Enzyme assays in the management of pregnancy

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The occurrence of increased serum enzyme activity in patients with diseases involving tissue damage such as myocardial infarction suggested that placental damage may also give rise to characteristic changes in maternal serum enzymes, which might be useful diagnostically.

One of the problems in obstetrics is the recognition of placental 'dysfunction' or 'insufficiency', terms which are used by obstetricians to indicate a process of impaired transmission of nutrients resulting in retarded fetal growth, and progressing eventually to impaired oxygen transfer resulting in fetal hypoxia and possibly death from anoxia.

The diagnosis depends on the demonstration of fetal distress during labour, or on the delivery of a baby with a low Apgar¹ score, or a birth weight below the 10th centile for that duration of pregnancy. These criteria have only recently been applied to the evaluation of tests of placental function.

It has been established that a number of enzymes in maternal serum remain unchanged in normal pregnancy, but that others increase either during normal or abnormal pregnancy or in both (Table I).

Serum enzymes which do not change during normal pregnancy are cholinesterase (EC 3.1.1.8) (Meade and Rosalki, 1963; Rimbach and Figge, 1966), 2-hydroxybutyrate dehydrogenase (EC 1.1.1.27) (Meade and Rosalki, 1963), and alanine and aspartate aminotransferases (EC 2.6.1.2.4.2.6.1.1) (Meade and Rosalki, 1963; Szekely and Fritzsh, 1969). Studies of lactate dehydrogenase (EC 1.1.1.27) and isocitrate dehydrogenase (EC 1.1.1.42) have given less uniform results. Whereas most authors have found them to remain unchanged, an increase in lactate dehydrogenase at term was found by Pulkkinen and Willman (1968) and a marked increase in isocitrate dehydrogenase was found at 39-40 weeks by Kraussold (1969).

Serum β -glucuronidase (EC 3.2.1.31), hyaluronidase (EC 3.2.1.35) and β -acetylglucosaminidase (EC 3.2.1.30) have all been described as increasing late in normal pregnancy (Platt and Platt, 1968).

¹The Apgar score is based on assessment of the baby's colour, heart rate, respirations, muscle tone, and the response to a catheter in the nostril. The best prognosis is indicated by a score of 10 and the worst by a score of 0.

In abnormal pregnancy, many of the above enzymes have been found to increase. Thus increased levels of both aminotransferases were found in two out of 14 patients with mild pre-eclampsia, and 11 out of 12 with eclampsia (Dass and Bhagwani, 1964). These results were taken to mean that toxæmia is associated with liver damage, and it was suggested that estimations of serum aminotransferases might be of prognostic value in toxæmia. Similarly isocitrate dehydrogenase may show transient increases in pre-eclampsia or accidental haemorrhage (Jeacock, Morris, and Plester, 1962; Meade and Rosalki, 1963; Pulkkinen and Willman, 1968), possibly due to placental infarction (Dawkins and Wigglesworth, 1961). Pulkkinen and Willman (1968) also found increased levels of lactate dehydrogenase in four patients with hypertension.

Plasma renin (EC 3.4.4.15) levels were found to be increased during the third trimester in nine normal women (Geehoed and Vander, 1968), but the mean value at 13-27 weeks in 10 women who subsequently developed toxæmia was greater than that in 48 in whom pregnancy remained uneventful.

None of the above enzymes has been used to assist in the management of pregnancy. However, diamine oxidase (EC 1.4.3.6.), 'oxytocinase', and the heat-stable isoenzyme of alkaline phosphatase (EC 3.1.3.1) all of which increase during normal pregnancy, have been found useful in the management of pregnancy and will be discussed in greater detail.

Diamine Oxidase

Southren and Weingold (1966), Southren, Kobayashi, Weingold, and Carmody (1966), and Southren, Weingold, Kobayashi, Sherman, Grimaldi, and Gold (1968) studied the serum levels of diamine oxidase, using putrescine labelled with isotope as the substrate. They showed that there is a fairly rapid increase in activity during the first trimester followed by a rather slower rise during the rest of the pregnancy, with a wide range of values. The outcome of pregnancies complicated by threatened abortion, by a history of habitual abortion, or missed abortion appeared to be related to the serial changes in

Enzyme	Normal Pregnancy	Abnormal Pregnancy
Alanine aminotransferase	No change	May increase
Aspartate aminotransferase	No change	May increase
Alkaline phosphatase (heat-stable)	Increased	May increase further or decrease
B-acetylglucosaminidase	Increased	—
Cholinesterase	No change	—
Diamine oxidase	Increased	May fall
B-glucuronidase	Increased	—
Hyaluronidase	Increased	—
2-Hydroxybutyrate dehydrogenase	No change	—
Isocitrate dehydrogenase	No change	May increase
Lactate dehydrogenase	No change	May increase
Oxytocinase	Increased	May increase further or decrease
Renin	Increased	May increase further

Table 1 Changes in maternal serum enzymes during normal and abnormal pregnancy

diamine oxidase levels. When the levels increased steadily and remained within the normal range, the pregnancy continued into the third trimester. Conversely, a falling or persistently low level was associated with a significant increase in fetal wastage, although some did continue to term. In cases of pregnancy complicated by diabetes the abnormality of enzyme levels appeared to be related to the severity of the diabetes, but the relationship to fetal survival was poor. In 16 pregnancies complicated by hydatiform mole, serial diamine oxidase levels remained within the normal range during the first trimester, but fell to zero during the second trimester (Torok, Brewer, and Dolkart, 1970). The same authors were unable to detect any diamine oxidase in the serum of 71 out of 80 other patients with trophoblastic disease. Resnik and Levine (1969) confirmed that diamine oxidase activity rises rapidly during the first trimester, and remains elevated during the second and third trimesters; however in 30 patients with 'high risk' pregnancies they were unable to predict the outcome by means of serial assays.

'Oxytocinase'

'Oxytocinase' was first described by Fekete (1930) as an enzyme present in the plasma of pregnant women, which hydrolysed oxytocin and which was measured by the biological assay of the unhydrolysed hormone. Recently, Kleiner (1969) reviewed the evidence that 'oxytocinase' is an exopeptidase and discussed the different substrates which have formed the basis of its biochemical assay. He also reviewed the histochemical and biochemical evidence showing that the placenta is the main source of this enzyme. Many workers have studied 'oxytocinase' in the maternal serum during normal and abnormal pregnancies, using various substrates to assay the enzyme, such as L-leucyl- β -naphthylamide (Kleiner, 1969; Kleiner, Brouet-Yager, and Graff, 1969), leucine-p-nitroanilide (Rimbach and Schreiner, 1967), or L-cystine-di- β -naphthylamide (Babuna and Yenen,

1966a and b; Klimek, 1969; Poch, Abad Martinez, Holzer, and Hohlweg, 1970; Hensleigh and Krantz, 1970). There is universal agreement, regardless of the substrate used, that serum levels of this enzyme increase during normal pregnancy from the second trimester onwards, showing a fairly wide scatter. There is some evidence that serum oxytocinase levels may be related to the duration of pregnancy; thus, Lambrinopoulos (1964) found significantly higher levels in patients who were seven to 19 days past term than in controls at term. They may also be related to the duration of labour, for abnormally high levels have been found in uterine inertia (Babuna and Yenen, 1966a). High levels have also been found in twin pregnancies (Ichaliotis and Lambrinopoulos, 1965; Babuna and Yenen, 1966a), though this was not confirmed by Kleiner *et al* (1969). Serum oxytocinase levels have also been shown to correlate with placental weight (Klimek, 1969; Poch *et al*, 1970), fetal weight (Poch *et al*, 1970), and fetal surface area (Klimek, 1969).

The value of serum oxytocinase assays in the diagnosis of placental dysfunction is difficult to assess, since the latter is defined differently by different workers. Babuna and Yenen (1966) claimed that rising serum enzyme levels were usually a good sign indicating a favourable prognosis, whilst falling levels suggested either the imminent onset of labour or intrauterine death of the fetus. In a retrospective analysis of pregnancies grouped according to the state of the fetus at birth, Klimek (1969) found the mean value to be highest in the group with babies in good condition on delivery, lowest in the group with stillborn babies, and intermediate in the group with babies in poor condition at birth. However, in all groups there was fairly wide variation about the mean values. Similarly Kleiner *et al* (1969) found the mean enzyme level in 50 pregnancies ending in the birth of a dysmature baby¹ to be significantly lower than that for a control

¹One whose birth weight is more than two standard deviations below the mean weight for the duration of pregnancy.

group, but in only 18 were the levels below the lower limit of normal, so that only in this small group could the enzyme assay have been used to 'predict' that the baby would be born dysmature.

Hensleigh and Krantz (1970) estimated serum 'cystine aminopeptidase¹' in a heterogeneous group of 186 pregnant women with a variety of clinical abnormalities including hypertension, pre-eclampsia, and a bad obstetric history such as a previous still-birth. Twelve patients had abnormal serum levels and all showed acceptable evidence of inadequate placental function. In 174 patients the levels were normal, but seven of these also showed evidence of placental dysfunction. Thus, although no false positives were encountered there were seven false negatives, which the authors attributed to the lack of serial samples in these cases.

This review of the literature on oxytocinase leads to the conclusion that when serial assays of the enzyme are performed, a progressive rise of activity suggests that the pregnancy is likely to continue and the fetus is probably not at risk. Falling or abnormally low levels suggest either that the fetus is at risk, or that the spontaneous onset of labour is imminent.

Heat-stable Alkaline Phosphatase

Since Coryn (1934) first reported that maternal serum alkaline phosphatase is raised in late pregnancy, there have been numerous confirmatory reports including our own (Curzen and Morris, 1965). There is also considerable evidence that the placental content of alkaline phosphatase increases in late pregnancy and that it is relatively higher in pregnancy complicated by hypertensive disorders than in normal pregnancy (Jeacock, Morris, and Plester, 1963; Curzen, 1964 and 1967). This led to the suggestion that some of the increase in the serum alkaline phosphatase might be due to a placental component, and that the assay of this placental isoenzyme might be of clinical use. The discovery by Neale, Clubb, Hotchkis, and Posen (1964) that the placental isoenzyme is relatively heat-stable, and the demonstration by McMaster, Tennant, Clubb, and Neale (1964) that incubation of serum at 56°C for 30 minutes destroys most of the alkaline phosphatase from non-placental sources, provided a simple means of separating the placental isoenzyme. We studied serum heat-stable alkaline phosphatase (HSAP) levels in normal pregnancy, and found a fairly wide range of values, but with a progressive rise of mean values during the last twelve weeks of pregnancy (Curzen and Morris, 1966, 1968). Furthermore, HSAP levels tend to be abnormally

high in patients suffering from hypertensive disorders of pregnancy, and there appears to be a relationship between the severity of the hypertensive disorder and the extent to which serum HSAP levels are deranged. Hunter (1969) showed that incubating serum at 65°C for 30 minutes instead of 56°C entirely inactivated all non-placental isoenzymes of alkaline phosphatase. Using this method he showed that serial HSAP estimations underwent an abnormal rise in patients with pre-eclampsia. Furthermore, the abnormal elevation of HSAP seemed to precede the onset of clinical signs by two to three weeks. Several workers have suggested that serial HSAP determinations may sometimes provide an indication of placental dysfunction and fetal prognosis (Curzen and Morris, 1966, 1968; Messer, 1967; Hunter, 1969).

However, Watney, Hallu, Ladell, and Scott (1970) have shown that in a group of 29 patients whose babies had grossly abnormal placentae and weighed less than the 10th centile at birth, only 34% had abnormally high or low HSAP values in pregnancy compared with 83% who had abnormally low oestriol values. Furthermore these workers heated their sera at 56°C for 30 minutes before measuring HSAP activity, and therefore may not have completely inactivated non-placental isoenzymes.

We have recently been using a modified technique for assaying the placental isoenzyme of alkaline phosphatase. This is based on the extensive investigations of Fishman and his colleagues into the biochemical characteristics of the isoenzymes of alkaline phosphatase (Fishman and Ghosh, 1967; Fishman, Inglis, Green, Anstiss, Ghosh, Reiff, Rustigan, Krant, and Stolbaga, 1968a; Fishman, Inglis, and Ghosh, 1968b; Ghosh and Fishman, 1967). They have shown that incubating serum at 65°C for five minutes totally inactivates all isoenzymes other than those from the placenta, and also that the optimum pH and substrate concentration are higher for the placental isoenzymes than for other isoenzymes. We have therefore assayed the alkaline phosphatase activity remaining after heating the serum at 65°C for five minutes.

Using this method we determined the normal range of serum HSAP during the last 12 weeks of pregnancy on 204 samples from normal pregnant women (Fig. 1). Assays were also carried out in 50 pregnant women thought to be at risk from placental dysfunction. The clinical diagnoses in the latter are detailed in Table II. Each of these patients had simultaneous assays of serum HSAP and 24-hour urinary total oestrogens (Oakey, Bradshaw, Eccles, Stitch, and Heys, 1967) performed two or three times a week until delivery. Serum HSAP results

¹'Oxytocinase' estimated with cystine-di- β -naphthylamide as substrate.

Fig. 1 The normal range of serum HSAP during the last 12 weeks of pregnancy. The lines indicate 95% confidence limits. The HSAP unit = the amount of enzyme which will liberate 1 mg phenol from 36 mM disodium phenylphosphate in 15 minutes at 37°C and in bicarbonate buffer at pH 10.7.

Diagnosis	Number of Patients
Pre-eclampsia	26
Hypertension	12
'Small-for-dates'	10
Albuminuria	1
Twins	1

Table II Clinical diagnosis in patients in whom serum heat-stable alkaline phosphatase was estimated because of the possibility of placental dysfunction

were considered abnormal when they were above or below the limits of normal, or when two successive determinations showed a reduction in activity even though they were within normal limits.

Normal and abnormal results were related to birth weight (Table III), objective signs of fetal distress, ie, a fetal blood pH of less than 7.2, or delayed onset of fetal bradycardia (Table IV), and the Apgar score at one minute (Table V). From these results it can be seen that serial total urinary oestrogen determinations correctly predicted fetal dysmaturity in 64.7% of cases ($p = < 0.001$), fetal distress in 64.3% of cases ($p = < 0.001$), and a poor Apgar

score in 61.5% of cases ($p = < 0.01$). In contrast, serum HSAP determinations were of little value in this respect.

The correlation of serum HSAP levels and urinary oestrogen output was poor ($r = 0.13$, $SE = 0.06$); the correlation of serum HSAP or urinary oestrogen output with either birth weight or placental weight was even worse ($r \geq 0.14$).

These results indicate that serial assays of serum HSAP are of no value for determining fetal prognosis, even when great care is taken to assay only the placental isoenzyme. It may be that abnormally high serum HSAP levels represent placental damage, and that abnormally low levels indicate poor placental development. However, there does not seem to be any strong evidence to support this idea at the present time. It is perhaps of interest to note that serial estimations of urinary total oestrogen output were also associated with a fairly high incidence of inaccurate predictions of the outcome of pregnancy.

The conclusion seems to be that assays of single enzymes are of little value in the management of pregnancy; however, assays of several enzymes may prove to be more useful.

Birth Weight	No. of Cases	Serum HSAP		Urinary Oestrogen	
		Normal	Abnormal ¹	Normal	Abnormal
< 10th percentile	17	15 (88.2%)	2 (11.8%)	6 (35.3%)	11 (64.7%)
> 10th percentile	33	28 (84.8%)	5 (15.2%)	28 (84.8%)	5 (15.2%)
Total	50	43 (86%)	7 (14%)	34 (68%)	16 (32%)
		$\chi^2 = 0.01, p = 0.9$		$\chi^2 = 15.04, p < 0.001$	

Table III Abnormalities of maternal serum heat-stable alkaline phosphatase (HSAP) and urinary oestrogen output for infant birth weights above and below the 10th centile

¹ Usually high, but sometimes low.

	No. of Cases	Serum HSAP		Urinary Oestrogen	
		Normal	Abnormal ¹	Normal	Abnormal
Fetal distress	14	12 (85.7%)	2 (14.3%)	5 (35.7%)	9 (64.3%)
No fetal distress	36	31 (86.1%)	5 (13.9%)	29 (80.1%)	7 (19.4%)
Total	50	43 (86%)	7 (14%)	34 (68%)	16 (32%)
		$\chi^2 = 0.24, p > 0.5$		$\chi^2 = 11.49, p < 0.001$	

Table IV Abnormalities of maternal heat-stable alkaline phosphatase (HSAP) and urinary oestrogen output according to the presence or absence of fetal distress

¹ Usually high, but sometimes low

	No. of Cases	Serum HSAP		Urinary Oestrogen	
		Normal	Abnormal ¹	Normal	Abnormal
Apgar (0-5)	13	13 (100%)	0	5 (38.5%)	8 (61.5%)
Apgar (6-10)	37	30 (81.1%)	7 (18.9%)	29 (78.4%)	8 (21.6%)
Total	50	43 (86%)	7 (14%)	34 (68%)	16 (32%)
				$\chi^2 = 9.0, p < 0.01$	

Table V Abnormalities of maternal heat-stable alkaline phosphatase and urinary oestrogen output according to Apgar score of a newborn infant

¹ Usually high, but sometimes low.

This work was supported by a grant from the Medical Research Council. It is a pleasure to acknowledge the help of Miss Helen Hensel who performed the biochemical assays, and Dr D. F. Hawkins of the Institute of Obstetrics and Gynaecology, who calculated the approximate fiducial limits for Figure 1.

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