increases, probably reflecting the placental production of this steroid.

In the initial stages of pregnancy, steroid determinations have scarce clinical significance. Their usefulness increases as pregnancy advances and they become determinant when the full foeto-placental interrelations are established.

In cases of suspected molar pregnancy, the determination of the ratio 16α-hydroxylated oestrogens/16-deoxyoestrogens may be useful as a low production of the first group of compounds is consistent with the absence of the foetus.

Blood levels and urinary excretion of steroids in pregnancies induced with exogenous gonadotrophins may differ considerably from those found in spontaneous pregnancies. These differences, when present, are specially marked in the first few weeks after conception. It is generally agreed that the elevated steroid levels usually found in the gonadotrophins induced pregnancies, are probably due to ovarian hyperstimulation resulting either in multiple corpora lutea or, perhaps, in hyperactivity of one corpus luteum.

The high oestrogen, progesterone and 17α-hydroxyprogesterone levels found in pharmacologically induced pregnancies associated with hyperstimulation, usually return to the levels normally found in the spontaneous pregnancies of the same age by the 8th-10th week of gestation and they are not correlated with the occurrence of multiple pregnancies.

42. Significance of HCG, HCS, progesterone, and estriol determinations during the first half of human pregnancy, I. GERHARD and B. RUNNEBAUM, Abteilüng für gynäkologische Endokrinologie, Universitäts-Frauenklinik, 69 Heidelberg, West Germany

This study was undertaken to ascertain if the determination of hormones in maternal blood gives reliable information about the functional state of the endocrine systems during early pregnancy. Using radioimmunoassay techniques, HCG, HCS, progesterone, and estriol were determined in 75 women with normal pregnancy and in 82 women with bleeding during pregnancy weeks 5–23. In women with normal pregnancy the hormones were assayed weekly, and in patients with vaginal bleeding usually 3 determinations per week were performed. Of 32 women with bleeding during week 5–10 of pregnancy, 11 went to term and 21 had an abortion. In the women with abortion, HCG was below the normal range in 8, HCS and progesterone in 10, and estriol in 6 women. Of 30 women with bleeding during week 11–14, 12 went to term and 18 had an abortion. In the women with abortion HCG and HCS were below the normal range in 10, progesterone in 9, and estriol in 12 women. Of 20 women studied during week 15–23, 3 went to term and 17 had an abortion. In the abortion group HCG was below the normal range in 6, HCS in 8, progesterone in 6, and estriol in 8 women. In most women with bleeding and abortion during the first half of pregnancy, the levels of the studied hormones were significantly below the normal range. In general, women with vaginal bleeding during early pregnancy have a good prognosis for normal termination, if repeated determinations of HCG, HCS, progesterone and estriol are within the normal range.

43. Steroids and protein hormones as indices of placental blood flow, P. G. CROSIGNANI, A. ATTANASIO, G. TURCONI, G. C. LOMBROSO, L. COMO, G. PARDI and E. TONANI, Department of Obstetrics & Gynecology, University of Milan, Italy

Toxemia and post-term pregnancy are two situations where placental blood flow may be impaired. The severe reduction in placental blood flow in part of toxemic patients is deduced from the fetal outcome (intrauterine death, retarded growth), while the fetal hypoxia occasionally present in post-term pregnancy is inferred from its manifestations in labor (stained liquor, altered patterns of fetal heart rate). Seventeen toxemic patients who delivered either a dead fetus or a small-for-date infant were studied by plasma measurement of free estradiol (E_2), free estriol (E_3), chorionic gonadotropin (HCG), and placental lactogen (HPL). Plasma estradiol was normal in all 7 patients in whom it was measured. Estriol was abnormally low (below 2 SD) in 2 of 9 patients. Chorionic gonadotropin was abnormally high (above 2 SD) in 7 of 13 patients. Placental lactogen was abnormally low (below 2 SD) in 11 of 17 patients. Five of the 7 women with high HCG showed low HPL as well. Intensive care and early intervention (7 caesarean sections in this series) probably anticipated further pathological changes in these indices and more critical conditions in the fetuses. Thus, in toxemia, placental protein hormones seem more prompt and reliable indices in reflecting impaired placental blood flow, compared with free E_2 and E_3. Twenty-five post-term patients were similarly studied. Twelve of these showed stained liquor and/or pathological changes in FHR during labor; all of them had normal E_3 and HPL levels, while as a group they had significantly higher HCG plasma concentrations. Thus, even the short lasting reduction in placental blood flow which characterizes a considerable number of post-term pregnancies seems effective in enhancing HCG production before or in the absence of E_2, E_3, and HPL changes.

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44. Estrogens and progesterone and other biochemical parameters in pathological pregnancies, RICHARD GOEBEL and ERICH KUSS, I. Frauenklinik der Universität Müchen, Germany

In late pregnancy, serum free estrone, estradiol-17β, estriol as well as total estrogens and progesterone were estimated and compared with urinary total estrogens. About 4000 specimen were analyzed during surveillance of more than 200 high risk pregnancies (114 toxemia, 41 low birth weights without signs of toxemia, 45 diabetes, 14 fetal deaths). The serum constituents were measured by radioimmunoassay, urinary total estrogens by photometry. The between assay precisions were characterized by VK's $<8\%$. Parallel assays of placental lactogen, phosphatase, cystine aminopeptidase and fetoprotein were run.

Results:

1. Toxemia
   (a) Normal weighted newborns were delivered mostly in cases of mild toxemia. The values of urinary estrogens and unconjugated serum estriol were found in the normal range, the other estrogens and progesterone frequently in the lower range.
   (b) Small-for-date babies were delivered in most cases of severe or superimposed toxemia. In about 75%, the growth retardation was indicated by low urinary total estrogen and by low serum unconjugated estriol. The other parameters were less clear-cut.
2. Intrauterine fetal retardation (without toxemia)
   In 86% of cases, fetal retardation was indicated by low serum unconjugated estriol and by low urinary total estrogens. The other parameters were again found to be less clear-cut.

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.