Hormone Excretion in Premenarcheal Girls

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Relatively little has been written on hormone excretion in premenarcheal girls compared with the enormous number of publications devoted to this subject in adults. Fitschen and Clayton (1965) studied gonadotrophin excretion in children, and summarized the findings of only 22 earlier papers on the subject since 1931; not all of these papers dealt with the problem in girls, and several measured excretion only in pooled specimens of urine. Kulin et al. (1967) have studied gonadotrophin excretion more recently. The earlier work on oestrogen excretion was carried out using biological methods of assay, but Bulbrook, Greenwood, and Snaith (1958) and Dewhurst (1963) have reported on excretion measured chemically. The excretion of 17-oxosteroids in girls was studied by Hamburger (1948), Norymberski, Stubbs, and West (1953), Levell et al. (1957), and Gardner and Snaith (1958). Pregnanediol excretion was reported on by Bergstrand and Gemzell (1957), while pregnanetriol has been estimated in both normal girls and girls with the adrenogenital syndrome by Morris (1959), Bell and Varley (1960), and more recently, using gas-liquid chromatography, by Kinoshita et al. (1966).

Further work was clearly necessary, and the present study was undertaken to measure the normal excretion of sex hormones during childhood, against which possible endocrine disorders might then be studied.

Materials and Methods

24-hour specimens of urine were obtained from normal girls between the ages of 4 weeks and 14 years who had not yet menstruated. Wherever possible 2 consecutive 24-hour specimens were obtained, one containing preservative to permit gonadotrophin assay, the remaining assays being performed on the other sample. Where this was not possible a single sample containing chloroform had to suffice. Some girls provided one or more samples at a later date after intervals varying from a few months to a few years. The older girls showed varying degrees of secondary sexual development, from mild to full (breast rating 2–5 [Tanner, 1962]), but were all premenarcheal. Very few samples could be obtained from clearly normal girls aged less than 3 years; this group was, therefore, enlarged by the inclusion of results from girls in this age-group from whom samples had been obtained for one or either assay to investigate a suspected endocrine fault when no fault was in fact discovered.

Samples of urine from each child were, whenever possible, assayed for oestradiol, oestrone and oestriadiol, total urinary gonadotrophin, 17-oxosteroids, 17-oxogenic steroids, pregnanediol, and pregnanetriol. The three oestrogens were assayed by the method of Brown (1955) and Brown, Bulbrook, and Greenwood (1957); pregnanediol by the method of Klopper, Michie, and Brown (1955) or Podmore (1966); 17-oxosteroids and 17-oxogenic steroids by the techniques of Appleby et al. (1955), as modified by the Medical Research Council Committee on Clinical Endocrinology (1963) and Gray et al. (1969), and urinary gonadotrophins by the method of Loraine and Brown (1959). It was generally possible to carry out all these assays on the sample or samples provided by each girl, but occasionally with small volumes involved one or other assay had to be omitted. Two girls aged 11 and 10 years, with early secondary sexual development, provided almost daily samples throughout one month, and similar studies were performed on each.

Fiducial limits have not been calculated for the gonadotrophin results obtained. However, the index of precision found for assays in our laboratory is between 0·2 and 0·3; while such an index is less precise than one below 0·2 (Gaddum, 1933) it can still be used with reasonable confidence. The figure generally given for the index of precision for the mouse uterine test employed here is between 0·2 and 0·3 (Loraine and Brown, 1954, 1956; Schmidt-Elmendorff, Loraine, and Bell, 1962; Borth and Menzi, 1964).

Results

Table I shows the results of all the hormone assays performed on girls of 3 or more years of age who provided samples solely for the purpose of this study. These, together with the urine from the younger patients in whom abnormality was sus-
### TABLE I

**Urinary Hormone Excretion in girls of 3 to 14 years**

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<tr>
<th>No.</th>
<th>Age (yr.)</th>
<th>Urinary Gonadotrophins (IU/24 hr.)</th>
<th>17-Oxosteroids (mg./24 hr.)</th>
<th>Oestradiol (ug./24 hr.)</th>
<th>Oestrone (ug./24 hr.)</th>
<th>Oestradiol (mg./24 hr.)</th>
<th>Pregnanediol (mg./24 hr.)</th>
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The figures in brackets against the chronological age of the older children indicate breast rating after Tanner.
Hormone Excretion in Premenarcheal Girls

**Fig. 1.**—Urinary oestriol, oestrone, and oestradiol excretion in premenarcheal girls according to age. ●—● mean; ×—× 1 SD; ○—○ 2 SD.

**Fig. 2.**—Urinary gonadotrophin excretion in premenarcheal girls according to age. —— mean; ....... 1 SD; ——— 2 SD. (Gonadotrophins were not detected on a number of occasions, as shown in Table I.)

**Fig. 3.**—Urinary 17-oxosteroid excretion in premenarcheal girls according to age. Key as in Fig. 1.

Oestrogen excretion in premenarcheal girls is shown in Fig. 1. Oestriol was present in all specimens and is not shown in the figure. Oestrone was detected in 23 samples and absent from 22. In girls with some signs of secondary sexual development (Stages 2–5, Tanner), gonadotrophin was present in 19 specimens and absent from 7; values as high as 7 IU (aged 12 years) and 8 IU (aged 13½ years) were recorded.

Oestrogens in one or other form were detected in all samples; oestriol was detected in all, oestrone in all but 2, both in the 0–3 years group, and oestradiol was detected in all but 5, of which 4 were in the 0–3 years group and the fifth was aged...
14 years. 17-oxosteroids were undetected in only 2 samples—from a 7-year-old-girl without secondary sexual development, and from a 10-year-old with early development; in all other samples examined, 17-oxosteroids and 17-oxogenic steroids were detected.

Pregnanediol was present in 36 samples and absent from 17, and pregnanetriol was present in 27 of 28 samples examined.

The daily variation in hormone excretion is revealed by the results of the study of the 2 girls aged 10 and 11 years, with early secondary sexual development (stage 3) (Fig. 7 and 8). These indicated moderate variation within the normal range for oestriol and oestrone; oestradiol excretion occasionally slightly exceeded the normal range. 17-oxosteroid values were well within the normal range for the 10-year-old girl, and within in all but one reading from the 11-year-old. 17-oxogenic steroids, pregnanediol, and gonadotrophin were within the normal range in all readings in both girls.

**Discussion**

The present study reveals some differences from and similarities with results of earlier studies.

**Gonadotrophins.** Early authors writing on urinary gonadotrophins reported no detectable excretion in younger girls (Fitschen and Clayton, 1965; Kulin et al., 1967). Johnsen (1959) even regarded the detection of gonadotrophin in the urine of a girl less than 9 years of age as abnormal, and he considered that gonadotrophin appeared in the urine some 2 or 3 years before the menarche. One older report (Catchpole and Greulich, 1943) and several more recent ones (Brown, 1958; Carletti and Kehyayan, 1962; Fitschen and Clayton, 1965; Kulin et al., 1967) suggested that the urine of some younger girls did contain detectable amounts of gonadotrophin; Kulin and his colleagues, for instance, demonstrated gonadotrophin in one-sixth of the specimens from girls and boys between the ages of 4 years and 10 years, most of whom were prepubertal.

Our study confirms these later papers, but indicates that gonadotrophin may be detected more frequently than has previously been believed. In our series, girls showing no secondary sexual development showed detectable gonadotrophin excretion in about 50% of the specimens examined; with the appearance of secondary sexual development in the older children, gonadotrophin was detectable more frequently still. The demonstration of gonadotrophin in some samples but not
in others from those girls providing several specimens indicates that little reliance can be placed upon the results of a single sample; Fitschen and Clayton draw attention to this point. The normal range for age (Fig. 2) cannot be considered other than an approximate one, in view of the variation observed, which may be partly explained by the essentially imprecise nature of this assay, and per-haps also by the small number of girls in some age-groups. It seems obvious that with the introduction of more sensitive radio-immunoassays (Wilde, Orr, and Bagshawe, 1965; Bagshawe, Wilde, and Orr, 1966) the detection of gonadotrophins in urine will be much facilitated.
Oestrogens. Earlier work on oestrogen excretion by bioassay suggested that only very small amounts were detectable in the urine of younger girls, considerably more being present shortly before the menarche. Bulbrook et al. (1958), using chemical methods of assay, were able to detect oestrogen in only 1 of 15 urine samples from girls between the ages of 3 and 10 years. Older girls showing some secondary sexual development but not having menstruated produced various levels of excretion between 2-9 and 7-1 μg./24 hours. Dewhurst (1963) reported oestriol excretion between 0-2 and 3-10 μg./24 hours, oestrone between 0 and 5-6 μg./24 hours, and oestradiol between 0 and 0-9 μg./24 hours in 11 girls aged between 5 and 8 years without secondary sexual development, and slightly higher levels in two 11-year-old girls with early secondary sexual development; these results fall within the normal range compiled in the present paper.

It is evident from the results quoted in this paper that it is possible to detect oestrogens in the urine of young girls on nearly all occasions. The graph of the normal range shows a trend upwards with increasing age and secondary sexual development, until the values obtained in the older girls are comparable with the 'off peak' times of the menstrual cycle reported by Brown (1955).

Pregnanediol. The excretion of pregnanediol in this study is somewhat higher than that noted by Bergstrand and Gemzell (1957) who measured excretion in 87 children aged from 3 to 15 years; they reported a mean excretion of less than 1 mg./24 hours in both boys and girls. Their results compare with the values in the 0–3 and 4–6-year-old groups studied here, but are less than the values obtained in the three older groups (Fig. 5). During the luteal phase of the menstrual cycle, Klopper (1957) reported values to the order of 1–6 mg./24 hours.

Pregnanetriol. The results of the pregnanetriol analysis fall broadly within the range of those previously reported. Morris (1959) reported an excretion of 0·1 mg./24 hours in a 17-month infant, and a range of values between 0·2–0·4 mg./24 hours in a 5–7-year-old group; at 10 years he reported a single value of 1·1 mg./24 hours.

17-oxosteroids and 17-oxogenic steroids. 17-oxosteroid excretion during childhood has previously been regarded as low. Levell et al. (1957) reported levels of between 1–5 mg./24 hours in children; Hamburger (1948) described a similar range of values, while Prout and Snaith (1958) showed mean excretions of 0·3 mg./24 hours (0–1 years), 0·8 mg./24 hours (1–5 years), 1–4 mg./24 hours (6–10 years), and 5·0 mg./24 hours (11–17 years). The present series confirms these findings. Slightly higher levels of 17-oxogenic steroid excretion were reported by Levell et al. (1957), and by Norymberski et al. (1953) and Gardner and Snaith (1958). These results too are confirmed by the present study.

Daily excretion. The two girls studied throughout one month provide important material for consideration against the background of the other results. Moderate day-to-day variation occurred in the values of all hormones measured. This variation, however, with very few exceptions, was within the normal range constructed from the other data. This suggests that values obtained from single 24-hour specimens may up to a point be helpful when applied to the normal graph though more than one sample would give more valuable information. It should be emphasized however that occasionally in the single samples from girls of different age-groups one or other hormone was not detectable at all. This was, commonest with gonadotrophin, especially in younger girls, but sometimes in older ones as well, in the daily assays of the two girls in question, gonadotrophin was undetectable only once in each girl, during the period studied (the mice died on the other blank days recorded in Fig. 8). This total absence of gonadotrophin from single samples
may be a reflection on the method of biological assay rather than a real absence of the hormone from the urine.

Oestradiol, oestrone, and oestriol were detected in all samples from both girls. 17-oxygenated steroids were undetectable in two samples from the 10-year-old, but all samples from both girls showed oxogenic steroids. Pregnanediol was undetected in 7 of 27 samples from the 10-year-old girl and in 8 of the 26 samples from the 11-year-old. Pregnanetriol excretion was not measured. These results are comparable with the frequency of detection in the isolated specimens examined from the girls of all ages, where only gonadotrophin and pregnanediol were undetectable with any frequency.

Finally, mention must be made of the method of obtaining samples from some of the younger children. Reliable samples from a child who is not fully continent can be obtained only by the application of an adhesive bag to the vulval area. Soreness, which can be marked, results in most cases, and we felt that only if some possible abnormality suggested it, was collection justified. We have tried to include only those children in whom no endocrine fault was seen but it must be admitted that the fact that such a fault was suspected at all suggests that the child may not be considered completely normal; until further data are available, however, this seems to be as near to normal as we can get.

Summary

The excretion of total urinary gonadotrophin, oestrogens, 17-oxygenated steroids, pregnanediol, and pregnanetriol has been investigated in a group of premenarchal girls aged between 4 weeks and 14 years. Daily assays of the excretion of the same hormones (pregnanetriol excepted) were carried out in two girls aged 10 and 11 years with early secondary sexual development. Gonadotrophin was present in 23 of 45 samples of urine from girls without secondary sexual development, and in 19 of 26 samples from girls with secondary sexual development of some degree. Oestrogens as oestradiol, oestrone, or oestriol were detectable in all samples. 17-oxygenated steroids were detectable in only two samples and 17-oxygenated steroids were detectable in all samples. Pregnanediol was present in 36 samples and absent from 17; pregnanetriol was present in 27 of 28 samples examined.

The daily excretion of these hormones by the girls aged 10 and 11 years showed moderate variation which, with few exceptions, was within the range of normal as computed from the other samples.

It is a pleasure to record our thanks for the cooperation of many paediatricians from the United Sheffield Hospitals and hospitals of the Sheffield Regional Board.

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The following articles will appear in future issues of this Journal:


Effects of Kwashiorkor on Cortical and Trabecular Bone. By Peter Adams and F. R. Berridge.


Pudendal Neurectomy in Management of Neurogenic Bladder in Myelomeningocele. By Gordon Stark.


Comparison of Arterial and Arterialized Capillary Blood in Infants with Respiratory Distress. By Ann Banister.


Gastric Atrophy in Childhood. By A. L. Vaterlaws.


Pilot Trial of an Infant Screening Programme for Cystic Fibrosis: Measurement of Parotid Salivary Sodium at 4 months. By David Lawson, Peter Westcombe, and Barry Saggars.

Proceedings of the Paediatric Research Society: Abstracts of papers read at a Meeting held at Sheffield University, March 28 and 29, 1969.

Lipoprotein Lipase Acticity in Marasmic Type of Protein-calorie Malnutrition. By C. T. Gürson and G. Saner.

Occipital Cranial Bifidum. By A. N. Guthkelch.

Serum Immunoglobulin and Transferrin Levels After Childhood Splenectomy. By M. J. Schumacher.


Sacrococcygeal Teratoma in Nigerian Children. By A. O. Williams, S. B. Lagundoye, and M. A. Bankole.

Hormone excretion in premenarcheal girls.

G. W. Pennington and C. J. Dewhurst

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