Journal of Steroid Biochemistry, Vol. 11, pp. 563 to 569
Pergamon Press Ltd 1979. Printed in Great Britain

17. Puberty
SEQUENTIAL HORMONAL CHANGES AND ACTIVATION OF THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS

J. R. DUCHARME, S. CATIN-SAVOIE, Y. TACHÉ, B. BOUREL and R. COLLU
The Endocrine Laboratory, Centre de Recherche Pédiatrique, L'Hôpital Sainte-Justine; and Department of Pediatrics, Université de Montréal, Montréal, Québec, Canada

SUMMARY

Plasma dehydroepiandrosterone (DHA) and its sulfate (DHAS), androstenedione (Δ), 17α-hydroxyprogesterone (17-OHP), testosterone (T), oestradiol (E_2) and LH were measured in cord plasma and in plasma samples obtained in normal infants and children 3–18 months and/or 3.5–16 yrs of age. DHAS, Δ, 17-OHP, and T were elevated in cord plasma and decreased to relatively constant levels by 3 months except for T in male infants which was higher at 3 months than in cord plasma. LH was also higher at 3 months in male infants than later up to 18 months. A significant rise in DHA and Δ was noted in girls between 6–8 and 8–10 years of age respectively while these hormones increased 1–2 years later in boys, prior to any significant rise in T and E_2. These studies confirm the reported activity of the hypothalamic-pituitary gonadal axis in early infancy together with the prepubertal adrenal activity leading to gonadotrophin release. Studies in the young lamb showed higher plasma LH and T values at 4–5 weeks and pulsatile release of LH, T and prolactin which were more important at 1 week and 8 weeks than at 4 weeks of age. *In vivo* and *in vitro* responsiveness to HCG was also more important at 1 week while the number of binding sites was identical. A complete dissociation between cAMP and T response to HCG was observed. It is suggested that the newborn lamb may be used as a model for the study of hypothalamic-pituitary gonadal relationships in man.

INTRODUCTION

The ontogeny of hypothalamic-pituitary gonadal relationships in man from fetal life through adolescence has been extensively studied in recent years. Three preferential phases involving gonadotrophin and gonadal steroid secretion are found: in the first trimester of pregnancy, in the second and third months of postnatal life and at puberty [1–7]. It is well recognized since the early work of Jost [8] that the fetal testis, which is under the stimulus of a placental trophoblastic hormone called chorionic gonadotropin or HCG, plays an essential role in masculine sexual differentiation. In addition, the endocrine function of the gonads is under the control of complex neuroendocrine regulatory mechanisms involving pituitary gonadotropins, LH-RH [9–10] and most likely other substances such as biogenic amines of the central nervous system [11]. While it is widely accepted that it is essentially circulating sex steroids which control LH secretion through negative feedback at the hypothalamic or pituitary level and hence gonadal steroid hormone secretion [5, 6], the negative feedback control of FSH is less well understood. A gonadal hormone called "inhibin", a polypeptide presumably secreted by the sertoli cells of the testis and by the ovarian follicles, appears to exert, at least in part, such a role [12].

In the human male infant studies by Forest *et al.*[4], later confirmed by Winter *et al.*[3], have conclusively shown that plasma testosterone levels are elevated at birth in both sexes and rapidly decline thereafter. However, a transient rise in plasma testosterone is observed after the first week in males, reaching maximum levels during the second and the third months of postnatal life, and decreasing to prepubertal levels by seven months of age [3, 4]. Serum androstenedione in the male infant shows a similar trend albeit less marked [3, 4]. Since unbound testosterone levels follow a similar pattern to that of total testosterone, these differences cannot be accounted for by an increase in testosterone binding protein and indicate a true increase in the circulating free steroid [13]. In addition, since the newborn testis is responsive to HCG administration which raises blood testosterone to levels observed in older children and in adults, and since the administration of fluoxymesterone suppresses circulating testosterone levels, it appears evident that an active gonadostat is operative during this period [14].

In order to study further the mechanisms which control early postnatal testicular activation in man, a suitable experimental model had to be found. Although the chimpanzee appears promising in this regard [2], its availability and cost preclude its use in most centres. In the immature male lamb, Lee *et al.*[15] have measured plasma testosterone, LH and FSH levels and have observed a transient rise in all these hormones at 5 weeks of age prior to any evidence of spermatogenesis [16], suggesting its usefulness as a model for the study of the activity of the hypothalamic-pituitary-gonadal axis in early postnatal life.

The present paper reports plasma levels of several hormones in children from birth to 18 months of life, in prepuberty and during pubertal development together with preliminary studies in young immature male lambs.

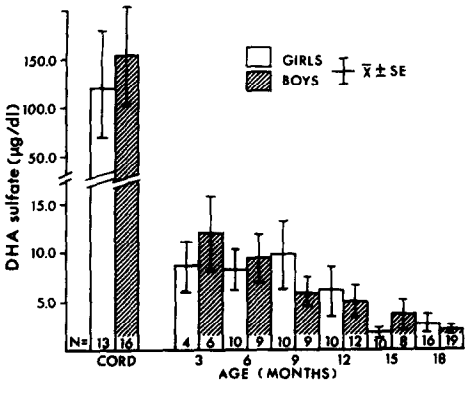

Fig. 1. Dehydroepiandrosterone Sulfate (DHAS) levels in mixed cord and peripheral plasma in female and male infants from 3–18 months of age. The number of subjects (N) is indicated at the bottom of each column. $\bar{X} \pm$ S.E. = Mean ± standard error.

MATERIALS AND METHODS

In the first part of this study, plasma levels of dehydroepiandrosterone sulfate (DHAS) [17], androstenedione (Δ) [18], 17-hydroxyprogesterone (17-OHP) [19], testosterone (T) [18] and LH [20] were measured by specific radioimmunoassays (RIAs) in normal children from birth to 18 months of age. Similarly, plasma dehydroepiandrosterone (DHA), Δ, T and oestradiol (E_2) were measured on a single blood sample obtained in normal children from 3.5 to 16 years of age as previously described [7, 18].

In the second part of this work, heparinized blood was obtained in the young lamb from birth till two months of life by jugular vein puncture and plasma T [18], FSH [21], LH [22] and prolactin [23] determined by specific RIAs.

In the third part of this study, the plasma response to HCG was evaluated in 1, 4 or 8 week old lambs which were subjected to hemicastration immediately prior to HCG administration (A.P.L. Ayerst Laboratories, 500 I.U./kg body weight). Three animals were studied at 1 week, 2 at 4 weeks and 4 at 8 weeks. The number of binding sites to HCG was determined and levels of cyclic adenosine monophosphate (cAMP) and T measured in isolated Leydig cells prepared from non stimulated testes after a 3 h incubation in buffer alone or with added HCG or choleratoxin essentially as described previously [24]. In the second testis obtained after *in vivo* HCG stimulation, cAMP and T content were determined in homogenates of pools obtained from each animal in each experimental group.

RESULTS

I. Hormone levels in normal children from birth through puberty

(A) *DHA and DHAS.* Figure 1 shows the plasma levels of DHAS in mixed cord blood and in plasma samples obtained at three months intervals to 18 months of age in both girls and boys. No sex difference was observed at any age, the very high concentration of DHAS in cord blood gradually decreasing throughout the period of study, a 10-fold decrease being already present by 3 months of age. DHA, which in general follows DHAS, was measured from 3.5 till 16 years and a significant increase was found as early as 6 to 8 years of age in girls and 8–10 years of age in boys [Fig. 2].

(B) *Androstenedione* (Δ). Androstenedione, another steroid mainly of adrenal origin [25], was also elevated at birth but levels equivalent to one fifth those found in cord plasma were already seen at 3 months and remained unchanged until 18 months (Fig. 3). In older children, a significant increase in Δ was found by 8–10 years in girls and 10–12 years in boys (Fig. 2).

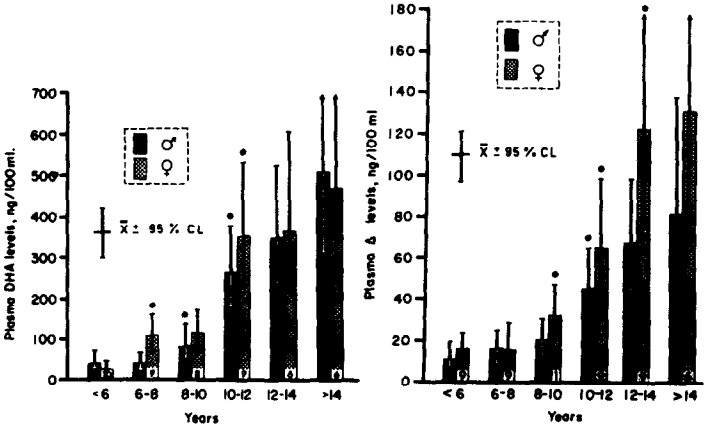

Fig. 2. Dehydroepiandrosterone (DHA) and androstenedione (Δ) in boys and girls from 3.5 to 16 years of age. The number of subjects is indicated at the bottom of each column; $\bar{X} \pm 95\%$ CL = Mean ± 95% confidential limits or intervals.

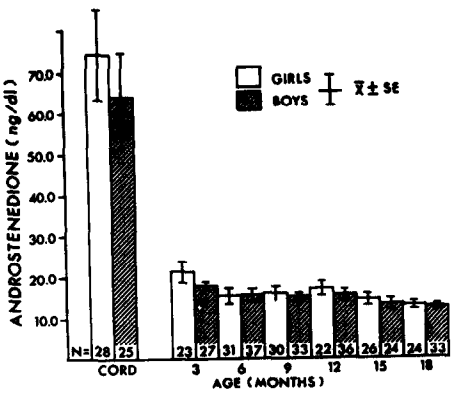

Fig. 3. Androstenedione (△) levels in mixed cord and peripheral plasma in female and male infants from 3 to 18 months of age. The number of subjects is indicated at the bottom of each column. $\bar{X}$ ± S.E. = Mean ± standard error.

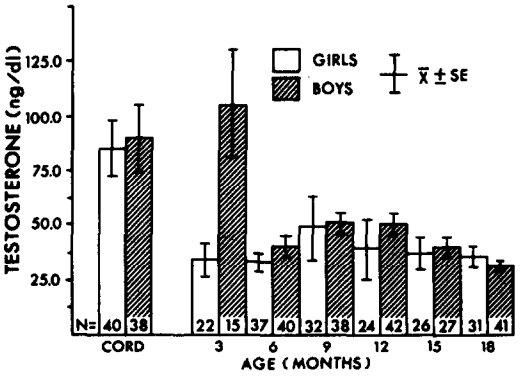

Fig. 5. Testosterone (T) levels in mixed cord and peripheral plasma in female and male infants from 3 to 18 months of age. The number of subjects (*N*) is indicated at the bottom of each column. $\bar{X}$ ± S.E. = Mean ± standard error.

(C) *17α-Hydroxyprogesterone (17-OHP).* 17-OHP was measured only through 18 months of age. Very high in cord plasma in both sexes, levels 10–12 times lower were already observed by 3 months, decreased again by 6 months and remained stable to 18 months (Fig. 4).

(D) *Testosterone (T).* Somewhat high in cord plasma of both sexes, levels fell by 3 months in girls and 6 months in boys and remained constant to 18 months (Fig. 5). In male infants however, the highest T values were found at 3 months.

In older children, plasma T remained low until 12–14 years of age in boys and girls at which time a statistically significant increase was observed (Fig. 6).

(E) *Oestradiol (E_2).* Plasma E_2 was measured from 3.5 to 16 years only (Fig. 6). A significant rise was seen in girls by 10–12 years of age while levels were somewhat high in boys after 14 years of age, although this difference was not statistically significant.

(F) *Luteinizing hormone (LH).* Plasma LH was measured from 3 to 18 months of age (Fig. 7). Although mean levels appeared higher at 3 months in boys, LH concentration varied little throughout the period of study.

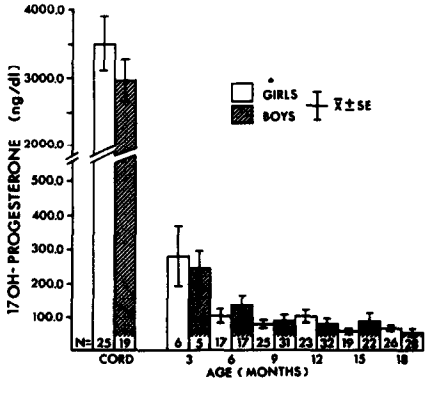

Fig. 4. 17α-hydroxyprogesterone (17-OHP) levels in mixed cord and peripheral plasma in female and male infants from 3 to 18 months of age. The number of subjects (*N*) is indicated at the bottom of each column. $\bar{X}$ ± S.E. = Mean ± standard error.

II. "In vivo" *and* "in vitro" *studies in male lambs*

As shown in Fig. 8, plasma testosterone (T) and LH concentrations measured in the developing ram from birth to 6 months of age were found higher around 4–5 weeks of age while these hormones were lower in the first and the eight weeks postnatally. Other LH and testosterone peaks were also noted at 20 weeks which probably coincide with the onset of puberty.

Figure 9 shows the pattern of prolactin (PRL), LH, FSH and T levels during the first 2 months of life as observed in a representative male lamb. Several LH and testosterone peaks were observed in this animal after the first week, up to the sixth week of age while FSH fluctuated little if any, and high and fluctuating levels of PRL were observed throughout the period of study. A close correlation between LH and testosterone peaks was found.

Representative ultradian variations of T, LH, FSH and PRL obtained at 30 min intervals for 6 h at 2 and 8 weeks of age are represented in Fig. 10. LH peaks followed by testosterone peaks were found at 2 weeks with fluctuating PRL levels. At 4 weeks, not represented in Fig. 10, in general no fluctuation of any hormone was found. At 8 weeks however, here again PRL levels fluctuated markedly while only little variations in FSH were observed. In addition, it should be noted that several LH peaks were found which were not consistently followed by testosterone peaks.

Figure 11 shows the plasma T response to HCG expressed as percentage increment of control levels. Although the resting levels of testosterone were much lower at 1 week than at 4 weeks the mean increase in plasma T under HCG stimulation was greater at 1

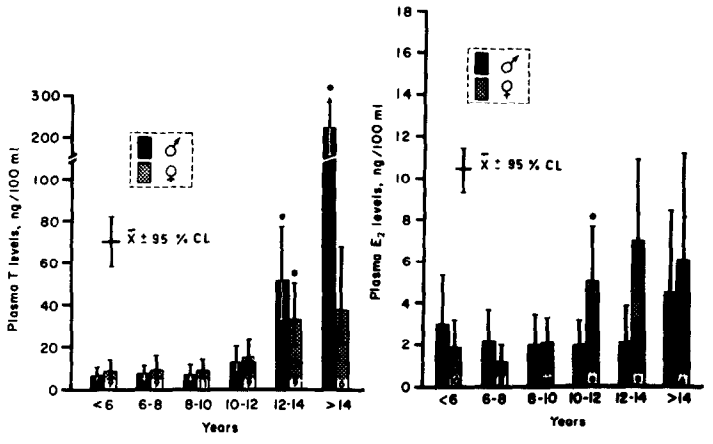

Fig. 6. Testosterone (T) and oestradiol (E_2) levels in boys and girls from 3.5 to 16 years of age. The number of subjects is indicated at the bottom of each column; $\bar{X} \pm 95\%$ CL = Mean $\pm$ 95% confidential limits or intervals.

week with a staistically significant difference between 1 and 8 weeks.

Percent increments of cAMP and T testicular content post HCG are represented in Fig. 12. Consistent with the plasma response to HCG, a higher testosterone percentage increase was observed at 1 week, while cAMP increased progressively from 1 to 8 weeks.

The results found with isolated Leydig cells prepared from the pools of testes obtained from each experimental group are presented in Fig. 13. When incubated in the presence of HCG, again the percentage increase of T production was much higher at 1 than at 4 and 8 weeks. However, the percentage increase of cAMP was similar in the three groups. Here again, a complete dissociation between cAMP production and T increments was observed *in vitro* as well as after HCG *in vivo* stimulation (Fig. 12). When Leydig cells were incubated in the presence of choleratoxin, T production followed a similar trend, albeit less marked. The binding of HCG to Leydig cell particles, shown in the bottom part of Fig. 13 was similar at 1, 4 and 8 weeks.

DISCUSSION

Our results of plasma steroids in human cord plasma and in peripheral plasma in children 3 to 18 months of age are in general agreement with those previously published [3, 4, 26, 27]. Indeed, in boys and girls, DHAS, Δ and 17-OHP are elevated in cord plasma and show an abrupt fall by 3 months, the levels remaining relatively stable until 18 months of age (Figs 1, 3 and 4). T levels fall by 3 months in girls. In contrast, T in boys is elevated in cord plasma and even higher in peripheral plasma at 3 months but values similar in both sexes are found from 6–18 months of age (Fig. 5). Plasma LH is somewhat higher in plasma from male infants at 3 months while similar levels are found in both sexes till 18 months of age. The elevated levels of LH, T and to a lesser degree, Δ [4] in male infants are in agreement with the view of an active hypothalamic-pituitary-gonadal axis at this age. It should be noted that our plasma

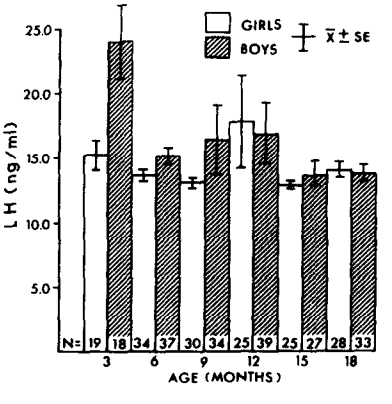

Fig. 7. Luteinizing hormone (LH) levels in peripheral plasma in female and male infants from 3 to 18 months of age. LER-907 was used as reference preparation. The number of subject (N) is indicated at the bottom of each column. $\bar{X} \pm$ S.E. = Mean $\pm$ standard error.

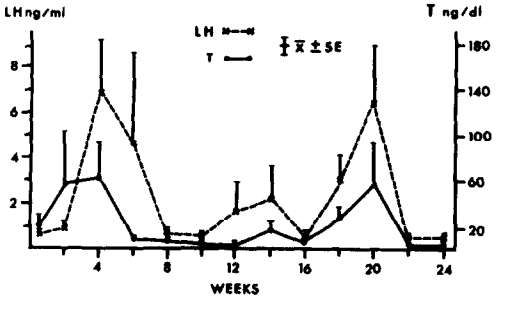

Fig. 8. Plasma LH and testosterone (T) in the developing ram at weekly intervals from the first to the 24th week of life. Each experimental value represents the mean $\pm$ standard error ($\bar{X} \pm$ S.E.) of 9 individual blood samples (specimens obtained thrice weekly in three animals.

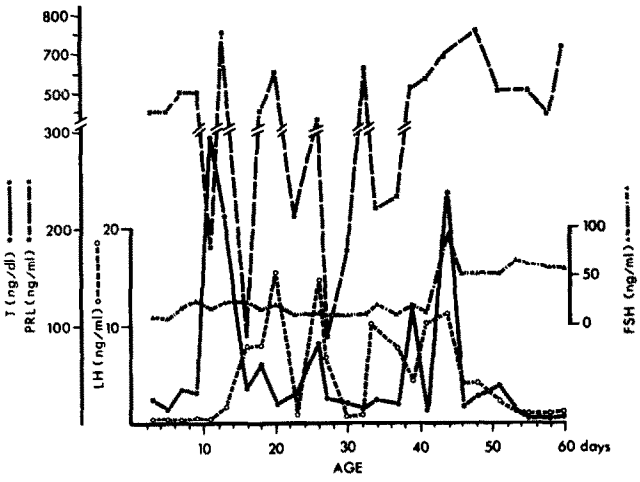

Fig. 9. Representative pattern of testosterone (T), LH, FSH and prolactin plasma levels obtained in one male lamb from birth to 2 months of age.

samples were obtained exactly at 3 months as part of a longitudinal study and therefore we were unable to determine whether peak levels coincided in each infant with the sample obtained.

In children 3.5–16 years of age, as previously published [7], DHA levels rose significantly in girls by 6 to 8 years and in boys by 8 to 10 years of age while Δ showed a significant rise approx. 1–2 years later in both sexes (Fig. 2), long before any increase in T or E_2 in boys and girls respectively (Fig. 6). This finding is interpreted as evidence of a role of adrenal androgens in the mechanisms involved in triggering the hypothalamic pituitary–gonadal axis at puberty.

The levels of plasma T reported in cord plasma, in infancy after the third month and in prepuberty are probably similar although the absolute values found are somewhat higher in the younger age group than in the latter than those previously published [3, 4]. This can probably be explained by slight differences in methodology and the different antibody used.

Our study carried out in the developing lamb also shows differences in pituitary-gonadal relationships from the 1st till the 8th week of postnatal life. Indeed, from the secretory profiles of different hormones in the developing ram from birth to 2 months and up to 6 months of age (Figs 8 and 9) and from the ultradian variations studied (Fig. 10), we can conclude that the newborn hypophysis of the male lamb is capable of secreting gonadotrophins and prolactin in significant amounts which can elevate plasma testosterone levels as early as the first week of life [28]. Significant episodic secretion of high quantities of LH and T, although not of FSH, would suggest the existence of an active hypothalamic-pituitary gonadal axis throughout the first 2 months of postnatal life [28]. The high and fluctuating levels of PRL however are of interest but so far their relationship to gonadostatic control remains to be elucidated.

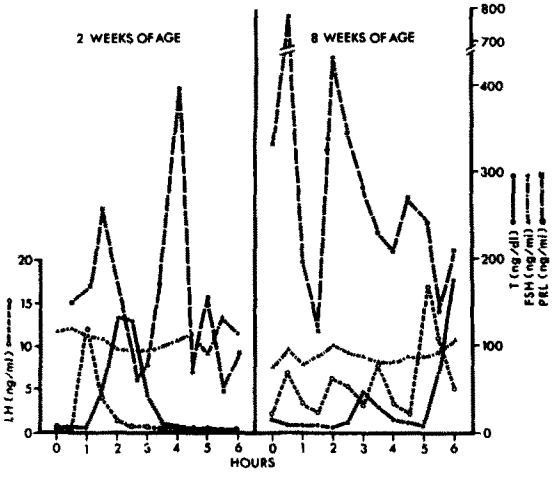

Fig. 10. Representative pattern of circulating levels of testosterone (T) (in ng/dl) and of LH, FSH, and prolactin (in ng/ml) during a 6 h period in one 2-week and one 8-week old lambs.

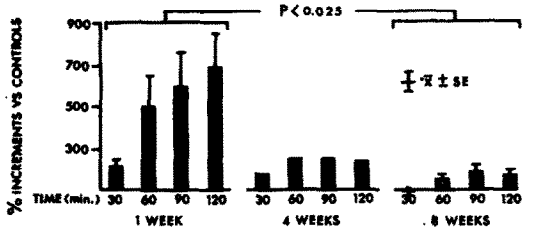

Fig. 11. Plasma testosterone (T) response to human chorionic gonadotropin (HCG) 500 I.U./kg b.wt. in 1, 4 and 8 week old lambs. The results are expressed as mean percentage increments over basal levels ± standard error ($\bar{X}$ ± S.E.). Three animals were studied at 1 week, two at 4 weeks and four at 8 weeks.

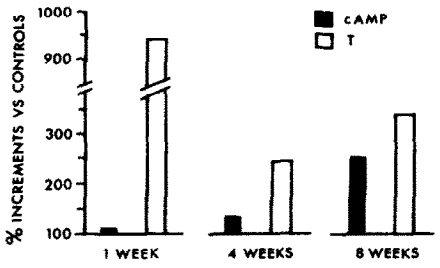

Fig. 12. Percentage increments above non stimulated levels of cAMP and testosterone (T) testicular content after human chorionic gonadotropin (HCG) in pools of testes obtained from three 1 week, two 4 week and four 8 week old lambs.

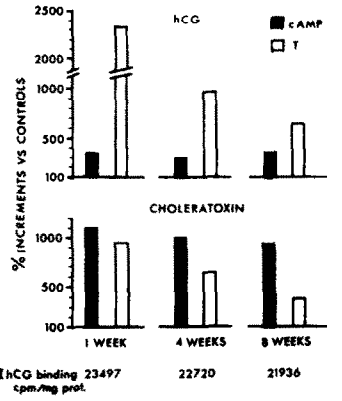

Fig. 13. *In vitro* production of cAMP and testosterone (T) response to human chorionic gonadotropin (HCG) and choleratoxin by isolated Leydig cells prepared from pools of testes obtained from three 1-week, two 4-week and four 8-week old lambs. The results are expressed as percentage increment from non stimulated control levels. The number of binding sites to HCG are expressed as c.p.m. of ^{125}I per mg of proteins.

The *in vivo* and *in vitro* response to HCG and other secretory stimuli, shows that plasma testosterone and LH are lower at 1 week, that testosterone production under HCG *in vivo* and *in vitro* is greater at 1 week and that cAMP production and HCG binding to Leydig cell particles are similar in all groups. The complete dissociation between cAMP production and T response to HCG indicates that the higher testicular responsiveness to HCG at 1 week than at 4 and 8 weeks is independent of the number of binding sites and of cAMP production. This suggests that a step beyond cAMP formation may be responsible for the difference in steroidogenesis observed. The lower T responsiveness of isolated Leydig cells to choleratoxin is not unexpected since it is known that choleratoxin acts through cAMP production and that its effect becomes apparent only after a certain lag period which may be of importance in a 3 h incubation study. In addition, the greater increment of cAMP under choleratoxin than under HCG can also be explained by the response of cells other than Leydig cells which are present in our preparation and which can increase their cAMP production under HCG stimulation.

Our studies in human infants are in keeping with increasing gonadostatic sensitivity to circulating sex steroids from fetal life through the first year postnatally except for a specific period of activation which is found around the second and third months in the human male infant. After a period of relative quiescence, adrenal androgens seem to be at play in the mechanisms which will eventually trigger gonadotrophin release and hence gonadal secretion. Whether the adrenals play any role in the early postnatal testicular activity observed is presently unknown. However, since no further activation appears evident until puberty, it is tempting to speculate that the early postanatal activation of the hypothalamic pituitary gonadal axis may also play a role in the processes which take place at the time of "adrenarche" and puberty and that even the early postnatal activity of the axis may itself result from prenatal programming. Further studies are needed particularly in the neuroendocrine aspects of gonadal development, in order to elucidate the mechanisms which preside testicular activation in early postnatal life in male infants. Although one cannot ascertain at this point whether the findings reported here in the newborn lamb are representative of postnatal testicular activity in the human, our results so far would tend to suggest that this animal can be a useful experimental model for the study of hypothalamic-pituitary-gonadal relationships at this age.

Acknowledgements—This work was supported by research grants from Health and Welfare Canada, La Fondation Justine Lacoste-Beaubien and NATO. The authors wish to thank doctors J. Saez, M. G. Forest and F. Haour for their participation in the *in vitro* studies and C. Faiman, G. D. Niswender and H. Guyda for some of the antisera used in these studies, the cooperation of the Centre de Croissance of the U. of Montreal, the expert technical assistance of Ms G. Alberti, J. Gaulin, D. Ivelja, H. Lepage and L. Royer and the secretarial help of Ms G. Jacob are gratefully acknowledged.

REFERENCES

1. Kaplan S. L., Grumbach M. M. and Aubert M. L.: The ontogenesis of pituitary and hypothalamic factors in the human fetus: maturation of central nervous system regulation of anterior pituitary function. *Rec. Prog. Horm. Res.* **32** (1976) 161–243.
2. Winter J. S. D., Faiman C., Hobson W. C., Prasad A. V. and Reyes F. I.: Pituitary-gonadal relations in infancy: 1. Patterns of serum gonadotrophin concentrations from birth to four years of age in man and chimpanzee. *J. clin. Endocr. Metab.* **40** (1975) 545–551.
3. Winter J. S. D., Hughes I. A., Reyes F. I. and Faiman C.: Pituitary-gonadal relations in infancy: 2. Patterns of serum gonadal steroid concentrations in man from birth to two years of age. *J. clin. Endocr. Metab.* **42** (1976) 679–686.
4. Forest M. G., Sizonenko P. C., Cathiard A. M. and Bertrand J.: Hypophyso-gonadal function in humans during the first year of life. 1. Evidence for testicular activity in early infancy. *J. clin. Invest.* **53** (1974) 819–828.

5. Kulin H. E., Grumbach M. M. and Kaplan S. L.: Changing sensitivity of the pubertal gonadal hypothalamic feedback mechanism in man. *Science* **166** 3908 (1969) 1012–1013.
6. Reiter E. O. and Kulin H. E.: Sexual maturation in the female. *Pediat. Clin. N. Amer.* **19** (1972) 581–603.
7. Ducharme J. R., Forest M. G., De Peretti E., Sempé M., Collu R. and Bertrand J.: Plasma adrenal and gonadal sex steroids in human pubertal development. *J. clin. Endocr. Metab.* **42** (1976) 468–476.
8. Jost A.: La fonction endocrine du testicule foetal. In *La Fonction Endocrine du Testicule.* Masson et Cie, Paris (1957) pp. 9–18.
9. Job J. C., Garner P. E., Chaussain J. L. and Milhaud G.: Elevation of serum gonadotropins (LH and FSH) after releasing hormone (LH-RH) injection in normal children and in patients with disorders of puberty. *J. clin. Endocr. Metab.* **35** (1972) 473–476.
10. Grumbach M. M., Roth J. C., Kaplan S. L. and Kelch L. P.: Hypothalamic pituitary regulation of puberty in man: evidence and concepts derived from clinical research. In *Control of the Onset of Puberty* (Edited by M. M. Grumbach, G. D. Grave and F. E. Mayer). Jolin Wiby, New York (1974) pp. 115–166.
11. Collu R.: Role of central cholinergic and aminergic neurotransmittors in the control of anterior pituitary hormone secretion. *Clinical Neuroendocrinology* (Edited by L. Martini and G. M. Besser). Academic Press Inc. New York (1977) pp. 43–65.
12. Baker H. W. G., Bremnen W. J., Burger H. G., De Krester D. M. A., Bulmanis L. W., Eddie H. B., Keogh E. J., Lee V. W. K. and Rennie G. C. Testicular control of follicle-stimulating hormone secretion. *Rec. Prog. Horm Res.* **32** (1976) 429–476.
13. Forest M. G., Cathiard A. M., Bourgeois J. and Genoud J.: Plasma androgens in normal and premature infants. Relation to hypothalamo-hypophyseal-gonadal axis maturation. In *Endocrinologie sexuelle de la Période Périnatale*, Colloque INSERM No. 32 (1974) pp. 315–335.
14. Forest M. G.: Ontogeny of gonadal steroids in the human infant and their neuroendocrine regulation. In *New Developments in Pediatric Research* (Edited by O. P. Ghai). Interprints New Delhi, Vol. 111 (1977) pp. 1039–1042.
15. Lee V. W. K., Cumming I. A., De Kretser D. M., Findlay J. K., Hudson B. and Keogh E. J.: Regulation of gonadotropin secretion in rams from birth to sexual maturity. 1. Plasma LH, FSH testosterone levels. *J. Reprod. Fert.* **46** (1976) 1–6.
16. Courot M.: Developpement du testicule chez l'agneau. Etablissement de la spermatogénèse. *Ann. Biol. Anim. Bioch. Biophys.* **2** (1962) 25–41.
17. Buster J. E. and Abraham G. E.: Radioimmunoassay of plasma dehydroepiandrosterone sulfate. *Anal. Lett.* **5** (1972) 543–551.
18. Forest M. G., Cathiard A. M. and Bertrand J.: Total and unbound testosterone levels in the newborn and in normal and hypogonadal children: use of a sensitive radioimmunoassay for testosterone. *J. clin. Endocr. Metab.* **36** (1973) 1132–1142.
19. Youssefnejadian E., Florensa E., Collins W. P. and Sommerville I. F.: Radioimmunoassay of 17-hydroxyprogesterone. *Steroids* **20** (1972) 773–788
20. Faiman C. and Ryan R. J.: Radioimmunoassay for human luteinizing hormone. *Proc. Soc. Exp. Biol. Med.* **125** (1967) 1130–1133.
21. Faiman C., Stearns E. L., Winter J. S. D., Reyes F. I. and Hobson W. C.: Radioimmunoassay for rhesus monkey gonadotropins. *Proc. Soc. Exp. Biol. Med.* **194** (1975) 670–676.
22. Niswender G. D., Reichert L. E., Midgley A. R. and Nalbandov A. U.: Radioimmunoassay for bovine and ovine luteinizing hormone. *Endocrinology* **84** (1969) 1166–1173.
23. Davis S. L., Reichert L. E. Jr and Niswender G. D.: Serum levels of prolactin in sheep as measured by radioimmunoassay. *Biol. Reprod.* **4** (1971) 145–153.
24. Saez J. M., Morera A. M., Haour F. and Evain D.: Effects of *in vivo* administration of dexamethasone, corticotropin and human chorionic gonadotropin on steroidogenesis and protein and DNA synthesis of testicular interstitial cells in prepuberal rats. *Endocrinology* **101** (1977) 1256–1263.
25. Baird D. T., Horton R., Longscope C. and Tait J. F.: Steroid dynamics under steady-state conditions. *Rec. Prog. Horm. Res.* **25** (1969) 611–664.
26. Forest M. G. and Cathiard A. M.: Ontogenic study of plasma 17α-hydroxy-progesterone in the human. 1. Postnatal period: Evidence for a transient ovarian activity. *Pediat. Res.* **12** (1978) 6–11.
27. DePeretti E. and Forest M. G.: Pattern of plasma dehydroepiandrosterone sulfate levels in humans from birth to adulthood: evidence for testicular production. *J. clin. Endocr. Metab.* **47** (1978) 572–577.
28. Foster D. L., Mickelson I. H., Ryan K. D., Coon G. A., Drongowski R. A. and Holt J. A.: Ontogeny of pulsatile luteinizing hormone and testerone secretion in male lambs. *Endocrinology* **102** (1978) 1137–1146.