Effect of Steroids upon Resistance of Skin to Intracutaneous Injection. (19362)

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Cortisone inhibits the spreading action of hyaluronidase in the skin(1,2). The physical properties of the ground substance of connective tissue are probably related to the metabolism of hyaluronic acid, and evidence has been presented that this is partly under control of the adrenocortical steroids(3,4). Atrophic changes in the skin and inhibition of hair growth have also been described in animals treated with cortisone(5,6).

The present investigation demonstrates the effect of hyaluronidase and of cortisone upon the resistance offered by the skin to the intracutaneous introduction of fluids, as measured by the amount of pressure required to perform an intracutaneous injection under carefully controlled conditions.

Material and methods. Young adult male Holtzman rats of about 200-250 g body weight were used. Observations were made under light ether anesthesia. Injections were made in comparable shaved areas of the skin of the back in all animals. The apparatus used is illustrated in Fig. 1. The syringes and Tygon tubing connections shown in stippling were filled with water. The solution to be injected was drawn into syringe B, and needle A was inserted into the skin, care being taken not to get the point of the needle into the subcutaneous tissue. The automatic infusion pump G was then started, and injection continued until 0.05 ml of fluid was delivered from syringe B. The speed of the infusion pump was set so that the plunger of syringe D moved at a rate of 22 mm per minute. When care was taken to keep the volume of injected fluid and the rate of injection constant, the pressure readings (obtained on drum F) were remarkably uniform among different animals treated in the same way on the same day. Variations in volume or speed of injection markedly affected the pressure readings obtained. The solution used in syringe B was either physiological saline or 0.33% testicular hyaluronidase (a commercial preparation purchased from The Treemond Co., New York) in saline. The pressure readings reported were obtained by subtracting the pressure required to extrude the fluid from the needle (*i.e.*, in air) from that obtained when the needle was in the skin. From 4 such determinations on each animal, the mean for each

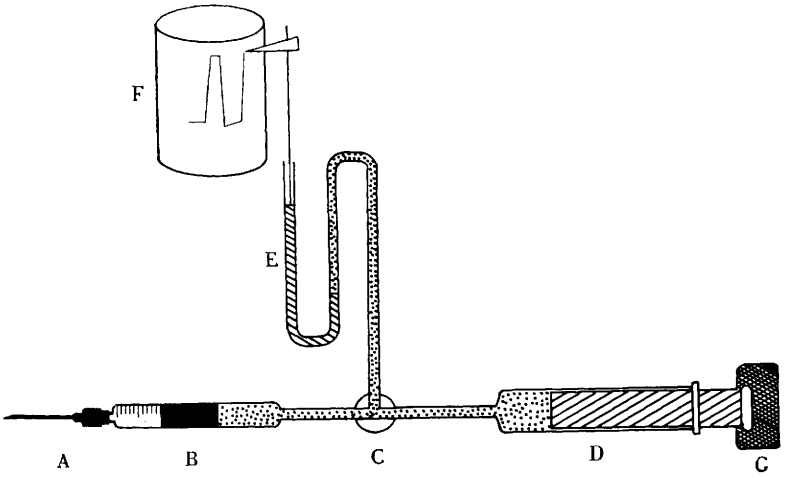

FIG. 1. Apparatus used for measuring intracutaneous pressure. A. 27 gauge ½-inch needle. B. Tuberculin 1 cc syringe with small segment of plunger (shown in black). C. 3-way stopcock. D. 5 cc syringe. E. Mercury manometer. F. Kymograph. G. Constant rate infusion pump.

TABLE I. Pressure in mm of Mercury Required to Introduce Fixed Volume of Fluid Under Controlled Conditions Intracutaneously in Rats.

Steroid	Inj. fluid	Day 1	2	3	4	Mean
None	S*	75.75	75.75	68.25	60	68.7
	H	56.25	37.25	49	38.25	45.2
Testosterone	S	82.50	70	66.25	49.25	67
	H	48.75	40.25	48	42.25	44.8
Desoxycorticosterone	S	75.50	75.25	64.75	53.75	67.3
	H	63.25	43	39	41.50	46.7
Compound S	S	61	69	58	58	61.5
	H	50.75	40.75	43.50	44.25	44.8
21-acetoxypregnenolone	S	78.50	71	62	58.50	67.5
	H	52.25	53.25	50.75	42.50	49.7
Cortisone	S	116	106.25	94	81	99.3
	H	84	63.25	70	67.25	71.1

Analysis of variance

Source of variation	df	F
(1) Days	3	3.68
(2) Hyaluronidase	1	32.71 s
(3) Error, hyaluronidase	3	1
(4) Steroids	5	50.42 v
(5) Error, steroids†	83	1
Total	95	

* S = Saline. H = Hyaluronidase.
† Including non-significant interactions.
Standard error for a single hyaluronidase observation = ± 18.4 mm Hg.
" " " " " steroid " = ± 6.72
s = significant (.01 < P < .05). v = very highly significant (P < .001).

group was calculated. Steroids were administered intramuscularly in saline suspension, 0.5 mg daily for 4 days, and the pressure measurements were made 2 hours after the last injection. The steroids used were cortisone acetate (Cortone Merck), desoxycorticosterone acetate (D.C.A.), 17 hydroxy-11-desoxycorticosterone acetate (Reichstein's Compound S), 21-acetoxy-pregnenolone (artisone), and testosterone propionate.

Experimental. Preliminary experiments demonstrated that cortisone alone of the steroids tested markedly increased the resistance of the skin to intracutaneous injection, that this effect was observed whether hyaluronidase was present in the injection fluid or not, but that the picture was complicated by the presence of considerable day-to-day variation. Therefore, an experiment was conducted according to an experimental design as detailed by Cochran and Cox(7), by means of which the effects of the various treatments of the animals could be assessed against the background of daily variation. The design of the experiment is clear from an inspection of Table I; each individual figure in the table is the average of 2 animals, so that a total of 96 animals was used, 16 on each steroid, 8 with hyaluronidase, and 8 without hyaluronidase in the fluid injected intracutaneously.

The data clearly demonstrate the following: 1) The amount of pressure required to make an intracutaneous injection under the conditions of these experiments, with saline as the injection fluid, varies from about 50 to 80 mm Hg, with an average of about 67 mm Hg. 2) If hyaluronidase is present in the injection fluid, the injection pressure is reduced by 21.5 mm Hg on the average, or about one-third. This finding is consistent with results of others(8,9). 3) Neither testosterone, D.C.A., Compound S, nor artisone has any effect upon the injection pressure, either in the presence or absence of hyaluronidase. 4) Cortisone increases the injection pressure by about 20 mm Hg, either in the presence or absence of hyaluronidase. The statistical

analysis shows no significant interaction between steroid treatment and hyaluronidase.* Therefore, the effect of the cortisone is independent of the effects of hyaluronidase.

Discussion. It is evident that cortisone has a highly specific effect in increasing the resistance offered by the skin to intracutaneous injection. Even though hyaluronidase lowered the injection pressure, and cortisone increased it, it is clear that this effect of cortisone is not due to its antagonism to hyaluronidase. This is evidenced by the fact that the cortisone had an equally great effect when hyaluronidase was not included in the injection fluid, and, as mentioned above, the independence between effects of steroid and effects of hyaluronidase. Furthermore, in previous experiments in this laboratory, some of which are published(2), it had been found that all the steroids used, with the exception of D.C.A. produced, when administered to intact rats, marked inhibition of hyaluronidase as measured by the spreading of intradermal fluids.

It must, therefore, be assumed that cortisone treatment produced some change within the tissue of the skin, presumably in the ground substance of the connective tissue, to account for the observed resistance of the tissue to introduction of fluid. Previous investigators have emphasized the influence of cortisone upon permeability(4,10) and probably the effect herein described is in some way related to that described by these authors, but the relative effects of the various steroids herein described are very different from those observed by Seifter and coworkers(10,11), who found D.C.A. to have an action opposite to that of cortisone, and artisone to be as effective as cortisone. The action of cortisone seen in our experiments is a more specific one than those observed by the authors cited; that is, a number of compounds which had an action similar to that of cortisone in Seifter's experiments were inactive in the present experiments. The collagen and amorphous ground substance of the skin are diminished and the synthesis of chondroitin sulfate is inhibited by cortisone(12). These metabolic changes may produce alterations in the physical properties of the skin which might account for the effects observed. Such alterations might include a change in the hydrophilic properties of the colloids in the skin so that aqueous fluids are less readily absorbed.

Summary. 1. Measurements have been made of the resistance offered by the skin to the intracutaneous introduction of fluid, as determined by the amount of pressure required to perform an intracutaneous injection under carefully controlled conditions in adult male rats. 2. Systemic administration of cortisone markedly increased the resistance of the skin to injection. Hyaluronidase, incorporated in the fluid administered intracutaneously, lowered the injection pressure. The effect of cortisone, however, was shown to be independent of the effect of the steroid upon hyaluronidase. Testosterone, desoxycorticosterone, compound S, and 21-acetoxypregnenolone were without effect under the conditions of these experiments.

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