THE DIAGNOSTIC VALUE OF URINARY GONADOTROPIC HORMONE ('FSH') ASSAYS *

B. M. BLOOMBERG, D. ALLDIS, R. JANKELOWITZ and I. WOLMER

Endocrine and Metabolic Unit, Department of Clinical Pathology, University of the Witwatersrand, Johannesburg

Gonadotropins or gonadotrophins are hormones producing specific stimulating effects on the gonads of both sexes. As far as is known at the present time, the *normal* production of gonadotropins is limited in man to two sites, viz. (*a*) the anterior pituitary gland and (*b*) the placenta. Gonadotropins are also produced by tumours of the placenta, ovary, testis and other organs, e.g. chorionepithelioma, hydatidiform mole, seminoma and teratoma. It is of interest that the production of gonadotropins by tumours of the pituitary gland has not been described.

The gonadotropins of the anterior pituitary gland consist essentially of 3 components, all of which are glycoproteins,[1] viz. (*a*) follicle-stimulating hormone (FSH) or gametokinetic hormone (GH), (*b*) luteinizing hormone (LH), which is similar to or identical with the interstitial cell-stimulating hormone (ICSH), and (*c*) luteotropic hormone (LTH), which is probably identical with the lactogenic hormone.

The scope of this paper is limited to a discussion of the clinical usefulness and application of the assay, in the urine of 50 males and non-pregnant females, of a gonadotropin presumably elaborated in the anterior pituitary gland. It is referred to as follicle-stimulating-hormone, or FSH, since the assumption is made in the assay method used in these experiments that FSH stimulates the production of oestrogens in the immature female mouse. The resultant increase in weight or size of the uterus is then used as the indicator of the FSH content of the extracts. This assumption rests on experimental work involving the use of impure gonadotropic extracts, and the demonstration that, as the Graafian follicles increase in size, the liquor folliculi which is formed contains oestrogens. However, using 'pure' FSH, Greep, van Dyke and Chow [2] reported in the hypophysectomized rat that the Graafian follicles increased in size, but did not secrete oestrogen as measured by the response of the uterus and vagina. The addition, however, of small quantities of luteinizing hormone (LH) immediately resulted in oestrin production.

Since the biological effect of FSH by definition is the stimulation of growth of Graafian follicles it would appear preferable to determine the extent of follicular growth and development as the index of FSH activity. However, here too difficulties arise. For example, contamination with other gonadotropins will augment the ovarian response to FSH,[3] and in the hypophysectomized rat the augmentation occurring at low doses shows first in enlargement of follicular size. Secondly, the crude nature of urinary extracts also introduces complications since they almost certainly contain a mixture of gonadotropins.

It is evident, therefore, that an assay procedure simple enough for clinical use would probably not be specific for follicle-stimulating hormone (FSH). The term 'urinary FSH assays' has, however, gained so wide an acceptance that it is convenient to retain this term for the present. The biological methods in present use are only roughly quantitative, owing to the inherent variability of animal response, particularly with urines of low FSH content, and the unknown degree of synergism between the gonadotropins extracted from the urine and the minute amounts present in the pituitary of the assay animal, even though this is immature. Howard *et al.*,[4] for example, feel that the minute amounts of LH necessary for the ovarian follicles to produce oestrogen may be already present in the pituitary of the infantile mouse used in the FSH assay. They do not, however, deny that LH is also present in at least small amounts in some, if not in all, of the urine extracts used.

EXPERIMENTAL

No claim is made for originality in the method described below but the procedure is described in some detail for the convenience of other clinical laboratories who may wish to use this technique. The extraction of the urine is based on Dekanski's modification of Scott's method,[5, 6] and in some of its details it follows the method in use at St. Thomas' Hospital, London. The method of bio-assay is essentially that carried out by Albright and his colleagues [7] at Boston, using the mouse uterine technique.

Principle. The gonadotropins are adsorbed on to kaolin at an

* A summary of this paper was presented at a meeting of the Transvaal Society of Pathologists held in Johannesburg on 11 March 1954.

acid pH, leaving some water-soluble toxic substances in the supernatant, which is discarded. The gonadotropins are then eluted from the kaolin by alkali. After neutralization the eluate is treated with cold acetone, which precipitates the protein gonadotropins, leaving steroids such as the sex hormones in the supernatant fluid, which is again discarded. The precipitate is dried, washed with ether, and dried until free of all traces of acetone. The dry brownish powder is dissolved in distilled water and suitable aliquots injected into mice. This method produces a relatively non-toxic extract and rarely causes death of the mice.

Details of Method. Collect a 24-hour specimen of urine without preservative. Measure the volume, and if less than 2 litres make up to 2 litres with water.

Extraction. Acidify with 20% HCl (1 part concentrated hydrochloric acid and 4 parts distilled water) to pH 4·0, using a pH paper of suitable range. Add 100 ml. of 20% aqueous kaolin (acid-washed) suspension per litre (of original volume) of urine. Allow to stand at room temperature for 1-2 hours, shaking at intervals. Leave overnight at approximately 4°C. Remove the greater part of the supernatant fluid with the suction pump and transfer the remainder with the kaolin sediment to 250 ml. centrifuge cups. Centrifuge for 15-20 minutes at approximately 2,000 r.p.m. and discard the supernatant fluid. Add to the kaolin deposit 50 ml. of N/10 NaOH per litre (of original volume) of urine. Grind up the sediment thoroughly in the NaOH solution with a glass rod, making sure that all lumps are completely broken up. Centrifuge again for 15-20 minutes at ± 2,000 r.p.m. Transfer supernatant to suitable container, e.g. a vaco-litre bottle, and adjust pH to 5.0 with 20% HCl. Measure the final volume and precipitate with 5 volumes of cold acetone. A flocculent precipitate should form almost immediately. Allow to stand for 4 hours, shaking at intervals. Remove part of the supernatant if possible as above and spin the remainder in a 250 ml. centrifuge cup for 10 minutes at 2,000 r.p.m. Decant supernatant carefully and discard. The precipitate is dried by blowing air gently over it for 30-60 minutes, or even longer, *until almost dry*. Approximately 10 ml. ether is then added to the precipitate and stirred for a few minutes, the ether decanted, and the drying process repeated. The ether wash is repeated if necessary *until all the acetone has been removed* and a dry powder remains. This powder is usually amorphous and brown-coloured.

As a routine, 8 immature female mice, approximately 20 days old and 6-8 g. in weight, are used for each assay. Two mice are used at assay levels of 6, 12, 24 and 48 units.

Bio-assay

Nine ml. of distilled water is added to the dried powder in the centrifuge bottle, stirred well with a glass rod, and allowed to stand overnight at approximately 4°C. Centrifuge, and decant the supernatant into a clean test-tube.

0·25 ml. of this supernatant is injected subcutaneously into the lower part of the back twice daily for 3 days into each of 2 mice. Each mouse thus receives 1·5 ml. of the total 9 ml. i.e. $\frac{1}{6}$ of the total extract. A positive response shown by the mice at this level means that 1 mouse unit is present in $\frac{1}{6}$ of the extract, and therefore 6 *mouse units in the total* 24-*hour specimen.*

Serial dilutions are prepared from 4 ml. of the above supernatant to give 1 : 1, 1 : 2 and 1 : 4 concentrations of the original extract. Positive results with these dilutions used in the above manner would then correspond respectively to 12, 24 *and* 48 *mouse units in the total* 24-*hour specimen.*

All the solutions are kept in the refrigerator at ± 4°C between injections. In case further dilutions may be required, 4 ml. of the last dilution are placed in the deep freeze or in the freezing unit of a refrigerator. If a positive result is found at the 48-unit level, this solution is diluted further to be tested at 96, 192, 384 and 768 unit levels. Where less than 6 units of urinary gonadotropins are found, a second 24-hour specimen may be extracted and tested at the 3-mouse-unit level by dissolving it in 4·5 ml. saline. This barely gives 3 ml. of supernatant and is usually just sufficient for injecting 2 mice.

The mice are killed by coal gas on the 4th day, and the uteri inspected. If required, the uteri are carefully dissected out, freed of connective tissue, gently pressed between filter papers to remove free fluid and then weighed. The uterine weight of a 7-g. mouse has usually been found to be just less than 6 mg. and always less than 7 mg.

We have not used the original Mouse Uterine Unit of Levin and Tyndale [8] but, following Klinefelter *et al.*,[7] *one Mouse Uterine Unit* is here taken to be present in the highest dilution of extract which, when injected twice daily for 3 days, produces, 72 hours after the first injection, an obvious enlargement of the uterus of the test animal. The uteri are weighed only when there is questionable enlargement. If the uterus weighs more than 7 mg. after the fluid has been expressed by pressure between layers of filter paper, it is considered to be enlarged.

We report 'FSH' in inverted commas, because it does not appear justifiable to assume at the present time that pure FSH, which stimulates follicle growth, is also responsible for the oestrogen production necessary to stimulate uterine growth.

The normal ranges reported for the various bio-assay methods used vary considerably. Evans and Simpson's review [3] should be consulted by those particularly interested in this aspect of the subject. Although we have not as yet examined an extensive series of normal subjects, the normal range for the method as described above in adult males and females appears to be 6-24 units. In children until the onset of puberty, gonadotropins are below 6 M.U. In view of the ranges quoted in the literature, it may be preferable at the present time to extend the extreme upper range for normal adults to 48 units.

RESULTS AND DISCUSSION

The results in 50 patients in whom 'FSH' assays were undertaken are shown in the following tables. The cases are classified according to the presenting clinical problem or most obvious endocrinopathic state. This method of presentation was selected so as to illustrate the circumstances in which 'FSH' assays may have considerable differential diagnostic value:

(1) Suspected hypopituitarism (Table I).
(2) Delayed adolescence (Table II).
(3) Amenorrhoea (Table III).
(4) Hypogonadism in males (Table IV).
(5) Sexual precocity (Table V).
(6) Hirsutism and/or virilism (Table VI).
(7) Sterility (Table VII).

(1) *Hypopituitarism* (Table I)

It has already been stated that no tumour of the anterior pituitary gland is known to produce excessive quantities of gonadotropin, nor has hyperpituitarism with hypersecretion of gonadotropins been described. In clinical states, however, in which *hypopituitarism* is suspected, FSH assays are of great importance, since it

TABLE I. SIX CASES OF SUSPECTED HYPOPITUITARISM

Case	*Initials*	*Age*	*Sex*	*Diagnosis*	*Urinary FSH (Mouse Uterine Units per 24 hours)**
1	E.S.	40	F	Pituitary myxoedema	− 6
2	J.O.	23	F	Sheehan's syndrome	− 3
3	P.	45	F	Calcified tumour of pituitary	− 6
4	A.A.	21	F	Early anorexia nervosa	+24 −48
5	H.P.	20	F	Anorexia nervosa with secondary hypopituitarism	− 6
6	I.J.	8	F	Dwarfism (? primordial, ? pituitary)	− 6

* In reporting results of FSH assays the highest level showing a positive response is preceded by a plus sign and the lowest level giving a negative response is preceded by a minus sign. If results were negative at all levels, the figure shown is the lowest at which bio-assay was performed.

is the most common of the 'tropic' hormones to fail. Such deficiency may be 'selective' or may be accompanied by evidence of deficient thyrotropic hormone (as in case 1) and deficient adrenocorticotropic hormone (ACTH) production, i.e. panhypopituitarism—e.g. Sheehan's syndrome (case 2) and Simmonds' disease.

In case 1, myxoedematous since childhood, the possibility of the myxoedema being pituitary in origin was considered on clinical and biochemical grounds. Evidence in favour of the hypothyroidism being due to hypopituitarism was an FSH excretion below the normal level since this is usually normal in hypothyroidism.[9] Case 2 was a typical example of Sheehan's syndrome [10]—post-partum necrosis of the pituitary gland. In this patient, where destruction of the anterior pituitary gland appeared fairly certain from the clinical features and history, FSH excretion could not be demonstrated even at the 3-unit level. With an organic lesion involving the pituitary gland, however, FSH excretion is again low. In case 3, where a calcified tumour of the pituitary was found at operation, no FSH could be demonstrated in the urine.

FSH assays are also of crucial importance in the differentiation of cases of anorexia nervosa (cases 4 and 5) from organic lesions producing hypopituitarism. In early cases FSH may be within normal limits (case 4), whereas in more advanced cases (case 5), evidence of secondary hypopituitarism is present, and the prognosis is correspondingly more serious. Since the approach to therapy in anorexia nervosa and hypopituitarism is fundamentally different, the importance of differentiating these two states is obvious.

Wilkins,[11] in his classification of the various causes of dwarfism, lists the types of dwarfism due to endocrine disturbances and genetic causes, and goes on to say that 'the distinction between patients with stunted growth and delayed adolescence, and those with pituitary deficiency or with genetic dwarfism is often exceedingly difficult to make during childhood'. No attempt can be made here to discuss the investigation of a case of dwarfism as such but, where it is impossible to determine the cause of the stunted growth, the observation of the pattern of sexual development in early adolescence will throw light on whether there is a genetic defect, pituitary deficiency or delayed adolescence. In primordial or genetic dwarfism, other than in the special type associated with ovarian agenesis (Turner's syndrome), in which very high titres of FSH are found (Case 28—Table III), sexual maturation is normal and FSH levels are normal. The pituitary dwarf on the other hand remains sexually infantile, and FSH is not demonstrable in the urine. In Case 6, the absence of FSH in the urine is to be expected in view of the age of the patient. Assay of FSH towards the time of puberty may, however, distinguish between primordial or genetic dwarfism and hypopituitarism in this case.

(2) *Delayed Adolescence and Sexual Infantilism* (Table II)

Although, by the method of bio-assay described here, FSH excretion cannot be demonstrated until puberty, there are nevertheless 3 clinical problems in childhood where information regarding the secretion of gonadotropins by the anterior pituitary gland may be sought. The 1st, which is the differential diagnosis of dwarfism in childhood, has already been discussed. The 2nd is the investigation of sexual infantilism persisting into adolescence (Table II). The 3rd problem is the differentiation of cases of sexual precocity (Table V). The first 2 problems may, and commonly do, overlap.

Sexual infantilism persisting into adolescence poses in the first place the problem of determining whether this is due to delayed puberty, in which case normal sexual maturation will eventually occur even as late as 17-18 years of age, or whether there is an organic disorder in the hypothalamus, the pituitary gland or the gonads. Wilkins [12] clearly indicates the value of FSH assays in these cases.

In the early 'teens the demonstration of an FSH excretion of 6-12 units would lead one to expect normal sexual maturation, and therefore to advise that treatment with gonadotropin preparations or sex hormones should be withheld. An absence of FSH excretion should be interpreted with caution until 16-17 years of age, after which time it can be considered to indicate sexual infantilism due to pituitary or hypothalamic disorders.

In cases 7, 8, 9 and 10 the presenting problem was to differentiate a delayed onset of adolescence from a more severe organic endocrinological disorder. Case 7 was tall and thin, with eunuchoid proportions and no secondary sex development. Case 8 was obese but again showed no secondary sex development. In neither case could urinary FSH be demonstrated. Case 9 also showed a delayed onset of puberty with an absence of demonstrable FSH in the urine but, since this patient was of short stature, the question arose as to whether there was also a lack of pituitary growth factor. In none of these cases could the available evidence exclude a 'delayed' adolescence. These cases were therefore advised to return for reassessment and a further FSH assay after one year. During this period hormonal therapy should be

TABLE II. EIGHT CASES IN WHICH THE DIFFERENTIAL DIAGNOSIS LAY BETWEEN DELAYED ADOLESCENCE AND SEXUAL INFANTILISM

Case	*Initials*	*Age*	*Sex*	*Diagnosis*	*Urinary FSH (Mouse Uterine Units per 24 hours)*
7	A.G.	14	M	? Delayed adolescence	− 6
8	I.A.	13	M	? Delayed adolescence	− 6
9	B.H.	14	F	Delayed adolescence +dwarfism (? hypopituitarism)	− 6
10	S.T.	16	F	? delayed adolescence	− 6
11	M.F.	16	M	Sexual infantilism, ? hypopituitarism	− 6
12	H.S.	16	M	Sexual infantilism, ? hypopituitarism (cyst of pituitary excised in childhood)	± 6 −12
13	S.	13	M	Obesity with ? small genitalia, ?? Froehlich's syndrome	± 6 −12
14	F.C.	12	M	Sexual infantilism due to primary testicular deficiency	+12 −24

withheld. Testicular biopsy, or a study of vaginal cytology, may also be necessary before a final diagnosis can be made.

Case 10 offered an interesting variant of this problem. This patient was a girl aged 16 years with primary amenorrhoea. Breast development and pubic hair growth, however, appeared to be normal. Primary ovarian failure (see section 3) was considered unlikely in view of the lack of demonstrable FSH in the urine. The presence of mammary growth suggested that some oestrogen was being produced by the ovaries, which were as yet perhaps being inadequately stimulated by gonadotropins, and a study of the vaginal cytology for evidence of oestrogen stimulation was therefore suggested but the patient's co-operation could not be obtained. Here again it would appear preferable to keep this type of case under observation for some while before having recourse to oestrogen therapy, which could conceivably still further depress endogenous ovarian function.

The remaining cases in Table II provide features contrasting with the cases already discussed. Cases 11 and 12, both 16 years of age, provided good clinical evidence of hypopituitarism with sexual infantilism—very small testes and no secondary sex characters, dwarfism, and general immaturity of appearance. In case 12 a cyst of the pituitary had been removed several years previously, and testicular biopsy now showed complete absence of spermatic tubules and interstitial cells. Since there was obviously no reasonable expectation of such testes ever producing androgens or spermatozoa, replacement therapy with testosterone was commenced, with excellent results, not only in the development of secondary sex characters but also in general well-being, initiative, and drive. Case 13, which should be contrasted with Cases 7 and 8, was an obese boy of 13 years whose genitalia were buried in the excess of fat around the pubis, and appeared small for his age. He serves as an example of a common clinical problem, viz. the fat boy who at puberty does not appear to be maturing normally and in whom the possibility of Froehlich's syndrome is inevitably raised. It should be remembered that Froehlich's syndrome is rare, and that fat boys usually mature normally, although puberty may be delayed. The differential diagnosis was made by FSH assay and, since this was normal, Froehlich's syndrome was excluded. Case 14 is included as providing a very interesting early example of the Klinefelter-Heller syndrome.[13, 14] Clinically this case appeared to be very similar to Case 13—a boy aged 12 years, stunted in growth, with small testes and no secondary sex characters. The *high FSH excretion for age*, however, excludes hypopituitarism and also excludes delayed adolescence. With this amount of FSH the testes should have shown normal pubertal development. The small testes were therefore taken to indicate a primary testicular deficiency or failure. In this case a testicular biopsy was unfortunately refused, but the diagnosis was confirmed by a further FSH assay 1 year later (when aged 13 years) which showed an increase in the excretion to 48 units. In this patient, therefore, replacement therapy with testosterone was advised, as endogenous production of testosterone could not be expected.

(3) *Amenorrhoea* (Table III)

We turn now to a discussion of 17 cases of amenorrhoea in which FSH studies were carried out. The differential diagnosis of amenorrhoea (or oligomenorrhoea) will not be considered here; but from the endocrinologist's point of view it is of interest and sometimes of importance to determine the level of the functional or organic disturbance in the endocrine system which leads to amenorrhoea. As far as is known today, it is probable that depressed ovarian function must be present in all cases (other than pregnancy or pathological over-production of oestrogens leading to endometrial hyperplasia and temporary suppression of menses). With a primary ovarian deficiency the pituitary-ovarian balance is disturbed, leading in many cases to excessive production of FSH. Where the ovaries fail to develop, e.g. in Turner's syndrome,[15] the level of FSH excretion may rise considerably (case 15). After oestrogen therapy this level drops rapidly (case 16). A follow-up of both these cases after a few months on oestrogen therapy showed a decrease in FSH to 6-12 units. Similarly an increase of FSH excretion occurs at or after the menopause, this again suggesting a primary ovarian failure (cases 17 and 18). In cases of premature onset of the menopause (cases 19 and 20) the FSH excretion rises to the upper limits of normal or beyond.

Cases of primary ovarian deficiency may occur without the other stigmata of Turner's syndrome, and such patients may present with the clinical features associated with eunuchoidism. The differentiation from eunuchoid-

TABLE III. SEVENTEEN CASES OF AMENORRHOEA IN WHICH THE DIFFERENTIAL DIAGNOSIS LAY BETWEEN A GONADOTROPIN DEFICIENCY, AN OVARIAN DEFICIENCY AND A NON-ENDOCRINOPATHIC ETIOLOGY

Case	*Initials*	*Age*	*Sex*	*Diagnosis*	*Urinary FSH (Mouse Uterine Units per 24 hours)*	
15	M.V.	19	F	Ovarian agenesis (Turner's syndrome)	+192	−384
16	C.M.	30	F	Ovarian agenesis (Turner's syndrome) (Previous therapy with oestrogens)	+48	−96
17	J.B.	59	F	Post-menopausal (normal control)	+96	−192
18	E.B.	65	F	Post-menopausal (normal control)	+24	−48
19	T.L.	36	F	Premature menopause	+48	−96
20	J.N.	29	F	Premature menopause	+48	−96
21	R.	35	F	Eunuchoidism due to primary ovarian deficiency	+192	−384
22	S.G.	32	F	Eunuchoidism due to specific gonadotropin deficiency		−12
23	D.E.	39	F	Chronic anxiety state	+ 6	−12
24	J.C.	30	F	Anxiety state		− 6
25	J.C.	26	F	Obesity of hypothalamic origin		−12
26	A.D.	18	F	Thyrotoxicosis		− 6
27	S.S.	39	F	Thyrotoxicosis	+24	−48
28	C.G.	30	F	? Cushing's syndrome	+24	−48
29	N.	±50	F	? Cushing's syndrome		− 6
30	M.M.	38	F	Obesity (? Cushing's syndrome)	+12	−24
31	W.B.	17	F	Obesity	+ 6	−12

ism due to hypopituitarism can then be made on the basis of a high FSH excretion. Two cases of this type are included in this series. Case 21 is an example of eunuchoidism due to primary ovarian deficiency (in view of the high FSH excretion), whereas case 22 may be an example of a primary gonadotropin deficiency,[16] since no FSH excretion could be shown at the lowest level of FSH which could be tested (more concentrated extracts being toxic to the test animals).

Klinefelter *et al.*[7] drew attention to these differences very clearly in 1943, and concluded their paper with the statement 'It would appear that with the tests for excretion levels of follicle-stimulating hormone, one can divide cases of hypo-estrinism into 3 categories: (*a*) ovarian hypoestrinism due to primary ovarian insufficiency and associated with increased excretion of follicle-stimulating hormone, (*b*) pituitary hypoestrinism due to primary lack of production of follicle-stimulating hormone and associated with decreased excretion of it, and (*c*) hypothalamic hypoestrinism due to disturbance in the hypothalamic-pituitary nervous pathway and associated with a normal excretion level of follicle-stimulating hormone. These authors suggest that lack of production of oestrogen with a normal excretion of FSH might be due to lack of production of LH by the anterior pituitary gland. They argue that by analogy with the conditions obtaining in other mammals such lack may be due to the failure of the hypothalamic-pituitary nervous pathways to release LH from the anterior pituitary. The complexity of the hypothalamic-pituitary pathways is still far from being clearly understood, but an awareness of such a third group is useful for the inclusion of cases of amenorrhoea or oligomenorrhoea which appear to be 'psychogenic' in type. Case 23 and cases 43-45 (Table VII) possibly fall into this group. In case 24 (psychogenic amenorrhoea) the pituitary depression has led apparently to a decrease in FSH as well as LH production. In case 25, where a diagnosis of hypothalamic obesity with amenorrhoea was made, FSH excretion was again low.

In other endocrine disorders and in simple obesity (cases 26-31) the results of the FSH assays are of no assistance in diagnosis. Escamilla [9] also reported normal FSH values in such conditions as thyrotoxicosis and Cushing's syndrome, but no mention is made whether amenorrhoea was present. In such cases the pathogenesis of the amenorrhoea has not, to our knowledge, been clearly established.

(4) *Hypogonadism in Males* (Table IV)

Klinefelter *et al.*[13] in 1942 described a hypogonadal syndrome with gynaecomastia, and an increased excretion of FSH. Heller and Nelson [14] in 1945 extended this work to include cases of hypogonadism without gynaecomastia.

These pioneering studies have been followed by many others which have considerably clarified the relationships between hypogonadism, bodily habitus, hormonal assays and testicular biopsy examinations. Sohval [17] offers a working classification in which cases of hypogonadism are grouped according to whether they originate in the testes or elsewhere, and whether they begin before or after the completion of puberty. In primary testicular failure FSH excretion is increased, whereas in hypogonadism due to extragenital causes FSH is low or absent. The level of FSH excretion therefore helps in determining the etiology of the hypogonadism and is a valuable pointer to treatment. Where the deficiency is shown to be primarily testicular (high FSH excretion) as in cases 32 and 33, substitution therapy with testosterone is indicated. Stimulation therapy with gonadotropins should however be tried where a low or absent FSH excretion suggests that testicular deficiency may be secondary to a lack of normally-produced gonadotropins.

TABLE IV. TWO CASES OF HYPOGONADISM IN MALES IN WHICH THE DIFFERENTIAL DIAGNOSIS LAY BETWEEN A PRIMARY GONADOTROPIN DEFICIENCY AND A PRIMARY TESTICULAR DEFICIENCY

Case	*Initials*	*Age*	*Sex*	*Diagnosis*	*Urinary FSH (Mouse Uterine Units per 24 hours)*
32	B.L.	25	M	Eunuchoidism due to primary testicular deficiency (Klinefelter-Heller syndrome)	+192 −384
33	F.K.	36	M	Primary testicular deficiency (Klinefelter-Heller syndrome)	+384 −768

(5) *Sexual Precocity* (Table V)

The value of FSH assays in the study of cases of sexual precocity is considered with reference to 4 cases. True or constitutional (usually idiopathic) precocity apparently results from pituitary gonadotropic stimulation of the gonads, but no tumour or other lesion in the pituitary gland has ever been found. In a small minority of cases, however, organic lesions in the region of the pineal gland and hypothalamus have been demonstrated. Since true precocity is due to secretion of pituitary gonadotropins, the presence of FSH in the urine establishes the diagnosis (case 34). In normal

TABLE V. FOUR CASES OF SEXUAL PRECOCITY

Case	*Initials*	*Age*	*Sex*	*Diagnosis*	*Urinary FSH (Mouse Uterine Units per 24 hours)*
34	K.T.	4	M	Isosexual precocity (constitutional or idiopathic)	+24 −48
35	E.R.	6	M	Isosexual precocity (suprarenal hyperplasia)	− 6
36	H.R.	9	F	Heterosexual precocity due to adrenogenital virilism (suprarenal hyperplasia)	− 6
37	E.G.	8	F	Isosexual precocity (pituitary tumour removed 4 years previous to test), hypothalamic obesity, diabetes insipidus	− 6

childhood FSH is not demonstrable in the urine. The diagnosis in case 34, a child aged 4 years, was confirmed by testicular biopsy, which showed active spermatogenesis and the picture of an early pubertal testis. In case 35, a boy aged 6 years (to be contrasted with case 34), the patient also showed isosexual precocity, but that this was a pseudo-precocious puberty was established by the absence of demonstrable FSH in the urine (less than 6 units), and confirmed by testicular biopsy, which showed a pre-pubertal testis approximately normal for the age of the patient, and by a high neutral 17-ketosteroid excretion for age (10-11 mg.) The latter finding almost certainly indicates a suprarenal hyperplasia or tumour, provided that an intestitial-cell tumour of the testis can be excluded. Case 36, a sister of case 35, also showed precocious sexual development, apparently from birth, but the chief manifestations were heterosexual in type. The final diagnosis in this case was suprarenal hyperplasia (neutral 17-ketosteroid excretion 17-18 mg. per 24 hours). Here again, since the origin of the 'sex hormones' was the suprarenal gland and not the gonad, absence of FSH in the urine could be anticipated. Case 37 is of considerable interest—isosexual precocity in a little girl aged 8 years, who 4 years previously had a pituitary tumour removed. Since the patient showed marked obesity, polyphagia, and diabetes insipidus, it appeared probable that a lesion (? traumatic) of the hypothalamus was responsible for the precocity. However, in this case the absence of urinary FSH led to an alternative possibility being considered, viz. the presence of gonadotropins in the whole-pituitary powder (administered by nasal insufflation) which was used to control the diabetes insipidus, since the daily dose of this powder was found to contain approximately 48 mouse units.

(6) *Hirsutism and/or Virilism* (Table VI)

Case 38, a case of female pseudohermaphroditism due to suprarenal hyperactivity (no tumour was demonstrable at operation) is included to show again that increased FSH excretion does not accompany suprarenal virilizing lesions and that the androgenic hormones produced in the suprarenal gland may in fact suppress gonadotropin production by the pituitary gland. Escamilla,[9] however, reported the presence of FSH in many of his cases with an actual slight increase in about half. Cases 39 and 40 were diagnosed clinically as cases of the hyperthecosis syndrome (Culiner and Shippel [18]) or Stein-Leventhal syndrome.[19] This was proved by ovarian biopsies, and both showed levels of FSH excretion at the upper limits of reported normals, and somewhat greater than the normal values usually found by us. The significance of this is not clear, but the increased FSH production may be associated with the enlarged cystic follicles sometimes found in the ovaries of these cases, rather than with the marked hyperplasia of theca-cell elements which is a feature of this syndrome (Shippel [20]). Escamilla,[9] however, reported normal FSH in 3 cases and an elevated FSH excretion in one patient. In a third case of marked hirsutism (case 41) a high normal FSH excretion was found. This patient had undergone unilateral adrenalectomy with only temporary relief. The recurrence of hirsutism and the level of FSH excretion may however indicate that the ovary and not the suprarenal gland is abnormal.

TABLE VI. FOUR CASES OF HIRSUTISM AND/OR VIRILISM

Case	*Initials*	*Age*	*Sex*	*Diagnosis*	*Urinary FSH (Mouse Uterine Units per 24 hours)*
38	W.	20	F	Adrenogenital virilism due to suprarenal hyperplasia	− 6
39	E.R.	33	F	Hyperthecosis syndrome	+48 −96
40	E.P.	31	F	Hyperthecosis syndrome	+48 −96
41	F.G.	25	F	Hirsutism (? adrenogenital syndrome. ? hyperthecosis syndrome)	+24 −48

(7) *Sterility* (Table VII)

Four cases of female sterility (cases 42-45) and 5 cases of male sterility (cases 46-50) are recorded, where the FSH excretion was determined as part of a study to discover any accompanying endocrinological abnormality. The 4 female cases showed no obvious endocrinopathy and the FSH excretion was normal. The possibility has not however been excluded that, despite the

TABLE VII. NINE CASES OF STERILITY WHERE GONADOTROPIN ASSAYS WERE DONE AS PART OF THE STUDY TO DETERMINE THE UNDERLYING ETIOLOGY

Case	*Initials*	*Age*	*Sex*	*Diagnosis*	*Urinary FSH (Mouse Uterine Units per 24 hours)*
42	Z.M.	33	F	Sterility. No obvious endrocrinopathy and menstrual history normal	+12 −24
43	B.D.	22	F	Sterility. No obvious endrocrinopathy but oligomenorrhoea present	+ 6 −12
44	S.R.	25	F	Ditto.	+12 −24
45	E.J.	27	F	Ditto.	+12 −24
46	J.H.	30	M	Azoospermia and eunuchoidism, small testes (? selective deficiency of Gonadotropins)	+ 6 −12
47	D.H.	24	M	Azoospermia, impotence, obesity (? small testes, selective deficiency of Gonadotropins)	± 6 −12
48	H.B.	±30	M	Oligospermia, normal-sized testes but testicular biopsy showed impaired spermatogenesis. No obvious endocrinopathy	+24 −48
49	L.J.	±30	M	Oligospermia, small testes (no biopsy permitted). No obvious endocrinopathy	+12 −24
50	C.	±25	M	Azoospermia (bilateral mumps orchitis 2 years ago), normal-sized testes	+48 −96

apparently normal production of FSH, there is insufficient LH to cause ovulation. However, from the standpoint of the present discussion it is apparent that in those cases of sterility where a normal FSH excretion is found the assay does not assist in explaining the etiology of the sterility. It does, however, suggest that no gross ovarian or pituitary abnormality is present.

In male sterility several studies attempting to correlate testicular deficiency with FSH excretion levels have been reported. Thus Howard *et al.*[4] recorded the clinical findings with testicular biopsy results and excretion levels of FSH in 141 men with testicular deficiency. They classified their cases into 3 groups—cases with low FSH, normal FSH and high FSH excretion respectively. Their classification of cases contributes in no small measure to our understanding of cases showing azoospermia. In some cases sufficient evidence can be obtained to indicate whether there is an associated endocrine disturbance or not, and whether this is a primary testicular or pituitary deficiency. Although testicular biopsy should always be advised and may provide crucial information, nevertheless permission for this procedure is often refused. Even in its absence, however, a reasonably accurate diagnosis may be provided by FSH assays. Thus a raised FSH excretion occurs after mumps orchitis, and in case 50, where azoospermia was present in an apparently normal, healthy, potent young male adult with a history of mumps orchitis 2 years before the assay, the excretion of 48 units is compatible with the diagnosis. The bodily habitus of cases 46 and 47, both of whom showed azoospermia, suggested some degree of hypopituitarism, and the low normal level of FSH excretion tends to confirm this impression. In cases 48 and 49, however, with a normal FSH excretion the etiological factors responsible for the sterility were not apparent, but neither case appears to be primarily due to endocrine defects.

SUMMARY AND CONCLUSIONS

Urinary gonadotropin ('FSH') assays are reported on 50 males and non-pregnant females, and the clinical applications of the results are discussed.

The assay method used is described in detail.

In suspected hypopituitarism, FSH assays are of importance since

(*a*) it is the most common of the 'tropic' hormones to fail, and

(*b*) it permits differentiation between anorexia nervosa and organic hypopituitarism.

In adolescence FSH assays are of value in investigating cases of dwarfism, in differentiating between delayed puberty and sexual infantilism, and in determining the type of sexual precocity.

FSH assays assist in determining the level of the functional or organic disturbance in the endocrine system which leads to amenorrhoea. In the study of hypogonadism in males, FSH assays are of fundamental importance.

Since true or constitutional precocious puberty is due to secretion of pituitary gonadotropins, the presence of FSH establishes the diagnosis, whereas the absence of FSH indicates a pseudo-precocious puberty. Similar considerations suggest that suppression of FSH may accompany suprarenal virilizing lesions.

In cases of sterility a normal FSH excretion suggests that no gross pituitary or gonadal abnormality is present. An abnormal excretion would however indicate that the sterility is only one manifestation of an endocrinopathy.

Our thanks are due to Dr. S. Sims, Dr. J. Gluckman and Dr. W. Lewin for providing many of the facilities used in this investigation; to Dr. S. Lopis, Endocrine and Metabolic Clinic, Johannesburg Hospital, for allowing us access to a number of the cases reported, and to our medical colleagues in practice for permission to report our FSH assays on their cases and for their cooperation in supplying us with the clinical notes.

It is also a pleasure to acknowledge our indebtedness to Dr. H. B. Stein, Acting Head of the Department of Clinical Pathology, for considerable help in the preparation of this paper.

REFERENCES

1. Soffer, L. J. (1951): *Diseases of the Endocrine Glands*, p. 25. Philadelphia: Lea and Febiger.
2. Greep, R. O., van Dyke, H. B. and Chow, B. F. (1942): Endocrinology, **30**, 635.
3. Evans, H. M. and Simpson, M. E. (1950): *The Hormones*, ed. by Pincus, G. and Thimann, K. V. Vol. II, chap. VI. New York: Academic Press.
4. Howard, R. P., Sniffen, R. C., Simmons, F. A. and Albright, F. (1950): J. Clin. Endocr., **10**, 121.
5. Dekanski, J. (1949): Brit. J. Exp. Path., **30**, 273.
6. Scott, L. D. (1940): *Ibid.*, **21**, 320.
7. Klinefelter, H. F., Jr., Albright, F., and Griswold, G. C. (1943): J. Clin. Endocrinol., **3**, 529.
8. Levin, L., and Tyndale, H. H. (1937): Endocrinology, **21**, 619.
9. Escamilla, R. F. (1949): Ann. Intern. Med., **30**, 249.
10. Sheehan, H. L. (1939): Quart. J. Med., **8**, 277.
11. Wilkins, L. (1950): *The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence*, p. 120. U.S.A.: Charles C. Thomas.
12. *Ibid.*, pp. 174 and 175.
13. Klinefelter, H. F., Jr., Reifenstein, E. C., Jr., and Albright, F. (1942): Endocrinology, **30**, 1033.
14. Heller, C. G. and Nelson, W. O. (1945): J. Clin. Endocrinol., **5**, 1.
15. Turner, H. H. (1938): Endocrinology, **23**, 566.
16. Wilkins, L. (1950): *Loc. cit.*,[11] pp. 178 and 179.
17. Sohval, A. A. (1951): in Soffer's *Diseases of the Endocrine Glands*, p. 457. Philadelphia: Lea and Febiger.
18. Culiner, A. and Shippel, S. (1949): J. Obstet. Gynaec. Brit. Emp., **56**, 439.
19. Stein, I. F., and Leventhal, M. L. (1935): Amer. J. Obstet. and Gynec., **29**, 181.
20. Shippel, S. J. (1955): J. Obstet. Gynaec. Brit. Emp. (in the press).