At birth, in cases of premature delivery, EPH gestosis, feto-placental insufficiency, retarded fetal growth and so on, lower values of excretion of these steroids are present. In the first few days of neonatal life we have observed an increasing trend of almost all these compounds. Generally, when low oestriol levels are present during fetal life, low levels of all 16-substituted steroids appear in newborn urine too. This fact confirms the opinion that, as hormonal activity, the newborn in the first few days of life would be a kind of fetus model.

72. Cortisol metabolism in the neo-natal period, C. H. L. SHACKLETON, J. W. HONOUR and N. F. TAYLOR, Division of Clinical Chemistry, Clinical Research Centre, Harrow, Middlesex, England

1β-Hydroxycortolone was recently shown to be an important urinary metabolite of cortisol in an infant with renal-tubule insensitivity towards aldosterone (Shackleton C. H. L. and Snodgrass G. H. A. I.: *Ann. clin. Biochem.* 11 (1974) 91) and this compound may well be identical to an unidentified metabolite isolated from infant urine by Danilescu-Goldenberg and Giroud (*J. clin. Endocr. Metab.* 38 (1974) 64) following administration of labeled cortisol. The present investigation was undertaken to ascertain the quantitative excretion of urinary 1β-hydroxycortolone relative to other cortisol metabolites in the normal and pre-term newborn. Total steroid extracts of urine were obtained following enzymic hydrolysis, Amberlite XAD-2 extraction and purification on Sephadex LH-20 columns (Shackleton C. H. L., Gustafsson J.-Å. and Mitchell F. L.: *Acta endocr., Copenh.* 74 (1974) 157). Methyloxime-trimethylsilyl ethers of the steroids were prepared and analysed by combined gas chromatography-mass spectrometry (GC–MS). Complete spectra in the mass range 100–800 nm were acquired at 10 s intervals throughout the GC–MS analysis. The mass spectra were stored in mass converted format on magnetic tape. The data were processed (DPLOT module Varian SpectroSystem 100 MS) and intensities of up to eight selected ions from the series of mass spectra were plotted in graphic form on an oscilloscope and photographed. The intensities of selected ions specific for cortisol and its metabolites (e.g. cortisone, 6β-hydroxycortisol, 6β-hydroxycortisone, 20-dihydrocortisol, 20-dihydrocortisone, tetrahydrocortisol, tetrahydrocortisone, cortolones, cortols, and 1β-hydroxycortolone) were determined and related to intensities of ions given by standard mixture of cortisol metabolites. The major metabolites of cortisol present in infancy urine were found to be tetrahydrocortisone, 6β-hydroxycortisol, cortolone and 1β-hydroxycortolone. The excretion of tetrahydrocortisol and *allo*-tetrahydrocortisol was found to be extremely low. A significant amount of cortisol was excreted unmetabolised.

73. Integrated serum gonatrophins and gonadal steroids during first weeks of life in premature male infants, A. ATTANASIO, E. STEIL, M. EICHNER, K. RAGER, H. MENTZEL and D. GUPTA, Departments of Diagnostic Endocrinology and Neonatology, University Children's Hospital, 74 Tübingen, Germany

Pituitary-gonadal relations in newborns and in infants have been investigated by several authors in the last years. Evidence for testicular activity in early infancy has been accumulated. While many authors evaluated hormonal data in infants in relation to later pubertal events, the significance of hormonal activity in the newborn period for the normal genital development has not been discussed so far. In this study, blood specimens from prematurely born male infants (27th to 39th week of gestational age) were obtained longitudinally. The specimens were assayed for plasma testosterone, serum LH and FSH by RIA. Most of the subjects were found to have undescended testes at the beginning of the observation period, and had them down in the scrotal position by the end of this period. During the longitudinal follow up, peak values of plasma testosterone, sometime reaching 2000 ng/100 ml, were observed, although the timing of the peak was individually variable. No such pattern was found for serum gonadotrophins, although, on the average, they were significantly elevated when compared to later developmental stages. The results of this longitudinal study show that the hypothalamo-pituitary-gonadal axis is highly active during this developmental period. The high gonadotropin values clearly demonstrate that the enhanced testicular activity found in these premature infants do not depend upon placental factors. Since descent of testis is known to occur between the 32nd and the 40th week of normal gestation, the pattern of plasma testosterone so far revealed in this longitudinal study suggests a relationship between high levels of circulating testosterone and descent of testis in the scrotal position.

74. Plasma dehydroepiandrosterone (DHEA) and pregnenolone (Δ_5P) in newborns after HCG stimulation, A. LUCISANO, G. TORTOROLO, E. ARNO and S. DELL'ACQUA, Instituto di Clinica Pediatrica e Istituto di Clinica Ostetrica e Ginecologica Università Cattolica del Sacro Cuore, Rome, Italy

The foeto-placental unit utilizes as estriol precursor mainly the DHEA, synthesized by foetal adrenals. The purpose of the present investigation was to elucidate the role of HCG in the regulatory mechanism of foetal DHEA synthesis.

In a group of 8 newborns, during the first days of the life, when the *paleocortex* is still present, total DHEA and total Δ_5P have been measured daily in plasma by means of a gas-chromatographic assay. The plasma levels of DHEA and Δ_5P decrease until the third day from birth, from 0·9 to 0·3 μg/ml for DHEA and from 0·8 to 0·4 μg/ml for Δ_5P. In a group of 6 newborn at third day after birth we injected 5000 IU of HCG and in the following 24 h we obtained a significant increase of plasma levels of DHEA and Δ_5P. These results suggest that as well as foetal ACTH, HCG can also be involved in the control of DHEA synthesis by foetal adrenals.

75. Perinatal adrenal anomaly associated with total absence of 3β-hydroxy-5-ene-steroids in the infants urine, K. CARLSTRÖM*, G. BJÖRK†, P. ENEROTH‡ and J.-A. GUSTAFSSON§, Hormone Laboratory* and Department of Obstetrics and Gynaecology†, Sabbatsbergs Sjukhus; Hormone Laboratory, Karolinska Sjukhuset‡ and Department of Chemistry §,Karolinska Institutet, Stockholm, Sweden

3β-Hydroxy-5-ene-steroids are the major steroids in the human foetus and in early infancy, and are excreted in large amounts in the infants urine. They are mainly synthesized by the foetal zone of the adrenal cortex and act as precursors for the foetoplacental oestrogens, notably oestriol. Foetal adrenal anomalies might therefore be accompanied by low maternal urinary oestriol excretion. As far as we know from the literature such

cases result in perinatal deaths or require cortisone treatment within some weeks after birth.

The present communication describes a case of otherwise uncomplicated pregnancy associated with an extremely low maternal oestriol excretion (1·9–3·3 mg/24 h during the 36–37th week), but with an excretion of oestrone + oestradiol-17β at the lower normal limit. A male infant was born which, with exception of a very mild ichtyosis, showed normal clinical findings at birth as well as 10 months later. There were no signs of cerebral dysfunction. The placental steroid sulphatase activity was normal. 5 days after birth the plasma levels of cortisol were within the lower limit of the normal values but raised later. Urinary pregnanetriol excretion and response to ACTH stimulation were normal.

Gas chromatographic – mass spectrometric analysis of the infants urine revealed a steroid pattern resembling that found in anencephaly with a total absence of 3β-hydroxy-5-ene-steroids. This particular child might therefore have lacked the foetal zone of the adrenal cortex, which is the case in anencephaly. Due to the normal clinical findings it might be speculated that the excessive production of 3β-hydroxy-5-ene-steroids in the foetal and early infancy stages is not of vital importance.

76. Urinary excretion of aldosterone, tetrahydrocortisone (THE), and tetrahydrocortisol (THF) in premature infants, W. RAUH, L. WILLE, P. VECSEI, W. VIELHAUER and H. WILL, Department of Pediatrics and Pharmacology, University of Heidelberg, Germany

Urinary excretion rates of aldosterone, THE, and THF were determined radioimmunologically in 7 premature infants during the first 10 days of life. Specific radioimmunoassays for THE and THF were developed. White New Zealand rabbits were immunized against complexes of THE- and THF-20-oximes and bovine serum albumine. After an immunization period of 6 months specific antibodies against THE and THF were obtained. Radioimmunochromatographic analysis showed that THE- and THF-antibodies also bind with the glucuronates of THE and THF. Urinary excretion rates of THE (0·002–0·1 mg/m^2/24 h) and THF (0·005–0·05 mg/m^2/24 h) were extremely low when compared with normal values of older children and adults (THE: 0·3–3·0 mg/m^2/24 h, THF: 0·3–2·5 mg/m^2/24 h). Aldosterone excretion ranged from 3·0–60·0 μg/m^2/24 h, the normal values for adults being 2·0–12·0 μg/m^2/24 h. There was a definite increase in THE excretion during the first 10 days of life, whereas THF and aldosterone excretion did not change during this period of time. In this study a special pattern of adrenal steroid excretion in premature infants is demonstrated. The differences between premature infants and older children can be explained by changes in adrenal function and hormone metabolism.

77. Role of aldosterone in sodium homeostasis in premature neonates, J. W. HONOUR, C. H. L. SHACKLETON and H. B. VALMAN, Northwick Park Hospital and Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, England

The urinary steroid excretion of pre-term infants (22–30 weeks gestation age) has been studied following the repeated observation of a persistent period of urinary salt loss and hyponatraemia developing between the first and third weeks of life. This investigation was therefore undertaken to study the production of steroids by pre-term infants during this period. Aliquots of 24 h urine collections from pre-term infants were hydrolysed enzymically, and the steroids extracted on Amberlite XAD-2 columns and fractionated by Sephadex LH-20 chromatography (Shackleton C. H. L., Gustafsson J.-A. and Mitchell F. L.: *Acta endocr., Copenh.* **74** (1973) 157). Steroids present in fractions were analysed as methyloxime-trimethylsilyl ethers using capillary column gas chromatography and gas chromatography-mass spectrometry (GC–MS). The steroid excretion was similar to that of full-term neonates, apart from the finding of relatively large amounts of tetrahydroaldosterone, the principal urinary metabolite of aldosterone. Selected ion-monitoring GC–MS was used for the specific quantitative determination of urinary tetrahydraldosterone, 3β-*allo*-tetrahydroaldosterone being used as internal standard (Shackleton C. H. L. and Honour J. W.: *Z. Klin. chem. klin. biochem.* **12** (1974) 259). A weak positive sodium balance was observed during the first weeks of life and this was associated with low plasma sodium and elevated urinary tetrahydroaldosterone excretion (100–300 μg/24 h). In full-term infants the excretion of tetrahydroaldosterone is between 1 and 25 μg/24 h. These results show that pre-term infants are capable of synthesizing aldosterone in response to low plasma sodium concentrations. An increasingly positive sodium balance was observed from the third week of life but tetrahydroaldosterone excretion remained high and was still elevated when normal plasma sodium concentration was established. The results suggest for the first time that the renal tubular response to aldosterone is low in pre-term infants although the juxta-glomerular apparatus is considered to be functional.

78. Steroid metabolism by mouse placental tissue *in vitro,* G. H. OKKER-REITSMA, Laboratory of Cell Biology and Histology, University of Leiden, Leiden, The Netherlands

It is known that the mouse placenta is capable of steroid metabolism at some time of its existence, but to what extent is still not clear. It is generally accepted that in the rodent placenta steroid formation is one of the functions of a particular cell type – the mononuclear giant cells. The present study was designed to look into the capacity for steroid synthesis of the mouse placenta during development, with special emphasis on the role of the giant cells. Mouse placental tissue of 10 and 15 days gestation was incubated for 4 h in 1 ml medium 199 with either [^{3}H]-progesterone or [^{3}H]-dehydroepiandrosterone. The placenta of 10 days gestation shows many well developed giant cells. The labyrinth and the "basal zone" are still very small; consequently a division of the placenta in parts with and without giant cells is not possible. The explants of placental tissue of 15 days gestation, however, consisted of a part of the placenta which contains many giant cells or a part of the labyrinth which should not contain any giant cell. At 15 days the giant cells are smaller than earlier in development. The steroid metabolites were extracted and subjected to paper- and thin layer chromatography. The identified metabolites implicate the presence of enzymes involved in steroid synthesis. In the placenta of 10 and 15 days 3β-hydroxysteroid dehydrogenase (3β-HSD), 5α-reductase and C_{21}-C_{19} lyase could be detected. Incubations of placental tissue of 10 and 15 days with giant cells showed 20α-HSD activity. Placental tissue of 15 days with giant cells showed also 17β-HSD activity. 21-Hydroxylase was found in the labyrinth of the placenta of 15 days gestation. From these results we can conclude that giant cells as well as the labyrinth of the mouse placenta are