a potent inhibitor, possibly a nucleotide, merits careful attention.

The results shown in Figure 1 provide no evidence for a dissociable inhibitor of GDH in Reye syndrome hepatocytes. The strict linearity between units of GDH activity added and units of activity observed over a wide range of protein concentrations, for Reye specimens measured singly or in the presence of GDH from normal liver, is incompatible with this type of inhibition. If the approximately 50% decrease in GDH activity in Reye syndrome is caused by the conversion of active enzyme into an enzyme.inhibitor complex, then the addition of 100 units of normal hepatic enzyme to the same cuvette should produce a similar degree of inhibition so that mixtures of control and Reye hepatic enzymes would display about two-thirds of the summated, individual activities. A deviation of this magnitude would be detected easily but did not occur.

Moreover, since GDH is only one of the mitochondrial enzymes known to be diminished in activity in Reye syndrome, if *in vivo* inhibition were to be a general explanation for the mitochondrial deficits in Reye syndrome, there would have to be an inhibitor for each affected enzyme, since the inhibitor described by Holt *et al.* (9) was specific for GDH. Although two of six serum samples obtained from Reye syndrome patients during neurologic deterioration contained a substance inhibitory to normal hepatic GDH, the other samples produced effects ranging from weak inhibition to stimulation.

We conclude that the decreased activity of hepatic GDH (and by analogy, other mitochondrial enzyme activities) is not the result of an intracellular inhibitor. The *in vitro* inhibition or stimulation of normal hepatic GDH by substances present in Reye syndrome or control serum is neither directly related to hepatic intracellular events nor to neurologic status.

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Evidence for Decreased Secretion of Gonadotropin-Releasing Hormone in Pubertal Boys during Short-Term Testosterone Treatment

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ABSTRACT. Information about the site(s) of action as well as the age-dependent effects of sex steroids on gonadotropin-releasing hormone and gonadotropin secretion during human puberty is limited. To begin to address these questions, we evaluated the effects of a depot preparation of testosterone (testosterone enanthate) on gonadotropin secretion and pituitary responses to synthetic GnRH in 10, early to mid-pubertal boys who had either isolated GH deficiency (*n*-2) or delayed adolescent maturation (*n*-8). Chronological and bone age ranges were 13 1/12–16 1/12 and 11-14 yr, respectively. Frequent blood withdrawal studies (every 20 min for 20 consecutive h) were performed in the Clinical Research Center over two consecutive weekends. Following each study, gonadotropin responses to GnRH (0.25 μg/kg iv bolus) were determined. During the initial study, all boys showed a sleep-entrained increase in luteinizing hormone (LH) and testosterone (T) secretion; mean nocturnal concentrations of LH and T were 2.3-fold greater than daytime values. At the end of the first study, testosterone enanthate was given im (0, 25, 50, or 75 mg/m^2). Six days later, mean plasma T concentrations were in the

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pubertal to mid adult male range and were constant throughout the day: 25 mg/m², 3.7 ± 0.4 (SE) ng/ml; 50 mg/m², 4.6 ± 0.2 ng/ml; and 75 mg/m², 6.7 ± 0.4 ng/ml. T treatment had no effect on pituitary responses to GnRH: mean LH increment was 8.5 mIU/ml before and 10.0 mIU/ml after T treatment. Plasma LH and follicle-stimulating hormone were dramatically suppressed by T; paired analyses, however, revealed the persistence of slightly greater LH values during sleep in four of the nine treated boys. During the control study, LH pulse frequency averaged 2.8 ± 0.3 pulses/6 h during the day and 3.8 ± 0.3 pulses/6 h during sleep. In boys who received 50 or 75 mg/m² of testosterone enanthate, gonadotropin secretion was profoundly suppressed and LH pulse frequency could not be accurately assessed. However, LH pulse frequency in three boys treated with 25 mg/m² was not different than their control values: control day, 2.6 ± 0.6; control night, 4.1 ± 0.6; treatment day, 2.1 ± 0.5; and treatment night, 3.9 ± 0.3 pulses/6 h. These results imply that reduction of GnRH secretion is the principal feedback mechanism of testicular steroids in pubertal boys. (*Pediatr Res* 19: 112–117, 1985)

Abbreviations

CRC, Clinical Research Center
DHEAS, dehydroepiandrosterone sulfate
E_2, estradiol
FSH, follicle-stimulating hormone
GH, gonadotropin hormone
GnRH, gonadotropin-releasing hormone
IRP, international reference preparation
LH, luteinizing hormone
T, testosterone

Pubertal maturation in man is preceded by a quiescent or inhibitory stage of development which extends from late infancy until the end of the first decade (1, 2). During childhood, or the inhibitory stage, the low rate of gonadotropin secretion is believed to be the result of suppression of GnRH secretion. Two postulated mechanisms for suppression of GnRH secretion have been: 1) increased sensitivity of the hypothalamus, and possibly the pituitary gland, to the negative feedback effects of gonadal steroids, and 2) inhibition by a sex steroid-independent system intrinsic to the CNS. Until recently, many investigators proposed that pubertal development was initiated by the onset of pulsatile secretion of GnRH during sleep (3). Certainly, it is well established that gonadotropin secretion is often strikingly greater during sleep than during the daytime in late prepubertal and early to mid pubertal children (4–11), but this probably represents sleep-related augmentation of previous low level GnRH secretion, rather than the *de novo* onset of GnRH release.

Little is known about the age-dependent effects of sex steroids on GnRH and gonadotropin secretion during human sexual maturation. This lack of information plus our continued interest in mechanisms of pubertal maturation, prompted us to begin to study the feedback effects of the principal sex steroids in pubertal children. This report summarizes results obtained after short-term treatment of boys with T enanthate.

MATERIALS AND METHODS

All studies were performed in the CRC after written informed parental and patient consent had been obtained. These studies were approved by the Human Investigation Committee of the University of Michigan.

Ten boys who had early to mid pubertal physical maturation were admitted to the CRC for two, consecutive weekend studies (Table 1). Eight boys had delayed adolescent development and normal GH secretion. Two boys had isolated GH deficiency and were receiving treatment with human GH which was supplied by the National Pituitary Agency of the University of Maryland and the NIAMDD. All boys were studied before and after T had been added to their therapeutic programs. The patients were in good general health and were shown to have normal thyroid and adrenal gland function. Skeletal maturation was assessed by the standards of Greulich and Pyle (12).

Patients were admitted to the CRC by 1800 h on a Friday afternoon before the first day to allow acclimatization to the unit (13). Patients were allowed to ambulate freely until 2200 h, when they were required to retire to their beds and room lights were turned off. Sleep was monitored visually by trained nursing personnel. A 21-gauge scalp vein needle (Butterfly infusion set, Abbott Laboratories, North Chicago, IL) was placed in a forearm vein at 0800 h on Saturday, the 1st study day, and was maintained patent by intermittent injections of a dilute solution of heparin in normal saline throughout the study. Blood samples were obtained every 20 min for 20 consecutive h between 1200 h (Saturday) and 0800 h (Sunday) for determination of LH and FSH. Plasma T and E_2 were measured at 4-h intervals throughout the study day. At 1200 h on Sunday, pituitary responsiveness to synthetic GnRH was assessed by administering 0.25 μg/kg (9 to 14 μg total dose) as an intravenous bolus; blood samples were obtained before injection and at +20 and +40 min. At the end of the GnRH test, T enanthate (0, 25, 50, or 75/mg/m²) was administered intramuscularly. Patients were readmitted on the next Friday afternoon and the study was repeated. Blood withdrawal did not exceed 5% of the patients' estimated blood volume during each weekend study.

LH and FSH radioimmunoassays were performed as previously described (14–16). All samples from an individual patient were analyzed in a single assay. Gonadotropin concentrations were reported as milliinternational units per milliliter of Second International Reference Preparation of human menopausal gonadotropin (2nd IRP-HMG) after conversion from the First International Reference Preparation (1st IRP) of pituitary FSH/LH (Medical Research Council 69/104) which was used as the assay standard. Plasma T, E_2, and DHEAS were measured by established radioimmunoassays (17–19). Calculations for all assays were performed with a computer program described by Duddleson *et al.* (20).

A computer program was developed and used to determine the intraassay variability of replicate samples from individual subjects. We defined a significant LH pulse as a rise from nadir to peak within 40 min which was at least twice as great as the intraassay coefficient of variation of replicate samples from each patient. Values below assay sensitivity were assigned the value of assay sensitivity. Student's unpaired or when appropriate, paired t test was used to determine significant differences ($p < 0.05$).

RESULTS

Table 1 lists selected characteristics of the study patients. Their mean chronologic age was 14.7 yr and their mean bone age was 12.8 yr. All patients had undergone adrenarche as indicated by their pubertal or adult concentrations of plasma DHEAS and all had a clear day/night difference in mean plasma T values.

Control study. Mean plasma concentrations of LH and T as well as LH responses to synthetic GnRH before and after treatment are listed in Table 2. Selected results from six patients are illustrated in Figures 1 and 2. All patients had significantly greater LH values at night before treatment; mean nocturnal LH values were 2.3-fold greater than daytime values (range 1.7- to 3.3-fold increase). Similarly, the mean nocturnal concentrations of T were 2.3-fold greater than daytime values (range 1.5- to 4.0-fold increase). During the control study, nocturnal concentrations of FSH were significantly greater than daytime values in six patients; in the remaining four patients, FSH values were often at or below assay sensitivity and no significant circadian pattern could be demonstrated. Plasma E_2 concentrations averaged 28

Table 1. *Characteristics of study patients*

Patient Number	Diagnosis	Chronological age/ bone age (yr)	T enanthate dosage (mg/m^2)	Plasma DHEAS ($\mu g/dl$)	Mean plasma T before treatment (ng/ml)	
					Day	Night
1	Delayed adolescence	14 3/12/11 6/12	0	152	0.3	1.0
2	Isolated GH deficiency	15 3/12/13	25	117	1.9	5.4
3	Delayed adolescence	15 2/12/14	25	157	0.7	1.4
4	Delayed adolescence	14 5/12/13	25	96	1.7	4.7
5	Isolated GH deficiency	16 1/12/14	50	220	1.8	4.2
6	Delayed adolescence	15 6/12/12 6/12	50	106	2.4	6.5
7	Delayed adolescence	15 8/12/13 6/12	75	242	2.2	3.2
8	Delayed adolescence	14 7/12/12 6/12	75	95	0.5	1.4
9	Delayed adolescence	13 6/12/13	75	245	0.6	0.9
10	Delayed adolescence	13 1/12/11	75	66	0.3	1.2

Table 2. *Plasma LH, T, and LH responses to synthetic GnRH (0.25 μg/kg iv) before and after T therapy*

Patient	T enanthate dosage (mg/m^2)	Pre			Post			
		LH (mIU/ml)*		ΔLH (mIU/ml)	LH (mIU/ml)*		ΔLH (mIU/ml)	Post treatment mean T (ng/ml)
		Day	Night		Day	Night		
1	0	2.0 ± 0.2	4.9 ± 0.4†	11.3	1.8 ± 0.1	4.6 ± 0.4†	9.9	0.5
2	25	3.7 ± 0.3	6.9 ± 0.4†	1.4	2.7 ± 0.3[b]	3.6 ± 0.3†‡	3.2	2.3
3	25	3.1 ± 0.2	6.2 ± 0.5†	23.8	2.8 ± 0.2	3.0 ± 0.2‡	28.8	5.5
4	25	2.3 ± 0.2	5.8 ± 0.4†	12.7	2.1 ± 0.1	3.8 ± 0.3†‡	17.2	3.3
5	50	5.5 ± 0.2	9.3 ± 0.7†	4.3	2.1 ± 0.1[b]	1.7 ± 0.1‡	4.6	3.9
6	50	2.3 ± 0.1	5.5 ± 0.4†	5.8	0.7 ± 0.1[b]	<0.7‡	5.7	5.3
7	75	3.6 ± 0.2	6.2 ± 0.4†	6.2	1.5 ± 0.1[b]	1.5 ± 0.1‡	4.4	7.6
8	75	1.9 ± 0.1	3.6 ± 0.2†	3.0	0.9 ± 0.1[b]	1.3 ± 0.1†‡	2.2	8.7
9	75	<1.6	5.1 ± 0.6†	15.7	1.6 ± 0.1	2.2 ± 0.1†‡	22.2	3.8
10	75	<0.7	2.3 ± 0.2†	3.8	<0.7	0.7 ± 0.1‡	2.2	7.7

* Mean ± SE.
† N > D, $p < 0.05$.
‡ Post < pre, $p < 0.05$.

pg/ml during the control period and were unchanged throughout the day.

Treatment study. During the treatment study, 6 days after administration of T enanthate, plasma T values (total hydrolyzed T) did not vary significantly throughout the day (Table 2). Mean plasma T was dependent on dosage: 25 mg/m^2, 3.7 ± 0.4 (SE) ng/ml; 50 mg/m^2, 4.6 ± 0.2 ng/ml; 75 mg/m^2, 6.7 ± 0.4 ng/ml. Plasma E_2 averaged 33 pg/ml and showed a slight but statistically insignificant dependence on T dosage.

LH and FSH secretion were profoundly suppressed by treatment with T. Indeed, overall mean LH was suppressed to below 2 mIU/ml in all boys who received either 50 or 75 mg/m^2 of T enanthate, treatment which effected mean plasma T concentrations in the low to mid-range of values for adult men. Persistence of a significant day/night rhythm in LH secretion was detectable in four of the nine treated boys (Table 2). Mean FSH values were suppressed to or below assay sensitivity in all nine boys. In contrast, the boy who did not receive T (patient 1) showed a remarkably consistent pattern of gonadotropin and sex steroid secretion during both studies.

LH pulse frequency and amplitude. Table 3 summarizes data on LH pulse frequency and amplitude analyses of the control study for all boys and of the treatment study for the boys who received the lowest dosage of testosterone enanthate, 25 mg/m^2. During the control study, the two youngest boys (patients 9 and 10) did not have discernible LH pulsations during the day and thus, data from those two time periods have been omitted. A significant nocturnal increase in both pulse frequency and amplitude was found during the control study. However, the lower LH amplitude and mean LH values found during the day would be expected to lead to an underestimation of LH pulse frequency.

After T treatment, only occasional, low amplitude LH pulses were detectable in the boys who received either 50 or 75 mg/m^2: approximately one pulse per every 6 h of observation; mean pulse amplitude 1.1 mIU/ml. Thus, the effects of T treatment on LH pulse frequency could not be accurately assessed in this group. However, all boys who received the lowest dose of T enanthate, 25 mg/m^2, had discernible LH pulses during both the day and night. In those boys (Tables 2 and 3), T treatment significantly suppressed LH secretion, especially nocturnal secretion; LH pulse frequency, however, was not affected.

Pituitary responses to GnRH. Pituitary responses to GnRH were highly variable among patients as is expected in boys this age, but responses were remarkably consistent within individual studies. T treatment had no significant effect on pituitary responses to GnRH: mean ΔLH before, 8.5 mIU/ml; mean ΔLH after, 10.0 mIU/ml. FSH responses were low and inconsistent as is also characteristic of early pubertal boys.

DISCUSSION

Earlier data supported the concept of decreasing hypothalamic-pituitary sensitivity to negative feedback by gonadal steroids as being part of the mechanism of pubertal maturation in man and in lower species (2, 21). However, detailed information about the site(s) of action as well as the age-dependent effects of sex steroids on gonadotropin secretion in children is limited. In this study, we determined the relatively short-term effects of administration of a depot form of T to early and mid-pubertal boys. The dosages of depot T were chosen to achieve mean concentrations of T in the mid-pubertal to average adult male range, 6 days after administration. Treatment with depot T caused a

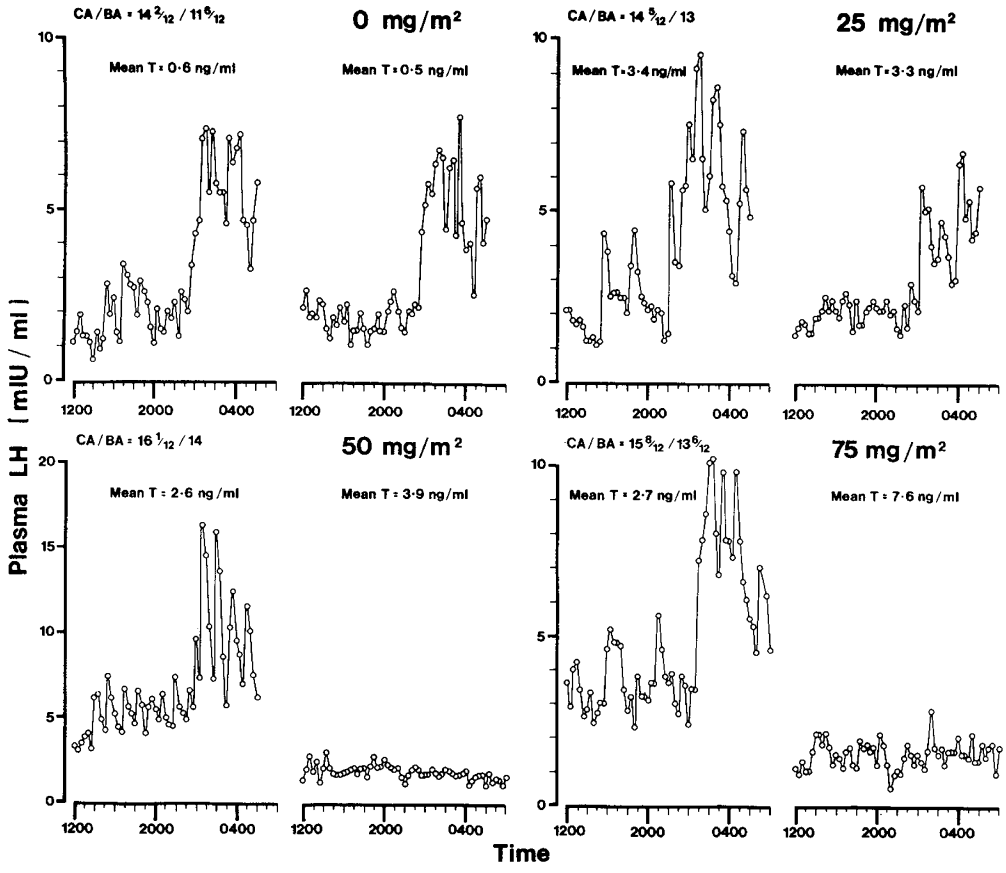

Fig. 1. Plasma LH patterns in four early pubertal boys before and 6 days after initiation of therapy with testosterone enanthate: patient 1, 0 mg/m² (*upper left*); patient 4, 25 mg/m² (*upper right*); patient 5, 50 mg/m² (*lower left*); and patient 7, 75 mg/m² (*lower right*). *CA/BA* indicates chronologic age/bone age in yr. (Refer to Materials and methods for further experimental details.)

striking reduction in gonadotropin concentrations but did not affect pituitary responsiveness to a modest dose of GnRH. These results clearly indicate that under these conditions, the CNS and not the pituitary gland is the principal site of the negative feedback action of sex steroids. Moreover, the principal feedback mechanism of testicular steroids appears to be reduction of GnRH secretion. Reduction of GnRH pulse amplitude seems to be most likely in view of the data obtained from the three boys who received the lowest dosage of T (25 mg/m²): T treatment decreased mean gonadotropin values significantly but did not affect LH pulse frequency.

In an earlier study, we treated similar boys with T enanthate; 2 wk later, we noted that the FSH and LH responses to a 4-h infusion of GnRH were decreased by 66 and 49%, respectively, and that pulsatile release of LH was not discernible during the control hour (22). However, other observations did not lead us to expect to find a major reduction in pituitary responses to GnRH in this study, even if GnRH secretion were decreased markedly by sex steroids (23, 24). For example, we demonstrated previously that abrupt withdrawal of pulsatile GnRH therapy in GnRH-deficient patients leads to subsequent augmentation of pituitary responsiveness (23). Furthermore, Camino-Torres *et al.* (24) demonstrated that T-induced inhibition of pituitary responses to GnRH occurs slowly, after several weeks.

Several groups of investigators have shown that the suppressive effects of T on GnRH responses in men occur slowly. Camino-Torres *et al.* (24) administered either 50 or 200 mg of T enanthate to normal men weekly, for 8 wk. They found that serum T must be increased to 150% of the mean adult male value for 28 days before gonadotropin responses to GnRH are significantly decreased. Furthermore, weekly administration of 50 mg had no effect on basal gonadotropins, and the 200-mg dose gradually decreased basal LH from 8.8 ± 1.3 (SE) to 4.9 ± 0.5 mIU/ml (2nd IRP-HMG) at the end of 8 wk. Comparison of these results with those of the current study clearly support the concept of decreasing sensitivity to negative feedback as being part of pubertal maturation.

In normal men, short-term intravenous infusion of T at twice the normal blood production rate decreased LH pulse frequency but had no effect on responsiveness to GnRH (25, 26). In contrast, short-term infusions of E_2, also at twice the normal blood production rate, have been shown to decrease LH pulse amplitude and gonadotropin responses to GnRH without affecting LH pulse frequency (25). These short-term studies, however, do not appear to reflect the longer term net feedback effects of the major sex steroids in men. After discontinuation of prolonged (88 h) pulsatile GnRH treatment that produced increased gonadotropin T, and E_2 concentrations in normal men, we observed that endogenous LH pulse amplitude was decreased by approximately 50%, but that LH pulse frequency and responses to GnRH were not altered (27). In the current study, T treatment which resulted in increases in plasma T and E_2 and undoubtedly, increased tissue exposure to both steroids, caused striking reductions in gonadotropin secretion but had no effect on pituitary responses to GnRH or discernible LH pulse frequency. These combined observations suggest that an increase in testicular steroids over a period of 3½ to 6 days, regulates LH secretion primarily by reducing GnRH pulse amplitude in both pubertal boys and men. However, as the effects of sex steroids are both time- and dose-dependent, it is important to emphasize that longer treatment with T might be expected to have direct and/or indirect suppressive effects on pituitary responsiveness to GnRH (24).

Results from this study and recent studies by us and others have increased our understanding about the neuroendocrine control of gonadotropin secretion during human sexual matu-

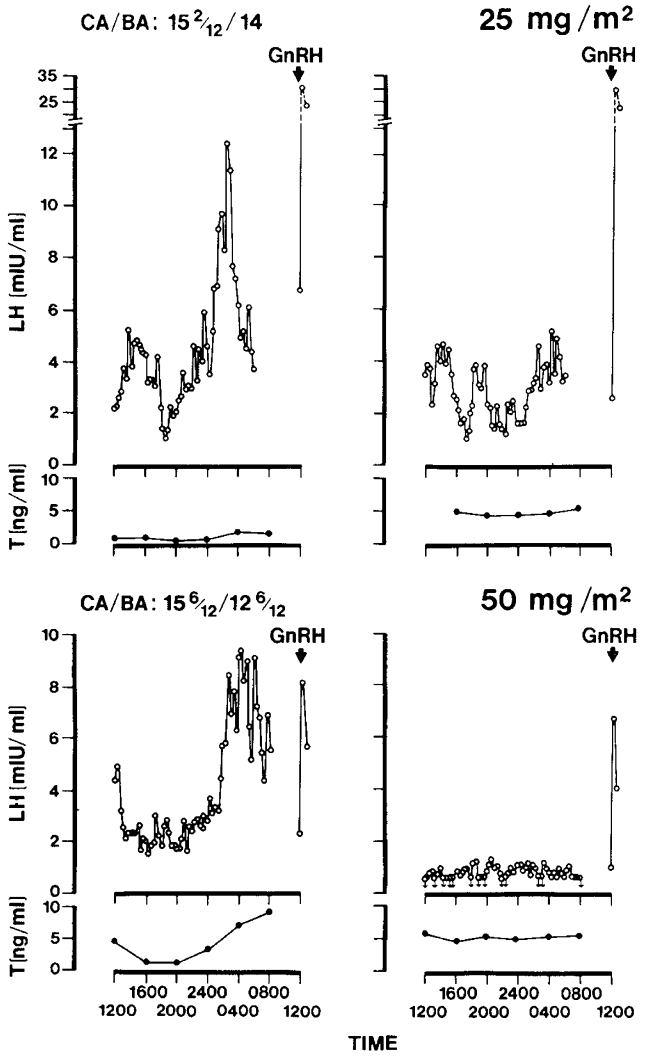

Fig. 2. Plasma LH, T, and LH responses to synthetic GnRH in two early pubertal boys before and 6 days after initiation of therapy with testosterone enanthate: patient 3, 25 mg/m^2 (*top*); and patient 6, 50 mg/m^2 (*bottom*). Synthetic GnRH (0.25 μg/kg) was given as an intravenous bolus at the *arrows*. *CA/BA* indicates chronologic age/bone age in yr. (Refer to Materials and methods for further experimental details.)

Table 3. *LH pulse amplitude and frequency in early pubertal boys (patients 1–10): before and after T enanthate (25 mg/m^2) therapy (patients 2–4)*

	Pulse amplitude (mIU/ml)		Pulse frequency (pulses/6 h)	
Patient	Day	Night	Day	Night
1 through 10:	1.6 ± 0.2	2.9 ± 0.4*	2.8 ± 0.3	3.8 ± 0.3*
2 through 4:				
Pre	1.9 ± 0.5	2.9 ± 0.3*	2.6 ± 0.6	4.1 ± 0.6*
Post	1.4 ± 0.4	1.9 ± 0.5†	2.1 ± 0.5	3.9 ± 0.3*

* N > D, $p < 0.05$.
† Post < pre, $p < 0.05$.

ration. First, pulsatile secretion of LH and presumably GnRH occurs in children (14, 28); in addition, the apparent LH pulse frequency seen in prepubertal and pubertal children is not greatly different than the frequencies reported in adult men and women (2). Second, a sleep-entrained increase in GnRH and gonadotropin secretion is present during childhood (14, 29); this pattern of gonadotropin secretion is augmented during early puberty. In most studies in adults, gonadotropin secretion is relatively similar during the day and during sleep. However, increased pituitary responsiveness to GnRH would make it difficult to detect a modest sleep-entrained increase in GnRH secretion in adults. Finally, endogenous opiates do not appear to be responsible for suppression of GnRH secretion during childhood or early puberty (2, 30). Despite these recent findings, the precise neuroendocrine mechanisms responsible for pubertal maturation remain unknown. The experimental paradigm developed in the current study may allow for additional approaches to further our understanding about the regulation of GnRH secretion. Since, in this model, GnRH secretion appears to be decreased without alteration of pituitary responsiveness to GnRH, studies aimed at stimulating GnRH secretion via selected neurotransmitters and/or opiate blockade may yield valuable information about sex steroid control of GnRH secretion during puberty.

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Red Cell Glycolytic Intermediates and Adenosine Triphosphate in Preterm Infants on the First Day of Life

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ABSTRACT. Red cell glycolytic intermediates and ATP were evaluated in 47 appropriate for gestational age preterm infants on the 1st day of life who were divided into three groups on the basis of gestational age: 28–30, 31–33, and 34–36 wk. The results were compared to those previously obtained in term infants. The concentrations of glucose-6-phosphate, total triose phosphates, and ATP were significantly higher than in term infants but appeared to be appropriately elevated for the young mean age of the red cell population. The concentration of red cell 2,3-diphosphoglycerate (2,3-DPG) was significantly decreased when compared to term infants and was lowest at 28–30 wk gestation. The content of red cell 3-phosphoglycerate was increased in term infants and was inappropriately elevated for the age of the red cell population at 28–30 wk gestation. This pattern of glycolytic intermediates was suggestive of a young red cell population metabolizing at an increased glycolytic rate with increased flow through the phosphoglycerate kinase step rather than the 2,3-DPG bypass in "normal" preterm infants. Two preterm infants of 28–30 wk gestation with low red cell intracellular pH were also evaluated and had markedly decreased concentrations of red cell 2,3-DPG and ATP and all phosphorylated intermediates distal to the phosphofructokinase reaction, indicative of a cross-over at the phosphofructokinase step secondary to acidosis. These studies demonstrate that the "normal" preterm infant has a decreased concentration of red cell 2,3-DPG in the steady state and in the presence of acidosis additional red cell metabolic perturbations occur which lead to a further fall in red cell 2,3-DPG and a decrease in the concentration of red cell ATP. (*Pediatr Res* 19: 117–121, 1985)

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Abbreviations

PFK, phosphofructokinase
G-6-P, glucose-6-phosphate
2,3-DPG, 2,3-diphosphoglycerate
TTP, total triose phosphates
P_i, inorganic phosphorus
AGA, appropriate for gestational age
F-6-P, fructose-6-phosphate
3-PG, 3-phosphoglycerate
2-PG, 2-phosphoglycerate
PEP, phosphoenolpyruvate
RBC, red blood cells
PK, pyruvate kinase
DPGM, diphosphoglycerate mutase
PGK, phosphoglycerate kinase

The pattern of glycolytic enzymes and intermediates in newborn red cells differs from that observed in subjects with a red cell population of a similar mean age (2, 4, 9, 10, 14, 15, 19, 31, 35). Metabolically, these cells appear to consume less glucose