Relation between Pituitary-Adrenal System and Excretion of Trypsin Inhibitor in Urine.* (23949)

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Various early investigators(1,2,3) have studied the trypsin inhibitor (T.I.) in human urine. They observed an increased excretion in cancer and infectious diseases. Dillard (4) resumed these investigations with modern methods in an effort to devise a cancer test, but found the increase of T.I. in urine to be too unspecific for neoplasms. It was accidentally found in this laboratory that urine from pregnant women contains large amounts of T.I. (Astrup and Storm, unpublished). We have since observed an increased excretion of T.I. during pregnancy, in artificial fever, in various infectious diseases and during gold therapy(5). Since stress probably is the common denominator in these different conditions, excretion of T.I. in urine could be a physiological response to stress. The present investigations were based upon this hypothesis.

Methods. 1. *The inhibitor* (T.I.) was estimated against trypsin on a casein substrate using the biuret reaction. *Reagents. Buffer I;* 0.1 M barbital sodium, pH 7.75 + NaCl to ionic strength 0.15. *Buffer II;* buffer I, containing 0.01 M $CaCl_2 \cdot 2H_2O$. Crystalline trypsin (24.4 A.U./g) was kindly provided by NOVO Laboratories, Copenhagen. Casein, 3% prepared after(6), but dissolved in buffer I. Biuret reagent, modified according to(7). 0.50 ml buffer II, containing 3.75 μg trypsin, is incubated at 37°C with 0.50 ml urine solution (appropriately diluted with buffer II). After 20 minutes, 2 ml casein solution is added, and the mixture incubated 40 minutes at 37°C, followed by addition of 1 ml 20% perchloric acid. The mixture is filtered after one hour and 2 ml of the filtrate is added to 1 ml 2 N NaOH and 3 ml biuret reagent. After 40 minutes incubation at 37°C, the optical density is determined at 545 mμ. In the blanks perchloric acid was added (after 20 minutes incubation of trypsin with urine dilution) before addition of the casein solution. Free trypsin is found by interpolation on a trypsin dilution curve, and bound trypsin calculated. Inhibition of trypsin follows a straight line until 85% inhibition, above which it deflects, indicating that the compound formed is dissociable. All measurements were performed on the straight part of curve, and a T.I. unit was defined as the amount which can inhibit one μg of crystalline trypsin (24.4 A.U./g). Further details of the method will be published elsewhere. 2. *Hormone determinations* were performed according to standard procedures(8) by the Hormone Department of the Statens Serum-institut through the kindness of Dr. Chr. Hamburger.

Results. Effect of adrenocorticotropic hormone. In 4 normal men and 4 normal women from 20 to 40 years of age, and in a 55-year-old male patient with aplastic anemia, the total daily excretion of T.I. in urine before and during treatment with ACTH was estimated. The normal subjects were given 25 i.u. ACTH i.m. on 2 successive days. The patient received daily doses of ACTH i.m. for 2 weeks. Table I and Fig. 1 demonstrate that T.I. excretion increases considerably during ACTH administration.

Effect of cortisone and hydrocortisone was studied in 10 normal persons (age: 20 to 55 years). The hormones were given orally (as acetates) in doses of 100 mg for 3 consecutive days (cortisone) or for 2 days (hydrocortisone). Cortisone and hydrocortisone, like ACTH, increase excretion of T.I. many times. Fig. 2 shows a typical curve. In only one (apparently normal) person administration of cortisone was for no obvious reasons not followed by an increased T.I. excretion.

17-ketosteroids, 17-ketogenic steroids and T.I. in urine. Urine from a normal woman

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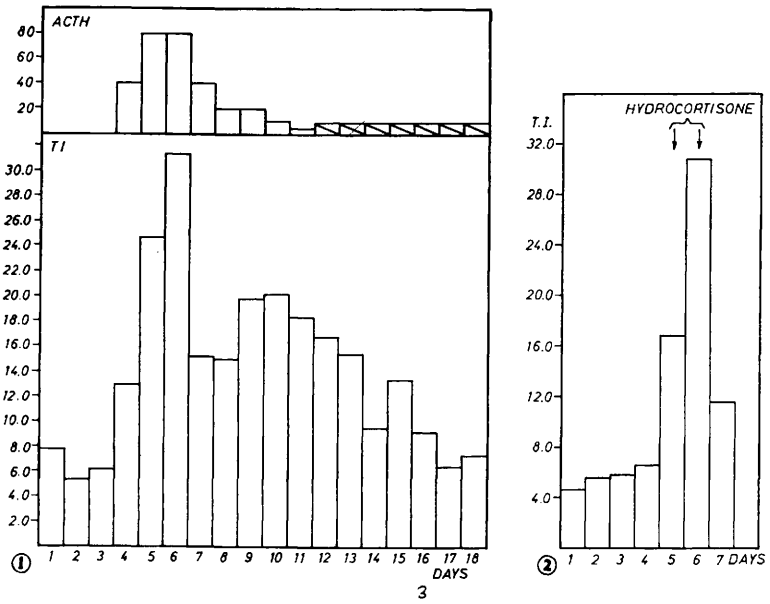

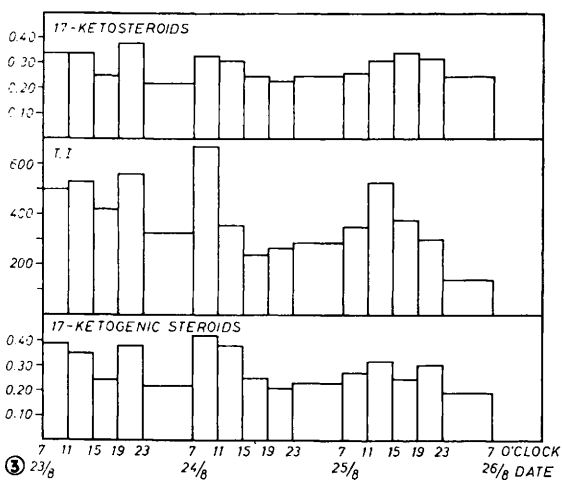

FIG. 1. Excretion of trypsin inhibitor in urine from a patient with aplastic anemia, before and during treatment with ACTH. *Abscissa:* Time in days. *Ordinates:* Trypsin inhibitor in units $\times 10^{-3}$, and dose of ACTH in international units. An ACTH preparation with protracted action was used in the last 7 days (columns marked with a diagonal).

FIG. 2. Effect of hydrocortisone on excretion of trypsin inhibitor. *Abscissa:* Days. *Ordinate:* Trypsin inhibitor in units $\times 10^{-3}$. The arrows indicate the days on which hydrocortisone acetate (20 mg 5 times daily) was given.

FIG. 3. Excretion of 17-ketosteroids, 17-ketogenic steroids and trypsin inhibitor in a woman. *Abscissa:* Date and hour. *Ordinates:* 17-ketosteroids and 17-ketogenic steroids in mg/hr. Trypsin inhibitor in units/hr.

was collected in samples every 4 hours during the day and once a night in 3 successive days. 17-ketosteroids, 17-ketogenic steroids and T.I. were estimated and the average excretion/hour calculated for individual samples. Fig. 3 shows that T.I. closely follows the steroid excretion.

Discussion. Since cortisone and hydrocortisone produce an increase of T.I. in urine, the increased excretion of T.I. in stress and during ACTH administration could be caused by the increased release of adrenocortical hormones in these conditions. The relation observed between the excretion of T.I. and of

TABLE I. Effect of ACTH on Excretion of Trypsin Inhibitor in 8 Normal Subjects.

	T.I. units × 10^{-3} excreted					Avg T.I. excretion/day		Increase during ACTH treatment, %
	No treatment			ACTH, 25 i.u. i.m.		Before ACTH treatment	During ACTH treatment	
Age, yr	Day 1	Day 2	Day 3	Day 4	Day 5			
30	.0	.0	.36	3.15	7.46	.12	5.31	4325
30	1.35	.90	.0	1.52	3.96	.75	2.79	272
30	.68	1.10	1.40	2.03	2.40	1.06	2.22	109
22	2.02	1.20	.90	4.14	4.71	1.37	4.43	223
40	3.10	2.88	1.51	5.11	11.79	2.50	8.45	238
29	4.73	4.04	7.96	13.73	16.77	5.58	15.25	174
24	7.80	5.07	5.70	16.17	29.04	6.19	22.61	265
40	10.54	5.98	7.35	15.39	20.68	7.96	18.04	127

17-ketosteroids and 17-ketogenic steroids in urine suggests a close connection between function of pituitary-adrenal system and excretion of trypsin inhibitor. The increase in T.I. could be caused either by increased production of inhibitor or by increased renal excretion. Though it is too early to consider any connections between the observations here presented and pathological states in the organism, it is interesting to note that some investigators have reported proteolytic activity in tissues and blood in conditions where ACTH and corticosteroids have a therapeutic effect, especially in anaphylactic reactions. The results support our hypothesis that the increase of trypsin inhibitor in urine in various physiological and pathological conditions is caused by a stress reaction.

Summary. 1. Administration of ACTH, cortisone or hydrocortisone to human beings produces an increase in excretion of trypsin inhibitor in urine. 2. Excretion of trypsin inhibitor during the day is nearly parallel to excretion of 17-ketosteroids and 17-ketogenic steroids. 3. The pituitary-adrenal system plays a role in regulation of excretion of trypsin inhibitor.

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Nutritional Studies of Hydroxyzine and Rauwolfia in Cattle and Lambs. (23950)

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Although tranquilizing drugs have been studied with monogastric animals, the levels of the drugs fed were within the sedating range. No reports of investigations of nutritional effects of tranquilizing drugs upon ruminants have been found. The present report compares effects upon growth, feed efficiency and carcass quality of tranquilizing drugs fed to beef steers and wether lambs in a range generally below the level at which sedation would be expected. The tranquilizers tested were crystalline pharmaceutical grade hy-