Simplified Method for Estimation of 11-Oxygenated Neutral 17-Ketosteroids in Urine of Individuals with Adrenocortical Hyperplasia.* (20905)

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In the adrenogenital syndrome due to adrenocortical hyperplasia as defined by Wilkins(1), there is a high urinary excretion of 17-ketosteroids. Chromatographic separation of the neutral 17-ketosteroids from the urine of individuals with this condition revealed a larger proportion of 11-oxygenated 17-ketosteroids (11-hydroxyandrostanolone) than is found in normal adults(2). The presence of the 11-oxygen group would indicate that these compounds are probably of adrenocortical origin and hence their relative increase with adrenocortical hyperplasia is not surprising. Following suppression of the total 17-ketosteroid excretion in this condition by 11-dehydro-17-hydroxycorticosterone (cortisone) described by Wilkins(1), the quantities of 11-oxygenated steroids become relatively less in relation to the total output.

Both the Zimmerman(3) and Pincus(4) reactions have been used for the determination of urinary 17-ketosteroids. The Zimmerman reaction with meta-dinitrobenzene produces a response in proportion to the total quantity of 17-ketosteroids present, and this response is equal for the 11-oxygenated and 11-desoxy 17-ketosteroids. However, the Pincus reagent, antimony trichloride, reacts to a considerably smaller degree with 11-oxygenated than with 11-desoxy compounds. In this laboratory, the color of 1 mg of 11-hydroxyandrostanolone corresponds to that of 0.4-0.5 mg of androsterone with the Pincus method. During the hydrolysis, with acid and heat, for the release of 17-ketosteroids in urine, the 11-hydroxy compounds may be converted in great part to Δ-9 steroids. Dingemanse(5) has shown that the latter also produces about one half the color with the Pincus reaction as compared to the Zimmerman, so that artefacts produced by hydrolysis are similar in their responses to these tests. Such artefacts, however, are not easily separated by chromatography from the 11-desoxy compounds so that their relative increase may not be apparent. Furthermore, chromatographic separations are time consuming and are not suitable for large-scale studies.

The application of both the Zimmerman and Pincus reactions to identical urinary extracts of normal adults and patients with adrenocortical hyperplasia has been found useful for the estimation of the relative quantities of 11-oxygenated steroids present. Androsterone was used as the standard for both tests and is known to react well with either. The results were then expressed as P/Z, or the proportion of Pincus reacting steroids as compared to the "total" (Zimmerman) compounds (Table I). When large quantities of 11-oxygenated steroids are present, it would be expected that the Pincus levels would be considerably lower than the Zimmerman.

The results obtained with normal adults reveal that the ratio of Pincus reacting steroids (P) to Zimmerman (Z), averaged 0.95 with a range of 0.74-1.2. Untreated individuals with adrenocortical hyperplasia were found to have ratios averaging 0.54 with a range of 0.35-0.72. The difference between the two groups is highly significant since P = less than 0.01. This would indicate that a large proportion of 11-oxygenated steroids, or their Δ-9 artefacts were present in the urine of individuals with adrenocortical hyperplasia. This conforms to the results of chromatographic separation on alumina(2). The ratio rises following suppression of the adrenal cortex with 11-dehydro-17-hydroxycorticosterone, indicating a greater suppression of 11-

* This work was made possible by a Grant-in-Aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, and research grant from Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

TABLE I.

	No treatment			On cortisone		
	17-ketosteroids (mg/24 hr)			17-ketosteroids (mg/24 hr)		
	Pincus	Zimmerman	P/Z	Pincus	Zimmerman	P/Z
Normal adults ♂	7.0	9.5	.74			
	10.8	9.1	1.2			
	7.6	6.8	1.1			
♀	3.2	3.9	.82			
	6.1	6.1	1.0			
	2.8	3.0	.93			
	2.3	2.4	.96			
	4.1	5.0	.82			
	6.9	6.9	1.0			
			Mean 0.95	SE ± 0.137		
Adrenal hyperplasia						
SW	2.7	6.0	.45	1.8	1.5	1.2
TD	.7	1.5	.47	.3	.1	3.0
PD	14.2	40.7	.35	8.4	9.0	.94
DD	10.0	26.6	.38	11.5	16.8	.69
SB	15.5	21.5	.72			
	13.0	24.2	.54	10.4	10.2	1.0
LB	25.4	55.0	.46			
	35.0	60.0	.58	5.4	6.0	.90
NW	29.2	47.0	.62	5.8	7.7	.75
	36.0	54.3	.66	16.8	18.2	.93
HW	22.1	33.3	.66	2.6	3.7	.71
			Mean 0.54	SE ± 0.118		
			P = <0.01			

oxygenated compounds. This has been noted in spite of the fact that a certain amount of the administered steroid might be excreted as 11-oxygenated substances.

The application of this combination of reactions to the study of adrenocortical hyperplasia offers a relatively simple system for estimating the proportion of 11-oxygenated 17-ketosteroids which are elevated in this condition, and their relatively greater suppression with treatment. One word of caution must be added: Dehydroepiandrosterone, which is usually excreted in large quantities by individuals with tumors of the adrenal cortex also reacts poorly with the Pincus reagent. However, two instances of tumor so studied indicated that the quantities of dehydroepiandrosterone excreted were so great that the P/Z ratio was less than 0.1.

Summary. There are differences in the reactions of 11-oxygenated and 11-desoxy 17-ketosteroids with the Pincus and Zimmerman reagents. Since those steroids with an 11-oxygen function are probably of adrenal cortical origin, it is possible to estimate the quantity of these two groups of compounds in extracts of urine by applying both tests. It has been shown that in urine from patients with adrenal cortical hyperplasia the ratio of Pincus to Zimmerman reacting 17-ketosteroids is low indicating a large proportion of 11-oxy compounds.

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Received February 9, 1954. P.S.E.B.M., 1954, v85.