Acta Paediatrica Academiae Scientiarum Hungaricae, Vol. 23 (2), pp. 151–165 (1982)

Dynamics of adrenal steroidogenesis in childhood: steroid excretion in prepubertal and pubertal girls

L. Kecskés, Zsuzsanna Juricskay, Zsuzsanna Tatai, Márta Szécsényi

Central Laboratory, University Medical School, Pécs

C_{19}—$C_{21}O_{2-3}$ steroid spectrum and $C_{21}O_5$ corticoids have been studied in the urine collected from 29 girls aged 2–14 years. A total of 34 investigations was carried out by means of simultaneous two-column gas chromatography. The values of steroid excretion have been expressed in terms related to body weight and body surface, respectively.

Before the age of seven the excretion of $C_{19}O_2$ steroids representing adrenal androgens and their metabolites did not exceed 7 μg/kg/day or 170 μg/m²/day ("infantile type androgen excretion"). Then it began to increase between the 7th and 9th years of life and preceded the appearance of the physical signs of adrenarche. The increase was accelerated during puberty and no value under 18 μg/kg/day, or 600 μg/m²/day, respectively, was found when the physical signs of adrenarche were present ("puberty type androgen excretion"). Excretion of 11-oxygenated C_{19} steroids, $C_{21}O_5$ corticoids and pregnenediol was also augmented during puberty; however, when compared with the increase in $C_{19}O_2$ excretion this augmentation was moderate. As a consequence of the marked elevation in $C_{19}O_2$ excretion, the ratio of $C_{19}O_2$ to $C_{21}O_5$, too, increased during puberty, indicating that the cortisol-androgen dissociation, otherwise characteristic of childhood, had ceased.

It is concluded that the most plausible explanation of the increased androgen production is the gradual development of the zona reticularis during puberty.

In addition to cortisol and aldosterone, the adrenal cortex of adults secretes significant amounts of cortical androgens, such as dehydroepiandrosterone, dehydroepiandrosterone sulphate and androstenedione. The production of dehydroepiandrosterone (DHA) and dehydroepiandrosterone sulphate (DHA–S) is very low in children under eight, and activation of adrenal steroid secretion sets in only during puberty. These adrenal androgens are responsible for the development of pubic and axillary hair in females. This effect of adrenal androgens on secondary sex character and the developmental process itself were recognized and termed adrenarche some 40 years ago (2).

Since that time much has been learnt about adrenarche. It has been demonstrated that in children cortisol secretion and also its secretion ratio are identical with those of adults; when calculated for body surface or body weight they do not show any significant elevation even during puberty (12, 14). Complex radioimmune assays have revealed the increase of DHA and DHA–S secretion to begin as early as around the 8th year of life; it precedes by

2–3 years the elevation of the serum level of gonadotropins and the appearance of the first physical signs of puberty (18, 19).

There is convincing evidence available that, in adults, the secretion of DHA is regulated by ACTH and that, under physiological conditions, the secretion of cortisol and that of DHA run parallel (17).

In contrast, another specific form of steroidogenesis occurs in childhood when cortisol and cortical androgens undergo dissociation. There is no generally accepted explanation of either these childhood characteristics of androgen relations or of their gradual disappearance during puberty. The increased serum pregnenolone level (3) and the increased urinary pregnenediol excretion (10) during puberty point to the importance of intraglandular factors, i.e. to an increased biosynthesising activity of the adrenals in the years preceding sexual maturation. Functional effects based on morphological factors may also be of importance since, as demonstrated in obduction material (5), development of the adrenal zona reticularis sets in around the 8th year of life and ceases at the end of puberty. Recently, the existence of a pituitary factor called cortical androgen stimulating hormone (16) has been put forward; the hormone might act jointly with ACTH (19).

In the present study, the steroid excretion of healthy girls has been studied. Based on the investigation of urinary steroid excretion, an attempt has been made to shed light on the characteristics of adrenal steroidogenesis and metabolism in childhood, and on their changes during puberty.

As a rule, gas chromatography does not determine the active hormones but their urinary metabolites. In spite of this fact gas chromatography should be regarded as a full-value method to explore the functioning of the zona reticularis and zona fasciculata, since the amount of metabolites is in direct proportion to the production rate of active hormones.

Gas chromatographic steroid spectrum analysis is one of the most up-to-date methods for determination of urinary steroids. It gives information on the excretion of steroid intermediates, too; thus it is a diagnostic procedure suitable to identify pathologic steroids in enzyme deficiency or in the case of suspicion of hormonally active tumours. The method is quick, with a sensitivity superior to that of the routine photometric methods used for determination of 17-ketosteroids and 17-hydroxycorticoids.

When hepatic or renal damage is associated with the endocrine disease, a steroid value below the physiological level must not in itself be considered a definite sign of endocrine hypofunction; the low excretion may be due to disturbed metabolism, an event leading to increased half life of the active hormone. Thus, when renal or hepatic damage is present, preference should be given to the determination of steroid levels in plasma.

Materials and Methods

Reference steroids were made available by the generous gift of Prof. D. N. Kirk (Medical Council Steroid Reference Collection, London).

Material

Twenty-four-hour urine samples were collected from 29 healthy girls on 34 occasions. No liver or kidney disease was mentioned in their history. The age distribution was, 12 girls between 2 and 7 years, 9 girls between 7 and 10, and 13 girls between 10 and 14 years of age. According to the puberty stage, of the girls between 10 and 14 years, 4 girls were in stage P-2, five in P-3 and four in P-4.

Analytic methods. Extraction, derivate formation and gas chromatographic analysis of C_{19}—$C_{21}O_{2-3}$ steroids were carried out according to the method previously described (9), with a slight modification allowing to investigate 16-oxysteroids (11). The steroids were identified after separation of their acetylated derivatives; the retention times refer to the retention time of 5-α-cholestane (RRT_{ch}).

Gas chromatographic determination of $C_{21}O_5$ corticoids. The metabolites of cortisol were analysed as a common component, after reduction of the ketonic groups by sodium borohydrate and after splitting off the C_{17-21} side chain by periodate (22). Removal of the side chain makes hydrolysis unnecessary, for the conjugating glucuronide will be eliminated together with the side chain. The method of Trafford and Makin (22) applied for derivative formation and gas chromatographic analysis had been modified in the sense that acetylation was used instead of formylation. Briefly, 10% of the daily urine is analysed under the age of four, 5% in the age group 4–10 years, and 2.5% above ten years. Two ml of 10% sodium borohydrate dissolved in 0.01 N sodium hydroxide was added to each 10 ml of the urine sample; the mixture was kept at 50°C for 15 min. The surplus of the reagent was decomposed by addition of 0.5 ml of glacial acetic acid; then, the incubation was continued for 15 further min. Thereafter, the pH of the urine was adjusted to 6.5 by addition of 8 ml of 10% sodium metaperiodate, a procedure followed by incubation anew at 50°C for 20 min. The steroids were extracted by two volumes of dichloromethane. The original extract was then washed with 1 N sodium hydroxide, 1% acetic acid and, finally, with distilled water. This was followed by dehydration by sodium sulphate; the residue was concentrated by means of a rotating evaporator. The concentrates were transferred quantitatively (by washing) into conic centrifuge tubes and evaporated under CO_2. Acetylation of the dry extract was performed at room temperature for 16 h.

A Packard 7300 two-channel gas chromatograph was used for analysis. The extract was applied simulteneously on two 1.82×2 mm columns. Column A with combined filling contained at the inlet end, 3% SP 2250 stationary-phase 78 cm in length on 100/120 mesh Supelcoport support, and at the detector end, 3% SP 2100 stationary-phase 102 cm in length on 80/100 mesh Supelcoport support. Column B contained 3% SP 2100 stationary-phase on 80/100 mesh Supelcoport support. Double flame ionization detector (temperature: 270°C) and as carrier gas, highly purified N_2 were used at 40 ml/min flow rate.

Analytic circumstances: On-column injection technique, heated inlet (270°C). Isothermic period at 250°C with a duration of three times the retention time of the previously injected 5α-cholestane; then the temperature was elevated at a rate of 1°C/min. Prior to analysis, 50 μg of cholesteryl propionate dissolved in 50 μl of ethanol-benzene was added to the extract.

This served as internal standard. On some occasions, after performing the whole procedure, we took linear calibration curves by adding to the urine 50, 100 and

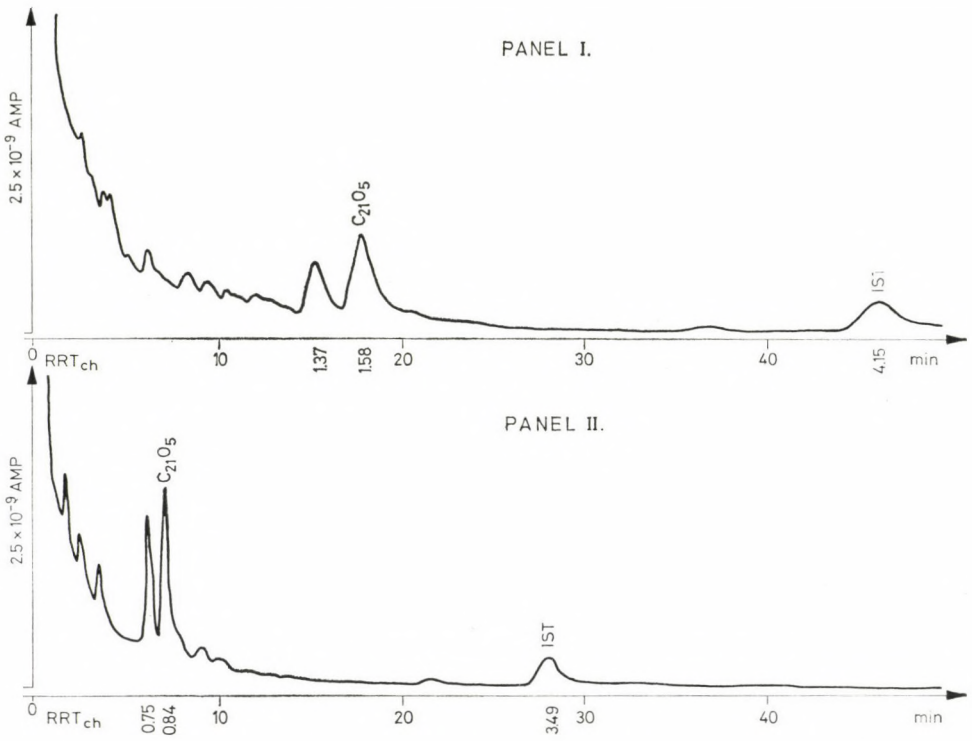

FIG. 1. Simultaneous gas chromatographic analysis of acetylated $C_{21}O_5$ corticoids obtained from the urine of a 9-year-old girl with adrenarche. Before extraction, the sample was reduced with sodium borohydrate and oxydized with periodate. Panel I: SP 2250/SP 2100 combined column; Panel II: SP 2100 column

200 μg of tetrahydrocortisol, tetrahydrocortisone and 20α-cortol, respectively. The slope of the curve was 0.46 ± 0.04 (S.D.). The reciprocal of this value was used as response factor during the analysis of $C_{21}O_5$ steroids.

$C_{21}O_5$ content of the urine in mg/day:

$$\frac{\text{area of } C_{21}O_5 \text{ peak}}{\text{area of the cholesteryl propionate peak}} \times \times \frac{50^*}{1000} \times \text{response factor} \times \times \frac{\text{24-h urine (ml)}}{\text{urine processed (ml)}}$$

Each determination was performed on two columns and the mean of the values obtained was used for further calculation. Of the $C_{21}O_5$ corticoids, 11-β-hydroxy-etiocholanolone (11-OH-E) and 11-β-hydroxy-androsterone (11-OH-A), both originating in the chemical procedures, were evaluated in one common peak (Fig. 1). They were not disturbed by 11-oxy-C_{19}-steroids of urine, since the latter are conjugated at the third carbon atom, and the 11-β-17-β-dihydroxy-5-α-/β-androstane-3-one, produced in the course of oxidation, has a retention time shorter than that of either 11-OH-E or 11-OH-A.

The accuracy of the method was checked

* Amount in μg of added cholesteryl proprionate.

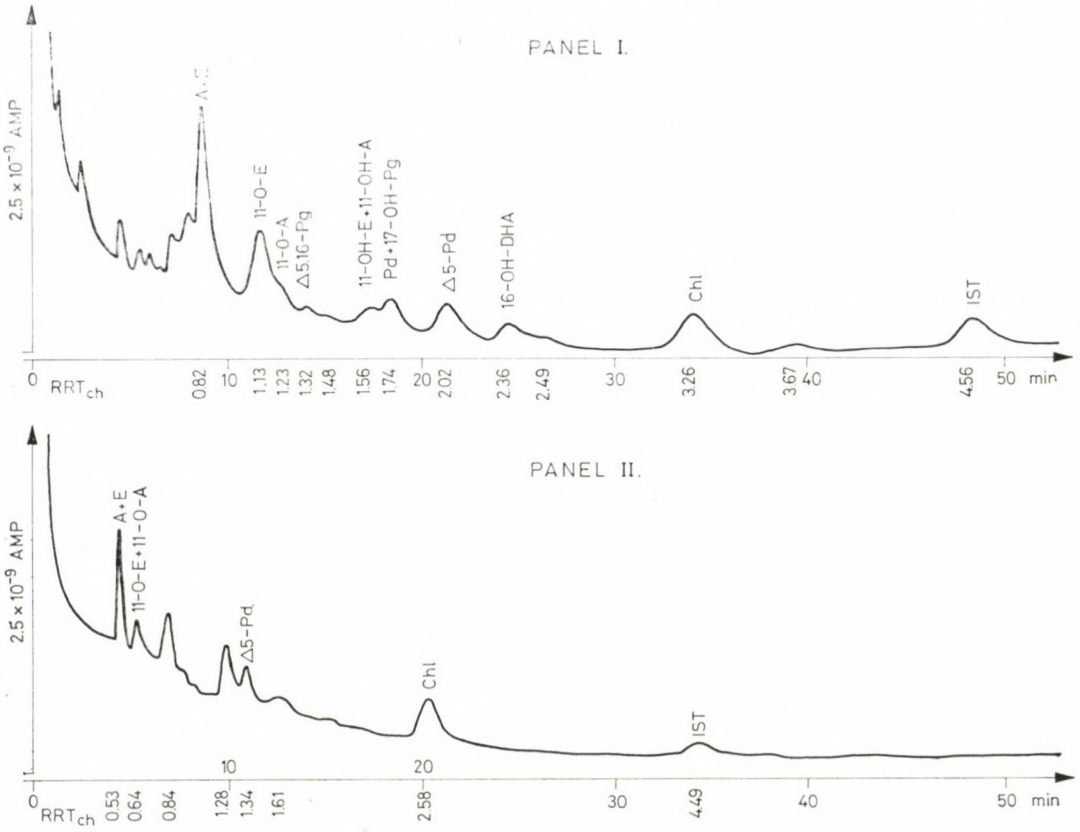

FIG. 2. Simultaneous gas chromatographic analysis of $C_{19}C_{21}O_{2-3}$ steroids obtained from the urine of a 9-year-old girl with adrenarche. Conditions of analysis: acidic hydrolysis, toluene extraction and formation of acetyl derivatives. Panel I: 2250/SP 2100 combined column; Panel II: SP 2100 column

on the basis of the percentage difference between the results of duplicate analysis of the urine of six children. Here, the formula $\sqrt{\frac{d^2}{2n}}$ was applied, where d means the difference in per cent between, and n the number of, the duplicate determinations. The difference amounted to 9.4%, a value to be considered statisfactory as to the reproducibility of the procedure used.

RESULTS

Figure 2 shows the urinary steroid spectrum of a 9-year-old girl with "adrenarche". The panel on the top was obtained from SP 2250/SP 2100 combined column, while the bottom one from SP 2100 column. The dominant peak on both columns was the common peak of androsterone (A) and etiocholanolone (E). Some further peaks were the common peak of 11-keto-etiocholanolone (11-O-E) and 11-keto-androsterone (11-O-A); that of pregnanediol (Pd), 17-hydroxy-pregnanolone (17-OH-Pg), pregnenediol (Δ-5-Pd), 16-α-hydroxy-DHA (16-OH-DHA) and 16-β-hydroxy-DHA, as well as the peak of cholesterol (Chl). The chromatogram also

TABLE I

Simplified scheme showing pathways of adrenocortical steroidogenesis and urinary steroids analysed by gas chromatography. The dotted line indicates a small fraction of pregnenolone

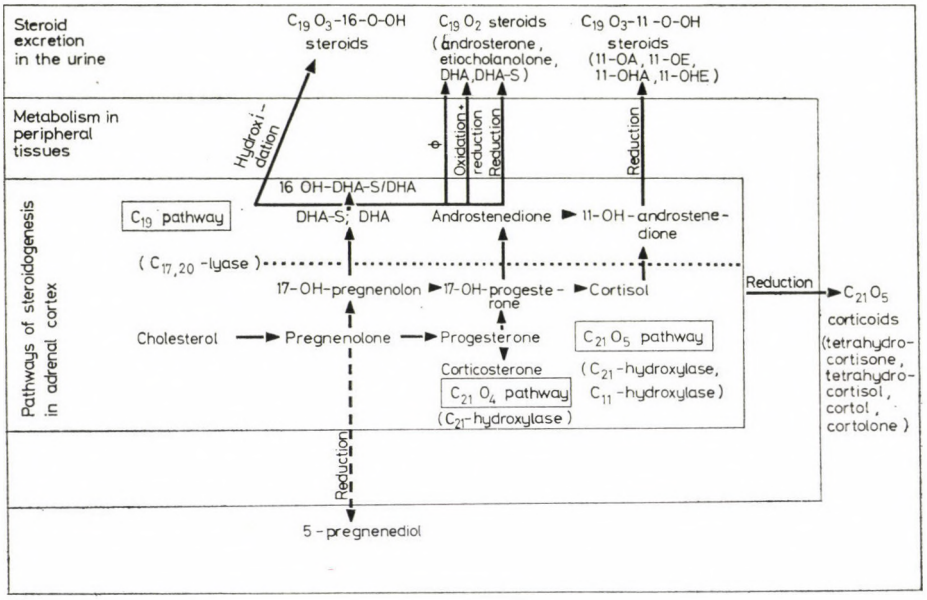

TABLE II

Excretion of C_{19} steroids, $C_{21}O_5$ corticoids and pregnenediol

A

Steroid / Age (year)	$C_{19}O_2$ Mean±S.D.	$C_{19}O_2$ Range	p	$C_{19}O_3$ (11-O-OH) Mean±S.D.	$C_{19}O_3$ (11-O-OH) Range	p	Mean±S.D.
2– 6	4.0± 1.7	2.1– 6.9	<0.02	5.5± 3.1	1.5– 9.3	<0.02	2.4± 2.7
7– 9	8.9± 4.9	3.1–13.8	<0.001	9.9± 8.0	2.2–22.8		3.2± 4.3
10–14	32.4±16.5	7.6–66.9		13.7±10.4	2.0–32.8		6.9±10.3

B

Steroid / Age (year)	$C_{19}O_2$ Mean±S.D.	$C_{19}O_2$ Range	p	$C_{19}O_3$ (11-O-OH) Mean±S.D.	$C_{19}O_3$ (11-O-OH) Range	p	Mean±S.D.
2– 6	97± 43	48– 167	<0.01	134± 80	36–265	<0.01	59± 69
7– 9	241±130	81– 476	<0.001	269±210	59–611		86±116
10–14	1048±597	231–2260		435±338	73–944		217±321

A = values in μg/kg/24 h;

allowed the identification of 11-β-hydroxy-etiocholanolone (11-OH-E) and the dehydrated form (Δ-5,16-Pg) of 16-α-hydroxy-5-pregnenolone. The results shown in Table I according to age group distribution were calculated for kg body weight and also for body surface; they were evaluated by biostatistical methods.

For the sake of an easier survey the steroids investigated had been divided in just four groups, according to the number of carbon atoms and of the functional groupings, respectively. Classification of urinary steroids as well as their connection to the pathways of adrenal steroidogenesis are summarized in Table I.

Group $C_{19}O_2$ contains DHA, androsterone and etiocholanolone. The two latter steroids are the metabolites of DHA, DHA-S and androstenedione. $C_{19}O_3$ steroids were divided in two different groups, based on the unlike position of the third functional group.

11-β-hydroxylation of C_{19} steroids takes place in the adrenals. These 11-β-hydroxy derivatives are the metabolites of androstenedione and, to a lesser extent, of cortisol and/or cortisone. The 16-oxy-C_{19}-steroids are produced not only in the adrenals, but also extraglandularly; their precursor is DHA. The $C_{21}O_5$ corticoids are the metabolites of cortisol and cortisone; their amount corresponds to about 80% of the total corticoid excretion. Pregnenediol represents the first steroid intermediate originating from cholesterol; it is the metabolite of pregnenolone.

The values for steroid excretion have been expressed in μg/kg/day and μg/m^2/day. In addition to the

in healthy girls of different age groups

$C_{19}O_3$(16-O-OH)		$C_{21}O_5$			Δ-5-Pd			No. of cases
Range	p	Mean±S.D.	Range	p	Mean±S.D.	Range	p	
0.3– 6.8		66.4±20.4	39.9– 94.4		3.4±1.8	0.9– 7.2		12
0.2–12.0	n.s.	56.3±23.0	34.4–100.0	<0.01	6.4±5.4	2.3–19.0	<0.01	9
0.1–30.4		91.4±32.8	48.9–159.5		9.0±6.1	0.5–21.7		13

$C_{19}O_3$(16-O-OH)		$C_{21}O_5$			Δ-5-Pd			No. of cases
Range	p	Mean±S.D.	Range	p	Mean±S.D.	Range	p	
6–172		1599±482	1022–2400		84± 50	22–205		12
5–327	n.s.	1536± 602	899–2680	<0.01	174±174	67–509	<0.01	9
4–961		2860±1015	1560–5154		280±201	314–626		13

B = values in μg/m^2/24 h.

mean values, standard deviations as well as upper and lower limits are given in Table II. Of the C_{19}-steroids, the metabolite occurring in the highest amount was 11-oxy-C_{19} in the 2 to 6 year age group. Here, the ratio of $C_{19}O_2$ to $C_{19}O_3$ was 0.72. This ratio increased to about 1.0 in the 7–9 age group and to 2.7 in puberty, due to increasing $C_{19}O_2$ steroid excretion.

Significant differences were found between the amounts of $C_{19}O_2$ steroids excreted in the three age groups. An increasing tendency was seen also in the amount of 11-oxy-C_{19} steroids, even though the difference from the age group of 2–6 years became significant in age group of 10–14 years only. Excretion of 16-oxy-C_{19}-steroids did not differ significantly in the three age groups.

The amount of $C_{21}O_5$ corticoids was increased in the age group of 10–14 years; the difference from the age group of 7–9 years was significant

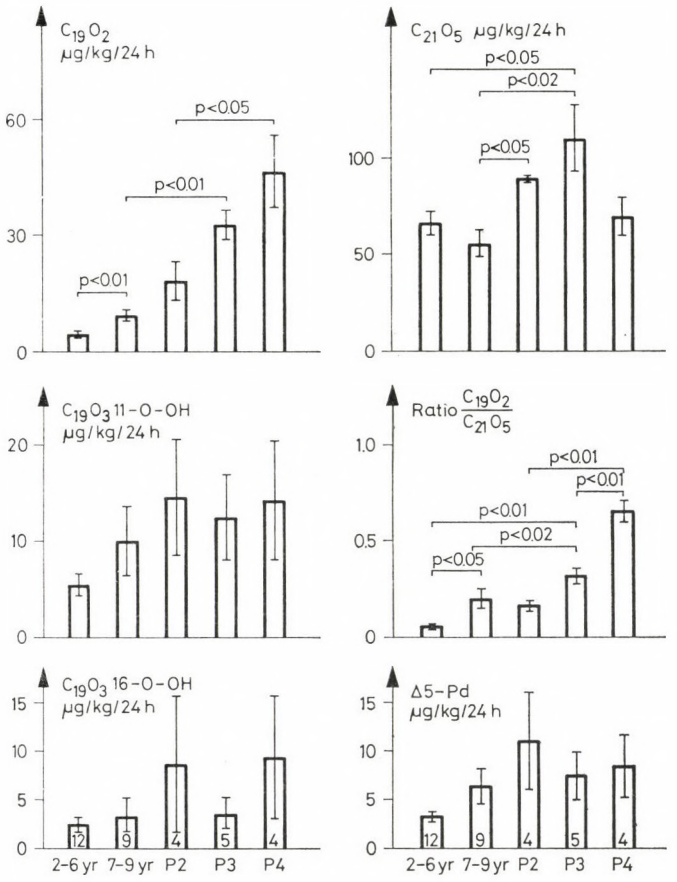

FIG. 3. Excretion values in µg/kg/day of $C_{19}O_2$, 11-oxy-C_{19}, 16-oxy-C_{19} steroids, $C_{21}O_5$ corticoids and pregnenediol according to age group distribution and puberty stages

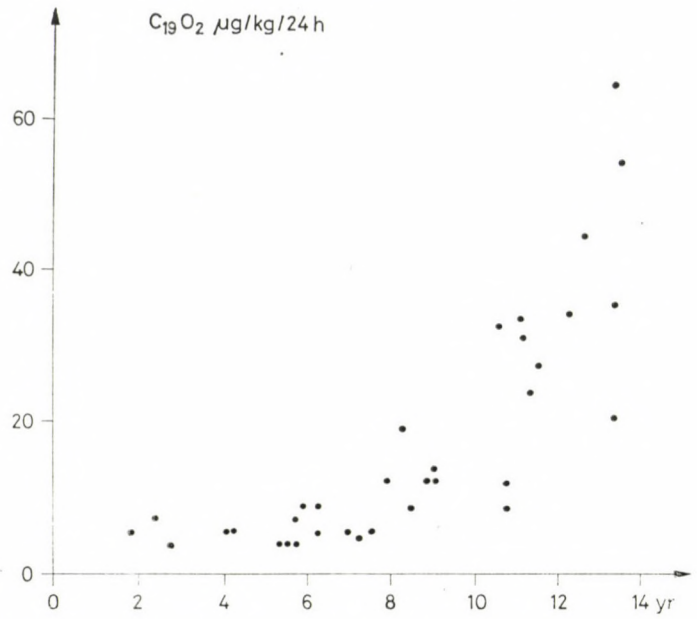

FIG. 4. Excretion in µg/kg/day of $C_{19}O_2$ steroids in 29 girls 2 to 14 years of age

statistically. Pregnenediol excretion had a tendency to increase in the age group of 7–10 years only; however, when compared with the age group of 2–6 years, the increase reached statistical significance in the 10–14 year age group.

All members of the age group of 10–14 years showed the physical signs of puberty. Therefore, this group was also evaluated according to the stage of puberty (21). These values, together with the S.E.M. and the probability level of the significant differences are shown in Fig. 3. As seen, the excretion of $C_{19}O_2$ steroids increased significantly already in the 7–9 age group, when compared with the age group of 2–6 years, even though the former group did not exhibit any physical sign of puberty. The increase continued also during puberty; the differences were significant between age group 7–9 and P-3, as well as between P-2 and P-4.

Excretion of $C_{19}O_2$ steroids in µg/kg/day is also expressed as a function of age (Fig. 4). Values over 7 µg/kg/day never occurred in the age group of 2–6 years ("infantile type adrenal androgen excretion"). The values in the age group of 7–9 years varied between 4 and 18 µg/kg/day. All the values below 10 µg/kg/day occurred in the 7th and 8th year of life. A $C_{19}O_2$ steroid excretion over 18 µg/kg/day was considered "puberty-type androgen excretion". In the age group of 10–14 years, the excretion of $C_{19}O_2$ steroids was below 18 µg/kg/day in two cases only. In these two girls the pubic hair was missing; the patients had been classi-

TABLE III

Quotient of $C_{19}O_2/C_{21}O_5$ excretion in healthy girls of different age groups

Ratio $C_{19}O_2/C_{21}O_5$ / Age (year)	Mean ± S.D.	Range	p	No. of cases
2– 6	0.06 ± 0.03	0.02–0.12		12
7– 9	0.17 ± 0.09	0.06–0.34	< 0.01	9
10–14	0.38 ± 0.22	0.10–0.85	< 0.01	13

fied in stage P-2 on the basis of breast development.

Excretion of 11-oxy-C_{19} steroids seemed to increase continuously till the second stage of puberty, but this increase was not significant statistically. There was an increase in the second and fourth stages of puberty, which, however, was not significant statistically (Fig. 3).

When compared with the age group of 7–9 years, a statistically significant elevation in $C_{21}O_5$ excretion was found in the second and third stages of puberty, but not in the fourth.

Pregnenediol excretion, though exhibiting a slight increase in stage P-2, did not significantly differ from the values found in the two age groups or in the different puberty stages.

In order to follow the corticoid-androgen dissociation we calculated and evaluated the ratio of $C_{19}O_2$

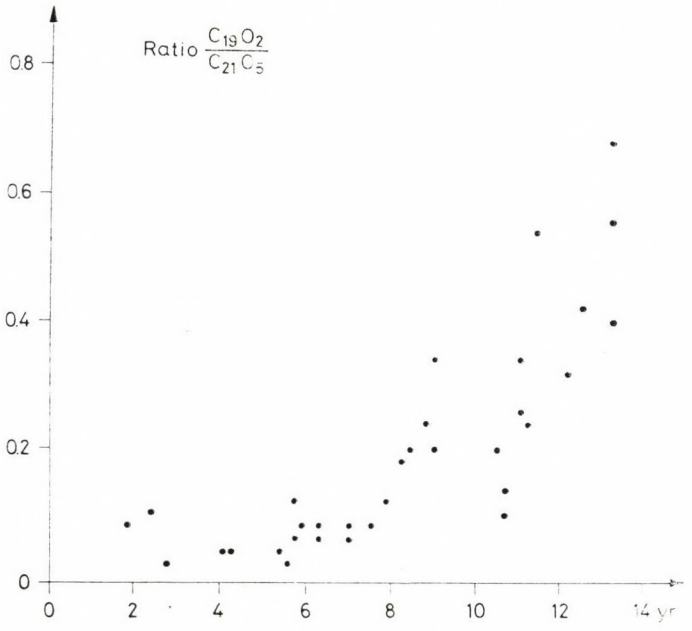

FIG. 5. Ratio of $C_{19}O_2$ to $C_{21}O_5$ corticoids in 29 girls 2 to 14 years of age

to $C_{21}O_5$ (Table III). The average ratio was 0.06 in the age group of 2–6 years. This means that at that age $C_{19}O_2$ steroid excretion only amounts to 6% of cortisol excretion. This ratio gradually increased and reached a value of 0.66 at the fourth stage of puberty, a finding indicative of a decreased androgen-cortisol dissociation during puberty. The distribution of individual values for the ratio was similar to those for $C_{19}O_2$ steroid excretion (Fig. 5).

The correlation between the excretion of pregnenediol and of the other steroid groups was also investigated. Pregnenediol showed a positive correlation with all steroids except $C_{21}O_5$

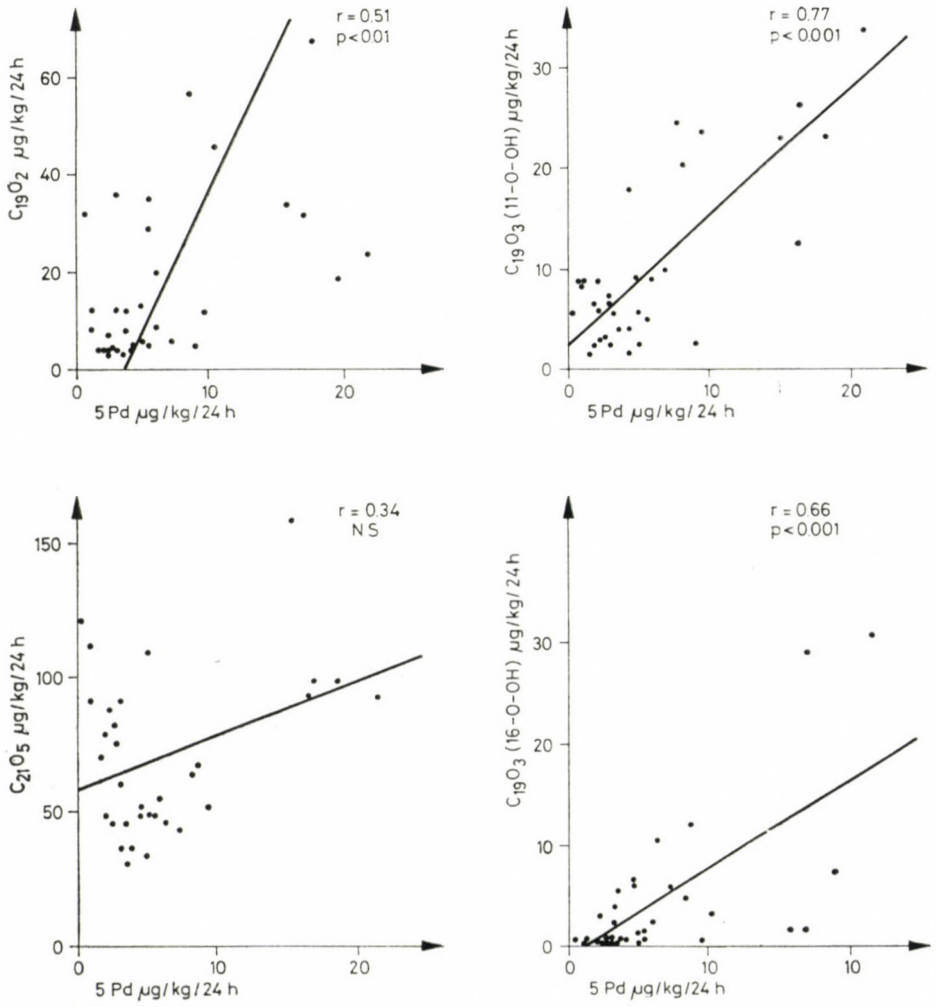

FIG. 6. Correlation between the excretion of C_{19}-steroids, $C_{21}O_5$ corticoids and pregnenediol in 29 girls on 34 occasions. r = correlation coefficient; p = statistical significance of the correlation

corticoids (Fig. 6). The best correlation was found with the 11-oxy-C_{19} and the 16-oxy-C_{19} steroid groups.

The 16-α-hydroxy metabolites of pregnenediol (i.e. 16-α-hydroxy-pregnenolone and 5-pregnene-3-β, 16-α, 20-α-triol) could be demonstrated in 20 out of the 34 cases. Thus, the total amount of the two steroids was between 0.5 and 7.4 μg/kg/day in the age group of 2–6 years (seven cases), 0.8 and 16 μg/kg/day in the age group of 7–9 years (eight cases) and 1.6 and 6.5 μg/kg/day in the age group of 10–14 years (eight cases).

Discussion

To obtain reliable results the amounts of steroids excreted were calculated for kg body weight and for sq.m. body surface. Supposing a physiological development, both procedures of calculation should eliminate any inhomogeneity due to differences in either body weight or body surface. A factor causing inhomogeneity in the 7–9 year age group was the onset of adrenarche the early stage of which, still free of physical signs, was first observed in girls over eight. Four out of the nine members of this group were under eight years at the time of the investigation (Fig. 4). In these girls the daily excretion in μg/kg of $C_{19}O_2$ steroids did not significantly differ from that of the 5–6-year-old children which means that these girls were still before the adrenarche. From the age of eight on, $C_{19}O_2$ steroid excretion surpassed the value of 10 μg/kg/day. This finding can be regarded as the sign of the physically still non-observable adrenarche. $C_{19}O_2$ steroid values between 10 and 18 μg/kg/day are regarded as prepubertal-type androgen excretion. Gonadarche and adrenarche do not always run parallel (18, 19); this was seen in two pubertal girls between 10 and 11 years of age (Fig 4) who on the basis of breast development were classified into the second stage of puberty. Their $C_{19}O_2$ steroid excretion was 7.6 and 14 μg/kg/day, respectively, and, on the basis of the absence of pubic hair they ought to have belonged to stage one.

Our observations on the onset of adrenarche are in agreement with both the classical findings based on investigation of 17-ketosteroids (7, 20) and the most recent results obtained by DHA and DHA-S determination (1, 6, 13, 15, 18, 19).

The augmentation of 11-oxy-C_{19} steroids is less marked than that of the $C_{19}O_2$ steroids. This difference, increasing with the progression of puberty, had previously been expressed by the ratio of 11-deoxy-17-KS to 11-oxy-17-KS, a value obtained during the evaluation of 17-ketosteroid fractionation. The value is 0.7–0.8 in childhood and increases in girls to 1.8–2.7 by the end of puberty (8, 23).

In contrast with data in the literature we observed an increased $C_{21}O_5$ steroid excretion during puberty when the values were calculated on body weight basis. This increase seemed

to be transient if the girls were divided into puberty subgroups. In view of the few cases studied, no final conclusion could be drawn from this observation.

There are few data on 16-hydroxy steroid excretion during childhood. Its excretion, though displaying a tendency to increase, did not significantly differ in the various age groups.

The increase of pregnenediol excretion was near the significance level between the age groups of 2–6 and 7–9 years; it became statistically significant during puberty. Here, however, the pregnenediol originating from the ovaries should also be taken into account. Most probably, the increase in pregnenediol excretion would have been significant in the prepubertal group, too, had the lower limit been set at the eighth instead of the seventh year of life.

ACTH is known to stimulate steroidogenesis by acting on cholesterol-pregnenolone transformation. Investigation of the correlation between pregnenediol and other steroids revealed a positive correlation between the elevation of the pregnenediol level and that of all other steroids except $C_{21}O_5$ corticoids. Pregnenediol is the immediate metabolite of pregnenolone; thus, the elevation in pregnenediol excretion allows the conclusion that the pregnenolone pool is increased also in the adrenals. We do not, however, regard this increased pregnenolone pool as a decisive factor in the augmentation of $C_{19}O_2$ steroids, for the correlation with pregnenediol excretion is lower than that of the slightly increasing 11-oxy-C_{19} steroids.

A prominent sign of steroidogenesis during puberty is the great increase in urinary $C_{19}O_2$ steroid excretion, an event causing the childhood cortisol-androgen dissociation to disappear. The augmentation in $C_{19}O_2$ secretion indicates the opening of a new way of steroidogenesis. This event can be brought into connection with an intraglandular factor, i.e. with the development of the zona reticularis.

The significance of intraglandular factors was pointed out also by Dhom (5) who observed in a large obduction material obtained from unexpectedly died subjects that the first signs of the formation of a continuous zona reticularis appeared in girls as early as at 6–8-years of age, even though full development of the zone was reached after the 13th year of life.

A zona reticularis focal in character was found in half of the subjects at the age of five; an even lower incidence could be observed in children under five. Other authors also regard the zona reticularis as rudimentary between the 3rd and 10th years of life; it reaches full development during puberty (4).

The role of extraglandular factors (FSH, LH, STH and oestrogens) in the activation of adrenal androgens during puberty was investigated by several authors (16). It was found that the above factors do not affect the adrenal. On the other hand, bovine pituitary extract possessed an androgen activating effect higher than that expected on the basis of the ACTH

content. Parker (16) ascribed this effect to an unknown pituitary factor. The existence of an "androgen activating hormone" acting together with ACTH was suggested also by other investigators (19) in connection with adrenarche.

On the other hand, the possibility of an intraglandular inhibition of adrenal androgen steroidogenesis has scarcely been dealt with, even though the existence of such a functional mechanism may be assumed. C_{21}-hydroxylase-deficient patients on corticoid substitution excrete C_{19}-steroids in amounts which is several-fold the physiological level, a fact indicating that the reticular zone of the hyperplastic adrenal cortex is active. In spite of this, there is no elevation in the excretion of $C_{19}O_2$ (otherwise characteristic of puberty) before the sixth year of life, nor are any physical signs of puberty present (11). Probably both phenomena are due to the inactivating effect of the relatively more intensive 16-α- and 11-β-hydroxylations. Another observation (19) has, in accordance with this assumption, shown that an early sexual development starting before the age of six is not accompanied by a change in the serum DHA–S level that would result in values differing significantly from the physiological ones.

Acknowledgements

The authors are indebted to Prof. D. N. Kirk (Medical Council Steroid Reference Collection, London) for a generous gift of reference steroids. Thanks are due to Dr Livia Palatinus (Municipal Institute for Care of Mother and Child, Pécs) for paediatric investigations as well as to Mrs Edith Kiss and Miss Ildikó Böröcz for skilful technical assistance.

This work was supported by a grant (3–13–0302–01–2/K) of the Scientific Council of the Hungarian Ministry of Health.

References

1. Akamine, Y, Kato, K, Ibayashi, H: Studies on changes in the concentration of serum adrenal androgens in pubertal twins. *Acta Endocrinol* 93:356, 1980
2. Albright, F, Smith, PH, Fraser, R: A syndrome characterised by primary ovarian insufficiency and a decreased stature: report of 11 cases with a digression on hormonal control of axillary and pubic hair. *J Med Sci* 204:625, 1942
3. Apter, D, Vihko, R: Serum pregnenolone, progesterone, 17-hydroxyprogesterone, testosterone and 5α-dihydrotestosterone during female puberty. *J Clin Endocrinol Metab* 45:1039, 1977
4. Bargmann, W: Histologie und mikroskopische Anatomie. 2. Aufl. Georg Thieme, Stuttgart 1956
5. Dhom, G: The prepuberal and puberal growth of the adrenal (adrenarche). *Beitr Pathol* 150:357, 1973
6. Ducharme, JR, Forest, MG, De Peretti, E, Sempe, M, Collu, R, Bertrand, J: Plasma adrenal and gonadal sex steroids in human pubertal development. *J Clin Endocrinol Metab* 42:468, 1976
7. Hamburger, C: Normal urinary excretion of neutral 17-ketosteroids with special refer to age and sex variations. *Acta Endocrinol* (Kbh) 1:19, 1948
8. Kádár, A, Fehér, T, Koref, O: Urinary excretion of individual 17-ketosteroids by normal children. *Arch Dis Child* 39:257, 1964
9. Kecskés, L, Juricskay, Zs: Steroid spectrum in human urine as revealed by gas chromatography I. Qualitative analysis of C_{19}–$C_{21}O_{2-3}$ steroids. *Acta Biochim Biophys Acad Sci Hung* 10: 37, 1975
10. Kecskés, L, Juricskay, Zs, Czira, G: Steroid spectrum in human urine as revealed by gas chromatography III. Identification and quantitation of pregnenediol at different stages of bodily development. *Acta Biochim Biophys Acad Sci Hung* 15:49, 1980

11. Kecskés, L, Juricskay, Zs, Kosztolányi, Gy, Szécsényi, M: Steroid spectrum in human urine as revealed by gas chromatography IV. Changes in the excretion of 16-oxygenated neutral steroids by children with 21-hydroxylase deficiency during different stages of development. *Acta Biochim Biophys Acad Sci Hung* 16:57, 1981
12. Kenny, FM, Gancayco, GP, Heald, FP, Hung, W: Cortisol production rate in adolescent males in different stages of sexual maturation. *J Clin Endocrinol Metab* 26:1232, 1966
13. Korth-Shutz, S, Levine, LS, New, MI: Serum androgens in normal prepubertal and pubertal children and children with precocious adrenarche. *J Clin Endocrinol Metab* 42:117, 1976
14. Migeon, CJ, Green, OC, Eckert, JP: Study of aderenocortical function in obesity. *Metabolism* 12:718, 1963
15. Parker, LN, Sack, J, Fischer, DA, Odell, WD: The adrenarche: prolactin, gonadotropins, adrenal androgens and cortisol. *J Clin Endocrinol Metab* 46: 396, 1978
16. Parker, LN, Odell, WD: Evidence for existence of cortical androgen-stimulating hormone. *Am J Physiol* 236:E 616, 1979
17. Rosenfeld, RS, Hellman, L, Roffwarg, H, Weitzman, ED, Fukushima, DK, Gallagher, TF: Dehydroisoandrosterone is secreted episodically and synchronously with cortisol by normal man. *J Clin Endocrinol Metab* 33:87, 1971
18. Sizonenko, PC, Paunier, L: Hormonal changes in puberty III. Correlation of plasma dehydroepiandrosterone, testosterone, FSH and LH with stages of puberty and bone age in normal boys and girls and in patients with Addison's disease or hypogonadism or with premature or late adrenarche. *J Clin Endocrinol Metab* 41:894, 1975
19. Sklar, CA, Kaplan, SL, Grumbach, MM: Evidence for dissociation between adrenarche and gonadarche: Studies in patients with idiopathic precocious puberty, gonadal dysgenesis, isolated gonadotropin deficiency and constitutionally delayed growth and adolescence. *J Clin Endocrinol Metab* 51:548, 1980
20. Talbot, NB, Butler, AM, Berman, RA, Rodrigues, PM, Mac Lachlan EA: Excretion of 17-ketosteroids by normal and by abnormal children. *Am J Dis Child* 65:364, 1943
21. Tanner, IM: *Wachstum und Reifung des Menschen.* Thieme, Stuttgart 1962
22. Trafford, DJH, Makin, HLJ: Rapid quantitative gas-chromatographic fractionations of the major urinary 17-oxosteroids and 17-oxogenic steroids. *Clin Chim Acta* 40:421, 1972
23. Vestergaard, P: Urinary excretion of individual 17-ketosteroids in children. *Acta Endocrinol* 49:436, 1965

Received 27 April 1981

L. Kecskés, M. D.
Szigeti út 12
H-7624 Pécs, Hungary