Individual Variability in Relationship of Human Ketosteroid Excretion To Urine Volume.* (25735)

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(Introduced by J. P. Marbarger)
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Numerous investigators(1-3) demonstrated that changes in urine volume do not significantly alter human 17-ketosteroid (17-KS) excretion. Others suggested that both 17-KS (4,5) and corticoid(6,7) excretions are dependent on urine volume. Similar discrepancies have been observed in rabbits(8,9) and guinea pigs(10,11) but not in rats(12) and swine(13). Prior to successful use of urinary steroid output as index of adrenocortical activity at least 4 variables must be considered: 1) urine volume, 2) steroids, 3) individual variability and 4) species differences. This study was undertaken to determine possible relationship of total ketosteroids (TKS), 17-ketogenic steroids (17-KG), 17-ketosteroids (17-KS) and dehydroepiandrosterone (DIA) to urine volume.

Materials and methods. Two experiments were carried out. In the first accurate 24-hour urine specimens were collected by 13 healthy men (20-38 years). Each subject acted as his own control while maintaining a record of daily *ad lib* fluid intake. Subsequently for 3 consecutive days (forced diuresis), subjects drank water at least twice that consumed during control period. Neither control nor experimental days coincided for all subjects, thus minimizing environmental effects. Aliquots of urine were refrigerated at 4°C 2-3 weeks prior to analysis for 17-KS by the method of Vestergaard(14). The second experiment determined spontaneous variability in urine volume and steroid excretion. Ten males (25-36 years), 6 of whom were subjects on first experiment, collected urine specimens for 7 to 34 days. As in previous experiment no attempts were made to control diet or to influence subjects' way of living. They recorded quantities of fluid and solids ingested during normal daily routine. Aliquots of daily urine samples were hydrolyzed and extracted for TKS, 17-KG, and 17-KS by procedure of Norymberski(15), then assayed for ketosteroids by Zimmermann reaction as modified by Holtorff and Koch(16). DIA determinations(17) were performed on aliquots of neutral 17-KS fraction.

Results. Ingestion of large quantities of fluid caused 3-fold increase in urine and mean 17-KS increase of 6% from control mean. Marked increases in urinary 17-KS were found in subjects 5 (34%) and 13 (27%), both had 2 of highest increases in urine. On the other hand maximum depressions of 25% were observed on third day of forced diuresis in subjects 10 and 16. Statistical analyses revealed that group changes in 17-KS excretion for any control day did not differ significantly ($P > 0.5$) from other days, whereas changes in fluid intake and urine volume were highly significant ($P < .001$). Four (2, 5, 8 and 13) of 13 subjects exhibited significant positive correlations, while 6 of the remaining insignificant correlations tended to be slightly negative (Table I). Nevertheless, probability levels of coefficients of correlation within samples ($r = +.42$), between subjects ($r = +.39$) and for total population ($r = +.27$) were obtained at $<.01$, $<.05$, and $<.05$ levels, respectively.

Spontaneous variations in coefficients of correlation between urine volume and ketosteroid excretion are presented in Table I. Urine for individual subjects varied from 0.81 to maximum of 1.99 liters/day, with group range of 0.52 to 3 liters/day. Unlike the forced diuresis experiment, 3 significant negative correlations (Subj. 1, 15, 17) in addition to one significant positive correlation (Subj. 17 for 17-KG) were observed. When all data for a specific group of steroids were combined,

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TABLE I. Individual Variability in Ketosteroid Excretion and Coefficients of Correlations.

Forced diuresis		Daily fluctuations in ketosteroid excretion							
17-KS		Urine vol	17-KS		TKS		17-KG		DIA	
Mean*	r†	range, l/day	Mean	r	Mean	r	Mean	r	Mean	r
28.1 ± 1.7	−.01	1.04-1.85	30.5 ± 1.2	−.63	44.4 ± 2.4	−.80‡	13.9 ± 2.1	−.61	10.4 ± .6	−.52
25.4 ± 2.8	.83‡									
17.8 ± 1.2	−.02									
		1.35-2.56	17.6 ± 1.4	.45	27.9 ± 1.3	.08	10.3 ± 3.4	−.42	3.7 ± .3	−.56
23.4 ± 2.3	.97‡	.52-1.57	21.8 ± 3.4	−.37	31.1 ± 2.5	−.23	9.3 ± 2.5	−.06	5.5 ± .3	−.23
14.2 ± 1.4	−.35									
10.3 ± 1.2	−.47									
18.7 ± 1.4	.82‡	.70-1.54	16.2 ± 1.1	.35	25.7 ± 1.3	−.23	9.5 ± 1.7	.37	4.2 ± .2	.41
12.9 ± .5	.05	.76-2.26	14.8 ± .9	.48	25.6 ± 3.2	.18	10.8 ± 3.	.02	3.5 ± .3	−.05
9.2 ± 1.4	−.02									
23.4 ± 1.6	.50	1.26-2.	24.6 ± 1.5	.05	36.4 ± 1.5	.15	11.8 ± 1.5	.14	2.6 ± .2	.08
11.2 ± .9	.04									
29.3 ± 2.5	.96‡	.56-2.34	28.7 ± 2.3	.47	39.2 ± 1.4	.06	10.5 ± 2.4	.41	7.9 ± .3	.30
		1.09-3.	15.5 ± 1.3	−.24	28.5 ± 1.5	−.09	13.1 ± 1.9	.18	2.8 ± .3	.43
		1.12-2.	20.4 ± 2.4	.39	32.0 ± 2.2	−.27	11.6 ± 1.7	−.91‡	5.1 ± .5	.46
12. ± 1.7	−.14									
		.70-1.68	13.9 ± .9	−.33	26.9 ± 2.2	.22	13.1 ± 2.6	.35‡	4.9 ± .5	−.47‡

* mg/24 hr ± S.D. † Coefficient of correlation. ‡ P .05.

only 17-KS excretion appeared dependent on urine volume ($r = +.49$; $P < .05$).

In 3 (1, 9, 11) of 6 subjects who participated in both experiments, 17-KS excretion was not related to urine volume in either experiment; the remaining 3 subjects (5, 8, 13) although exhibiting significant positive correlations during forced diuresis, showed no such correlation in the second experiment.

Discussion. Twenty-four hour excretions of TKS, 17-KS, 17-KG and DIA when correlated with subject's 24-hour urine volume varied not only from individual to individual, but also from time to time in the same individual. Of 53 coefficients of correlation, 5 were significantly positive (4 for 17-KS; 1 for 17-KG) and 3 were significantly negative (1 for TKS; 1 for 17-KG; 1 for DIA). The latter indicate that in some individuals increased urine volume may be associated with marked depression in ketosteroid output. However in most cases excretion of these steroids remained relatively constant although urine volume varied by a liter or more.

Diuresis, as a result of forced fluid intake, had a variable effect on individual urinary 17-KS excretion; in some instances it increased, in others remained relatively constant, and in a few decreased. All 4 positive 17-KS correlations were observed during forced diuresis. Since 3 subjects showed no correlation when placed on second experiment, forced fluid intake may have imposed some stress. It is apparent that when studying adrenocortical function results may be dependent on type and number of subjects. This was further demonstrated when data for all subjects in the second experiment were combined. A significant positive correlation is observed only for the 17-KS fraction. However in view of variability in individual coefficients of correlation these data suggest that subjects who normally excrete large quantities of urine tend to be high 17-KS excretors and *vice versa*; but it does not necessarily follow that titers of 17-KS are dependent upon fluctuations in urine within the same individual.

Discrepancies in the literature relative to effect of urine volume on ketosteroid excretion in normal humans may be the result of: 1) stress response to severe hydration or dehydration to give wide variations in urine; 2) limited number of subjects; 3) neglect of individual variability, and 4) grouping of all subjects' data for coefficients of correlation. Our experiments indicate that variations in urine as a result of relatively large fluctuations in daily fluid intake do not significantly alter excretion of ketosteroids except in some cases where stressful factors (forced diuresis, etc.) are superimposed. Whether this also holds true for other species awaits further experimentation.

Summary. Concentrations of total keto-

steroids, 17-ketosteroids, 17-ketogenic steroids, and dehydroepiandrosterone were determined in urine collected from healthy young men who ingested various quantities of fluid. Individual coefficients of correlation between urine and ketosteroid output varied from significantly positive to negative, with the majority showing no correlation. These results suggest that in most men daily variations in urine volume do not influence excretion of ketosteroids. However, group coefficients of correlation reveal that a significant positive correlation was obtained only for 17-ketosteroids. These data suggest that 1) men who normally produce relatively large volumes of urine also excrete large quantities of this steroid, and 2) type and number of subjects must be considered when calculating group coefficients of correlation.

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Effect of Co^{++}, Ni^{++}, and Zn^{++} on Corticoid Excretion by the Guinea Pig.* (25736)

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The guinea pig is useful for study of factors which influence ACTH release(1,2,3) since urinary corticoids are readily measurable in this species. The effect of Co^{++} was investigated because it has been suggested that events leading to establishment of polycythemia by this substance follow from production of a relatively anoxic state(4). It was observed that increased corticoid excretion followed intraperitoneal (but not subcutaneous or intramuscular) administration of Co^{++}. Increased corticoid excretion was also induced by intraperitoneal injection of Ni^{++} and Zn^{++}.

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Methods. The salts employed were $FeCl_2 \cdot 4H_2O$, $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, $HgCl_2$, $ZnCl_2$, $PbCl_2$, $CuCl_2 \cdot 2H_2O$. 300-350 g animals of either sex were injected intraperitoneally with various divalent ions, in 5 ml of saline; control animals received 5 ml saline as well. Co^{++} was also administered along with 2.5 mg morphine sulfate/100 g according to procedure previously described(2). Urine was collected for 6 hours(3). Urinary corticoids were determined by methods of Silber and Porter(5). When Co^{++} was administered subcutaneously or intramuscularly it was administered in 0.25 ml saline. To compare toxicity of these ions Co^{++}, Ni^{++}, Fe^{++}, Cu^{++} and Zn^{++} were administered twice daily at