Monitoring the fetoplacental unit—the placenta

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Summary

The non-steroidal tests available to determine the well-being of the fetoplacental unit are reviewed. The author's experience with measurements of human placental lactogen (HPL) is described in detail since this measurement has provided a great deal of new and useful information.

WITH the exception of congenital abnormalities and Rhesus iso-immunization, the primary pathology in most conditions which threaten the life or well-being of the fetus lie within the placenta. 'Placental insufficiency', a poorly defined but very useful term, characterizes specific conditions such as pre-eclampsia, diabetes and placental abruption, together with non-specific conditions having as their final common denominator the delivery of a fetus which is dead, or small, or shows evidence of hypoxia during labour and at the time of delivery. The pre-eminent position of the placenta in these abnormalities is argument enough for 'placental function tests' to be carried out in parallel with 'fetal function tests'; furthermore, in the majority of cases the implications of the two types of test are identical.

The placenta has two functions: synthesis, and transfer. From the point of view of the fetus the latter is, on present information, much the most important, since the placenta is required to act as lung, gut and kidney to the developing child. Synthesis, by contrast, seems almost irrelevant. Thus, while the placental hormones play an important if ill-defined role in the maintenance of pregnancy, they do so in a manner which seems wasteful when compared with adult endocrinology. The production of placental lactogen, for instance, exceeds in a single week of late pregnancy the secretion of insulin in a lifetime, yet the function of placental lactogen, if any, is unknown.

Most tests of placental function are tests of synthesis. The clinical assumption underlying such tests is that synthesis, being a function of exactly the same tissue, will reflect transfer. In other words, a high level of a placental product such as HPL will imply a large and efficient exchange surface for the passage of nutrients to the fetus. This assumption may well

be true, but it should be remembered that direct supporting evidence is almost totally non-existent. A similar argument can be levelled against specific tests of the fetus; a procedure which measures the activity of one metabolic pathway, such as the production of oestriol, is not necessarily guaranteed to measure the function of the fetus as a whole.

Tests of the placenta

The commonly used tests of placental synthesis are considered in detail below. Tests of placental transfer have been very little explored, chiefly because they present severe problems of technical execution. Best known of these is the selenomethionine uptake procedure (Garrow and Douglas, 1968). The amino-acid methionine, labelled with radioactive selenium, is injected into the mother, and its accumulation in the fetoplacental unit is followed with a scintillation counter. Useful results were claimed in a limited series of cases, but the procedure has not achieved widespread acceptance. Furthermore, it is not clear to what extent the technique would measure placental transfer *per se*, as opposed to accumulation of the isotope in the myometrium or the choriodecidual space. Studies on the placental clearance of another diffusible isotope, inhaled xenon-133, have failed to show any difference between normal and hypertensive subjects (Jacoby *et al.*, 1972).

Although not usually considered as a test of the placenta, the study of the coagulation system in relation to abnormalities of pregnancy may reflect a primary phenomenon occurring in the placenta itself. Evidence of intravascular fibrin deposition, as reflected by an elevation of the circulating levels of fibrin degradation products, can be found in placental abruption (Basu, 1969), pre-eclampsia (Bonnar, McNicol and Douglas, 1971), and placental insufficiency not associated with specific signs in the mother (Frigoletto *et al.*, 1971). In each of these conditions, the initial pathology, and much of the fibrin deposition can be found in the placenta. Thus, in many cases, the measurement of a parameter of fibrin deposition can be considered as a test of the placenta. The introduction of new methodology in

this area (Gordon *et al.*, 1973) holds much promise for future developments.

Tests of placenta synthesis

The most commonly used tests for monitoring the placental aspect of the fetoplacental unit are those which depend on the measurement, in the maternal circulation or urine, of specific and identifiable products of the placenta. These are shown in Table 1. In principle, any one might be appropriately used as a test of placental function. In practice, certain criteria must be met which are set out in Table 2, and of these possibly the most important is the fourth: that it should be possible to measure the substance in question rapidly, precisely, and in large numbers.

TABLE 1. Specific placental products which might be measured as a test of placental function

Hormones:	Human chorionic gonadotrophin (HCG)
	Human placental lactogen (HPL)
	Human chorionic thyrotrophin (HCT)
	Progesterone
Enzymes:	Heat stable alkaline phosphatase (HSAP)
	Oxytocinase (cystine aminopeptidase—CAP)

TABLE 2. The criteria for a test of placental function

1. The material estimated should be specific, either qualitatively or quantitatively, to pregnancy. The maternal contribution should be limited to metabolism and excretion.
2. The levels should not vary from day to day, should not show a nyctohemeral rhythm, and should not vary with common physiological events such as eating or exercise.
3. The half-life should be short, so that only a brief time elapses between a fall in production and a decrease in circulating levels.
4. The measurement technique should be rapid, precise, and applicable to large numbers of samples at low unit cost.

The choice of material and method apart, there are various research criteria which must be taken into account before any technique can be applied to routine clinical practice (see Chard, 1974). The fact that these criteria have so rarely been met with tests of the fetoplacental unit does not mean that they should be ignored in future work. Furthermore, failure to observe simple statistical concepts in the evaluation of data has often led to the enthusiastic introduction and acceptance of a test, which is followed by disappointment when the test is applied in routine clinical practice.

Of the specific placental products listed in Table 1, all but chorionic thyrotrophin (HCT) have been studied as possible tests of fetoplacental function. But only one, placental lactogen (HPL), has been evaluated on a sufficient scale to provide an adequate basis for clinical use. In the discussion which follows,

chorionic gonadotrophin (HCG), alkaline phosphatase, oxytocinase and progesterone will be briefly considered, and HPL in more detail.

Chorionic gonadotrophin (HCG)

Human chorionic gonadotrophin (HCG) is a glycoprotein, similar to pituitary luteinizing hormone (LH), which is synthesized by the placenta. In contrast to the other hormones of the fetoplacental unit, the levels reach a peak at around the 7th–8th week of pregnancy; there is a small secondary peak between the 32nd and the 36th weeks.

Immunological assays for HCG have largely replaced the earlier biological assays. Simple agglutination systems are widely used in the diagnosis of early pregnancy, but, for a test of placental function, in which precision is all important, radioimmunoassay is probably the method of choice. A system capable of precise estimation of large numbers of samples has been described (Butler *et al.*, 1971), but this is largely used in the diagnosis and management of chorioncarcinoma. Surprisingly, there is virtually no information on the use of HCG levels as a test in late pregnancy (Brody, 1969), although in principle its significance is likely to be similar to that of other placental products, such as HPL and progesterone. It is interesting to note that, as with HPL, the levels are elevated in Rhesus iso-immunization (Fairweather *et al.*, 1972), reflecting the increased mass of the placenta in this condition.

Heat stable alkaline phosphatase (HSAP)

An isoenzyme of alkaline phosphatase, which is not inactivated by heating serum at 65°C, occurs in pregnancy as a specific product of the placenta. The levels in the mother increase progressively during gestation to reach a peak at term. Excessive release of this enzyme into the maternal circulation may occur in association with placental damage, in a manner comparable to that of transaminases in liver disease; elevated levels have been reported in association with pre-eclampsia, and the delivery of 'small-for-dates' infants (Merrett and Hunter, 1973). However, it does not seem to be clear at the present time whether the critical change is towards abnormally high or abnormally low levels, and other workers have not been impressed with the value of this determination in the diagnosis of fetal size or condition (Elder, 1971; Curzen and Varma, 1971).

Oxytocinase

This is another specific placental enzyme, which is known by a variety of names including 'cystine aminopeptidase'. Preliminary clinical experience with determinations in maternal serum has been encouraging (Tovey, 1969; Hensleigh and Krantz, 1970; Tovey, Dawson and Fellowes, 1973; Petrucco, Cellier and

Fishtall, 1973), but considerable further work will be needed before final conclusions can be drawn. Should the estimation prove to be of value, it has the great advantage that large numbers of samples can be processed at relatively low cost (Tovey *et al.*, 1973).

Progesterone

Progesterone, in contrast to the oestrogens, is produced exclusively by the placenta. About 15% of that secreted into the mother is excreted in urine as pregnanediol; measurement of the latter has been the most commonly used as a test of placental function, although it seems likely that determination of circulating progesterone by means of radioimmunoassay would be a more efficient procedure.

Rather surprisingly, since the technique has been available for many years, the literature on the clinical significance of urinary pregnanediol concentrations is sparse. In severe cases of pre-eclampsia, the levels are said to be low, and a failure of the normal rise is thought to be characteristic of the small-for-dates infant (Rawlings, 1965). In Rhesus iso-immunization the levels may be elevated (Fairweather *et al.*, 1972), a finding similar to those made with estimation of another specific product of the placenta, HPL (Ward *et al.*, 1974). There is little information on the clinical application of the measurement of circulating progesterone, though it may be anticipated that its significance will be very similar to that of HPL. For example, raised levels are found in association with Rhesus disease (Tulchinsky *et al.*, 1972).

Human placental lactogen

'Human placental lactogen' (HPL) is now the preferred term, replacing the inelegant and cumbersome 'human chorionic somatomammotrophin' (HCS). HPL is a protein produced only by the placenta, and whose general properties are very similar to those of pituitary prolactin and growth hormone. The amount synthesized near term is of the order of 1–3 g/day, yielding high concentrations (up to 20 $\mu\text{g/ml}$) in the mother, and levels a thousand-fold less in the fetus. Virtually nothing is known of its control mechanism or its physiological function, though it has been suggested that it may play a part in the altered carbohydrate metabolism of pregnancy, and in the development of the breast. The fact that the levels show no nyctohemeral rhythm (Pavlou, Chard and Letchworth, 1972) and are not obviously influenced by normal physiological events (Pavlou *et al.*, 1973) indicates that clinical samples may be collected at any time of the day. Estimation is by radio immunoassay, and a semi-automated procedure has been described which permits the processing of many hundreds of samples a day (Letchworth *et al.*, 1971).

The clinical applications of HPL determinations

have been extensive, and a large literature has become available in a relatively short time.

(1) Normal range: HPL can be detected from a very early stage of pregnancy, in some cases before the first missed period. The levels rise progressively to reach a plateau during the last 5–6 weeks (Fig. 1). It should be noted that the distribution of levels in a normal population is skewed, a factor which is particularly critical in the analysis of results (Chard, 1974).

FIG. 1. The range of circulating HPL levels in normal pregnancy ± 2 s.d. Note that the distribution around the mean is skewed.

(2) Threatened abortion: in cases of vaginal bleeding in early pregnancy in which the outcome for the fetus is uncertain, determination of HPL levels can be a valuable aid to prognosis (Genazzani *et al.*, 1969; Niven, Landon and Chard, 1972) (Fig. 2a, b). Thus, a relatively inexpensive test (approximately 40p per sample) may obviate the need for a costly hospital stay by indicating those cases in which an early evacuation of the uterus may be safely performed.

(3) The 'small-for-dates' infant: HPL levels are related to fetal weight (Letchworth *et al.*, 1971). This relationship is secondary to that between the weight of the fetus and the weight of the placenta, which may explain why HPL levels are not considered to be a

FIG. 2. (a) HPL levels in normal subjects (solid lines), and in subjects with vaginal bleeding and an intact pregnancy (broken lines and shaded area). (b) HPL levels in normal subjects (solid lines) and in subjects with vaginal bleeding who subsequently aborted (broken lines and shaded area). (From Niven, Landon and Chard, 1972.)

FIG. 3. (a) HPL levels in normal subjects (solid lines), and in diabetic subjects with a favourable fetal outcome (solid lines and hatched area). (b) HPL levels in normal subjects (solid lines), and in diabetic subjects with an unfavourable fetal outcome (solid lines and hatched area). (From Ursell, Brudenell and Chard, 1973.)

particularly efficient means for 'weighing' the fetus *in utero* in an individual patient.

(4) Pre-eclampsia: the levels of HPL tend to be reduced in this condition, but the relationship to severity or to fetal outcome is uncertain. Letchworth and Chard (1972a) found that the lowest levels were found in the mild cases and were, in general, unrelated to fetal risk. But other authors have presented a more optimistic picture. Thus, Spellacy and his colleagues (1971) defined a 'fetal danger zone': patients with hypertension and an HPL value of less

than $4 \mu\text{g/ml}$ after the 30th week had a fetal mortality of 50%. In addition, Keller and his colleagues (1971) have reported that HPL levels are of greater significance than either urinary oestrogens or heat stable alkaline phosphatase in the management of hypertensive pregnancies.

(5) Diabetes mellitus: HPL levels are generally elevated in this condition (Fig. 3a; Ursell, Brudenell and Chard, 1973). In those cases in which fetal outcome is unsatisfactory, the levels are reduced (Fig. 3b). It is important to recognize that 'abnormal' levels

in diabetic pregnancies must be judged in relation to elevated 'normal' levels. Thus, while 4 $\mu\text{g/ml}$ is a useful dividing line in other conditions, in diabetes the critical level becomes 5 $\mu\text{g/ml}$.

(6) Rhesus iso-immunization: in cases in which the child is severely affected, HPL levels are elevated, probably reflecting the hyperplacentosis which is associated with this condition (Ward *et al.*, 1974; Fig. 4). A reading more than two standard deviations above the normal mean before the 26th week suggests a 90% probability that the case is severe. The clinical value of this observation is that it provides an index of fetal outcome at a stage when other parameters, such as liquor bilirubin, may be unreliable.

FIG. 4. HPL levels in normal subjects (—), in mild (---) and moderate cases of Rhesus iso-immunization (- · - · -) and in severe cases (·····). (From Ward *et al.*, 1974.)

(7) Fetal death: in many, but not all cases, HPL levels are low or falling before fetal death occurs. This is not surprising when it is remembered that many such cases are based primarily on placental insufficiency. Similar observations have been made with urinary pregnanediol (Booth *et al.*, 1965).

(8) Fetal distress and neonatal asphyxia in an otherwise uncomplicated pregnancy: in some 10% of apparently normal pregnancies, fetal complications develop during labour or at the time of delivery.

A proportion of such cases can be predicted by serial estimation of HPL (Table 3; Letchworth and Chard, 1972b; England *et al.*, 1974). For instance, if a clinically normal patient has three or more levels below 4 $\mu\text{g/ml}$ in the last 6 weeks of pregnancy, the risk of fetal complications is 71%. The importance of these results is that they suggest that HPL might be a routine screening test in all pregnancies.

TABLE 3. The risk of fetal distress and/or neonatal asphyxia in clinically normal pregnancies as predicted by serial measurements of HPL

HPL levels	Risk of fetal distress and/or neonatal asphyxia (%)
Below 4 $\mu\text{g/ml}$	
1 or more	30
2 or more	50
3 or more	71
Above 5 $\mu\text{g/ml}$	
1 or more	8
2 or more	6
3 or more	4

Modified from Letchworth and Chard, 1972b.

Conclusions

In principle, the measurement of any of the products shown in Table 1 might provide an efficient test of placental function, and it seems likely that the clinical significance of any one of these tests will prove to be identical to the others. At the present time, HPL is the leader in this particular field, a fact which can be attributed to the ease with which it is measured, and the consequent accumulation of a large body of reasonably clearcut information. But if the availability of appropriate methodology is the sole criterion, there seems little reason why oxytocinase should not provide a strong competitor.

The welfare of the unborn child is one of the most important and underexplored subjects in present-day medicine. The gains from the complete or partial elimination of genetic disease and perinatal brain damage are potentially enormous. To study the fetus, a patient whom we can neither examine nor question, requires sophisticated scientific tools; biochemical monitoring of the placenta is only one of these, but has, in the guise of placental lactogen determinations, already made a significant impact on clinical obstetrics. Measurement of HPL or other placental products in no sense replaces existing techniques. Instead, it should complement them, and for the future it is possible to predict that it will be one among a battery of biochemical and electronic tests which will be applied not just to selected cases, but to every antenatal patient.

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