

# Endocrine Assessment of Feto-Placental Growth

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Intrauterine growth retardation (IUGR) accounts for increased perinatal morbidity and mortality, and the early identification of the growth-retarded fetus remains a key factor in achieving the most favorable outcome for the infant and the mother.

IUGR implies the operation of certain factors (such as hypertension, toxemia, and infection) which inhibit the achievement of the full genetic potential of the fetus. It is necessary to consider what reduction in the potential weight of the full-term fetus can be described as pathological. On the basis of a study on the siblings of small-for-gestational age (SGA) babies, this reduction has been estimated at 653 g (1), but it is evident that there is an overlap between SGA and normal or appropriate birth-weight for gestation age (AGA).

It thus appears that the diagnosis of IUGR is difficult. However, in this situation hormonal assays will be useful because they allow us to perform longitudinal studies in patients who are considered by conventional risk factors to be at high risk.

This chapter will describe some of the biochemical indices which have been developed to overcome the inadequacy of clinical diagnosis and to establish a strategy for use when the fetus is considered to be at risk.

There is good evidence from hemodynamic studies that the blood supply to the feto-placental unit is impaired in most pregnancies with IUGR, even when they are not associated with hypertensive disorders of pregnancy. Gross and microscopic examination of placentas from SGA pregnancies shows diverse abnormalities; villous dysmaturity, abnormal configurations, widespread subchorionic thrombosis, and infarction. The functioning mass in terms of parenchymal and cellular content is markedly decreased in the small placenta of the SGA infant (2). This may account for the high incidence of fetal distress during the intrapartum period in pregnancies complicated by fetal growth retardation and for the low hormonal levels in the maternal blood.

## **HUMAN PLACENTAL LACTOGEN**

Human placental lactogen (hPL), also called chorionic somatomammotrophic hormone (hCS), is a peptide produced in large amounts by the placenta (approxi-

mately 2 g/day) and secreted mainly into the maternal circulation. Its short half-life (15–25 min) and ease of analysis (usually by radioimmunoassay) explain why the determination of hPL has commonly been used for routine screening in pregnancy. Another advantage of hPL measurement is the absence of a diurnal variation in plasma levels. Serum samples also retain hPL activity for up to 3 days at ambient temperature, allowing central collection and assay. When frozen, activity remains for at least 6 months. Synthesis of hPL is related to placental mass and so a correlation is to be expected between hPL levels and placental weight. Most researchers have also shown a significant correlation between hPL and birthweight (3). An association between low maternal hPL levels and low birthweight in IUGR has been reported as early as 28 to 32 weeks gestation and has been found even more frequently after 36 weeks. Spellacy et al. (4) suggested that hPL might be a useful measurement for detection of the fetus at risk, and subsequently suggested that hPL concentration should be greater than 4  $\mu\text{g/ml}$  after 30 weeks gestation (Fig. 1); the area below this on a plot of hPL against gestational age was called the "fetal danger zone." In a retrospective analysis of hPL measurement in 2,318 high risk pregnancies, Spellacy et al. (5) have been able to relate perinatal mortality after 30 weeks to hPL values in the fetal danger zone. There were 71 fetal deaths, and in 36 of these hPL was in the danger zone. However, low hPL was found in only 20% of women whose infants died in the neonatal period, and hPL in the danger zone was observed more frequently in patients with severe toxemia.

In a 3-year prospective study, these authors (5) included all high-risk patients, who were divided into two groups: one in which the hPL values were not reported, and delivery was planned when the clinician thought appropriate by other criteria; and the second in which hPL values less than 4  $\mu\text{g/ml}$  were specially reported. If it was considered that the fetus might be mature, amniocentesis was performed and delivery was carried out if the lecithin/sphingomyelin ratio was mature. Of the 2,733 patients, 230 had hPL in the fetal danger zone; the perinatal death rate was significantly lower in the treatment group than in the control group (15% versus 3.4%) (Table 1).

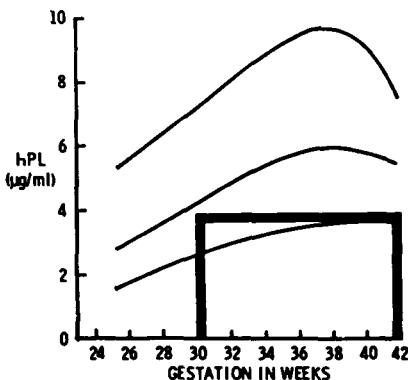


FIG. 1. Fetal danger zone for hPL concentrations. (From ref. 4.)

TABLE 1. *Human placental lactogen in fetal danger zone in 230 patients*

	Unreported group (%)	Treatment group (%)
Proportion of group in fetal danger zone	8.2	8.6
Fetal death rate	14.2	2.6 ( $p < 0.003$ )
Perinatal death rate	15.0	3.4 ( $p < 0.005$ )
Neonatal death rate	0.9	0.9 NS

From ref. 5.

Our first study in 1974 concerned 123 women in whom fundal height was less than would be expected for gestational age at two consecutive antenatal visits (6). After delivery, birthweight percentiles were calculated from Lubchenco's data (7), infants having a birthweight below the 10th percentile being classified as small for gestation (SGA). This corresponds approximately to the 3rd percentile in Paris, thus giving a high-risk newborn population. Most of the mothers were normotensive and without associated pathology. One-third of their infants (39/123) were SGA and two-thirds (84/123) were appropriate-weight-for-gestation (AGA). We excluded from this study diabetic and multiple pregnancies because in such cases the hPL levels are higher than in normal singleton pregnancies (8). In the study pregnancies, the mean values of hPL were lower than in normal pregnancies in both the SGA and the AGA groups, but the mean hPL value plateaued at 4  $\mu\text{g/ml}$  at 28 to 30 weeks in the SGA pregnancies, whereas it continued to increase in the AGA group until the 39th week. Most of the values were lower than the mean for normal pregnancies; in 38 assays from the SGA pregnancies (70%) the values fell between the mean and the minus 1 SD level. We therefore felt that fetal growth retardation was unlikely when hPL values were higher than the population mean.

We observed a correlation between birthweight and the hPL value obtained between 4 weeks and 5 days before delivery. In 11 assays performed in women who delivered a newborn weighing less than 1,800 g after 35 weeks, hPL values were lower than 3  $\mu\text{g/ml}$ . There was also a correlation between hPL and placental weight; all hPL values  $>4 \mu\text{g/ml}$ , except for one, corresponded to a placental weight of  $>400 \text{ g}$ .

These results are similar to those reported by other workers (3-5) who have observed a significant trend toward low mean hPL values in IUGR pregnancies, and have found it possible to differentiate AGA from SGA pregnancies as early as 33 weeks gestation. However, many authors have pointed out that there is a considerable false positive rate; for example, Letchworth et al. (9) found a normal outcome in as many as 57% of pregnancies with hPL levels below 4.3  $\mu\text{g/ml}$ . In a study by Morrison et al. (10), on the other hand, there was a high false negative rate, with only 41% of the cases of growth retardation being predicted by low hPL values. These workers concluded that hPL would appear to have a limited role in antepar-

tum evaluation, and that screening should be limited to pregnancies associated with hypertension, to help reduce the risk of stillbirth and fetal distress occurring specifically in the IUGR fetus.

Recently Obiekwe et al. (11) measured hPL in serial blood samples obtained from 663 women at weekly intervals from 30 to 40 weeks gestation, including 231 with preeclampsia. The hPL levels were significantly lower in pregnancies associated with fetal growth retardation, but the clinical value of this observation appeared to be greater in multigravidae than in primigravidae, suggesting that in the latter group maternal hPL levels reflected both the pathology of the disease and the condition of the fetus, and that in primigravidae with preeclampsia the cutoff point between normality and abnormality should be slightly raised.

In IUGR pregnancies, measurements of mRNA sequence coding for hPL with an hPL complementary probe have indicated that the hPL mRNA concentrations are similar to normal pregnancies and that low hormonal levels associated with growth-retarded fetuses can be explained by their lower placental weights, which correlate with their total RNA content. The total capacity of *in vitro* hPL production per placenta is significantly lower than normal, but without basic intracellular disturbance in hPL synthesis (12).

It is interesting to note that normal pregnancies associated with partial or complete absence of hPL have been described. This abnormality is extremely rare and is due to gene deletion (13).

Other protein specific for pregnancy, such as alpha-feto-protein (AFP), which is a major constituent of the circulating proteins in early fetal life, and pregnancy-specific  $\beta$ -glycoprotein SP<sub>1</sub>, have also been assayed in IUGR but their predictive value remains to be established.

## ESTROGENS

The measurement of estrogens in pregnancy has largely been employed as an index of fetoplacental function to identify the fetus at risk. The basic physiology underlying this practical application is the concept of the fetoplacental unit described several years ago (14).

Estrogen, and chiefly estriol (E<sub>3</sub>), is secreted by the placenta from androgenic precursors initially synthesized in the fetal adrenal gland and then further processed by the fetal liver. Estrogen production in pregnancy has frequently been measured in 24-hr urine collection and it is generally necessary to control for the completeness of the collection by simultaneously estimating creatinine. In most routine laboratories, estrogen is measured by the Brown colorimetric method (15) or by fluorometry (16).

The normal limits for estrogen excretion in the second half of pregnancy have been defined. Between 30 and 40 weeks the lower limit can adequately be described by a straight line joining 8 and 12 mg/24 hr, and this defines a zone of fetal risk (17). Estrogen excretion is very variable, both between women and daily in the same woman. Many factors are known to influence the urinary excretion of estrogens,

some of which greatly reduce the value of this estimation in clinical practice. Nevertheless, many researchers have observed a correlation between urinary estrogen excretion ( $E_{ur}$ ) and birthweight, and have claimed that the test is a useful one.

We have assayed urinary estrogen by the Ittrich method (16) in 123 women with suspected IUGR who delivered 39 SGA and 84 AGA infants (18). In women who delivered infants of normal birthweight, the urinary estrogen excretion was within the normal range. In the group delivering SGA infants, the mean urinary estrogen values were lower, with a plateau after the 32nd week instead of the normal increase, a phenomenon previously described by Klopper (19).

Beisher et al. (20) measured urinary estrogen excretion in 597 women; they reported that the perinatal mortality was 4.4% in the low estrogen group (<10th percentile) compared to 0.4% in the group with normal estrogens, and that birthweight was significantly reduced in the low estrogen group. The low estrogen group contained 21.2% infants with IUGR compared to 3.1% in the group with normal estrogens. However, only 54% of growth-retarded fetuses were detected by measurement of urinary estrogens in this study. False positive (low) results have been observed, particularly in preeclampsia, indicating that when estrogen excretion is low the clinical problem is usually obvious.

Advances in radioimmunoassay techniques have facilitated routine determination of unconjugated ( $E_3$ ) or total estriol ( $E_3T$ ) in plasma, and the ease of blood sample collections compared with 24-hr urine collection has led to increased interest in the clinical use of estrogen measurements. However, differences in methods for measurement of  $E_3$ , together with the extent of diurnal and random fluctuations in plasma  $E_3$  concentrations, as well as the limited extent of current appraisal of fetal outcome have obscured the value of this method as an aid in the management of complicated pregnancies. Few data have been analyzed appropriately to evaluate whether the test contributes any useful clinical information, and it has never been established whether it is more valuable to determine the unconjugated estriol fraction ( $E_3$ ) or the total plasma estriol ( $E_3T$ ), which is ten times higher. Both have similar individual and diurnal variations.

We have been performing routine assays of  $E_3T$  since 1977, using an RIA kit (IM 82) at the Radiochemical Centre (Amersham, England), and we shall report here some characteristic observations, together with those results that have been subjected to computer analysis (21).

We have established  $E_3T$  levels for normal pregnancies from longitudinal values obtained in 88 patients who were judged to be free of complications, as determined by the course of pregnancy and delivery of a term singleton infant of normal birthweight, who did well during labor and the neonatal period. In the same group of women we also measured total estrogens in urine and hPL in plasma. We then studied 141 high-risk pregnancies between 31 and 39 weeks of gestation, complicated by idiopathic IUGR or with arterial hypertension and various other disorders or factors predisposing to fetal growth retardation. Fetal distress was confirmed in 15 suspected cases (11%). Total plasma estriol and 24-hr urinary estriol (expressed as mg estrogen/g creatinine) were correlated with each other when the samples were col-

lected on the same day, and the results have been grouped into 3-week periods. It appears from Table 2 that the correlations are highly significant between  $E_3T$  and  $E_{ur}$  in all the groups except in preeclampsia. The general shape of the curves obtained by sequential determination of  $E_{ur}$  and  $E_3T$  is similar. This similarity holds true for the increasing levels observed in high-risk patients where pregnancy was normal, as well as for the sustained falls corresponding to *in utero* deaths in severe hypertension or preeclampsia.

In cases of fetal distress the estrogen values were lower than normal, but were not significantly different from those of the high-risk group without fetal distress. It therefore appears that the same information can be derived from  $E_3T$  plasma assays as from urinary estrogen analysis, but that a single determination could not give unequivocal information on the state of the fetus, better information being provided by sequential determination of estrogens, either in serum or urine.

The predictive value of estrogen measurements in cases of suspected IUGR seems to be better. In a group of 222 patients who delivered of 152 AGA infants and 70 SGA infants (mean birthweight 2,002 g at 37.8 weeks), there appeared to be a significant difference between the two populations for both variables except at the end of pregnancy in the case of  $E_3T$  (Table 3).

It should be noted that while 53% to 82% of  $E_3T$  values were low in SGA pregnancies, 28% to 41% of the values in AGA pregnancies were also low. The predictive value of a low  $E_3T$  was 41% to 45% according to the gestational age. The predictive value of  $E_{ur}$  was lower at 37% to 41%.

We were interested to see whether the combination of the two variables increased the precision of the diagnosis. We did not observe any SGA infants with normal  $E_3T$  and  $E_{ur}$ , or with low  $E_3T$  and normal  $E_{ur}$ . Fetal growth retardation was most likely to result when both of the tests were low at the same time, but the combination of  $E_3T$  and  $E_{ur}$  did not improve the predictive value, demonstrating once again that the same

TABLE 2. Correlation between total plasma estriol ( $E_3T$ ) and 24-hr urinary estrogen ( $E_{ur}$ ) (expressed as mg/g creatinine)

	No. of patients	Weeks of pregnancy					
		34-36				37-39	
		No. of paired samples*	<i>r</i>	<i>p</i>	No. of paired samples*	<i>r</i>	<i>p</i>
Hypertension	33	6	0.85	< 0.05	49	0.31	< 0.05
Toxemia	8	6	0.49	NS	—	—	—
Hypertension + IUGR	21	15	0.58	< 0.05	22	0.68	< 0.001
IUGR	22	28	0.67	< 0.001	18	0.52	< 0.05

IUGR, intrauterine growth retardation.

\*Paired samples  $E_3T/E_{ur}$ .

From ref. 21.

TABLE 3. Percentage of normal and low plasma total estriol and urinary estrogens in suspected intrauterine growth retardation with (SGA) and without (AGA) birth of small-for-age neonate

	Weeks of pregnancy					
	31-33		34-36		37-39	
	AGA	SGA	AGA	SGA	AGA	SGA
$E_3T$	n = 40	n = 11	n = 58	n = 23	n = 47	n = 17
Normal ( $\geq$ 10th percentile)	72	18	59	26	72	47
	$p < 0.01$		$p < 0.01$		NS	
Low (< 10th percentile)	28	82	41	74	28	53
$E_{ur}$	n = 59	n = 31	n = 85	n = 49	n = 73	n = 38
Normal ( $\geq$ 50% of mean)	76	58	69	41	67	47
	NS		$p < 0.01$		$p < 0.05$	
Low (< 50% of mean)	24	42	31	53	33	53

SGA, small for gestational age; AGA, appropriate for gestational age;  $E_3T$ , total plasma estriol;  $E_{ur}$ , 24-hr urinary estrogens.

Boxed numbers, %.

From ref. 21.

information can be derived from  $E_3T$  plasma assays as from the urinary estrogen analysis (21).

In a prospective study of 1,042 women with singleton pregnancies and known length of gestation, Nielsen (22) has tried to determine the prognostic value of  $E_3T$ . In high risk pregnancies, a low  $E_3T$  value (<2.5th percentile) was associated with a 42% risk of delivery of an infant with perinatal complications and a 17% risk of an SGA infant. Perinatal complications had been forecast or detected in 7% of these infants and fetal growth retardation in 19% by routine clinical screening. In a subgroup of 800 pregnancies considered normal according to given criteria, a low  $E_3T$  level involved a 17% risk of perinatal complications and a 15% risk of an SGA infant. In this group, 4% and 14%, respectively, had been detected by routine screening. He concluded that there should be a low threshold for measuring  $E_3T$  levels, but that the test was of limited value in pregnancies that are clinically quite normal.

Evans et al. (23) have carried out a prospective study of the clinical usefulness of plasma estriol determinations in predicting "light-for-date" infants before birth. Maternal estriols were measured at 35 to 36 weeks of gestation and the 10th percentile for  $E_3T$  and unconjugated  $E_3$  values were used as cutoff points. Low estriol values were identified in less than a third of all low weight babies, and less than a third of pregnancies with low estriol values were associated with birth of light-for-date infants.

Chard et al. (24) have compared hPL and unconjugated  $E_3$  in the prediction of IUGR. They measured hPL and  $E_3$  in 392 women at weekly intervals from 36 to 40 weeks of gestation and found reduced levels of both in subjects delivering an SGA infant. The clinical significance of this observation was similar for the two compounds, with a marginal advantage to hPL. There was a higher incidence of falling

levels of hPL and  $E_3$  in cases of growth retardation. However, the findings had very little predictive value in the individual patient. In a further publication (25), these authors discuss the evidence that maternal  $E_3$  levels are related to fetal growth and well-being, and conclude that this is likely to be a reflection of placental function, which is the final and probably rate-limiting step in the synthetic pathways from fetal precursors. This explains why measurements of  $E_3$  seem remarkably similar to those of placental products such as hPL.

We should not forget the possible occurrence of sulfatase deficiency (26) in the case of very low levels of plasma or urinary estrogen. This is an interesting anomaly of placental steroidogenesis observed only in the male fetus, indicating that this deficiency is under control of a sex-linked recessive character (27). *In vivo* loading tests with dehydroepiandrosterone sulfate (DHA-S) allow an antenatal diagnosis of this condition, which may be confirmed by *in vitro* experiments showing zero or virtually zero placental sulfatase activity toward  $\Delta_5$ -pregnenolone or DHA-S.

We shall not dwell on the various general mechanisms which may result in a decrease of plasma or urinary levels of estrogen, such as antibiotics (e.g., ampicillin) which disturb intestinal glycoconjugation or corticosteroids which inhibit the adrenal secretion of  $C_{19}$  steroids, but we wish to mention our personal observation of a significant decrease of unconjugated estradiol ( $E_2$ ) and  $E_3T$  in plasma, and of  $E_{ur}$ , in patients treated with oral chlormadinone acetate for threatened premature labor. This phenomenon is due to a temporary inhibition of placental sulfatase by the synthetic steroid agent, without any noticeable fetal effects (28).

### Metabolic Clearance of DHA-S

Measurements of metabolic clearance rate of DHA-S suggest that decreased utero-placental blood flow is the main factor contributing to reduced estrogen production and to prolonged DHA-S half-life (29). The *in vivo* placental clearance of DHA-S through  $E_2$  is especially flow-dependent (30). There has been some controversy about the use of the DHA-S loading test (DLT) as described by Lauritzen (31) for the diagnosis of IUGR. We have studied the half-life of injected DHA-S and have found this variable to be more reliable than estrogen determinations in the diagnosis of IUGR (32). The half-life of DHA-S (50 mg intravenously) (DHA-S t 1/2) was calculated by least square analysis in the plot of log DHA-S concentration versus time at several intervals. We performed 102 tests in pregnant women between 30 and 41 weeks of gestation (Table 4).

We then compared the predictive value of (a) plasma  $E_3T$ , (b) the maximal  $E_2$  increase after DLT ( $E_2$ ), (c) its rate of increase per minute during the first 15 min after DLT ( $V E_2$ ) (33), and (d) that DHA-S is the main precursor of estrogens in pregnancy. DHA-S first undergoes  $16\alpha$ -hydroxylation in the fetal liver, then in the placenta a conversion to estriol DHA-S t 1/2. As shown in Table 4, the maximal  $E_2$  was similar in the control and the AGA groups, but was significantly lower in SGA pregnancies.  $V E_2$  was also similar in control and AGA groups and significantly lower in



TABLE 4. Results of dehydroepiandrosterone sulfate (DHA-S) loading test in suspected intrauterine growth retardation and controls

	Control	AGA	SGA
Number of cases	43	39	20
Birthweight (g) (mean $\pm$ SEM)	3,066 $\pm$ 65	2,730 $\pm$ 68	1,881 $\pm$ 89
Maximal E <sub>2</sub> increase (ng/ml) (mean $\pm$ SEM)	34.4 $\pm$ 2.45 (36) <sup>a</sup>	33.7 $\pm$ 2.9 (26)	19.5 $\pm$ 1.92 (17), $p < 0.001$ <sup>b</sup>
Rate of increase of E <sub>2</sub> (ng/ml) (mean $\pm$ SEM)	1.86 $\pm$ 0.21 (30)	1.73 $\pm$ 0.20 (26)	1.08 $\pm$ 0.15 (16), $p < 0.02$
DHA-S t <sub>1/2</sub> (hr) (mean $\pm$ SEM)	2.90 $\pm$ 0.12 (43)	3.24 $\pm$ 0.12 (39)	5.05 $\pm$ 0.20 (20), $p < 0.001$

AGA, appropriate for gestational age; SGA, small for gestational age; E<sub>2</sub>, maximum increase in plasma estrogen after DHA-S loading test.

<sup>a</sup>The numbers of assays in each category are given in parentheses.

<sup>b</sup>Student's t-tests were used to compute the significance of the difference between the AGA and SGA group.

fetal growth retardation. The mean value of DHA-S t<sub>1/2</sub> was similar in control and AGA groups. In SGA pregnancies the DHA-S t<sub>1/2</sub> was longer and the difference between AGA and SGA groups was highly significant. All the SGA babies except one had DHA-S t<sub>1/2</sub> >4.29 and most of the normal babies had values lower than this. A value lower than 3.75 always corresponded to normal birthweight for gestational age.

The E<sub>3</sub>T was not a good criterion, as previously described. A low level was observed in 50% of the cases with normal birthweight. An E<sub>2</sub> value lower than 25 ng/ml was observed in 15 out of 17 cases of fetal growth retardation versus 7 out of 26 AGA pregnancies and 11 out of 37 control pregnancies.  $\Delta$  E<sub>2</sub> was less discriminant, but had a high sensitivity: 13 of 16 determinations were <1.50 ng/ml per min in SGA pregnancies. However, the specificity was low, a slow increase being observed in 50% of AGA pregnancies and 36% of controls.

In a complementary approach we performed sequential assays of steroid intermediates after DLT in clinically suspected cases of IUGR. We observed that plasma levels of  $\Delta_4$ -androstenedione and testosterone were significantly increased and remained elevated for 2 to 3 hr in SGA pregnancies whereas in the AGA group these values were increased only for a short period of time after the injection (34). *In vitro* perfusions of the delivered placentas of those patients given DHA-S have shown that a low estrogen production coincides with an accumulation of neutral steroids in the perfusate and it appears that the placental conversion of DHA-S into estrogen may be slowed at the aromatization step in some case of IUGR (35). This phenomenon is independent of the placental blood flow, since each perfusion is performed

under standardized conditions of pressure and flow. It suggests that an impairment of placental metabolism could be superimposed on the hemodynamic alteration.

### **PREDICTIVE VALUES OF THE ENDOCRINE ASSESSMENT OF FETOPLACENTAL GROWTH**

Echographic measurements of fetal development have been steadily improving over several years. Nevertheless, attempts are still being made to define the clinical usefulness of estriol assay for diagnosing fetal growth retardation (23) and to determine cutoff points which discriminate between normal and abnormal hPL values in the prediction of IUGR. For example, Litford et al. (36) determined the effects of changing the cutoff points on the sensitivity, specificity, and predictive value of the test. When the 10th percentile of hPL values was used, 29% of all growth-retarded fetuses were identified and 91% of all normal fetuses were excluded. The 15th and 25th percentiles yielded improved sensitivities of 37% and 50%, respectively, but specificity was reduced. They proposed the 10th percentile as the best compromise between sensitivity on the one hand, and predictive value on the other, and suggested that this concept can also be applied to other biochemical or non-biochemical tests of fetal well-being.

Aickin et al. (37) analyzed urine and plasma  $E_3$  data obtained during 608 pregnancies together with plasma progesterone, hPL,  $SP_1$ ,  $\beta$ -glycoprotein, and serum cystyl aminopeptidase. The predictive accuracy of low values for the identification of SGA infants was assessed for each test at various gestation ages. Dates were analyzed to obtain 10th to 90th percentile values for each test from 28 weeks to delivery. Groups with values under different percentile levels were compared; those under the lower percentiles had higher proportions, but smaller absolute numbers of SGA infants than those under higher percentiles. No test was superior to the others at all percentiles and gestations, and it was concluded that biochemical screening of pregnant populations to identify women requiring intensive monitoring has limited potential.

The problem is complicated by the fact that racial differences may interfere with the determination of reference standards of intrauterine growth. It has been shown that Asian mothers have higher hPL levels between 28 and 38 weeks than European mothers, and in IUGR their hPL levels were only low in the last trimester, and urinary estriol excretion was normal (38).

Two years ago we tried to define a strategy for the use of the biochemical assays (hPL,  $E_3T$ ,  $E_{ur}$ , and  $E_2$  increase and DHA-S t 1/2 after loading test), based on a study of 178 women admitted to hospital for suspected IUGR during a period of two years, and who had also ultrasonic determination of biparietal and transverse abdominal diameters between 27 and 40 weeks of gestation (*unpublished observations*).

These women delivered 52 SGA infants (29%), indicating a high risk of fetal hypotrophy in the group. Table 5 shows that four measurements had diagnostic value:

TABLE 5. Distribution of echographic variables and hormonal estimations in suspected intrauterine growth retardation (SGA) and control (AGA) groups

	SGA		AGA		p
	n	%	n	%	
Biparietal diameter					
> 10th percentile	26	50	92	73	< 0.01
≤ 10th percentile	26	50	34	27	
Transverse abdominal diameter					
> 10th percentile	21	42	86	69	< 0.001
≤ 10th percentile	29	58	38	31	
hPL					
Normal	15	31	66	53	
Low	34	69	58	47	< 0.01
E <sub>3</sub> T					
> 10th percentile	28	56	78	61	NS
≤ 10th percentile	22	44	49	39	
E <sub>ur</sub>					
Normal	12	36	49	51	NS
Low	21	64	48	49	
DHA-S t <sub>1/2</sub>					
< 4.29 hr	23	45	91	71	< 0.01
> 4.29 hr	28	55	37	29	

SGA, small for gestational age; AGA, appropriate for gestational age; hPL, human placental lactogen; E<sub>3</sub>T, total plasma estriol; E<sub>ur</sub>, 24-hr urinary estriol excretion; DHA-S t<sub>1/2</sub>, half life of dehydroepiandrosterone sulfate after 50 mg loading dose.

Boxed numbers, %.

biparietal and abdominal diameters, hPL and DHA-S t<sub>1/2</sub>. Biparietal diameter had the highest specificity and hPL the highest sensitivity. The predictive value of these two criteria was identical.

In an attempt to choose the best cutoff point for DHA-S t<sub>1/2</sub> we determined the sensitivity and the specificity for different values. It appears that the value 4.29 hr, which had previously been chosen, is a good compromise, the sensitivity being 54.9%, and the specificity being 71%. The two echographic variables (biparietal and abdominal diameters) both had a sensitivity of 76%, using the 10th percentile as cutoff point, and a specificity of 68%. Due to the low cost of hPL versus DHA-S t<sub>1/2</sub>, we consider that the former is a better complementary biochemical variable.

In a more theoretical study we integrated these results to establish the predictive value of the different tests for screening a general population where the prevalence of IUGR is 5%, and a high-risk population with a prevalence of 20%. Whatever the a priori risk of IUGR (5% or 20%), the presence of at least two abnormal results gives a high probability of identifying fetal growth retardation (between 36% and 92%).

It thus appears that in the low-risk population it is not necessary to undertake

TABLE 6. Probability of small-for-gestational-age (SGA) infants in a population having a low risk of fetal growth retardation (5%)

Transverse abdominal diameter		n	Probability (%)
< 10th percentile	hPL low	294	68
	hPL normal	945	11
≥ 10th percentile	hPL low	1,000	15
	hPL normal	7,761	1

hPL, human placental lactogen.

DHA-S t 1/2 determination when transverse abdominal diameter and hPL are both normal or both low, and that this latter screening procedure allows us to isolate a group including 3% of the population in which the risk of fetal growth retardation is high (68%) and a group at very low risk (<1%) which includes 78% of the population (Table 6).

Since 1983 we have also observed in our hormone laboratory, as in others, that there are important correlations between various biochemical indices in high-risk pregnancies and the non-invasive determination of utero-placental blood flow using either 113 indium and a computer-linked gamma camera (39) or, more commonly, blood velocity measured by the Doppler effect on ultrasound (40), both of which are capable of predicting the severity of fetal growth retardation.

Nevertheless, it is evident that assays of hPL, and to a lesser extent estrogens, may have a place in the prediction of IUGR, especially if used in conjunction with ultrasonography, providing that they can be repeated at least twice between the 30th and the 36th week of pregnancy to detect stationary or falling levels.

## REFERENCES

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## DISCUSSION

*Dr. Bossart:* When you did the loading test did you only look at estriol or did you look at other hormones such as estrone? When we looked at estrone with the same loading test we found it gave more significant results than estriol. And what about Schwangerschaft's protein 1 (SP 1, a placental glycoprotein specific for pregnancy), oxytocinase, and so on? Are they as good as human placental lactogen (hPL), or better?

*Dr. Cédard:* We did not look at estrone, but I believe the results would be the same. With regard to the second question, we have done some SP1 assays which seem to give the same results as hPL. However, it is possible that some countries might be more interested in SP1 because it can be used throughout the course of pregnancy. This would have obvious advantages in terms of the cost of analytic equipment, since it could replace two assays (human chorionic gonadotropin (hCG) estimations at the beginning of pregnancy and hPL towards the end).

*Dr. Seeds:* Do you think any of these hormonal tests are likely to be useful for the detection of high-risk groups in developing countries? What do you think of the possible use of hPL or serum estradiol as screening tests in such areas?

*Dr. Cédard:* I think hPL is the more robust test because there is no diurnal variation and because the gestational increase in values is less than for estriol. It is thus possible to define an absolute fetal danger zone, which can be set at 4 µg per ml irrespective of definite knowledge of gestation. I think values below that level will define most cases of severe IUGR.

*Dr. Marini:* I know two factors which support your view that hPL is better than estriol. First, there has been a recent study from Padua in which it was demonstrated that hPL was a good predictor of IUGR; and second, we know that estriol is unreliable when the mother is given steroids to prevent hyaline membrane disease in the baby. This causes a large fall in estriol.

*Dr. Cédard:* It is true that estriol values are modified by many substances, and especially corticoids. hPL, on the other hand, is not subject to hormonal regulation and remains very stable, with little diurnal variation.

*Dr. Wharton:* Do you find that the etiology of IUGR has different effects on the biochemical measurements? In our population in Birmingham, the indigenous white mothers have con-

ventional reasons for IUGR, such as smoking, preeclampsia, and so on (with a large number of “unknowns”), and in these both estriol and hPL are reasonably predictive—not very good but reasonable. On the other hand, in our large Asian population, where we think malnutrition is the major cause of IUGR, estriol has no predictive value at all, though hPL is still useful (1).

*Dr. Cédard:* We have not shown a difference between different populations in estriol measurements. But we do find that hPL is more predictive for the whole population. The main problem arises when you are dealing with a fetus with a malformation syndrome. In these babies the hPL may be normal in spite of pronounced IUGR. Thus, where there is a discrepancy between ultrasound findings and hPL values, we suspect fetal malformation.

*Dr. Wharton:* Does hPL tell us anything about the function of the placenta or is the level in the plasma simply proportional to placental size?

*Dr. Cédard:* It is related to functional placental mass.

*Dr. Wharton:* I believe there has been some recent work suggesting that hPL measured early in pregnancy is highly correlated with postconceptional age and so could be used as a substitute for ultrasound in developing countries to confirm the length of gestation. Could you comment on this?

*Dr. Cédard:* I know that this has been done but cannot comment on its reliability.

*Dr. Dias-Correa:* In my city it is extremely difficult to get women to do complete 24 hr urine collections. Do you also find this difficulty?

*Dr. Cédard:* I agree there are considerable difficulties. This is why tests relying on urine collection are generally bad. There are too many sources of error.

*Dr. Dias-Correa:* What about a random urine sample related to creatinine?

*Dr. Cédard:* I don't think a random sample is reliable, even if related to creatinine excretion. Correction using creatinine may be useful if you suspect that a 24 hr collection is not complete but not if there is preeclampsia or hypertension, which affect the degree of creatinuria. Personally, I think that the results obtained using plasma estrogen measurements are the same as using urinary estrogens, so you might as well take the plasma sample and avoid the uncertainty of incomplete urine collections.

*Dr. Belizan:* My advice is that, if you want to detect or monitor IUGR, you should not bother with any of these hormonal measurements. You are wasting time and money, and maybe even life. This is not only my experience but the experience of a large body of published work. I have used these tests for 10 years but I have now abandoned them because they do not help.

*Dr. Cédard:* I must disagree. I think they do work, especially hPL, which is more robust than estriol for various reasons I have already discussed.

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