JCI The Journal of Clinical Investigation

Suppression of Urinary and Plasma Follicle-Stimulating Hormone by Exogenous Estrogens in Prepubertal and Pubertal Children

R. P. Kelch, … , S. L. Kaplan, M. M. Grumbach

J Clin Invest. 1973;52(5):1122-1128. https://doi.org/10.1172/JCI107278.

Research Article

Clomiphene citrate, an “anti-estrogen” with mild estrogenic properties, inhibits rather than stimulates gonadotropin excretion in prepubertal and early pubertal children. These and other data suggest that the sensitivity of the hypothalamic-pituitary “gonadostat” decreases at the onset of puberty. To test this hypothesis further, the daily excretion of urinary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) was determined in 19 children (5 “short normals” and 14 with isolated human growth hormone (HGH) deficiency) who were given ethinyl estradiol (EE) 1.4-14.7 $\mu g/m^2$ per day (2-10 μg/day) for 4 to 7 days. In addition, plasma and urinary gonadotropins and plasma estrogens were serially determined in two prepubertal females(with isolated HGH deficiency) given two injections (24 h apart) of estradiol benzoate, 10 μg/kg. FSH and LH concentrations in plasma and kaolin-acetone urinary concentrates and plasma 17β-estradiol (E_2) and estrone (E_1) were measured by radioimmunoassays. 2-3 $\mu g/m^2$ per day of EE significantly suppressed urinary FSH (and LH when detected in the control period) in two out of six prepubertal children, while all doses >5 $\mu g/m^2$ per day suppressed urinary gonadotropins to undetectable levels in eight prepubertal subjects. In early to midpubertal subjects. 2-10 $\mu g/m^2$ per day of EE produced a slight suppression of urinary FSH, but failed to suppress to undetectable levels. Two subjects in late puberty (stage 4) did not suppress their urinary FSH […]

Suppression of Urinary and Plasma Follicle-Stimulating Hormone by Exogenous Estrogens in Prepubertal and Pubertal Children

R. P. KELCH, S. L. KAPLAN, and M. M. GRUMBACH

From the Department of Pediatrics, University of California at San Francisco, California 94122

ABSTRACT Clomiphene citrate, an "anti-estrogen" with mild estrogenic properties, inhibits rather than stimulates gonadotropin excretion in prepubertal and early pubertal children. These and other data suggest that the sensitivity of the hypothalamic-pituitary "gonadostat" decreases at the onset of puberty. To test this hypothesis further, the daily excretion of urinary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) was determined in 19 children (5 "short normals" and 14 with isolated human growth hormone (HGH) deficiency) who were given ethinyl estradiol (EE) 1.4–14.7 $\mu g/m^2$ per day (2–10 μg/day) for 4 to 7 days. In addition, plasma and urinary gonadotropins and plasma estrogens were serially determined in two prepubertal females(with isolated HGH deficiency) given two injections (24 h apart) of estradiol benzoate, 10 μg/kg. FSH and LH concentrations in plasma and kaolin-acetone urinary concentrates and plasma 17β-estradiol (E_2) and estrone (E_1) were measured by radioimmunoassays. 2–3 $\mu g/m^2$ per day of EE significantly suppressed urinary FSH (and LH when detected in the control period) in two out of six prepubertal children, while all doses > 5 $\mu g/m^2$ per day suppressed urinary gonadotropins to undetectable levels in eight prepubertal subjects. In early to midpubertal subjects, 2–10 $\mu g/m^2$ per day of EE produced a slight suppression of urinary FSH, but failed to suppress to undetectable levels. Two subjects in late puberty (stage 4) did not suppress their urinary FSH while on 7 and 8.3 $\mu g/m^2$ per day. In both subjects treated with estradiol benzoate, plasma FSH promptly decreased after the first injection. Urinary FSH was suppressed to < 0.1 IU/day on day 2 and urinary and plasma gonadotropins remained suppressed for the duration of the study (3 days). Plasma E_2 and E_1 rose from prepubertal values to peak concentrations of 150 to 250 pg/ml (E_2), and 50 and 100 pg/ml (E_1) at approximately 36 h. We conclude that the hypothalamic-pituitary-gonadal axis is operative in the prepubertal child and that the sensitivity of the hypothalamic-pituitary center(s) which control the secretion of FSH and LH decreases at the onset of puberty in man.

INTRODUCTION

Mounting evidence suggests that the gonads in the prepubertal human being are not quiescent and, in fact, play a significant role in regulating the release of gonadotropins (1–4). Previous reports from this laboratory (5, 6) demonstrated that clomiphene citrate, an "anti-estrogen" with mild estrogenic properties, suppresses the excretion of urinary follicle-stimulating hormone (FSH) in prepubertal children. As little as 1 mg/m^2 per day of oral clomiphene citrate decreases urinary FSH in prepubertal children, while approximately 100 mg/m^2 per day is required to inhibit FSH in early to midpubertal subjects. These and other data (summarized in reference 1) suggest that a negative feedback system is operative in prepubertal children and that a decrease in the sensitivity of the negative feedback center is the initiating factor governing the onset of puberty in man. The purpose of the present study was to test this hypothesis further by administering various dosages of potent estrogens to prepubertal and pubertal children.

METHODS

The daily excretion of urinary FSH and luteinizing hormone (LH) was determined in 19 children for 2 to 4 days before and 4 to 7 days during the oral administration of

Dr. Kelch is a trainee in Pediatric Endocrinology under a program sponsored by the National Institute of Arthritis and Metabolic Diseases.

Received for publication 25 April 1972 and in revised form 10 January 1973.

TABLE I

Clinical Characteristics of Study Subjects

Patient	Sex	CA/BA* (yr-mo/yr-mo)	Pubertal stage	Diagnosis and therapy‡
1 A. M.	M	11-2/8	1	Isolated HGH deficiency: Off treatment for 5 mo
2 I. H.	M	8-1/5	1	" " " On treatment for 19 mo
3 J. R.	M	9-5/7	1	" " " On treatment for 34 mo
4 E. P.	M	11-11/10-6	1	" " " On treatment for 27 mo
5 D. P.	M	6-7/5	1	" " " On treatment for 9 mo
6 M. R.	M	13-4/10-9	1	" " " On treatment for 13 mo
7 W. W.	M	9-3/6	1	" " " Pretreatment
8 M. O.	M	9-8/5	1	" " " Pretreatment
9 L. A.	F	10-9/8-10	1	Short normal
10 S. D.	M	8-9/4-6	1	Isolated HGH deficiency: Pretreatment
11 T. F.	F	9-4/7-10	1	" " " Pretreatment
12 E. M.	M	11-6/8	1	Short normal
13 C. N.	F	8-1/5-9	1	Isolated HGH deficiency: Pretreatment
14 R. G.	M	7-5/3	1	Short normal
15 G. K.	M	13-8/11	2	Isolated HGH deficiency: On treatment for 33 mo
16*a* J. S.	M	13-11/13	2	" " " On treatment for 42 mo
b J. S.	M	14-10/13-6	3	" " " On treatment for 53 mo
17 T. K.	M	14-2/11	3	Short normal
18 K. M.	M	14-10/15	4	" "
19 A. G.	F	13-9/13	4	Isolated HGH deficiency: Pretreatment
20 J. R.	F	12-8/7-10	1	" " " On treatment for 17 mo
21 S. H.	F	10-1/8-10	1	" " " On treatment for 5 yr

* Chronologic age/bone age.

‡ HGH-deficient patients are treated with 2–4 mg HGH, i.m., 3 times/wk.

ethinyl estradiol (1.4–14.7 $\mu g/m^2$ per day).[1] The study group (Table I) consisted of 5 healthy but "short normal" children and 14 children with an isolated deficiency of growth hormone documented as previously described (7, 8). Thyroid and adrenal function studies were normal in all of the subjects. No differences in gonadotropin excretion or in the plasma concentrations of LH and FSH have been noted between normal children and children with isolated growth hormone deficiency. Further, spontaneous pubertal development has occurred in seven out of eight of our patients with isolated growth hormone deficiency who are 14 yr or older (unpublished data).

Pubertal development was carefully assessed in each patient and staged as previously described (1, 9, 10): P1, prepubertal, through stage P5, adult body contours and secondary sex characteristics. One-half of the total daily dose of ethinyl estradiol was given every 12 h throughout the treatment period. In addition, the plasma concentrations of LH, FSH, estrone (E_1),[2] 17β-estradiol (E_2), and the excretion of urinary gonadotropins were serially determined in two prepubertal females with isolated human growth hormone (HGH) deficiency (patients 20 and 21—Table I) who were given two intramuscular injections (24 h apart) of estradiol benzoate, 10 $\mu g/kg$ per injection.

[1] Ethinyl estradiol was selected because it is a potent estradiol derivative, which is effective when administered orally.

[2] *Abbreviations used in this paper:* EE, ethinyl estradiol; E_1, estrone; E_2, 17β-estradiol.

All studies were carried out in the Pediatric Clinical Research Unit under close supervision. Completeness of the urine collections was verified by determining the daily excretion of creatinine. Informed parental consent was obtained. Bone age estimates were determined according to the standards of Greulich and Pyle (11). All statistical analyses were performed by the Mann-Whitney modification of the Wilcoxon-Rank test (12).

FSH and LH concentrations in plasma and kaolin-acetone urinary concentrates were determined by radioimmunoassay as previously described (5, 6, 13, 14). In brief, the highly purified pituitary gonadotropins, LER-869-2 (FSH) and LER-960 (LH) were used for iodination with ^{131}I in both the plasma and urinary radioimmunoassays and as the standards for the plasma specimens: the 2nd International Reference Preparation-human menopausal gonadotropin (IRP-HMG) was used as the urinary standard. 90-ml aliquots of the 24-h urine samples were concentrated to 2.0 ml by a modification of the kaolin-acetone technique of Albert (15). All plasma samples and urinary concentrates were assayed at two or more different dilutions to insure parallelism with their respective standards. Urine samples from each individual were concentrated at the same time and processed in the same assay. To examine the variability of this technique, two 24-h urine collections from two normal adults and one patient with the syndrome of gonadal dysgenesis were processed completely in triplicate. This gave an overall average intraassay coefficient of variation of 9.3% for FSH and 13.8% for LH. The interassay coefficient of variation of a high titer urinary concentrate (eight assays) is

TABLE II

Elution Pattern of Estrone and 17β-Estradiol on Sephadex LH-20 "Microcolumns"

Fraction‡	Benzene:methanol 85:15	Content of eluates
	ml	
A	0.1 × 2	Used for application of plasma extracts
B	0.9	Plasma lipids
C	1.0	C19 and C21 plasma steroids
D	1.0	Estrone area
E	0.4	"Overlap" area
F	1.5	17β-estradiol area

* 9.2 × 0.5 cm (ID), approximately 0.7 g of Sephadex LH-20.
‡ Fractions A, B, and E are discarded. Columns were run at 5.0 ml/h.

11.4% for FSH and 13.9% for LH. In this laboratory, 1 ng of FSH (LER-869) is equivalent to 100 ng LER-907 and 1 ng of LH (LER-960) is equivalent to 40 ng LER-907.

Although estrone cross-reacted significantly (30–60%) in our previously described 17β-estradiol radioimmunoassay (9, 16), we were unable to measure estrone accurately in plasma samples because of low recoveries of estrone and high methodologic blanks. To circumvent these problems, the assays were modified as follows:

After the addition of 1000 cpm each of [^{3}H]estrone (sp act 95 Ci/mM) and [^{3}H]17β-estradiol (sp act 95 Ci/mM), 0.1–2.0-ml plasma portions were extracted twice with 7.0 ml of redistilled, anesthetic-grade diethyl ether.

The combined ether extracts were evaporated to dryness and submitted to column chromatography on 9.2 × 0.5 cm (ID) Sephadex LH-20 "microcolumns" with benzene:methanol, 85:15, as the solvent system (modified after the techniques of Mikhail, Wu, Ferin, and Vande Wiele (17) and Wu and Lundy (18)). The columns were prepared by shortening a thick-walled, Pasteur pipette (12-1301, Bellco Glass, Inc., Vineland, N. J.) and fitting the tip snugly into a Tomac 3-way stopcock (17108, K-75, American Hospital Supply, Evanston, Ill.). A 3 mm diameter silicone bead and a small pledget of glass wool were used to support the Sephadex LH-20 columns. The elution pattern was remarkably constant and is summarized in Table II. These columns have been reused for up to 4 mo, but they must be washed with freshly prepared solvent for at least 90 min before each assay. The appropriate column eluates were evaporated to dryness and dissolved in 1.0 ml of spectrograde methanol; 0.2 ml was removed to monitor procedural losses and the remainder was dried down and submitted to radioimmunoassay. The average recoveries of [^{3}H]estrone and [^{3}H]17β-estradiol were 71.5 and 71.4%, respectively.

A simulated column eluate (1.0 ml for the estrone assay and 1.5 ml for the 17β-estradiol assay) and 0.8 ml of methanol were incorporated into the standard curve tubes. These modifications greatly decreased the methodologic blanks. 2-ml deionized water blanks (N = 17) averaged 4.4±1.1 pg SEM in the estrone assay and 3.0±0.8 pg SEM in the 17β-estradiol assay; blank values less than 5 pg are not subtracted from reported values. Occasionally, blank values greater than 5 pg are obtained and in those instances the blanks are subtracted before correcting for procedural losses.

Previously, we used 0.1 ml of a 1/500,000 dilution of a rivanol-treated and bovine serum albumin-absorbed sheep antiserum to 17β-estradiol-17-succinyl-BSA (kindly supplied by Dr. Raymond Vande Wiele, Columbia University) in the radioimmunoassay. However, the dilute antibody solution

TABLE III

Effect of Ethinyl Estradiol on Urinary Gonadotropins in Prepubertal Children

Patient no.	Dose			Urinary gonadotropins, *IU/day**										
				Control days				Treatment days								
				−4	−3	−2	−1	1	2	3	4	5	6	7	*P*‡	
	μg/day	*μg/m² per day*														
1	2	2.2	FSH			1.10	1.54	1.07	0.33	0.34	<0.05				0.05	
			LH			0.55	0.49	0.49	0.14	<0.1	<0.1				0.05	
2	2	2.6	FSH				0.13	0.98	0.25	0.91	0.43				NS	
3	2	2.3	FSH		0.67	0.65	1.13	0.81	0.23	0.37	0.24				0.05	
4	2	2.0	FSH		0.38	0.21	0.90	0.38	0.64	0.63	0.45				NS	
5	2	2.9	FSH		0.32	0.89	0.15	0.49	0.16	0.26	0.21				NS	
6	2	2.0	FSH		1.13	1.03	0.64	0.26	0.45	0.39					NS	
7	5	6.0	FSH		0.23	0.16	0.11	0.13	0.10	<0.04	→				<0.01	
8	5	5.7	FSH			0.45	0.49	0.37	0.34	0.12	0.08	<0.07			0.05	
9	5	5.6	FSH		0.23	0.77	0.61	<0.1	→						<0.1	>0.05§
10	5	7.1	FSH		0.47	0.31	0.24	0.28	0.16	0.15	0.06	0.09	0.05	0.06	<0.01	
11	5	6.5	FSH		0.52	0.33	0.15	0.11	0.07	0.05	<0.06	→			<0.025	
12	10	10.6	FSH	0.24	0.63	0.20	0.43	0.36	0.07	<0.06	→				<0.025	
			LH	0.38	0.28	0.23	0.32	0.16	<0.1	→					<0.025	
13	10	11.4	FSH		0.12	0.08	0.07	<0.04	<0.02	→					0.05	
14	10	14.7	FSH		0.10	0.07	0.22	0.29	0.06	<0.03	→				<0.01	
Mean ±SEM of control urines			FSH	0.46±0.06												

* LH values are listed only when consistently detected.
‡ Rank test: one-sided alternative used to test for significance of suppression (treatment day (1) is combined with control days).
§ The rapid response on treatment day (1) decreased the level of significance.

was unstable and binding decreased significantly after several days. Addition of Knox gelatin (0.1% wt/vol) to the 0.1 M phosphate-saline buffer (pH 7.0), used to dilute the antibody, increased antibody-binding approximately twofold and assured stability for at least 6 mo. Currently, a final antibody dilution of $1/10^7$ (0.1 ml of $1/10^6$ in a 1.0 ml incubation vol and 12,000 cpm of either [^{3}H]E_1 or [^{3}H]E_2 are used in the radioimmunoassays. This concentration of antibody binds 35% of [^{3}H]E_1 (17 pg) or 50% of [^{3}H]E_2 (25 pg) in the absence of "cold" estrogens. Duplicate determinations of an adult female plasma pool (10 assays) averaged 87±8.6 pg SD/ml for E_1 and 123±14.1 pg SD/ml for E_2. The average and the range of values found in 15 normal adult males were 40±14.5 pg SD/ml (range 20–69 pg/ml) for estrone and 26±7.2 pg SD/ml (range 14–37 pg/ml) for 17β-estradiol. The addition of 100 pg of estrone and 17β-estradiol to duplicate 2.0-ml samples of a charcoal-adsorbed plasma pool from adult males (six assays) gave recoveries of 107±10.8 pg SD (E_1) and 100±8.6 pg SD (E_2).

RESULTS

The effects of ethinyl estradiol on gonadotropin excretion in prepubertal children are summarized in Table III. Urinary FSH decreased in four of the six prepubertal boys who were given 2 μg/day, but the changes were statistically significant in only two patients (1 and 3). In contrast, urinary FSH excretion was suppressed to $<$ 0.1 IU/day in all of the patients who were given 5 or 10 μg/day of ethinyl estradiol; ethinyl estradiol also suppressed the excretion of urinary LH when it was consistently detected in the control period (patients 1 and 12).

The response of pubertal children to ethinyl estradiol treatment was less constant (Table IV). Patients 15 (FSH and LH) and 18 (LH) had apparent increases in gonadotropin excretion. Although all doses of ethinyl estradiol greater than 5 μg/m^2 per day produced sustained suppression of urinary FSH ($<$ 0.1 IU/day) in prepubertal patients, similar amounts (7.0–9.5 μg/m^2 per day) produced statistically significant suppression of urinary FSH in only one of four pubertal patients (*16b*).

The effects of parenteral estradiol benzoate on plasma and urinary gonadotropins in patients 20 and 21 are illustrated in Figs. 1 and 2. In both patients, plasma FSH decreased significantly on the 1st day and remained suppressed for the duration of the study. Plasma LH concentrations also decreased slightly in these patients. Significant suppression of urinary FSH did not occur until the 2nd treatment day. As in most prepubertal subjects, urinary LH was not consistantly detectable by our radioimmunoassay technique which uses a 90 ml portion of the 24 h urine collection. Plasma estrogen concentrations rose from prepubertal levels ($<$ 10 pg/ml E_2, $<$ 20 pg/ml E_1) to peak values of approximately 150 and 250 pg/ml for E_2, and 50 and 100 pg/ml for estrone on day 2.

DISCUSSION

These studies indicate that the hypothalamic pituitary negative feedback area(s) ("gonadostat") in prepubertal

TABLE IV

Effect of Ethinyl Estradiol on Urinary Gonadotropins in Pubertal Children (P2–P4)

Patient no.	Pubertal stage	Dose			Urinary gonadotropins, *IU/day*										
					Control days				Treatment days							
					−4	−3	−2	−1	1	2	3	4	5	6	7	*P**
		μg/day	*μg/m² per day*													
15	P2	2	2.0	FSH			1.07	0.83	0.14	1.65	2.59	2.82				NS‡
				LH			0.11	0.12	<0.1	0.59	0.55	0.97				NS‡
16*a*	P2	2	1.7	FSH				1.73	2.04	0.93	0.65	1.64				0.1
				LH				1.17	2.3	0.73	0.77	1.75				NS
17	P3	10	9.5	FSH		0.88	—	2.0	2.1	1.05	—	0.75	0.21			0.1
				LH		0.24	—	0.63	0.57	0.38	—	0.26	0.16			NS
16*b*	P3	10	7.6	FSH			2.26	4.27	3.54	1.59	2.01	1.95	1.77			0.05
				LH			1.02	2.43	2.06	0.73	1.07	1.76	1.29			
	Mean ±SEM of control urines			FSH	1.86±0.45											
				LH	0.82±0.31											
18	P4	10	7.0	FSH	1.30	0.42	0.55	1.03	0.52	1.75	1.66	0.92	0.66	0.60		NS
				LH	3.54	2.66	2.73	2.75	1.74	3.20	3.60	4.26	3.66	3.50		NS‡
19	P4	10	8.3	FSH	4.03	4.53	5.84	3.08	1.85	0.75	1.21	1.38	3.47	3.53	3.60	NS
				LH	1.12	2.00	2.20	0.90	0.65	0.97	0.55	0.33	—	1.29	1.27	NS
	Mean ±SEM of control urines			FSH	2.60±0.73											
				LH	2.24±0.31											

* Rank test: one-sided alternative used to test for significance of suppression (treatment day [1] is combined with control days).

‡ Treatment values are greater than controls, $P < 0.05$.

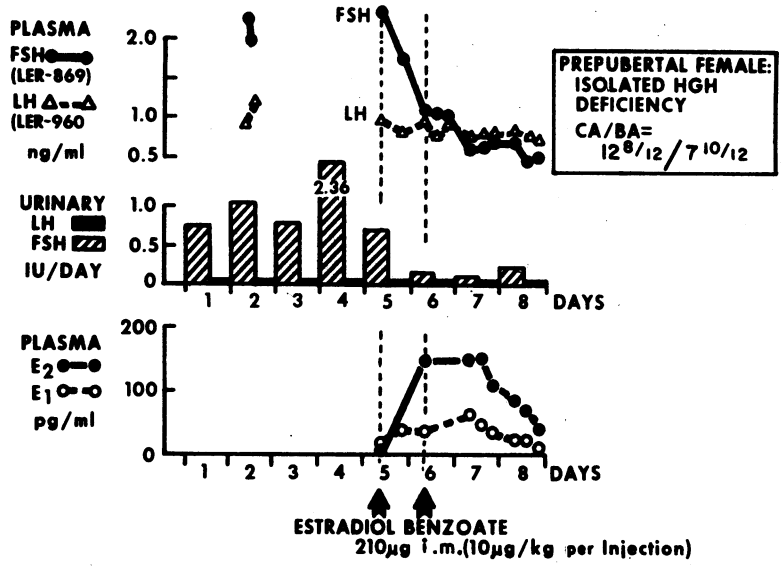

FIGURE 1 The effects of intramuscular estradiol benzoate, 10 μg/kg per dose (arrows), on plasma and urinary gonadotropins and plasma estrogens in a $12\frac{8}{12}$-yr old prepubertal female with isolated HGH deficiency (patient 20). (Suppression of plasma FSH, $P < 0.01$, plasma LH, $P < 0.05$, and urinary FSH, $P < 0.05$.)

humans is highly sensitive to the suppressive effects of ethinyl estradiol. Additional evidence for an intact negative feedback system between the prepubertal gonad and the "gonadostat" was reported from this laboratory by Kulin, Grumbach, and Kaplan (5, 6) who found that as little as 1 mg/m² per day of oral clomiphene citrate, an "anti-estrogen" with weak estrogenic activity, suppressed urinary FSH excretion in prepubertal children. Clomiphene has since been shown to decrease plasma testosterone in prepubertal boys (19) and to suppress plasma gonadotropins in adult males with "hypogonadotropic eunuchoidism" (20). The unexpected finding of gonadotropin suppression in contrast to the stimulatory response elicited in adults given clomiphene citrate (21–24), was attributed to the intrinsic estrogenic properties of clomiphene citrate (25). The current results with ethinyl estradiol, a potent semisynthetic estrogen, support this interpretation.

Suppression of plasma gonadotropins in prepubertal children (Figs. 1 and 2) has not been demonstrated previously. Indeed, the low levels of plasma FSH and LH in prepubertal children and the relative insensitivity of the radioimmunoassays for plasma gonadotropins make it difficult to correlate changes in urinary gonadotropins with plasma values in this age group. Although patients 20 and 21 had no physical signs of pubertal development, they both had consistently detectable plasma FSH concentrations within the broad pubertal range (9). In cross-sectional studies, a significant increase in plasma FSH has been found in females just before the onset of secondary sex characteristics (3). In view of the plasma FSH values, our two patients might be expected to enter into puberty in the near future. Nonetheless, these studies demonstrate that the "gonadostat" in the prepubertal child responds to the natural sex steroids, 17β-estradiol and estrone.

Evidence for an operative negative feedback system in the prepubertal rat and for a decrease in the sensitivity of the "gonadostat" at the onset of puberty has been well established for many years (26–30). Until recently, however, data in man have been lacking. Fitschen and Clayton (31) and Rifkind, Kulin, and Ross (32) clearly demonstrated, by bioassay methods, that prepubertal children excrete FSH and LH. Radioimmunoassay results of plasma and urinary gonadotropins have also demonstrated that normal children have detectable gonadotropins, significantly greater than hypopituitary subjects (13, 14, 33, 34). Penny, Guyda, Baghdassarian, Johanson, and Blizzard (3) detected increased concentrations of serum FSH and statistically significant, but less pronounced increases of serum LH in 5–10-yr old patients with gonadal dysgenesis. Results from this laboratory (1) indicate that plasma FSH is elevated in infancy in patients with gonadal dysgenesis, but plasma LH concentrations do not reach castrate levels until after 11 yr of age. Further, Laron and Zilka (2) noted that "compensatory hypertrophy" occurs in descended testes of prepubertal males with unilateral cryptorchidism. These observations, in light of the current data, strongly support the concept that the gonads regulate the secretion

of gonadotropins in the prepubertal human and that the prepubertal "gonadostat" is highly sensitive to the negative feedback effects of sex steroids.

The effect of EE in the early to late pubertal patients supports the concept that the sensitivity of the "gonadostat" decreases at the onset of puberty. Kulin, Grumbach, and Kaplan (6) found a 100-fold decrease in the sensitivity of the "gonadostat" to the suppressive effects of oral clomiphene citrate. Although we did not determine the amount of EE required to uniformly suppress urinary FSH in pubertal subjects, the relative change in sensitivity to EE seems to be less than that seen with clomiphene citrate. It has been found recently that 40 μg or more of EE is needed to significantly suppress gonadotropin excretion in adult males (Kulin, H., personal communication). Further, we have found that EE (50 μg/day for 5 days) fails to suppress plasma or urinary gonadotropins to within the normal range in adolescent patients (16 to 20-yr old) with the syndrome of gonadal dysgenesis (unpublished data).

Considerable evidence, as recently reviewed by Vande Wiele et al. (35), indicates that estrogens have both negative and positive feedback effects on gonadotropin release in adult females. Yen and Tasi (36) detected a "biphasic" pattern in serum FSH and LH concentrations in seven postmenopausal women treated with 400 μg/day of EE for 5–8 days; initially EE suppressed both serum FSH and LH concentrations, but on the 3rd or 4th day increased gonadotropin concentrations, especially LH, were seen in all seven subjects. Positive responses," or increased gonadotropin excretion, was not observed in any of the prepubertal patients in this study. Patients 15 (P2) and 18 (P4) did have significantly greater urinary FSH and LH excretion while on EE, but these may have been chance occurrences. The absence of positive feedback responses in prepubertal children and the "positive" feedback effects of clomiphene in two girls with advanced puberty (6) suggest that the potential for a positive feedback response appears after midpuberty.

Several points must be considered when interpreting the results of this study. First, urinary excretion has been used as a reasonable and practical index of the pituitary secretion of gonadotropins; striking day to day variations in urinary LH and FSH were noted in this and previous studies (5, 6, 31). Changes in the metabolic clearance of gonadotropins cannot be ruled out, but it seems highly unlikely that they could account for the current findings. Second, since only two of the prepubertal children had detectable urinary LH throughout the control period, only tentative conclusions can be drawn about the relative sensitivity of the areas governing LH secretion in prepubertal and pubertal children. Finally, although this study supports the concept of decreasing sensitivity of the "gonadostat," it does not exclude the presence of other, physiologically important changes at puberty. Indeed, recent work from this laboratory indicates that pituitary responsiveness to synthetic LRF increases at puberty (37).

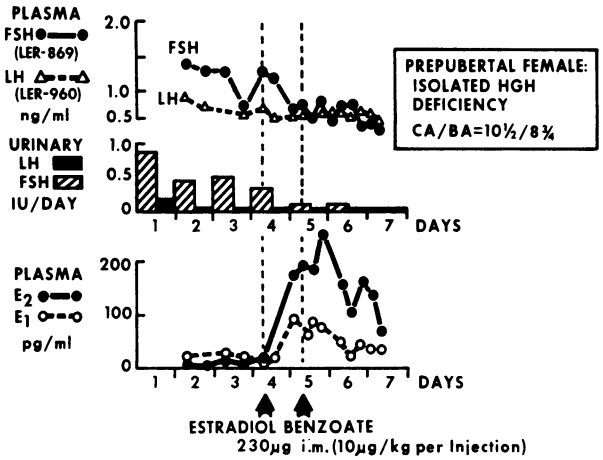

FIGURE 2 The effects of intramuscular estradiol benzoate, 10 μg/kg per dose (arrows), on plasma and urinary gonadotropins and plasma estrogens in a 10½-yr old prepubertal female with isolated HGH deficiency (patient 21). (Suppression of plasma FSH, $P < 0.01$ and urinary FSH, $P < 0.05$.)

ACKNOWLEDGMENTS

We thank the National Pituitary Agency, National Institutes of Health, and the British Medical Research Council for providing purified LH and FSH, FSH antiserum, and 2nd IRP-HMG. The technical assistance of A. Jardiolin, I. Holst, and J. Fruchtman, the secretarial assistance of K. Hayward, and the cooperation of the physicians and nurses of the Pediatric Clinical Research Center are gratefully acknowledged.

This work was supported in part by grants from the National Institutes of Child Health and Human Development, and the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.

REFERENCES

1. Kelch, R. P., M. M. Grumbach, and S. L. Kaplan. 1972. Studies on the mechanism of puberty in man. *In* Gonadotropins. B. B. Saxena, C. G. Beling, and H. M. Gandy, editors. John Wiley & Sons, Inc., New York. 524.
2. Laron, Z., and E. Zilka. 1969. Compensatory hypertrophy of testicle in unilateral cryptochidism. *J. Clin. Endocrinol. Metab.* **29**: 1409.
3. Penny, R., H. J. Guyda, A. Baghdassarian, A. J. Johanson, and R. M. Blizzard. 1970. Correlation of serum follicular stimulating hormone (FSH) and luteinizing hormone (LH) as measured by radioimmunoassay in disorders of sexual development. *J. Clin. Invest.* **49**: 1847.
4. Faiman, C., and J. S. D. Winter. 1971. Sex differences in gonadotropin concentrations in infancy. *Nature (Lond.)*. **232**: 130.

5. Kulin, H. E., M. M. Grumbach, and S. L. Kaplan. 1969. Changing sensitivity of the pubertal gonadal hypothalamic feedback mechanism in man. *Science (Wash. D. C.)*. **166**: 1012.
6. Kulin, H. E., M. M. Grumbach, and S. L. Kaplan. 1972. Gonadal-hypothalamic interaction in prepubertal and pubertal man: effect of clomiphene citrate on urinary follicle-stimulating hormone and luteinizing hormone and plasma testosterone. *Pediatr. Res.* **6**: 162.
7. Goodman, H. G., M. M. Grumbach, and S. L. Kaplan. 1968. Growth and growth hormone. II. A comparison of isolated growth hormone deficiency and multiple pituitary hormone deficiencies in 35 patients with idiopathic hypopituitary dwarfism *N. Engl. J. Med.* **278**: 57.
8. Youlton, R., S. L. Kaplan, and M. M. Grumbach. 1969. Growth and growth hormone. IV. Limitations of the growth hormone response to insulin and arginine and of the immunoreactive insulin response to arginine in the assessment of growth hormone deficiency in children. *Pediatrics.* **43**: 989.
9. Jenner, M. R., R. P. Kelch, S. L. Kaplan, and M. M. Grumbach. 1972. Hormonal changes in puberty. IV. Plasma estradiol, LH and FSH in prepubertal children, pubertal females, and in precocious puberty, premature thelarche, hypogonadism and in a child with a feminizing ovarian tumor. *J. Clin. Endocrinol. Metab.* **34**: 521.
10. August, G. P., M. M. Grumbach, and S. L. Kaplan. 1972. Hormonal changes in puberty. III. Correlation of plasma testosterone, LH, FSH, testicular size, and bone age with male pubertal development. *J. Clin. Endocrinol. Metab.* **34**: 319.
11. Greulich, W. W., and S. I. Pyle. 1959. Radiographic Atlas of Skeletal Development of the Hand and Wrist. Stanford University Press, Stanford. 2nd edition.
12. Tate, M. W., and R. C. Clelland. 1957. Nonparametric and Shortcut Statistics in the Social, Biological, and Medical Sciences. Interstate Printers & Publishers, Inc., Danville, Ill. 89.
13. Burr, I. M., P. C. Sizonenko, S. L. Kaplan, and M. M. Grumbach. 1970. Hormonal changes in puberty. I. Correlation of serum luteinizing hormone and follicle stimulating hormone with stages of puberty, testicular size and bone age in normal boys. *Pediatr. Res.* **4**: 25.
14. Sizonenko, P. C., I. M. Burr, S. L. Kaplan, and M. M. Grumbach. 1970. Hormonal changes in puberty. II. Correlation of serum luteinizing hormone and follicle stimulating hormone with stages of puberty and bone age in normal girls. *Pediatr. Res.* **4**: 36.
15. Albert, A. 1956. Human urinary gonadotropin. *Recent Prog. Horm. Res.* **12**: 227.
16. Kelch, R. P., M. R. Jenner, R. Weinstein, S. L. Kaplan, and M. M. Grumbach. 1972. Estradiol and testosterone secretion by human, simian, and canine testes, in males with hypogonadism and in male pseudohermaphrodites with the feminizing testes syndrome. *J. Clin. Invest.* **51**: 824.
17. Mikhail, G., C. H. Wu, M. Ferin, and R. L. Vande Wiele. 1970. Radioimmunoassay of plasma estrone and estradiol. *Steroids.* **15**: 333.
18. Wu, C. H., and L. E. Lundy. 1971. Radioimmunoassay of plasma estrogens. *Steroids.* **18**: 91.
19. Cathro, D. M., J. M. Saez, and J. Bertrand. 1971. The effect of clomiphene on the plasma androgens of prepubertal and pubertal boys. *J. Endocrinol.* **59**: 387.
20. Santen, R. J., J. M. Leonard, R. J. Sherins, H. M. Gandy, and C. A. Paulsen. 1971. Short- and long-term effects of clomiphene citrate on the pituitary-testicular axis *J. Clin. Endocrinol. Metab.* **33**: 970.
21. Roy, S., R. B. Greenblatt, V. B. Mahesh, and E. C. Jungck. 1963. Clomiphene citrate: further observations on its use in induction of ovulation in the human and on its mode of action. *Fertil. Steril.* **14**: 575.
22. Thompson, R. J., and R. C. Mellinger. 1965. The effects of clomiphene citrate in patients with pituitary-gonadal disorders. *Am. J. Obstet. Gynecol.* **92**: 412.
23. Bardin, C. W., G. T. Ross, and M. B. Lipsett. 1967. Site of action of clomiphene citrate in men: a study of the pituitary-Leydig cell axis. *J. Clin. Endocrinol. Metab.* **27**: 1558.
24. Peterson, N. T., Jr., A. R. Midgley, Jr., and R. B. Jaffe. 1968. Regulation of human gonadotropins. III. Luteinizing hormone and follicle stimulating hormone in sera from adult males *J. Clin. Endocrinol. Metab.* **28**: 1473.
25. Wood, J. R., T. R. Wrenn, and J. Bitman. 1968. Estrogenic and antiesterogenic effects of clomiphene, MER-25, and CN-55,945-27 on the rat uterus and vagina. *Endocrinology.* **82**: 69.
26. Kallas, H. 1929. Puberté précoce parparabiose. *C. R. Seances Soc. Biol. Fil.* **100**: 979.
27. Donovan, B. T., and J. J. Van der Werff ten Bosch. 1965. Physiology of Puberty. Hormones and the Time of Puberty. Edward Arnold Publishers Ltd., London. 38.
28. Ramirez, D. V., and S. M. McCann. 1963. Comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult rats. *Endocrinology.* **72**: 452.
29. Smith, E. R., and J. M. Davidson. 1967. Differential responses to hypothalamic testosterone in relation to male puberty. *Am. J. Physiol.* **212**: 1385.
30. Smith, E. R., and J. M. Davidson. 1968. Role of estrogen in the cerebral control of puberty in female rats. *Endocrinology.* **82**: 100.
31. Fitschen, W., and B. E. Clayton. 1965. Urinary excretion of gonadotropins with particular reference to children. *Arch. Dis. Child.* **40**: 16.
32. Rifkind, A. B., H. Kulin, and G. T. Ross. 1967. Follicle stimulating hormones (FSH) and luteinizing hormone (LH) in the urine of prepubertal children. *J. Clin. Invest.* **46**: 1925.
33. Rifkind, A. B., H. E. Kulin, P. L. Rayford, C. M. Cargille, and G. T. Ross. 1970. 24-hour urinary luteinizing hormone (LH) and follicle stimulating hormone (FSH) excretion in normal children *J. Clin. Endocrinol. Metab.* **31**: 517.
34. Blizzard, R. M., A. Johanson, H. Guyda, A. Baghdassarian, S. Raiti, and C. J. Migeon. 1970. Recent developments in the study of gonadotropin secretion in adolescence. *In* Adolescent Endocrinology. F. P. Heald and W. Hung, editors. Appleton-Century-Crofts, New York. 1.
35. Vande Wiele, R. L., J. Bogumil, I., Dyrenfurth, M. Ferin, R. Jewelewicz, M. Warren, T. Rizkallah, and G. Mikhail. 1970. Mechanisms regulating the menstrual cycle in women. *Recent Prog. Horm. Res.* **26**: 63.
36. Yen, S. S. C., and C. C. Tsai. 1971. The biphasic pattern in the feedback action of ethinyl estradiol on the release of pituitary FSH and LH. *J. Clin. Endocrinol. Metab.* **33**: 882.
37. Roth, J. C., R. P. Kelch, S. L. Kaplan, and M. M. Grumbach. 1972. FSH and LH response to luteinizing hormone-releasing factor in prepubertal and pubertal children, adult males and patients with hypogonadotropic and hypergonadotropic hypogonadism. *J. Clin. Endocrinol. Metab.* **35**: 926.