The Endocrinologic Evaluation of Amenorrhea* **

CHARLES B. HAMMOND, M.D., R. HERBERT WIEBE, M.D.,
LEE TYREY, PH.D., DAVID W. SCHOMBERG, PH.D.

Division of Endocrinology and Infertility, Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, North Carolina

Introduction. The purpose of this paper is to review the endocrinologic evaluation of 58 patients who presented to our institution this past year with a chief complaint of absent or irregular menses. The primary goal for presenting these patients is to review the results of several newer studies as well as older and more classical studies which are useful in the differential diagnosis of amenorrhea. Such newer studies not only take advantage of the more current knowledge of the ovulatory process but also utilize recently developed and highly sensitive techniques of hormonal assay. It is hoped that this will provide the reader with some suggestions for a current and efficient method to evaluate the several causes of amenorrhea.

Materials and Methods. Amenorrhea is usually categorized as being either primary or secondary, and the patient's age or duration of absent menses varies among the several definitions available. For the purpose of this paper the following definitions will be utilized:

Primary Amenorrhea. Failure to have initiated menstrual bleeding by age 18.

Secondary Amenorrhea. Cessation of previously established menses for an interval of more than one year.

Oligomenorrhea. Spontaneous, irregular menses occurring at intervals of not less than three months.

Fifty-eight patients were studied and were divided into three major diagnostic categories according to their presenting menstrual patterns. Eight patients had *primary amenorrhea,* forty-six had *secondary amenorrhea,* and four patients had *oligomenorrhea.* Other patients with demonstrable uterine causes of amenorrhea are excluded from this study. This included patients with Asherman's syndrome, müllerian abnormalities in which no uterus was present, and testicular-feminization patients. Thus, all patients included had uteri capable of normal menstruation, and this was evidenced by progesterone-induced withdrawal bleeding, endometrial biopsy, estrogen-progesterone withdrawal bleeding, and/or hysterosalpingogram. Patients with easily diagnosable abnormalities of thyroid or adrenal function also are not included, as early outpatient studies usually revealed the etiology of their amenorrhea and appropriate therapy resulted in prompt return of menstrual function. For these reasons one can not draw valid incidence frequencies from this referral-practice population of 58 amenorrheic patients.

Studies Performed. All 58 patients were hospitalized on the Clinical Research Ward of our institution and underwent study. Tests included a thorough general and endocrine history, complete physical and pelvic examination, Papanicolaou smear, vaginal cytologic maturation index, complete blood count, urinalysis, Chem-18, anteroposterior (AP) and lateral skull x-rays, chest x-ray, and visual fields by perimeter.

All 58 patients also underwent a series of

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baseline and dynamic hormonal testing. Serum total estrogen (1), progesterone (2), follicle stimulating hormone (FSH) (3), luteinizing hormone (LH) (4), and growth hormone (5) were all performed in duplicate by standard radioimmunoassay techniques. Serum prolactin was initially done in the laboratory of Dr. Henry Friesen and later by Dr. Stuart Handwerger, both using radioimmunoassay (6). Serum T_4 and T_3 Uptake were done by standard techniques, as were urinary 17-hydroxycorticoids and 17-ketosteroids.

All patients also underwent the following three dynamic tests of pituitary function:

1. Hypoglycemia was induced by the intravenous administration of 0.1 units insulin per kilogram with blood glucose and human growth hormone (radioimmunoassay) determined at intervals over 90 minutes. Blood glucose had to achieve at least 50% reduction from fasting levels to be considered an adequate stimulus for the release of growth hormone. A positive growth hormone response to insulin-induced hypoglycemia consisted of a peak value of growth hormone greater than 10 ng/ml (7).

2. Control 24-hour urine collections were obtained for 17-hydroxycorticoids (17OH) and 17-ketosteroids (17KS). If normal hydroxycorticoids were present, the patient was then given Metopirone®, 750 mg orally every 4 hours for 6 doses. After the last dose of Metopirone®, another 24-hour urine collection was made. A normal response of pituitary ACTH was considered present if the levels of 17-hydroxycorticoids doubled (8).

3. The ability to release gonadotropins was tested with LH-FSH Releasing Hormone. Baseline FSH and LH were determined, then the patients received 100 μg SC of AY 24031, Ayerst Laboratories LH-FSH Releasing Hormone. Blood samples were then taken at frequent intervals over a period of 6 hours, and duplicate samples were assayed by radioimmunoassay for FSH and LH. Serum total estrogens were determined by radioimmunoassay on the control and 6-hours samples. Currently we are evaluating a variety of response parameters, and we realize that our own criteria for a positive response may be altered. However, in the interim, a positive response to LH-FSH Releasing Hormone was considered present if *both* serum LH rose by more than fourfold over baseline and serum FSH at least doubled (9).

Other Studies. As patients were evaluated by the techniques listed previously, many were found to have significant abnormalities which required further investigation. Such studies included:

Patients with *prolactin excess* (on duplicate samples) usually underwent further testing of prolactin kinetics with L-dopa, chlorpromazine, water load, and other studies. If the excess secretion was not readily explainable, then sella turcica tomography and pneumoencephalography were usually performed.

Evidence of clinical *androgen excess* or significant laboratory documentation of this usually led to determination of serum testosterone, urinary pregnanetriol, and appropriate dexamethasone suppression. If an ovarian source was presumed, then laparoscopy and ovarian biopsy were carried out.

Elevated 17-hydroxycorticoids led to the determination of plasma cortisol and dexamethasone suppression, with pituitary evaluation if pituitary tumor was suspected.

Abnormal *thyroid function* was evaluated by traditional methodology.

Patients who had stigmata of gonadal dysgenesis, or primary amenorrhea which seemed of gonadal origin (such as evidenced by elevated gonadotropins), were then evaluated by buccal smear and karyotype of peripheral leukocytes. If there was evidence of significant early estrogen production or a "Y" chromosome on karyotype, then laparoscopy and gonadal biopsy with both histopathologic study and gonadal karyotype were done. Gonads with "Y" chromosome were surgically removed.

If patients had abnormal skull x-rays or significant endocrinologic evidence of hypopituitarism or pituitary tumor, then tomograms of the sella turcica, pneumoencephalography, and/or carotid arteriography were performed.

Once a diagnosis was achieved, appropriate therapy was carried out. No patient suffered any significant side effects from any of these diagnostic studies.

Results. After these 58 patients had completed the previously listed diagnostic studies, a final diagnosis was assigned. The tables which follow (Tables 1, 2, 3) are each arranged with patients grouped by these final diagnoses and the results of the various diagnostic tests listed below. In this fashion one can not only study the results of the several tests performed in patients with a particular cause of amenorrhea but also evaluate how a specific test may provide useful data in the several groups of patient diagnoses.

TABLE 1
Primary Amenorrhea
(8 Patients)

Diagnosis (No.)	Constitutional Delay (3)	Hypothalamic Cause (2)	?Congenital Absence LH (2)	Gonadal Dysgenesis (1)
Family Hx.	+	–	–	–
Estrogen	↓	↓ sl.	↓	↓
FSH-LH	FSH-Normal LH-sl. ↑	FSH-Normal LH-sl. ↑	FSH-Normal LH-sl. ↓	↑↑
HGH, Prolactin, 17OH, 17KS, Thyroid	–	–	–	–
LRH Test	FSH + LH +	FSH + LH +	FSH + LH –	FSH + LH +
Insulin-HGH	+	+	+	+
Metopirone*	+	+	+	+

+ positive response, or normal test result.
– negative response, or reduced test result.
(See text for details.)

Primary Amenorrhea (Table 1). Eight patients presented with primary amenorrhea. Three patients were felt to have constitutional delay of menarche. Family history was positive in all three patients, and, while all had had a sequential development of secondary sex characteristics, all had reduced estrogen levels. Levels of FSH were normal and LH levels were minimally elevated in all three patients. Baseline testing of other hormones was normal. There was a significant response in all patients to LH Releasing Hormone, Insulin-Growth Hormone, and Metopirone®. The patients' ages were 18, 19, and 23 years. All have subsequently begun spontaneous menses.

Similar findings to those mentioned in the previous category were present in the testing of two patients thought to have "hypothalamic" primary amenorrhea, except there was no family history of delayed menarche.

Two patients had low baseline serum LH and failed to elevate LH in response to LH-Releasing Hormone on either of two occasions. This suggested to us a reduced ability to produce, or release, or a congenital diminution in LH.

Finally, one patient had Turner's syndrome (gonadal dysgenesis) which was proven by karyotype. Serum FSH was quite high, but there still existed a capacity of the pituitary to increase gonadotropin release when LH-Releasing Hormone was given.

Secondary Amenorrhea-Organic (Table 2). Sixteen patients presented with secondary amenorrhea which was finally shown to be of an organic etiology.

Four patients had pituitary tumors; one, a patient with a microadenoma and hyperprolactinemia, one with clinical signs of acromegaly. Three of the four patients had abnormal skull x-rays and reduced estrogen production. Two patients had significantly low gonadotropin values. Baseline hormonal testing revealed an elevation of growth hormone in the patient with acromegaly. Other baseline testing was normal. Two of the four patients failed to have an adequate response by LH (one borderline, however) while two had a positive response of FSH to LH-Releasing Hormone. One of the four patients had a negative growth hormone test. All had surgically proven lesions.

Two patients had craniopharyngiomas. Both had positive skull x-rays and decreased estrogen and gonadotropins. One patient had reduced baseline 17-hydroxycorticoids. One patient had a modest response to LH-Releasing Hormone while the other had no response. Neither patient responded to Insulin-HGH testing. Metopirone® testing was not done in one patient, but ACTH testing was normal.

Two patients had the "empty sella" syndrome. Again, skull x-rays were abnormal in both patients and estrogen and gonadotropins were low. Other baseline hormonal testing was normal. Both patients

responded to Insulin-HGH and Metopirone*, but one patient responded poorly to LH-Releasing Hormone. Both patients had this diagnosis confirmed by pneumoencephalography.

Two patients had Sheehan's syndrome with postpartum pituitary necrosis. One patient was clinically panhypopituitary, finding confirmed by low test results of estrogen, gonadotropins, thyroid, and adrenal function. Neither patient responded positively with both LH and FSH to LH-Releasing Hormone or Insulin-HGH. Metopirone* testing was not performed when low serum cortisols were detected. Adrenal response to ACTH was normal.

Four patients had clinical signs of androgen excess with significant hirsutism. Clinical estrogenization was good in all four, but, while baseline FSH was normal, the baseline LH was mildly elevated in all. Laboratory evidence of excess androgen was present in all four patients and was reflected by elevated 17-ketosteroids. Serum testosterone was elevated in two patients with Stein-Leventhal syndrome, and pregnanetriols were elevated in the other two patients who were felt to have adult-onset adrenogenital syndrome. In all four patients there was a normal response of FSH to LH-Releasing Hormone, but an impaired LH response. Other testing was normal.

There were two other patients with organic causes of secondary amenorrhea, one with hypothyroidism and one with premature ovarian failure. Test results are as listed.

Secondary Amenorrhea-Functional (Table 3). Thirty patients had secondary amenorrhea which was felt to be of functional etiology. Patients are divided into diagnoses of anorexia nervosa (4 patients) and "hypothalamic" causes (26 patients). This latter large group is subdivided into patients who had rapid weight loss or gain, stable weight, or had developed amenorrhea after utilizing oral contraceptives. Skull x-rays and physical examinations were negative except for that of weight change, and for four patients who had galactorrhea.

All patients with anorexia nervosa and hypoestrogenism had decreased gonadotropins. Thyroid function was reduced slightly in two

TABLE 2
Secondary Amenorrhea-Organic
(16 Patients)

Diagnosis (No.)	Pituitary Tumor (4)	Cranio-pharyngioma (2)	"Empty" Sella (2)	Sheehan's Syndrome (2)	Androgen Excess (4)
P. Exam	Acromegaly (1) Galactorrhea (1)	V. Fields (1)	–	Hypopit. (1)	Hirsutism (4)
Skull x-ray	+(3)	+	+	–	–
Estrogen	↓ (3)	↓	↓	↓	–
FSH-LH	↑(2)	↓	Normal	↓	FSH Normal LH↑ (4)
HGH, Prolactin, 17OH, 17KS, Thyroid	HGH↑ (1) Prolactin↑ (1)	17OH↓(1)	Normal	17OH↓(1) Thyroid↓ (1)	↑ 17KS (4) ↑ Testos. (2) ↑ PTriol (2)
LRH Test	FSH +2 LH +2	–(1)	sl.↓ (1)	–	FSH+ LH–
Insulin-HGH	–(1)	–	+	–(1)	+
Metopirone*	+	–	+	–	+

Others:
Hypothyroid (1)—Galactorrhea; decreased estrogen, FSH, LH, thyroid studies. Other testing normal.
Premature ●varian Failure (1)—▶ecreased estrogen; elevated FSH and LH. Other testing normal.
+ positive response, or normal test result.
– negative response, or reduced test result.
(See text for details.)

TABLE 3
Secondary Amenorrhea-Functional
(30 Patients)

Diagnosis (No.)	Anorexia Nervosa (4)	"HYPOTHALAMIC CAUSES" (26)				
		Rapid wgt. Loss (4)	Rapid wgt. Gain (3)	Stable wgt. (15)	"Post Pill" (4)	Group Total (26)
Skull x-ray P. Exam	–	–	–	Galactorrhea (3)	Galactorrhea (1)	(4)
Estrogen	↓	↓ (1)	Normal	(4)	(2)	↓ (7)
FSH-LH	↓	↓ (3)	↓ (2)	sl.↓ (2)	↓ (2)	sl.↓ (2) ↓ (7)
HGH, Prolactin, 17OH, 17KS, thyroid	Thyroid sl. ↓ (2)	Normal	Normal	Thyroid sl. ↓ (3)	Prolactin ↑ (1)	Thyroid↓ (3) Prolactin↑(1)
LRH Test	−(3)	−(3)	−(1)	+ +(5) sl.↓ (6)	−(1) sl.↓ (1)	+ +(5) sl.↓ (7) − (5)
Insulin-HGH	+	−(1)	+	+	+	−(1)
Metopirone*	+	+	+	+	+	+

\+ positive response, or normal test result.
− negative response, or reduced test result.

patients. Three of the four patients failed to respond to LH-Releasing Hormone, but all patients had normal responses to insulin by growth hormone and to Metopirone®.

The four patients with rapid weight loss, but not anorexia nervosa, showed better estrogenization, but low gonadotropins also. Similar poor responsiveness to LH-Releasing Hormone was present.

A greater percentage of patients who had rapid weight gain had normal estrogenization, baseline gonadotropins, and normal LH-Releasing Hormone response.

Patients with stable weight who were felt to have hypothalamic amenorrhea were basically normal in estrogenization and baseline gonadotropins. All patients had at least some response to LH-Releasing Hormone, and five had excessive responses. Patients who developed amenorrhea after using oral contraceptives were similar.

The 26 patients with hypothalamic amenorrhea, as a group, were better estrogenized, had more normal gonadotropins, and responded more normally or excessively to LH-Releasing Hormone than did the patients with anorexia nervosa or patients with organic causes of secondary amenorrhea.

Oligomenorrhea. Four patients had oligomenorrhea, two with evidence of ovarian androgen excess. Baseline estrogen, gonadotropins, and other hormone testing were normal except for one patient who was hypothyroid and two hirsute patients in whom there were elevated 17-ketosteroids and serum testosterone. All four patients had positive responses to LH-Releasing Hormone, although in two of these patients the response of LH was borderline. All patients had normal responses to Insulin-HGH and Metopirone®.

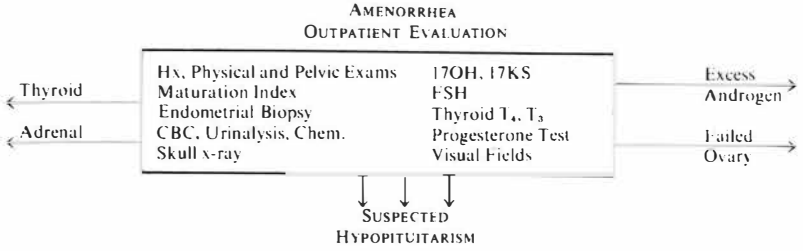
Summary of Studies. In an attempt to summarize these diagnostic studies, one can draw the following conclusions from this group of 58 amenorrheic women:

Skull x-rays are very useful, if positive.

Physical examination is useful when obvious signs of endocrinopathy are present, including acromegaly, hypopituitarism, galactorrhea, or signs of androgen excess. Negative findings do not exclude significant organic pathology, however. Findings suggesting deficiency of a "target gland" (for example, adrenal, thyroid) may be reflections of pituitary-hypothalamic dysfunction rather than peripheral gland abnormality.

Patients with organic causes of amenorrhea are more likely to have clinical hypoestrogenism, and it will tend to be more severe than in patients with "functional" or nonorganic causes. The exception is anorexia nervosa, certainly a severe functional disorder.

FIGURE 1



Baseline serum FSH is of use in identifying gonadal failure, or dysgenesis, when FSH is very high. In problems of androgen excess and functional causes of primary amenorrhea, LH seems likely to be slightly elevated with FSH normal. Gonadotropins are low in patients both with central organic causes and with the more severe functional causes of this problem.

Baseline measurements of growth hormone, prolactin, 17-hydroxycorticoids and 17-ketosteroids, and thyroid hormone all are of great use if abnormal, but usually will require further evaluation.

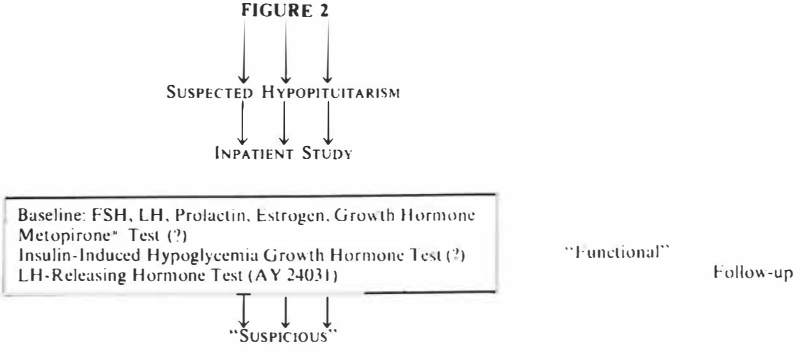
Testing with LH-Releasing Hormone does provide useful information. Patients with organic causes of amenorrhea, other than gonadal failure or androgen excess, were more likely to respond poorly to LH-Releasing Hormone; however, a significant proportion of these patients showed some response with regard to LH, or FSH, or both gonadotropins. Patients with functional disorders more likely had normal or excessive response to LH-Releasing Hormone while those classified as having more severe functional disorders responded very poorly. Growth hormone testing with insulin and Metopirone® testing were of relatively little diagnostic aid unless a major organic etiology was present; an etiology likely demonstrable on skull x-rays, clinical examination, or in patients with more severe abnormalities in baseline hormonal testing.

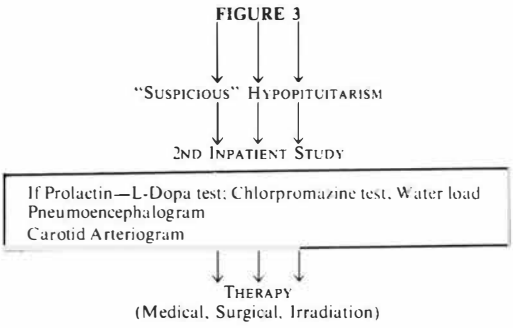
We would again emphasize that these are only *trends* in the results of diagnostic studies in patients who present with amenorrhea. Larger group studies are needed to evaluate the full statistical significance of these data. We would also again emphasize the need for a thoughtful, individualized, sequential evaluation of each patient who presents.

Comment. To reach the goal of individualization in the evaluation of the amenorrheic patient we suggest several possibilities:

Figure 1 illustrates what we consider an adequate screening study for the amenorrheic patient which usually can be done in the outpatient setting. Based on these results, and the clinical findings present, one can categorize problems under thyroid, adrenal, ovarian androgen, or gonadal failure for further studies which can usually be done in the out-

FIGURE 2





patient setting. Some patients will have suspected hypopituitarism and require hospitalization for further study.

Patients with the problem of suspected hypopituitarism should undergo further testing (Fig. 2) as evidenced by the preliminary studies, which should at least include baseline hormonal testing and testing with LH-Releasing Hormone. Growth hormone testing with insulin and/or Metopirone® testing may be indicated, but this must be individualized. Patients who are felt to have "functional" disorders causing amenorrhea are begun on appropriate therapy or follow-up, while patients who are strongly suspected of hypopituitarism usually are studied further.

Further studies (Fig. 3) include tests such as prolactin kinetics, pneumoencephalogram, and possibly arteriogram. If pituitary tumor, craniopharygioma, or other etiology of hypopituitarism is found, then appropriate medical, surgical, or irradiational therapy is initiated.

In this fashion we have tried to present to you the summation of data in 58 patients who presented with amenorrhea, and the endocrinologic testing results that have led to the establishment of a diagnosis. Again, it should be stressed that a systematic, individualized work-up is mandatory for the adequate endocrinologic work-up of the amenorrheic patient.

REFERENCES

1. PUPKIN MJ, SCHOMBERG DW, NAGEY DA, ET AL: Effect of exogenous dehydroepiandrosterone (DHEA) upon the fetoplacental biosynthesis of estrogens and its effect upon uterine blood flow in the term pregnant ewe. *Am J Obstet Gynecol* (in press).

2. ABRAHAM GE, SWERDLOFF R, TULCHINSKY D, ET AL: Radioimmunoassay of plasma progesterone. *J Clin Endocrinol Metab* 32:619–624, 1971.

3. ODELL WD, ROSS GT, RAYFORD PL: Radioimmunoassay for luteinizing hormone in human plasma or serum: Physiological studies. *J Clin Invest* 46:248–255, 1967.

4. ODELL WD, PARLOW AF, CARGILLE CM, ET AL: Radioimmunoassay for human follicle-stimulating hormone: Physiological studies. *J Clin Invest* 47:2551–2562, 1968.

5. ROTH J, GLICK SM, YALOW RS, ET AL: Hypoglycemia: A potent stimulus to secretion of growth hormone. *Science* 140:987–988, 1963.

6. FRIESEN H, HWANG P, GUYDA H, ET AL: A radioimmunoassay for human prolactin. Prolactin and carcinogenesis. Proc. 4th Tenows Workshop. Boyns AR and Griffiths K (eds). Cardiff, Wales, Alpha Omega Alpha Pub. 1972, pp. 64–80.

7. ROTH J, GLICK SM, YALOW RS, ET AL: Secretion of human growth hormone: Physiologic and experimental modification. *Metabolism* 12:577–579, 1963.

8. BRINCK-JOHNSEN T, SOLEM JH, BRINCK-JOHNSEN K, ET AL: The 17-hydroxycorticosteroid response to corticotrophin, metopiron and bacterial pyrogen. *Acta Med Scand* 173:129–140, 1963.

9. FLEISCHER N, GUILLEMIN R: Clinical applications of hypothalamic-releasing factors, in Stollerman, GH (ed): *Advances in Internal Medicine*, Vol. 18, Chicago, Year Book Medical Pub., 1972, pp. 303–323.