Concentrations of Plasma Luteinizing Hormone in Infants, Children, and Adolescents with Normal and Abnormal Gonadal Function

ALLEN W. ROOT^[71], THOMAS MOSHANG, Jr., ALFRED M. BONGIOVANNI and WALTER R. EBERLEIN

Division of Endocrinology, The Children's Hospital of Philadelphia,
and Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

Extract

This communication reports the development of a sensitive, reproducible, and specific radioimmunoassay for luteinizing hormone (LH) in plasma and the application of this method to a cross-sectional survey of plasma LH concentrations in 243 infants, children, and adolescents with normal and abnormal growth and sexual development. One nanogram of Hartree purified human pituitary LH standard was immunologically equivalent to 13.7 mIU of the Second International Reference Preparation of Human Menopausal Gonadotropin. The lower limit of sensitivity of the assay varied between 0.025 and 0.05 ng/ml. Specimens of plasma containing <0.25 ng/ml (<3.7 mIU/ml) have been considered indistinguishable from 'zero'.

Plasma LH concentrations increased after 9 years of age in normal boys and after 6 years of age in normal girls. In males with constitutional delay in growth and development, LH levels were generally lower than in normal boys of comparable ages (figs. 3 and 4). The plasma LH concentration also increased with advancing sexual maturation (fig. 5).

Levels of LH were <0.25 ng/ml in 15 of 18 males (6-20 years old) and in 10 of 11 females (6-19 years old) with hypopituitarism and documented lack of human growth hormone response to hypoglycemia. In three such patients, plasma LH concentrations correlated with their degrees of sexual maturation, but in a 16-year-old clinically prepubertal male, *patient SA*, with hypopituitarism secondary to a craniopharyngioma, LH levels higher than normal male values were noted.

Plasma LH concentrations were 1.2 and 1.45 ng/ml in 2 of 3 girls less than 4 years old, with gonadal dysgenesis, and ranged between 0.33 and 3.6 ng/ml in 10 of 15 older patients with this diagnosis (table III). The plasma LH concentration was 1.7 ng/ml in a 3-month-old male pseudohermaphrodite. Plasma LH concentrations ranged between 0.38 and 0.88 ng/ml in five of seven children with true sexual precocity. In 13 of 15 girls with precocious thelarche, and in all of 10 patients with premature pubarche, LH levels were <0.25 ng/ml. The intravenous infusion of conjugated equine estrogens was followed by a prompt decline in plasma LH levels in patients with gonadal dysgenesis and Klinefelter's syndrome, but failed to alter significantly LH values in *patient SA* (fig. 6A, B, C). Administration of clomiphene citrate and cisclophene citrate increased plasma LH concentrations in normal males, but failed to do so in patients with hypopituitarism, including *patient SA* (fig. 7A, B). The intramuscular administration of medroxyprogesterone acetate was associated with suppression of plasma LH levels in two patients with true sexual precocity (fig. 8).

Speculation

The hypothalamic-pituitary-gonadal axis is active in the prepubertal human subject, and is responsive to abnormal function in one segment of the system.

Introduction

Since the development of the radioimmunoassay for plasma insulin by YALOW and BERSON [50], these techniques have been applied to the measurement of many other protein and polypeptide hormones [4, 28, 35, 47]. Recently, radioimmunoassays for the measurement of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) have been developed and utilized for the investigation of gonadotropic function in adults [3, 7, 10, 11, 23, 24, 27, 38, 39, 41-43, 45, 46], and several investigators have also applied these methods to the study of gonadotropin concentrations in the sera of children [1, 10, 15, 19, 20, 27, 34, 39, 40, 45, 53]. We are reporting our experience with a specific, precise, and sensitive radioimmunoassay for LH and the results of a cross-sectional survey of plasma LH concentrations in 243 infants, children, and adolescents with normal and abnormal growth and sexual maturation.

Materials and Methods

Radioimmunoassay of LH [33]

The antigenic similarity between pituitary LH and placental human chorionic gonadotropin (HCG) has been documented [6, 29, 48]. Antisera to HCG were prepared in guinea pigs by the subcutaneous administration fortnightly of 3,000 IU of HCG [54] dissolved in 1 ml of saline and emulsified in 1 ml of complete Freund's adjuvant [55]. Serum was harvested by cardiac puncture after the fifth injection and stored at -20° . The antiserum selected for use in the assay was that which exhibited the greatest degree of dissociation from tracer $\text{LH-}^{125}\text{I}$ with the smallest quantity of unlabeled LH [5]. Hartree human pituitary LH [56] was labeled with ^{125}I [12, 27, 57] to specific activities of 250-500 $\mu\text{Ci}/\mu\text{g}$. Hartree LH also was used as standard in the assay. It was compared immunologically with the Second International Reference Preparation of Human Menopausal Gonadotropin (2nd IRP-HMG) [58] and to LER 907, the gonadotropin reference standard recently distributed by the National Pituitary Agency [59].

The radioimmunoassay was performed in disposable glass test tubes (10 mm by 75 mm) to which were added: 0.5 ml of diluent [60] or Hartree LH standard in diluent (0.025-2.5 ng/ml); 0.1 ml of unknown plasma

or plasma from a patient with hypopituitarism; 0.1 ml of $\text{LH-}^{125}\text{I}$ (approximately 0.05-0.1 ng, 15-20,000 cpm); and 0.1 ml of guinea pig anti-HCG serum (1:80,000). Tubes for the determination of total radioactive counts added and of nonspecifically precipitated counts were also prepared. After incubation for 96 h at 4° , 0.1 ml of normal guinea pig serum (1:200) in 0.1 M sodium ethylenediaminetetraacetate (EDTA) and 0.1 ml of rabbit anti-guinea pig gamma globulin (1:2) were added. The incubation was continued for 72 h at 4° . On day 8, the tubes were transferred directly from the refrigerator to precooled centrifuge cups and centrifuged for 30 min, at 3,000 rpm and 4° . The supernatant was discarded and the precipitated (antibody-bound) $\text{LH-}^{125}\text{I}$ was counted in an automatic gamma spectrometer [61] employing a background subtract correction for the nonspecifically precipitated counts. More than 90% of $\text{LH-}^{125}\text{I}$ was precipitable by excess anti-HCG serum in the absence of unlabeled LH.

The standard curve was constructed by plotting the percentage of antibody-bound $\text{LH-}^{125}\text{I}$ against the standard concentrations of LH. The LH content of an unknown specimen was determined directly from the standard curve and expressed in nanograms of Hartree LH per milliliter of undiluted plasma. All standards and unknown specimens were analyzed in triplicate. Longitudinal study specimens were analyzed in the same assay to minimize interassay variability.

Subjects

Blood was drawn in heparinized syringes and the plasma stored at -20° . Specimens were obtained randomly from the following types of subjects:

Normal males ($N = 61$), 1 month-19 years of age, without evidence of disease of the central nervous system or of the pituitary-testicular axis; males with constitutional delay in growth and development [49] ($N = 44$), 6-16 years of age; normal females ($N = 48$), 2 months-15 years of age; all were premenarchal and without evidence of disease of the central nervous system or of the pituitary-ovarian axis; patients with hypopituitarism and documented lack of growth hormone response to hypoglycemia [35] ($N = 29$), 6-20 years of age; phenotypic females with gonadal dysgenesis ($N = 18$), 3 weeks-18 years of age; anorchic males ($N = 6$), 11-22 years of age; male pseudohermaphrodites ($N = 5$), 3 months-15 years of age; and subjects with precocious sexual development ($N = 32$), 1-11 years of age.

Plasma LH concentrations were also determined in seven normal adult males, five males with Klinefelter's syndrome and three subjects with XYY sex chromosome constitution. Informed consent was obtained [68].

Special Studies

The effect of intravenously administered conjugated equine estrogens [62] upon plasma LH levels was determined in subjects with gonadal dysgenesis, Klinefelter's syndrome, and hypopituitarism. Clomiphene citrate and cisclophene citrate [63] were administered orally to three normal adult males and to five males with hypopituitarism, and the effect on plasma LH concentrations studied. The plasma LH response to the intramuscular administration of medroxyprogesterone acetate was investigated in two patients with true sexual precocity.

Results

Radioimmunoassay of LH

Figure 1 presents a representative standard curve for Hartree LH and for the 2nd IRP-HMG. A similar curve was obtained when HCG or LER 907 served as standard. One nanogram of Hartree LH was equivalent immunologically to 13.7 mIU of the 2nd IRP-HMG and to 100 ng of LER 907. The immunological potency of Hartree LH relative to the 2nd IRP-HMG was derived from 22 assays utilizing data between 'zero' and 1.0 ng/ml Hartree LH and 'zero' and 10.0 mIU/ml 2nd IRP-HMG. The range of potencies in these assays was 11.8–16.6 mIU/ng (SD 1.3). This immunologic potency is higher than that reported by ODELL *et al.* [27] for a similar Hartree pituitary LH preparation. The authors have no explanation for this discrepancy. Hypopituitary plasma from three patients significantly increased the binding of LH-¹²⁵I by anti-HCG serum (fig. 2, table I) and was, therefore, added to all tubes containing standard Hartree LH as indicated in *Methods*. This phenomenon was not due to the salt content of the plasma, inasmuch as the effect was not altered by dialysis of the plasma. A similar effect has been noted with horse serum [45]. Human growth hormone [64], porcine adrenocorticotropin [65], porcine insulin [66], and plasma from patients with primary hypothyroidism, presumably containing high levels of thyrotropin, did not displace LH-¹²⁵I from the anti-HCG serum utilized, as reported previously [33]. Anti-HCG serum bound insignificant amounts (<1%) of HFSH-¹²⁵I (LER-869-2) [59].

The lower limit of sensitivity of the standard curve varied between 0.025 and 0.05 ng/ml. Plasma specimens containing <0.25 ng/ml of LH have been con-

Fig. 1. Standard curves for Hartree pituitary LH and for the Second International Reference Preparation of Human Menopausal Gonadotropin (2nd IRP-HMG).

Fig. 2. Effect of plasma from a subject with hypopituitarism upon the standard curve for Hartree LH.

Fig. 3. Plasma LH concentrations in normal males (●) and in males with constitutional delay in growth and development (○). (One nanogram Hartree LH immunologically equivalent to 13.7 mIU of 2nd IRP-HMG and 100 ng LER 907.)

sidered indistinguishable from 'zero'. The mean LH concentration of a plasma specimen from one adult male determined 12 times in the same assay, was 0.90 ng/ml (SD 0.09), while the mean concentration of this specimen measured in 8 consecutive assays was 0.94 ng/ml (SD 0.06). Approximately 90% (70–116%) of pituitary LH added to hypopituitary plasma at concentrations between 0.25 and 8.0 ng/ml was recovered. Assay of varying volumes (up to 0.1 ml) of plasma containing a high concentration of LH did not alter the calculated LH concentration of the specimen. Table II

Table I. Effect of plasma from a subject with hypopituitarism upon the binding of LH-¹²⁵I by anti-HCG

	Percentage binding of LH- ¹²⁵ I to anti-HCG	
	Without plasma (20) ¹	With plasma (19) ¹
Mean	27.5	30.7 ²
SD	0.9	0.6

¹ Numbers in parentheses indicate number of tests.

² $t = 22.2442$; $P < 0.005$.

Table II. Correlation between radioimmunoassay and bioassay of gonadotropin preparations

Preparation	FSH/LH	Radio-immuno-assay	Bio-assay	Index of discrimination, immunopotency/biopotency
Urinary				
PR2101	0.59	0.783 ¹	1.7 ¹	0.5
PR2128	0.89	0.997 ¹	1.12 ¹	0.9
PR2075	0.9	0.998 ¹	1.1 ¹	0.9
PR2119	2.9	0.093 ¹	0.35 ¹	0.3
PR2167	50.0	0.748 ¹	0.60 ¹	1.2
Pituitary				
LER907	0.4	28.0 ²	48.0 ²	0.6

¹ Luteinizing hormone, international units of 2nd International Reference Preparation Human Menopausal Gonadotropin (2nd IRP-HMG) per milliliter.

² Luteinizing hormone, international units of 2nd IRP-HMG per milligram. (Preparations and bioassay of Cutter Laboratories, Berkeley, California, supplied by Dr. G.T. Ross, National Cancer Institute, National Institutes of Health.)

indicates that a relatively good correlation existed between levels of LH determined by bioassay and by radioimmunoassay for several urinary gonadotropin preparations with varying FSH/LH ratios. High FSH levels did not seem to interfere with the LH assay.

Plasma LH Concentrations

Normal subjects. Figure 3 presents the plasma LH concentrations recorded in normal males and in boys with constitutional delay in growth and development. Generally, the plasma LH level tended to increase after 9 years of age, although 4 of 11 infant boys had measurable plasma LH. In males with constitutional delay in growth and development, LH concentrations were usually lower (i.e., were undetectable in a relatively greater number of subjects) than in normal boys. Figure 4 presents similar data obtained in normal girls, all of whom were premenarchal. Concentrations of LH increased after 6 years of age in this group. No infant girl had measurable levels of LH. Fifty-four boys and

Fig. 4. Plasma LH concentrations in normal premenarchal females.

Fig. 5. Relation of the stage of sexual maturation to the plasma LH concentration in males (●) and premenarchal females (□).

21 girls were classified by the criteria of TANNER [44] into five stages of sexual maturation. Figure 5 indicates that increasing concentrations of LH in plasma were recorded with advancing stages of sexual development in both sexes, but that even in some subjects with significant sexual maturation plasma LH concentrations were below the limit of sensitivity of the present assay.

Hypopituitarism. Concentrations of LH were <0.25 ng/ml in 15 of 18 boys (6–20 years old) and in 10 of 11 girls (6–19 years old) with documented absence of human growth hormone [35]. In two males, 14.5 and 16 years of age, with LH concentrations of 0.53 and 0.35 ng/ml, respectively, and in a 17-year-old girl with an LH level of 1.0 ng/ml, sexual maturation occurred. In another subject, SA, a 16-year-old clinically prepubertal boy with hypopituitarism secondary to removal of a craniopharyngioma, LH levels persistently higher than those recorded in our normal male population were observed. This patient will be discussed in greater detail later in the presentation.

Gonadal dysgenesis. It will be noted in table III that plasma LH concentrations were 1.2 and 1.45 ng/ml in two of three girls less than 4 years of age with XO

chromosome constitution. Plasma LH levels ranged between 0.33 and 3.6 ng/ml in 10 of 15 subjects over 5 years of age. The plasma LH concentration was below detectable limits in two subjects with XO/XX sex chromosome mosaicism (NS and DB), in two subjects with XO sex chromosome constitution (DS and SM) and in a subject with a chromatin-negative buccal smear in whom peripheral karyotyping had not been obtained (PM).

Males with abnormal sexual development. Table IV presents data on a group of males with varied abnormalities of sexual development. Of note is the plasma LH concentration of 1.7 ng/ml recorded in a 3-month-old male pseudohermaphrodite (DR). Elevated plasma LH levels were also noted in two older males (EC and GW) with anorchia and in three of five adult males with Klinefelter's syndrome. Testicular biopsy of these

Table III. Plasma concentrations of luteinizing hormone in subjects with gonadal dysgenesis

Patient	Age, yr	Luteinizing hormone, ng/ml	Sex chromosome constitution
LS	3 wk	1.2	XO
DR	3	1.45	XO
MB	4	<0.25	XO
NS	5	<0.25	XO/XX, trisomy 21
CR	6	0.35	XO
PM	8	<0.25	**1
VM	10.5	0.4	XO
SF	10.5	0.4	XO
DW	11	0.33	**1
AB	11	0.78	XO
SD	11	0.4	XO/XY
AP	12	2.83	XO
DB	13	<0.25	XO/XX
MK	14	3.6	XO
DS	15.5	<0.25	XO
DT	16	2.64	XisoX
SM	16.5	<0.25	XO
PA	18	1.65	XO

¹ Buccal smear, 100% chromatin negative.

Table IV. Plasma concentrations of luteinizing hormone in males with abnormalities of sexual development

Patients and diagnosis	Age, yr	Luteinizing hormone, ng/ml	Comment
Klinefelter's syndrome			
JL	20	2.58	XX
E	28	0.38	XXY
EC	28	5.30	XXY
F	46	4.85	XXY
C	47	1.05	XXY
XYY sex chromosome constitution			
B	18	0.50	Prison inmate
AB	38	1.05	Prison inmate
A	62	0.75	Prison inmate
Male pseudohermaphroditism			
DR	0.25	1.7	XY
KW	10.5	0.25	XY, feminizing testes
SD	11	0.25	XY, feminizing testes
JB	13	1.12	XO/XY
GO	15	0.35	XO/XisoY/XYisoY
Anorchia			
MM	11	0.27	Congenital
JA	11.5	0.25	Congenital
SK	12	0.25	Congenital
WH	17	1.3	Congenital
EC	19	3.4	Congenital
GW	22	4.37	Traumatic

five adult males was consistent with the diagnosis of Klinefelter's syndrome in these patients (table IV). Three males with XYY sex chromosome constitution [67] had plasma LH concentrations that were within our normal adult range for the male (0.5–0.9 ng/ml).

Precocious sexual development. The concentrations of LH recorded in seven children with true sexual precocity [49] are shown in table V. Plasma LH levels ranged between 0.38 and 0.88 ng/ml in five children, but were below detectable levels in two (*KP* and *JW*). Plasma LH concentrations were <0.25 ng/ml in 13 of 15 girls with precocious thelarche [49] less than 8 years of age. Levels of 0.28 and 0.35 ng/ml were recorded in two such subjects, 1.6 and 4.5 years of age, respectively. These children were considered to have precocious thelarche rather than true sexual precocity because

A

B

C

Fig. 6. The response of plasma LH to the intravenous administration of conjugated equine estrogens in: A (*PA*, 18 years, gonadal dysgenesis); B (*JL*, 20 years, Klinefelter's syndrome); and C (*SA*, 16 years, hypopituitarism).

Table V. Plasma concentrations of luteinizing hormone in patients with true sexual precocity

Patient	Chronologic age, yr	Height age, yr ¹	Bone age, yr ¹	Luteinizing hormone ng/ml	Comments
Females					
<i>LB</i>	4	5.5	10	0.38	Idiopathic
<i>BS</i>	6	9.5	11	0.4	Posttuberculous meningitis
<i>KP</i>	6	7	11	<0.25	Idiopathic; medroxyprogesterone acetate
<i>JW</i>	7	8.5	11.5	<0.25	Idiopathic; medroxyprogesterone acetate
<i>MB</i>	7	11.5	13	0.53	Idiopathic; medroxyprogesterone acetate
Males					
<i>JS</i>	4	2.5	6.5	0.88	Poststreptococcal meningitis
<i>TD</i>	11	11	13	0.45	Cyst of the cavum velum interpositum

¹ From reference [49].

skeletal maturation was consistent with chronologic age, vaginal cytology revealed no evidence of estrogen effect, and there was no progression of sexual development during prolonged periods of observation. Levels of LH were below the limits of sensitivity of the assay in all of 10 children with premature pubarche [49].

Special Studies

The intravenous infusion of conjugated equine estrogens was followed by a prompt decline in plasma LH concentrations in patients with gonadal dysgenesis (PA) and Klinefelter's syndrome (JL) (fig. 6A, B). When conjugated equine estrogens were administered

Table VI. Radioimmunoassayable luteinizing hormone concentrations in plasma of normal subjects as reported by other authors

Reference	No.	Age, yr	Sex	Luteinizing hormone, mIU 2nd IRP-HMG/ml	
				Mean	Range
Adult males					
Present	7	30-75		8.8	6.9- 12.3 ¹
(7)	40	20-50		23.0	8.0- 48.0
(10)	114	20-49		15.2	<8.0- 47.2
	44	> 50		27.2	<8.0-110.4
(15)	35	—		10.9	6.2- 23.0
(27)	28	—		14.4	6.4- 25.6
(38)	27	20-45		14.0	2.5- 31.5
(41)	10	18-22		10.5	—
(44)	16	—		9.4	1.7- 24.1
Children					
Present	26	0.2- 9	M	—	<3.7- 6.6
	21	10-12	M	—	<3.7- 7.2
	14	14-19	M	—	<3.7- 19.9
	25	0.2- 9	F	—	<3.7- 7.1
	19	10-13	F	—	<3.7- 14.1
	4	14-15	F	—	3.7- 19.9
(10)	15	—	—	12.8	3.2- 23.2
(15)	20	5- 8	M	3.4	2.5- 4.5
	10	9-10	M	4.8	3.5- 7.0
	20	11-12	M	6.8	4.0- 12.0
	40	13-16	M	9.2	4.0- 14.0
	77	2- 9	F	2.7	1.1- 4.0
	39	10-12	F	4.4	1.6- 12.0
(19)	100	< 10	—	3.0	0.0- 7.0
(20)	—	1-10	M	2.8	—
	—	1-10	F	2.7	—
(27)	—	1- 8	—	8.0	4.8- 12.8
(38)	6	3- 8	—	10.2	2.5- 15.0
(40)	61	< 10	M	5.0	—
			F	6.0	—
(45)	17	—	—	6.8	1.3- 14.9
(53)	34	8-10	M	0.9	—
	38	11-13	M	2.2	—
	21	14-15	M	4.1	—
	28	8-10	F	2.3	—
	26	11-13	F	5.5	—

¹ Data are expressed utilizing the directly reported data or conversion factors suggested by the authors.

A

B

Fig. 7 A. The response of LH plasma to orally administered clomiphene citrate (a) and cisclophene citrate (b) in normal adult males: 1 (NF, 28 years); 2 (HS, 28 years); and 3 (WS, 30 years).

B. The response of plasma LH to cisclophene citrate in males with hypopituitarism: 4 (JM, 15 years, chromophobe, 1-7 days); 5 (SA, 16 years, craniopharynx, 1-7 days); 6 (RS, 20 years, idiopathic, 1-10 days); 7 (DG, 16 years, chromophobe, 1-7 days); and 8 (TS, 20 years, idiopathic, 1-6 days).

intravenously to SA, the subject with hypopituitarism secondary to a craniopharyngioma, the plasma LH response was erratic, but no significant suppression of plasma LH levels was noted (fig. 6C).

Figure 7A depicts the plasma LH response to the oral administration of clomiphene citrate and cisclophene citrate in normal adult males, while figure 7B presents the plasma LH response to orally administered cisclophene citrate in five boys with hypopituitarism. It will be noted that plasma LH concentrations increased promptly in response to both drugs in normal adult males, but there was no significant effect upon plasma LH levels in somewhat younger subjects with hypopituitarism. Subject SA again presented elevated levels of LH which did not change greatly during the administration of cisclophene citrate.

The intramuscular administration of medroxyprogesterone acetate was followed by a decline in plasma LH concentrations in two patients with true sexual precocity (fig. 8).

Discussion

The radioimmunoassay for luteinizing hormone (LH) reported in this communication is sensitive, reproducible, and apparently specific for materials with luteinizing properties. The lower limit of sensitivity of the assay was 0.25 ng of Hartree pituitary LH/ml of undiluted plasma (3.7 mIU of 2nd IRP-HMG/ml) and is comparable to the assay sensitivities reported by MIDGLEY and JAFFE [24] and YEN *et al.* [52]. The intra-assay variability was 10%, while the interassay variability was approximately 7%. Specificity of the anti-HCG serum employed in the assay was demonstrated by failure of several other polypeptide hormones to interfere with the anti-HCG:LH-¹²⁵I reaction, and by the correlation between LH levels determined by bioassay and by radioimmunoassay of several urinary gonadotropin preparations with widely varying FSH-LH contents.

The cross-sectional survey of plasma LH levels revealed that in the majority of prepubertal subjects, LH concentrations were below the lower limits of sensitivity of the assay. The plasma LH level tended to increase with advancing age and stage of sexual maturation. In several subjects with early sexual development, however, LH was not measurable. Table VI compares our data with those of other investigators, several of whom [10, 27, 39, 45] have reported higher plasma LH concentrations in children than observed

Fig. 8. The response of plasma LH to the intramuscular administration of 100 mg of medroxyprogesterone acetate in subjects with true sexual precocity.

here. It is difficult to analyze some of these data inasmuch as the numbers of subjects studied by each group have been small and little information concerning age and stage of sexual maturation has been presented. JOHANSON *et al.* [15], KENNY *et al.* [19], LEE *et al.* [20], and YEN *et al.* [53] have reported the presence of immunoreactive LH in the plasma of most normal prepubertal children; the levels reported, however, were below the lower limit of sensitivity of the method reported here in the majority of instances. Exceedingly wide ranges of plasma LH concentrations were found in older children. JOHANSON *et al.* [15] demonstrated a significant correlation between stage of sexual maturation and the plasma LH concentration for both sexes. These investigators [15] employed a nonequilibrium radioimmunoassay together with larger quantities of plasma (0.2, 0.3 ml) than used here (0.1 ml). When it was attempted to increase the volume of plasma in our system, it became impossible to maintain acceptable standards of precision. The reason for this was not apparent. Within the limits of sensitivity of the assay our data are compatible with those of JOHANSON *et al.* [15], KENNY *et al.* [19], LEE *et al.* [20], and YEN *et al.* [53].

It was of interest to observe that several youngsters with abnormalities of gonadal structure or function (gonadal dysgenesis, male pseudohermaphroditism) had relatively elevated plasma LH concentrations. In subject LS, the LH concentration of 1.2 ng/ml at 3 weeks of age is unlikely to represent residual placental HCG inasmuch as this material should have been eliminated by this time [25, 52]. GUYDA *et al.* [13] have also reported recently a significant increase in the mean plasma LH concentration of prepubertal children with gonadal dysgenesis. The finding of high LH concentrations in the plasma of some children with abnormal gonadal development or function suggests that in the prepubertal human the pituitary-gonadal axis is dynamically active. Evidence that supports this hypothesis includes: recent detection of both FSH and LH in the urine of prepubertal children [2, 30, 32], and reports of elevated urinary gonadotropin excretion in prepubertal children with gonadal dysgenesis [17]. Furthermore, there is an increase in the circulating gonadotropin level of the prepubertal animal following castration [9, 26, 51].

The secretion of LH is regulated in part by the hypothalamic luteinizing hormone-releasing factor [22]. Estrogens and androgens are thought to inhibit the release of LH through an effect on hypothalamic receptors [22]. If the hypothalamic-pituitary-gonadal axis functions in the prepubertal human, then puberty represents an increase in the activity of this system rather than *de novo* initiation of function. The mechanisms responsible for pubertal changes in the activity of the system remain unknown. DONOVAN and VAN DER

WERFF TEN BOSCH [9] proposed that pubertal activity of the hypothalamic-pituitary-gonadal axis ensued when 'maturational' changes in the central nervous system decreased the sensitivity of the hypothalamic receptors to the inhibiting effects of gonadal secretions. The nature of these changes has not been clarified but they may involve cellular alterations, or the removal of extrahypothalamic inhibitory influences (pineal [9], amygdala [8]).

Slightly elevated plasma LH concentrations were noted in five of seven children with true, complete sexual precocity, but not in subjects with premature thelarche or precocious pubarche. In the former subjects, inordinately premature 'maturation' of the hypothalamic-pituitary-gonadal axis has apparently occurred, while the latter conditions may represent local tissue responses to unknown stimuli. GUYDA *et al.* [13] and KENNY *et al.* [15] have also noted elevated plasma LH levels in subjects with sexual precocity. Although KENNY *et al.* [18] suggested that plasma LH levels may be slightly increased in females with premature thelarche, neither the data of GUYDA *et al.* [13] nor the present data substantiate this impression. In two patients with sexual precocity, a progestogen depressed plasma LH levels acutely, implying that inhibitory hypothalamic receptors remained intact. RIFKIND *et al.* [31] have reported that excretion of LH and FSH in urine decreased in two of five children with sexual precocity following the administration of medroxyprogesterone acetate.

The 16-year-old male with hypopituitarism, SA, is of interest because he had persistently high concentrations of plasma LH. There was no significant plasma LH response to either the suppressive effects of estrogens or to the stimulating effects of cisclomiphene citrate, suggesting the presence of autonomous LH-secreting cells. Despite these high plasma LH levels, SA remained clinically prepubertal. This may have been due to 1) lack of FSH secretion, a necessary synergistic stimulus to testicular maturation [16, 36]; or 2) absence of biological activity in an immunologically reactive LH molecule. Although the first explanation appears most plausible, direct measurements of plasma FSH concentrations will be necessary to confirm this hypothesis.

The advantages of the radioimmunoassay for the measurement of gonadotropins in plasma include: (immunologic) specificity, sensitivity, reproducibility and precision, relative technical simplicity, and the employment of small sample volumes, thus permitting repetitive sampling of the same subject. The utilization of these techniques, together with the competitive protein-binding assays for gonadal secretions [21, 37], will permit longitudinal studies to correlate pituitary and gonadal function with somatic and sexual maturation.

Summary

The plasma concentration of radioimmunoassayable luteinizing hormone was determined in 243 infants, children, and adolescents. In normal subjects, plasma LH levels tended to increase with age and stage of sexual maturation, but there was wide individual variation. In several children with abnormal gonadal function or structure (gonadal dysgenesis, male pseudohermaphroditism), plasma LH levels were quite high. Luteinizing hormone was measurable in the plasma of five of seven subjects with true sexual precocity, but was not detectable in most subjects with precocious thelarche or premature pubarche. In a 16-year-old male with hypopituitarism secondary to a craniopharyngioma, persistently elevated plasma LH concentrations were recorded. Estrogens did not suppress nor did cislomiphene citrate significantly increase these levels, suggesting the presence of autonomous LH-secreting cells.

The results of the present study, and those of other investigators, tend to support the suggestion that the hypothalamic-pituitary axis may be sensitive to altered gonadal function in the prepubertal child. In the normal subject, puberty may occur when the equilibrium established between the hypothalamus, pituitary, and gonads is altered by decreasing sensitivity to gonadal secretions.

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55. Difco, Detroit, MI.
56. Provided by Dr. A. S. HARTREE, University of Cambridge, England (10-24-64) (14). Biologic potency: 5.7 (3.6-9.2) times NIH-LH-S3; 7.2 (5.6-22.5) times NIH-LH-S6. (Approximately 4.7 times NIH-LH-S1; 2,770 IU 2nd IRP-HMG/mg.)
57. Iso-Serve, Cambridge, MA.
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71. Request for reprints should be addressed to: A. W. ROOT, M.D., Division of Pediatrics, Albert Einstein Medical Center, York and Tabor Roads, Philadelphia, PA 19141 (USA).
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