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SECTION MEETINGS

IOWA	
State University of Iowa	March 29, 1954
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Comparison of 17-Ketosteroid and Uropepsin Data on 69 Healthy Adult Males.* (20994)

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During the past few years a number of investigators(1,2) have suggested that uropepsin excretion is an indicator of adrenal cortical activity. Others have questioned this assumption. Having collected considerable data on uropepsin and 17-ketosteroid excretion in the same individuals during the course of another type of study, we decided to analyze our data to see whether any correlation exists between these two measures. It is recognized generally that the larger part of the urinary 17-ketosteroid excretion is derived from the adrenal cortex, with the lesser part coming from the gonads(3). Many workers have shown that 17-ketosteroid excretion in normal individuals is increased following ACTH administration(3,4). The complete relationship between cortiscosteroids and 17-ketosteroids is not yet known, but there is evidence which indicates that normal C_{21}-11-desoxy and C_{21}-11-oxygenated hormones of the adrenal cortex contribute to the titer of urinary 17-ketosteroids(4). In the opinion of investigators who have made an extensive study of the methods of extraction and assay of the urinary steroids related to the adrenal cortex, the methods for the urinary corticosteroids do not give dependable, quantitative data(5), whereas those for the urinary 17-ketosteroids are generally reliable(6). As a consequence, in the present study the measurement of 17-ketosteroid excretion was selected as the index of adrenal cortical activity.

During the course of an investigation of certain biochemical factors in healthy male sex offenders data were collected on the 17-ketosteroid and corresponding uropepsin levels

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TABLE I. Urinary Excretion of 17-Ketosteroids and of Uropepsin.

Age, yr	No. of persons	Avg day to night ratios	No. of persons	Avg excretion, mg/24 hr	Median values mg/24 hr	Chi square	Probability level
17-Ketosteroids							
17-35			46	10.97	10.4	5.67	.017*
36-58			23	8.24	7.1		
17-30	35	1.30	37	10.68	10.4		
31-40	14	1.69	16	10.55	9.4	4.69	.096
41-58	14	1.30	16	8.12	6.7		
Uropepsin			West, units/hr		West, u/hr		
17-35			46	36.13	29.3	.09	.800*
36-58			23	42.24	26.5		
17-30	35	1.38	37	37.26	29.5		
31-40	14	1.39	16	41.00	36.5	.57	.720
41-58	14	1.47	16	37.44	24.5		

* Difference between age groups may be expected to be due to chance 17 and 800 times, respectively, in 1000 samples.

of 69 individuals. Since administration of ACTH increases the excretion of both 17-ketosteroids(3) and uropepsin(2) in healthy individuals it seems logical to assume that a correlation might exist between the excretion levels of these two entities without the administration of ACTH. Such evidence would strengthen the belief that the determination of uropepsin can be used as a measure of adrenal cortical activity. The purpose of this paper is to present the results of a comparison of these data.

Subjects. The present study utilized 2 groups of male subjects. The first of these was composed of 55 healthy sex offenders who had been committed to Metropolitan State Hospital for a period of observation and treatment under the California Sex Psychopath Act. All persons used for this study had been given routine physical examinations including chest x-rays by the hospital staff. Those who showed evidence of organic disease were excluded. A further check was made by determination of white blood counts and sedimentation rates immediately prior to the collection of the urine samples to be used for the 17-ketosteroid and uropepsin determinations. The second group was composed of 14 male medical students and research workers at the University of California at Los Angeles. For the purpose of comparing the 17-ketosteroid excretion with uropepsin output these two groups of subjects were combined.

Methods. One to 3 pairs of consecutive 12-hour urine specimens were collected on each subject. The first morning specimen of urine was discarded. The day collection was begun immediately thereafter and continued for 12 hours. Collection of the night specimen was then begun and continued through the first morning specimen voided. Care was taken to protect these specimens from light and to keep them refrigerated. Steroid and uropepsin determinations were run on the same specimen; the uropepsin determinations were done within 36 hours of the start of collection and the steroid extraction within 50 hours. 17-ketosteroid determinations were made by a method of hydrolysis and extraction perfected in this laboratory(7). In this extraction process 6 N sulfuric acid is used in place of concentrated hydrochloric acid for the hydrolysis of the conjugates. The assay method used is a modification(7) of the Callow absolute alcohol technic for the Zimmerman determination. Uropepsin determinations were made according to the method of West *et al.*(8). The endpoint of this method is the clotting of milk by the urinary enzyme at pH 4.9. West's method is rapid, reproducible, and extremely sensitive. The results reported in this paper in units per hour are 1/24th of the total 24-hour excretion per person. Output of uropepsin per hour for normal individuals falls between 10 and 40 West units.

Results. In order to determine the relationship between 17-ketosteroid and uropepsin excretion, comparisons were made of the 24-

hour excretion values and of the day to night ratios, respectively, of the two measures. In all instances, data for both measures were obtained from the same urine specimen. It should be noted that the values for both measures cover a 24-hour period for the absolute excretion values and a 12-hour period for the calculation of the day to night ratios (essentially waking-sleeping ratios). Urine samples collected over much shorter periods of time might give erroneous relationships between 2 or more biochemical measures because of different rates of reaction during the interval between the initiation of stress and the collection of the specimen.

a. 24-Hour excretion values. The average and median values of the data obtained in this study are given in Table I. In agreement with the findings of others the 17-ketosteroid data(3,9) are seen to vary significantly with age, whereas the uropepsin values(10) show no such variation. Because of this variation in 17-ketosteroids with age, the data obtained were originally divided into two categories; one for individuals 17 to 35 with a median age of 25, the other, 36 to 58 with a median age of 43. Further analysis of the data, however, disclosed that whereas this division into two age groups shows the only statistically significant difference in 17-ketosteroid excretion values with age, it appears to mask the relationship between 17-ketosteroid and uropepsin excretion. This could be due either to the lack of variation in uropepsin excretion with age, or to the influence on uropepsin excretion of factors which have no special bearing on 17-ketosteroid excretion. Accordingly, the data were divided into 3 age groups for comparison of these measures. It seems probable that the reduction in 17-ketosteroid excretion with age is due to both decreased adrenal cortical activity and gonadal activity. Pincus(9) has reported that while the difference he found between the 17-ketosteroid excretion of young and old men might be attributed to testis precursors, the quantitative relationship (young and old men *vs* true eunuchs) is not entirely reconcilable with this explanation. He suggests that either an adrenal cortical 17-ketosteroid precursor is produced in lesser amounts in older men,

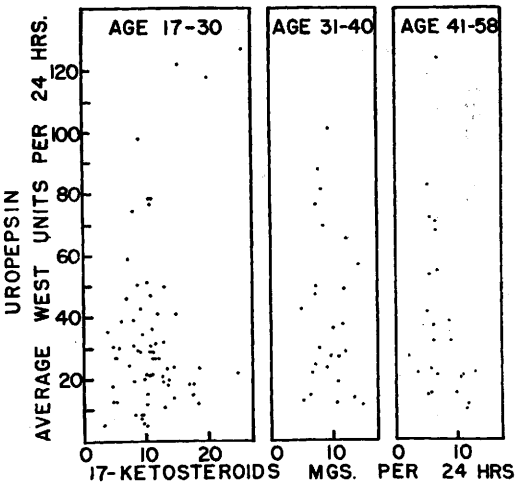

FIG. 1. Comparison of 17-ketosteroid and uropepsin excretion levels in 3 age groups of healthy males. Both determinations were made on the same 24-hr urine specimen in each case.

or that these precursors are differently metabolized in the two age groups. Several comparisons have been made of the 24-hour data obtained. Scattergrams (Fig. 1) of the individual 17-ketosteroid and uropepsin excretion values (true pairs) show the relationships of these values. The correlations for these data are given in Table II A. It will be noted that a significant correlation is obtained for each of the extreme groups, a low positive one for the youngest group and a higher negative one for the oldest group. The intermediate group shows a transitional state.

b. Day to night ratios. In agreement with other investigators(3,11) we have obtained day to night variations in 17-ketosteroid levels. Using the West milk-clotting method,

TABLE II. Pearson Correlation Coefficients for 17-Ketosteroid and Uropepsin Values.

	No. of persons	No. of values	r found	Probability level
A. 24-hr values				
17-30 yr group	37	69	+.261	.032
31-40	16	28	—.101	.582
41-58	16	26	—.469	.012*
B. Day to night ratios				
17-30 yr group	35	67	+.500	<.001*
31-40	14	26	+.828	<.001*
41-58	14	24	—.162	.453

* Difference between age groups may be expected to be due to chance 12 times and less than once, respectively, in 1000 samples.

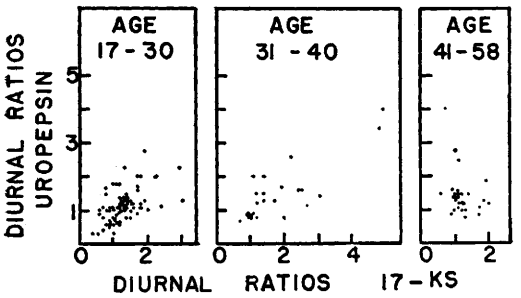

FIG. 2. Comparison of diurnal variations in 17-ketosteroid excretion with corresponding diurnal variations in uropepsin levels. Both determinations were made on the same 12-hr urine specimen in each case.

we have also found day to night variations in uropepsin output. This is in contrast with the findings of Mirsky *et al.*(12) who used a hemoglobin digestion method for uropepsin determinations.

Day to night ratios for 17-ketosteroid excretion are believed to reflect the difference in the amount of stress to which the individual is subjected during the waking period as compared with the sleeping period(3). For most individuals this ratio is greater than unity. Some show the reverse picture. Since no special stress situations were employed during the collection of these data, the day to night ratios may reflect to some extent the normal diurnal variations in metabolism.

The day to night ratios were divided into three groups according to the age of the subject, the age limits being the same as those used for the 24-hour values. When the ratios for the youngest group are compared (Table II B), a significant positive correlation is obtained. An even higher positive correlation is obtained for the intermediate group. The ratios for the oldest age group show a negative relationship of no significance. Distribution of the day to night ratios for these 2 measures is shown in Fig. 2.

Discussion. The low positive correlation of the 24-hour excretion levels of 17-ketosteroids and uropepsin for the 17-30-year group may indicate that a reactive adrenal cortex can exert a measurable influence on the level of uropepsin excretion. This suggestion is substantiated by the high positive correlations of the day to night ratios for these two measures for the two age groups, 17-30 and 31-40. If this hypothesis is correct, then the high negative correlation of the 24-hour values for the oldest group (41-58 years) may mean that the lessened activity of the adrenal cortex (indicated by the declining 17-ketosteroid excretion) is no longer effective in affecting the level of uropepsin secretion, and that the sustained or increasing uropepsin levels in these persons are primarily under some other influence. An alternative explanation for this negative correlation might be that a decline occurs in the secretion of adrenal cortical 17-ketosteroid precursors by the 41-58-year group without a decline in the adrenal cortical factor affecting gastric secretory activity. In a study of stress responsivity in normal, healthy men, Pincus (9) found that the total adrenocortical responsivity of the older group (median age 77) was generally slightly, though not significantly, lower than that of the younger group (median age 32).

Attention should be called to the data for the 31-40-year group. The 24-hour values for the two measures show no relationship, a finding which is in contrast with the significant positive correlation of the day to night ratios. The diurnal excretion of both 17-ketosteroids and uropepsin is very high, but *only in relation to the nocturnal excretion* since the 24-hour excretion level of both of these measures is average.

There appear to be 2 pathways by which stress of various types may influence gastric secretory activity. Stimuli from the cerebral cortex via the hypothalamus may be relayed to the stomach either by neural pathways to the vagal centers, or by hormonal pathways through the pituitary and adrenal cortical glands(13). Some workers(14) have found that uropepsin excretion does not follow gastric pepsin secretion, whereas others(15) have obtained evidence that they are directly related, at least under basal conditions. It seems reasonable to assume that some relationship does exist since the administration of ACTH or cortisone increases uropepsin excretion, and may cause reactivation or perforation of peptic ulcers(13). There is considerable evidence that stress as well as exogenous ACTH increases the secretion of the

TABLE III. Data on Patient M.P., A Suspected Hypopituitary Case (Age 29). 7 samples.

17-KS, mg/12 hr*	Uropepsin, units/hr	Sedimentation rate†	Eosinophile count	Leucocyte count
—	11	3.5	1606	9600
—	26	2.0	1573	9300
—	33	3.0	1507	10300
—	49	6.0	1355	8800
—	60	—	—	—
.00	37	—	—	—
.10	20	—	—	—

* A value less than 0.50 mg is questionable. This probably does not represent ketosteroid material.

† Normal range: 0-10.

adrenal cortex(3,13). The data presented here appear to indicate that uropepsin excretion in young men is measurably influenced by stimuli carried over hormonal pathways via the adrenal cortex. In older men the negative correlation may mean a difference in the production of the adrenal cortical factors which affect uropepsin and 17-ketosteroid excretion, or it may mean that stimuli affecting the production of uropepsin are carried primarily over neural pathways.

Special mention might be made of one young subject who was not included in the over-all study (Table III). This individual showed no 17-ketosteroid excretion although his uropepsin excretion ranged from 11 to 60 West units per hour. Four abnormally high eosinophile counts, taken on different days, substantiated the indication of adrenal cortical insufficiency. This case is mentioned since it shows the possibility of a normal uropepsin excretion in the complete absence of 17-ketosteroid output.

Summary. 24-hour uropepsin excretion levels were found to show a low positive correlation with 24-hour 17-ketosteroid excretion levels in healthy young men. To an even greater degree this is true of the day to night excretion ratios of these two biochemical measures. Since 17-ketosteroid excretion is believed to reflect adrenal cortical activity, the findings point to the possibility that adrenal cortical activity may exert a controlling effect on uropepsin excretion in young men. Older men were found to show a negative correlation in the 24-hour excretion levels of these two measures with a trend toward a similar relationship between the day to night ratios.

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