0013-7227/81/1093-0914$02.00/0
Endocrinology

Vol. 109, No. 3
Printed in U.S.A.

Endocrine Correlates of Sexual Development in the Male Monkey, *Macaca fascicularis**

ROBERT A. STEINER AND WILLIAM J. BREMNER

Departments of Obstetrics and Gynecology (R.A.S., W.J.B.), Physiology and Biophysics (SJ-40) (R.A.S.), and Medicine (W.J.B.), and the Regional Primate Research Center (R.A.S., W.J.B.), University of Washington, and the U.S. Veterans Administration Hospital (W.J.B.), Seattle, Washington 98195

ABSTRACT. To increase our understanding of the physiology of sexual maturation in the primate, we have studied various endocrine correlates of reproductive development in the male monkey, *Macaca fascicularis*. In a cross-sectional study, we examined 80 monkeys, ranging in age from 2 weeks to more than 8 yr. We weighed, measured testis size, and obtained a single morning blood sample from all animals. Serum LH and testosterone (T) levels were measured by bioassay and RIA, respectively. Based on the criteria of age and testis size, we classified the monkeys into five study groups: neonates, older infants, juveniles, peripubertals, and adults. To characterize hormone secretory patterns and to search for evidence of episodic LH and T secretion during development, we selected animals at random ($n \geq 6$) from each study group and bled them at 20- to 30-min intervals during evening hours (between 1900–2300 h). To assess pituitary responsiveness to LHRH during development, we administered LHRH (5 μg/kg, iv bolus) to groups of neonatal, older infant, juvenile, and adult animals ($n \geq 4$). LH and T concentrations were integrated over the 90-min postinfusion period. Analysis of serum LH and T levels measured in single morning blood samples showed significantly higher levels of both LH and T in neonatal and older infants, peripubertals, and adults compared to juveniles ($P < 0.02$). The LH responses to LHRH infusions were significantly greater in neonates and adults compared to those in older infants and juveniles ($P < 0.0006$). Neither the neonatal *vs.* the adult nor the older infant *vs.* the juvenile LH responses differed significantly from one another ($P > 0.05$). All peripubertal and adult animals showed evidence of episodic LH and T secretion during the evening sampling interval; each T secretory episode was either coincidental with, or preceded by, a LH increment with a lag time of 30 min or less. Six of six infants and three of eight juveniles demonstrated evidence of episodic variation in serum LH concentrations over the sampling interval; five of six infants and seven of eight juveniles showed episodic variation in serum T levels. These data suggest that episodic LH secretion occurs throughout the life cycle, and that amplitude modulation of episodic LH secretion, caused by a changing amplitude of episodic LHRH secretion, is an important characteristic of the shifts between developmental stages. (*Endocrinology* **109:** 914, 1981)

THE DEVELOPMENTAL patterns of gonadotropin and gonadal steroid levels have been described for several species, as have various changes in body characteristics, such as testicular size, hair growth, and breast development (1, 2). Pubertal development in human beings is more similar to that in other primate species than to that in rats, sheep, and other nonprimates (2–5). Because nonhuman primates appear to offer an important experimental model for human puberty, we have initiated studies of one species of these animals, the crab-eating macaque, *Macaca fascicularis*. To gain an understanding of the endocrine correlates of sexual development in this monkey, we measured LH and testosterone (T) levels from birth to adulthood in normal males. We report evidence for greater secretory activity of the pituitary-gonadal axis of neonatal, older infant, peripubertal, and adult monkeys compared to that of juvenile animals. In addition, we found evidence for the episodic release of LH and T in neonatal, older infant, and juvenile as well as in peripubertal and adult animals.

Received October 9, 1980.

Address requests for reprints to: Dr. Robert A. Steiner, Department of Physiology and Biophysics, SJ-40, University of Washington School of Medicine, Seattle, Washington 98195.

* This work was supported by USPHS Grants 1-RO1-HD12625-01, RR-00166, and P50-HD-12629 and Graduate School research grants from the University of Washington (63-1422 and BMS-301), and the U.S.V.A. Parts of this work were presented at the 62nd Annual Meeting of The Endocrine Society, Washington, D.C., 1980 (Abstract 535).

Materials and Methods

Monkeys

We studied 80 male monkeys of the species *Macaca fascicularis*. The animals ranged in age from 2 weeks to greater than 8 yr (weight range, 0.3–9.6 kg) and were housed at constant temperatures (23 ± 2 C) in cages sealed from outside light, either individually or with their mothers in the case of infants less than 4 months of age. All animals were maintained on a 12-h light, 12-h dark cycle (lights on at 0600 h), and all studies were performed between February and June. We captured the animals by hand after inducing light anesthesia with ketamine-HCl (10 mg/kg, im; Parke-Davis Co., Detroit, MI). Blood samples were obtained either through acutely placed iv catheters or by venipuncture. During all experimental blood-sam-

pling intervals, overhead fluorescent lights were turned on to facilitate blood sampling. Blood samples were permitted to clot overnight at 6 C and then were centrifuged at 1500 × *g* for 30 min at 4 C. Serum was separated, then stored at −20 C. We gauged testis size by measuring the length (l) and width (w) of the left testis with a pair of fine calipers and used the formula for an ellipsoid [volume = ($\pi w^2 l/6$)] to estimate testis volume.

Experimental design

Based on the criteria of age and testis size, we classified the monkeys into five study groups: neonate (age, <3.5 months), older infant (age, 3.5–8 months; testis volume, <0.3 cm^3), juvenile (age, 8–36 months; testis volume, 0.3–3.0 cm^3), peripubertal (age, 36–52 months; testis volume, 3.0–20.0 cm^3), and adult (age, >52 months; testis volume, >20.0 cm^3). To describe the relationship among the developmental factors of age, body weight, testis size, and basal serum LH and T concentrations, we obtained single blood samples from all animals during the morning (0800–1200 h). To characterize hormone secretory patterns and to search for evidence of episodic LH and T secretion during sexual development, we selected animals at random ($n \geq 6$) from each developmental stage and bled them at 20- to 30-min intervals during the evening (1900–2300 h). We deduced from our previous work in adults that sampling during the evening would maximize our chances of seeing episodic hormone patterns if they occurred (6). To assess pituitary and testicular responsiveness to LHRH during development, we administered LHRH (5 μg/kg, iv bolus) between 0800–1000 h to neonatal (n = 4), older infant (n = 4), juvenile (n = 8), and adult animals (n = 4), selected at random. We obtained blood samples at −30, 0, 15, 30, 40, and 90 min relative to LHRH injection.

Hormone assays

We measured T by RIA using reagents supplied by the WHO Matched Reagent Programme. The antiserum was raised against T linked at the 3 position by carboxymethyl-oxime to bovine serum albumin. This antiserum exhibited cross-reactivity of 14% with 5α-dihydrotestosterone and 6% with 5α-androstanediol. Cross-reactivity was 2% or less with the range of other steroids tested. We extracted all serum samples with ether and used dextran-coated charcoal to separate bound from free hormone. All samples were run in duplicate. The assay sensitivity was less than 10 pg/tube (0.1 ng/ml). The intra- and interassay coefficients of variation were 5.1% and 9.8%, respectively.

We measured serum LH concentrations by bioassay (6), using a modification of the procedures described by Van Damme *et al.* (7) and Dufau *et al.* (8). This assay is based on the measurement of T production by dispersed immature mouse Leydig cells. Serial dilutions of serum samples containing high LH levels were shown to be parallel to the standard, LER 1909-2 (relative potency, 0.0032 × NIH-LH-S1). The minimally detectable amount of LH was 3.0 ng/incubation tube (0.15 μg/ml; n = 19). This represents the LH value read from the Leydig cell blank plus 5% of the maximum T produced with excess standard. All samples were run in triplicate in 20-μl volumes. The mean intra- and interassay coefficients of variation for pooled serum from male *Macaca fascicularis* were 14.4% and 24.3%, respectively (n = 12).

Data analysis

Differences in basal serum hormone values among the developmental groups were assessed by analysis of variance on log-normalized transforms of raw data; specific comparisons were made with a Duncan's multiple range test ($P < 0.05$). Determination of significant variations in serum hormone concentrations, within individuals over time, was assessed by one-way analysis of variance with repeated measures (assay replicates). If the variability across time was statistically significant by the test, we termed the pattern episodic. Using a trapezoid area approximation, we integrated (over 0–90 min) the serum LH and T responses to LHRH and subtracted the integrated baseline value. All data are presented as the sample mean ± SE.

Results

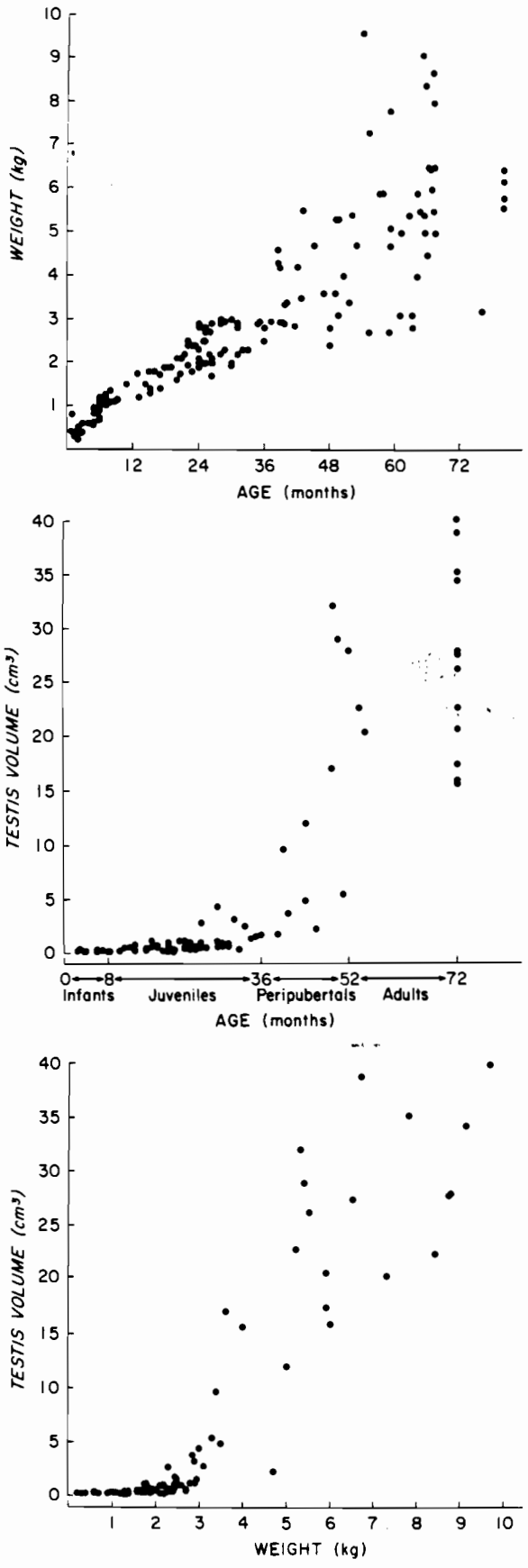
Body weight, testis size and position, genitals, and age

The increases in body weight and testis size that occurred with aging are shown in Fig. 1. The rate of increase in body weight (Fig. 1, *top panel*) was essentially linear until approximately 36 months of age, when the variability increased markedly. The rate of increase in testis size (Fig. 1, *middle panel*) was low and stable until approximately 36 months, when a marked increase in the rate of testis growth occurred. The percentage increase in testis size exceeded that of body weight for these animals, when their weights were between approximately 3.0–5.5 kg (Fig. 1, *bottom panel*). Using these data, we classified the animals into four groups, as described in *Materials and Methods* and as shown in the *middle panel* of Fig. 1.

In animals less than 3 months of age (<0.6 kg), the testes were located in a position low in the inguinal canal near the upper border of the scrotum. The scrotum of these neonatal animals was swollen and exhibited conspicuous folds of darkened skin; the penis was also mildly pigmented. In animals between 6–15 months of age (between 1.2–1.5 kg), the testes were located high in the inguinal canal. The scrotum showed less folding, and the penis and scrotum were less pigmented than those in the neonates. By approximately 22 months of age (~2.0 kg), the testes had again descended down toward the lower reaches of the inguinal canal, and by 28 months (~2.8 kg), the testes approximated the scrotal border. Beginning at approximately 44 months of age, the testes of most animals had descended into the scrotum, which again had developed a pigmented appearance and showed conspicuous rugae, reminiscent of the neonates.

Basal serum LH and T levels

Serum LH and T concentrations, measured in single morning blood samples from each of the developmental



stages, are shown in Fig. 2 (*top panel*). The neonatal and older infants, peripubertals, and adults all showed significantly higher mean serum LH levels than juveniles ($P = 0.02$). Analysis of T concentrations among groups required the exclusion of the peripubertals because they apparently did not represent a homogeneous population, as indicated by the variance for that group. For mean serum T concentrations, each group (excluding peripubertals) differed significantly from all others ($P < 0.0001$). Individual serum T values from 9 of 16 infants fell into the adult range (>1.5 mg/ml), whereas only 1 of the 45 values for juveniles were in the adult range. Basal serum LH concentrations were significantly higher in adults than in all other groups, which did not differ from one another.

Pituitary responsiveness to LHRH

Integrated LH and T responses to LHRH injections are shown in Fig. 2 (*bottom panel*) for groups of neonatal and older infant, juvenile, and adult animals. The LH and T responses of the neonatal and adult groups were significantly greater than those of the older infant and juvenile groups ($P < 0.0001$). The T response of the adult group was significantly greater than those of all other groups ($P = 0.006$). No other differences were demonstrable.

Patterns of circulating LH and T secretion

Examples of the evening pattern of LH and T in serum from infant, juvenile, peripubertal, and adult animals are shown in Fig. 3. Hormone concentrations are presented on a logarithmic scale which diminishes the apparent amplitude of changes, but allows comparison of wide-ranging values among groups. In this format, the range of the vertical excursion of each line graph delineates the same relative change, regardless of the baseline value.

All peripubertal and adult animals demonstrated evidence of episodic LH and T secretion over the 3- to 4-h sampling interval. In these groups, each T secretory episode was preceded by or coincident with an unequivocal LH increment, with a lag time of 30 min or less between the first recognizable increase in LH and that of T. Six of six infants and three of eight juvenile animals demonstrated significant variation in serum LH concentrations over the sampling interval; five of six infants and seven of eight juvenile animals showed episodic variation in serum T levels.

FIG. 1. *Top panel*, Increase in body weight with age in normal male *Macaca fascicularis*. *Middle panel*, Increase in volume of left testis with age. *Bottom panel*, Increase in volume of left testis with weight. Each *point* depicts the value for one monkey.

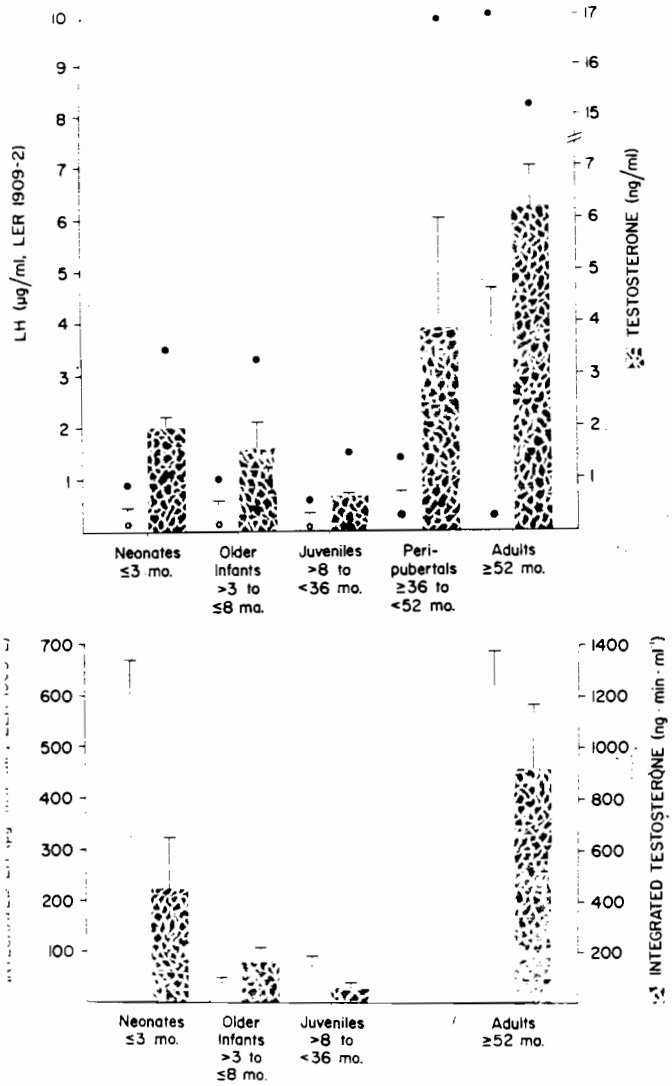


FIG. 2. *Top panel*, Mean (±SE) serum LH and T levels measured in ngle morning blood samples in groups of neonates (n = 12 and 9 for H and T, respectively), older infants (n = 11 and 7), juveniles (n = 45 ıd 40), peripubertals (n = 9 and 7), and adult animals (n = 16 and 20). ', Range of values observed. *Bottom panel*, Integrated (0-90 min) LH ıd T responses to LHRH (5 μg/kg, iv bolus; n = 4 in all cases except ıveniles, where n = 8). The *vertical lines above the bars* show the SE.

Discussion

Our results document the changes in body weight, ·stis size, and serum LH and T levels that occur between irth and adulthood in the *Macaca fascicularis*. Testis ze increases gradually with age and body weight until pproximately 36 months and 3 kg, when a phase of ıuch more rapid growth begins. This rapid growth phase ısts until the animals are approximately 52 months old ıd weigh about 5.5 kg. We have used these changes in ·stis size and age to define stages of development and to escribe basal and LHRH-stimulated LH and T levels at ıe various stages. Our observations on the position of

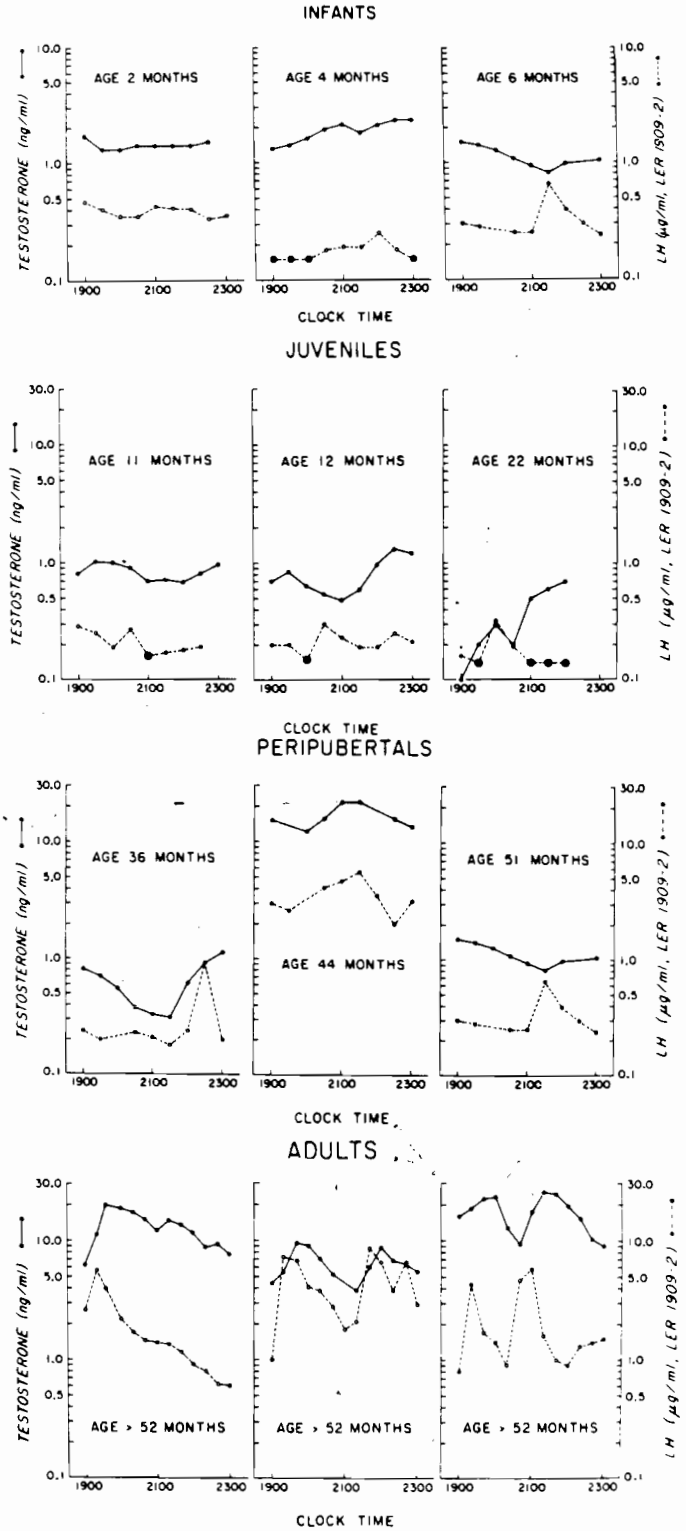


FIG. 3. Examples of the evening patterns of serum LH and T levels in infant, juvenile, peripubertal, and adult animals. ⊙, Serum values that were at or below the minimum detectable limits of the LH bioassay.

the testes show that in the neonatal *Macaca fascicularis*, concomitant with the neonatal activation of the pituitary-testicular axis, the testes are located very near the

scrotal border. Within a few months of birth, the testes ascend into the upper reaches of the inguinal canal. Then, concomitant with increasing plasma LH and T levels, the testes descend permanently into the scrotum. In the early postnatal period, the penis and scrotum appear mildly pigmented, and the scrotum shows conspicuous rugae. Within a few months, the scrotal folds become flattened and the pigmentation disappears until gonadal reactivation at the time of puberty, when the pigmentation reappears, and the scrotum again develops conspicuous rugae. These findings are similar to the early descriptions by Wislocki (9) of genital development of an unidentified species of macaque.

Our findings suggest that during the neonatal period, the testis exhibits a phase of augmented secretory activity. This early postnatal activation of the gonad is also characteristic of human development (10, 11). Similar data have been reported by Robinson and Bridson (12) in the pigtail monkey *Macaca nemestrina*, by Frawley and Neill (13) in the rhesus monkey, *Macaca mulatta*, and by Winter *et al.* (11) in the chimpanzee, *Pan troglodytes*. Plant (14) demonstrated that castration of neonatal rhesus monkeys results in an abrupt increase of circulating LH levels, which subsequently decline by the fifth postnatal month to near-nondetectable values. Together, these reports document the similarity among primate species in the increased activity of the pituitary-testicular axis during the neonatal period and in the marked decline of this secretory activity within the first several months of postnatal life.

Neonatal animals exhibited large LH and T responses to LHRH. These responses in the youngest animals were greater than those in older prepubertal animals, and for LH, were as great as those found in adults. A possible explanation of these data is that there may be a relatively high production of endogenous LHRH in neonatal animals, which leads to an increase in the acutely releasable pool of LH (15, 16). Animals of all ages exhibited unequivocal LH secretory episodes in response to exogenous LHRH administration. These data demonstrate that the pituitary, even in the juvenile animals, is capable of responding to LHRH. The fact that juvenile animals exhibit lower LH levels and lack discrete LH secretory episodes of the type seen in adults is consistent with the possibility that the amplitude of episodic LHRH secretion is lower in juvenile than in adult animals.

We (6) and Plant (17) have demonstrated previously that normal adult male monkeys show clearly episodic LH and T secretion, with each LH increment eliciting a T secretory episode. In the present work, we have performed frequent sampling over several hours in animals at each developmental stage. We found clear evidence for episodic LH secretion in peripubertal as well as adult animals, with increases in blood T levels after the LH secretory episodes. In the neonatal, older infant, and juvenile animals, statistically significant variations in plasma LH and T levels occurred over the sampling interval. The fact that only three of eight juvenile animals demonstrated significant variability in plasma LH levels may have been due to the fact that their LH levels were frequently near or below the sensitivity of the assay. This time course of LH and T levels in the infant and juvenile animals suggests that endogenous LHRH stimulation of the pituitary may be occurring in an episodic fashion in the prepubertal state, as is generally accepted to be the case in older animals. If this were true, it would suggest that amplitude modulation of episodic LHRH secretion is an important characteristic of the shifts between developmental stages. For example, although pituitary responsiveness to LHRH is equal in the neonatal and adult animals, spontaneous LH pulses are of much lower amplitude in the neonatal animals. This implies that the amplitude of endogenous LHRH pulses is lower in neonatal animals than in adults. In support of the concept of amplitude modulation of endogenous LHRH production are the data of Penny and coworkers (18) demonstrating episodic LH secretion in prepubertal children at a frequency similar to that in adults, and the observations of Bourguignon and coworkers (19) showing low but measureable quantities of LHRH-like material in the urine of prepubertal children. It is also possible, however, that the variability we have demonstrated in LH and T levels in prepubertal animals could reflect intrinsic pituitary and gonadal variabilities and may not be a function of episodic endogenous LHRH secretion.

Acknowledgments

We thank Pat Pieters, Pat Payne, Judy Tsoi, and Vasumathi Sundarraj for their excellent technical support and Pat Roberts, Pam Nichols, Lan Nguyen, and Sylvia Cotton for their fine photographic, secretarial, and editorial assistance. We are indebted to Stan Crossman, Glen Knitter, and Bill Morton of the staff of the Regional Primate Research Center and to Tim Beamer, Joe Bielitzki, and Jerry Blakely on the staff of the Primate Field Station at Medical Lake, WA, for their cooperation. We also thank Ayerst Laboratories (Rouses Point, NY) for their kind gift of LHRH. We are grateful for the help of Helen Conner, Mike Gilbert, and Wade Nishimoto, who were undergraduates at the University of Washington.

References

1. Styne DM, Grumbach MM 1978 Puberty in the male and female: its physiology and disorders. In: Yen SSC, Jaffe RB (eds) Reproductive Endocrinology. Saunders, Philadelphia, p 189
2. McCann SM, Ojeda S, Negro-Vilar A 1974 Sex steroids, pituitary and hypothalamic hormones during puberty in experimental animals. In: Grumbach MM, Grave GD, Mayer FE (eds) Control of the onset of puberty. Wiley and Sons, New York, pp 1–31
3. Schwartz NB, Dierschke DJ, McCormack CE, Waltz PW 1977 Feedback regulation of reproductive cycles in rats, sheep, monkeys, and humans, with particular attention to computer modeling. In: Greep RO, Koblinsky MA (eds) Frontiers in Reproduction and

Fertility Control. MIT Press, Cambridge, p 55
4. Ramirez VD 1973 Endocrinology of puberty. In: Greep RO (ed) Handbook of Physiology. Williams and Wilkins, Baltimore, p 1
5. Bremner WJ, Cumming IA, Williams DM, deKretser DM, Lee VWK, The effect of intrauterine castration on fetal and neonatal gonadotropin secretion in the lamb. J Reprod Fertil, in press
6. Steiner RA, Peterson RP, Conner Yu, JYL, H, Gilbert M, ter-Penning B Bremner WJ 1980 Ultradian LH and testosterone rhythms in the adult male monkey, *Macaca fascicularis*. Endocrinology 107:1489
7. Van Damme MP, Robertson DM, Diczfalusy E 1974 An improved *in vitro* bioassay method for measuring luteinizing hormone (LH) activity using mouse leydig cell preparations. Acta Endocrinol (Copenh) 77:655
8. Dufau ML, Hodgen GD, Goodman AL, Catt DJ Bioassay of circulating luteininzing hormone in the rhesus monkey: comparison with radioimmunoassay during physiological changes. Endocrinology 100:157
9. Wislocki GB 1933 Observation on the descent of the testes in the macaque and the chimpanzee. Anat Rec 57:133
10. Forest MG, Sizonenko PC, Cathiard AM, Bertrand J 1974 Hypophysiogonadal function in human during the first year of life. I. Evidence for testicular activity in early infancy. J Clin Invest 53: 819
11. Winter JSD, Faiman C, Hobson WC, Prasa AV, Reyes FI 1975 Pituitary-gonadal relations in infancy. Patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. J Clin Endocrinol Metab 40:545
12. Robinson JA, Bridson WE 1978 Neonatal hormone patterns in the macaque, I. Steroids. Biol Reprod 19:773
13. Frawley LS, Neill JD 1979 Age related changes in serum levels of gonadotropins and testosterone in infantile male rhesus monkeys. Biol Reprod 20:1147
14. Plant TM 1980 The effects of neonatal orchidectomy on the developmental pattern of gonadotropin secretion in the male rhesus monkey (*Macaca mulatta*). Endocrinology 106:1451
15. Bremner WJ, Paulsen CA 1974 Two pools of luteinizing hormone in the human pituitary: evidence from constant administration of luteinizing hormone-releasing hormone. J. Clin Endocrinol Metab 39:811
16. Bremner WJ, Fernando NN, Paulsen CA 1977 The effect of luteinizing hormone-releasing hormone in hypogonadotrophic eunuchoidism. Acta Endocrinol (Copenh) 86:1
17. Plant TM 1980 Neuroendocrine basis of the diurnal variation of testicular testosterone secretion in the adult rhesus monkey (*Macaca mulatta*). In: Steinberger A, Steinberger E (eds) Testicular Development, Structure and Function. Raven Press, New York, p 419
18. Penny R, Olambiwonnu NO, Frasier SD 1977 Episodic fluctuations of serum gonadotropins in pre- and postpubertal girls and boys. J Clin Endocrinol Metab 45:307
19. Bourguignon J-P, Hoyoux, C, Reuter A, Franchimont P 1979 Urinary excretion of immunoreactive luteinizing hormone-releasing hormone-like material and gonadotropins at different stages of life. J Clin Endocrinol Metab 48:78