URINARY EXCRETION OF GONADOTROPHINS WITH PARTICULAR REFERENCE TO CHILDREN

BY

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Until recently there has been fairly general agreement that gonadotrophins are not detectable in the urine during childhood. Table 1 summarizes the results of previous investigators in this field. Of the early workers, only Catchpole and Greulich (1943) have, in serial determinations, detected gonadotrophins in young girls. More recently, studies performed on pools of urine have indicated that gonadotrophins are excreted as early as 3 to 5 years in boys (Knappe, Dörner, and Stahl, 1961); and possibly in girls (Carletti and Kehyayan, 1962a, b), though the latter workers used combined pools for boys and girls. Full experimental details have not been published to substantiate the claim of Minkina (1961) that gonadotrophin excretion in girls aged 1-3 years is very much higher than in girls at or near the menarche.

In 1960, Landau, Schwartz, and Soffer suggested that children's urine contained an inhibitor for gonadotrophins. They observed inhibition of the response when extracts of children's urine were combined with extracts of known gonadotrophic potency. A later report (Soffer, Futterweit, and Salvaneschi, 1961) substantiated these observations, particularly with regard to children under 6 years of age. Soffer, Salvaneschi, and Futterweit (1962) claimed to have detected a factor in all age-groups that inhibited the action of human chorionic gonadotrophin (HCG), but failed to inhibit the uterine response to oestrone.

In view of the paucity both of positive results in young children and of results in terms of standard reference preparations, it seemed of value to investigate again the excretion of gonadotrophins in childhood. This was an essential preliminary to assessing gonadotrophin excretion in pathological conditions.

The increase in uterine weight of the immature mouse is regarded as the most sensitive end-point for the routine bio-assay of total urinary gonadotrophins (Rosemberg, 1961). Though methods commonly

employed have involved multiple injections over a three-day period (Loraine and Brown, 1959), it was thought that the use of a single injection method such as that of Claringbold and Lamond (1957) might afford a more sensitive assay because a threshold dose would not be subdivided into sub-threshold doses. Since Lamond and Claringbold (1958) failed to demonstrate synergism between various gonadotrophins in an assay depending on the early response of the uterus after a single injection, it is likely that such an assay will more accurately reflect the total gonadotrophin present (follicle stimulating hormone (FSH) and luteinizing hormone (LH)). Brown and Billewicz (1962) showed that if enough time were allowed for growth and differentiation of the ovary (as in the usual three-day assay), the same uterine response was achieved by a wide variation in the total dose of gonadotrophin, using different proportions of FSH and LH. Thus extracts containing unknown proportions of FSH and LH could not be reliably assayed in an assay allowing extensive differentiation of the ovary, except at very low levels when the uterine response to FSH and LH was similar. Taking these facts into account, a more sensitive method for the assay of total urinary gonadotrophins has been developed.

In addition, luteinizing hormone (LH) has been estimated in children's urine using the ovarian ascorbic acid depletion assay, as modified by Schmidt-Elmendorff and Loraine (1962).

Material and Methods

Hormones Used in Assays. These are listed below.

- (1). Human menopausal gonadotrophin. International Reference Preparation (I.R.P.) (*Bull. Wld Hlth Org.*, 1960).
- (2). Human chorionic gonadotrophin (HCG). The preparation used was gonadotrophin LH (Paines and Byrne, Ltd.).
- (3). Human menopausal gonadotrophin (LAB-G). This preparation was extracted and purified from post-menopausal urine by the method of Loraine and Brown (1959). It was assayed in terms of I.R.P. using the modification of the mouse uterus method as described in this paper, and the following data were obtained.

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TABLE 1
GONADOTROPHINS IN THE URINE OF CHILDREN

Author	Response Used For Measurement	Urinary Gonadotrophins (units/24 hr.)	
		Boys	Girls
Schörcher (1931)	Ovarian hyperaemia in immature mouse	Undetectable in boys and girls up to 20 yr.	
Saethre (1933)	Ovarian hyperaemia in immature mouse		Undetectable in girls aged $11\frac{1}{2}$ to 14 yr.
Katzman and Doisy (1933, 1934)	Signs of oestrous in vagina and uterus	Undetectable in prepubertal boys and girls	
Catchpole, Greulich, and Sollenberger (1938)	Increase in mouse uterine weight	< 11 yr.: undetectable 12-16 yr.: 2-10 m.u.	
Nathanson, Towne, and Aub (1941)	Follicle growth in the immature mouse	< 13 yr.: undetectable	< 11 yr.: undetectable
Greulich, Dorfman, Catchpole, Solomon, and Culotta (1942)	Increase in mouse uterine weight	< 11 yr.: undetectable 13-14 yr.: 50% of cases gave a positive response 14 + yr.: 7-20 m.u.	
Catchpole and Greulich (1943)	Increase in mouse uterine weight		Detectable in serial determinations on girls aged $4\frac{1}{2}$ -14 yr.
Pedersen-Bjergaard and Tønnesen (1948a, b)	Changes in rat uterus	< 8 yr.: undetectable rising to 6 r.u. at 15 yr.	3-12 yr.: 1 or < 1 r.u. 12-16 yr.: rising to 12 r.u.
Albert (1956)	Changes in immature rat ovary	Undetectable in prepubertal boys and girls	
Schwenk and Ohndorf (1957)	Changes in mouse uterus	< 12½ yr.: undetectable	< 10 yr.: undetectable
Brown (1958)	Increase in mouse uterine weight	Pooled specimens 10-11 yr.: 2.9 mg. HMG 20A/l. 12-15 yr.: 3.5 mg. HMG 20A/l. Interstitial cell stimulating hormone: positive in 2 boys aged 6 and 9 yr. Undetectable at 10 yr. Interstitial cell stimulating hormone: undetectable in boys and girls of 7-9 yr. < 10½ yr.: undetectable	Pooled specimens 10-11 yr.: 8.2 mg. HMG 20A/l. 12-13 yr.: 7.3 mg. HMG 20A/l. 14-15 yr.: 14 mg. HMG 20A/l. Interstitial cell stimulating hormone: positive in 1 girl aged 7 yr. Undetectable at 9 yr. Undetectable at 10 yr.
McArthur, Ingersoll, and Worcester (1958)	Repair of the ventral prostate in hypophysectomized rats		
Johnsen (1959)	Changes in mouse uterus		
Rosemberg (1960)	Changes in mouse uterus		
Morato-Manaro, Cerviño, and Maggiolo (1960)	Mitosis count in the seminal vesicles of hypophysectomized rats		
Kehyayan (1960)	Changes in immature mouse and rat uteri		
Carletti, Kehyayan, and Perletti (1961)	Changes in immature mouse and rat uteri		
Carletti and Kehyayan (1962a, b)	Changes in rat uterus		
Knappe <i>et al.</i> (1961)	Changes in rat uterus		
Minkina (1961)	Changes in mouse uterus	Pooled 3 to 5 yr.: detectable	Activity in girls 1 to 3 yr., much higher than in girls 12 to 13 yr.

m.u. = mouse unit; r.u. = rat unit.

Relative potency: 1.00 mg. LAB-G = 0.77 mg. I.R.P.*Errors:* Fiducial limits 0.60 to 0.93 mg. I.R.P. ($p = 0.95$).Index of precision, $\lambda = 0.164$.*Validity test:* t test for parallelism, $t = 0.216$ (i.e. no significant deviation).*Index of significance of slope b :* $g = 0.083$ (i.e. significant).

(4). Luteinizing hormone (NIH-LH-S-1).*

Extraction Method. Extracts were prepared from complete 24-hour urine specimens (or multiples thereof) and from pooled random specimens of urine by the kaolin-acetone method of Albert (1956) as modified by

* Supplied by the Endocrinology Study Section, National Institutes of Health, Washington D.C., U.S.A., and prepared from sheep pituitary hormone.

Borth, Lunenfeld, and Menzi (1961), but using the adjustments of pH as described by Loraine and Brown (1959). Extracts were purified by salt-ethanol fractionation (stage 2, Albert, Kobi, Leiferman, and Derner, 1961).

For the assay of total urinary gonadotrophins and luteinizing hormone, the extracts were dissolved in 0.1 M borate buffer (pH 9) and 0.9% NaCl respectively, so that each mouse or rat received 0.25 or 0.2 ml., respectively.

0.1 M borate buffer: 100 ml. boric acid (6.18 g./l.) mixed with 42.6 ml. sodium hydroxide (4 g./l.).

Animals. Female mice, strain T/o, 19 to 22 days old and weighing 9 to 11 g., and female Sprague-Dawley rats aged 25 days and weighing 65 to 80 g. were obtained from Animal Suppliers (London) Ltd. They were maintained on a synthetic diet prepared from casein, lard, sucrose, salt mixture, and vitamins (H. Saunders, 1958, personal communication).

Statistical Evaluation of Results. Slope (b), relative potency, fiducial limits of error, index of precision (λ), index of significance of regression, and validity of parallelism were calculated according to Gaddum (1953a, b) and Borth, Diczfalussy, and Heinrichs (1957).

Genital and Secondary Sex Character Rating. This was assessed according to Tanner (1955), but for various reasons it was not possible to do this in every child.

Bio-assay of Total Urinary Gonadotrophins

Extracts were assayed by a modification of the mouse uterus test of Klinefelter, Albright, and Griswold (1943).

Each mouse received 0.1 i.u. HCG subcutaneously in 0.1 ml. 0.9% NaCl; 24 hours later the standard or extract was administered as a single injection, and the mice were killed by decapitation 40 to 44 hours later. The uteri were dissected out, placed in Bouin's fluid for 24 hours, cleaned, blotted dry, and weighed to the nearest 1 mg. on a torsion balance.

The design of the assays is described in the sections relating to the clinical investigations performed, dose-response curves for Standard being constructed using total dosages of LAB-G of 0.39 to 1.34 mg./mouse.

Development of Method.

(1). *Determination of the time of maximum response after a single injection of LAB-G:* in three separate investigations, a total of 68 mice were injected with 1.0 mg. LAB-G and killed in groups of four at intervals between 10 and 70 hours after injection.

The uterine responses obtained are shown in Fig. 1.

FIG. 1.—Determination of the time of maximum response after a single injection of LAB-G. Three separate investigations were performed. Each point = mean for 4 mice $\pm$ standard deviation. ----- = control uterine weight.

TABLE 2
UTERINE WEIGHTS 40 TO 44 HOURS AFTER
SINGLE INJECTION OF HCG

Dose per Mouse (i.u.)	Mean Uterine Weight (mg. $\pm$ S.E.M.)
0.10	3.8 $\pm$ 0.26
0.25	5.5 $\pm$ 1.07
0.50	6.0 $\pm$ 0.74
0.75	7.3 $\pm$ 0.71
1.0	9.7 $\pm$ 0.32
Saline	4.0 $\pm$ 0.32

The maximum response occurred at 40 hours, and in all subsequent experiments involving single injections the mice were killed 40 to 44 hours after injection of the material to be assayed.

(2). *Comparison of the size of the uterine response after single, as compared with multiple, injections:* 0.60 mg. LAB-G was injected into 2 groups of 8 mice. Group A received the total amount divided into 5 doses; 2 injections were given on both the first and second days, and one on the third day. The mice were killed 72 hours after the first injection. Group B received the total amount as a single injection, and the mice were killed 40 to 44 hours later. A control group of 5 mice, injected as in Group B, received saline only.

The mean uterine weights ($\pm$ standard errors of the means) were as follows. Group A: 4.7 $\pm$ 0.4 mg.; Group B: 7.6 $\pm$ 0.3 mg.; and saline controls: 4.4 $\pm$ 0.4 mg.

The uterine weights in Group B were significantly greater than those of Group A ($p < 0.005$).

(3). *Determination of a satisfactory priming dose of HCG:* mice, in groups of 5, were given single injections of HCG in doses of 0.10 to 1.0 i.u. per mouse; they were killed 40 to 44 hours later. The uterine weights are shown in Table 2. There was a significant increase ($p = 0.05$) in uterine weight with 0.50 but not 0.25 i.u. per mouse, as compared with the saline-injected controls. The threshold dose therefore lay between 0.25 and 0.50 i.u. per mouse.

(4). *Effect of the length of time between the priming dose of HCG and injection of the test substance:* groups of 5

FIG. 2.—Effect of the length of time between the priming dose of HCG and a single injection of LAB-G. All points are means for 5 mice $\pm$ standard deviation.

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TABLE 3
EFFECT OF SIZE OF PRIMING DOSE OF HCG

Dose of HCG (i.u./mouse)	Dose of LAB-G (mg./mouse)	Uterine Weight $\pm$ S.E.M.	
0.05	0.7	8.8 $\pm$ 1.3	Not significantly different ($p \approx 0.15$)
0.10	0.7	11.4 $\pm$ 1.6	
0.10	1.0	14.5 $\pm$ 1.9	Not significantly different ($p \approx 0.15$)
0.25	1.0	13.7 $\pm$ 1.5	

mice were primed with 0.10 i.u. HCG at intervals of 0 to 32 hours before a standard dose of 0.70 mg. LAB-G. The results are shown in Fig. 2. Maximum potentiation of response was achieved when the interval between HCG and LAB-G was 16 and 24 hours. In all subsequent assays, 24 hours was used as the priming interval.

(5). *Effect of the magnitude of the priming dose of HCG:* groups of 5 mice were primed with 0.05, 0.10, or 0.25 i.u. HCG, and 24 hours later 0.7 or 1.0 mg. LAB-G was injected. The results, given in Table 3, showed that variations in the size of the priming dose tested did not affect the uterine responses significantly. 0.10 i.u. HCG was selected as the priming dose in all subsequent experiments.

(6). *To study three different schedules of injection:* using 5 mice per group, LAB-G was injected in a total dosage of 0.2 to 1.0 mg. per mouse according to one of three schedules.

Schedule A. A single injection of LAB-G was given to mice pretreated 24 hours previously with 0.1 i.u. HCG. The mice were killed 40 to 44 hours after the last injection.

Schedule B. LAB-G was given in a single injection to untreated mice which were killed 40 to 44 hours later.

Schedule C. The total dose of LAB-G was divided into five, and administered to untreated mice. Two injections were given on both the first and second days, and one during the third day, and the mice were killed 72 hours after the first injection.

The results are given in Table 4, and recorded diagrammatically in Fig. 3, in which the logarithm of the total dose of LAB-G is plotted against the percentage increase in uterine weight, the control uterine weight being taken as 100%.

It was concluded that maximum sensitivity and the steepest dose-response line at low doses was achieved by using a single injection of LAB-G following pretreatment

FIG. 3.—The effect of three different schedules of injection. The logarithm of the total dose of LAB-G is plotted against the percentage increase in uterine weight, the control uterine weight being taken as 100%.

with HCG. Schedule (a) has been used in all subsequent assays of total urinary gonadotrophin.

(7). *Ovarian histology with HCG, LAB-G, and HCG + LAB-G:* mice were injected and killed in groups of 4 according to the schedule illustrated in Fig. 4.

On sacrifice, the ovaries were fixed in 10% (v/v) formalin in saline, sectioned, and stained with haematoxylin and eosin. Ovaries from mice injected with 0.1 i.u. HCG only, showed slight follicular ripening at all time intervals compared with those from saline-injected controls. A similar but more marked effect was obtained with LAB-G at 24 hours. At 48 hours after LAB-G, extensive follicular stimulation and luteinization was seen. No difference in ovarian histology was noted between the groups injected with LAB-G and killed after the same time interval, with and without pretreatment with HCG.

(8). *Accuracy:* children's urine, in which no total gonadotrophin activity could be detected, was pooled. 20.0 mg. LAB-G dissolved in 2.0 ml. 0.1 M borate buffer was added to each of three 1 litre aliquots. These samples and a further 1 litre to which no LAB-G had been added, were extracted and purified. The purified extract from each sample was dissolved in 2.5 ml. 0.1 M borate buffer

TABLE 4
UTERINE RESPONSES USING THREE DIFFERENT SCHEDULES FOR INJECTION

Total Dose LAB-G (mg./mouse)	Uterine Response (mg. $\pm$ S.E.M.) Schedule			Probability that Schedules Give Different Responses	
	A	B	C	A as compared with B	A as compared with C
0	4.0 $\pm$ 0.4				
0.3	6.6 $\pm$ 0.7	4.2 $\pm$ 0.2	3.8 $\pm$ 0.2	≈ 0.01	≈ 0.005
0.45	8.0 $\pm$ 0.5	5.0 $\pm$ 0.5	4.2 $\pm$ 0.8	≈ 0.002	≈ 0.005
0.67	11.0 $\pm$ 1.6	7.4 $\pm$ 0.5	6.6 $\pm$ 1.6	≈ 0.025	$\approx 0.025-0.05$
1.00	14.4 $\pm$ 2.0	9.8 $\pm$ 0.9	7.8 $\pm$ 1.2	≈ 0.025	≈ 0.01

FIG. 4.—The schedule for injecting and killing the mice. × = killed; → = injection.

and assayed against LAB-G using 10 mice per group and a (2 + 1) assay design.

No gonadotrophic activity was detected in the urine containing no added LAB-G. Recoveries of added LAB-G are shown in Table 5.

Four samples of LAB-G, each of 10.0 mg., were purified by the salt-ethanol fractionation procedure, and assayed against LAB-G itself using 5 mice per group and a (2 + 1) assay design. Results are shown in Table 5.

TABLE 5

Recovery (LAB-G in mg.)	95% Fiducial Limits	Percentage Recovery
<i>Recovery of LAB-G after extraction and purification (20.0 mg. LAB-G added to each litre of urine)</i>		
10.5	5.5-17.7	52.5
11.5	6.2-17.4	57.5
13.4	6.4-21.8	67.0
<i>Recovery of LAB-G after purification (10.0 mg. samples of LAB-G were purified)</i>		
7.1	2.9-12.5	71
8.1	3.3-14.9	81
8.5	4.2-14.3	85
10.5	4.5-18.1	105

In view of the number of steps involved and the errors inherent in the bio-assay, the recoveries were considered reasonably satisfactory.

(9). *Precision*: a pool of 4 litres of urine from normal men was divided into 4 aliquots which were extracted and purified. Each extract was assayed against LAB-G using a (2 + 1) assay design and 10 mice per group. The results, shown in Table 6, indicated satisfactory reproducibility.

The precision of the assay was satisfactory, Gaddum's λ being < 0.2 except on two occasions. Fiducial limits for assays are given in the relevant sections on clinical application of the method.

(10). *Toxicity of the purified extract*: no toxic effects were observed when purified extracts (equivalent to a 24-hour urine output) from children and adults were injected into individual mice. It was frequently possible to inject twice the equivalent of a 24-hour urine from a child into each mouse.

TABLE 6
REPRODUCIBILITY OF METHOD FOR ASSAYING
TOTAL URINARY GONADOTROPHINS

Urine Sample	Total Gonadotrophins in LAB-G (mg./l.)	Fiducial Limits of Assay ($p = 0.95$)	λ
1	3.56	1.47-6.25	0.139
2	3.35	1.36-6.08	0.151
3	3.92	1.89-6.57	0.135
4	4.04	1.71-7.19	0.151

Mean = $3.72 \pm \text{S.E.M. } 0.15$

(11). *Sensitivity*: over a period of two years, the mean control uterine weight of mice which received 0.1 i.u. HCG only, was consistently between 3.5 and 4.5 mg. Data with respect to three standard curves, selected at random from the results available, are shown in Table 7.

Bio-assay of Luteinizing Hormone in Urine

Luteinizing hormone in urine was assayed by the method of ovarian ascorbic acid depletion, as modified by Schmidt-Elmendorff and Loraine (1962) using NIH-LH as a reference preparation. Symmetrical four-point assays with two dose levels of standard (S) and unknown (U) respectively were used, the log dose interval being $\log_{10} 3$ (0.4771): 4 or 5 rats (8 or 10 ovaries) were used at each dose.

TABLE 7
DATA ON THREE STANDARD CURVES

Dose/mouse (mg. LAB-G)	Mean Uterine Response for Group of 5 Mice $\pm$ Standard Deviation		
	Curve 1	Curve 2	Curve 3
0.39	11.3 $\pm$ 2.1	10.6 $\pm$ 1.9	7.5 $\pm$ 2.9
0.59	17.1 $\pm$ 4.1	14.2 $\pm$ 3.8	10.5 $\pm$ 3.1
0.88	17.7 $\pm$ 6.2	17.9 $\pm$ 5.9	12.6 $\pm$ 2.9
1.34	27.1 $\pm$ 8.7	27.6 $\pm$ 7.0	20.6 $\pm$ 6.1
Saline	4.5 $\pm$ 1.0	5.7 $\pm$ 0.9	5.7 $\pm$ 1.2
b	28.24	30.75	24.34

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TABLE 8
ASSAYS OF TOTAL URINARY GONADOTROPHIN EXCRETION

Urine	Gonadotrophin Excretion per 24 hr. (mg. I.R.P.)	Fiducial Limits (p = 0.95)	λ	g	p
Adult male	6.1	4.5-8.1	0.12	0.0126	0.320
Adult female	6.7	4.5-9.9	0.13	0.4995	0.230
Post-menarchal girls (pooled urine, 14-15 yr.)	0.8	0.6-1.3	0.15	0.8190	0.138
Pre-menarchal girls (pooled urine, 10-10½ yr.)	0.3	0.2-0.5	0.20	0.1410	0.083
Pre-adolescent boy, 8½ yr.	0.5	0.3-0.9	0.23	0.1490	0.284

Clinical Investigations and Results

Four-point Assays for Total Gonadotrophins on Urine from Normal Subjects. Assays using a (2 + 2) design and 5 mice per group were performed on extracts of urine from healthy subjects, both adults and children. All the assays were statistically valid and results are shown in Table 8.

Excretion of Total Urinary Gonadotrophins in Normal Men and Women. Pools of urine comprising 2 to 6 complete 24-hour collections from each subject were assayed for gonadotrophic activity using a 3 (standard) + 1 (unknown) design with 5 mice per group.

In 12 healthy men aged 18 to 40 years, total urinary gonadotrophins ranged from 3.8 to 18.1 mg. I.R.P./24 hr. with a mean of 7.7. Eight healthy women aged 18 to 35 years were studied, and 24-hour urine collections were made at random during the cycle and pooled for each subject, no attempt being made to follow changes during individual menstrual cycles. The mean excretion of total urinary gonadotrophins was 4.9 mg. I.R.P./24 hr. with a range of 2.7 to 7.05.

Effect of Extracts from Urine of Children on Assay of Total Urinary Gonadotrophin. In order to determine whether children's urine extracted by the method described contained an inhibitor to gonadotrophic activity, extracts of urine were added

to a standard amount of 1.0 mg. LAB-G as shown in Table 9, and the mixture was assayed according to the usual procedure.

The uterine weights obtained are shown in Table 9. The extracts alone did not cause any increase in uterine weight. When combined with LAB-G they did not significantly alter the response to 1.0 mg. LAB-G. None of the mice showed evidence of toxicity in this experiment. There was no evidence to suggest that an inhibitor was present in extracts prepared from children's urine by the method already described.

Total Urinary Gonadotrophin Excretion in Children Without Endocrinological Abnormalities. The majority of assays were of 4 (standard) + 1 (unknown) design, using 5 mice at each dose level for the standard and 3 to 5 for the unknown, depending on the amount of extract available. The limit of sensitivity for each assay varied according to the amount of extract injected and the sensitivity of the mice. If the largest dose of extract injected failed to cause a positive response, the result was recorded as 'undetectable activity and less than x mg. I.R.P./24 hr.', the value of x varying with the sizes of the responses to the standard and the amount of extract injected. No toxic effects were observed when purified extracts equivalent to a 24-hour urine collection were injected into each mouse.

TABLE 9
EFFECT OF EXTRACTS FROM CHILDREN'S URINE ON TOTAL GONADOTROPHIC ACTIVITY

Test	Material Assayed		Number of Mice Tested	Mean Uterine Weight (mg. $\pm$ S.E.M.)	p
	LAB-G Added (mg.)	Urinary Extract			
1	1.0	Nil	10	10.3 $\pm$ 0.90	For (1) and (2) $\approx$ 0.3 For (1) and (3) $\approx$ 0.25 For (1) and (4) $\approx$ 0.4
2	1.0	From 24-hr. urine of 3-month-old boy	5	9.6 $\pm$ 1.47	
3	1.0	From 400 ml. pooled urine from girls 7-9 yr.	10	9.1 $\pm$ 1.18	
4	1.0	From 400 ml. pooled urine from boys 7-9 yr.	10	10.6 $\pm$ 0.98	
5	Nil	From 400 ml. pooled urine from girls 7-9 yr.	5	4.1 $\pm$ 0.34	
6	Nil	From 400 ml. pooled urine from boys 7-9 yr.	5	4.5 $\pm$ 0.41	
7	Nil	Nil	5	4.0 $\pm$ 0.35	

TABLE 10
EXCRETION OF TOTAL URINARY GONADOTROPHIN IN BOYS

Patient	Chronological Age (yr.)	Genital and Secondary Sex Character Rating		Total Urinary Gonadotrophin (mg. I.R.P./24 hr.)	Fiducial Limits (p = 0.95)
		Genital	Pubic Hair		
P.L.	3/12	1	1	< 0.3	
R.A.	6/12	1	1	< 0.3	
P.W.	9/12	1	1	< 0.92	0.60-1.42
R.W.	1 11/12	1	1	< 0.3	
P.W.	3 10/12	1	1	< 0.76	0.30-1.12
G.S.	4 6/12	1	1	< 0.7	
G.H.	5 2/12	—	—	< 0.5	
D.S.	5 2/12	1	1	< 0.3	
M.G.	5 6/12	—	—	< 0.59	0.25-0.87
L.B.	5 9/12	1	1	< 0.4	
S.B.	6	1	1	< 0.3	
M.P.	7	—	—	< 0.4	
M.J.	7	1	1	< 0.3	
M.Hu.	7	—	—	3.14	2.04-5.50
M.Hs.	7 3/12	1	1	< 0.04	
M.R.	7 3/12	1	1	< 0.7	
A.S.	7 4/12	1	1	< 0.5	
R.S.	7 9/12	1	1	0.76	0.49-1.18
A.W.	8 3/12	1	1	0.77	0.32-1.12
G.S.	8 5/12	1	1	0.80	0.58-1.25
A.M.	8 6/12	—	—	0.80	0.51-1.26
L.L.	8 6/12	1	1	0.94	0.71-1.16
G.M.	9	—	—	< 0.3	
R.D.	9 6/12	—	—	0.32	0.13-0.45
I.B.	9 7/12	—	—	< 0.5	
P.F.	9 9/12	3	1	< 0.4	
C.M.	9 11/12	1	1	< 0.3	
A.H.	10	—	—	0.43	0.19-0.87
I.W.	10 2/12	2	1	0.67	0.44-0.97
A.G.	11	—	—	< 0.5	
A.B.	11 2/12	2	1	< 0.4	
D.F.	11 9/12	—	—	< 0.4	
A.J.	12	—	—	6.72	4.43-9.65
R.D.	12 2/12	—	—	0.31	0.09-0.49
P.B.	12 2/12	—	—	2.90	2.14-4.53
A.M.	12 7/12	5	4	1.86	1.32-2.65
J.P.	13	—	—	< 0.4	
R.O.	13	—	—	4.23	2.32-7.52
K.B.	13	—	—	< 0.5	
J.B.	13 1/12	—	—	1.36	0.66-2.28
M.S.	13 6/12	4	5	1.05	0.68-1.62
S.B.	13 9/12	—	—	0.59	0.28-0.88
S.H.	14 6/12	—	—	1.02	0.77-1.34
J.S.	14 7/12	4	4	0.58	0.34-0.80
C.L.	14 8/12	—	—	0.72	0.52-1.12
P.D.	15	5	4	3.30	2.42-4.72

— indicates 'not determined'.

Assays were performed on urine from 46 boys and 58 girls aged 3 months to 15 years 8 months. The results are given in Tables 10 and 11, and are shown diagrammatically in Fig. 5.

Measurable activity was obtained as early as 9 months in a boy and 1 year 10 months in a girl.

Serial determinations were made on 2- or 3-day pools of complete 24-hour urines, as follows.

(a). 5 premenarchal girls (M.M., L.O., A.P., A.H., and Y.Y.) all of whom had pre-adolescent secondary sex character rating.

(b). 1 post-menarchal girl (M.D.) with breast development rating, 3; and pubic hair rating, 4.

(c). 1 pre-adolescent boy (A.W.).

(d). 1 adolescent boy (J.S.) with genital development rating, 4; and pubic hair rating, 4.

In Y.Y. (7 yr. 10 mth. of age) no gonadotrophin activity was detected throughout a 30-day period of study. Results for M.M., L.O., A.H., M.D., A.W., A.P., and J.S. are shown in Fig. 6.

Urinary Excretion of Luteinizing Hormone by Children without Endocrinological Abnormalities.

A series of assays was performed on pooled collections of urine from children of various age-groups. There were 6 to 8 children in each age-group, and the results are shown in Table 12. Toxic reactions were produced with urine from the higher age-groups, both boys and girls, even though the amount of extract injected was generally equivalent to less than 24-hour urine. In the lower age-groups the toxic effect (if any) was much less marked, even though the amount of extract injected into each rat exceeded the equivalent of 24-hour urine.

Assays were also performed on individual pools of 24-hour urines obtained from 12 children: 4 assays were invalid because of significant deviation from parallelism and 2 assays showed no depletion of ovarian ascorbic acid at the highest dose injected. Results of valid assays are shown in Table 12.

URINARY EXCRETION OF GONADOTROPHINS

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TABLE 11
EXCRETION OF TOTAL URINARY GONADOTROPHIN IN GIRLS

Patient	Age (yr.)	Secondary Sex Character Rating		Total Urinary Gonadotrophin (mg. I.R.P./24 hr.)	Fiducial Limits (p = 0.95)
		Breast Development	Pubic Hair		
M.M.	1 10/12	1	1	0.51	0.14-0.80
L.O.	2 2/12	1	1	0.36	0.17-0.69
L.H.	3 2/12	1	1	1.07	0.60-1.87
A.S.	3 2/12	1	1	< 0.3	
S.E.	3 4/12	1	1	< 0.47	0.21-0.81
C.E.	4	—	—	< 0.4	
C.O.	5 1/12	1	1	0.34	0.13-0.50
A.P.	5 3/12	1	1	0.38	0.18-0.64
A.A.	5 8/12	1	1	0.51	0.28-0.89
O.C.	5 11/12	1	1	0.43	0.20-0.72
S.O.	6 3/12	1	1	0.42	0.28-0.67
S.C.	6 5/12	1	1	0.5	
K.W.	6 7/12	1	1	>>> 0.3	
Y.Y.	6 11/12	1	1	>>> 0.3	
B.C.	7 1/12	1	1	>>> 0.5	
B.O.	7 2/12	1	1	>>> 0.45	0.26-0.62
L.N.	7 2/12	1	1	0.45	0.23-0.77
B.D.	7 6/12	1	1	0.37	0.14-0.55
S.R.	7 11/12	1	1	>> 0.3	
L.S.	8 2/12	1	1	>> 0.3	
I.C.	8 4/12	1	1	>> 0.59	0.62-0.85
C.B.	8 6/12	1	1	> 0.4	
M.W.	8 10/12	1	1	> 0.42	0.26-0.80
K.F.	9	—	—	> 0.4	
J.M.	9 3/12	—	—	> 0.44	0.23-0.78
M.O.	9 5/12	1	1	> 0.5	
A.H.	9 5/12	1	1	> 0.51	0.28-0.91
E.E.	9 5/12	1	1	0.76	0.50-1.17
P.B.	9 5/12	2	1	0.38	0.18-0.57
C.B.	9 7/12	1	1	0.29	0.16-0.52
T.S.	9 10/12	—	—	>> 0.3	
D.C.	10	—	—	>> 0.3	
Y.B.	10	—	—	>> 0.34	0.17-0.55
J.B.	10 1/12	—	—	0.59	0.43-0.73
M.A.	10 2/12	1	1	0.32	0.15-0.51
C.E.	10 3/12	1	1	< 0.5	
D.H.	10 4/12	1	1	< 0.51	0.34-0.91
J.G.	10 6/12	—	—	< 0.3	
P.B.	10 8/12	—	—	< 1.10	0.82-1.71
P.O.	10 8/12	1	1	< 0.4	
M.K.	10 9/12	3	4	1.03	0.66-1.56
S.B.	11 2/12	—	—	0.50	0.15-0.93
C.W.	11 10/12	—	—	0.76	0.56-1.08
L.W.	12	2	2	< 0.4	
C.P.	12 1/12	1	1	< 0.52	0.26-0.88
C.R.	12 2/12	2	3	< 0.5	
H.R.	12 3/12	—	—	< 0.52	0.34-0.75
P.W.	12 3/12	—	—	0.75	0.41-1.34
M.D.	12 5/12	3	4	1.24	0.55-2.54
S.I.	12 9/12	4	3	< 0.3	
A.L.	13	—	—	< 2.68	1.63-4.18
P.R.	13 4/12	—	—	>> 0.5	
J.T.	13 11/12	—	—	>> 0.5	
E.G.	14	4	3	>> 0.80	0.53-1.16
M.B.	14 4/12	4	4	0.85	0.40-1.27
C.W.	14 11/12	—	—	0.38	0.18-0.57
D.H.	15	—	—	0.98	0.73-1.52
B.S.	15 8/12	—	—	0.51	0.29-1.89

— indicates 'not determined'.

Discussion

The immature mouse uterus has been used as a reliable and sensitive end-point for the bio-assay of total urinary gonadotrophins. The extract has been administered as a single injection following pre-treatment of the mice with a sub-threshold dose of HCG.

From results published by other investigators (Borth *et al.*, 1961), it is clear that many strains of mice have a greater sensitivity to human menopausal gonadotrophin than the strain available for the

present study, so that the method described could have even greater sensitivity.

The mechanism of potentiation with HCG remains obscure. The fact that simultaneous administration of HCG and LAB-G did not show any synergistic interaction raises the possibility that this phenomenon is only demonstrable when HCG is administered before an injection containing follicle-stimulating hormone. This aspect appears to have been overlooked in most studies on synergism because assays extending over several days have been used (Evans and Simpson, 1950).

FIG. 5.—Total urinary gonadotrophin excretion in boys and girls.

● Total urinary gonadotrophin in mg. I.R.P./24 hours. ○ No gonadotrophin detected, and excretion is less than the value indicated.

The extraction and assay used in the present study gave reproducible results of reasonable accuracy. Recovery experiments showed results similar to those reported by Loraine and Brown (1959) and Heinrichs and Eulefeld (1960). This type of recovery can be criticized (Albert, 1956), because it is not certain that gonadotrophin added to raw urine or extracts will behave in the same way as it did initially in the original urine. The four-point assays with extracts of urine from pre-adolescent children and adults showed satisfactory similarity of response with the laboratory standard LAB-G which was obtained from post-menopausal urine. The levels found in men and women were similar to those reported by other investigators who used similar extraction procedures (Apostolakis, 1961; Brown, 1959a, b; Loraine and Bell, 1963).

Of 33 boys aged less than 12 years, measurable excretions of total urinary gonadotrophins were

found in 13 of them, using pools of 24-hour urines from single subjects. The youngest boy with detectable amounts was only 9 months. In older boys aged 12 to 15 years, total urinary gonadotrophin was measurable in 12 of the 14 investigated. Serial determinations in a pre-adolescent boy (A.W.) indicated intermittent and/or considerable fluctuation in excretion, whereas a fairly constant excretion was found in an older adolescent boy (J.S.). Certainly below the age of 12 years, no clinical significance can be attached to a failure to demonstrate gonadotrophin in the urine, though a positive result may be of value.

Similar results were found in the girls though levels were generally lower than in the boys. Detectable excretion was found in 75% of the girls in the 0-6 year age-group. The more frequent undetectable levels (compared with boys) in the older age-groups may be due to the greater fluctua-

FIG. 6.—Serial determinations of the excretion of total urinary gonadotrophin in 7 children. Results are in mg. I.R.P./24 hours.

The solid black area is total urinary gonadotrophin. The shaded area indicates no gonadotrophin, and excretion less than the value indicated.

tion in excretion as indicated by the serial determinations. Intermittent excretion was seen in M.M. aged 1 year and 10 months, and also in the post-menarchal subject studied (M.D., 12 years and 5 months). As with the boys, assays of total urinary gonadotrophin must be interpreted with these limitations in mind. In general, results for total

gonadotrophin excretion in children have not been related to a reference preparation. Brown (1958) studied children from 10 to 15 years and gave his results in mg. HMG 20/l. urine. He used pools composed of random samples of urine, so that his results cannot be interpreted in terms of individual 24-hour excretions. In contrast to the present results, he found more gonadotrophin in girls than in boys, his results for boys being similar to the ones reported in this study.

The ovarian ascorbic acid depletion assay of urinary extracts containing low levels of LH presented considerable difficulties. The large amount of extract that had to be injected produced toxic effects and resulted in death of some of the animals. The rather flat slope of the dose-response curve usually resulted in an unsatisfactory index of precision and wide fiducial limits. This is in accord with the results published by Schmidt-Elmendorff and Loraine (1962), though Bell, Mukerji, and Loraine (1964) have recently published a modification of the method overcoming all these difficulties.

In pooled urine from boys, measurable LH was found in all age-groups, the amount increasing with age. Similar results were seen in girls but the assay on the 0 to 6 year age-group was invalid. The results suggest that there may be a correlation between the onset of puberty and the excretion of LH, and this would be worth further investigation using the improved method of Bell *et al.* (1964).

No evidence suggesting a gonadotrophin inhibitor in children's urine has been found. The method of extraction and purification used in the present study is different from that of Soffer *et al.* (1961, 1962), and perhaps this explains the conflicting results.

The values, in terms of a reference preparation for gonadotrophin excretion in normal children, will enable the results found in pathological states, e.g. precocious puberty, to be better evaluated. Failure to demonstrate gonadotrophin in the urine of any particular child must be interpreted in the light of the findings in the normal child, and the other clinical and laboratory findings must be taken carefully into account.

Summary

The excretion of total urinary gonadotrophin was studied in endocrinologically normal boys and girls, and the results were expressed in terms of a standard reference preparation. Low amounts were frequently found in children's urine even before the age of puberty, the youngest boy being 9 months and girl 1 year and 10 months. The inability to detect gonadotrophin in any particular child must be interpreted with caution and in the light of the other clinical and laboratory findings. No inhibitor to

TABLE 12
ASSAY OF LUTEINIZING HORMONE IN
CHILDREN'S URINE

Patient	Age (yr.)	Excretion of Luteinizing Hormone (μ g. NIH- LH/24 hr.)	Fiducial Limits ($p = 0.95$)	λ
Boys:				
Pool	0-6	0.89	0.35-1.47	0.28
Pool	6-8	0.26	0.04-0.53	0.34
Pool	10-12	1.14	0.29-2.16	0.33
Pool	12-15	6.59	2.38-11.70	0.34
C.J.	2	1.68	0.52-2.92	0.25
A.B.	11	2.30	1.04-3.98	0.24
Girls:				
Pool	0-6	Invalid assay: significant deviation from parallelism		
Pool	6-8	0.25	0.06-0.52	0.25
Pool	10-12	1.50	0.54-2.55	0.31
Pool	12-15	2.46	0.95-4.35	0.34
M.M.	2	1.06	0.27-1.97	0.29
L.H.	3 2/12	<0.5	—	—
S.B.	11	1.98	1.11-3.56	0.21
Y.B.	12	2.09	1.14-3.10	0.25
A.L.	13 6/12	<0.5	—	—

urinary gonadotrophic activity could be demonstrated with the method of extraction and purification used in this study.

A method specific for luteinizing hormone was applied to pools of children's urine, and detectable amounts were present both before and after puberty. There was considerable variation in excretion among the few individuals studied separately.

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