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% CO_2 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.

TABLE I. Effect of Corticosteroids Dissolved in Ethanol on Contractility of Cat Papillary Muscle at 27°C in 0.63 mM Calcium Buffer.

		Steroid-treated		Ethanol-treated
Steroid	N	Concentration (μg/ml)	% Δ CF	% Δ CF
Prednisolone	8	.5 *	+ .5 ± 2.4	+ .3 ± 2.5
	8	2.5	— 8.8 ± 3.0	— 8.0 ± 3.4
	8	10.0	—40.1 ± 3.8	—41.3 ± 4.5
Methylprednisolone	4	.5	+ .4 ± 2.6	+ .6 ± 2.7
	4	2.5	—10.2 ± 3.8	—12.6 ± 3.7
	4	10.0	—44.6 ± 7.1	—46.2 ± 6.4
Deoxycorticosterone	8	.5	+ .9 ± 3.3	+ 2.0 ± 3.8
	8	2.5	—13.3 ± 4.7	—14.8 ± 3.0
	8	10.0	—45.0 ± 6.9	—49.8 ± 6.5
Betamethasone	6	.5	+ 3.8 ± 1.2	— 1.3 ± 3.8
	6	2.5	— .2 ± 3.0	— 2.3 ± 2.4
	6	10.0	—29.8 ± 6.2	—30.0 ± 5.9
2 α-methyl-9 α-FF	4	.1	.0 ± .0	.0 ± .0
	4	.25	+ 3.3 ± 1.6	.0 ± .0
	4	.5	+ 3.0 ± 2.6	— .5 ± .2
	4	2.5	— 6.3 ± 6.3	— 5.0 ± 2.0
	4	10.0	—33.8 ± 3.5	—31.5 ± 6.2
Substance "S"	8	.5	+ 6.9 ± 4.3	— .5 ± 2.9
	8	2.5	—11.5 ± 3.5	—10.9 ± 4.3
	8	10.0	—45.6 ± 3.2†	—58.1 ± 3.8
Hydrocortisone	8	.1	+ 2.0 ± 2.0	+ 1.0 ± 2.0
	8	.5	+ 3.0 ± 3.6	+ 3.3 ± 3.8
	8	2.5	— 8.5 ± 2.4	— 7.3 ± 1.8
	8	10.0	—39.0 ± 5.8	—38.5 ± 3.2
Dexamethasone	4	.1	+ 2.5 ± 2.2	+ 5.2 ± 4.4
	4	.5	.0 ± 4.1	— 1.5 ± 3.4
	4	2.5	— 8.3 ± 2.4	—13.3 ± 2.8
	4	10.0	—40.0 ± 2.9	—44.5 ± 3.5
Aldosterone	6	.25	+ 3.2 ± 1.5	+ .7 ± 2.8
	6	1.0	+14.7 ± 3.5†	— 3.1 ± 1.6
	6	2.5	+27.7 ± 4.2†	— 8.7 ± 3.5
	10	5.0	+20.9 ± 2.5†	—11.4 ± 3.2

All values are means ± standard errors.
% Δ CF = percent change in contractile force.
* The same values apply to that of the ethanol concentration expressed in μl/ml. Thus if a steroid-treated papillary muscle received 0.5 μg/ml of a steroid, the vehicle control muscle received 0.5 μl/ml of ethanol.
† Significant from vehicle control ($p < .01$).

and the osmolality is 285 mOsm. Papillary muscles ranging from 0.7 to 1.1 mm in diameter were used. These muscles exhibited developed tensions of 1.8 to 2.5 g/mm^2 measured at the peak of the length-tension curve. Osmolality was maintained with sucrose in the low sodium solution. All steroids were obtained in the crystalline state and dissolved in ethanol. In addition, dexamethasone, hydrocortisone, prednisolone and methylprednisolone were also obtained in aqueous solution.

Results. Table I is a summary of the inotropic responses to a variety of corticosteroids. At least 3 different concentrations were tested for each steroid. Each response to an addition of steroid to the bath was compared with the response to an addition of a corresponding amount of ethanol. Measurements were made after a period of 30 minutes. Under the conditions of the experiments, contractile force randomly changed only an average of 2% over 30 minutes with no addition to the bath. It can be seen that at the higher steroid concentrations, (2.5 μg/ml and above), a prominent negative inotropic response was observed for all steroids used except aldos-

TABLE II. Inotropic Effect of Aqueous Solutions of Corticosteroids in Cat Papillary Muscles.

Steroid	Concentration (μg/ml)	N	Steroid (% Δ CF)	Aqueous vehicle (% Δ CF)
Hydrocortisone	.1	6	+ 4.5 ± 2.8	+1.8 ± 1.0
	.5	8	+ 8.5 ± 3.8	+3.0 ± 2.4
	2.5	8	+11.8 ± 1.7*	— .6 ± 1.1
	10	8	+20.1 ± 3.8*	+ .8 ± 1.5
	25	6	+13.2 ± 2.0*	— .5 ± 2.6
Dexamethasone	.5	6	+ 2.4 ± 2.5	+2.3 ± 1.2
	2.5	8	+ 8.1 ± 6.0	+4.8 ± 5.1
	10	9	+21.0 ± 2.5*	+2.3 ± 3.0
	25	8	+22.6 ± 5.4*	+3.8 ± 3.3
Prednisolone	.5	7	+ 2.9 ± 2.9	+1.2 ± 1.6
	2	7	+ 3.2 ± 2.0	—1.4 ± 2.2
	10	7	+ 6.4 ± 2.9	+2.6 ± 2.1
Methylprednisolone	.5	7	+ 1.9 ± 2.7	—1.3 ± 2.1
	2	7	+ 3.9 ± 6.8	+2.1 ± 3.0
	10	7	+ 3.0 ± 2.6	+1.6 ± 2.2

All values are means ± standard errors.
% Δ CF = percent change in contractile force.
* $p < .01$ from vehicle control.

terone. This negative inotropic effect could be entirely accounted for by the ethanol in the solution. The only instances of a significant positive inotropic effect of a corticosteroid were found at 1.0, 2.5, 5.0 μg/ml of aldosterone. At 10 μg/ml, Substance S provided a significantly smaller negative inotropic effect when compared with the ethanol response ($p<0.01$) but this is difficult to equate with a positive inotropic response.

There does not appear to be any obvious relationship between mineralocorticoid potency and inotropic activity of the corticosteroid tested. Thus, 2 α-methyl-9 α-fluorocortisol, which has comparable mineralocorticoid activity to aldosterone, was completely devoid of inotropic activity. Reichstein's Substance S, which at high concentrations has moderate mineralocorticoid activity, had only a slight effect at a relatively high concentration (10 μg/ml). DOC elicited no response nor did any of the glucocorticoids used.

Since ethanol depressed the contractile force of the papillary muscle, four glucocorticoids, hydrocortisone and dexamethasone, prednisolone, and methylprednisolone, were administered in a water soluble solution. Dexamethasone phosphate (Decadron; Merck, Sharp and Dohme), hydrocortisone sodium-succinate (Solu-Cortef; Upjohn), prednisolone acetate (Meticortelone; Schering) and methylprednisolone acetate (Depo-medrol; Upjohn) were used. These are among the most commonly used corticosteroids and are the only water soluble corticosteroids commercially available. Table II shows the results of these experiments. It is of interest that in these experiments, both hydrocortisone and dexamethasone increased contractile force by about 20% at comparable concentrations, whereas prednisolone and methylprednisolone were without significant effect. None of the aqueous vehicles significantly depressed nor stimulated contractility at any dose level administered.

In addition to the experiments performed at a reduced calcium concentration, studies on the inotropic effects of aldosterone and hydrocortisone were performed in a low sodium (100 mM) and a low potassium (2.0 mM) Krebs-Henseleit solution. These studies were performed at 27 and at 37°C. No significant inotropic effects were observed at any concentration used (same range as that shown in Table I). Therefore, decreasing the sodium or the potassium concentration does not sensitize cardiac muscle to the inotropic effects of corticosteroids as does lowering the calcium concentration.

Discussion. These findings indicate that glucocorticoids are capable of exerting modest positive inotropic effects if they are administered in a vehicle which itself does not exert a

negative inotropic effect. Other substances used to dissolve steroids such as methylene chloride and propylene glycol also markedly depress the contractile force of the papillary muscle. Therefore, ethanol is not unique among steroid solvents in depressing cardiac contractility.

Ethanol, however, appeared to decrease the responsiveness of cardiac muscle to corticosteroids. This can be inferred from the fact that the steroid treated muscles showed the same degree of depression as the ethanol treated muscles in almost all cases. If the ethanol merely depressed contractility, one would expect the steroid treated muscles to show less depression than their corresponding vehicle controls, particularly in the cases of dexamethasone and hydrocortisone.

This study calls attention to the fact that the manner in which corticosteroids are solubilized may play an important role in determining their eventual effect. Certainly, if the vehicle exerts an effect opposite in direction to that of the steroid, the steroid effect may be masked by the vehicle. This appears to be the case with the inotropic effect of corticosteroids dissolved in ethanol.

The mechanism for the enhancement of corticosteroid induced inotropic responses in a low calcium medium remains unanswered. Since lowering the sodium or the potassium concentration of the medium did not enhance inotropic responsiveness to corticosteroids, some specific role of calcium (*e.g.*, in relation to excitation-contraction coupling) may explain this phenomenon. No obvious correlation of inotropic activity with mineralocorticoid, glucocorticoid or anti-ouabain potency exists. Therefore, it seems likely that the inotropic effect exerted by some corticosteroids is brought about by mechanisms other than those involved in the more classic actions of these hormones.

Summary. A series of 9 corticosteroids dissolved in ethanol were studied in the isolated cat papillary muscle preparation maintained at 27°C in a reduced calcium buffer. Only aldosterone exerted a significant positive inotropic effect. The magnitude of this positive inotropic effect was larger than previously observed at 37°C in a normal calcium buffer. The ethanol was markedly depressant to the papillary muscles. Hydrocortisone and dexamethasone, in aqueous solutions, also exerted positive inotropic effects, whereas prednisolone and methylprednisolone were ineffective. Lowering the sodium or the potassium concentration of the medium did not result in significant inotropic responses to aldosterone or hydrocortisone.

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Received December 2, 1966. P.S.E.B.M., 1967, v125.

Comparison of Endogenous and Exogenous Creatinine Clearances in Man.* (32050)

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Shannon(1) demonstrated that the excretion of exogenous creatinine in man is greater than can be expected from glomerular filtration alone. He concluded from these and

* Supported in part by USPHS HE-07852, Los Angeles County Heart Assn. and Southern California Chapter, National Kidney Foundation.