HORMONES AS INDICES OF FETO-PLACENTAL FUNCTION

by

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and

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Western countries have succeeded in reducing the perinatal mortality rate within the last few decades to a great degree, but in our country the rate still remains considerably high. The little decline in perinatal mortality that has taken place in this country may be attributed to better antenatal care. Diagnostic aids represent a valuable aspect of the antenatal care. An accurate assessment of the feto-placental function during gestation is essential to determine the optimal chances of fetal survival if the pregnancy has to be terminated.

Considerable information is available on various aspects of the endocrinology of pregnancy. Human pregnancy is accompanied by a spectacular rise in both the steroid and non-steroidal hormones. Since the work of Halban (1905) and Philip (1936) evidence indicating that the contents of the pregnant uterus are the most likely source of these hormones, has been reviewed extensively (Zondek and Sulman, 1945; Diczfalusy and Troen, 1961; Brody, 1969; Diczfalusy and Mancuso, 1969; Solomon and Younglai, 1969 and Lauritzen, 1971). Based upon this information, a series of procedures, measuring the secretions of the feto-placental unit, have been introduced in the last few decades to evaluate the feto-placental function during pregnancy. In addition to physical approaches, these include a large number of prognostic tests on both the enzymatic and hormonal products of feto-placental origin. However, hormones having a considerably faster disappearance rate (in minutes) than the enzymes (in days) have been generally preferred to minimize the time lost between feto-placental compromise and its earliest possible detection.

Since estrogen production depends on both the fetus and the placenta (Diczfalusy and Mancuso, 1969) tests for this group of hormones are useful in a variety
of fetal complications. The estimation of estriol has been usually preferred over estrone (E₁) and Estradiol (E₂), because its production predominantly depends on the fetal function. However, the fact that about 60 per cent of the total E₂ synthesized by the placenta during pregnancy is derived from fetal precursors (Siiteri and MacDonald, 1966 and Jaffe et al, 1968), the estimation of E₂ as fetoplacental function test is being evaluated (Townslay et al, 1973 and Rahman et al, 1974a and d).

Unlike estrogens, the human placental lactogen (HPL), human chorionic gonadotrophin (HCG) and progesterone are specific products of placenta. Therefore, the estimation of these hormones in the maternal circulation reflects the fetal function indirectly. It is now recognised that the fetus and placenta are interdependent and some of the abnormalities of pregnancy are due to placental insufficiency. It is, therefore, not surprising to note that in the prediction of fetal risk the estimation of placental hormones compares favourably well with estrogens (Beischer et al, 1968).

In this paper, various hormonal parameters used as fetoplacental function indices are critically reviewed.

**Human Chorionic Gonadotrophin as an index of fetoplacental function**

The estimation of human chorionic gonadotrophin (HCG) either by immuno-, bio- or radioimmunoassay, during pregnancy, appears to carry little or no clinical value as a fetoplacental function index (Loraine and Mathew, 1950 and Samaan et al, 1969). Although high levels of serum and urinary HCG in pregnancies associated with toxemia and diabetes have been reported (Smith and Smith, 1934 and 1948; Loraine, 1958 and White, 1959), a significant increase in HCG level has only been confirmed in the case of severe toxemia (Loraine and Mathew, 1950 and Samaan et al, 1969).

In cases of threatened abortion, the serum HCG determination appears to have some diagnostic value. Hon and Morris (1956); Vermelin et al (1957) and Brody and Carlstrom (1962) have shown that reduction in HCG level is invariably associated with abortion. However, in cases of intra-uterine fetal death without placental damage, the HCG levels may remain within the normal range for some time (Klopper, 1969). Moreover, a large category of abortions may not be associated with a fall in HCG early enough for such assays to be of any clinical value (Samaan et al, 1969).

**Human Placental Lactogen as an index of fetoplacental function**

Human placental lactogen (HPL) is a protein hormone synthesized by the syncytiotrophoblast cells of the chorionic villi. In recent years, much interest has centered around the estimation of HPL in the serum of pregnant women as a possible means of monitoring the placental endocrine function (Saxena et al, 1969; El-Tomi et al, 1970; Spona and Janisch, 1971; Varma et al, 1971; Rahman et al, 1974b and Spellacy et al, 1974). HPL is measurable in maternal serum as early as three to four weeks after conception. The levels show a progressive increase until 33 to 34 weeks of pregnancy and then plateau to term (Table I).

Serial estimation of serum HPL levels in toxemic pregnancies as compared with the range in normal pregnancies...
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TABLE I
The Levels of Urinary Estriol, Serum Free Estradiol-17β and Human Placental Lactogen in Normal Pregnancy

<table>
<thead>
<tr>
<th>Pregnancy in weeks</th>
<th>Urinary Estriol (mg/24 hour)</th>
<th>Estradiol-17β (ng/ml serum)</th>
<th>Human placental Lactogen (μg/ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-14</td>
<td>1.98 ± 0.35</td>
<td>3.47 ± 0.59</td>
<td>0.99 ± 0.15</td>
</tr>
<tr>
<td>15-16</td>
<td>2.10 ± 0.21</td>
<td>4.31 ± 0.88</td>
<td>1.59 ± 0.27</td>
</tr>
<tr>
<td>17-18</td>
<td>2.64 ± 0.22</td>
<td>4.44 ± 0.54</td>
<td>1.30 ± 0.21</td>
</tr>
<tr>
<td>19-20</td>
<td>4.00 ± 0.33</td>
<td>5.54 ± 1.02</td>
<td>2.10 ± 0.25</td>
</tr>
<tr>
<td>21-22</td>
<td>5.14 ± 0.23</td>
<td>7.84 ± 0.65</td>
<td>2.55 ± 0.27</td>
</tr>
<tr>
<td>23-24</td>
<td>6.47 ± 0.32</td>
<td>7.74 ± 0.56</td>
<td>3.10 ± 0.31</td>
</tr>
<tr>
<td>25-26</td>
<td>7.56 ± 0.40</td>
<td>10.96 ± 0.96</td>
<td>2.67 ± 0.29</td>
</tr>
<tr>
<td>27-28</td>
<td>9.76 ± 0.68</td>
<td>13.36 ± 0.94</td>
<td>3.99 ± 0.25</td>
</tr>
<tr>
<td>29-30</td>
<td>9.44 ± 0.59</td>
<td>15.73 ± 1.25</td>
<td>4.45 ± 0.24</td>
</tr>
<tr>
<td>31-33</td>
<td>11.72 ± 1.18</td>
<td>16.72 ± 1.68</td>
<td>4.56 ± 0.30</td>
</tr>
<tr>
<td>33-34</td>
<td>14.66 ± 1.48</td>
<td>17.97 ± 1.46</td>
<td>5.41 ± 0.29</td>
</tr>
<tr>
<td>35-36</td>
<td>18.99 ± 1.46</td>
<td>20.65 ± 1.46</td>
<td>5.64 ± 0.37</td>
</tr>
<tr>
<td>37-38</td>
<td>18.75 ± 1.46</td>
<td>21.38 ± 2.63</td>
<td>5.49 ± 0.43</td>
</tr>
<tr>
<td>39-40</td>
<td>21.79 ± 1.61</td>
<td>23.52 ± 1.72</td>
<td>5.97 ± 0.34</td>
</tr>
</tbody>
</table>

All values are expressed as Mean ± S.E.

shows low levels with a gradual but slow rise in pregnancies terminating with a live birth (Fig. 1). In cases of severe toxemia leading to intra-uterine fetal death, the HPL level shows a sharp fall after fetal death. However, a sharp fall in HPL level prior to the fetal death is not a consistent finding (Samaan et al, 1969). In our series of four cases of intra-uterine fetal death, only in one case (M.D.) the HPL level showed a decline prior to detectable fetal death (no heart sound). Although, higher values of HPL in toxemia than in normal pregnancies have been reported (Singer et al, 1970), it is generally agreed that serum HPL level is a good indicator of placental function (Saxena et al, 1969; Spellacy et al, 1971; Letchworth and Chard, 1972; Rahman et al, 1974b and d and Lindberg and Nilson, 1973). We found that in toxemia, the fetal death occurred only in cases where maternal HPL values were less than 3.0 μg/ml serum after the 30th week of pregnancy (Fig. 1). The level of HPL in the maternal serum may be regarded as a true reflection of the endocrine functional integrity of the placenta throughout pregnancy. However, the serum HPL level may not reflect the true fetal conditions. The estimation of HPL would be most useful in pregnancies where a primary defect in the placental function is endangering the fetal health.

In pregnancies complicated with anemia, we found low levels of serum HPL compared with the range in normal pregnancy (Fig. 2). On the other hand, elevated levels of serum HPL in both the uncomplicated diabetic and twin pregnancies was observed (Fig. 2). Although, no significant difference in maternal serum HPL levels in diabetic compared with the normal subjects has been reported (Beck et al, 1965 and Spona and Janisch, 1971), yet higher levels of serum HPL in diabetic and twin pregnancies is a consistent finding (Saxena
et al, 1968 and 1969 and Varma et al, 1971). It may be concluded that the serum HPL level is closely related to the growth and size of the placenta. Since fetal death is known to result from metabolic changes besides placental dysfunction (Samaan et al, 1971), a simultaneous determination of estrogens, in addition to serum HPL, in high-risk pregnancies may be useful.

The fact that a rapid rise in serum HPL starts about 5-6 weeks earlier than a similar rise in estrogens (Table 1) suggests that the estimation of HPL may be useful in case of spontaneous abortion in the early stages of pregnancy. The results of Samaan et al (1969); Saxena et al (1969); Spona and Janisch (1971) and Ylikorkala and Jouppila (1973) have shown a good correlation between decreased HPL levels and the outcome of pregnancies threatened with abortion. Thus, the estimation of serum HPL appears to be a good prognostic aid in the follow up of early pregnancy.

**Progesterone and its metabolites as index of feto-placental function**

The assay of the excreted progesterone metabolite—pregnandiol in the urine as an index of feto-placental function during pregnancy, has been used extensively in the past. Reliable methods for the estimation of serum progesterone are also available. However, the estimation of neither urinary pregnanediol nor serum progesterone has gained much importance.

In a large number of toxemic women blood progesterone level failed to show any difference from normal, regardless of the severity of the condition (Eton and Short, 1960). Subnormal levels of progesterone before fetal death have been observed in two cases of pre-eclampsia (Coyle et al, 1962). However, the values did not show any fall after fetal death. In these cases pregnanediol excretion in urine also did not provide any guidance to the eminence of fetal death. Johansson (1969) and Jonasson Johansson (1971) have concluded that the measurement of progesterone, either in plasma or in the amniotic fluid did not provide any help in the evaluation of fetal status in the last trimester of pregnancy.

The estimation of both serum progesterone (Robertson et al, 1971) and urinary pregnanediol (Rawlings and Kriger, 1959; Shearman, 1959 and Patti et al, 1963) has been used in predicting the outcome of threatened abortion in early pregnancy. As a diagnostic aid in late pregnancy, the estimation of urinary pregnanediol, has been used extensively in the past with little success. It is doubtful if urinary pregnanediol can provide useful information regarding the fetal health more accurately than progesterone itself (Samaan et al, 1969 and 1971). Russel et al (1957) and Coyle et al (1962) have concluded that the measurement of urinary pregnanediol did not provide any accurate information on the fetal health.

**Serum estradiol as an index of feto-placental function**

The estimation of serum estradiol (E2) as an index of feto-placental function has not gained much importance because of the general belief that E2 synthesis depends predominantly on the placental rather than fetal function. However, it needs to be emphasized that about 60 per cent of the total E2 synthesized by the placenta during pregnancy is derived from fetal precursors (Sitteri...
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and MacDonald, 1966 and Jaffe et al, 1968). The usefulness of serum E₂ as a feto-placental function test has been evaluated in the last few years when radioimmunoassay methods for the estimation of E₂ have become available.

Fig. 3 shows the level of free E₂ in the serum of toxemic pregnancies as compared with the normal range. The low levels of E₂ in toxemic pregnancies with gradual though slow rise in pregnancies terminating with live births may be observed. In cases of severe toxemia leading to intra-uterine fetal death, the E₂ level declined sharply before detectable fetal death (no fetal heart sound). Similar results have been reported by Tulchinsky and Korenmann (1971); Fischer-Resmusson (1971) and Sybulski and Maughan (1972). In a recent study, Townsley et al (1973) have examined the relationship of maternal E₂ to total serum E₃, 24 hour urinary E₃ and urinary E₃/creatinine ratio, in normal and complicated pregnancies. A good agreement between E₂ and other estrogen indices was observed in general. Thus, the serum E₂ level appears to reflect the feto-placental function quite satisfactorily.

Figure 4 shows the levels of serum free E₂ in pregnancies associated with anemia, diabetes and twin pregnancies. The levels of serum E₂ closely follow the pattern of urinary E₃ in normal and in pregnancies associated with various pathological conditions. Moreover, the lowered serum E₂ levels in toxaemic pregnancies have been shown to reflect the true steroid synthesizing ability of the toxaemic placenta (Rahman et al, 1974c and 1975). Thus it may be concluded that serum E₂ estimation is as useful as urinary E₃ and a serial estimation would indicate whether fetal jeopardy is present.

Serum estriol as an index of feto-placental function

The measurement of estriol (E₃) levels in maternal circulation is an acceptable guide to the evaluation of fetal health (Schiffer et al, 1970; Corker and Neftolin, 1971; Tulchinsky et al, 1971; Mathur et al, 1973). It is generally agreed that a change in serial plasma E₃ concentrations precedes the clinical manifestations of toxemia and that the severity of the condition and fetal prognosis can be predicted from plasma E₃ concentration (Masson, 1973 and Mathur et al, 1973).

The additional advantage of serum over urinary E₃ estimation as the index of feto-placental function is that serum E₃ levels are relatively independent of alteration in the kidney clearance of E₃. However renal involvement in toxemia may also increase the plasma E₃ due to a diminished renal clearance and therefore, may not reflect the severity of the placental insufficiency accurately (Roy et al, 1964; Nachtigall et al, 1968; Selinger and Levitz, 1969 and Carrington et al, 1970). This is also true with class D diabetes as a result of the diabetic nephropathy (Ratnasopa et al, 1967). It may be concluded that the estimation of blood E₃ level would be an additional help in high-risk pregnancies, especially when the urinary E₃ is undependable. However, it would still be unreasonable to replace the urinary E₃ with plasma E₃ estimation. Detailed comparative investigation of plasma E₃ assay vis-a-vis the urinary E₃ estimation as diagnostic tool is needed.

Urinary estriol as an index of feto-placental function

The estimation of the urinary estriol
(E₃) as a feto-placental function index has been used extensively (Banerjea, 1962; Martin and Hahnel, 1964; Klopper, 1965 and 1968; Laumas and co-workers, 1966, 1968 and 1974; Frandsen, 1969; Galbraith et al., 1970 and Ostergard, 1973). It is generally believed that urinary E₃ levels show a good correlation with the fetal health, especially in the last trimester of pregnancy. However, a variation of about 30 per cent even in normal pregnancies has been recorded (Klopper, 1964) and therefore, a serial estimation may be essential.

During toxaemia of pregnancy, the reduction in the urinary E₃ excretion is generally related to the outcome of the pregnancy. As might be expected, not only the decline of E₃ in toxaemia, but also the degree to which it may be declined is related to the severity of the disease (Fig. 5). The cases with abnormally low levels of urinary E₃ may indicate severe foetal distress and placental dysfunction. Such cases lead to the intra-uterine fetal death. Klopper (1965) has reported that the reduction in mild toxæmia was slight but in severe toxæmia the excretion of urinary E₃ fell to 47 per cent of the normal 7-10 days before the death of the fetus in utero.

In pregnancies complicated with anæmia and diabetes the pattern of E₃ excretion correlates well with the outcome of pregnancy (Fig. 6). A normal E₃ level is a reliable indicator of intra-uterine fetal well being. A variable number of fetal death have been encountered with a decrease in urinary E₃ excretion (Beling, 1963; Corson and Bolognese, 1968; Frandsen, 1969 and Schwarz et al., 1969).

The critical question is the clinical usefulness of urinary E₃ assays for evaluating the health of the fetus in utero. It is quite unusual for intra-uterine fetal demise to be associated with a normal urinary E₃ level. The determination of current and future fetal well being may be the most important use of urinary E₃. Thus the estimation of urinary E₃ would be useful in knowing when the pregnancy needs to be terminated in order to save the fetus.

**Simple and rapid colorimetric method for estimation of urinary estriol**

During the course of our study, urinary estriol has been estimated by a simple and rapid colorimetric method for pregnancy urine (Laumas et al., 1966). The method is a modification of Brown (1965) and Rouke et al., (1968).

**Extraction:** An aliquot of a 24 hour urine was taken in Kober tube and diluted with equal amount of water. Concentrated HCl was added until the pH was 2 or below (pH paper), 2.5 g of NaCl was then added. After thoroughly mixing on vortex for 2 minutes ethyl acetate (6.0 ml) was added and the mixture was vortexed again. After centrifugation at 2000 rpm for 10 minutes at 4°C, duplicate aliquots of the solvent phase were transferred to Kober tubes and dried under nitrogen.

**Kober colour reaction:** To each tube, 0.2 ml of 2% quinol in ethanol was added and dried under nitrogen. 2.1 ml Kober reagent (2% purified quinol in 76% H₂SO₄) was added, heated in boiling water bath for 20 minutes and cooled in ice bath. 1.1 ml of distilled water was added, heated for 6 minutes and cooled again for 10 minutes in ice bath. 3.5 ml of distilled water was added to each tube. The tubes were left standing in the ice bath for 3 minutes, shaken and left for another 3 minutes. 4.0 ml chloroform containing 2% p-nitrophenol and 1% ethanol...
was added, left for 3 minutes in the ice bath, vortexed for 20 seconds and immediately centrifuged at 2000 rpm for 2 minutes at 4°C. The aqueous phase was removed and optical density (O.D.) of the solvent phase recorded at 506, 538 and 576 nm. The corrected O.D. was calculated by the following formula: Corrected O.D. = 2 x O.D. at 538 - (O.D. at 500 + O.D. at 576).

**Standard curve:** Estriol in concentration of 0, 1, 5, 10, 15, 20 and 25 μg was transferred in duplicate to Kober tubes. Kober colour reaction performed and the optical density was recorded as mentioned above. The corrected optical density was plotted against the various doses of estriol on a simple graph paper. A new standard curve was plotted on every 15 days interval.

**Quantitation of estriol:** With each set of pregnancy urine samples, a tube containing 10 μg of standard estriol was taken and processed exactly as the sample. The corrected O.D. obtained for the urine sample was read from the standard curve and corrected for recovery.

**Evaluation of the urinary estriol assay method**

**Sensitivity:** The linearity of the relationship between graded doses of E3 and the corrected O.D. has been presented in Fig. 7. The calculation of least detectable dose showed that 1 μg of E3 was the lower limit of sensitivity.

**Accuracy:** 5 to 20 μg of standard E3 was added to aliquots of pregnancy urine. After Kober colour reaction, the developed colour was quantitated as mentioned earlier. The amount of E3 already present in each aliquot of urine was subtracted from each observation. Figure 8 shows the amount of E3 measured for every addition of standard E3. It may be observed that the method is quite accurate.

**Precision:** Multiple determinations of urinary E3 in 4 samples from different weeks of pregnancy have been presented in Table II. The co-efficient of variation, ranging between 4.7 to 10.4 per cent may show a satisfactory precision.

**TABLE II**

<table>
<thead>
<tr>
<th>Pregnant women</th>
<th>Mean</th>
<th>S.D.</th>
<th>C.V.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.S.</td>
<td>4.7</td>
<td>0.49</td>
<td>10.4</td>
</tr>
<tr>
<td>S.D.</td>
<td>7.1</td>
<td>0.69</td>
<td>9.7</td>
</tr>
<tr>
<td>G.A.</td>
<td>15.4</td>
<td>0.91</td>
<td>6.9</td>
</tr>
<tr>
<td>K.R.</td>
<td>20.0</td>
<td>0.94</td>
<td>4.7</td>
</tr>
</tbody>
</table>

* C.V. = Co-efficient of variation = S.D./mean x 100.

**Concluding Remarks**

The different hormonal indices may provide useful guidelines for proper management and control of pregnancies associated with various pathological conditions. The use of human placental lactogen, serum estradiol-17β and urinary estriol estimations have been extensively practiced by us with considerable improvement in antenatal care.

The fact that a rapid rise in serum HPL starts about 5-6 weeks earlier than a similar rise is noted in both urinary E3 and serum E2; the estimation of HPL appears to be more useful in cases of spontaneous abortion in the early stages of pregnancy. The estimation of HPL would also be valuable in pregnancies where a primary defect in the placental function is endangering the fetal health.

The level of serum E2 or E3 closely follows the pattern of urinary E3 in normal pregnancy and in pregnancies associated with various pathological condi-
tions. Therefore, it is evident that serum E₂ or E₃ is as useful as urinary E₃. Serial measurement of the hormones would indicate whether fetal jeopardy is present, and timely intervention may be done to save the fetus. Thus the measurement of serum HPL, E₂ or E₃ would be of additional help in high-risk pregnancies as well as in instances when the urinary E₃ is suspected of providing a misleading information.

The estimation of circulating hormone levels, involves radioimmunoassay techniques. The technical skill and sophisticated instruments required for this technique may limit its utility to only those laboratories where these facilities exist. On the other hand, the estimation of urinary E₃ is simple, rapid and does not require any major instrumentation. The collection of urine sample is easy and venepuncture is not required. Moreover, a single technical hand can process as many as 10 urine samples every day. It is concluded that the urinary E₃ estimation by a simple and rapid method, such as the one used by us, may be more practical and useful as a routine diagnostic test for monitoring feto-placental function for any hospital or clinical laboratory.

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See Figs. on Art Paper I-II