specific gas chromatographic procedure. It is a specific and rapid procedure that allows for a great number of assays to be carried out in a short time. The method also seems amenable to further refinement, e.g., the introduction of radioactive iodine into the tracer might not only reduce counting time but also make possible the direct counting of the particles without excessive loss of either sensitivity or precision.

50. Control of aldosterone secretion in mother and newborn under delivery and in childbed, J. NUSSBERGER, H. BUCHER, J. SCHMID, U. SCHMIED, J. MICHELI, D. MIETH, G. DUC, W. SIEGENTHALER and W. VETTER, Departments of Medicine, Obstetrics and Neonatology, Kantonsspital, University of Zürich, Switzerland

Plasma aldosterone (PA), plasma cortisol (PC), angiotensin II (A II) and renin activity (PRA) were determined in 6 healthy women during and one week after delivery, in umbilical blood and in the 6 newborns one week after birth. PA, A II and PRA were measured by radioimmunoassays. PC was determined by the protein binding method. Diazepame and N_2O were the only therapeutic regimens used.

In the mothers, mean PA, PC, A II and PRA decreased significantly ($P < 0{\cdot}001$) within one week from elevated levels under delivery to normal or even subnormal values: PA from 873 ± 470 ($\pm$ S.D.) to 58 ± 8 pg/ml, PC from 53 ± 25 to 13 ± 2 μg/100 ml, A II from 46 ± 18 to < 6 pg/ml and PRA from $11{\cdot}5 \pm 7{\cdot}5$ to $0{\cdot}6 \pm 1{\cdot}2$ ng/ml3h. PA correlated significantly with A II ($P < 0{\cdot}001$), PRA ($P < 0{\cdot}01$) and PC ($P < 0{\cdot}01$).

In the umbilical vessels, PA, PC, A II and PRA showed no significant arteriovenous difference (artery: PA 589 ± 300 pg/ml, PC 11 ± 4 μg/100 ml, A II 73 ± 45 pg/ml and PRA $15{\cdot}5 \pm 22$ ng/ml·3h; vein: PA 632 ± 391 pg/ml, PC 11 ± 4 μg/100 ml, A II 87 ± 53 pg/ml and PRA $16 \pm 22{\cdot}6$ ng/ml·3h). Under these conditions no significant correlations were found between PA and PRA, PA and A II and between PA and PC.

In the newborns, one week after birth, lower PA, PC, A II and PRA values were observed than in umbilical blood (PA 437 ± 404 pg/ml, PC 4 ± 4 μg/100 ml, A II 30 ± 22 pg/ml and PRA $12 \pm 22{\cdot}3$ ng/ml·3h). Under these conditions significant correlations were found between PA and PRA ($P < 0{\cdot}001$) and between PA and A II ($P < 0{\cdot}05$), whereas PA did not correlate with PC.

Our results indicate that in the mother both the renin angiotensin system and ACTH influence the secretion of aldosterone. In the newborn, adrenal aldosterone release seems to be predominantly controlled by renal renin secretion. The inability to correlate umbilical PA with PRA, A II or PC might be caused by the following reasons: (1) Aldosterone crosses the placenta and (2) The placenta seems to produce renin.

K. Sexual steroids in the neonatal period, MAGUELONE G. FOREST and JEAN BERTRAND, Unité de Recherches Endocriniennes et Métaboliques chez l'Enfant, INSERM U. 34, 29 Rue Soeur Bouvier, 69322 Lyon Cedex 1, France

It is now well established that the gonadal endocrine function is under the control of a complex neuroendocrine system and that in adults the regulatory mechanisms are different in male and female. From experimental studies in the last decade, it became clear that the cybernetical hypothalamic-pituitary-gonadal system was not fully mature at birth. Recent technical improvements led to precise measurement of sexual and pituitary hormones. It has been established that the pituitary gonadal system functions in childhood and also in infancy.

Although it is often difficult to extrapolate studies between species particularly since the maturation of the central nervous system at birth is quite different from one species to the next, experimental studies have considerably advanced our comprehension of the establishment, maturation and change with age, in the regulatory mechanisms controlling the gonadal function.

The purpose of this presentation is to report our ongoing studies of the ontogenesis of plasma concentration of gonadal steroid hormones in human and particularly the chronology of their secretion by the neonate and the infant as one aspect of development and maturation of the hypothalamo-pituitary-gonadal axis in the neonatal period.

We have developed sensitive and specific radioimmunoassay technics to quantify plasma levels of testosterone, [1] 4-androstenedione and [2] 17-hydroxyprogesterone (present). Blood was obtained from the cord at 105 normal full term babies at the time of spontaneous vaginal delivery and from a peripheral vein of 245 normal infants aged 0 to 361 days. Normal children and adults were also studied for comparison.

A. *Testicular function in the neonatal period*

Total unconjugated testosterone. In a previous study of 81 normal neonates we have shown that cord blood levels of testosterone were significantly higher in males ($33{\cdot}8 \pm 9{\cdot}5$ ng/100 ml; $n = 35$) than in female newborns ($26{\cdot}4 \pm 7{\cdot}4$ ng/100 ml; $n = 46$) [3]. The simultaneous measurement of testosterone (T), 4-androstenedione (Δ) and 17-hydroxy-progesterone (17-OH-P) was made in another smaller group of normal neonates and results are given in Table 1. Although mean values in T levels are slightly higher in this second group the same sex difference is observed. Our results strongly suggest that testicular activity is present at birth. However in none of the available studies in the literature was a sex difference in cord T levels demonstrated. We therefore studied androgens in the peripheral veinous blood of normal neonates. Results are given in Table 1. In male newborns on the first day of life, T plasma concentrations are considerably higher in experipheral blood than in cord blood and the sex difference is even more significant. These results further evidence the fetal and testicular origin of T at birth. In female newborns, circulating levels of T are slightly but significantly higher than in cord plasma. These mean T levels of $46{\cdot}3 \pm 13{\cdot}9$ ng/100 ml, comparable to those we observed in adult females ($37{\cdot}2 \pm 9{\cdot}6$ ng/100 ml), decrease rapidly within the first two weeks of life to low values and remain constant throughout the first year of life ($7{\cdot}1 \pm 3{\cdot}3$ ng/100 ml).

In contrast the pattern in T plasma concentration is quite different in male infants and follows a triphasic evolution. The high T levels present at birth (228 ng/100 ml, comparable to those of boys at stage P_3 of puberty), decrease very sharply within the first week of life to a nadir observed at 5–7 days of age when values average 31 ng/100 ml. T plasma concentrations increase thereafter rapidly to peak values of $265 \pm 31{\cdot}3$ ng/100 ml reached between 30 and 60 days of age. From the second to the seventh month of life T values decrease again, more slowly, and correlatively with time. From 7 months of age onwards T levels remain low in male infants ($7 \pm 4{\cdot}7$ ng/100 ml) and identical to those of female infants 1 to 12 months old and to those of prepubertal children of both sexes ($6{\cdot}7 \pm 2{\cdot}5$ ng/100 ml).

The binding capacity of plasma proteins for testosterone was measured by equilibrium dialysis. At birth this binding capacity is low in both sexes and similar in cord and peripheral bloods. It increases very rapidly after birth, reaching prepubertal values in 2–3 weeks. The unbound

fraction of T represents 2·9 ± 0·6% and 3·0 ± 0·5% of the total T levels in male and female cord blood respectively. After 1 month of age onwards the percentage of total T which is not bound to plasma proteins equals 0·71 ± 0·2 and 0·70 ± 0·19 in male and female infants respectively, that is to say, is similar to that observed throughout the prepubertal period (0·7 ± 0·2%).

The changes with age in the unbound concentration of testosterone reflect the blood changes in the plasma binding capacity. In cord unbound T levels are higher ($P < 0.001$) in male (1·1 ± 0·4 ng/100 ml) than in female newborns (0·89 ± 0·29 ng/100 ml). In the latter sex these levels decrease within the first 2 weeks of life to mean values of 0·04 ± 0·02 ng/100 ml, comparable to those of prepubertal children of both sexes.

In male infants at birth and unbound T concentrations are extremely high in peripheral blood (range = 3 to 8 ng/100 ml, comparable to that of adult males). These levels drop within the first week of age to a nadir of 0·33 ± 0·1 ng/100 ml. However, due to the rapid neonatal increase in the binding capacity of plasma proteins the postnatal peak in unbound T concentration is of a lesser amplitude (mean ± 1 S.D. = 1·6 ± 0·2 ng/100 ml). After 7 months of age unbound T levels are comparable in both sexes.

4-androstenedione. Androstenedione concentrations found at birth in cord and peripheral plasmas are given in Table 1. In both sexes these levels are in the adult range in the cord but 2 to 3 times higher in systemic blood with no sex difference. Circulating levels of Δ decrease rapidly within the first week of life to mean values of 39·4 ± 22·1 and 28·8 ± 13 in female and male neonates respectively. While in female Δ plasma concentrations continue to decrease more slowly, these levels increase slightly but significantly from 10 to 30 days of age in male infants (44 ± 18 ng/100 ml) before decreasing again. Between 1 to 6 months of life values are lower in female than in male. However, the sex difference is significant only between 1 and 3 months of age where Δ levels average 34 ± 11 and 19 ± 4 ng/100 ml in male and female infants respectively. After 6 months of age circulating levels of Δ are identical in male (10·9 ± 2·7 ng/100 ml) and female respectively. After 6 months of age circulating levels of Δ are identical in male (10·9 ± 2·7 ng/100 ml) and female (11 ± 1 ng/100 ml) infants and similar to those of prepubertal children of both sexes 1 to 7 years old (11·5 ± 6·2 ng/100 ml).

These data evidence the existence of a postnatal transient testicular activity under the control of a hypothalamo-hypophyseal activity as demonstrated by high levels of circulating pituitary gonadotropins (LH and FSH) in this period [2]. Although the pattern varies from one species to the next a postnatal activation of the pituitary-testicular axis has been demonstrated in rat, guinea-pig, sheep, pig, bovine and Rhesus monkey.

B. *Ovarian function in the perinatal period*

A similar postnatal activity of the hypothalamo-pituitary system was observed in female infants [2]. However in human, as well as in many species, very little evidence is available concerning the secretory capacity of the ovary in this period. We tried to investigate whether the gonadal response to the physiological neuroendocrine stimuli was different with sex in the neonatal period. Due to the relatively large volume of plasma still required to measure oestrogen levels accurately and by ethical limitation in the quantity of blood obtainable from small babies we chose to study 17-hydroxyprogesterone as our indicator of gonadal activity.

17-hydroxy-progesterone (17-OH-P). "At birth". Concentrations of 17-OH-P in peripheral and cord plasmas are given in Table 1. As expected 17-OH-P levels are considerably higher in cord than in systemic blood with no sex difference. Circulating levels of 17-OH-P at birth are several times higher than in normal adult males (127 ± 48 ng/100 ml) or normal adult females (follicular phase: 63 ± 16 ng/100 ml; luteal phase: 211 ± 106 ng/100 ml). During the first day of life a significant ($2\alpha < 0.001$) correlation ($r = -0.608$) between 17-OH-P and age was found. This hormone further decreases during the first week of life to mean values of 85 ng/100 ml in female and 77 ng/100 ml in female neonates.

"In male infants" a secondary increase in 17-OH-P levels is thereafter observed. Mean peak values of 188 ng/100 ml are observed between 30 and 60 days of age. A progessive decline follows (mean values of 83 ng/100 ml at 60–90 days). 17-OH-P levels average 46 ng/100 ml between 7 to 12 months and 34 ng/100 ml at 1–2 years of age. However median values are similar in these two groups (27–31). Although the age-related variations in 17-OH-P levels are of a lesser amplitude than those of T and despite a rather wide range in 17-OH-P values, the similarity in the general pattern during infancy between these two hormones is evident.

"In female infants" a somewhat wider range of values is observed throughout the first year of life. Mean and median were however comparable in all age groups. The second week of life, mean = 143 ng/100 ml. From then until the end of the 3rd month of life mean 17-OH-P levels plateau at 103–110 ng/100 ml decreasing proges-

Table 1. Plasma concentrations (in ng/100 ml) of testosterone, 4 androstenedione and 17-hydroxy-progesterone in mixed cord blood and in peripheral venous blood of normal newborns

		Male		Female	
		Cord	Peripheral vein	Cord	Peripheral vein
Testosterone	mean ± 1 SD	39·4 ± 11*	228 ± 128·7*	29·2 ± 7·6*	46·3 ± 13·9*
	median	39	201	29	45
	n	25	19	21	21
	P	<0·005		<0·005	
Androstenedione	mean ± 1 SD	86·7 ± 30·2**	197 ± 92·2**	92·6 ± 37·9**	173·8 ± 75·4**
	median	85	183	80	156
	n	25	19	21	21
	P	<0·01		<0·005	
17-Hydroxy-progesterone	mean	1841**	410**	2582**	393**
	(range)	(931–4121)	(153–886)	(919–5483)	(200–506)
	median	1752	416	2278	385
	n	21	15	20	16

*Sex difference ($P < 0.0025$)
**No sex difference.

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sively thereafter. However, between 3 to 12 months of life mean values mostly remain higher (66 to 87 ng/100 ml) than in male infants of the same age and also higher than in girls aged 1 to 2 years (40 ng/100 ml), period at which 17-OH-P levels are similar in both sexes.

"Is 17-OH-P a good index of gonadal activity?" Although 17-hydroxy-progesterone is a hormone of dual origin, the testicular or ovarian participaton to the plasma pool of 17-OH-P is important at periods of gonadal activity in maturity. Plasma 17-OH-P levels also increase in children with precocious puberty and reach adult male values in prepubertal boys after human chorionic gonadotropin stimulation. As we expected, the pattern observed for 17-OH-P in male infants correlates with that of testosterone.

In contrast we did not expect to observe such a pattern in female infants. From the above data we would conclude that a certain ovarian activity is present in infancy and moreover opposite to male infants lasts (episodically or as surges?) until late infancy.

In addition the pattern of 17-OH-P levels found in female infants resembles closely that described by Bidlingmaier *et al.* for oestradiol [4] and supports the findings and conclusions of these authors.

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L. **Prenatal and neonatal steroid influences,** J. H. LEATHEM, S. W. C. CHAN, A. W. JORDAN and B. N. GARNER, Bureau of Biological Research, Rutgers University, New Brunswick, New Jersey 08903, U.S.A.

Placental growth is of biological significance in that placental tissue promotes prenatal life and pregnancy maintenance whereas placental suppression can be adverse. Uniquely, however, DNA synthesis stops on day 18 of the 21 day rat gestation period but cell hypertrophy continues. Nevertheless, placental weight is known to vary inversely in the rat relative to the number of fetuses but normal placental growth is not modified by exogenous estrone, progesterone or provera but may be retarded by prednisolone. However, the interrelationship among maternal, fetal and placental endocrine systems has had only modest consideration relative to placental growth. Thus it was of interest to observe a marked placental overgrowth in the rat, if ovariectomized on day 10 and injected with estrogen and progesterone to sustain pregnancy. This overgrowth resulted in an increase in DNA that did not stop on day 18 and also in Ribosomal RNA. Furthermore, the excess growth occurred in the absence of the adrenal and pituitary. An ovarian control over placental growth was indicated and in an inhibitory manner as "giant placentae" do not develop when ovaries are intact. Efforts to elucidate the potential placental growth antagonist has centered on estradiol. Wide variations in serum progesterone did not relate to the degree of hypertrophy while reductions in serum estradiol promoted, whereas elevated estradiol levels suppressed excessive placental growth. Unilateral ovariectomy favors placental overgrowth and the response is enhanced by administration of anti-estradiol serum. The estrogen–progesterone ratio is also important to the growth induced. Then too, aminoglutethimide (10 mg/100 gm) modifies steroid synthesis so that placental overgrowth could be induced with estrone and progesterone in the presence of the ovaries.

The independent development of the placenta after fetectomy is well known but fetectomy before day 16 in the rat sharply retards placental growth. However, the sustaining of placental weight results in growth of the endocrine elements. Fetectomy on day 14 restricted placental growth to 100–150 mg and the organ was unresponsive to exogenous steroids. However, in the absence of the ovary, placental weight increased to 700 mg in response to estrone and progesterone with little evidence of an influence of steroid ratio. Curiously, 20α-dihydro-progesterone provided an enhanced growth response. The significant placental growth invoked by steroids was associated with a marked increase in total protein and DNA but RNA and Ribosomal RNA failed to increase in the absence of the fetus.

The steroid synthetic competence of the rat placenta has been reinvestigated to compare the normal and giant placenta. Incubation of basal zone placental tissue with [7α-H^3]-pregnenolone as the substrate resulted in significant progesterone synthesis (30·3%) and with 17-hydroxyprogesterone, androstenedione and testosterone being identified. Incubation of whole placentae produced only a 4% conversion to progesterone and the 5α-reduced products predominated. Addition of 200 iu HCG *in vitro* to basal zone tissue significantly enhanced the production of progesterone, 17-hydroxyprogesterone, androstenedione and 5α-pregnan-3,20-one. The basal zone of "giant" placentae was significantly greater than normal in mass. However, incubation of this tissue with pregnenolone yielded significantly less progesterone (17%) and more androstenedione than normal tissue. Furthermore unlike normal placental tissue no stimulation of steroid formation was obtained in response to hCG when giant placenta basal zone was used. These data then suggest that it may be premature to conclude that placental hypertrophy is merely accelerated growth consequential to the removal of any growth inhibitor due to ovariectomy.

Following parturition, a critical period of sensitivity to steroids occurs in some rodents from birth to day 10. Indeed, a single injection of androgen on day 5 can permanently sterilize a female mouse or rat. Similar results were noted with estradiol but less consideration was given to this effect in view of the attractive studies relative to the impact of early androgen on the development of a genetic female. Nevertheless, the potential importance of estrogen effects was emphasized by the recent evidence that testosterone is converted to estradiol by the brain. Furthermore, the dosages used for sterilization may not be pharmacologic as previously suggested in that receptors are few in the neonate brain.

Estradiol dipropionate (10 μg) administered to Swiss–Webster mice at either 5 or 20 days of age caused vaginal opening within 3 to 6 days. Beginning at 30 days of age, vaginal smears of mice injected at age 5 days revealed irregular cycles for 30 days and periods of prolonged diestrus thereafter. The steroid did not alter the pattern of estrous cycles when injected at 20 days of age.

Morphologically the ovaries of mice injected with 5 μg of estradiol diproprionate fail to form corpora lutea. When treatment was delayed until 10 days of age, no corpora lutea developed in 60 day old mice until the dosage was reduced to 1 μg. Another measure of ovarian development was the distribution of 5-ene-3β-hydroxysteroid dehydrogenase. In the mouse ovary,