URINARY EXCRETION OF INDIVIDUAL 17-KETOSTEROIDS BY NORMAL CHILDREN

BY

A. KÁDÁR, T. FEHÉR and O. KOREF

From the Second Department of Pediatrics, and the First Department of Medicine, University Medical School, Budapest, Hungary

(RECEIVED FOR PUBLICATION AUGUST 20, 1963)

Our knowledge of the functions of the normal and diseased adrenal cortex in adults is still slight and we know even less about it in newborn babies and in children.

In the past few years an increasing number of methods for examining adrenal function have been described. In addition to the determination of the total amount of 17-ketosteroids (17-KS) and of 17-hydroxycorticoids (17-OH) excreted in urine, various methods for fractionating these compounds have been used, which are more suitable for studying normal function and various pathological mechanisms of the adrenal gland and of the gonads.

Although data on adults exist, there is little information available on the determination of individual 17-KS from the urine of children. Therefore, it seemed to us useful to determine the urinary steroids in healthy children of various ages.

Method

The total amount of 17-KS excreted in urine was determined by Zimmermann's (1955) method. The amount of individual 17-KS was measured by our method (Fehér, Koref and Holló, 1962). The denomination and abbreviation of the six fractions obtained are compiled in Table 1. For determining the total number of 17-OH steroids we used the modified Reddy method (Reddy, 1954; Koref, Fehér, Juvancz, Foldes and Fischer, 1964).

Our measurements refer to 52 children from our clinic (29 boys and 23 girls), who had no endocrine disorders.

Results

The cases are grouped according to sex and age, with due regard to the beginning of puberty as judged by the appearance of pubic and axillary hair in boys and the same in girls plus the beginning of breast development. The grouping according to age was affected by the difference in the time when puberty began in the two sexes. The results are

included in Tables 2 and 3. These show that the total amount of 17-KS and 17-OH excreted in urine increases with age in both sexes. Among the 17-KS, the HOE + HOA does not change with age; the amount of OE + OA increases slightly. The excretion of 11-desoxy-17-KS is negligible in infants but increases considerably in later years: this is particularly striking in the E and A.

The Tables also show that the 11-oxy/11-desoxy ratio decreases with age owing to the slight increase in the 11-oxy-17-KS and the greater increase in the 11-desoxy-17-KS. Owing to the slight and identical increase in E and A, the E/A ratio remains the same in both sexes after infancy, from about 1 up to 5 years. Between 5 years and the beginning of puberty, owing to the increase of E, the E/A ratio increases, then with an unchanged E value at the beginning of puberty the amount of A increases. Hence the decrease in the E/A ratio.

TABLE 1
LIST OF 17-KETOSTEROIDS

No.	Systematic Name*	Abbreviation
1	5β-androstane-3α, 1β-diol-17-one (11-OH-etiocholanolone)	HOE
	5α-androstane-3α, 1β-diol-17-one (11-OH-androsterone)	HOA
2	5β-androstane-3α-ol-11, 17-dione (11-keto-etiocholanolone)	OE
	5α-androstane-3α-ol-11, 17-dione (11-keto-androsterone)	OA
3	androst-5-ene-3β-ol-17-one (dehydroepiandrosterone)	DEA
4	5β-androstane-3α-ol-17-one (etiocholanolone)	E
5	5α-androstane-3α-ol-17-one (androsterone)	A
6	Not identified steroids, artefacts, etc.	Not identified

* After International Union of Pure and Applied Chemistry (1958).

TABLE 2: THE INDIVIDUAL 17-KS EXCRETED IN URINE OF BOYS

Subject	Age (yr.)	Total 17-KS (mg./24 hr.)	17-KS Fractions (mg./24 hr.)						11-oxy./11-des-oxy.	E/A	Total 17-OH (mg./24 hr.)
			HOE HOA	OE OA	DEA	E	A	Not Identified			
1	<1	0.5	0.2	0.3							0.8
2	<1	0.3		0.1		0.1		0.1	1.0		0.5
3	<1	0.2	0.1	0.1							0.6
4	<1	0.4	0.1	0.1		0.1	0.1		1.0	1.0	1.1
5	1	1.0	0.2	0.2		0.2	0.1	0.3	1.3	2.0	2.8
Range ..	..	0.2-1.0	-0.2	0.1-0.3		-0.2	-0.1	-0.3	1.0-	1.0-	0.5-2.8
6	3	1.2	0.1	0.1	0.1	0.2	0.1	0.6	0.5	2.0	1.7
7	3	1.0	0.1	0.2		0.3	0.3	0.1	0.5	1.0	3.8
8	3	0.3		0.1		0.1	0.1		0.5	1.0	3.1
9	4	0.4	0.1	0.1		0.1		0.1	2.0		4.9
10	5	1.6	0.2	0.2	0.1	0.4	0.5	0.2	0.4	0.8	1.9
11	5	3.2	0.3	1.0	0.2	0.5	0.4	0.8	1.2	1.2	—
Range ..	..	0.2-3.2	-0.3	0.1-1.0	-0.2	0.1-0.5	-0.5	-0.8	0.4-2.0	0.8-	1.7-4.9
12	6	1.0	0.1	0.1	0.1	0.3	0.1	0.3	0.4	3.0	4.1
13	7	1.5	0.2	0.4	0.1	0.2	0.2	0.3	1.0	1.0	3.9
14	7	1.7	0.1	0.2		1.0	0.1	0.3	0.3	10.0	4.7
15	8	2.9	0.2	1.1	0.2	1.0		0.2	0.9		4.5
16	8	1.5	0.1	0.3	0.2	0.6	0.1	0.2	0.4	6.0	1.6
17	9	1.4	0.2	0.3	0.2	0.3	0.1	0.3	0.8	3.0	2.4
18	9	3.5	0.2	0.6	0.3	1.0	0.6	0.8	0.4	1.7	2.8
19	9	2.4	0.2	1.0	0.1	0.5	0.2	0.4	1.5	2.5	5.8
20	10	2.0	0.2	0.3	0.1	0.4	0.4	0.6	0.6	1.0	4.0
21	10	3.1	0.4	0.6	0.2	0.6	0.6	0.7	0.7	1.0	5.2
22	11	2.6	0.2	0.3	0.5	0.7	0.3	0.6	0.3	2.4	5.6
Range ..	..	1.0-3.5	0.1-0.4	0.1-1.1	-0.5	0.2-1.0	-0.6	0.2-0.8	0.3-1.5	1.0-	1.6-5.8
23	12	5.1	0.2	1.2	0.2	2.1	0.7	0.7	0.5	3.0	4.8
24	12	2.7	0.3	0.6	0.4	0.8	0.3	0.3	0.6	2.7	5.4
25	12*	3.7	0.2	0.6	0.5	1.0	1.0	0.4	0.3	1.0	5.7
26	13	2.6	0.4	0.3	0.7	0.7	0.4	0.1	0.5	1.8	3.0
27	13*	3.8	0.2	0.6	0.5	1.1	0.5	0.9	0.4	2.2	2.6
28	13*	3.6	0.3	0.5	0.3	1.0	0.9	0.6	0.4	1.1	4.1
29	13*	1.2	0.2	0.5	0.1	0.1	0.1	0.3	0.2	1.0	2.6
Range ..	..	1.1-5.1	0.2-0.4	0.3-1.2	0.1-0.5	0.1-2.1	0.1-1.0	0.1-0.9	0.2-0.6	1.0-3.0	2.6-5.7

* These boys showed signs of puberty.

Blanks indicate values not measurable by our method.

Values not determined are marked by the sign —.

TABLE 3: INDIVIDUAL 17-KS EXCRETED IN URINE OF GIRLS

Subject	Age (yr.)	Total 17-KS (mg./24 hr.)	17-KS Fractions (mg./24 hr.)						11-oxy./11-des-oxy.	E/A	Total 17-OH (mg./24 hr.)
			HOE HOA	OE OA	DEA	E	A	Not Identified			
1	3	1.1	0.2	0.2	0.1	0.1	0.1	0.4	1.3	1.0	3.4
2	3	2.0	0.3	0.6	0.1	0.2	0.1	0.7	2.2	2.0	6.8
3	4	0.7	0.1	0.2		0.1	0.1	0.2	1.5	1.0	3.1
4	5	1.1	0.1	0.4		0.1	0.1	0.4	2.5	1.0	2.3
5	6	1.6	0.2	0.2		0.8	0.2	0.2	0.4	4.0	5.3
6	6	0.8	0.2	0.2		0.1	0.2	0.1	1.3	0.5	0.7
7	8	1.5	0.1	0.2	0.3	0.3	0.3	0.2	0.3	1.0	3.4
8	8	2.1	0.3	0.7	0.1	0.1	0.3	0.6	2.0	0.3	3.9
9	8	1.2	0.1	0.2		0.3	0.5	0.1	0.4	0.6	2.1
Range ..	..	0.7-2.1	0.1-0.3	0.2-0.7	-0.3	0.1-0.8	0.1-0.5	0.1-0.7	0.3-2.5	0.3-4.0	0.7-6.8
10	9	5.6	0.1	2.4	1.1	1.0	0.6	0.4	0.9	1.7	1.6
11	9*	3.0	0.1	0.4	0.4	0.6	0.4	1.1	0.4	1.5	2.6
12	10*	1.3	0.2	0.3		0.5	0.2	0.1	0.7	2.5	3.0
13	10*	4.7	0.2	1.5	0.2	1.1	1.4	0.5	0.6	0.8	5.6
14	10	5.9	0.5	1.1	0.4	1.5	1.1	1.3	0.5	1.4	6.5
15	11*	6.0	0.4	0.5	0.3	3.0	1.5	0.3	0.2	2.0	3.1
16	11*	3.7	0.2	0.5	0.4	1.4	0.8	0.4	0.3	1.8	4.6
17	11*	4.0	0.2	0.3	0.1	1.9	1.0	0.5	0.2	1.9	6.5
Range ..	..	1.3-6.0	0.1-0.5	0.3-2.4	-1.1	0.5-3.0	0.2-1.5	0.1-1.3	0.2-0.9	0.8-2.5	1.6-6.5
18	12*	4.0	0.3	0.4	0.1	1.7	1.3	0.2	0.2	1.3	5.9
19	13*	1.4	0.1	0.2	0.1	0.4	0.4	0.2	0.3	1.0	2.3
20	13*	3.6	0.1	0.7		1.1	1.5	0.2	0.3	0.7	7.4
21	14*	5.4	0.4	0.8	1.0	1.4	0.8	1.0	0.4	1.8	5.7
22	14*	2.3	0.2	0.5	0.2	0.7	0.5	0.2	0.5	1.4	3.3
23	14*	4.2	0.4	0.4		1.9	1.0	0.3	0.3	1.9	4.5
Range ..	..	1.4-5.4	0.1-0.4	0.2-0.8	-1.0	0.4-1.9	0.4-1.5	0.2-1.0	0.2-0.5	0.7-1.9	2.3-7.4

* These girls showed signs of puberty.

Blanks indicate values not measurable by our method.

Discussion

In spite of the wide scattering and differences in method, there are many unequivocal and concurring data on the number of urinary 17-KS fractions and on the values of their ratios in adults (Johnsen, 1956; Faredin and Blahó, 1958; James, 1961). On the other hand there is little information for children (Devis, 1951; Eagle, 1952; de Courcy, 1954; Bergstrand, Birke, Plantin and Zetterström, 1954; Ulstrom and Doeden, 1956; Huis in't Veld, 1957; Masuda and Holmes, 1957; Paulsen and Sobel, 1960; Birchall, Cathro, Forsyth and Mitchell, 1961; Beas, Zurbrügg, Cara and Gardner, 1962). The authors generally report on a small number of cases and most of them pool the 17-KS into groups but fail to divide them into individual steroids. Their data are contradictory in many instances. Eagle (1952) was unable to demonstrate the presence of DEA in infants' urine. Birchall *et al.* (1961) found the amount of this steroid in the urine of newborn babies to be 12-29 $\mu\text{g}/24$ hours. Masuda and Holmes (1957) found smaller amounts of 11-desoxy-17-KS than other authors (Huis in't Veld, 1957; Birchall *et al.*, 1961). Huis in't Veld (1957), on the other hand, found higher levels of 11-oxy-17-KS than did others.

According to Prout and Snaith (1958), to Wilkins (1962) and to our own measurements, the total amount of 17-KS and 17-OH in children reaches adult values gradually. Regarding the 17-KS, we have observed a similar development: the number of fractions generally grows with age, the 11-oxy-17-KS showing a slight, and the 11-desoxy-17-KS a very significant, increase. The differences observed within the various age-groups can be better expressed by the changes in the values of the various ratios.

The 17-KS excreted in urine in adults are known to consist in the main of 11-desoxy-17-KS (up to 60-80%). Although the quantity of the 11-oxy-17-KS is much smaller, their importance is considerable. In young adults the value of the 11-oxy/11-desoxy ratio is 0.2-0.4, increasing to about 0.4-0.6 in old age (Fehér *et al.*, 1962). Owing to a minimum excretion of 11-desoxy-17-KS, the highest value of this ratio was seen in infancy. Between the ages of 3 and 8 this ratio is a multiple of the adult value in both sexes; between 8 and the beginning of puberty it is usually double and then attains a value similar to or identical with that in adults. Although at this age the ratio is nearly identical to that in adults, the absolute quantity of the two 17-KS fraction groups is still considerably lower.

According to Gallagher (1957), the 11-oxy-17-KS (their precursors) are indispensable, while the

11-desoxy-17-KS are of secondary importance. This has been confirmed by our findings. The relatively high quantities of 11-oxy-17-KS in infancy are reached and even exceeded by the 11-oxy-17-KS values in puberty. In the urine of adults the E and A represent the largest fractions, which are eliminated in nearly equal amounts, and the ratio of E/A is therefore about 1 (Fehér *et al.*, 1962). According to our data, this ratio is usually much higher in children. Immediately before puberty the value of the E/A ratio shows a considerable increase, especially in boys. The number of precursors of 17-KS formed in the endocrine organs and the principal ones governing their transformation in the liver (kidney) into urinary end-products are known (Dorfman, 1954, 1959). Of the precursory substances of the two largest 17-KS fractions (E and A), the precursors of E, as compared with those of A, are, under normal conditions, formed in greater quantities in the adrenal cortex than in the gonads. Hence the E/A values permit us to draw certain conclusions concerning the production of adrenal androgens, i.e. pre-androgens. If this is so, the high E/A values before puberty confirm the assumption that the appearance of the external signs of puberty are preceded by increased production of adrenal pre-androgens, and the functioning of the gonads starts considerably later. (Cf. the striking decrease in the E/A ratio and the changes to the values of adults.) This problem requires further investigation.

Increased separation of the very important 11-oxy-17-KS, detailed examination of steroid metabolism during the period immediately preceding puberty, and steroid analysis of endocrine disorders in children, will greatly contribute to our knowledge of the normal and pathological function of the adrenal cortex in children.

Summary

The individual 17-KS in the urine of 52 normal children of different age and sex have been determined by the authors by means of the paper chromatographic method. Of the six fractions that can be separated by this method, hardly any quantitative change occurs with increasing age in the elimination of the 11-oxy-17-KS. On the other hand the quantity of 11-desoxy-17-KS rises significantly with age. Of the ratios computed from the various groups of the 17-KS fractions which reflect the function of the adrenal cortex, the 11-oxy-17-KS/11-desoxy-17-KS ratio is very high in infancy and decreases to the adult value at the advent of puberty. The E/A value is usually higher in children than in adults, and the high E/A value measured

immediately before puberty seems to indicate an increased production of pre-androgens in the adrenal cortex. The total amount of 17-KS and of 17-OH determined simultaneously reaches the values of adults after a gradual increase beginning in infancy.

For their technical assistance we are indebted to Miss Kornélia Adok and Miss Mária Kazik.

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Erratum

It is regretted that in the paper by J. Lister on 'Blood Supply and Oesophageal Atresia', which appeared in the previous issue (*Arch. Dis. Childh.* (1964), **39**, 131), Figs. 8 and 9 on page 135 should have been labelled Figs. 10 and 11, and Figs. 10 and 11 on page 136 should have been labelled Figs. 8 and 9.