3. Diabetes
   Urinary total estrogens were proven to be a useful tool in managing diabetic pregnancies. However, in cases of minor decreases more useful information was obtained from serum unconjugated estriol levels. The other parameters were found to be without diagnostic value.
4. Intrauterine death
   In all cases of fetal death the event was preceeded by a sharp decrease of serum unconjugated estriol. The indication of fetal death by the other parameters was less stringent.

Conclusion: Of the steroids (and proteins) investigated, unconjugated serum estriol values proved to be the biochemical parameter most closely correlated to "fetal well-being".

45. *In vitro* and *in vivo* adrenal cortical steroid production by foetal sheep – effect of angiotensin II, sodium deficiency, ACTH., E. M. WINTOUR, E. H. BROWN, K. J. HARDY, J. G. McDOUGALL, C. J. ODDIE and G. T. WHIPP, Howard Florey Institute of Experimental Physiology and Medicine, Dept. Physiology, Dept. Surgery, University of Melbourne, Victoria, Australia

In 1974 we reported that the ovine foetal adrenal cortex was capable of secreting aldosterone, corticosterone, and cortisol as early as the 40th day of a 145–150 day gestation period. ACTH was shown to be a potent stimulus to all three steroid secretions from adrenals incubated *in vitro*, particularly in the <90 day old animals. The experiments have been further extended by studying the effects of angiotensin II, and sodium deficiency on *in vitro* steroid production, and by the study of ACTH infused into chronically-catheterized foetuses from 100–150 days. ACTH infused into chronically-catheterized foetuses (5 I.U/h for 90 min) produced an approximate doubling of peripheral blood aldosterone, corticosterone, and 11-deoxycorticosterone concentrations, with no change in 11-deoxycortisol concentrations. From 110 days→term, control cortisol values increased from 0·05→0·5 μg/100 ml, and acute ACTH infusion induced 3–10-fold elevations on this baseline. Forty-one pregnant ewes provided foetuses for the angiotensin II study. Angiotensin II (2·5 μg/ml) added to the incubation medium increased the production rates of aldosterone (1½–2-fold), corticosterone (2–9-fold) and cortisol (2–8-fold) from adrenals of foetuses up to 100 days gestation. After 120 days angiotensin II was not a significant stimulus to steroid production *in vitro*. When adrenals of foetuses, 125–127 days gestation were incubated in low sodium (130 mol/l) buffer aldosterone production was not increased. 8 ewes were made severely sodium deficient by uncompensated loss of parotid saliva for 10 days. The adrenals of their foetuses, when incubated *in vitro*, did not produce substantially increased quantities of aldosterone. Despite demonstrated steroidogenic capacity foetal adrenal cortical cells younger than 80 days contained insigificant amounts of agranular endoplasmic reticulum.

J. Steroids in late pregnancy, ARNOLD KLOPPER, University of Aberdeen, Scotland

Although the foeto-placental unit produces a great array of steroids, clinical interest lies mainly with progestagens and oestrogens. This review will be confined to these two groups, – their precursors, the active hormones and their metabolites. Urinary steroid assays have been done for many years, plasma measurements are new; attention will be directed to plasma assays. The concentration of a steroid in blood is a different concept from urinary steroid excretion. These differences will be examined and models for the control of steroid hormone concentration proposed.

Data concerning the range of steroid concentration in normal subjects in late pregnancy will be produced. These show that plasma concentration, as with urinary excretion may vary greatly from one healthy woman to another. So large is the normal range that there is a considerable overlap with the values found in a variety of obstetric diseases. It will be demonstrated that steroid assays have little diagnostic value; they cannot be used to diagnose the presence of retarded foetal growth or other obstetric complication. In this event the main clinical application for steroid assay is to delimit changes of steroid concentration with time in the same subject. Day-to-day variability of steroid concentration in the same subject and the factors which may affect this, becomes the central criterion in the application of hormone assays and it is intended to present some evidence concerning the time-to-time variability of plasma steroid concentration.

The use of plasma hormone estimations is based on the assumption that the maternal plasma concentration of a steroid reflects its rate of production by the foeto-placental unit, i.e., the activity of a variety of biosynthetic enzymes in the foetus and placenta. Evidence will be presented concerning the activity of such placental enzyme systems as 3β-hydroxysteroid dehydrogenase and ring A aromatase when precursors such as dehydroepiandrosterone sulphate or pregnenolone sulphate, are injected intravenously into the mother in late pregnancy.

Accurate information concerning the range of steroid concentration at various stages of pregnancy is an essential prerequisite to the application of steroid assays in the assessment of foeto-placental function. The normal levels of a variety of steroids will be reviewed and the order of change in obstetric pathology demonstrated. The changes with time in the same patient will be explored and an attempt made to correlate these with changes in the pathological state and the outcome of the pregnancy.

The evidence to be presented will tend to show that none of the steroid assays presently in use are wholly satisfactory and some speculations will be offered concerning particular steroid assays with a larger potential.

46. Identification and measurement of three oestetrols and two oestriolones in late pregnancy urine, N. F. TAYLOR and C. H. L. SHACKLETON, Division of Clinical Chemistry, Clinical Research Centre, Harrow, Middlesex HA1 3UJ, England

Two oestriolones have been identified by gas chromatography–mass spectrometry in extracts of late pregnancy urine. Sodium borohydride reduction of these steroids gave compounds with mass spectra identical to 15- and 18-hydroxy-oestriol respectively. It was concluded that they had the structures 3,15ξ16ξ-trihydroxy-oestratrien-17-one and 3,16ξ,18-trihydroxy-oestratrien-17-one and therefore might be intermediates in the placental conversion of foetal 3β,16α or β,18-trihydroxy-5-androsten-17-one and 3β15α or β,16α-trihydroxy-5-androsten-17-one to the oestetrols which occur in pregnancy urine (Taylor N. F. and Shackleton C. H. L.: *Steroids* **24** (1974) 185). In order to assess the importance of these new oestrogens they have been quantified in extracts of late pregnancy urine. Steroids were recovered from urine by enzymic hydrolysis, Amberlite XAD-2 extraction and Sephadex LH-20 chromatography (Taylor N. F. and Shackleton C. H. L.: *J. Endocr.* **64** (1975) 8P). Following derivatization the oestetrols and oestriolones were quantified by gas chromatography and mass fragmentography. See Table.

The correlation coefficient between 15α-hydroxy-oestriol and its epimer was 0·88 and with total oestrogens determined by the Kober reaction, 0·69, while that with 18-hydroxy-oestriol was −0·03. The poor correlation between 18-hydroxy-oestriol and 15α-hydroxy-oestriol may signify an important difference in the pathways of their production. Measurement of 18-hydroxy-oestriol may prove to be of more clinical significance in pregnancy monitoring that 15α-hydroxy-oestriol has so far proved to be .

47. Plasma levels of estriol, estradiol-17β, progesterone, 17-hydroxy-progesterone and prostaglandins-$F_{1\alpha}$ and $F_{2\alpha}$ in pregnant women near term, S. DELL'ACQUA, A. MONTEMURRO, A. LUCISANO, C. PATRONO, D. GROSSI-BELLONI, E. PARLATI, B. CINQUE, E. ARNO and A. BOMPIANI, Instituto di Clinica Ostetrica e Ginecologica e Istituto di Farmacologia, Università Cattolica del S. Cuore, Roma, Italia

It is still not clear whether the levels of circulating progestins and estrogens in late pregnancy can influence the beginning of labour. The purpose of the present investigation was to determine the plasma levels of these steroids in normal pregnant women near term through a serial sampling and to correlate them with the plasma levels of prostaglandins-$F_{1\alpha}$ and -$F_{2\alpha}$. In a group of normal pregnant women plasma samples have been collected every 24 h at 38th week of gestation, every 12 h at 39th week of gestation and then every 6 h until labour began. Prostaglandins-$F_{1\alpha}$ and -$F_{2\alpha}$ and estriol, estradiol-17β, progesterone and 17-hydroxy-progesterone in free form have been measured in plasma samples by means of specific RIA.

From the analysis of the profiles obtained and the ratiòs between the different compounds studied we could not demonstrate any significant variation in the steroids and prostaglandins plasma levels before the beginning of labour.

48. Effect of ACTH administration into the fetus, on onset of labour and on maternal plasma steroid levels, O. GAMISSANS, E. DAVI, E. PEREZ-PICAÑOL, P. PUGOL-AMAT and G. R. WILSON, Department of Obstetrics and Gynaecology, School of Medecine, Universities of Barcelona (Spain) and Aberdeen (Scotland)

In a previous study (Gamissans *et al.*, Acta Endocrinologica Congress, Amsterdam, 1975) it was shown that the intraamniotic injection of β-metasone (20 mg) is ineffective in precipitating the onset of labour in humans. However, the following modifications in maternal serum steroid levels were found as a result of the corticosteroid administration: a decrease in total immunoreactive oestrogens and of unconjugated estriol. In an attempt to study further the role of the fetal adrenal gland on the mechanisms of onset of labour, synthetic ACTH (Synacthen depot 1 mg) has been injected into the fetal breech, in a group of nine pregnant women at 38–41 weeks. In a control group of eight pregnant patients, 1 ml of isotonic saline was also injected into the fetal breech. In all patients blood samples were taken daily, for two before injection into the fetus, until delivery. Injection-delivery interval and blood pH of umbilical vessels at delivery were recorded. Maternal serum progesterone, total immunoreactive oestrogens, unconjugated oestriol and oestradiol-17-β were measured by R.I.A. The results did not show any significant difference between treated and control groups recording injection-delivery interval, and umbilical artery and vein pH at delivery. Maternal serum total immunoreactive oestrogens and progesterone levels did not show, after ACTH injection, a different pattern than that observed in the control group. Results on unconjugated oestriol and oestradiol-17-β will also be presented. The ACTH injection into the fetus has no influence on the onset of labour in the conditions and dose level used in this study.

49. Monitoring of foetal well-being by the determination of estriol-16α-glucuronide in urine and plasma, HERMAN ADLERCREUTZ, TESSA LEHTINEN and KATARINA BIRATH, Department of Clinical Chemistry, University of Helsinki, Meilahti Hospital, SF-00290 Helsinki 29, Finland

A rapid and specific radioimmunological method for the assay, in pregnancy, of estriol-16α-glucuronide in urine and plasma has been developed. No extraction or purification is necessary. Antisera to estriol-16α-glucuronide, raised in two sheep and coupled to Sepharose particles, showed good specificity. Both antisera cross-reacted less than 4% with unconjugated estradiol and estriol, and less than 1% with unconjugated estrone. When tested against a number of conjugated estrogens, the antisera cross-reacted less than 1% with all except one. The exception was 17-epiestriol-16-α-glucuronide with which one antiserum cross-reacted about 17% and the other not at all. The method calls for samples of plasma to be diluted, 1:100 (v/v), samples of urine, 1:5000–100,000. Antibody diluted to give a 20% binding in the absence of unlabelled steroid, is added, and the samples are incubated while gently rotated for 30 min at room temperature. [^{3}H]-estriol-16α-glucuronide in buffer is added and incubation is continued for 1·5 h at room temperature and, while the tubes are still rotated, for 1 h at +4°C. After centrifugation the particles are washed three times with saline. Radioactivity, released from the particles by shaking with 1 M HCl, is then counted. This step enhances the sensitivity of the method 40-fold as compared to a direct counting of the particles. The coefficient of variation calculated from 30 duplicate urine samples was 8·7%. The limit of detection is 10 pg. Some preliminary data indicate that the day-to-day variation in urinary output of estriol-16α-glucuronide is about 7 to 13% and is hence smaller than that reported for total estriol. Preliminary clinical results suggest that, because of this smaller variation, the determination of estriol-16α-glucuronide is more useful than the measurement of total estriol in monitoring of foetal well-being. The results obtained with this method correlated well with those obtained with a

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Structure	Mean mg/24h	Range mg/24h	No.
Oestratriene-3,15ξ,16ξ,17ξ-tetrol	0·74	0·3 –3·0	20
Oestratriene-3,15α,16α,17β-tetrol	1·69	0·75–4·1	20
Oestratriene-3,16ξ,17ξ,18-tetrol	0·39	0·2 –1·5	20
3, 15ξ,16ξ-trihydroxy oestratriene-17-one	0·08	0·05–0·10	3
3,16ξ, 18-trihydroxy oestratriene-17-one	0·07	0·05–0·10	3