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Hyaluronidase Inhibitor and Trypsin Inhibitor in Preparations from Human Urine.* (27005)

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Shulman(1) and Astrup et al.(2) have elaborated methods for isolating the trypsin inhibitor in human urine, and preparations with high inhibitor content have been obtained. These preparations also inhibit chymotrypsin and plasmin.

Recently it was observed that the hyaluronidase in human urine is strongly inhibited at pH 4.63 by a substance in the urine(3). The present communication shows that hyaluronidase is also inhibited by preparations purified by Astrup et al., and that concentrations of trypsin inhibitor and hyaluronidase inhibitor in preparations of different degrees of purification, parallel each other.

Methods. Trypsin inhibitor concentrations were estimated on casein by the biuret method (4). One trypsin inhibitor unit is the amount which inhibits 1 μg crystalline trypsin, containing 24.4 Anson units per gram (Novo Laboratories, Copenhagen).

Five preparations containing varying concentrations of trypsin inhibitor were prepared from human pregnancy urine and placed at our disposal by Dr. Tage Astrup. Prep. I contained 56.4, Prep. II 187.5, Prep. III 234, Prep. IV 333 and Prep. V 612 trypsin inhibitor units per mg. Preparations I to IV were prepared as before(2). Preparation V had been further purified (Astrup, to be published).

Hyaluronidase activity was estimated viscosimetrically(3), using USP Reference Standard hyaluronidase (bovine testis). Thirty μg of hyaluronidase preparation and different amounts of Prep. V (Fig. 1) or of preparation I to IV (Table 1), dissolved in 30 μl acetate buffer (pH 4.63) containing albumin, were added to 150 μl of the substrate (details in (3)).

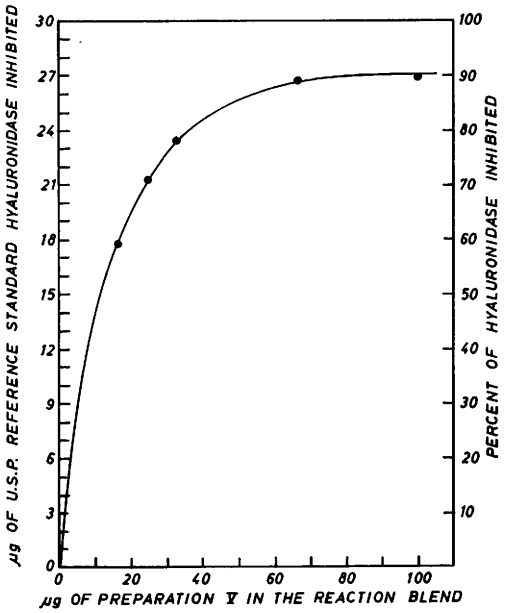

FIG. 1. Inhibition of USP Reference Standard Hyaluronidase by different amounts of a purified urinary trypsin inhibitor preparation containing 612 trypsin inhibitor units per mg (Preparation V). *Abscissa,* μg of trypsin inhibitor preparation in the reaction blend. *Ordinate,* Inhibited amount of hyaluronidase expressed in % of total amount present and μg of hyaluronidase inhibited.

Results. Fig. 1 shows that preparation V, the purest of the trypsin inhibitor preparations, inhibits testis hyaluronidase. The curve indicates a dissociable binding between hyaluronidase and hyaluronidase inhibitor. It is inter-

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TABLE I. Contents of trypsin inhibitor and hyaluronidase inhibitor in preparation I to IV.

Trypsin inhibitor preparation	Amt. of preparation in reaction blend, mg	Units of trypsin inhibitor in reaction blend a	μg of hyaluronidase inhibited b	Amt of hyaluronidase inhibitor* in reaction blend c	c/a
I	.100	5.6	11.4	8	1.4
IV	.030	10.00	18.5	18	1.8
II	.100	18.8	23.3	32	1.7
III	.100	23.4	24.9	42	1.8

* expressed as μg of preparation V as estimated by interpolation on the curve in Fig. 1. (c/a for preparation V is 1.6).

esting that hylauronidase present in human urine also is influenced by an inhibitor in a dissociable manner(3).

Table 1 compares concentrations of trypsin inhibitor in Preparations I to IV with estimated amounts of hyaluronidase inhibitor. Column *a* shows amounts of trypsin inhibitor present in the reaction mixtures calculated from the activities of the trypsin inhibitor preparations. Column *b* shows amounts of hyaluronidase inhibited as estimated in the experiments. Column *c* shows amounts of hyaluronidase inhibitor present as calculated from the values in column *b* by interpolation on the curve in Fig. 1 and expressed as μg of preparation V. The last column shows the proportion between amounts of hyaluronidase inhibitor found and trypsin inhibitor present in the reaction mixtures. It is seen that approximately identical proportions are obtained.

It seems permissible to conclude that concentrations of trypsin inhibitor and hyaluronidase inhibitor parallel each other in the different preparations. Apparently the hyaluronidase inhibitor and trypsin inhibitor behave identically during the process of purification, suggesting that the two effects are caused by a single compound. This possibility is interesting in view of the relationship between urinary trypsin inhibitor excretion and pituitary-adrenal hormones as demonstrated previously (4,5).

Summary. Trypsin inhibitor preparations isolated from human pregnancy urine inhibit hyaluronidase. The activity curve indicates formation of a dissociable compound. The trypsin inhibitor concentration in preparations of different purity parallels the content of hylauronidase inhibitor suggesting identity of the 2 inhibitors.

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Acute Effects of 9α-fluorohydrocortisone and 2-methyl-9α-fluorohydrocortisone on Renal Clearances of Sodium and Potassium in Man.* (27006)

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Before beginning a study of the modifying effects of sodium-retaining steroids upon mercurial diuresis, it was necessary to establish the usual pattern of response to the steroids in normal human subjects. The steroids used were 9 ∝-fluorohydrocortisone (FF) and 2-methyl-9 ∝-fluorohydrocortisone (2MeFF). The latter compound has been shown by Liddle and his associates(1) to exert a more potent sodium-retaining effect than aldosterone. The acute

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