

# SERUM OXYTOCINASE AS AN INDEX OF PLACENTAL FUNCTION

by

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## *Introduction*

During the course of pregnancy, particularly in the human, the placenta plays a major role in exchanging the material between mother and foetus and producing the vitally needed hormones and enzymes for the successful maintenance of pregnancy. Quite a few complications of pregnancy have been found to be very often associated with placental dysfunction. The correct and continuous study of placental capacity is thus, the sine qua non for closely following the pregnancy and forestalling its abnormal trends.

The clinical methods developed so far for estimation of the functional capacity of the placenta are hardly reliable and satisfactory. The suitable confirmation of any clinical suspicion about the functional insufficiency of placenta by reliable laboratory techniques would greatly help the obstetrician in deciding the best time for delivery in a number of abnormal cases.

Several laboratory methods have been put forward for evaluating the placental competence. The urinary estriol and pregnanediol determinations have proved to be valuable in predicting foetal viability

and distress in pregnancy. These methods, however, suffer from certain drawbacks which considerably reduce their utility in routine check-ups. Besides being costly, the methods involve lengthy procedures and most of the tests need 24-hour collected urine specimens. This makes these tests irksome to patients and unsuitable for emergent conditions.

The human placenta, in addition to estrogens and progesterone, contains a complex of enzymes which are also present in the maternal serum. Most of these enzymes are also produced by certain tissues other than the placenta and therefore, their levels during pregnancy may not necessarily reflect the true state of placental function. This impasse has been got over with the finding that the enzyme oxytocinase is largely, if not solely, produced by the placenta. The studies of this enzyme bear comparatively bright prospects of better accuracy in the investigation of functional capacity of human placenta. Oxytocinase estimation can be done from a small amount of serum with better accuracy and practical advantages over the hormonal estimations. Because of its low cost, easy technique and better accuracy, it could be developed as one of the routine tests for assessment of placental function.

## *Chemical Method of Assay of Oxytocinase*

Chemical methods of assay of oxy-

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tocinase has been proposed by Tuppy and collaborators. This procedure is based on determinations of the rate of cleavage of the substrate L-cystine-di-B-naphthylamide, a synthetic compound with the same configuration as the amino terminal half cystinal residue of the oxytocin molecule. Chemical method of assay of oxytocinase is superior to biological method of assay of oxytocinase in precision and practicability. Its use as a method for assays of plasma oxytocinase activity is based on the assumption that the inactivation of oxytocin by plasma is due mainly to a special enzyme with a marked affinity to cystinyl-peptides. The enzyme has been termed cystine aminopeptidase (CAP) or Oxytocinase.

#### *Review of Literature*

In most of the reports concerning the plasma oxytocinase activity in normal human pregnancy, it has been noted that the activity starts to rise at about the beginning of the fourth month, that there is a continuous curvi-linear increase during pregnancy and that the highest value is reached around term. (Woodbury *et al*, 1948; Kanazawa, 1954; Dicker and Whyley, 1959; Muller-Hartburg *et al* 1959; Riad, 1962; Melander, 1965; Babuna and Yenen 1966). Semm (1961) found a slight increase in the oxytocinase activity from 2nd to 7th month of gestation and a steep increase during the 8th to 9th months.

Plasma oxytocinase activity has been determined prior to the onset of spontaneous labour, during labour and at various time intervals following delivery and during postpartum period (Werle and Effkemann, 1949; Page, 1947; Werle *et al* 1950; Semm, 1958; Beller *et al* 1957; Periti *et al* 1961; Semm, 1961; Fylling, 1963). No decline in the plasma oxytocinase value has been noticed just before or at the start

of delivery. Neither has any correlation been noticed between the stages of delivery and plasma oxytocinase values. Riad (1962) noticed that the levels obtained in early labour show only minor changes from the late pregnancy readings. The enzyme level was seen to have dropped to approximately 40% of the labour value within one week after delivery and returning to the normal non-pregnancy level by six weeks postpartum.

Klimek (1964) reported that enzyme estimations could be a useful guide in planning induction of labour. (Timoshenko 1959; Ichaliotis and Lambrinopoulos (1965) and Babuna *et al* (1966) observed higher enzyme activity with uterine inertia than in normal cases but Dicker (1959) failed to demonstrate any such changes. Riad (1966) found no correlation between the enzyme level and the dose of oxytocin required in the induction of labour.

Josephides and Turkington (1967) carried out a study to find out if serial estimations of cystine aminopeptidase could serve as an index of placenta function. They also attempted to compare the usefulness of such estimations with that of urinary estriol estimations. They found that there is a considerable range in both but the estriol levels vary more widely.

#### *Material and Method*

Thirty-four pregnant women either attending antenatal clinic or hospitalised in the obstetric wards of the All-India Institute of Medical Sciences Hospital, New Delhi, were selected for study. Six non-pregnant women from gynaecological outpatient department were also studied.

#### *Selection of cases*

**GROUP A: (normal pregnancy)—6 patients**

The criteria of normality were:

(i) No abnormality of obstetric, family or personal history.

(ii) Blood pressure below 130/90 mm. of Hg.

(iii) Absence of oedema and proteinuria.

Serum oxytocinase estimations were done from early pregnancy (8-15 weeks) till term. Sera were tested at 4 weekly intervals from the time of observation till the 36th week and thereafter at weekly intervals till term.

**GROUP B: (abnormal pregnancy)—26 patients**

Criteria for various abnormal groups were as follows:

**(1) Toxaemia of pregnancy—15 patients**

Toxaemia of pregnancy was defined as disease occurring during gestation or in early puerperium and characterised by hypertension, oedema and proteinuria separately or together.

Hypertension was defined when systolic blood pressure exceeded 140 mm. Hg. and the diastolic 90 mm. Hg. and neither was below these figures and the blood pressure was persistently elevated over a period of 48 hours.

**(2) Poor Obstetric History—5 patients**

This group included patients who had previous history of stillbirths, intra-uterine deaths, abortions, difficult labours, postmaturity, Rh-immunisation, syphilis or foetal abnormalities.

**(3) Repeated premature labour: 6 patients**

Premature labour was defined as onset of labour, a minimum of three weeks prior to the expected date of delivery but after the 28th week of pregnancy.

**GROUP C: (postpartum)—6 patients**

Serum Oxytocinase estimations were

done on samples of blood taken during labour, daily on the first four postpartum days and final sample four weeks postpartum. Four of these 6 patients were under study during pregnancy also.

**Method**

The chemical method of assay for estimation of oxytocinase was used in the present study. The colorimetric method for determining the serum oxytocinase was first described by Tuppy and Nesvadba (1957). It is based on the hydrolysis of the substrate L-cystine-di-B-naphthylamide by the enzyme oxytocinase in the serum, and liberation of B-naphthylamine (a colourless product) at the end of the reaction. This B-naphthylamine is diazotised with  $\text{NaNO}_2$ , excess of sodium nitrite being destroyed with ammonium sulphamate. The diazotised naphthylamide is subsequently converted into an azo dye with naphthyl-ethylene-diamine, which (azo dye) can be measured with a spectrophotometer.

In the present study the modified method of Babuna *et al* (1966) was used. The optical density is found to be directly proportional to the amount of B-naphthylamine freed by enzymatic reaction. Results when expressed in optical density, give better chances to detect small variations in oxytocinase activity than those expressed in mg. of b-naphthylamine, since the optical density figures are twenty times higher than the absolute figures. The serum oxytocinase levels are expressed as the number of the net optical density reading. The readings were in 3 decimal points; these were, therefore, multiplied by 1000 and the resultant values expressed as units of serum oxytocinase.

**Observations**

Serum Oxytocinase level was studied in

6 non-pregnant women. The mean value was 34 units (Range 14 to 56 units).

The mean values of serum oxytocinase levels during last 4 weeks of pregnancy in cases of normal and abnormal pregnancy are shown in Fig. 1. In abnormal preg-

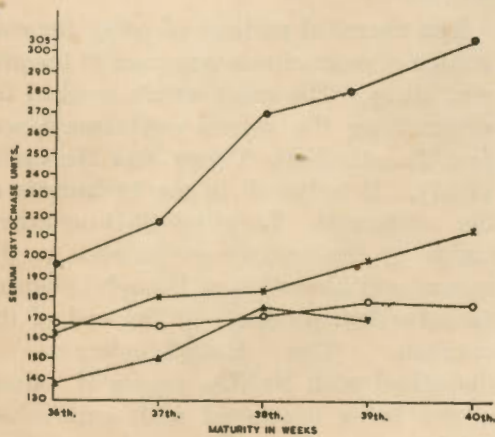


Fig. 1

Mean values of serum oxytocinase levels at 36 to 40 weeks of gestation in normal and abnormal pregnancy

- \* Normal pregnancy
- o Repeated premature labour
- x Toxaemia
- Poor obstetric history.

nancy, the enzyme levels are lower than in the case of normal pregnancy.

**Group A—Normal Pregnancy**

Serial samples of blood from early pregnancy to term were studied from 6 normal gravid women. Serum oxytocinase levels showed a progressive rise from early pregnancy to term (Fig. 2). During the first 4 postpartum days there is marked decrease in the level of the enzyme. The serum oxytocinase level 4 weeks postpartum is comparable to the level in the non-pregnant women.

**Group B—Abnormal Pregnancy**

**1. Toxaemia:**

Serial specimens obtained from 15 cases

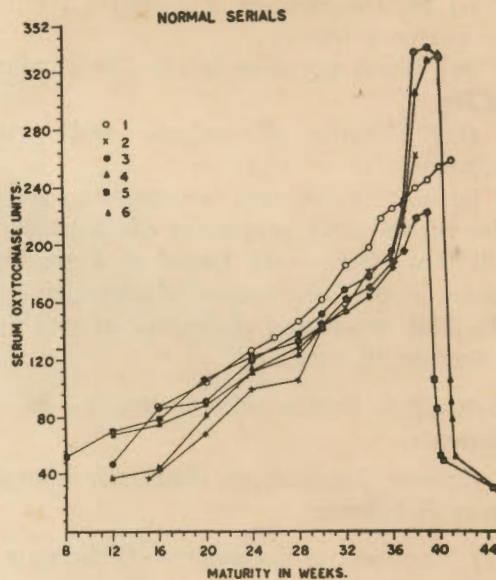


Fig. 2

Serial serum oxytocinase levels in 6 cases of normal pregnancy from early pregnancy (8-15 weeks) to term postpartum values of cases 5 and 6 are also shown.

of toxaemia were investigated for serum oxytocinase activity. Oxytocinase levels were lower than in normal pregnancy specially during the last 4 weeks of pregnancy.

**Case Report**

S. M., 28 years old, gravida 4, para 2 + 1 was under study from 16 weeks of gestation till term. Serial oxytocinase levels showed a steady rise but the value at any stage of pregnancy was lower than in the case of normal pregnancy (Fig. 3). At 40 weeks, serum oxytocinase level was 197 units which is much lower than the mean value of 304 units in normal pregnancy. She had a spontaneous vaginal delivery. A live baby weighing 3300 mgs. was born.

**2. Poor Obstetric History**

Five subjects in this category were studied. They had a history of abortions, stillbirths, intrauterine death of the foetus, postmaturity, Rh-immunisation, syphilis

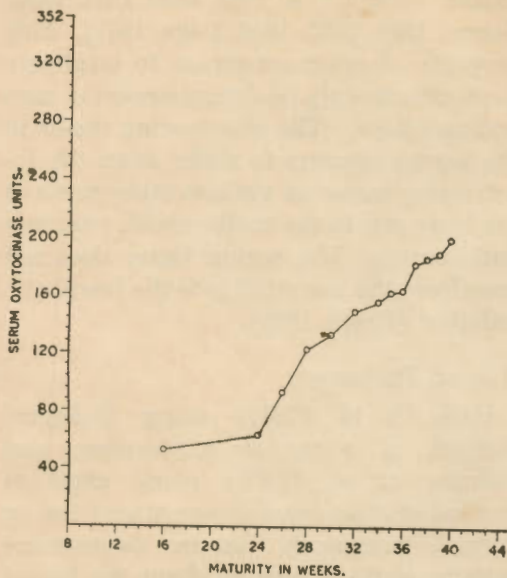


Fig. 3

Serial serum oxytocinase values in a case of toxæmia.

or foetal abnormalities. Serial oxytocinase levels were lower than in normal pregnancy. A decrease in enzyme level at any stage of pregnancy was suggestive of threatening intrauterine foetal death.

#### Case Report

M. K. age 33 years, gravida 4, para 1 + 2 gave a history of one premature delivery and 2 abortions. Serial oxytocinase levels were done at weekly intervals. The enzyme level at 24 weeks of gestation was 124 units. She reported diminished foetal movements at 27 weeks. Serum oxytocinase level at 28 weeks showed a fall in the level to 65 units (Fig. 4). At this time, she had a spontaneous delivery and was delivered of a macerated foetus weighing 2500 gms.

#### 3. Repeated Premature Labour

Six pregnant women with history of repeated premature deliveries were studied. Serum oxytocinase levels were lower than the corresponding values in normal pregnancy.

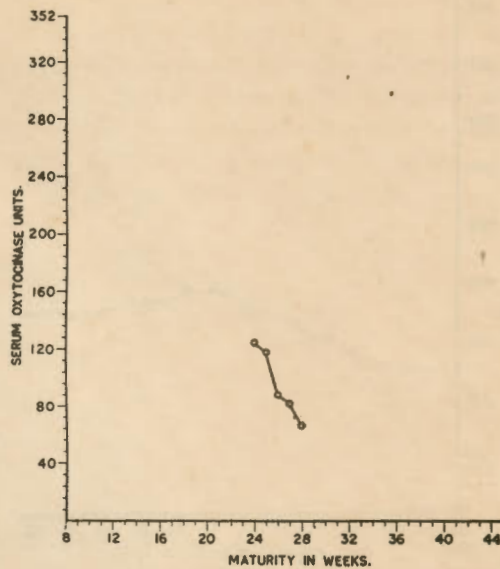


Fig. 4

Serial serum oxytocinase values in a case of intrauterine death of the foetus. Oxytocinase levels at 28 week fell to 65 units.

#### Case Report

S. S., age 30 years, gravida 7, para 6 + 1 gave a history of 6 premature deliveries at 30 to 34 weeks of pregnancy. Serum oxytocinase levels were studied from 28 weeks to 39 weeks. Serial estimations showed rather a gradual rise in the level of the enzyme upto 35 weeks. Samples of blood were studied at interval of 3 or 4 days. Serum oxytocinase level decreased after 35 weeks and then remained more or less stationary (Fig. 5). She was kept on bed rest from 35 weeks of gestation till the onset of labour. At 39 weeks she was delivered of a live baby weighing 2900 gms.

#### Group C—Postpartum

Serial oxytocinase levels on 4 consecutive days postpartum were studied in 6 women after delivery. A sample of blood was later taken 4 weeks postpartum. There was a rapid decline in the serum oxytocinase during immediate postpartum period. The enzyme level at 4 weeks postpartum was compared to the oxytocinase level in the non-pregnant women.

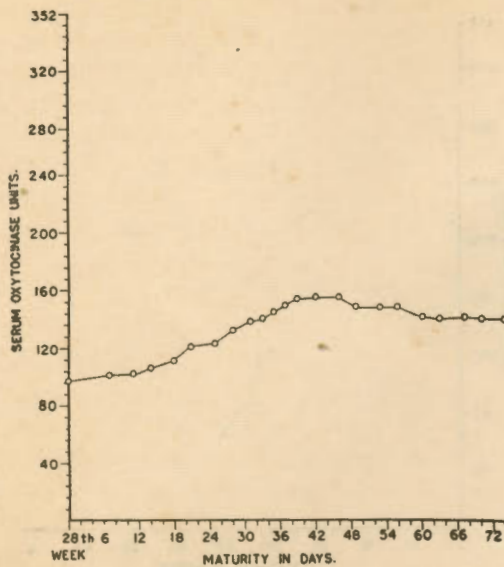


Fig. 5

Serial serum oxytocinase values in a case of repeated premature labour. Oxytocinase levels decreased after 35 weeks and the level is more or less stationary till onset of labour. Maturity in days indicates period of gestation (28 weeks + number of days).

### Discussion

Oxytocin is a hormone secreted by the posterior pituitary. It is eliminated by inactivation or excretion. Muller-Hartburg *et al* (1959) and Caldeyro-Barcia and Poseiro (1959) experimentally proved that oxytocin is rapidly metabolised. It is known that part of the injected oxytocin is excreted in the urine (Larson, 1935) but most of it would appear to be inactivated in the kidney tissues, as is the case with injected vasopressin which is inactivated by the kidneys and by the organs of the splanchnic vascular area (Ginzburg and Smith 1958).

A factor inactivating oxytocin can be demonstrated in human placenta (Hawker 1956). Further work has been done in this field on the assumption that the inactivation is due to an enzyme "oxyto-

cinase" (Werle *et al* 1949, 1950, 1951, 1956; Semm, 1953, 1955, 1958; Page, 1947). This property of pregnant serum to inactivate oxytocin can only be demonstrated in man and monkeys. The inactivating factor in the serum appears to differ from the inactivating factor in various other parts of the body, e.g. in the erythrocytes, pancreas and ovaries. The serum factor does not pass from the maternal into the foetal circulation (Tuppy 1960).

### Normal Pregnancy

Page *et al* (1947) using biological method of assay of oxytocinase and Babuna *et al* (1966) using chemical method of assay have demonstrated that in normal pregnancy serum oxytocinase activity starts to rise at about the beginning of the 4th month, that the highest value is reached around term. Following delivery a sharp fall in oxytocinase level was observed by Page *et al* using chemical method of analysis, Riad (1962), Fylling (1963), Melander (1965) Kukuchi (1967) found low but clearly demonstrable oxytocinase activity in the non-pregnant.

In the present study, 6 non-pregnant women were included. Serum oxytocinase levels ranged from 14 to 56 units. Serial oxytocinase estimations done in 6 normal pregnant women showed a progressive rise in enzyme activity from early pregnancy reaching maximum level at term. There was a rapid fall of oxytocinase activity during the first 4 days postpartum. Value of serum oxytocinase level 4 weeks postpartum was almost the same as in the non-pregnant.

### Abnormal Pregnancy

Dieckman *et al* (1950) suggested that there might be some relationship between the ability of blood of pregnant women to inactivate oxytocin and antidiuretic

hormone of posterior pituitary and the occurrence of pre-eclampsia and eclampsia. If the degree of inactivation of both oxytocin and antidiuretic hormone is parallel, then toxæmia patients may be expected to have low oxytocinase levels. Riad (1962) and later Semm (1965) and Bernhard (1967) and Ichaliotis and Lambrinopoulos (1965) confirmed this hypothesis practically. They found considerably lower levels of serum oxytocinase in cases of toxæmia of pregnancy as compared to the normal pregnant.

Aragon and Page (1948) found no correlation between oxytocinase levels and toxæmia of pregnancy. Josephides and Turkington (1967) reported that there is no distinct effect on the level of serum oxytocinase in mild toxæmia and uncomplicated essential hypertension as compared to normal.

In the present study of 15 cases of toxæmia of pregnancy, oxytocinase levels were lower than those in normal pregnancy during the last 4 weeks of gestation. Previous to the 36th week, values were comparable to those of normal pregnancy. The comparison of serum oxytocinase levels during 36 to 40 weeks of pregnancy in a normal pregnant and in toxæmia is well illustrated in Fig. 1.

In case of the patients with the poor obstetric history the oxytocinase levels obtained were lower than those in the normal pregnant. In cases of intrauterine death serum oxytocinase levels go on falling. The levels are much lower as compared to those of normal pregnancy at corresponding period of gestation (Fig. 1). Once the level start decreasing the fall is progressive and there is no rise in the level of oxytocinase at any stage.

The levels of serum oxytocinase were lower in patients with history of repeated premature deliveries than the corresponding values in normal pregnancy as

would be evident from (Fig. 1). Similar results were obtained by Mendez-Bauer *et al* (1961). Melander (1965), however, found no difference in the oxytocinase values in 6 cases of premature labour and those of normal pregnancy.

It appears that serial determinations of serum oxytocinase values are necessary for accurate diagnosis of placental function, while a single estimation may only provide a rough estimate of placental efficiency. When performed daily or even at 2 to 3 days intervals, the repetitive serial estimations may afford a clue to the best time for terminating pregnancy in cases of placental insufficiency. A steady decrease or a sharp fall in enzyme activity indicates a disturbance in placental function. The pregnancy should be terminated in such a case if foetal size is estimated to be adequate.

#### Conclusions

1. The chemical method for estimation of serum oxytocinase first described by Tuppy and Nesvadba (1957) and subsequently modified by Babuna *et al.* (1966) has been found to be of practical value because of reasonable rapidity, accuracy and cheapness of the modified method.

2. Total of 286 serum oxytocinase estimations were done in 34 pregnant and 6 non-pregnant women. The pregnancies studied were grouped as normal and abnormal. In the abnormal group were included cases of (a) toxæmia of pregnancy, (b) poor obstetric history and (c) repeated premature labours.

3. In normal pregnant women serum oxytocinase was estimated once during first trimester, then every 4 weeks till the 28 weeks then every 2 weeks till the 36th week and thereafter at weekly intervals till term.

4. In 6 non-pregnant cases studied,

serum oxytocinase levels ranged from 14 to 56 units and corresponded to values obtained one month postpartum ranging from 15 to 67 units.

5. Serial oxytocinase estimations in 6 normal pregnancy cases depicted a progressive rise from early pregnancy (8-15 weeks) reaching maximum value at term.

6. Estimations of serum oxytocinase were also done in 6 postpartum cases. Following delivery there is a rapid fall in the oxytocinase activity during the first week and then there is gradual fall to the non-pregnant levels about one month postpartum.

7. Oxytocinase levels in 15 cases of toxæmia of pregnancy were found lower than those in normal pregnancy during the last 4 weeks of gestation, though the levels were almost similar prior to 36 weeks.

8. In 5 cases of poor obstetric history, the serum oxytocinase levels were found lower than those in normal cases from 32 weeks onwards.

9. In 6 cases of delivery of premature births, the levels of serum oxytocinase were found lower than the corresponding values of normal pregnancy from 32 weeks onwards.

10. In 2 cases of intrauterine foetal death there was a marked fall in serum oxytocinase levels, even before actual foetal death occurred.

11. The postpartum drop in the serum oxytocinase levels in one case of toxæmia and one case of poor obstetric history was found comparable to that in postpartum cases of normal pregnancy.

12. Serial estimation of serum oxytocinase levels is of value in the diagnosis of placental function. Thus the impending foetal death in certain pathological pregnancies can be predicted. A decrease or sudden fall in the oxytocinase level is indicative of placental insufficiency and

the pregnancy should be terminated if the foetal size is estimated to be adequate.

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