significant increases occur in the theca and interstitium between days 10 and 25 of age whereas the granulosa exhibits an enxyme decline after day 15. Dehydroepiandrosterone (DHA), pregnenolone, 17-hydroxypregnenolone and 16-dehydropregnenolone served as substrates. Administration of 5 μg of estradiol benzoate at 5 days of age delayed the development of the enzyme to day 20 in the interstitium and to day 30 in the theca regardless of the substrate but at age 40 days substrate differences were noted. When estradiol was injected on day 10, enzyme development in the interstitium was actually enhanced through day 40.

Prior studies have indicated that 10 μg of estradiol diproprionate administered to the neonate will invoke infertility if injected at 5 days of age and that this dosage is partially effective at 10 days of age when studied in mice 100 days old. Administering 5 or 10 μg of estradiol benzoate at 5 days of age resulted in 29 of 30 mice being infertile, although cohabitated with males for 90 days. Littermate controls proved fertile in 23 or 24 cases with litters being delivered in 24–31 days after admission of the male. When comparable steroid treatment was given at 10 days of age, 50% of the mice in each group proved infertile. Furthermore, litter size was subnormal and cohabitation time with the male was extended from 24 days (control) to 41 days (5 μg) to 57 days (10 μg) with each time period being significantly different. Finally, the influence of aging and neonatal steroid effects were examined 16 to 17 months after 5 μg of estradiol benzoate was administered on day 5 (7 expt., 9 controls) or day 10 (6 expt., 8 controls). After 100–150 days in breeding n4 pregnancies resulted in neonatal steroid treated rats whereas 3 controls produced small litters. The impact of neonatal steroid treatment on aging is unknown but does deserve consideration.

**51. Physiologically available cortisol in the human fetus and mother,** B. E. PEARSON MURPHY and A. C. CAMPBELL, Reproductive Physiology Unit, Montreal General Hospital, and Department of Experimental Medicine, McGill University, Canada

In order to interpret the relationships between total cortisol levels in maternal serum, cord arterial and venous serum and amniotic fluid, it is necessary to consider the fraction of cortisol which is physiologically available to the tissues, i.e. that which is not bound to transcortin. This was measured at 37°C by equilibrium microdialysis of 0·1 ml sample against 0·1 ml saline containing a concentration of albumin equal to that of the sample. Total cortisol was measured by a specific non-chromatographic radiotransinassay. Physiologically available cortisol was then calculated as % unbound x total cortisol. Subjects were studied at 12 to 20 weeks gestation. (6 elective hysterectomies) and at term (8 elective Caesarean sections, 14 induced vaginal deliveries, and 18 spontaneous-onset vaginal deliveries. The two vaginal delivery groups were matched for gestational age and duration of labour. The % unbound cortisol in maternal serum ranged from 17 to 29% while that in cord serum ranged from 34 to 76%. Cord arterial and venous values were similar in all instances. Lowest percentages were found at 12 to 20 weeks and highest at spontaneous vaginal delivery. Levels of physiologically available cortisol at term were all higher than at 12 to 20 weeks gestation. Cord arterial levels were consistently higher than venous levels in all groups, but especially in the spontaneous group ($P < 0.01$). Maternal levels were higher than cord levels but the amount crossing the placenta, corrected for 85% conversion of cortisol to cortisone, as estimated previously in our laboratory (*Am. J. obstet. Gynec.* **118** (1974) 538), was lower than cord levels in all instances ($P < 0.01$). Spontaneous-onset cord arterial levels (61 ± 3 ng/ml) were higher ($P < 0.05$) than induced cord arterial levels (43 ± 5 ng/ml) or Caesarean section cord arterial levels (36 ± 8 ng/ml). Amniotic fluid levels were about half those of cord levels and correlated well with cord arterial levels but poorly with maternal venous levels. These studies provide evidence that (1) the placenta, by converting maternal cortisol to cortisone, acts as a barrier to prevent fetal pituitary-adrenal axis suppression, and (2) there is a surge of fetal cortisol which precedes the onset of spontaneous-onset labour which may be important in triggering parturition in man.

**52. Urinary steroid metabolites in the human newborn,** R. A. ANDERSON, G. DEFAYE, C. MADANI, E. M. CHAMBAZ, C.H.U. Grenoble (France), and C. J. W. BROOKS, Chemistry Department, University of Glasgow, Scotland

Urinary steroid separations using gas phase analysis (g.l.c. and GC–MS) have shown that the main metabolites excreted in the newborn period might have a different biological significance, according to their mode of conjugation. Steroid sulfates were of the 5-ene-3β-hydroxy type, increased after ACTH stimulation and were absent in a case of anencephaly. By contrast, the glucuronide fraction contained mainly saturated pregnane structures, were not influenced by ACTH and disappeared within the first 10 days of life.

Methods were developed to obtain satisfactory group separations of steroid metabolites using lipophilic substituted dextran gels, either by direct or reversed phase elution (Anderson *et al.: J. Chromatog.* 99 (1974) 485).

A number of polyhydroxylated 5-en-3β-hydroxy-$C_{19}$ compounds were synthesized, either by biological or chemical routes: 3β,15α-dihydroxy-5-androsten-17-one; 5-androstene-3β,7α (and 7β), 17β-triol; 3β,16β,17β-trihydroxy-5-androsten-7-one; 5-androstene-3β,15α,16α,17β-tetrol; 5-androstene-3β,15β,16β,17β-tetrol; 3β, 18-dihydroxy-5-androsten-17-one; 5-androstene-3β,17β,19-triol.

After preliminary group fractionation, 15α-hydroxy-DHA (trace) and 5-androstene-3β,15α,16α,17β-tetrol (average 110 μg/24 h) could be identified in the sulfate fraction in the newborn period.

The same methodology could be applied to the study of steroid in amniotic fluid; in a case of sulfatase defect, values within the normal range were obtained for the major 5-ene-3β-hydroxy steroids which could be measured.

**53. Pattern of plasma concentration of dehydroepiandrosterone during the neonatal period and the first year of life in human,** EVELINE DE PERETTI and MAGUELONE G. FOREST, Unité de Recherches Endocriniennes et Métaboliques chez l'Enfant, INSERM-U.34 Hôpital Debrousse, 29, rue Soeur Bouvier, 69322 Lyon Cedex 1, France

A specific and sensitive radioimmunoassay (RIA) for measuring unconjugated plasma dehydroepiandrosterone (DHA) has been developed. Specific antibodies have been obtained in rabbit immunized with a DHA-17 (O-carboxymethyl) oxime – BSA complex. Plasma was extracted by diethyl ether and the dry extract purified on a celite column. At the end of the RIA, bound and free fractions were separated using a Dextran-charcoal solution. In mixed cord blood, the mean values were 593·3 ng/100 ml ± 186·5 (range 248–1493) in 21 females, and 712·7 ng/100 ml ± 190·9 in 18 males (range 179–1367). These values were within adult range (642 ± 112 ng/100 ml in male and 515 ± 107 ng/100 ml in female). During the first day of life the peripheral

plasma concentration of DHA was 917·6 ± 317·8 ng/100 ml (range 324–3575) in 22 females and 922·65 ± 290 ng/100 ml (range 161–1841) in 17 males. From 2 to 30 days of age, plasma DHA levels were significantly lower: 344·4 ± 93·7 ng/100 ml (range 99–696) in 18 girls and 229·81 ± 81·11 ng/100 ml (range 54–685) in 18 boys. A progressive decline was observed throughout the first year of life; from 1 to 6 month of age mean values were 147·1 ± 53·6 ng/100 ml (range 43–409) in 16 girls and 151·6 ± 62·7 ng/100 ml (range 16–614) in 28 boys, and from 6 to 12 months of age they were 90·92 ± 43·3 ng/100 ml (range 20–218) in 11 girls, and 68·14 ± 30·9 ng/100 ml (range 8–330) in 24 boys. The adrenal origin of plasma DHA was confirmed by the sharp rise observed during stress and acute or chronic ACTH stimulation. In boys the testicular origin was attested by the increase noted under HCG stimulation.

**54. Plasma aldosterone concentrations during the neonatal period and the first year of life,** M. C. RAUX, M. T. PHAM-HUU-TRUNG, D. MARREC, F. GIRARD, J. HERVE and J. SALAT-BAROUX, Laboratoire de physiologie endocrinienne infantile, Hop. Trousseau, Paris, France Maternité Hôpital Rothschild, Paris, France

A direct radioimmunoassay for plasma aldosterone (PA) was applied to 85 normal newborns and infants, using a highly specific antibody. Blood samples were taken from the antecubital vein of the resting babies, 3 to 5 h after feeding, between 9 and 11 a.m. The 0–7-day-old babies had PA levels ranging from 0 to 330 ng/100 ml (65 determinations). In most cases, the levels could be considered high since only 3 values were found to be within the range of those of the non-stimulated normal adults (<3·5 ng/100 ml) and 60% of the values were higher than those of the stimulated normal adults (>30 ng/100 ml). In the first 3 to 5 days, the weight loss was between 3 and 10·5% and thereafter occurred a normal weight increase. There was no correlation between PA levels and the variations in weight. Sodium intake was evaluated for breast fed (0·1 to 0·3 mEq/kg), bottle fed (0·5 to 1 mEq/kg) and sodium loaded (3·5 to 4·5 mEq/kg) newborns. No correlation was found between PA and the sodium intakes. When the mother was submitted to a salt restricted diet during the late pregnancy, the PA level of the newborn was unaffected. In comparison with newborns, lower PA values and narrower ranges were found in older babies (3 to 80 ng/100 ml in the group aged 2 to 8 weeks, n = 12 and 3 to 12 ng/100 ml in the 2 to 12-month-old babies, n = 9). After the first year of life, the observed levels were within the range of those of adults. Some values reaching 5 to 42 ng/100 ml were observed in newborns suffering from adrenal diseases with acute salt loss. These values might reflect a limited aldosterone biosynthesis. Infants with salt losing kidney diseases had PA levels over 320 ng/100 ml. From this study, it appears that within the first year of life, PA levels must be interpreted with caution.

**55. Ovarian-placental dependency in rat: estrogen biosynthesis by the rat ovary in late pregnancy,** ROMAN REMBIESA, MARIA MARCHUT and AMELIA WARCHOL, Department of Endocrinology, Institute of Pharmacology, Pollish Academy of Sciences, Kiaków, Poland

While the biosynthesis of estrogens from progesterone has been adequately documented in the ovaries before placental development, evidence for the ability of the luteinized rat ovary to form estrogens in the late state of pregnancy is lacking. Observations in a number of laboratories suggested that the placenta could not take over the endocrine function of ovaries. Our observation confirmed this conclusion because no estrogens were isolated from rat placental tissue. However, we have observed conversion of C-21 steroids to androgens by placental tissue. This observation suggests that in rat during the second half of pregnancy ovarian-placental dependency may exist in the synthesis of estrogens. (Steroids 1972, **19**, 65). Thus, it was proposed that pregnant rat ovaries could synthesize estrogens from androgens.

To evaluate the ovarian-placental dependency hypothesis, we examined: (1) whether rat ovaries collected during late pregnancy were capable of removing the 17-side-chain of progesterone and of aromatizing the cleavage product and (2) whether rat ovaries were capable of aromatizing C-19 precursors. Our results indicated that rat ovary during late pregnancy is capable of forming estradiol-17$\beta$ *in vitro* from 4-androstendione but not from pregnenolone, progesterone and 17-hydroxyprogesterone. Hence it appears that during pregnancy, placental androgens may be a precursor for the synthesis of estrogens by the ovary.

**56. Comparison of plasma hormone levels in the first trimester of normal human pregnancy with these same levels in pregnancies following induction of ovulation,** J. R. T. COUTTS, P. ENGLAND and W. P. BLACK, Department of Gynaecology Research, Glasgow University, Glasgow Royal Infirmary, 106 Castle Street, Glasgow, Scotland

The corpus luteum in the human secretes relatively large amounts of hormones including progesterone, 17-hydroxyprogesterone and oestradiol-17$\beta$. In anovulatory women in whom ovulation was stimulated by treatment with human menopausal gonadotrophin (HMG) and human chorionic gonadotrophin (HCG), the ovarian production of these hormones was increased. When conception occurs the corpus luteum secretes increasing amounts of these hormones and maintains the pregnancy in the first trimester. The stimulus for luteotrophic activity is probably HCG synthesized by the products of conception. Serial weekly plasma samples have been collected in two groups of pregnant women from 5 to 16 weeks after their last menstrual period. The first group consisted of 12 apparently normal pregnancies and the second group of 8 pregnancies in women following induction of ovulation with HMG and HCG. Plasma levels of HCG, progesterone, 17-hydroxyprogesterone and oestradiol-17$\beta$ were determined by specific radioimmunoassays in each of the plasma samples. The results of these assays in each of the 2 groups of patients were averaged and these results show that the corpus luteum of pregnancy has a functional life of 8 to 10 weeks; thereafter maintenance of the pregnancy is the responsibility of the trophoblast. Comparison of the hormone levels in the 2 groups of patients confirmed the increased ovarian activity as a result of induction of ovulation. Comparison of the profiles of these hormones in peripheral plasma in the 2 groups of patients will be made and the hormone levels will be related to the outcome of the pregnancy in successful and unsuccessful cases.

**57. Monitoring early pregnancy with RIA of steroids. Prognostic value of estrogens and progesterone.** R. MONDINA, P. CAPETTA, G. MOJANA, E. ANTIFORA, M. MEILLE and F. POLVANI, II° Clinica Ostetrico Ginecologica della Università di Milano, Laboratorio di chimica degli ormoni del C.N.R. di Milano, Italy