Amniotic, urinary and serum hormones in fetal diagnosis

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Until recently the fetus was thought to be at best a passenger, at worst a parasite, drowsing in the tropical wet heat of the womb at the receiving end of a simple filter providing, in Professor McCance's words, the essential services of supply and sewage. Now it seems we have done him an injustice because, weightless in his sterile command module, he can monitor a flow of complex signals and make some effective contribution to mission control and possibly to his own safe splashdown. The fetus and the placenta each lack key enzymes owned by the other so that steroids pass back and forth before the finished product is released into the mother or the amniotic fluid. Failure or aberration in one or other component of this fetal-placental unit may release a hormonal warning signal which may be interpreted in the laboratory. This involves a search for readily detectable hormones which result from fetal and not just placental activity. Many steroid compounds are involved in pregnancy and only those which are readily measurable are discussed (Table I).

Presented at the Heinz Seminar on Antenatal Pediatrics held in Winnipeg, Manitoba, July 1970 and at the invitation of the Canadian Paediatric Society.

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Human chorionic gonadotrophin (HCG)

HCG in the mother's serum and urine and in the amniotic fluid has been used to assess the condition of the conceptus, but since it is a purely placental product it may not reflect fetal problems, and Michie[26] reviews the case against its present reliability as a measure of the effects, for example, of erythroblastosis. High levels, of course, can indicate twin pregnancy as well as hydatidiform mole.[19]

Human placental lactogen (HPL)

In normal pregnancy serum levels of HPL are believed to be low in the first trimester, to rise fairly acutely in the second and to continue at an elevated level to term. Samaan, Bradbury and Goplerud[33] found it provided no useful prognostic information in a number of common abnormalities of pregnancy.

Pregnanediol

Progesterone, too, is mainly placental in origin so that maternal urinary pregnanediol (plasma levels vary too much to be of value[12]) may also be an unsatisfactory measure of fetal health, although Klopper and Stephenson[25] have reported very high levels in some severely affected cases. Acevedo *et al.*[1] believe that normal urinary values of pregnanolone and pregnanediol in the first trimester exclude the likelihood of abortion even when clinical symptoms suggest it, and attribute the reliability of their results to improved methodology.

Estriol

In normal women

Estriol can be measured in the mother's serum and urine and in the amniotic fluid in such quantities that it is easier to estimate than estrone and 17-β estradiol. Amniotic fluid[6] and urine levels escape the more rapid diurnal variations found in serum.[36]

Maternal estrogen has as its principal precursor in pregnancy, dehydroepiandrosterone, from the fetal adrenal gland, although some precursor may be derived from the mother's own adrenal cortex.[11] The dehydroepiandrosterone sulphate is converted in the fetal liver to 16 α-hydroxydehydroisoandrosterone sulphate which passes to the placenta, where it is aromatized and emerges as 16 α-hydroxylated estrogen. The importance of the fetal component means that maternal urinary estriol excretion falls if the fetus is in jeopardy long enough to make the *change* in estriol apparent. It is this last point which makes Michie and others[2, 29] insist on the importance of *serial* measurements.

In anencephaly

Women carrying an anencephalic fetus, which has little or no hypo-

TABLE I
Summary of hormonal antenatal tests described as useful

Threatened abortion	Urine	Pregnanolone, pregnanediol—low
Fetal death	Urine	Estriol—low
Anencephaly	Urine	Estriol (and pregnanetriol)—low
	Amniotic fluid	Estriol—low
Intrauterine growth retardation	Urine	Estriol—falling to low
Erythroblastosis, untreated	Urine, Amniotic fluid	Estriol—can fall to low
Intrauterine transfusion	Urine	Estriol—falls immediately but rises again if fetus improves
Severe toxemia	Urine	Estriol can be low
Renal insufficiency	Urine	Estriol can be low
Diabetes mellitus	Urine	Falling estriol indicates bad prognosis
Steroid-treated mothers	Urine	Low-estriol may mean suppressed fetal adrenal cortex
Congenital adrenocortical hypoplasia	Urine, ?Amniotic fluid	Estriol—low
Congenital adrenocortical hyperplasia	Urine	Estriol possibly raised in 11 β- and 21-hydroxylase deficiency

thalamo-hypophyseal-adrenocortical system, have much reduced urinary estriol levels in spite of demonstrable fetal life.[16] Michie and Livingstone[29] have established that this is also true for amniotic fluid levels, and Michie[26] has shown that an intra-amniotic injection of dehydroepiandrosterone sulphate corrects both, although this obviously has no therapeutic value. It, like the intra-amniotic injection of estriol sulfate,[24] is, however, used as a test of placental adequacy, and I shall refer to it again.

In intrauterine growth retardation (IGR)

Although a clear direct correlation between birth weight and maternal urinary estriol is disputed, both Klopper[23] and Michie[27] believe that normal excretion figures near term make unlikely the birth of an unusually light-for-dates baby, a stillbirth or neonatal death. Conversely, Ferdman and Belits[14] found that maternal urinary estriol levels *always* fall in IGR but without positive correlation with vaginal cytology. The studies of Galbraith, Low and Boston[17] have been rather less certain in that mothers of only 70% of IGR babies had low urinary estriol levels, while only 60% of mothers yielding low results produced IGR infants.

In other conditions associated with fetal risk

(a) *Erythroblastosis fetalis*—Earlier observations by Klopper and Stephenson[25] and by Schindler *et al.*[34] showed that normal values of *urinary* or *plasma* estriol could be found when the fetus was severely affected. In *amniotic fluid*, Schindler and his colleagues and Berman *et al.*[5] found low estriol levels in severe erythroblastosis fetalis. Michie[28] has used amniotic fluid estriol levels (falling or static in serial specimens over several weeks) to predict successfully imminent fetal death but not to predict the complexity of treatment in severely affected infants after delivery. Aleem, Pinkerton and Neil[2] found low amniotic fluid levels to be significant at 33 to 36 weeks and highly so at 37 to 40 weeks. Serial maternal urinary estriol levels have also been used to monitor intrauterine transfusions in that a sharp fall after the procedure (attributed to fetal upset) is followed by a more or less satisfactory rise where the prognosis is good. A continued fall, even if the levels are still within normal limits, is thought to indicate continued fetal deterioration.[7]

(b) *Toxemia*—Mild and moderate toxemia does not generally depress fetal growth. Thomson and Billewicz[3] and Michie[27] found it unassociated with lowered maternal urinary estriol unless the baby suffered also from IGR or was dead. Michie found low levels in severe toxemia which were associated with a higher incidence of perinatal death, but there is some evidence that they may be due in part to impaired renal clearance in women with gross proteinuria.

(c) *Diabetes mellitus*—Experience of estriol levels in diabetic pregnancy varies and will be discussed separately.

(d) *Prolonged pregnancy*—A Czech study of women pregnant for 288 days or more showed no correlation between maternal urinary estriol excretion and the duration of pregnancy or the ultimate result.[37] Beischer *et al.*[4] found excretion to be low in less than 4% but it correlated with fetal distress. However, fetal death was uncommon unless there was associated cephalopelvic disproportion.

(e) *Steroid-treated mothers*—High-dosage steroid therapy may be associated with low maternal urinary estriol excretion because of suppression of the fetal and/or maternal adrenal cortex or by interference with the function of placental enzymes.[32, 35, 38] Low levels may nevertheless be important in drawing attention to the need for possible replacement therapy in the live-born baby.

False results

Negative—Apart from the different interpretation which is required when the mother is treated with steroids and the possible depressed excretion in renal insufficiency (e.g. chronic pyelonephritis) and in toxemia with heavy proteinuria (above), urinary levels can be reduced to those usually indicating fetal death or anencephaly when the mother is being treated with menthenamine mandelate (Mandelamine). It is thought that this agent or its breakdown product (formaldehyde) can destroy urinary estriol but serum levels remain unaffected.[13]

Positive—Michie[28] draws attention to the relatively high amounts of estriol in meconium and to the resultant danger of false readings where amniotic fluid is meconium-stained, as it may well be in fetal distress.

Low estriol values and condition at birth—Having recognized the association of low maternal estriol and perinatal loss and morbidity, it is not surprising that there is also an association with fetal acidosis, and Fliegner *et al.*[15] recommend fetal pH monitoring where the estriol has been reported as low.

Low estriol values and later progress—The original paper by Wallace and Michie,[30] describing the follow-up of babies born to mothers with low estriol excretion during pregnancy and surviving at least until examined

in the second year of life, reported a high proportion of neurological and psychological abnormalities. Greene *et al.*[18] have recently reviewed their experience, and while more than half their babies, many of whom were small, had perinatal problems, they found both the incidence and the severity of later problems to be relatively low and unrelated to the kind of abnormal estriol pattern obtained. The series was not much larger than the Edinburgh one, however, and because of this the results should be accepted with caution although a more optimistic view is attractive.

Pregnanetriol

Pregnanetriol is increased in pregnancy urine as a result of increased precursor from the fetal adrenal cortex[20] and decreased when the fetus is anencephalic,[21] but the distinction is not so sharp as it is with estriol.

Future possibilities

Michie[28] states that future developments in hormonal monitoring of the fetus, particularly in rhesus-immunized pregnancies, depend on more precise and accurate methodology. The measurement of a specifically fetal hormone metabolite (e.g. 15-α-hydroxyestriol) and the possible separation of the various estrogen conjugates may prove possible and better.

The prenatal diagnosis of adrenal abnormality in the fetus

A prenatal diagnosis of congenital adrenocortical hyperplasia was made by Jeffcoate *et al.*[22] from an examination of the amniotic fluid for 17-oxysteroids and pregnanetriol. In spite of ample opportunity to extend this observation, little if anything has been published since. This may be due in part to a better understanding of the complexity of steroid metabolism in the fetus and infant, reviewed by Mitchell and Shackleton.[31] It seems as though the maturation of the fetal adrenal cortex is such that it may not yet be possible to unmask the defect in prenatal life in all or many cases.

Cathro[8] describes the difficulty of establishing the diagnosis when the fetus is suspected of having the very rare congenital lipoid hyperplasia of the adrenal glands in which the conversion of cholesterol to pregnanolone is blocked. Maternal urinary estriol excretion is akin to that in IGR, i.e. subnormal, but above that occurring in anencephaly for reasons that remain arguable, while pregnanediol is high normal perhaps because of intensive pregnanolone production in maternal steroidogenic glands.

Cathro has not had the opportunity to study a mother suspected of carrying a fetus with congenital adrenocortical hyperplasia, but he guesses at the following results from maternal urine:

21-hydroxylase deficiency: estriol above normal range for a single fetus

11-β-hydroxylase deficiency: estriol high

Δ^5-3 β-ol-dehydrogenase deficiency: estrogens, pregnanediol and pregnanetriol—all probably normal but a high output of estrogens and pregnanetriol is feasible.

Lastly, Cathro and Coyle[9, 10] successfully detected the existence of congenital adrenal hypoplasia in a fetus whose mother had previously produced a child with this abnormality. Maternal urinary estriol excretion was very low.

I am indebted to my colleagues, Dr. Eileen Michie and Dr. Angus Harkness, for help in the preparation of this paper.

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This undesirable result can be avoided by (a) making certain that the recipient holes are deep enough to accept the entire donor plug so that it does not project and (b) taking care during the boring initially to cut through the epidermis vertical to the skin surface and then to angle the cut as described earlier. The fit of donor plug to recipient site is thereby rendered more accurate and no excess epidermis results.

Sparse growth

Some plugs produce an insufficient number of hairs. Generally, any plug growing less than four hairs should be replaced. Usually any plug growing less than five hairs is partially replaced by a 3-mm. plug which overlaps the deficient one.

Loss of sensation

Temporary loss of sensation nearly always occurs in the donor and recipient areas, and is the result of the severing of nerves by the punch as it bores out donor and recipient sites. Patients usually notice this, but rarely complain about it. Sensation returns over a period of six to 12 months after the procedure is completed.

Illustrative case histories

Case 1

A 26-year-old single man, employed in advertising and sales for a metal works company, was concerned about his rapid loss of scalp hair over a period of approximately five years (Fig. 3).

Examination of the scalp revealed a large area of partial alopecia in the typical physiological hair loss pattern. Hair was virtually absent anteriorly but satisfactory posteriorly.

The anterior portion of the area of alopecia received 550 plugs in 15 sessions over a nine-month period. Figs. 4 and 5 were taken 3½ months after the last transplanting session. This patient was extremely pleased with the result and felt that he could do without filler plugs, preferring to save them for his crown, which was rapidly thinning. I would have preferred to use 10 to 20 plugs to thicken out what I considered to be an inadequate growth of hair on the left side anteriorly near the midline, but the patient refused.

No complications occurred during the course of his transplanting sessions.

Case 2

A 33-year-old married blue-collar worker was constantly getting into fights with fellow workers about the way he wore his hair to cover a rather large area of alopecia (Fig. 6). This patient had spent over $750 at a "hair studio" before being referred.

Examination of the scalp revealed a wide rim of moderately thickly growing hair surrounding the denuded areas. He also had severe seborrheic dermatitis. After the latter condition had been controlled by regular shampooing, 525 plugs were inserted in the area of alopecia in 17 sessions over a 14-month period. Two filler sessions were carried out six months later, adding 48 plugs to the total (Figs. 7 and 8). No complications occurred during the course of his sessions.

The course of treatment was longer than usual because he was reluctant to miss work on a regular basis, and preferred not to go to work wearing a dressing. His entire personality has changed since his alopecia was corrected. Where previously he was tense and introspective, he now is more easy-going and personable.

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