Enzymic evaluation of thiamin, riboflavin and pyridoxine status of parturient women and their newborn infants

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(Received 24 June 1975 – Accepted 8 October 1975)

1. Thiamin, riboflavin and pyridoxine status of ‘low-income-group’ mothers and their newborn infants was assessed by analysing paired samples of maternal and umbilical cord blood for erythrocyte transketolase (EC 2.2.1.1) (ETK), erythrocyte glutathione reductase (EC 1.6.4.2) (EGR), and erythrocyte aspartate aminotransferase (EC 2.6.1.1) (EAA) activities.

2. The vitamin status of the infants was better than that of the mothers.

3. Most of the mothers and some of the infants had biochemical evidence of thiamin and riboflavin deficiency.

4. The pregnant women had a higher EAA activity and also higher stimulation with pyridoxal-5-phosphate than the non-pregnant women of the same community.

5. There was a significant correlation between maternal and umbilical blood samples for ETK and EGR activities, but not for EAA activity or any of the coenzyme stimulation tests.

The results of studies in animals indicate that deficiencies as well as excess of vitamins during gestation affect foetal growth and development (Jennings, 1970). Information correlating maternal vitamin status and the outcome of pregnancy in humans is equivocal (Chaudhuri, Halder, Chaudhuri & Bagchi, 1969; Chaudhuri, 1971; Gaynor & Dempsey, 1972; Heller, Salkeld & Korner, 1973, 1974a, b; Reinken & Gant, 1974).

The increase in the requirement for B-vitamins during pregnancy, particularly in the final trimester, may be due partly to sequestration of vitamins by the foetus and placenta, and partly to the metabolic effects of high circulating levels of female sex hormones. The latter possibility is suggested by the results of recent studies in women using oral contraceptives (Nutrition Reviews, 1972b; Ahmed, Bamji & Iyengar, 1975; Wynn, 1975).

Several workers have reported that the concentration of B-vitamins is higher in umbilical cord blood than in maternal blood. According to a recent report, the value for the ratio, maternal:umbilical cord blood for thiamin and riboflavin is 1:2−1:5, but for pyridoxine it is 1:1 (Baker, Frank, Thompson, Langer, Munves, De Angalis & Kaminetzky, 1975). Lust, Hagerman & Vilee (1954) found that while the total riboflavin concentration is markedly higher in the umbilical cord blood, that of FAD is twice as great in maternal blood.

Functional tests based on the activities of enzymes in erythrocytes and their in vitro stimulation with coenzyme have proved to be very valuable in assessing thiamin, riboflavin and pyridoxine status of man (Brin, 1962, Nutrition Reviews, 1972a; Sauberlich, Dowdy & Skala, 1973). Information on maternal and foetal vitamin status using these functional tests is meagre (Tripathy, 1968; Cooperman, Cole, Gordon & Lopez, 1973; Migasena, Chongbumrung, Supawan & Limtrakarn, 1974).
This report deals with the enzymic evaluation of the thiamin, riboflavin and pyridoxine status of mothers belonging to low-income groups in Hyderabad, India, and their newborn infants.

EXPERIMENTAL

The women included in this study belonged to low-income (monthly income < Rs. 500) families in Hyderabad, India. The habitual diets of this group of women are known to be deficient with respect to many nutrients (Gopalan, Balasubramanian, Ramasastry & Rao, 1972). Paired samples of maternal and umbilical cord blood were collected in heparinized bottles at parturition from only apparently healthy women whose delivery was normal. Some women had mild lesions of the mouth such as angular stomatitis and glossitis (particularly the latter) which are very common among pregnant women of this population (L. Iyengar, personal communication). As the blood specimens had to be sent to the laboratory within 3 h of collection, only women who delivered between 08.00 and 11.00 hours could be included; the samples were placed in ice within 5 min of collection. Otherwise, the selection of the subjects was random.

The following enzymic tests were used for determining thiamin, riboflavin and pyridoxine status.

Erythrocyte transketolase (EC 2.2.1.1) (ETK) activity and its in vitro stimulation with thiamin pyrophosphate (TPP) (TPP effect) were measured by the method of Bamji (1970).

Erythrocyte glutathione reductase (EC 1.6.4.2) (EGR) activity and its in vitro stimulation with FAD (FAD effect) were measured by the method of Bamji (1969).

Erythrocyte aspartate aminotransferase (EC 2.6.1.1) (EAA) activity and its in vitro stimulation with pyridoxal phosphate (PLP) (PLP effect) were measured by the method of Cabaud, Leeper & Wroblewski (1956).

Each of these assays was done on a total of fifteen mothers and their newborn infants. In eleven specimens erythrocyte riboflavin concentration was also measured fluorometrically (Sharda & Bamji, 1972).

The results were analysed using paired t tests.

RESULTS

Values for ETK activity are given in Table 1. The blood of eleven of the fifteen women studied had values for TPP effect of greater than 15% stimulation and six of them had values greater than 25% stimulation. Of the remaining four subjects, three had low values for ETK activity even though values for TPP effect were low (Table 1b). Subjects 10 and 24 had unusually high values for TPP effect (Table 1b) but did not show clinical signs of thiamin deficiency. It should be noted that the total enzyme activity (activity in the presence of TPP), was rather high in these blood samples and the infants born to these women had low values for TPP effect (Table 1b).

In all instances, the umbilical cord blood had higher endogenous as well as TPP-stimulated (total) ETK activity. The TPP effect in the blood of the newborn infant
Table 1. *Erythrocyte transketolase* (EC 2.2.1.1) (ETK) activity (µg sedoheptulose formed/ml erythrocytes per 30 min) and the effect of thiamin pyrophosphate (TPP) in *vitro*, in blood of 'low-income-group' Indian mothers and their newborn infants

(a) Mean values with their standard errors and ranges for fifteen mothers and their newborn infants

<table>
<thead>
<tr>
<th>ETK activity</th>
<th>Mother</th>
<th>Newborn infant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SE</td>
<td>Range</td>
</tr>
<tr>
<td>-TPP</td>
<td>480</td>
<td>42.0</td>
</tr>
<tr>
<td>+TPP</td>
<td>640</td>
<td>70.6</td>
</tr>
<tr>
<td>TPP effect (% stimulation)</td>
<td>34</td>
<td>5.8</td>
</tr>
</tbody>
</table>

(b) Individual results for four mothers whose blood had a TPP effect <15% stimulation, and for two mothers whose blood had TPP effect >80% stimulation

<table>
<thead>
<tr>
<th>ETK activity</th>
<th>Mother</th>
<th>Newborn infant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject no.</td>
<td>-TPP</td>
<td>+TPP</td>
</tr>
<tr>
<td>5</td>
<td>352</td>
<td>364</td>
</tr>
<tr>
<td>7</td>
<td>203</td>
<td>213</td>
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<tr>
<td>14</td>
<td>425</td>
<td>454</td>
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<tr>
<td>15</td>
<td>661</td>
<td>718</td>
</tr>
<tr>
<td>10</td>
<td>478</td>
<td>970</td>
</tr>
<tr>
<td>24</td>
<td>650</td>
<td>1170</td>
</tr>
</tbody>
</table>

Differences between mean values for maternal and neonatal blood were statistically significant:
- *P<0.05;  **P<0.02;  ***P<0.001.
- † For details of experimental procedures, see p. 260.

Tended to be lower than that of the mother. Five samples of umbilical cord blood had values for TPP effect of greater than 15% stimulation and two of these had values greater than 25% stimulation. Two other infants, (nos. 5 and 14, Table 1b) had low values for TPP effect but the values for ETK activity were also low.

The values for EGR activity are given in Table 2. The blood of thirteen of the fifteen mothers had values for FAD effect of greater than 25% stimulation. The umbilical cord blood had higher EGR activity and lower values for FAD effect than the mothers' blood. The total enzyme activity (FAD-stimulated activity) however, was not significantly different in the maternal and umbilical cord blood. Five samples of umbilical cord blood had values for FAD effect of greater than 25% stimulation, and the mothers of these five infants had values for FAD effect exceeding 75% stimulation. However, in some instances even though values for FAD effect in mothers' blood were very high the umbilical cord blood had values for FAD effect of less than 25% stimulation.

The erythrocyte riboflavin concentration in the umbilical cord blood was similar to, or higher than that of maternal blood (Table 2).

The values for EAA activity, also given in Table 2, suggested that the umbilical cord blood had higher EAA activity and lower values for PLP effect than the maternal blood. The total enzyme activity (PLP-stimulated activity) was not significantly different in the maternal and umbilical cord blood. The EAA activity of the pregnant
Table 2. *Erythrocyte glutathione reductase (EC 1.6.4.2) (EGR) activity (mg reduced glutathione formed/ml erythrocytes per 15 min), and the effect of FAD in vitro, erythrocyte aspartate aminotransferase (EC 2.6.1.1) (EAA) activity (μmol pyruvate formed/ml erythrocyte per 30 min), and the effect of pyridoxal phosphate (PLP) in vitro, and erythrocyte riboflavin content (mg/l)†, in blood of 'low-income-group' Indian mothers and their newborn infants

(Mean values with their standard errors and ranges for fifteen mothers and their newborn infants)

<table>
<thead>
<tr>
<th></th>
<th>Mother</th>
<th></th>
<th>Newborn infant</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>EGR activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>− FAD</td>
<td>7.8</td>
<td>0.97</td>
<td>1.6 - 14.2</td>
<td>12.6</td>
</tr>
<tr>
<td>+ FAD</td>
<td>12.1</td>
<td>0.35</td>
<td>3.2 - 17.4</td>
<td>14.2</td>
</tr>
<tr>
<td>FAD effect (%)</td>
<td>67.2</td>
<td>8.5</td>
<td>100 - 117.7</td>
<td>18.9</td>
</tr>
<tr>
<td>EAA activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>− PLP</td>
<td>60.7</td>
<td>7.52</td>
<td>22.7 - 115.8</td>
<td>81.7</td>
</tr>
<tr>
<td>+ PLP</td>
<td>82.1</td>
<td>9.22</td>
<td>37.5 - 168.1</td>
<td>98.9</td>
</tr>
<tr>
<td>PLP effect (%)</td>
<td>41.1</td>
<td>6.73</td>
<td>10.7 - 111.1</td>
<td>21.7</td>
</tr>
<tr>
<td>Erythrocyte riboflavin (mg/l)†</td>
<td>145</td>
<td>14.6</td>
<td>60 - 120</td>
<td>179</td>
</tr>
</tbody>
</table>

Differences between mean values for maternal and neonatal blood were statistically significant:
• P<0.05;  ** P<0.01;  *** P<0.001.
† For details of experimental procedures, see p. 260.
‡ Values for eleven samples.

...women was markedly higher than that of non-pregnant women studied previously by us (Ahmed et al. 1975).

There was a significant positive correlation between maternal and umbilical cord blood for ETK activity ($r + 0.63, P < 0.02$), and EGR activity ($r + 0.81, P < 0.001$), but not for EAA activity. The in vitro stimulation tests (TPP, FAD and PLP effects) however, did not show a significant correlation between maternal and umbilical cord blood. There was a significant positive correlation between maternal and umbilical cord erythrocyte riboflavin ($r + 0.68, P < 0.05$).

**DISCUSSION**

A value for TPP effect of greater than 15% stimulation is considered to be indicative of low thiamin status ('medium risk'), whereas a value for TPP effect of greater than 20 or 25% stimulation indicates thiamin deficiency (Brin, 1962; Sauberlich et al. 1973). By these criteria most of the mothers and a few of the newborn infants studied had inadequate thiamin status, even though they did not show clear clinical evidence of thiamin deficiency. Low values for TPP effect, even though enzyme activity was low in some blood samples, is suggestive of low apoenzyme ETK (apoETK). Low levels of apoETK have been found in patients with liver diseases (Fennelly, Frank, Baker & Leevy, 1967). Prolonged thiamin deficiency may also reduce apoETK level (Bamji, 1970). These women did not have any evidence of liver disease, and hence it is possible that their low ETK activity was due to chronic thiamin deficiency.
The exceptionally high values for TPP effect in subjects 10 and 24 (Table I) may be due to relatively higher apoETK levels (high total activity) rather than a very severe state of thiamin deficiency. The factors which increase apoETK levels are not known. Vitamin B₁₂ deficiency has been found to increase erythrocyte transketolase activity (Wells, Baylis, Holoway & Marks, 1973). Information on vitamin B₁₂ status of the women studied by us is not available. However, they did not have any clinical complications, and delivered infants whose thiamin status was satisfactory. In the author's opinion both ETK activity and TPP effect should be taken into consideration when assessing an individual's thiamin status (see also Bamji, 1970; Ahmed et al. 1975).

Clinical as well as biochemical thiamin deficiency is rare in our population (Bamji, 1970; Ahmed et al. 1975). This suggests that biochemical evidence of thiamin deficiency found in this study, was primarily associated with pregnancy. However, it should be pointed out that beri-beri is not found even among the pregnant women or infants of our community and the clinical implications of this state of biochemical thiamin inadequacy in mothers is not clear.

Higher ETK activity in umbilical cord blood compared to maternal blood has been found by Tripathy (1968) in American women and by Migasena et al. (1974) in Thai women.

FAD stimulation greater than 25% (activation coefficient 1.25) is considered to be indicative of riboflavin deficiency (Bamji, 1969; Sauberlich et al. 1973). By this criterion most women included in this study had biochemical riboflavin deficiency. In our community, lesions of the mouth, e.g. angular stomatitis and glossitis, have been found to respond to treatment with riboflavin (Bamji, 1969; Krishnaswamy, 1971b; Iyengar, 1973; Laxmi & Bamji, 1974) or pyridoxine (Krishnaswamy, 1971b; Iyengar, 1973). Biochemical as well as clinical riboflavin deficiency is frequently found in the Hyderabad area (Bamji, 1969; Iyengar, 1973; Ahmed et al. 1975). This existing state of deficiency may get worse in pregnancy. The mild oral lesions observed in some women may have been the result of riboflavin deficiency.

The high value for the erythrocyte riboflavin concentration ratio, maternal:umbilical cord blood reported by other workers was not found in the women studied here (Table 2). This may be due to the poor riboflavin status of mothers, or impaired transport across the placenta. According to Baker et al. (1975), the value for the vitamin concentration ratio, maternal:umbilical cord blood was maintained even when maternal hypovitaminaemia existed. However, there was no riboflavin deficiency in the population studied by them.

In the transport of riboflavin across the placenta, FAD in the maternal circulation is converted by the placenta into free riboflavin and then transferred to the foetus (Lust et al. 1954). Defective conversion of FAD to free riboflavin by the placenta could also affect the transport mechanism. In our population, B. V. Ramasastry (unpublished results) has found the umbilical cord:maternal serum folate ratio to be 2:1–4:1 and serum vitamin B₁₂ ratio to be around 1:5:1. This suggests that the transfer of folate and vitamin B₁₂ across the placenta are not affected in these malnourished women.

While the values for EAA activity in non-pregnant women, found by us in an earlier
study (Ahmed et al. 1975), are comparable to those reported by Rose, Strong, Folkard & Adams (1973) who used a similar colorimetric procedure, we did not find high values for PLP stimulation similar to those reported in the literature (Heller et al. 1973; Rose et al. 1973; Sauberlich et al. 1973). A value for PLP effect of up to 50–70 % stimulation has been regarded as normal (Salkeld, Knorr & Korner, 1973; Sauberlich et al. 1973), but this criterion may not be valid for our subjects and it is difficult to comment on the extent and severity of pyridoxine deficiency in the mothers and newborn infants studied. The pregnant women had higher values for PLP effect than those reported by Krishnaswamy (1971 a) for healthy Indians and by Ahmed et al. (1975) for non-pregnant women, even though the values found for EAA activity were higher.

The higher values for EAA activity among pregnant women (Table 2) may be due to the effect of hormones, as higher values for EAA activity have been found in women on oral contraceptives (Aly, Donald & Simpson, 1971; Rose, Strong, Adams & Harding, 1972; Ahmed et al. 1975). Riboflavin deficiency also tends to increase EAA activity (Krishnaswamy, 1971 b).

Since the EGR and EAA apoenzyme levels (total enzyme activity) in the umbilical cord blood were not significantly higher than those in maternal blood, it can be argued that better apoenzyme–coenzyme binding is responsible for the higher enzyme activities in umbilical cord blood. The riboflavin concentration of umbilical cord blood was not very much higher than that of maternal blood (Table 2), and pyridoxine levels are also reported to be the same in umbilical cord and maternal blood (Baker et al. 1975).

The positive correlation between ETK and EGR activities of maternal and umbilical cord blood, but not in vitro stimulation effects (TPP effect and FAD effect) suggests that the apoenzyme levels in the blood of mother and newborn infant are not correlated. The reason for this is not clear. Lack of correlation between EAA activity in the blood of mothers and newborn infants suggests that factors regulating the activity of this enzyme are complex and not understood.

Due to practical difficulties the sample volume could not be increased, but the results of this pilot study indicate that though the foetus tends to be protected against maternal hypovitaminosis, in a population where maternal nutritional status is poor, at least some infants (almost 30 %) may be born with biochemical evidence of thiamin and riboflavin deficiency. Baker et al. (1975) have also commented that a foetus can be born with hypovitamininaemia due to maternal hypovitamininaemia. However, the clinical implications of this biochemical hypovitamininaemia in mothers and in infants at birth remains to be established.

The higher enzyme activities found in the erythrocytes of newborn infants may be due to a higher concentration of immature cells in the blood.

The author is grateful to Dr S. G. Srikantia (Director, National Institute of Nutrition, Jamai-Osmania, Hyderabad—500 007) and Dr B. V. Ramasastry (Assistant Director, National Institute of Nutrition) for useful discussions. Dr Leela Iyengar’s help in obtaining the clinical material and Mr Surya Prakash’s technical assistance are gratefully acknowledged.
REFERENCES