SUMMARY

The plasma pyridoxal phosphate concentrations and folic acid concentration in packed red blood cells have been studied in 58 women during pregnancy and in their children at birth. The material was divided into three groups receiving no, 2 mg and 10 mg extra pyridoxine per day. The study showed a highly significant relationship between the mean plasma pyridoxal phosphate concentration in mother and child at delivery. Addition of 2 mg extra pyridoxine per day was found insufficient to keep the mean pyridoxal phosphate concentration level constant during pregnancy for which addition of between 2 and 10 mg extra pyridoxine per day was found necessary. It was found necessary to add between 2 and 10 mg extra pyridoxine per day to significantly increase the saturation of aspartate aminotransferase with pyridoxal phosphate. The mean value of pyridoxal phosphate concentration of the newborn children was significantly increased by 2 mg pyridoxine but no further increase was observed by 10 mg pyridoxine per day. No correlation was found between blood pyridoxal phosphate concentration and folic acid concentration. It is recommended to add 10 mg pyridoxine extra per day during pregnancy.

INTRODUCTION

In earlier investigations decreasing concentration of pyridoxal phosphate in plasma and increasing excretion of xanthurenic acid in urine after tryptophan load tests has been noted. The abnormality in tryptophan degradation has been supposed to depend on a combined defect of pyridoxal phosphate deficiency and hormonal imbalance during gestation.

Wachstein et al. found higher pyridoxal phosphate concentrations in cord blood than in maternal blood. He also determined the daily need of pyridoxine during pregnancy to normalize the tryptophan load test to be about 10 mg. The reliability of the tryptophan load test as a test of vitamin B₆ deficiency has, however, been questioned. Altman and Greengard have shown that there is a correlation between

the xanthurenic acid excretion during a tryptophan load test and the liver activity of
tryptophan pyrrolase and that the activity of this enzyme can be increased by hor-
monal influence. Therefore we have considered it to be of interest to determine the
need of pyridoxine during pregnancy by measuring the pyridoxal phosphate con-
centration in plasma. It has been shown that in certain patients with vitamin B₆
deficiency there is an inverse correlation between the plasma pyridoxal phosphate
concentration and the saturation of aspartate aminotransferase with pyridoxal phos-
phate.

Some authors have found that the folic acid concentration in whole blood de-
creases during pregnancy on ordinary food intake.

After resorption folic acid is transformed to various metabolically active forms,
among others to 5-hydroxymethyltetrahydrofolic acid, which is considered to be the
storage form of folic acid. One metabolic step in the formation of 5-hydroxymethyl-
tetrahydrofolic acid is the pyridoxal phosphate-dependent hydroxymethyltransferase
(ref. 10). We have therefore considered it to be of interest to study whether the de-
creasing pyridoxal phosphate concentration could influence this enzyme and be re-
sponsible for the folic acid deficiency. In a few cases an increase of folic acid concentra-
tion in whole blood has been noticed by administration of pyridoxine to folic acid-
deficient patients.

MATERIAL

Sixty-three pregnant women were enlisted for the experiment on their first
visit to the prenatal care clinic. They were divided into three groups according to time
of arrival. Number 1, 4, 7, etc. were given no extra pyridoxine (group A), number 2,
5, 8, etc. were given 2 mg extra pyridoxine per day (group B), and number 3, 6, 9, etc.
to mg extra pyridoxine per day (group C). Three women moved to other parts of the
country, one was taking extra vitamins and one had an early abortion. These five were
excluded from the study.

The remaining 58 patients completed the investigation. Blood was taken from
the pregnant women at their first, second and third visit to the doctor and at delivery.
The first visit was made on day 51-146 after the last menstrual period. The second
and third visit about 6 and 9 months after the last menstrual period.

Cord blood was taken at delivery, except in two cases of technical failure. In
one case, a stillbirth, no blood was taken for psychological reasons. No extra folic
acid was given to any group, but all received iron supplementation. Except the case
of stillbirth, no complications were registered in the 58 cases studied.

METHODS

1. Pyridoxal phosphate concentration in plasma. Venous blood samples were
drawn into tubes containing Mg K₂-EDTA. Pyridoxal phosphate concentration in
plasma was determined according to Hamfelt.

2. Folic acid concentration in blood. Folic acid concentration in serum and whole
blood was determined microbiologically with Lactobacillus casei. (The determinations
were kindly performed by A. Killander, M.D., Uppsala.)

3. Aspartate aminotransferase activity in erythrocytes. The aspartate amino-

transferase activity was determined according to Karmen-Wróblewski with and without pyridoxal phosphate added in excess.

4. Hematological determinations. The hematological determinations were performed according to ordinary laboratory methods. The hemoglobin concentration was performed according to the cyanmethemoglobin method, the red blood cell count was performed with an automatic cell counter. The hematocrit determinations were performed by a microcentrifuge. The white blood cells were counted microscopically.

5. Statistical methods. The significance of differences was calculated according to both Student’s t test and \( \chi^2 \) methods.

RESULTS

1. Mean pyridoxal phosphate concentration in plasma

The mean values of the pyridoxal phosphate concentrations in plasma during various periods of pregnancy are tabulated in Table I for patients with no extra

<p>| TABLE I |</p>
<table>
<thead>
<tr>
<th>PYRIDOXAL PHOSPHATE CONCENTRATION IN PLASMA (( \mu )g/ml) DURING PREGNANCY IN WOMEN NOT RECEIVING EXTRA PYRIDOXINE (GROUP A) AND THEIR NEWBORN CHILDREN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significance of difference in mean values at various stages of pregnancy.</td>
</tr>
<tr>
<td>Gestational age Cord blood Months</td>
</tr>
<tr>
<td>M</td>
</tr>
<tr>
<td>SEM</td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>Significance of difference</td>
</tr>
<tr>
<td>M = Mean value</td>
</tr>
<tr>
<td>SEM = Standard error of the mean</td>
</tr>
<tr>
<td>N = Number of cases</td>
</tr>
</tbody>
</table>

| TABLE II |
| PYRIDOXAL PHOSPHATE CONCENTRATION IN PLASMA (\( \mu \)g/ml) DURING PREGNANCY IN WOMEN RECEIVING 2 \( \mu \)G EXTRA PYRIDOXINE PER DAY (GROUP B) AND THEIR NEWBORN CHILDREN |
| Significance of difference in mean values at various stages of pregnancy. |
| Gestational age Cord blood Months | 3 | 6 | 9 | 10 |
| M | 6.82 | 5.1 | 3.61 | 4.47 | 26.6 |
| SEM | 1.31 | 0.98 | 0.45 | 0.76 | 4.08 |
| N | 20 | 20 | 19 | 19 | 18 |
| Significance of difference | \( \rho \) < < 0.05 | 0.025 < \( \rho \) < 0.0125 | \( \rho \) < < 0.05 | 0.0005 < \( \rho \) | 0.005 < \( \rho \) |

_M. S. CLIN._

TABLE III

PYRIDOXAL PHOSPHATE CONCENTRATION IN PLASMA (ng/ml) DURING PREGNANCY IN WOMEN RECEIVING 10 mg EXTRA PYRIDOXINE PER DAY (GROUP C) AND THEIR NEWBORN CHILDREN

Significance of difference in mean values at various stages of pregnancy.

<table>
<thead>
<tr>
<th>Gestational age</th>
<th>Months</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>10</th>
<th>Cord blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>SEM</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.60</td>
</tr>
<tr>
<td></td>
<td>1.12</td>
<td>19</td>
<td>19</td>
<td>16</td>
<td>19</td>
<td>11.40</td>
</tr>
<tr>
<td></td>
<td>2.64</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.74</td>
</tr>
<tr>
<td></td>
<td>1.40</td>
<td></td>
<td></td>
<td></td>
<td>1.40</td>
<td>8.09</td>
</tr>
<tr>
<td></td>
<td>1.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30.4</td>
</tr>
<tr>
<td></td>
<td>1.26</td>
<td></td>
<td></td>
<td></td>
<td>1.26</td>
<td>5.49</td>
</tr>
</tbody>
</table>

Significance of difference:
- \( p < 0.05 \)
- \( p < 0.05 \)
- \( p < 0.005 \)
- \( p < 0.0005 \)
- \( p < 0.0005 \)

Pyridoxine addition, in Table II when 2 mg was added and in Table III when 10 mg was added. In Table IV, the significance in the difference between the mean values in the different groups is shown.

2. Pyridoxal phosphate concentration in plasma

Pyridoxal phosphate concentration in plasma during various stages of pregnancy in women who were given varying amounts of extra pyridoxine is shown in Figs. 1-3, where the values of the pyridoxal phosphate concentration in cord blood are also noticed.

3. Plasma pyridoxal phosphate concentration related to initial value

The difference between the individual plasma pyridoxal phosphate concentra-
### TABLE IV
Plasma Pyridoxal Phosphate Concentrations (ng/ml) in the Three Groups A, B and C with 0 mg, 2 mg and 10 mg extra pyridoxine per day at various stages of pregnancy and in cord blood

<table>
<thead>
<tr>
<th>Gestational age Months</th>
<th>Added pyridoxal/day mg</th>
<th>0</th>
<th>2</th>
<th>10</th>
<th>0</th>
<th>2</th>
<th>10</th>
<th>0</th>
<th>2</th>
<th>10</th>
<th>0</th>
<th>2</th>
<th>10</th>
<th>Cord blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>6.24 0.79 19</td>
<td>6.82 1.31 19</td>
<td>7.60 1.12 19</td>
<td>2.80 0.63 19</td>
<td>5.1 0.98 19</td>
<td>11.4 2.64 18</td>
<td>1.44 0.49 18</td>
<td>3.61 1.40 17</td>
<td>8.74 0.52 17</td>
<td>2.43 0.75 17</td>
<td>4.47 1.40 17</td>
<td>8.09 1.94 17</td>
<td>7.69 4.08 17</td>
<td>30.4 5.49 17</td>
</tr>
<tr>
<td>SEM</td>
<td>0.79 0.46 0.79</td>
<td>0.29 0.29 0.79</td>
<td>1.31 0.29 0.79</td>
<td>0.56 0.29 0.79</td>
<td>0.98 0.29 0.79</td>
<td>2.64 0.29 0.79</td>
<td>0.63 0.29 0.79</td>
<td>0.34 0.29 0.79</td>
<td>0.98 0.29 0.79</td>
<td>1.40 0.29 0.79</td>
<td>2.64 0.29 0.79</td>
<td>0.34 0.29 0.79</td>
<td>0.56 0.29 0.79</td>
<td>0.56 0.29 0.79</td>
</tr>
</tbody>
</table>

Significance of difference
- $p < 0.05$
- $0.05 < p < 0.025$
- $0.005 < p < 0.0125$
- $0.0005 < p < 0.025$
- $0.0005 < p < 0.0125$
- $p < 0.05$

### TABLE V
Serum Folic Acid Concentration (ng/ml) during pregnancy at varying additions of pyridoxine

<table>
<thead>
<tr>
<th>Gestational age Months</th>
<th>Added</th>
<th>0</th>
<th>2</th>
<th>10</th>
<th>0</th>
<th>2</th>
<th>10</th>
<th>0</th>
<th>2</th>
<th>10</th>
<th>0</th>
<th>2</th>
<th>10</th>
<th>Cord blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>6.31 0.46 19</td>
<td>5.22 0.29 19</td>
<td>5.72 0.56 19</td>
<td>5.34 0.52 19</td>
<td>4.53 0.34 19</td>
<td>4.95 0.56 18</td>
<td>4.28 0.37 18</td>
<td>3.21 0.17 18</td>
<td>3.59 0.32 18</td>
<td>3.43 0.27 18</td>
<td>3.31 0.24 18</td>
<td>2.97 0.21 18</td>
<td>17.21 1.07 17</td>
<td>15.01 1.16 17</td>
</tr>
<tr>
<td>SEM</td>
<td>0.46 0.29 0.46</td>
<td>0.29 0.29 0.46</td>
<td>0.56 0.29 0.46</td>
<td>0.52 0.29 0.46</td>
<td>0.34 0.29 0.46</td>
<td>0.56 0.29 0.46</td>
<td>0.37 0.29 0.46</td>
<td>0.17 0.29 0.46</td>
<td>0.32 0.29 0.46</td>
<td>0.27 0.29 0.46</td>
<td>0.24 0.29 0.46</td>
<td>0.21 0.29 0.46</td>
<td>1.07 1.07 1.07</td>
<td>1.16 1.16 1.16</td>
</tr>
</tbody>
</table>

| N                      | 19 20 19 | 20 20 19 | 19 19 19 | 18 18 18 | 19 19 18 | 16 16 18 | 18 18 18 | 17 17 18 | 16 16 18 | 18 18 18 | 17 17 18 | 16 16 18 | 18 18 18 | 18 18 18 | 18 18 18 | 18 18 18 |
### TABLE VI

**Whole Blood Folic Acid Concentration (ng/ml) During Pregnancy at Varying Additions of Pyridoxine**

<table>
<thead>
<tr>
<th>Gestational age Months</th>
<th>Added pyridoxal/day mg</th>
<th>0</th>
<th>2</th>
<th>10</th>
<th>0</th>
<th>2</th>
<th>10</th>
<th>0</th>
<th>2</th>
<th>10</th>
<th>0</th>
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<th>0</th>
<th>2</th>
<th>10</th>
<th>0</th>
<th>2</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>135.2</td>
<td>137.5</td>
<td>137.5</td>
<td>161.2</td>
<td>139.5</td>
<td>145.8</td>
<td>149.4</td>
<td>129.6</td>
<td>134.3</td>
<td>111.5</td>
<td>128.8</td>
<td>132.5</td>
<td>283.2</td>
<td>226.9</td>
<td>298.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>9.49</td>
<td>9.40</td>
<td>7.90</td>
<td>16.15</td>
<td>15.07</td>
<td>12.01</td>
<td>15.3</td>
<td>11.34</td>
<td>10.42</td>
<td>10.0</td>
<td>9.74</td>
<td>12.98</td>
<td>22.7</td>
<td>23.5</td>
<td>24.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>19</td>
<td>20</td>
<td>19</td>
<td>15</td>
<td>20</td>
<td>19</td>
<td>18</td>
<td>19</td>
<td>18</td>
<td>18</td>
<td>17</td>
<td>14</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

### TABLE VII

**Per cent Increase of Aspartate Aminotransferase (GOT) activity after Addition of Pyridoxal Phosphate to Saturate the Apo-enzyme**

<table>
<thead>
<tr>
<th>Gestational age</th>
<th>Added pyridoxal/day mg</th>
<th>0</th>
<th>2</th>
<th>10</th>
<th>0</th>
<th>2</th>
<th>10</th>
<th>0</th>
<th>2</th>
<th>10</th>
<th>0</th>
<th>2</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>52.1</td>
<td>37.8</td>
<td>34.9</td>
<td>60.7</td>
<td>43.8</td>
<td>42.4</td>
<td>36.3</td>
<td>44.3</td>
<td>27.0</td>
<td>59.1</td>
<td>54.4</td>
<td>42.8</td>
<td>27.2</td>
</tr>
<tr>
<td>SEM</td>
<td>7.6</td>
<td>3.4</td>
<td>5.4</td>
<td>16.4</td>
<td>8.2</td>
<td>8.5</td>
<td>5.8</td>
<td>6.1</td>
<td>5.7</td>
<td>10.3</td>
<td>19.8</td>
<td>8.2</td>
<td>5.0</td>
</tr>
<tr>
<td>N</td>
<td>19</td>
<td>20</td>
<td>18</td>
<td>19</td>
<td>20</td>
<td>18</td>
<td>17</td>
<td>19</td>
<td>18</td>
<td>17</td>
<td>18</td>
<td>18</td>
<td>14</td>
</tr>
</tbody>
</table>

Pyridoxal phosphate added to the incubation mixture. Karmen units/ml

Significance of difference

\[ p = 0.0005 \]

\[ 0.025 < p < 0.0125 \]
Fig. 2. Pyridoxal phosphate concentration in plasma during pregnancy in women receiving 2 mg extra pyridoxine per day, and their newborn children (group B).

Fig. 3. Pyridoxal phosphate concentration in plasma during pregnancy in women receiving 10 mg extra pyridoxine per day and their newborn children (group C).

tions and the corresponding initial values were calculated. The mean values and standard errors of the mean of these differences were determined and the results at different gestational ages for groups A, B and C are given in Fig. 4.

![Graph](image)

Fig. 4. Changes during pregnancy in plasma pyridoxal phosphate concentration related to initial values. Mean values and standard errors of the mean.

4. **Folic acid concentration in blood**
   
   Folic acid concentration in serum is shown in Table V, and the folic acid concentration in packed red blood cells is shown in Table VI.

5. **Aspartate aminotransferase activity in erythrocytes**
   
   The percent increase of aspartate aminotransferase activity when pyridoxal phosphate was added is shown in Table VII.

6. **Pyridoxal phosphate concentration in cord blood and in maternal venous blood**
   
   The pyridoxal phosphate concentration in cord plasma was compared with the concentration in maternal blood plasma as shown in Fig. 5.

7. **Folic acid concentration in cord blood and in maternal blood**
   
   The folic acid concentration in cord blood compared with the concentration in maternal blood is shown in Fig. 6.

8. **Pyridoxal phosphate concentration in plasma and folic acid concentration in red blood cells**
   
   The concentrations of pyridoxal phosphate in plasma and folic acid in red blood cells are compared in Fig. 7.

Fig. 5. Plasma pyridoxal phosphate concentration ($y$) in cord blood related to maternal plasma pyridoxal phosphate concentration ($x$) at delivery. The equation of the regression line is: $y = 2.767x + 6.233$. $N = 48$, $r = 0.6835$.

Fig. 6. Folic acid concentration in packed red cells in cord blood ($y$) related to maternal venous blood ($x$) at delivery. The equation of the regression line is: $y = 0.494x + 380.4$. $N = 42$, $r = 0.265$.  

9. Packed red cell folic acid concentration, plasma pyridoxal phosphate concentration and birth weight

The maternal folic acid concentration in packed red cells at 3, 6, 9 and 10 months have been related to children's birth weights in the whole material independent of amount of extra pyridoxine given. Birth weights were in the range 2750–4500 g, folic acid concentration between 75 and 1050 ng/ml packed red cells. In this range there was no correlation between folic acid concentration and birth weight. Three women had values under 100 ng/ml packed red cells, their children weighed 3200–3750 g. Cord blood folic acid concentration has also been related to birth weight in the whole material. No correlation was found. In the same way the pyridoxal phosphate concentration in maternal blood plasma at 3, 6, 9 and 10 months and in cord blood plasma was compared with birth weight, but no correlation was found.

DISCUSSION

The mean values of the pyridoxal phosphate concentrations in plasma decreased during pregnancy in the groups of women who received no and 2 mg extra pyridoxine per day, respectively. In the first group the deviation from the initial value of plasma pyridoxal phosphate concentrations was statistically significant ($p < 0.0025$) at 6, 9 and 10 months, while in the second group (with 2 mg extra pyridoxine) the deviation was statistically significant ($p < 0.025$) only at 9 months.

In the group receiving 10 mg extra pyridoxine per day no decrease in the mean value of pyridoxal phosphate concentration was noticed. The pyridoxal phosphate
concentration in cord blood in the group given no extra pyridoxine did not deviate significantly from the maternal plasma values at about 3 months' pregnancy. In the other groups receiving extra pyridoxine the cord blood plasma concentration of pyridoxal phosphate was significantly higher than the 3 months' maternal plasma values, while there was no difference in cord blood concentration between the groups receiving 2 and 10 mg respectively.

As shown in Fig. 5 there was a significant correlation between the pyridoxal phosphate concentrations in maternal plasma and in cord blood plasma. This has not been shown earlier for pyridoxal phosphate. For folic acid such a correlation has been discussed but never shown\(^{13}\). We could find no correlation (Fig. 6) for blood folic acid concentration between mother and child.

Earlier investigations on folic acid concentration during pregnancy have shown decreasing serum folate levels already in the second trimester\(^{9,14}\) or only in the third trimester\(^9\). Studies on the clinically more significant whole blood folic acid have shown less pronounced changes and almost all of them only in the third trimester\(^8\) and in puerperium\(^8\). We found unchanged red blood cell folic acid concentrations until 9 months (36 weeks) but a significant decrease during the 10th month (Tables V and VI).

The 5-methyltetrahydrofolic acid has been considered to be the main storage form of folic acid in the body. The main pathway for forming 5-methyltetrahydrofolic acid is from serine and hydroxymethyltetrahydrofolic acid through participation of serine hydroxymethyltransferase, a pyridoxal phosphate-dependent enzyme. Because of this metabolic connection between folic acid and pyridoxal phosphate it was theoretically possible that low pyridoxal phosphate concentrations would give rise to decreased stores of folic acid in the body. The pyridoxal phosphate concentration in plasma was therefore compared with the folic acid concentration in serum and red blood cells and as shown in Fig. 7, no correlation was found in the different groups receiving various amounts of extra pyridoxine (Tables V and VI).

As pyridoxal phosphate is a coenzyme for aspartate aminotransferase, a deficiency of this vitamin could give rise to a decreased saturation of the enzyme with its coenzyme and therefore also a decreased enzyme activity. This has been shown in certain vitamin \(B_6\) deficiency cases\(^7\). No such correlation between pyridoxal phosphate concentration and the enzyme saturation with its coenzyme could be demonstrated. As shown in Table VII, no connection between gestational age, pyridoxine addition and the enzyme saturation with its coenzyme could be traced in the whole material. The reason for this is obscure. But during pregnancy other factors affect the result of deficiency tests—such as histidine load tests\(^{13}\) and tryptophan load tests\(^4\)—which might be originating in endocrine changes during pregnancy and could also influence the aspartate aminotransferase activity. The finding in Table VII of a significant "unsaturation" of aspartate aminotransferase in the groups who received no or only 2 mg extra pyridoxine per day compared to the group receiving 10 mg pyridoxine per day at 9 months of gestation should be compared to the finding of decreased plasma pyridoxal concentrations at 9 months in the same groups (Table IV, Fig. 4). This finding might indicate metabolic significance of the decreased plasma pyridoxal phosphate concentration, even when 2 mg extra pyridoxine per day had been added.
ACKNOWLEDGEMENTS

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