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Received November 18, 1966. P.S.E.B.M., 1967, v125.

Studies of Insulin Effect on Permeability of Dermal Connective Tissue. (32048)

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Our previous studies have shown that numerous metabolites and other compounds affect the permeability of dermal connective tissue(1-4). The changes in permeability appeared to be due to alterations in the physico-chemical properties of hyaluronic acid, which is the main constituent of the ground substance of soft connective tissue(1-3). Since insulin has been reported to be an important agent regulating the permeability of cell membrane towards glucose(5) and proteins(6), it suggested the investigation of the effect of this hormone on the permeability of soft connective tissue, *i.e.*, dermis.

The studies were performed on 3- to 4-month-old albino rats of both sexes. They concerned two types of experiments: the effect of local application of insulin (U-40 Regular Iletin, Insulin Injection, U.S.P., Eli Lilly & Co.) and its systemic effect by intraperitoneal injection.

Materials and methods. The permeability was measured by one of our previously

described methods(1,2) based on the rate of dermal diffusion of dye alone or combined with test substance. The procedure consists of intradermal injection of 0.05 ml of a 0.4% solution of Evans blue in physiological saline (pH 7.1) with or without test substance. Three injections on each animal are made for the control (generally dye alone) and 3 for the test compound. Thus, each animal serves as its own control. The contours of the blue spots formed are traced onto semi-transparent paper at 30-, 60-, 120-, and 180-minute intervals after injection. The areas of the spots traced on the semi-transparent paper are cut out, weighed, and calculated as mm². The area of each spot is entered as an individual result in the calculation of the average value and standard deviation. Reproducibility of results and tests obtained by this method were discussed elsewhere(1,2,4).

Results and discussion. Two concentrations of insulin, 1.6 and 5 units per ml were used in local applications injected intradermally along with the dye (Table I). In the study of the systemic effect of the hormone, the rats were treated for 3 weeks with daily intraperitoneal administration of 1 unit insulin. Then the effects of dye alone, ascorbic acid and ascorbic acid combined with thiourea

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hyaluronic *in vitro* enhanced skin 'permeability' *in vivo* and compounds which inhibited its depolymerization acted also as inhibitors of this permeability(1,2,7,8).

Thus, the alterations in connective tissue permeability appear to be due to changes in the degree of polymerization of hyaluronic acid, the major component of the ground matrix of soft connective tissue. And since the ground substance is essentially responsible for the extravascular transport of metabolites to and from the cells,[§] the changes in connective tissue permeability are measurements of the rate of intercellular movements of biological substances.

Conclusion. The present results with insulin seem to indicate that the hormone, at the doses used in the present work, is without local and systemic effect on the intercellular transport of metabolites, at least if tested by

the rate of intradermal diffusion of Evans blue dye.

The authors wish to thank Mr. Donald E. Hinton for his valuable assistance.

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[§] Paper in preparation.

Received November 29, 1966. P.S.E.B.M., 1967, v125.

Factors Influencing the Inotropic Effect of Corticosteroids.* (32049)

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Certain corticosteroids exert a positive inotropic effect on isolated cardiac muscle as well as in intact animals(1,2,3). Previous work(4,5) has shown that aldosterone does not exert a positive inotropic effect in cat papillary muscles maintained at 37°C in buffer containing normal concentrations of calcium (2.54 mM), whereas aldosterone increased contractile force by about 10 to 12% when the calcium concentration was reduced to 0.63 mM(5,6). Hydrocortisone, (cortisol), a glucocorticoid, does not exert significant

inotropic effects at either calcium concentration. Preliminary studies revealed that hypothermia also enhanced the responsiveness of cat papillary muscles to aldosterone(6). The present study was undertaken in order to determine whether papillary muscles maintained at 27°C in a low calcium medium would show an altered inotropic response to corticosteroids other than aldosterone and whether alterations of the concentrations of other cations could modify the inotropic response to corticosteroids.

Methods. The isolated cat papillary muscle preparation previously described(6) was used exclusively in this investigation. A modification of Krebs-Henseleit solution previously described(6) was used as the bathing medium. The solution was gassed with 95% + 5% CO₂ at a temperature of 27 ± 1°C or of 37 ± 0.2°C. The pH of this solution is 7.3

* This investigation was supported by Nat. Heart Inst. Grant HE-09924. The author wishes to thank Mr. Thomas F. Inge, Jr., for technical assistance, Mrs. Mary S. Duggan, Upjohn Co., Kalamazoo, Mich. for a generous supply of Solu-Cortef, and Dr. Thomas A. Barry, Merck Inst. for Therapeutic Research, Rahway, N. J. for a generous supply of Decadron.