Output of Luteinizing Hormone in the Urine of Normal Children and Those with Advanced Sexual Development

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Buckler, J. M. H., and Clayton, B. E. (1970). *Archives of Disease in Childhood*, **45**, 478. **Output of luteinizing hormone in the urine of normal children and those with advanced sexual development.** Estimations by radioimmunoassay of the 24-hour excretion of LH in normal children (152 boys and 104 girls) have shown high values in the first six months of life in boys, and possibly girls, and then low levels until the age of about 6 years. There is then a gradual rise in LH excretion in both sexes which continues for the 3–4 years which precede the onset of puberty, at which age a more marked increase occurs. 13 cases of true precocious puberty were all found to be associated with increased urinary excretion of LH, a feature that was seldom shown with other forms of advanced sexual development. Measurement of urinary LH excretion provides a useful tool in the differentiation of such conditions.

Much still remains to be known about the mechanisms for the initiation and regulation of puberty. Until recently there had been few studies of gonadotrophin levels in childhood and adolescence, and these few were by bioassay methods not sufficiently sensitive to detect minor changes. Since the introduction of the radioimmunoassay, more sensitive and accurate determinations have become feasible, but most studies in children refer to gonadotrophin levels in serum. The present study reports levels of luteinizing hormone (LH) in 24-hour collections of urine from normal children of all ages, and from children with precocious puberty. These results throw some light on one of the probable endocrine activities that precedes the more obvious changes at puberty.

Subjects and Methods

Controls. The study was conducted on 152 boys (aged from 3 days to 12½ years) and 104 girls (aged 3 months to 14 years) who were considered as normals. 122 of these boys and 78 of the girls were under the age of 9 years, and had no evidence of onset of puberty. The remaining children were assessed for staging of pubertal development by the method of Tanner (1962), but there were insufficient numbers for satisfactory analysis. These children were mostly hospital in-

patients undergoing treatment for various conditions, but were not acutely ill or suffering from endocrine disorders. Many were undergoing minor surgical procedures, such as operations on the ears, nose, or throat.

Children with advanced sexual development. 5 boys and 8 girls with features of true precocious puberty (i.e. with evidence of advanced gonadal development) were included in this study, and the details of these children are included in Table I. In addition, there were 2 boys and 9 girls with various features of advanced sexual development but lacking the full picture of true precocious puberty, and one true hermaphrodite (Table II). Many of these children were undergoing investigations as hospital in-patients but a few collected urine as out-patients. Cases of precocious sexual development known to be of adrenal origin have been excluded from the study.

Estimation of LH in urine. 24-hour collections of urine were preserved with merthiolate or chloroform and stored at –20 °C. before assay of LH content. Repeat collections on different days were obtained from several children. The urine samples were processed by the ethanol precipitation method described by Raiti and Blizzard (1968). The dried precipitates were redissolved in buffer of such a volume as effectively to concentrate the urine fourfold. The LH in the urine was estimated by a double antibody radioimmunoassay (Buckler and Clayton, 1970). The antiserum chosen for use in this assay was raised to human chorionic

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FIG. 1.—Amounts of LH (in terms of 2nd IRP) in 24-hour collections of urine from normal boys. Interrupted lines join values for repeat estimations on the same children. The exceptional result in one of the 5-year-old boys has been excluded from Table III.

FIG. 2.—Amounts of LH (in terms of 2nd IRP) in 24-hour collections of urine from normal girls. Interrupted lines join values for repeat estimations on the same children.

Features and Findings in Ca-

Case No.	Age When LH Estimated (yr.)	24-hr. Urinary LH (i.u. 2nd IRP)	First Symptoms		Clinical Features		
			Age (yr.)		Secondary Sex Characteristics		
					Breast Development (Staging according to Tanner, 1962)	Genital Development (Staging according to Tanner, 1962)	Pubic Hair (Staging according to Tanner, 1962)
<i>Boys</i>							
1	5.9	16.1	0.2	Pubic hair		5	5
2	2.7 } 3.6 }	7.3 } 17.8 }	0.5	Rapid growth; genital development		5	5
3	4.0 } 4.4 }	6.8 } 8.2 }	2.0	Pubic hair and large penis		5	5
4	4.7	14.1	2.8	Rapid growth; acne		4	4
5	6.2	2.5	3.0	Rapid growth		4	4
<i>Girls</i>							
6	5.5	2.1	Birth	Breasts	3	Large labia	5
7	2.0	10.6	0.8	Vaginal bleeding	3	Large labia	5
8	4.2	20.6 }	1.0	Breasts	3		5
9	4.8	27.2 }	1.0	Rapid growth; vaginal bleeding	3		5
10	4.9	7.7 } 7.4 } 3.7 }	1.5	Breasts; pubic and axillary hair	2-3	Normal	5
11	9.7	12.3	2.5	Breasts; vaginal discharge	5		5
12	4.0	5.8	3.0	Rapid growth	2		5
13	4.1	14.7	3.0	Breasts; pubic hair	2-3		5

*NS = not studied.

gonadotrophin (HCG) in a guinea-pig and shown to cross-react only slightly with follicle stimulating hormone (FSH) and thyrotrophic hormone (TSH). The antiserum had been treated with extracts from the urine of young children to remove non-specific cross reactants.

Results given are the mean of duplicate assays at 3 dilutions (undiluted and concentrated twofold and fourfold) and are reported in terms of the Second International Reference Preparation of Human Menopausal Gonadotrophin (2nd IRP) (kindly supplied by Dr. D. R. Bangham, National Institute for Medical Research, London). Estimations on repeated urine collections from the same children on different days (often before and after minor surgery) have usually shown only minor variations in LH content.

Sixteen recovery experiments have been included in the course of these studies and the values for recovery of added standard hormone (2nd IRP) averaged 103%, with a standard deviation of 10.3%.

Results

LH content of 24-hour collections of urine from normal children. The 24-hour urinary LH output for the normal boys is shown in the scatter diagram (Fig. 1) and for normal girls in Fig. 2. The mean values for the various age-groups

before the onset of puberty are shown for boys in Table III and for girls in Table IV.

After the first 6 months of life, urinary LH output in both sexes remains low until about 6 years of age, when levels rise slowly but steadily. This increased LH output is shown to be significant statistically, particularly in boys. As the time of puberty approaches, and throughout puberty, these LH levels rise more steeply towards adult values, and become much more scattered.

During the first 6 months of life, LH levels in 24-hour urine collections of boys are higher than throughout the rest of prepubertal childhood. There are insufficient estimations to draw the same conclusion about girls.

LH levels in 24-hour urine collections from children with abnormal sexual development. The 24-hour urinary LH values obtained in children with true precocious puberty together with details of their clinical features and histories are shown in Table I. All of these 13 cases have levels in excess (usually considerable) of the normal range for the appropriate age (as assessed by the mean control values reported plus 1 standard deviation).

The results of the other 12 children with abnormal

True Precocious Puberty

Time of LH Estimation			Bone Age (yr.)	Chrono-logical Age (yr.)	Oestrogen Effect	Pathological Cause
aracteristics	Centiles					
Vaginal Bleeding	Height	Weight				
	> 97	> 97	6-8 } 14-15 }	2.2 } 5.9 }		Idiopathic
	97	> 97	3 } 10 }	2.6 } 4.0 }		Suprasellar tumour; (?hamartoma)
	> 97	> 97	12.0	4.0		Idiopathic
	> 97	> 97	13.5 } 16.5 }	4.1 } 6.0 }		Idiopathic
	> 97	> 97	14	6.2		Idiopathic
Every 6 mth. since 1.7 yr.	90-97	90	9	5.4	*NS	Albright's syndrome
Every 2 mth. since 0.8 yr.	> 97	> 97	> 4	2.0	+	Hypothalamic tumour; ?hamartoma
—	> 97	> 97	10	4.2	+	Chronic subdural haematoma
3 episodes at 1.2, 2, and 3.5 yr.	> 97	> 97	12.5	4.7	*NS	?Ovarian cyst (ruptured at 3½ yr.) ?idiopathic
—	> 97	90-97	8	4.9	+	Idiopathic
Monthly since 7 yr.	75-90	75	6 } 14-15 }	3.5 } 9.8 }	*NS	Third ventricular tumour
Monthly since 3.7 yr.	> 97	> 97	8 } 10 }	4.0 } 4.9 }	+	Suprasellar mass (?hypothalamic tumour)
—	> 97	> 97	7-8	4.1	+	Idiopathic

sexual development are similarly shown in Table II. In most of these, the LH levels are within the normal range, and the nature of the abnormality is apparent, but in 2, a diagnosis of idiopathic precocious puberty could not be excluded. The 2 eldest children of this group did have a high LH output, and were probably entering normal adolescence at a rather early stage.

Discussion

By bioassay methods, detectable low levels of gonadotrophins have been found in pooled samples of urine collected from children over several days (Fitschen, 1963; Fitschen and Clayton, 1965), though Kulin *et al.* (1967) found considerable variation from day to day. There are only a few reports of radioimmunoassays being applied to the estimation of LH levels in children's urine (Bagshawe, Wilde, and Orr, 1966; Kulin, Rifkind, and Ross, 1968). Estimations on untreated urine samples give values that are much higher than those found with processed urines or bioassays (Kulin *et al.*, 1968; Stevens, 1969). The present study reports the results with urine samples treated by ethanol precipitation.

Though a number of workers have reported LH estimates in sera of children (e.g. Kenny *et al.*, 1968, 1969; Root *et al.*, 1968; Schalch *et al.*, 1968b; Johanson *et al.*, 1969), the variation in results is considerable, and many reports indicate no significant differences between the values of adults and children. We have found that with the low levels occurring in children, we are not satisfied with any of the methods at present available for estimating these values in sera. Serum estimations reflect only random samples and are not necessarily an indication of the mean values over 24 hours. In addition, there is at present no suitable standard on which to base serum LH estimations, and values given are reported in terms of pituitary reference materials or 2nd IRP, neither of which are likely to be equivalent immunologically to the form of LH that occurs in serum.

That LH output increases at the time of puberty is well recognized, and bioassay studies had suggested that the rise in LH at that time was more marked than that of FSH (Brown, 1958; Rifkind, Kulin, and Ross, 1967). The report of Johanson *et al.* (1969) on serum LH levels in children suggested that this rise could be correlated with the

Features and Findings in Cases with

Case No.	Age When LH Estimated yr.	24-hr. Urinary LH (i.u. 2nd IRP)	First Symptoms		Clinical Features		
			Age (yr.)		Secondary Sexual Characteristics		
					Breast Development (Staging according to Tanner, 1962)	Genital Development	Pubic Hair
Boys							
1	1.2	0.7	Birth	Slow weight gain; lipodystrophy		Large penis and scrotum; testes slightly enlarged	Large penis
2	9.3	3.3	3	Rapid growth; behaviour disorder		Large penis	
Girls							
3	1.3	0.4	Birth	Slow weight gain; enlarged labia and clitoris	2	Moderate enlargement of labia and clitoris	
4	0.9	0.4-1.0	0.5	Pubic hair	2	Slight clitoral enlargement	
5	0.9	1.0 } 2.1 }	0.6	Pubic hair; breasts	2		
6	2.8	1.1	2.0	Breasts	2	Normal	
7	7.9	2.1	4.0	Vaginal bleeding	2	Normal	
8	6.5	0.5	6.0	Vaginal discharge; body odour	1	Normal	
9	6.5	1.2	6.0	Pubic hair	1		
10	9.4	4.6	7.0	Pubic hair	1	Slight clitoral and labial enlargement	
11	9.0	1.7	8.6	Pubic hair	1		
Hermaphrodite							
12	0.5	0.4	Birth	Ambiguous genitalia	1	Enlarged clitoris and labia; single urogenital opening	

*NS = not studied.

stage of pubertal development. There have been insufficient pubertal subjects in the present series for such a correlation to be demonstrated significantly, though this trend is suggested. However, the results reported here show that a significant rise in LH output in urine occurs 3 to 4 years before the first signs of puberty develop. The findings of Johanson *et al.* in sera suggested that a rise in LH levels might occur in the year preceding puberty in boys, but no significant change before that time was detectable. It appears from the present findings that an endocrine mechanism, reflected in increasing LH production is active long before the first signs and symptoms of puberty develop.

The high levels of urinary LH excreted in the first 6 months of life by boys and possibly girls, are very striking. These findings do not, however, appear consistent with the report of Kaplan, Grumbach, and Shepard (1969), who, though demonstrating high levels of LH and FSH in the sera of fetuses at the time of mid-gestation, were unable to detect either at term in specimens of cord serum. Schalch, Bryson, and Lee (1968a) in contrast reported high serum FSH levels in normal newborn children, but they did not comment how long these persisted. Our findings correlate with the marked Leydig cell activity that has been reported on histological examination of testes of newborn

me Abnormal Sexual Characteristics

Time of LH Estimation			Bone Age (yr.)	Chronological Age (yr.)	Oestrogen Effect	Pathological Cause
Characteristics	Centiles					
Vaginal Bleeding	Height	Weight				
	> 97	25	0·8 } 1·2 }	0·7 } 1·1 }		Associated with lipodystrophy
	75	> 97				?Early normal puberty; (testes undescended—normal at operation)
—	<3	<3	1·2	1·3	*NS	?Oversensitivity of target organs; ?premature thelarche
—		25	1·0	1·0	*NS	?Premature thelarche and adrenarche
—	25–50	50	1·2	1·2	—	?Premature thelarche
slight episodes since 2·5 yr.	10	25–50	3·5	2·8	+	?Idiopathic precocious puberty
t 4, 4·5 and 6 yr.; monthly afterwards	97	> 97	8·5	7·9	+	?Idiopathic precocious puberty
times in 6 weeks from 6·3 yr.	25	> 90	6·0	6·5	—	Unknown cause of bleeding
—	50–75	> 97	8	6·5	*NS	?Premature adrenarche
—	50	25	10–11	9·4	*NS	?Premature adrenarche then early normal puberty
—	> 97	97	12	9·0	*NS	?Premature adrenarche or early normal puberty
—	25	50	1·0	0·5		True hermaphrodite (confirmed by biopsy)

children (Schonfeld, 1943). However, these cells disappear after 4 to 6 weeks and have been attributed to maternal pituitary or chorionic gonadotrophin stimulation.

Reports based on bioassay studies of cases of precocious puberty have given varied results, as have also those depending on radioimmunoassay of serum LH. Schalch and Bryson (1967) reported no increase in serum LH in 3 girls with true precocious puberty, and Root *et al.* (1968) found high serum LH levels in only 2 out of 22 children with sexual precocity (of various kinds), and these 2 were due to intracranial lesions. Yet Kenny *et al.* (1969) showed raised plasma LH and FSH levels in

20 girls with idiopathic precocious puberty (but not in 2 when the cause was a granulosa cell tumour of the ovary). These workers found slight increases in LH and FSH also in 26 cases of premature thelarche, but somewhat lower than normal levels in 5 girls with premature adrenarche.

The present report suggests that cases of true precocious puberty (involving increased activity of gonads, adrenal and pituitary), whether these are neurogenic in origin or idiopathic, are usually associated with raised levels of urinary LH. When the full picture including gonadal activity is not found, there is usually no increase in LH output. Such children show features of premature sexual

TABLE III

LH Levels in Urine of Normal Prepubertal Boys

Age (yr.)	No. Tested	Urinary LH i.u. 2nd IRP/24 hr. ± 1 SD	Statistical Significance of Differences
0-0.5	15	3.1 ± 2.1	Compared with 0.5-6 yr. $p < 0.001$
0.5-1	5	0.9 ± 0.21	
1-2	3	0.8 ± 0.21	
2-3	3	1.07 ± 0.61	
3-4	9	1.00 ± 0.42	
4-5	22	1.13 ± 0.41	
5-6	16	1.03 ± 0.43	
0.5-6	58	1.04 ± 0.42	$p < 0.02$
6-7	15	1.37 ± 0.45	
7-8	19	1.58 ± 0.59	
8-9	15	2.05 ± 0.61	
7-9	34	1.80 ± 0.59	Compared with 6-7 yr. $p < 0.01$

development but lack evidence of spermatogenesis or oestrogen excess (as reflected by cornification of the vaginal mucosa), and often, as in premature thelarche, have no advancement of bone age or stature. These findings indicate the value of radioimmunoassay of LH levels in urine as a diagnostic aid in differentiating cases of precocious puberty and those with other advanced sexual characteristics.

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TABLE IV

LH Levels in Urine of Normal Prepubertal Girls

Age (yr.)	No. Tested	Urinary LH i.u. 2nd IRP/24 hr. ± 1 SD	Statistical Significance of Differences
0-0.5	3	1.7 ± 0.46	$p < 0.1$
0.5-1	2	1.0 ± 0.14	
1-2	4	1.05 ± 0.13	
2-3	4	0.9 ± 0.45	
3-4	4	1.05 ± 0.48	
4-5	12	1.12 ± 0.42	
5-6	15	1.17 ± 0.53	
0.5-6	41	1.10 ± 0.43	$p < 0.4$
6-7	16	1.36 ± 0.51	
7-8	10	1.52 ± 0.35	
8-9	8	1.85 ± 0.69	
6-8	26	1.42 ± 0.45	Compared with 0.5-6 yr. $p < 0.01$

(LH) and follicle-stimulating hormone (FSH) in normal children and various abnormal conditions. (Abstr.) *Journal of Pediatrics*, **72**, 565.

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