A comparative study of five laboratory tests for foeto-placental dysfunction in late pregnancy

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SYNOPSIS Five foeto-placental function tests were studied in parallel in normal and abnormal late pregnancies with a view to establishing which test or tests is most satisfactorily able to identify the mother whose foetus is in danger.

A critical examination of the levels in blood serum of two enzymes, placental phosphatase isoenzyme and cystine aminopeptidase, the polypeptide hormone placental lactogen (chorionic somatomammotrophin), and the oestrogen oestriol-17 β is described.

A correlation was attempted between clinical data and the results of the above laboratory analyses and also with the daily urine oestrogen output. The plasma and urine oestriol levels proved generally to be the more useful warning tests in late pregnancy, whilst the plasma cystine aminopeptidase was the least sensitive indicator of foeto-placental dysfunction.

Applying organic chemical analysis, Brown, in 1955, succeeded in producing the first practical and objective test of early foeto-placental dysfunction—the daily urine oestrogen output. Since that time several other clinical chemistry techniques have been developed for the measurement and monitoring of placental function. Urine pregnanediol excretion was one of these early tests. Even though known to be normal in many cases of intrauterine death, pregnanediol assays have only comparatively recently lost favour among some obstetricians. Tests employing blood rather than 24-hr urine collections would seem to have obvious advantages if they are to provide a quick warning or confirmation that the foetus may be in danger. In the present study we looked at the reliability of blood analyses for four substances¹—placental phosphatase isoenzyme and cystine aminopeptidase (oxytocinase), placental lactogen (chorionic somatomammotrophin), and oestriol-17 β . Total oestrogen excretion was also measured. Comparative data were collected for both normal and complicated terminal pregnancies. All results were correlated with the subsequent conditions

of the foetus and placenta in order to evaluate the predictive efficacy of the various tests.

Patients

Women attending antenatal afternoon clinics at two district hospitals volunteered to make part of this study possible. Fifty-one women were selected on grounds of convenience (local residence, etc). Each donated blood and 24-hr urine specimens before and after delivery. Between four and 10 blood and urine specimens were collected at weekly intervals to term and two or three specimens at two-weekly intervals after delivery.

Of the group studied, 49 produced healthy babies, one delivered a pale infant, unusually shocked at birth (Apgar score 2.2.2.6.8). However, the birth and placental weight were normal and the child subsequently thrived. There had been gross congenital abnormalities in a previous delivery. Another woman's infant died eight hours after birth, probably due to the respiratory distress syndrome. The placenta weighed only 230 g.

There were four inductions for postmaturity, also three lower segment caesarian sections. Mild to moderate foeto-placental dysfunction was assumed in seven instances of macroscopic infarction of the placenta (with or without calcification, grittiness,

¹Non-standard abbreviations: h-PL = human placental lactogen; E3 = oestra-1,3,5 (10)-triene-3, 16 α , 17 β -triol; PA P'ase = placental alkaline phosphatase; CAS = cystine amino peptidase (nomenclature recommended by the International Union of Biochemistry, *J. clin. Path.* (1971) 24, 656).

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etc), and in one case with a low Apgar score after birth.

Additionally, two pregnant women admitted for hypertension, toxæmia, and poor obstetric history were also investigated, and serial blood and urine collections made in the same manner for the analysis of serum placental alkaline phosphatase, placental lactogen, cystine aminopeptidase and oestriol 17- β , and urine total oestrogens. There was evidence of severe foeto-placental distress in each case. Each showed abnormal dipping of the foetal cardiotocograph. In one a large retroplacental clot was present. The placenta of the other weighed 240 g.

Methods

All 24-hr urine specimens were analysed for creatinine and total oestrogens and a 10 ml aliquot was stored at -28°C for repeat analyses. During the first year of this study the method of Brown, MacLeod, Macnaughtan, Smith, and Smyth (1968) was used. Later analyses were made by the method of Oakey, Bradshaw, Eccles, Stitch, and Hayes (1967). All blood specimens were centrifuged after 30-40 min, separated, and the serum re-centrifuged. Cell-free sera were stored in plastic pots at -28°C until analysed.

Before determining placental alkaline phosphatase an aliquot of each thawed and well mixed serum was heated in a stoppered tube at $59^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for exactly 30 min. Subsequently, the phosphatase isoenzyme activity was measured at pH 10.1 by the method of Kind and King (1954).

Serum placental lactogen was determined in duplicate by radioimmunoanalysis using the reagents and technique supplied by Pharmacia. The procedure is based on that of Letchworth, Boardman, Bristow, Landon, and Chard (1971). Preliminary experiments had shown the results obtained to be comparable though slightly lower than those found using reagents from the Radiochemical Centre, Amersham.

Serum cystine aminopeptidase activity was measured by a modification of the method of Tovey (1969) and recently reported in summary (Watson and Gibbard, 1973). Kinetic measurement of the enzyme is made using Pye Unicam automation equipment AC 60/61 SP 1800 and recorder AR 25.

Serum (100 μl) is mixed with 1.0 ml of buffer at pH 7.0 (50 mM KH_2PO_4 -NaOH) and 100 μl of substrate (6.0 mmol, S-benzyl L-cysteine-4' nitro-anilide per litre of 2-methoxyethanol) is added. The reaction rate if followed graphically over a two min-period beginning one min after the addition of substrate. Increase in absorbance due to p-nitro-aniline liberation is measured at 405 nm. The reaction temperature is 30°C . One IU/1 produced

Δ /minute of 0.00082 under test conditions (calculated from the molar extinction coefficient of p-nitro-aniline, 9 900). Therefore Δ absorbance/min $\times$ 1210 equals serum cystine aminopeptidase in IU/1.

Serum oestriol 17- β (E3) was estimated by a radioimmunoassay technique also developed in this laboratory. The method is summarized in figure 1.

Fig 1 Flow sheet for determination of serum oestriol by radioimmunoassay (RIA).

For preparation of the standard curve, each final count is corrected for background and expressed as a percentage of the total counts per assay tube. This gives the percentage of bound E3 for each tube. The amount of E3 in each standard and control tube is corrected by multiplying by the recovery factor ($0.627 \pm 4\%$). Percentage bound E3 is plotted against mass of E3. The unknown calculated percentage of bound E3 is entered in the graph to give the mass of E3. This (pg/10 μl) is then corrected by dividing by the recovery factor and expressed as ng/ml serum by multiplying by 0.1.

Results

RELIABILITY OF ASSAYS

The heat-stable alkaline phosphatase of one mid-pregnancy serum was determined 23 times in one morning. The values ranged from 28 to 36 IU/l with a coefficient of variation of 9%. A similar experiment for cystine aminopeptidase showed a coefficient of variation of 3.6%, range 140-158 IU/l, and for h-PL a 2.0%, range 3.7-4.4 µg/ml. Serial analyses performed on three patients failed to show any rhythmic variation during a 48-hour period. Typical results in one case are shown in table I.

The plasma concentration of oestriol in a control serum was estimated in 10 consecutive radioimmunoassays and the observed coefficient of variation was 7.1% (mean 114 ng/ml). Intra-assay variation was estimated by the duplicates method of Snedecor (1962) and for the whole range of oestriol levels encountered was 5.6%.

One urine specimen was analysed as a control with each batch of specimens over a period of one month. The range of oestriol values was 24-32 mg/24 hr, SD = 2.6, coefficient of variation = 9%. Over a period of a year, random urine oestriol analyses were checked by independent reference laboratories. The values found in this laboratory were 6, 11, 20, and 37, the mean of 12 results being 19.4; the reference values for the same urine samples were 6.2, 12.9, 21.1, and 40.4, the mean of 12 results being 19.59 mg.

When stored at -28°C, the serum h-PL and

oestriol content was stable for at least one year and was uninfluenced by freezing and thawing up to four times. Loss of activity of serum cystine aminopeptidase occurred at -20°C. The rate of deterioration was about 6% per 10 weeks.

RESULTS IN NORMAL PREGNANCIES

The mean levels and actual ranges found for serum placental A P'ase, CAS, and h-PL in 34 normal pregnancies are given in table II. It will be seen that during late pregnancy the two enzymes show a similar steady increase in concentration, while the placental lactogen remains at a fairly constant level. By way of illustration of the latter finding, serum placental lactogen levels during the last six weeks of pregnancy were for one volunteer 3.7, 3.4, 4.1, 3.4, 3.6, and 3.4, and for another 3.1, 4.8, 3.1, 3.0, 3.0, and 2.7 µg/ml.

The correlation coefficient relating serum A P'ase and CAS during the week before delivery was calculated to be 0.511. Correlation between the enzymes can be seen by inspection of table II. No corresponding relation exists between h-PL and either of the enzymes.

The scatter of values in terminal pregnancy is greater for the enzymes than for h-PL, there being a five- to six-fold difference between the lowest and highest serum enzyme values and less than three-fold difference between the lowest and highest serum h-PL level.

The mean 24-hr urine 'oestriol' outputs for un-

Time of Specimen	Serum Placental Alkaline Phosphatase (IU/l)	Serum Cystine Aminopeptidase (IU/l)	Serum Placental Lactogen (µg/ml)	Serum Protein (g/l)	Serum Albumin (g/l)
Day 1 8.00	87	163	5.6	76	40
12.00	86	170	5.45	75	41
16.00	86	170	5.35	73	38
20.00	86	154	5.2	73	41
Day 2 8.00	86	184	5.4	72	38
12.00	83	173	5.15	71	42
16.00	87	168	5.25	68	40
20.00	86	143	5.6	75	42

Table I Serum placental A P'ase, CAS, h-PL, and proteins in late pregnancy at intervals during two consecutive days

	Weeks before Delivery					
	5-6	4-5	3-4	2-3	1-2	0-1
Placental alkaline phosphatase (IU)	54 (23-109)	55 (22-102)	58 (23-126)	64 (27-134)	70 (30-137)	76 (25-124)
Cystine amino-peptidase (IU)	94 (37-205)	96 (42-215)	99 (51-214)	110 (57-213)	117 (60-232)	121 (62-240)
Placental lactogen (µg/ml)	4.9 (3.1-6.4)	5.0 (3.4-7.1)	5.0 (3.1-8.0)	5.0 (3.0-6.8)	5.0 (3.0-6.8)	5.0 (2.7-7.8)

Table II Serum values (mean and actual ranges) for placental alkaline phosphatase, cystine amino peptidase, and placental lactogen in normal pregnancy (34 women)

Gestation Period in Weeks	Foeto-placental Dysfunction											
	Mild-moderate								Moderate-severe			
	Cb	Jm	Lj	Rj	Wa	Wi	Jh	Ms	Fm	Sw	Fj	Od
31												14
32												17
33												5
34	58	23	48	48		45	22	27	50		36	21
35	55	33	32	48		70	20	33	—		40	
36	62	40	40	57		67	22	43	60	22		
37	72	38	48	57	89	88	22	33	76	25		
38	74	38	60	67	108	90	36	38	88	25		
39	77	43	67	65	117		45	46	90			
40	79	45	84	72	122							

Table III Serum placental A-P'ase activities (IU) in 12 cases of foeto-placental dysfunction

Gestation Period in Weeks	Foeto-placental Dysfunction											
	Mild-moderate								Moderate-severe			
	Cb	Jm	Lj	Rj	Wa	Wi	Jh	Ms	Fm	Sw	Fj	Od
31												45
32												53
33												48
34						85	75	103	99		93	30
35	118	48	114	84	120	87	81	105	—		95	
36	125	63	—	102	141	88	69	119	114	111		
37	123	54	135	99	144	91	84	132	126	96		
38	144	63	165	105	180	93	105	125	144	123		
39	156	60	154	116	176		117	133	159			
40	165	66	156	109	192							

Table IV Serum cystine aminopeptidase level (IU) in 12 cases of foeto-placental dysfunction

Gestation Period in Weeks	Foeto-placental Dysfunction											
	Mild-moderate								Moderate-severe			
	Cb	Jm	Lj	Rj	Wa	Wi	Jh	Ms	Fm	Sw	Fj	Od
31												1.7
32												2.1
33												1.6
34						4.5	4.2	5.6	6.2		4.1	1.3
35	4.8		6.0		4.9	4.9	4.9	5.5	—		3.8	
36	4.9	3.7	—	4.9	5.6	4.4	5.1	5.3	6.4	4.2		
37	4.9	5.0	5.8	5.1	5.9	4.6	5.3	5.2	6.7	4.4		
38	4.9	4.7	5.8	5.1	6.8	4.4	5.8	5.3	6.5	4.8		
39	5.1	4.7	5.8	5.0	4.6		6.6	5.1	6.6			
40	4.9	4.5	6.2	5.1	6.0							

Table V Serum placental lactogen ($\mu\text{g/ml}$) in 12 cases of foeto-placental dysfunction

eventful pregnancies were calculated for 34, 35 . . . 39 weeks as 16 (range 8-28) mg, 18 (9-34) mg, 19 (9-34) mg, 21 (10-39) mg, 22 (11-38) mg, and 24 (11-35) mg.

The corresponding mean plasma oestriol concentrations at weeks 34, 35 . . . 39 were: 91 (range 38-216), 113 (49-260), 145 (69-308) 147 (64-334), 176 (85-363), and 179 (77-413) ng/ml.

RESULTS IN OTHER PREGNANCIES

There were five cases of prolonged, but otherwise normal, pregnancy (42 weeks or more). The serum values measured showed no consistent deviation from the normal.

Mild foeto-placental dysfunction was apparent in eight pregnancies, but of these, only one (Jh)

showed definite laboratory evidence of abnormality before delivery; tests giving abnormally low results were serum PAP'ase and oestriol and urine oestriol during the period three to six weeks before delivery. The other tests were normal (table III, V).

Of the four cases where foeto-placental dysfunction was more severe, one (Od) showed abnormality in all five tests. Another (Fj) showed low values for serum oestriol doubtful h-PL values, with the other tests normal. The mother (Sw) of the non-surviving infant presented abnormally low figures for the urine oestriol output, serum oestriol, and placental A P'ase, the other tests being normal. In the remaining case (Fm) the only test abnormality found was in the serial serum oestriol levels. These results are shown in tables III-V and figure 2. The degree of correlation between the serum and the urine oestriol values was $r = 0.387$ for the normal and $r = 0.484$ for the abnormal pregnancies (fig. 3).

Discussion

The value of a biochemical test in late pregnancy lies in its ability to select for special attention the mother whose foetus is in jeopardy. In this series the serum cystine aminopeptidase (CAS) and the placental A P'ase were the least frequent forecasters of foeto-placental insufficiency. The serum PAP'ase provided warning in two cases and the CAS in only one of 12 'abnormal' pregnancies. The positive correlation found between the levels of these substances was not unexpected in view of their common origin of synthesis in the syncytiotrophoblast. However, the wide range of values found for each of the two enzymes in normal terminal pregnancy makes their discriminative use difficult in practice. For example, the term values for CAS were arithmetically distributed in the range 60-240 IU/l (and similarly for serum PAP'ase at exactly half this level). If one puts the lower CAS limit at 70 IU, this will exclude 10% of the normal pregnancies and bring only one more of the cases with placental infarction into the 'abnormal' category. We considered using a temporary fall in serum activity (in excess of analytical variation) as the indicator of abnormality, but this proved unsatisfactory in its discriminative capacity (see tables III and IV). Thus we have no reason to doubt an earlier finding (Watson, Weston, and Porter, 1965) that the plasma placental A P'ase activity is a poor indicator of placental dysfunction. The other enzyme studied (CAS) proved to be of no prognostic value.

A similar impression emerges when serum h-PL levels are considered, viz, only one of 12 mothers with foeto-placental dysfunction had h-PL values below the lower limit of $3 \mu\text{g/ml}$ during the five weeks before term (table II). This finding was disappointing in

Fig 2 Serum oestriol in 12 cases of foeto-placental inadequacy. Normal values are shown by the thick line (mean values) and the dotted thick and thin lines (2 standard deviation above and below mean values).

view of the greater test sensitivity reported by others (Saxena, Emerson, and Selenkow, 1969; Keller, Baertschi, Bader, Gerber, Schmid, Saltermann, and Kopper, 1971). However, Josimovich, Kosor, Boccella, Mintz, and Hutchinson (1970) were unable to find any correlation between serum h-PL levels and foetal outcome in patients with small-for-dates babies or with toxemia. While there is no doubt about the existence of a significant correlation between serum h-PL levels and placental weight (Saxena *et al*, 1969), we think our results reflect the large capacity of the infarcted or abnormally small placenta to continue to produce considerable quantities of h-PL.

A novel feature of the present investigation was the concurrent measurement of serum total oestriol levels. Near term the arithmetic mean serum level of oestriol was 190 ng/ml (SD 60) which is similar to the concentrations reported by other workers (table VI) using alternative methods. At each week of gestation the distribution of serum oestriol levels was observed to be logarithmic and the range of normal values ($\pm 2\text{SD}$) was calculated accordingly (fig 2).

Fig 3 Scattergram of 24 hr urine excretion values and serum oestriol values on each of 131 women during late pregnancy. Normal; $r = 0.387$, $n = 88$, $P < 0.001$: foeto-placental inadequacy; $r = 0.484$; $n = 43$, $P < 0.001$.

Seven of the eight mild-moderate foeto-placental dysfunction cases had serum oestriol levels within the normal range (fig 2). The exception (Jh) showed abnormally low serum and urine oestriol levels at 34-37 weeks, becoming low normal before caesarian section for placenta praevia at 39 weeks. Each of the four patients with more severe foeto-placental dysfunction presented serum oestriol levels at or below $-2SD$ from the relevant normal mean. However, one of the patients (Sw) at three days before delivery showed a low normal serum E3 level following the previous week's subnormal value. The corresponding urine levels were all subnormal. In another (Fj) a normal excretion of 'oestriol' was

observed (10, 10, 8, and 14 mg at 34-35 weeks) whilst the serum oestriol concentrations were abnormally low (36 and 37 ng/ml). The lack of total agreement between plasma and urine oestriol levels is demonstrated in figure 3. The degree of correlation observed in the present study is much lower than that reported by other workers (eg, $r = 0.69$, Taylor, Hagerman, Betz, Williams, and Grey 1970). Part of the discrepancy may be the lack of specificity of the urine 'oestriol' method in addition to the observation that each woman exhibits a variable fluctuation in the level of total blood oestriol (Masson and Wilson, 1972). It is thus apparent that with the current assay procedures neither the urine nor the plasma level alone is able to predict all the cases of severe foeto-placental dysfunction.

In conclusion, of the five biochemical parameters studied in late pregnancy, those of placental origin (CAS, PAP'ase, and h-PL) appear to have no prognostic value in cases of mild placental inadequacy. Determination of the maternal blood or urine oestriol—to which the foetus contributes—proved to be the better indicator of foeto-placental dysfunction, particularly in the more severe cases. Nevertheless, assuming that infarction reduces placental adequacy then some 60% of the women with foeto-placental inadequacy did not present abnormal values in any of the tests studied. There would seem to be a pressing need for a more definitive laboratory test of foetal well-being.

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Investigator	Type of Method Used	No. of Women and Single Analyses	Total Oestriol (range, ng/ml)
Taylor <i>et al</i> (1970)	Fluorimetric	4, 30	50-600
Nachtigall <i>et al</i> (1971)	Fluorimetric	22, 148	100-550
Cooper <i>et al</i> (1971)	Gas liquid chromatography	5, 24	180-510
Fischer-Rasmussen (1970)	Gas liquid chromatography	21, 134	40-300
Macourt <i>et al</i> (1971)	Comp-prot-binding	60, 400	100-320
Present work	Radioimmunoassay	34, 149	63-497

Table VI Serum total oestriol levels in normal late pregnancy (34-40 weeks) determined by recent non-colorimetric methods

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