Effect of Anti-Hyaluronidase Compounds upon the Response of the Rat to Pituitary Trophic Hormones. (20749)

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The parenteral administration of aqueous solutions of protein hormones usually results in responses which are submaximal. This phenomenon has been ascribed to rapid absorption and dissipation from the injection site, increased excretion or inactivation. Attempts have been made to circumvent these suboptimal effects by combining these hormones with agents that will protect them from rapid absorption and/or inactivation. Preparations which have decreased the rate of absorption or dissipation of protein hormones have been oil (1,2), magnesium stearate pellets(3), colloids such as polyvinyl pyrrolidone, gelatin, acacia (4.5) and chemical adducts such as tannates (6).

Recently, Hamburger(7) and Cohen *et al.* (8) have described the use of anti-enzyme agents to enhance the activity of corticotrophin. Hamburger(7) used polyphloretin phosphate and attributed the enhancing effect primarily to the anti-proteolytic properties of the compound. Cohen *et al.*(8) used phosphorylated hesperidin and ascribed the enhancement to the anti-hyaluronidase activity of the material, since other substituted hesperidins (methyl chalcone) which had anti-proteolytic activity but no anti-hyaluronidase action did not enhance the activity of corticotrophin. It should be pointed out that the latter two groups of investigators used different corticotrophin preparations. Hamburger(7) employed a crude preparation, whereas the other group(8) administered an oxycellulose purified corticotrophin which has been shown to be relatively resistant to tissue inactivation.

The present communication deals with the effects of antihyaluronidase agents upon the action of exogenous pituitary gonadotrophin. Further observation on the effects of some of these compounds upon ACTH is also presented.

Material and methods. Immature female rats (Blue Spruce Farms) weighing 35-45 g were used. A purified preparation of pituitary gonadotrophin (Armour)* was incorporated into solutions of the substance to be tested. 0.5 ml injections were given subcutaneously twice daily for a period of 3½ days. On the fifth day the animals were sacrificed and their ovaries removed and weighed. The total dose of the gonadotrophin administered was 0.25 units and 1.0 unit (Armour). Hypophysectomized animals were also employed and consisted of immature female rats weighing 50-60 g. Hypophysectomy was accomplished by the parapharyngeal approach. They were injected 3 days after the removal of the pituitary. Since each substance was tested at a different time, FSH in distilled water was run concurrently with the FSH in the menstruum being tested. The following compounds were investigated for their effect on the activity of pituitary gonadotrophin. 1) 2% phosphorylated hesperidin† 2) 2.5% d-alpha tocopherol phosphate‡ 3) 1.25% d-alpha tocopherol phosphate 4) 5% Chondroitin sulfate 5) 10% Chondroitin sulfate 6) 3% sodium alginate 7) 5% hesperidin methyl chalcone. For the corticotrophin studies, hypophysectomized Sprague-Dawley male rats, 110-115 g body weight, were used 24 hours after surgery. Injections were given subcutaneously in 0.5 ml volume. Three hours after the injection the adrenals were removed and the adrenal ascorbic acid determined by the method of Mindlin & Butler(9). Purified corticotrophin (Princeton Laboratories), 25 U.S.P. units/mg was used.

* The pituitary gonadotrophin was kindly furnished by Dr. Albert H. Holland, Armour Laboratories, Chicago, Ill.

† Phosphorylated hesperidin was kindly supplied by Dr. G. Martin, The National Drug Co., Philadelphia, Pa.

‡ Dr. S. Evert Svenson of Hoffman-LaRoche, Inc., made available the d-alpha tocopherol phosphate.

TABLE I. Effect of Anti-Hyaluronidase Compounds upon Response of Rat Ovary to Follicle Stimulating Hormone.

%	Vehicle	No. animals	Armour units	Ovarian wt, mg
	A. Intact animals			
.9	Distilled water	15	.25	16 ± 1
.9	" "	21	1.0	40 ± 2
2.0	Hph	9	.25	23 ± 3
2.0	"	10	1.0	89 ± 13
.9	Distilled water	9	.25	20 ± 2
.9	" "	14	1.0	46 ± 4
2.5	Tph	11	.25	64 ± 10
2.5	"	14	1.0	105 ± 13
2.5	"	3	0	12 ± 1
1.25	"	7	.25	45 ± 7
1.25	"	12	1.0	94 ± 12
.9	Distilled water	4	.25	12 ± 1
5.0	C.S.	8	.25	38 ± 5
10.0	"	5	.25	32 ± 10
3.0	A	5	.25	18 ± 2
2.0	H.m.c.	5	.25	12 ± 1
	B. Hypophysectomized animals			
.9	Distilled water	6	1.	41 ± 8
1.25	Tph	6	1.	80 ± 12
2.5	"	6	1.	85 ± 23

All values are means ± stand. error.
A = sodium alginate; Tph = d-alpha tocopherol phosphate; Hph = phosphorylated hesperidin; C.S. = Chondroitin sulfate; H.m.c. = hesperidin methyl chalcone.

Results and discussion. The data presented in Table I indicate that the activity of the follicle stimulating hormone when administered together with anti-hyaluronidase agents, was enhanced approximately 4 times as compared to the hormone activity of the gonadotrophin administered in distilled water. This is evident from the observation that 0.25 units of the gonadotrophin in conjunction with d-alpha tocopherol phosphate, phosphorylated hesperidin or chondroitin sulfate induced ovarian hypertrophy comparable to the response obtained with 1.0 unit of the hormone in distilled water.

All the compounds tested and found active in enhancing the effectiveness of the gonadotrophin were chosen because of their strong hyaluronidase-inhibiting properties(10-12). Since some of these substances also possess anti-proteolytic properties, it was deemed important to determine whether the enhanced activity was a result of delayed absorption due to the anti-diffusion property of the compounds, or to an inhibition of proteolytic destruction at the site of injection or in the blood stream. To separate these effects the methyl chalcone of hesperidin was employed. This compound possesses no anti-hyaluronidase activity(10) but does have considerable anti-proteolytic activity when tested *in vitro*(8). As may be observed from the data, this compound exerted no enhancing effect upon the exogenous FSH. This finding confirmed the hypothesis that the enhancing effect of the active compounds was due primarily to their anti-hyaluronidase properties. McShan & Meyer(13) had earlier described the augmenting effect of hemin upon sheep pituitary gonadotrophin, but offered no explanation for this phenomenon. Recently(14) hemin was found to possess a strong anti-hyaluronidase effect, which property may explain the earlier observations of McShan & Meyer(13).

Enhancement noted in the hypophysectomized animals with 1.25 and 2.5% d-alpha tocopherol phosphate indicated that the augmented ovarian weight so obtained could not be ascribed to increased endogenous pituitary gonadotrophic release or secretion. In addition, administration of the anti-hyaluronidase preparations without FSH failed to induce any gonadal stimulation, indicating that these agents do not possess inherent gonadotrophic activity or potentiality.

One should note that of the three anti-hyaluronidase agents used in this study and in the doses employed, phosphorylated hesperidin produced the greatest degree of local tissue reaction§ while no reaction was noted after injection of the chondroitin sulfate menstruum. The local tissue irritative response to d-alpha tocopherol phosphate was less than that noted with phosphorylated hesperidin.

Chondroitin sulfate was of interest because it offered an opportunity to observe a compound which inhibits hyaluronidase by competitive inhibition(12). Its effect upon corticotrophin was also examined. The result obtained (Table II) indicated that it was effective in enhancing the activity of corticotrophin as well as FSH.

The augmented response noted after incorporating FSH or ACTH compounds with

§ This irritative reaction may be due to contaminants.

TABLE II. Effect of Chondroitin Sulfate upon ACTH Induced Adrenal Ascorbic Acid Depletion, 3 Hr Post Injection. 5 animals in each series.

%	Vehicle	Dose ACTH, U.S.P. units	Adrenal ascorbic acid, mg/100 g adrenal gland
.9	Saline	0	371 ± 10
.9	"	1.0	273 ± 10
8.0	C.S.	1.0	155 ± 4
16.0	Gelatin	.1	236 ± 17
8.0	C.S.	.1	248 ± 7

preparations inhibiting hyaluronidase directly or by competitive inhibition may be ascribed to decreased absorption of the trophic material from the injection site. That the decreased rate of absorption from the site of injection may play a part in augmentation of pituitary gonadotrophic hormone was shown by the studies of Meyer and Hertz(15). They demonstrated that hourly injection of pituitary gonadotrophins over a 4½-day period produced a marked augmentation of response over that noted when the same dose was given at 12-hour intervals.

Summary. Anti-hyaluronidase agents, *i.e.* phosphorylated hesperidin, d-alphatocopherol phosphate, and chrondroitin sulfate were shown to be capable of increasing the effectiveness of administered pituitary trophic hormones such as follicle stimulating hormone (FSH) and corticotrophin. A four-fold increase in effectiveness of FSH was attained when the hormone was administered together with the anti-hyaluronidase agents as compared to the injection of the gonadotrophin alone. A similar degree of enhancement was obtained with corticotrophin. The augmentation induced by the anti-hyaluronidase compounds is attributed to the decreased rate of absorption of the hormone from the site of injection.

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Effect of Metabolites on Accumulation of Citric Acid in Fluoroacetate-poisoned Rats.* (20750)

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This study was undertaken in connection with our work on the effect of Krebs cycle inhibitors and metabolites on the performance and metabolism of isolated dog hearts, (heart-lung preparations). On adding dicarboxylic acids of the Krebs cycle into the blood stream in such preparations the problem of permeability arose. It cannot be assumed *a priori* that such highly charged dicarboxylate ions as are normally formed and metabolized within the cell will diffuse into the cell as readily as the glucose molecule. To gain more information on this point we have undertaken to study the organs of small animals *in situ,* using in the experiments here reported the fluoroacetate

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