PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 169, 494–500 (1982)

Sex-Dependent Gonadotropin Concentrations in Infant Chimpanzees and Rhesus Monkeys[1] (41380)

G. B. FULLER,* C. FAIMAN,†‡ J. S. D. WINTER,§ F. I. REYES,‖ AND W. C. HOBSON*

Reproductive Biology Division, Primate Research Institute, New Mexico State University, Holloman AFB, New Mexico 88330; Departments of §Paediatrics, †Physiology, and ‡Medicine, University of Manitoba, Winnipeg, Manitoba R3E 0Z3, Canada; and ‖Division of Reproductive Endocrinology, Department of Obstetrics & Gynecology, SUNY Downstate Medical Center, Brooklyn, New York 11203

Abstract. Concentrations of circulating FSH and LH were determined serially during the first year of life in chimpanzees and rhesus monkeys. FSH levels rose soon after birth and subsequently declined to baseline during the first year in both species, but the magnitude and duration of the FSH peak was less in the monkey. In both species during the first 6 months after birth, FSH levels were significantly higher in females than males. LH levels tended to parallel the FSH pattern in the infant chimpanzees but the greatest postnatal rise was found in males rather than in females. In rhesus monkeys, immunoreactive LH levels remained constant in both sexes during the first year of life. These results support the hypothesis that important developmental endocrine changes occur during infancy in primates. The relationship of these events to later reproductive development and to the "normality" of adult reproductive function remains to be established.

A substantial amount of evidence suggests that the hypothalamic–pituitary–gonadal axis undergoes profound maturational changes during infancy in children. In a cross-sectional study, Winter *et al.* (1) described sex-specific gonadotropin patterns during infancy. Serum FSH concentrations in infant girls were low at birth, rose rapidly during the first 2 weeks until they reached adult castrate-like levels, and eventually declined to a prepubertal nadir by 2–3 years of age. A similar FSH pattern was observed in male infants except that the postnatal FSH rise was significantly smaller and of shorter duration than in the females. Serum LH levels followed a similar course as FSH levels, but showed no sex differences. Other authors have reported similar findings (2). These observations, derived from single serum samples from individual human subjects, were confirmed in a preliminary study utilizing serial samples from one male and one female infant chimpanzee (1).

The existence of a sex-specific pattern of gonadotropin secretion shortly after birth in humans has led to speculation that abnormal patterns found at this time may have their roots in events which occurred during prenatal life and also that abnormalities found in later sexual development might have their genesis in the infant environment (3). Both of these relationships require extensive investigation. Since detailed studies of both normal endocrine patterns and hormone feedback dynamics requiring serial sampling are practically impossible to conduct in normal human infants, the search for an appropriate endocrine animal model of the human infant is necessary. In this communication we describe normal gonadotropin patterns in infant rhesus monkeys and extend our previous observations in infant chimpanzees.

Materials and Methods. *Animals.* Infant chimpanzees were removed from their mothers at birth and maintained in an intensive-care nursery. Birth weights of the animals ranged from 1400 to 1900 g. The conditions of care and feeding in the nursery were similar to those of a hospital nurs-

[1] Supported in part by the Federal Department of Research and Technology of the Federal Republic of Germany, under a coordinated research program entitled "Ecologic–Toxicologic Effects of Foreign Compounds in Non-Human Primates and Other Laboratory Animals."

0037-9727/82/040494-07$01.00/0

ery. Infant rhesus monkeys were left with their mothers in individual cages until weaning at 4 months of age. Birth weights ranged from 400 to 550 g.

Blood samples (3 ml) were collected from the brachial vein of the five male and five female infant chimpanzees on Day 4 after birth and at 14-day intervals thereafter for 1 year. Samples (2 ml) were similarly collected from the infant monkeys (12 of each sex) on Days 4 and 14 after birth, at 14-day intervals until Day 84 and at 28-day intervals thereafter until 1 year of age.

Radioimmunoassays. Chimpanzee LH and FSH radioimmunoassay methods have been described previously (4). These assays are adaptations of homologous human radioimmunoassays. Dilutions of adult and infant chimpanzee sera were parallel in all cases to the standard curve utilizing the human pituitary standard LER 907 (5, 6).

Rhesus LH and FSH assays were those described by Faiman *et al.* (7). Serum concentrations were expressed in terms of the crude rhesus pituitary standard LER 1909-2. The minimum amount of this standard which can be detected by these assays ranges from 100 to 200 ng per tube, or 1–2 μg/ml serum in these studies. Because others (8, 9) using a different radioimmunoassay (10) have reported high levels of a biologically inactive LH-like substance in the serum of pubertal monkeys, we were concerned that the present assay might also be detecting some of this substance. This seems unlikely since the levels measured in the present study are comparatively low and because dilutions of sera from prepubertal rhesus monkeys were parallel to the standard curve. However, pending confirmation of these results by bioassay, we are conservatively calling the substance measured "immunoreactive LH." Assays were evaluated by the quality control system of Rodbard *et al.* (11).

Results. Mean serum FSH concentrations in the infant female chimpanzees were low at birth but rose rapidly by the second week of life and peaked at 2–3 months of age (Fig. 1). There was a gradual decline in FSH levels over the next several months reaching a nadir at approximately 7–8 months of age, after which the levels remained relatively stable. FSH patterns in the male chimpanzees showed little or no change during the first 2 months following birth and then declined to a nadir and remained low for the duration of the study. Peak levels in the males were significantly ($P < 0.05$) lower than females (17.4 vs 50.0 μg/dl, respectively).

In general LH patterns paralleled those of FSH in the infant chimpanzees (Fig. 2). However, the greatest postnatal peak was found in the males (9.6 μg/dl) whose levels were significantly higher ($P < 0.05$) than in the females (3.7 μg/dl) for the first 2 months following birth. Afterward LH levels declined in both sexes and remained unchanged up to one year of age.

The pattern of FSH in infant rhesus monkeys was qualitatively similar to that of the chimpanzees but was compressed in terms of magnitude and time (Fig. 3). In the females FSH peaked during the first month then gradually declined to a nadir by the fifth month following birth. In the males the peak also occurred within the first month but was much lower ($P < 0.05$) than in females (5.8 vs 20.7 μg/ml, respectively), and declined more rapidly. Mean immunoreactive LH levels remained relatively constant in both sexes during the first year of life (Fig. 4).

Discussion. Gonadotropin patterns, especially FSH, during infancy in chimpanzees and rhesus monkeys appear to be remarkably similar to those previously reported in humans (3). The longitudinal data derived from serial sampling of chimpanzees and rhesus monkeys in the present study resemble closely the patterns found by cross-sectional sampling of human infants. An immediate postnatal rise and subsequent fall of FSH levels occurs during infancy in all three species. The timing of the peak and nadir varies among the three species but appears to be correlated with both the length of childhood and life span of the species. The sex difference in FSH levels within the first 6 months of life (higher in females than in males) was present in both chimpanzees and rhesus monkeys as has been reported in human infants (1).

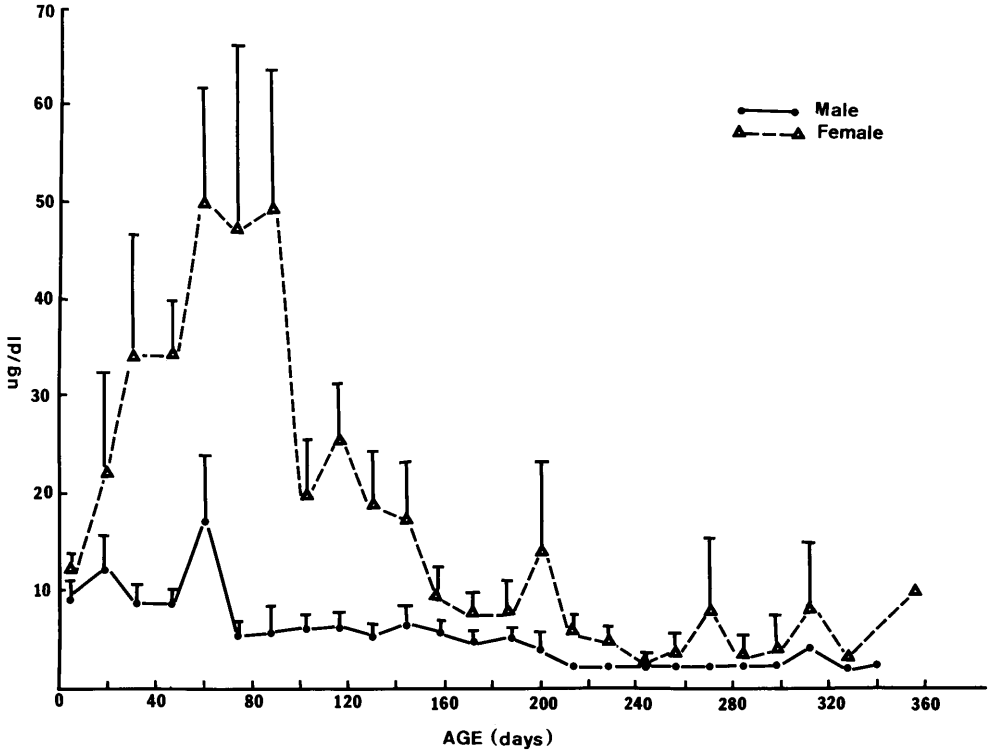

FIG. 1. Serum FSH levels (mean ± SEM) in male and female chimpanzees during the first year of life. Standard errors not shown indicate only one observation point.

Luteinizing hormone levels were elevated in male chimpanzees the first 2 to 3 months following birth as has been observed previously in human males (1). The LH pattern in female chimpanzees was also similar to that in humans, but of lesser magnitude. In contrast, male and female rhesus monkeys exhibited no change in immunoreactive LH levels throughout the first year following birth. Others (12, 26), however, found elevated LH levels during the first 4 months after birth in male rhesus monkeys which subsequently declined to undetectable levels by 6 months. The difference between these and our own results is probably best explained by differences in the assay systems used. It is possible that the assay used in the present study cannot detect changes at the low levels found in infants or, alternatively, that some cross-reaction with the "LH-like substance" is obscuring its ability to see changes in authentic LH.

With the possible exception of LH levels, it is interesting to note that in spite of different rearing conditions (chimps—bottle feeding and long-term nursery care; monkeys—mother rearing; humans—short-term nursery care followed by breast or bottle feeding) neonatal gonadotropin patterns are remarkably similar.

The mechanism responsible for the early postnatal, sex-dependent rise in gonadotropins and their subsequent decline is obscure. The neonatal rise in LH and FSH may be ascribed to the loss of placental steroid suppression. The elevated gonadotropin levels lead to increased gonadal activity in both females and males, as indicated by the finding of increased serum levels of testosterone in male rhesus monkeys (12), chimpanzees (13), and humans (14–16), and by increased estradiol levels in female infants (17, 18). The subsequent decline in gonadotropin levels presents an enigma. Although it has been suggested that

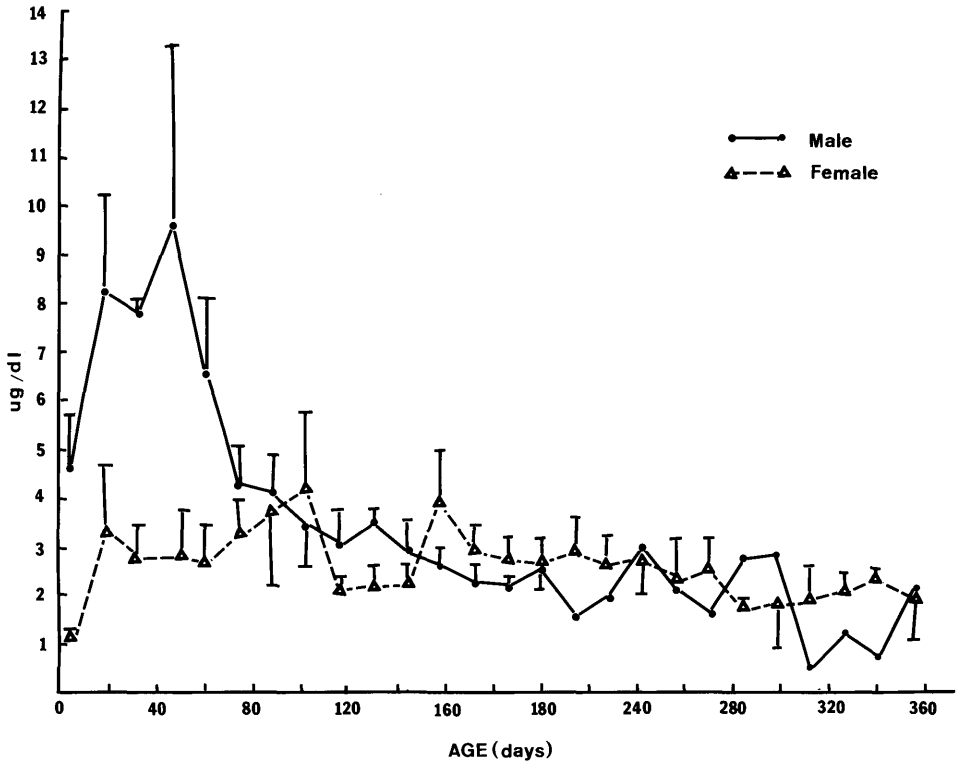

FIG. 2. Serum LH levels (mean ± SEM) in male and female chimpanzees during the first year of life. Standard errors not shown indicate only one observation point.

the decrease is due to an increased sensitivity of the hypothalamic–pituitary axis to gonadal steroids (1, 19–21), the evidence is not entirely convincing. Kelch *et al.* (19) found that estrogen suppressed urinary gonadotropin excretion in prepubertal children whereas they were unable to find consistent suppression of serum LH or FSH levels. Although we have found that estradiol-17β or diethylstilbestrol can suppress chimpanzee and monkey serum FSH levels within the first 4–6 months following birth, there is little or no effect in older infants (unpublished observations).

The rise and fall of serum gonadotropins during infancy appears to correlate well with pituitary responsiveness to gonadotropin-releasing hormone (GnRH). Huhtaniemi *et al.* (22) found that injection of GnRH (10–20 μg/kg) was followed by serum LH increments in infant male rhesus monkeys of 592, 862, 174, and 16% at ages 4, 18–89, 102–218, and 236–392 days, respectively. Plasma testosterone levels increased roughly parallel to LH. Thus, the fall in gonadotropin concentrations during late infancy appears to be, at least in part, due to loss of pituitary sensitivity to GnRH.

Interestingly, there are two other lines of evidence which suggest that gonadal steroids may not play the major role in control of gonadotropin secretion during the period between infancy and puberty. One is that agonadal children exhibit a pattern of gonadotropin secretion similar to normal children from infancy through puberty at a higher level (23, 24). Another is the finding (21, 25) that castration of prepubertal rhesus monkeys older than a year of age does not lead to an immediate rise in immunoreactive LH levels as seen in adults. However, when infant male rhesus mon-

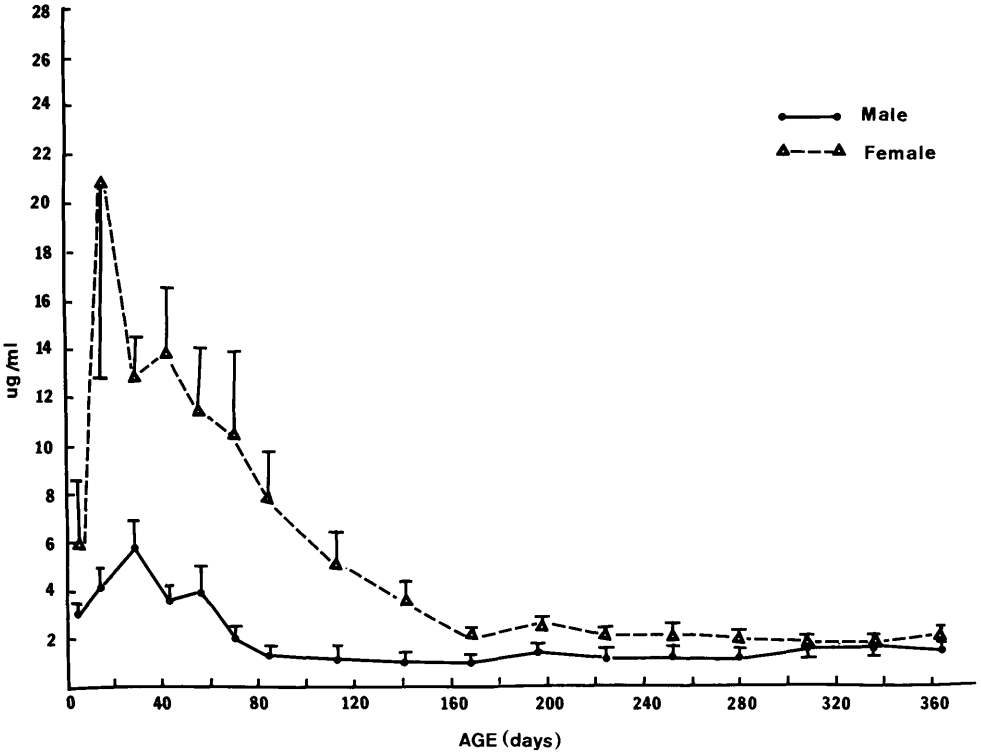

FIG. 3. Serum FSH levels (mean ± SEM) in infant male and female rhesus monkeys.

keys are castrated at birth, an adult-like postcastration increase in LH and FSH levels follows immediately. It is significant, however, that levels return to baseline by 8 months (26).

Our present observations, coupled with previously reported human and chimpanzee infant data indicate a definite sex difference in gonadotropin regulation in infant primates. In addition, these results suggest a

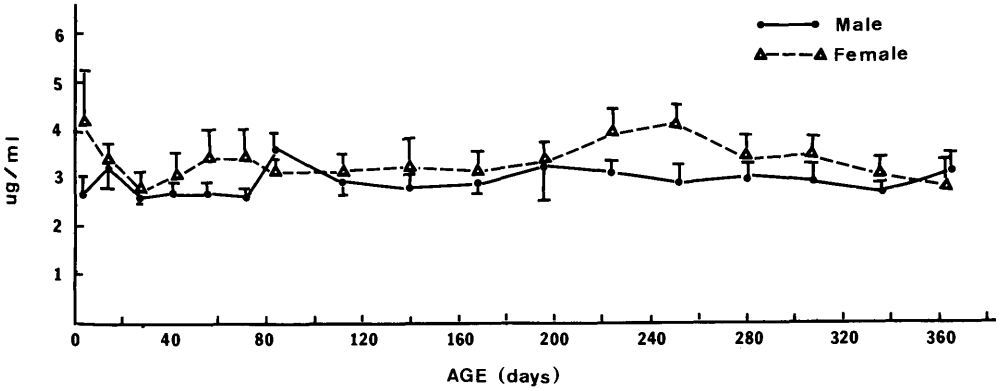

FIG. 4. Serum immunoreactive LH levels (mean ± SEM) in infant male and female rhesus monkeys.

transition from an active endocrine state during infancy to a lengthy quiescent stage which precedes puberty. This change appears to be a reversal of endocrine events during early infancy which seem to be rapidly progressing toward puberty (1). For instance, the rise of gonadotropin and sex steroids is reversed, sensitivity to exogenous LHRH is lost, and the postcastration gonadotropin surge is blunted. The existence of a period of hormonal transformation makes the chimpanzee and rhesus monkey excellent models to assess infant neuroendocrine development. Knowledge of infant endocrinology could permit identification and treatment of sexual and developmental disorders that may have their roots during this period of life.

1. Winter JSD, Faiman C, Hobson WC, Prasad AV, Reyes FI. Pituitary–gonadal relations in infancy. I. Patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. J Clin Endocrinol Metab **40**:545–551, 1975.
2. Penny R, Olambiwonnu NO, Frasier SD. Serum gonadotropin concentrations during the first four years of life. J Clin Endocrinol Metab **38**: 320–321, 1974.
3. Winter JSD, Faiman C, Reyes FI, Hobson WC. Gonadotropins and steroid hormones in the blood and urine of prepubertal girls and other primates. J Clin Endocrinol Metab **7**:513–530, 1978.
4. Howland BE, Faiman C, Butler TM. Serum levels of FSH and LH during the menstrual cycle of the chimpanzee. Biol Reprod **4**:101–105, 1971.
5. Faiman C, Ryan RJ. Radioimmunoassay for human follicle stimulating hormone. J Clin Endocrinol Metab **27**:444–447, 1967.
6. Faiman C, Ryan RJ. Radioimmunoassay for human luteinizing hormone. Proc Soc Exp Biol Med **125**:1130–1133, 1967.
7. Faiman C, Stearns EL, Winter JSD, Reyes FI, Hobson WC. Radioimmunoassay for rhesus monkey gonadotropins. Proc Soc Exp Biol Med **149**:670–676, 1975.
8. Peckam WD, Foster DL, Knobil E. A new substance resembling luteinizing hormone in the blood of rhesus monkeys. Endocrinology **100**:826–834, 1977.
9. Neill JD, Dailey RA, Tsou RC, Reichert LE Jr. Immunoreactive LH-like substances in serum of hypophysectomized and prepubertal monkeys: Inactive in an in vitro LH-bioassay. Endocrinology **100**:856–857, 1977.
10. Niswender GD, Monroe SE, Peckham WD, Midgley AR Jr, Knobil E, Reichert LE Jr. Radioimmunoassay for rhesus monkey luteinizing hormone (LH) with anti-ovine LH serum and ovine LH-^{1} ^{31}I. Endocrinology **88**:1327–1331, 1971.
11. Rodbard D, Rayford PL, Cooper JA, Ross GT. Statistical quality control of radioimmunoassays. J Clin Endocrinol Metab **28**:1412–1418, 1968.
12. Frawley LS, Neill JD. Age related changes in serum levels of gonadotropins and testosterone in infantile male rhesus monkeys. Biol Reprod **20**:1147–1151, 1979.
13. McCormack SA. Plasma testosterone concentration and binding in the chimpanzee: Effect of age. Endocrinology **89**:1171–1177, 1971.
14. Forest MG, Cathiard AM, Bertrand JA. Total and unbound testosterone levels in the new born and in normal and hypogonadal children: Use of a sensitive radioimmunoassay for testosterone. J Clin Endocrinol Metab **36**:1132–1142, 1973.
15. Hammond GL, Koivisto M, Kouvalainen K, Vihko R. Serum steroids and pituitary hormones in infants with particular reference to testicular activity. J Clin Endocrinol Metab **49**:40–45, 1979.
16. Pang S, Levine LS, Chow D, Sagiani F, Saenger P, New MI. Dihydrotestosterone and its relationship to testosterone in infancy and childhood. J Clin Endocrinol Metab **48**:821–826, 1979.
17. Bidlingmaier F, Versmold H, Knorr D. Plasma estrogens in newborns and infants. In: Proceedings, INSERM Symposium on Sexual Endocrinology of the Perinatal Period, pp299–314, 1974.
18. Marshall WA. Steroids after birth-puberty. Postgrad Med J **52**:620–624, 1976.
19. Kelch RP, Kaplan SL, Grumback MM. Suppression of urinary and plasma follicle-stimulating hormone by exogenous estrogens in prepubertal and pubertal children. J Clin Invest **52**:1122–1128, 1973.
20. Grumback MM, Roth JC, Kaplan SL, Kelch RP. Hypothalamic–pituitary regulation of puberty: Evidence and concepts derived from clinical research. In: Grumback MM, Grave GD, Mayer FE eds. Control of Onset of Puberty. Wiley, New York, pp115–166, 1974.
21. Winter JSD, Ellsworth LR, Faiman C, Reyes FI, Hobson WC. Studies of the primate gonadostat: Evidence of secretion of gonadal steroids before puberty and their role in feedback suppression of gonadotropin release. 49th Annual Meeting of the Endocrine Society, 1977 (Abstract).
22. Huhtaniemi IT, Koritnik DR, Korenbrot CC, Mennin S, Foster DB, Jaffe RB. Stimulation of

pituitary–testicular function with gonadotropin releasing hormone in fetal and infant monkeys. Endocrinology **105**:109–114, 1979.
23. Winter JSD, Faiman C. Serum gonadotropin levels in agonadal children and adults. J Clin Endocrinol Metab **35**:561–564, 1972.
24. Conte FA, Grumback MM, Kaplan SL. A diphasic pattern of gonadotropin secretion in patients with the syndrome of gonadal dysgenesis. J Clin Endocrinol Metab **40**:670–674, 1975.
25. Dierschke DJ, Weiss G, Knobil E. Sexual maturation in the female rhesus monkey and development of estrogen-induced gonadotropic hormone release. Endocrinology **94**:198–206, 1974.
26. Plant TM. The effects of neonatal orchidectomy on the developmental pattern of gonadotropin secretion in the male rhesus monkey (macaca mulatta). Endocrinology **106**:1451–1454, 1980.

Received October 20, 1981. P.S.E.B.M. 1982, Vol. 169.