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HUMAN URINARY GONADOTROPHINS

I. IN RELATION TO PUBERTY

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SUMMARY

Urinary gonadotrophins were assayed in terms of the reference substance HMG 20 A by methods believed to measure follicle stimulating hormone (FSH) directly and interstitial cell stimulating hormone (ICSH) indirectly.

In normal girls aged 10–11 and 14–15 years urinary FSH was equivalent to 26 and 14 mg HMG 20 A/l. Gonadotrophin from the older girls was qualitatively similar to HMG 20 A, but that from the younger girls contained relatively little ICSH. Relative deficiency of ICSH also occurred in some older girls with delayed puberty. Young girls with precocious isosexual development resembled older girls of the 14- to 15-year group by excreting gonadotrophins qualitatively like HMG 20A but in smaller amounts.

In normal boys the maximum urinary FSH was only 8·8 mg/l., P: and there was no evidence of relatively low concentrations of ICSH before normal puberty nor when it was abnormally delayed.

The physiological and statistical significance of the demonstrated qualitative differences in urinary gonadotrophins is discussed.

It is generally accepted that two gonadotrophins, defined as follicle stimulating hormone (FSH) and interstitial cell stimulating hormone (ICSH), can be separated from pituitary tissue. There are, however, differences of opinion about the existence of two hormones as separate entities in human urine. One view (for discussion see Loraine [1956]) is that urinary gonadotrophins are qualitatively constant, and the other is that FSH and ICSH vary independently in gonadotrophic extracts from urine. If there is qualitative constancy then different extracts should have constant relative potencies when assayed by various methods differing in their specificity for FSH and ICSH. If qualitative differences occur different methods of assay should sometimes give significantly different estimates of relative potency. The evidence for qualitative constancy is necessarily negative in that its supporters show no differences in the results of different assays. The evidence for qualitative differences consists in the positive demonstration of contrasting results by different assays. This evidence is accumulating steadily and has been obtained by comparing urinary gonadotrophins from different non-pregnant subjects [Brown, 1956*a*, 1957; Fletcher & Brown, 1957; Hamburger & Johnsen, 1957] or material prepared differently from human urine [Walter, 1956, 1957; Brown, 1956*b*; Butt & Ingram, 1957].

In the present series of investigations, urinary gonadotrophins from subjects in various physiological and pathological states are being assayed by two methods. One method is believed to measure FSH directly. The other measures the combined

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effect of FSH and ICSH so that by comparing the results of the two assays the content of ICSH in the extract can be inferred. Such investigations are necessary because much theorizing about human gonadotrophins has been based on the results of imprecise and non-specific assays. They also provide a more useful approach to the demonstration of qualitative differences in urinary gonadotrophins because these can be assessed in their physiological context. The present paper deals with urinary gonadotrophins in relation to normal and abnormal puberty.

MATERIALS AND METHODS

Normal subjects

Urine was collected from boys and girls aged 10–15 years who had either recovered from acute illnesses and were convalescent in hospital or were attending clinics. The specimens averaged < 110 ml. each and were pooled according to age-groups as they were collected.

The gonadotrophins were extracted and the dried extracts collected into six lots corresponding to age groups: 10–11, 12–13 and 14–15 years for each sex. When sufficient material had accumulated the extracts in each lot were dissolved and re-precipitated to ensure complete mixing. At least thirty children contributed to each of the six lots. In all cases only casual specimens of urine were collected and in each lot a similar proportion of the specimens came from the various sources of supply. It was hoped that the use of a large number of small specimens would give a more general picture of gonadotrophic activity than the use of larger specimens from fewer subjects. This seemed particularly important in the case of the older girls where various stages of the menstrual cycle might be represented.

Abnormal subjects

Urine was collected from six patients with precocious sexual development (Table 3). Three girls had isosexual development of the 'constitutional' type. Two others had heterosexual development and were typical examples of congenital adrenal hyperplasia with pseudohermaphroditism: the younger had never had treatment with cortisone and the elder none for 8 months. The boy had generalized neurofibromatosis and an intracranial lesion confirmed by a filling defect in the third ventricle shown radiologically.

Urine was also collected from individual patients with delayed sexual development and deficiency of secondary sexual characteristics (Table 4). Some children aged 13 years were also included because signs of sexual development might be expected at this age, although their absence would not necessarily be abnormal. Some of the patients with delayed development were also dwarfed or had associated general disease. Some had gonadal dysgenesis diagnosed clinically but their sex chromatin pattern was not examined: their gonadotrophins were assayed against the 'female standard' (see below).

Treatment of urine

Most of the abnormal subjects were in a metabolic ward and the urine was refrigerated directly after collection. Periods of collection ranged from 3 to 9 days and the several extracts from one subject were finally pooled. The urine from normal

subjects was refrigerated shortly after collection or preserved with chloroform if it had to be left for more than a few hours at room temperature.

Gonadotrophins were extracted by the modified kaolin-acetone method used in previous investigations [Brown, 1956*a*]. In this method elution from the kaolin is at pH 10·5 and further treatment of the extract with calcium phosphate is not used.

Methods of assay and standards

The gonadotrophins were assayed as 'total gonadotrophin' and as follicle stimulating hormone (FSH) by the methods previously described [Brown, 1955]. The specificity of the assay of FSH has been discussed elsewhere [Brown, 1956*c*], and the term 'FSH' is used bearing in mind that specificity can only be proved when human FSH has been isolated. The term 'total gonadotrophin' is used to imply that the combined action of FSH and ICSH is being measured. It is used because it is widely accepted in this sense, but it has the disadvantage of seeming to imply that results expressed as total gonadotrophin should equal the total amount of FSH and ICSH if assayed separately. This is not the case. If the ratio of FSH to ICSH is the same in the unknown sample as in the standard, the result given by both the assay for total gonadotrophin and for FSH will be identical when expressed in terms of the standard. If there is relatively more FSH than ICSH in the unknown the result will be relatively higher as FSH than as total gonadotrophin in terms of the standard: if the ratio of the hormones is reversed the assays will give the opposite result. In interpreting the results of the two assays used in the present investigation, the assay of FSH, being relatively specific, may be taken as giving a direct measure of FSH, and when it is compared with the assay for total gonadotrophin the relative concentration of ICSH may be inferred.

In the assays of total gonadotrophin from normal children and patients DF and K 86 the results were calculated from the graded (as opposed to quantal) response of the uterus. Calculation in this way has only become consistently possible with animals from this colony in the past 6 months [e.g. Butt, 1958]. Unless otherwise stated, at least two dose levels of both standard and unknown were used in each assay. Results were calculated by the methods of Gaddum [1953] and fiducial limits ($P = 0{\cdot}95$) are given in brackets. All results are expressed in terms of HMG 20A (Organon), the reference substance made for the Medical Research Council, which contains both FSH and ICSH. This material or one of two substandards was used in each assay. The substandard for the males was prepared by the kaolin-acetone method (see above) from pooled urine of normal adult men and that for the females from the urine of normal postmenopausal women. Details of standardization of these extracts are given in Table 1. The amount of HMG 20A required to double uterine weight in the mice used in this investigation is usually near 0·7 mg.

RESULTS

The assays of the substandards (Table 1) did not show qualitative differences between them and HMG 20A. All results were therefore expressed in terms of the latter, the same conversion factor being used for one substandard assayed by either method. Extracts from some of the patients were of low potency and there was sufficient only for 3-point assays or for upper limits to be set on potency because the highest doses

Table 1. *Details of assays of the two substandards*

Sub-standard	Statistical data* and potency relative to HMG 20A: Total gonadotrophin P_G	Total gonadotrophin P_H	Total gonadotrophin Potency	FSH P_G	FSH P_H	FSH Potency	Potency accepted for conversion
'Male'	0·4–0·5	0·3–0·4 0·6–0·7	0·033 (0·027–0·041)	0·8–0·9	—†	0·022 (0·012–0·048)	1/30
'Female'	0·3–0·4	0·4–0·5 0·6–0·7	0·50 (0·39–0·57)	0·3–0·4	0·5–0·6 0·5–0·6	0·42 (0·31–0·56)	1/2

* Notation of Gaddum [1953]: P_G tests parallelism; P_H tests linearity. † 2+2 assay (all others 3+3).

caused no response. With one exception, parallelism was satisfactory in all assays in which it could be tested with $P_G > 0{\cdot}1$ in the notation of Gaddum [1953]. In the sixteen pairs of assays with good parallelism there were six pairs in which the estimates by the two methods differed and the fiducial limits did not overlap, suggesting significant qualitative differences. In four of these six pairs there was a more than threefold difference in the two estimates of potency.

Normal children (Table 2; Fig. 1)

In the *girls* the potency of the urinary extracts was surprisingly high. In the 14- to 15-year group the extract was equally potent by both methods of assay and this implies that it was qualitatively similar to the standard. In the younger age-groups there was a progressive decline in the potency measured as total gonadotrophin compared with that measured as FSH: in the 10- to 11-year group there was a more than threefold difference in the estimates with widely separated fiducial limits. This implies that there was less ICSH in the urine of the younger children. The absolute quantity of FSH/l. urine was already high in the youngest age-group and it fell with increasing age. This may imply a greater output in the younger children or it may simply reflect different urinary volumes in children of different ages. The results show that FSH is excreted early and that ICSH is excreted in progressively greater proportion as the girls pass through the stages of puberty.

The results for the *boys* were very different. The general level of gonadotrophin was lower and there was no evidence of an increasing proportion of ICSH with increasing age. In the 10- to 11-year group the potency estimated by both assays was already the same, and in the older group the estimate as total gonadotrophin was relatively lower. In the 12- to 13-year group there was departure from parallelism

Table 2. *Potency of urinary extracts from normal children*

Sex	Age-group (years)	Potency of extract as mg HMG 20A/l. urine*: FSH	Potency of extract as mg HMG 20A/l. urine*: Total gonadotrophins	Ratio of potencies: total gonadotrophin/FSH
F.	10–11	26 (17–42)	8·2 (7·3–9·3)	0·32/1·0
F.	12–13	17 (12–25)	7·3 (6·4–8·8)	0·43/1·0
F.	14–15	14 (12–18)	14 (13–17)	1·0/1·0
M.	10–11	2·9 (2·0–4·4)	2·9 (2·0–4·3)	1·0/1·0
M.	12–13	6·8 (4·5–9·6)	Departure from parallelism	—
M.	14–15	8·8 (5·3–13·4)	3·5 (2·6–5·5)	0·40/1·0

* 95% Fiducial limits shown in parentheses.

($P_G < 0{\cdot}01$) in the assay of total gonadotrophin. The concentration of FSH in the boys' urine increased with increasing age, whereas that of the girls had fallen.

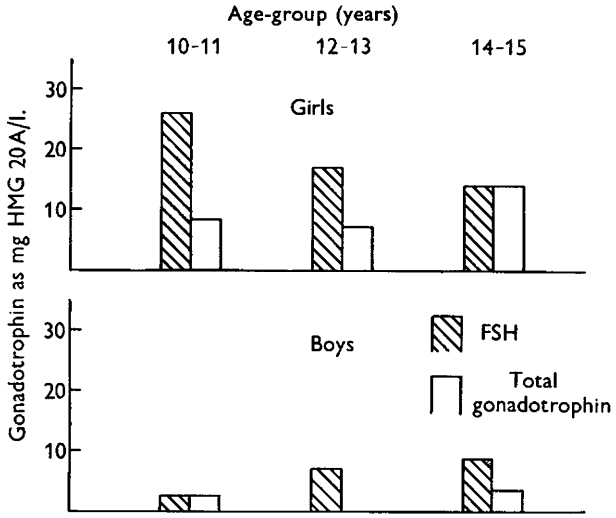

Fig. 1. Concentration of gonadotrophin in urine of normal children. (Fiducial limits are shown in Table 2.)

Precocious sexual development (Table 3)

In both the girls and the boy showing isosexual development the urinary gonadotrophins were low and the concentration in urine was lower than the levels for normal children near puberty. In interpreting these findings the small size and urinary volume of these patients must be considered. Another factor is that the symptoms were intermittent in some patients and urine may not have been collected during the active phases. In the girls there was no clear evidence of the relative preponderance of FSH found in normal children before puberty.

Table 3. *Data from subjects with precocious sexual development*

			Sexual development			Gonadotrophin excretion (as mg HMG 20A/day)	
Patient	Age (years)	Sex	Type	Symptoms	Duration (years)	FSH	Total gonadotrophin
B40	5	F.	Isosexual	Intermittent vaginal bleeding and breast enlargement	4½	1·8 (1·4–2·1)*	1·5 (0·4–3·3)*
W42	6	F.	Isosexual	Breast enlargement and development of body hair	1½	< 1·0	< 1·0
H14	8	F.	Isosexual	Intermittent vaginal bleeding	1½	1·5 (0·6–2·1)*	< 1·0
WC7	4	F.	Heterosexual	Pseudohermaphroditism	4	6·5 (5·0–8·0)	2·1 (1·4–2·7)
WC4	7	F.	Heterosexual	Pseudohermaphroditism	7	7·5 (5·0–10·0)	3·9 (3·4–4·7)
RC7	7	M.	Isosexual	Development of primary and secondary sexual characteristics	1	1·6 (0·5–3·5)*	2·5 (1·8–3·9)*

* 2+1 assay.

The urinary gonadotrophins of the two girls with adrenocortical hyperplasia were high and may well have been higher than those normal for their ages, but no figures for the latter are yet available. The ratio of estimates as total gonadotrophin and FSH was similar to that for normal prepubertal material.

Delayed sexual development (Table 4)

In the one girl (M38) with no apparent cause for delayed puberty the FSH excretion was high and was more than three times greater than the excretion estimated as total gonadotrophin. The urinary gonadotrophin of this 17-year-old girl was therefore qualitatively similar to that of children in the 10- to 11-year group, and a failure of ICSH secretion is a likely mechanism for delayed puberty in this patient. One of the girls (DF) of the three in whom bronchiectasis or steatorrhoea was present showed levels which would be normal during the menstrual cycle [to be published] and the ratio of the two estimates showed qualitative similarity to HMG 20A. In the other two girls (B54 and T77) there was relative deficiency of ICSH: in one of them this might still have been normal as the girl was only 13 years old.

Table 4. *Data from subjects with delayed sexual development*

Patient	Age (years)	Sex	Causal or associated condition recognized	Height (cm)	Gonadotrophin excretion as mg HMG 20A/day	
					FSH	Total gonadotrophin
M38	17	F.	None	157	275 (182–452)	87 (58–139)
B54	16	F.	Bronchiectasis	145	6·0 (2·5–12)*	< 1·5
T77	13	F.	Steatorrhoea	127	7·5 (5·5–9·5)	4·3 (3·2–7·3)
DF	19	F.	Steatorrhoea	128	34 (23–48)	38 (22–53)
GO3	31	(F.)	Gonadal dysgenesis	136	186 (150–286)	624 (482–1451)
P25	16	(F.)	Gonadal dysgenesis	141	322 (230–461)	403 (273–819)
GO4	14	(F.)	Gonadal dysgenesis	125	228 (166–315)	169 (119–259)
S63	18	(F.)	Gonadal dysgenesis	139	133 (99–176)	184 (169–200)
S87	18	M.	None	146	1·8 (1·5–2·0)	2·7 (1·9–3·5)
K86	15	M.	None	149	1·3 (0·7–1·7)	1·2 (0·7–2·3)
AQ8	13	M.	None	120	0·7 (0·4–1·6)	< 1·6

*2 + 1 assay.

In patients with gonadal dysgenesis the gonadotrophins were high and in three the FSH and ICSH were equally raised. In one patient (GO3) there was a relative deficiency of FSH with a more than threefold difference in the potencies estimated by the two assays with clear separation of the fiducial limits.

All the boys had delayed somatic as well as sexual development. Their gonadotrophin excretion was low and there was no evidence of any relative deficiency of ICSH.

DISCUSSION

The concentration of gonadotrophin in the urine of normal young children was higher than had been expected although pilot experiments had shown its presence, and Bahn, Lorenz, Bennett & Albert [1953] have found gonadotrophins in the pituitary gland of a 4-year-old child. The concentration of FSH in the children's urine is similar to concentrations in that of adults [to be published], and the concentration in females relative to that in males is similar. In comparing results

it is important that the extracts are prepared by identical methods as different ones may recover very different amounts of gonadotrophin [Butt, 1958]. The recovery by the method used in the present investigation will be discussed in a later paper.

The results of the assays have been accepted as further evidence of qualitative differences in urinary gonadotrophins because in some cases the two assays gave widely divergent estimates of potency without overlap of the fiducial limits. It may be argued, however, that these limits, being based on the errors within assays, underestimate the variation likely to occur between replicate assays. The findings of Sheps & Munson [1957] are relevant in discussing this possibility. They found that in a series of replicate assays of androgens, the intra-assay statistics underestimated the inter-assay variation only if chemically dissimilar steroids were compared or if ether was used as the vehicle for administration instead of ethanol, presumably because it caused greater experimental errors in dosage. In assays of parathyroid hormone they found that the intra-assay statistics gave fairly reliable predictions of the observed variations between replicates. No similar results for assays of gonadotrophins are known to be available, and the possibility of excessive inter-assay variation must be kept in mind. It would seem most likely to occur when the use of highly purified extracts causes relatively greater errors in weighing doses or when qualitatively dissimilar gonadotrophins are being compared. The error from this source would presumably be random in direction and unlikely to be important if the differences between assays occurred in a constant pattern. The qualitative differences found in the present investigation show some such constancy, with different patterns for the two sexes.

In normal girls before puberty the excretion of FSH is high and of ICSH is low, ICSH increasing as puberty progresses. This normal pattern is reflected in the results from abnormal subjects. Young girls with precocious isosexual development excrete gonadotrophins qualitatively like those of older normal girls; when precocious development is heterosexual, being caused by an adrenal abnormality, the gonadotrophins remain of the prepubertal type; and in some girls with delayed puberty there is persistence of the relative deficiency of ICSH found in normal prepubertal girls. There is, therefore, a constancy of pattern throughout the results from the girls, and the results from the boys are clearly contrasted. They show no evidence of a progressive increase in ICSH excretion as puberty progresses nor of a relative deficiency of ICSH when puberty is delayed.

The results from the girls support the concept of FSH being secreted before ICSH and of puberty being initiated by a rising level of the latter. The results from the boys do not support this concept and call for caution in interpreting the histology of the developing testis as evidence for it [Albert, Underdahl, Greene & Lorenz, 1953]. This leads to the problem of how far urinary excretion reflects either pituitary secretion or gonadal utilisation of the gonadotrophins. It is hoped that the extension of studies like the present one to patients with testicular abnormalities may help to solve this problem.

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REFERENCES

Albert, A., Underdahl, L. O., Greene, L. F. & Lorenz, N. [1953]. *Proc. Mayo Clin.* **28**, 409.
Bahn, R. C., Lorenz, N., Bennett, W. A. & Albert, A. [1953]. *Endocrinology*, **52**, 605.
Brown, P. S. [1955]. *J. Endocrin.* **13**, 59.
Brown, P. S. [1956*a*]. *J. Endocrin.* **14**, 129.
Brown, P. S. [1956*b*]. *Nature, Lond.*, **178**, 315.
Brown, P. S. [1956*c*]. *J. Endocrin.* **14**, 257.
Brown, P. S. [1957]. *J. clin. Endocrin.* **17**, 805.
Butt, W. R. [1958]. *J. Endocrin.* **17**, 143.
Butt, W. R. & Ingram, J. D. [1957]. *J. Endocrin.* **16**, ii.
Fletcher, R. F. & Brown, P. S. [1957]. *Clin. Sci.* **16**, 669.
Gaddum, J. H. [1953]. *J. Pharm., Lond.*, **6**, 345.
Hamburger, C. & Johnsen, S. G. [1957]. *Acta endocr., Copenhagen*, **26**, 1.
Loraine, J. A. [1956]. *Vitam. & Horm.* **14**, 305.
Sheps, M. C. & Munson, P. L. [1957]. *Biometrics*, **13**, 131.
Walter, K. [1956]. *J. Endocrin.* **13**, xxvi.
Walter, K. [1957]. *J. Endocrin.* **15**, 119.