn from the experimental needle (abdomen) by aspiration with a 27-gauge needle, replaced by solution II, a 1/10

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[†] The reference junction may also be conveniently made by placing the shaved ear in a beaker of isotonic NaCl.

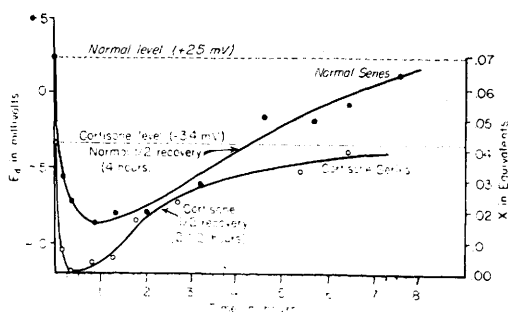


FIG. 1. Effect of hyaluronidase on dermal connective tissue in normal and cortisone treated rabbits. Each experimental point is mean of 5 or more observations. Standard deviations ranged from 1.0 to 2.4 in normal animals, and 0.5 to 1.7 in those receiving cortisone. Half recovery time in each series is estimated from mid-point between initial level and point of maximal displacement.

dilution (0.015 M NaCl), and the displaced potential (E') determined. Finally the dilute salt solution was replaced with 0.15 M NaCl, and the original baseline was restored. The difference, $E' - E$, is termed the dilution potential (E_d). After measurement of initial dilution potential, 5 turbidity reducing units (TRU) of a freshly prepared solution of testicular hyaluronidase[†] dissolved in 0.1 cc of 0.15 M saline was injected at the test site. Dilution potentials were determined at frequent intervals at first and later at more widely spaced times until the values approached their initial levels (Fig. 1). The relationship between the dilution potential and the density of immobile negative charge in the connective tissue (x) can be expressed by the approximation formula: $E_d = -12.3 + 215x$, where E_d is measured in millivolts and x is expressed as equivalents/kg of tissue water (1,5,6).

Results. The results of dilution potential determinations in the dermis of normal and cortisone treated animals are compared in Fig. 1. The average dilution potential in the normal rabbit was $+2.5 \pm 1.0$ mv corresponding to a colloidal charge density of 0.069 equivalents.[§] In animals pretreated with cortisone, the initial dilution potential averaged -3.4 ± 1.3 mv corresponding to a colloidal charge density of 0.041 equivalents,

or 60% of the normal level. After hyaluronidase action, dilution potentials decreased on the average to -8.5 mv for normal animals, and to -12.0 mv for cortisone treated rabbits. Return of dilution potentials to the initial state occurred more rapidly in the cortisone series. Half-recovery in this group required an average of about $2\frac{1}{2}$ hours as compared with 4 hours for normal animals.

Discussion. Continuous changes in the state of connective tissue can be studied by measuring dilution potentials as a function of time. The relation of such measurements to the concentration of negatively charged colloid has been established (5,6). In dense connective tissue such as bone and cartilage, where the colloid is considered to be highly aggregated, the dilution potential may attain values of $+25$ mv and x may be as high as 0.175 equivalents/kg of tissue water. In loose tissue, as for example synovial fluid, the values of E_d tend to approach the theoretical limit of -12.3 mv for the state where x approaches zero.

Certain hormones and enzymes alter the state of connective tissue colloids, increasing fraction of soluble colloids and lowering density of immobile negative charge. Dilution potentials may then tend to approach the lower theoretical limit. When these effects are reversed, potentials return to their initial levels. Accordingly, electrometric readings over an extended period of time offer an objective means of studying reversible responses of connective tissue.

Electrometric studies of rabbit skin indicate that normally the colloids of the dermis are not highly aggregated. Treatment with cortisone produces a decrease in E_d . As a result of this initial modification of connective tissue the effect of hyaluronidase in the cortisone treated animals is enhanced. Previous histochemical and electrochemical studies have shown that cortisone administration in the rat leads to the formation of a

[†] Wyeth and Co. contributed a generous supply of Wydase.

[§] Experiments in which liquid junctions are made directly to exposed dermis by means of moistened cotton have confirmed the existence of contiguous regions showing dilution potentials between -2 and $+2$ millivolts.

more loosely aggregated ground substance in the skin(3). Cortisone appears to increase the rate of formation of certain connective tissue colloids. However, these components have modified physicochemical properties. Stimulating effects of cortisone upon connective tissue have also been demonstrated in nasal mucosa altered by the allergic state(7) and in atopic dermatitis(8).

The rate at which connective tissue is disaggregated and restored following administration of hyaluronidase may be used as an index of its initial state and of its capacity for repair. It is probable that this response varies under the influence of many other hormones and drugs, as well as in disease states. The serial electrometric determination of these changes in connective tissue colloids may serve as a connective tissue "function" test, measuring the ability to maintain homeostasis.

Summary. Reversal of hyaluronidase effects on concentration of negatively charged colloids in the dermis of normal and cortisone treated rabbits was studied by serial measurement of liquid junction potentials. In normal animals, hyaluronidase lowers negative charge

density in the tissues; recovery is half completed in about 4 hours. In animals treated with cortisone initial charge density is lower than in normal animals. After hyaluronidase is injected into the dermis, the lowest levels are reached. Half recovery, however, occurs in $2\frac{1}{2}$ hours. Serial electrometric measurements following hyaluronidase injection are suggested as a means of studying hormone and drug effects, and disease states in connective tissue.

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Lack of Specificity of Insulin- I^{131} -Binding by Isolated Rat Diaphragm. (23075)

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Stadie and coworkers reported an increase in glycogen synthesis by the isolated rat diaphragm exposed briefly to solutions of insulin(1). It was suggested that insulin is bound firmly to tissues at or near sites of enzyme systems and that such fixation is necessary for its physiologic action. Stadie, Haugaard and Vaughn(2) later noted that, following brief immersion of diaphragm into solutions of S^{35} -labeled or I^{131} -labeled insulin, a small fraction of radioactivity remained fixed to the tissue even after repeated washing with saline solution, and with increasing concentrations of insulin there was a tendency for

bound insulin to reach a limiting value at about $1\frac{1}{2}$ to 2 $\mu\text{g/g}$ tissue.

In the course of studies with a variety of I^{131} labeled proteins from different sources, e.g., serum albumin, gamma globulin, insulin, glucagon, ACTH, diphtheria toxoid, etc., we observed that, at tracer levels, most of these proteins adsorb nonspecifically to almost any inert surface, and that such adsorption may be minimized by presence of high concentrations of almost any other protein. With insulin, particularly, adsorption to glass(3), and paper(4,5) has been reported previously. It therefore seemed of interest to reevaluate