CHAPTER IV

VITAMIN SUPPLEMENTS TO WOMEN USING ORAL CONTRACEPTIVES
The results reported in the previous Chapter as well as reports from other workers suggest that the ingestion of OC leads to alteration in the nutritional status of vitamins such as pyridoxine, riboflavin, thiamin, folic acid and perhaps vitamin A. The requirements of these vitamins seem to be higher in women using OC. In pregnancy too, vitamin requirements are known to go up, partly due to the hormone induced changes similar to those observed in women taking OC and partly due to the increased requirement of the growing fetus.

**Pyridoxine requirement of women using OC and pregnant women:**

Some studies have been carried out to determine the pyridoxine requirement of women using OC. However, no reports are available on the requirements of other vitamins like riboflavin, thiamin, vitamin A, and folic acid in these women. Large, daily doses of pyridoxine (from 20 to 100 mg) have been found to correct the abnormal tryptophan metabolism associated with OC use. Rose (1966) who was the first to report abnormal tryptophan metabolism in women using the pill also demonstrated that this could be corrected in one of the subjects by supplementing with 40 mg of vitamin B₆ for five days. The studies of Price *et al* (1967) showed that a daily dose of 100 mg of pyridoxine is required for the correction of tryptophan metabolism in these women. This study also showed that the excretion of 4-pyridoxic acid increased markedly following treatment with pyridoxine. Based on the studies
using various doses of pyridoxine Luhby et al (1971) suggested that a daily supplement of 25 mg of pyridoxine would be necessary to correct abnormal tryptophan metabolism in women taking OC. The spontaneous excretion of 3-hydroxyanthranilic acid which increases due to OC ingestion, was found to be corrected by a daily supplement of 20 mg of pyridoxine (Price et al, 1972).

Pyridoxine seems to play a role in the impaired glucose tolerance associated with OC use. Spellacy et al (1972c) showed that administration of 25 mg pyridoxine daily for a month improved the glucose tolerance of 8 out of 12 women taking OC. Recently Rose et al (1975) demonstrated that pyridoxine deficiency produced an impairment of glucose tolerance in women using OC, but not in those who do not use the pill. The ability of XA to complex with insulin and render it biologically inactive has been implicated in the impaired carbohydrate metabolism in OC users (Kotake and Murakami, 1971). It seems likely that administration of pyridoxine may release the inhibition of insulin by XA through the correction of abnormal tryptophan metabolism. Adams et al (1973) observed that a daily administration of 40 mg pyridoxine was necessary to correct the depression in women using the pill.

Abnormal tryptophan metabolism is also seen in pregnancy and has been shown to be corrected with pyridoxine supplementation (Sprince et al, 1951; Rose and Braidman, 1971).
Plasma and leucocyte PALP have been shown to decrease in pregnant women (Wachstein et al., 1960; 1967). Hamfelt and Hahn (1969) reported that for the same amount of PALP in blood, pregnant women show higher urinary XA excretion. Thus, in case of pregnancy, besides endocrine factors, deficiency of pyridoxine as a result of higher fetal requirement seems to be responsible for the abnormal tryptophan metabolism (Rose and Braidman, 1971). Shane and Contractor (1975) showed that blood PALP levels in pregnant women were lower than controls, indicating a relative pyridoxine deficiency.

Riboflavin requirement in pregnancy:

Urinary excretion of riboflavin has been shown to decrease during pregnancy, particularly in the third trimester (Brzezinski et al., 1952). It was also observed that subjects whose dietary intake of riboflavin was low developed clinical signs of riboflavin deficiency. Clarke (1971) demonstrated a lower whole blood riboflavin and mean corpuscular riboflavin concentration in 8 women during the last 6 weeks of pregnancy. These women had developed angular stomatitis and glossitis. Heller et al. (1974a) showed that the incidence of riboflavin deficiency increased from 25 per cent during first trimester of pregnancy to 40 per cent in the last trimester. The authors concluded that there is an increase in riboflavin requirement during pregnancy. The riboflavin requirement of pregnant women has been shown to increase by 0.2 to 0.3 mg daily compared to those of non-pregnant women (1.5 mg/day) (Narasinga Rao and Gopalan, 1968; Food and Nutrition Board, USA, 1974).
Thiamin requirement in pregnancy:

Incidence of thiamin deficiency is known to be higher during pregnancy. Urinary excretion of thiamin has been found to decrease during pregnancy (Lockhardt et al., 1943; Siddal and Mull, 1945). ETK activation test also indicates development of thiamin deficiency during pregnancy (Tripathy, 1968; Chong and Ho, 1970; Banji, 1970; 1976). Heller et al (1974b) recently observed that signs of thiamin deficiency develop early in pregnancy and remain constant throughout the gestational period. These findings were based on the TPP effect and further showed that 25 per cent of pregnant women develop thiamin deficiency. These results suggest that thiamin requirement increases during pregnancy (Narasinga Rao and Gopalan, 1968).

Vitamin A requirement in pregnancy:

Vitamin A requirement is also found to increase during pregnancy due to depletion of maternal stores (Srikantla, 1975). Serum vitamin A levels have been shown to fall progressively with gestational period in women of poor communities (Venkatechalam et al., 1972).

In pregnancy hormone induced changes also seem to contribute to the higher requirement of vitamins. The present study was, therefore, carried out to examine the additional requirement of pyridoxine, riboflavin and thiamin necessary to prevent a decline in the nutritional status of these vitamins in women using OC, Ovral. Since the use of OC increases plasma vitamin A levels, the effect of additional supplement of vitamin A on plasma levels of the vitamin was also studied.
Experimental

Thirty apparently healthy women belonging to the low-middle income group who had never before used OC and were not receiving any drugs or vitamins were examined for their vitamin nutritional status and then started on Ovral, along with vitamin supplements as described. Four women (Group 1) received 20 mg pyridoxine daily and were examined after 1 to 2 cycles of treatment. Sixteen women (Group 2) received a daily supplement of 10 mg of pyridoxine and were examined at one or more points of time during the first six cycles. A third group of 10 women (Group 3) received a multivitamin tablet containing thiamin - 3 mg, riboflavin - 2mg, nicotinamide - 20 mg, vitamin C - 30 mg, vitamin A - 5000 IU and vitamin D2 - 1000 IU, in addition to 10 mg pyridoxine daily and were examined at the end of 3 to 6 cycles.

Riboflavin, thiamin, pyridoxine and vitamin A status were assessed using the biochemical tests described in the previous Chapter.

The data were analysed by paired 't' test.

Results

The maximum excretion ofXA, following a tryptophan load, was found to be 36.4 μmoles/8 hours urine in women before starting on OC treatment. Hence, those subjects excreting greater than 36.4 μmoles/8 hours, following a tryptophan load, after the commencement of the treatment, were considered
to have abnormal tryptophan metabolism. The data given in Table 8 show that women who received 20 mg pyridoxine daily along with OC for 1 to 2 cycles (Group 1) excreted normal amounts of XA and KA after a tryptophan load. When 10 mg of pyridoxine was given along with Ovral, most of them showed 70 to 80 per cent increase in XA excretion following a tryptophan load after 1 to 2 cycles of treatment. After 3 to 6 cycles of treatment, 4 out of 13 did not show any rise in XA excretion, 4 had excretions which were slightly above normal and the rest of the subjects excreted from 50.6 to 140.3 μmoles of XA per 8 hours. Two of the women who were followed for 9 and 12 months showed a further rise in the excretion of tryptophan metabolites (Fig. 5). When a multivitamin tablet was supplemented along with 10 mg pyridoxine (Group 3), 4 out of 10 women showed no abnormality in tryptophan metabolism, 2 subjects had marginally elevated levels of XA, and 4 subjects excreted XA in amounts ranging from 46.4 to 145.3 μmoles/8 hours. (Table 9).

Supplements of 20 mg as well as 10 mg pyridoxine with or without multivitamin tablets led to an increase in the activity of EAspAT and a decrease in PALP effect in all the subjects investigated (Table 10). This improvement persisted in the 2 women who received 10 mg pyridoxine supplement and were followed for 9 and 12 months (Fig. 6).

Twenty mg of pyridoxine had no effect on riboflavin status of women using OC at the end of 1 to 2 cycles (Table 8).
### Table 8

**EFFECT OF 20 mg PYRIDOXINE SUPPLEMENTATION FOR 1 TO 2 MONTHS ON PYRIDOXINE, RIBOFLAVIN, THIAMIN AND VITAMIN A STATUS OF WOMEN TAKING OVRAL**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial (4)</th>
<th>After treatment (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XA /umoles/8 hr urine</td>
<td>26.9 ± 3.1</td>
<td>16.3 ± 3.2 **</td>
</tr>
<tr>
<td>KA /umoles/8 hr urine</td>
<td>21.9 ± 3.9</td>
<td>17.1 ± 5.1 +</td>
</tr>
<tr>
<td>EAspAT /umoles pyruvate formed/ml RBC/30 min.</td>
<td>37.6 ± 2.6</td>
<td>53.1 ± 2.5 **</td>
</tr>
<tr>
<td>PALP effect % stimulation</td>
<td>36.0 ± 2.6</td>
<td>26.7 ± 2.4</td>
</tr>
<tr>
<td>EGR mg GSH formed/ml RBC/15 min.</td>
<td>7.6 ± 0.9</td>
<td>7.3 ± 0.8</td>
</tr>
<tr>
<td>FAD effect % stimulation</td>
<td>97.5 ± 9.4</td>
<td>117.0 ± 9.4</td>
</tr>
<tr>
<td>RBC riboflavin /ug/100 ml</td>
<td>16.2 ± 2.5</td>
<td>14.0 ± 1.8</td>
</tr>
<tr>
<td>ETK /ug Su-7-P formed/ml RBC/30 min.</td>
<td>586.5 ± 77.0</td>
<td>621.7 ± 62.1</td>
</tr>
<tr>
<td>TPP effect % stimulation</td>
<td>14.7 ± 2.2</td>
<td>12.6 ± 3.3</td>
</tr>
<tr>
<td>Plasma vitamin A /ug/100 ml</td>
<td>36.3 ± 1.4</td>
<td>44.2 ± 1.6</td>
</tr>
</tbody>
</table>

*a - Mean ± SEM  + P < 0.1 * P < 0.05 ** P < 0.02

Figures in parentheses indicate number of subjects.
### Table 9

**EFFECT OF VITAMIN SUPPLEMENTATION ON PYRIDOXINE AND VITAMIN A STATUS OF WOMEN USING OVRAL**

<table>
<thead>
<tr>
<th>Treatment Duration</th>
<th>Parameter</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (8)</td>
<td>After treatment (8)</td>
<td>Initial (13)</td>
</tr>
<tr>
<td>1 to 2 months</td>
<td>XA /umoles/8 hr urine</td>
<td>29.9 ± 2.6</td>
<td>50.5 ± 10.8</td>
</tr>
<tr>
<td></td>
<td>KA /umoles/8 hr urine</td>
<td>26.7 ± 1.6</td>
<td>42.9 ± 8.3</td>
</tr>
<tr>
<td>3 to 6 months</td>
<td>EAspAT /umoles pyruvate formed/ml RBC/30 min.</td>
<td>33.9 ± 1.7</td>
<td>49.9 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>PALP effect % stimulation</td>
<td>35.6 ± 3.3</td>
<td>28.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>Plasma vitamin A /ug/100 ml</td>
<td>31.8 ± 1.8</td>
<td>45.8 ± 1.2</td>
</tr>
</tbody>
</table>

- Mean ± SEM  
- * P \( \leq 0.05 \)  
- ** P \( \leq 0.02 \)  
- *** P \( \leq 0.001 \)

Figures in parentheses indicate number of subjects per group.
### Table 10
**EFFECT OF VITAMIN SUPPLEMENTS ON RIBOFLAVIN AND THIAMIN STATUS OF WOMEN USING OVJRAL**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>After treatment</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(8)</td>
</tr>
<tr>
<td><strong>EGR</strong> (mg GSH formed/ml RBC/15 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.9 ± 0.9</td>
<td>8.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>7.7 ± 0.5</td>
<td>8.2 ± 0.5</td>
</tr>
<tr>
<td><strong>FAD effect % stimulation</strong></td>
<td>88.1 ± 13.2</td>
<td>117.1 ± 18.3</td>
</tr>
<tr>
<td></td>
<td>64.7 ± 8.2</td>
<td>34.6 ± 3.6</td>
</tr>
<tr>
<td><strong>RBC riboflavin /ug/100 ml</strong></td>
<td>17.1 ± 1.4</td>
<td>15.2 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>20.0 ± 1.1</td>
<td>19.5 ± 1.0</td>
</tr>
<tr>
<td><strong>ETK /ug Su-7-P formed/ml RBC/30 min.</strong></td>
<td>620.0 ± 31.9</td>
<td>641.6 ± 32.0</td>
</tr>
<tr>
<td></td>
<td>695.2 ± 40.4</td>
<td>710.7 ± 45.4</td>
</tr>
<tr>
<td><strong>TPP effect % stimulation</strong></td>
<td>5.0 ± 2.8</td>
<td>6.4 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>3.1 ± 1.3</td>
<td>2.0 ± 0.8</td>
</tr>
</tbody>
</table>

- a - Mean ± SEM
- @ P < 0.05
- * P < 0.02
- ** P < 0.01
- *** P < 0.001

Figures in parentheses indicate number of subjects per group.
Fig. 5: EFFECT OF 10 mg. PYRIDOXINE ON XA AND KA EXCRETION IN 2 SUBJECTS TAKING OVRAIL.
Fig. 6: EFFECT OF 10 mg PYRIDOXINE ON EAspAT ACTIVITY AND PALP EFFECT IN 2 SUBJECTS TAKING OVRAI.
Both red cell riboflavin and EGR showed a decline at the end of 3 to 6 cycles in Group 2 women who were supplemented only with 10 mg pyridoxine. This was associated with an increase in the FAD effect. When 2 mg riboflavin was given through the multivitamin tablet (Group 3), FAD effect significantly decreased and EGR activity as well as RBC riboflavin returned to normal values (Table 10).

Thiamin status of women taking OC was not altered when treated with 20 mg pyridoxine (Group 1) or 10 mg pyridoxine (Group 2) for 1 to 2 cycles. However, there was a decrease in EHK activity at the end of 3 to 6 cycles in Group 2 women. This decrease was not seen in Group 3 women who were treated with 3 mg thiamin through the multivitamin tablet (Tables 8 and 10).

Vitamin A levels showed a significant increase in all the three groups (Tables 8 and 9). Vitamin A supplements did not further increase the plasma vitamin A levels (Table 9).

Discussion

Pyridoxine status and requirement:

Luhby et al (1971) studied the effects of various doses of pyridoxine, 2 mg, 5 mg, 10 mg and 20 mg on XA excretion following a tryptophan load in women taking various OC preparations. In their study none of the women who were not taking OC excreted more than 35/umoles XA/8 hours and hence women excreting more than 35/umoles/8 hours following OC
treatment were considered to be abnormal. Based on this, they found that 2 mg and 5 mg pyridoxine supplements could correct only a small number of women. Ten mg corrected 75 per cent of women, and 20 mg, 86 per cent of women. In the present investigation, all the 4 women treated with 20 mg pyridoxine had normal tryptophan metabolism, despite simultaneous administration of Ovral. Ten mg pyridoxine could prevent the abnormality completely only in out of 8 women after 1 to 2 cycles and 4 out of 13 women after 3 to 6 cycles. However, it should be emphasised here that compared to the average, 10 to 15 fold increase in the excretion of tryptophan metabolites in women taking Ovral (Chapter III), the increase observed here was only marginal being 70 to 80 per cent (Groups 2; 3 to 6 months). Women who received additional supplements of multivitamins (Group 3) showed almost similar extent of increase in XA excretion.

Metabolic interrelationships are known to exist between vitamins of B-complex, particularly riboflavin and pyridoxine (Lakshmi and Bamji, 1974). Rose and McGinty (1968) observed that the administration of riboflavin and nicotinamide led to a decrease in urinary excretion of tryptophan metabolites following injection of cortisol to human subjects. It is possible that riboflavin increases the conversion of pyridoxine to PALP, thereby helping to saturate all the PALP dependant enzymes, including those of tryptophan-niacin pathway.

In the present investigation, most of the women were riboflavin deficient to start with and
this deteriorated further on treatment with Ovral and 10 mg pyridoxine (Group 2, 3 to 6 months)(Table 10). Two mg supplements of riboflavin could prevent the deficiency due to OC treatment, but could not correct the initial deficiency (Table 10). Higher daily supplements of riboflavin, which would mitigate even the initial state of deficiency, along with 10 mg of pyridoxine, would probably correct the abnormal tryptophan metabolism to a greater extent.

Bennink and Schreurs (1973) using different types of OC preparations showed that a daily dose of pyridoxine as large as 100 mg was necessary to correct the abnormal tryptophan metabolism seen in women using OC. An OC preparation, Steridil-d, similar to Ovral in composition, produced maximum excretion of XA in 1 to 2 years. It was observed earlier (Chapter III) that Ovral produced markedly less effect on tryptophan metabolism as indicated by the lower excretion of XA and KA compared to Ovulen-50. It was expected that the use of this preparation (Ovral) probably would require lower supplements of pyridoxine to correct the abnormal tryptophan metabolism than other preparations. However, in two of the women who were followed upto 9 and 12 months (with Ovral and 10 mg pyridoxine), there was further increase in the XA excretions. These results indicate that 10 mg pyridoxine may not be enough to correct tryptophan metabolism completely in women who use OC for longer periods of time.
Rose et al (1973a) observed increases in both EAIAT activity and EAspAT activity following the administration of 40 mg of pyridoxine daily for 4 to 8 weeks in women taking OC. The in vitro stimulation of these enzymes with PALP showed significant decrease. In our present study, women treated with 20 mg pyridoxine as well as 10 mg pyridoxine with or without multivitamin tablet showed increases in EAspAT activity and significant decrease in the PALP effect. The increase in the activity of EAspAT on pyridoxine treatment has been suggested to be due to the stabilization of the apoenzyme (Rose et al, 1973a). In two of the women who were followed for 9 and 12 months, there was further increase in the activity of EAspAT and progressive decrease in PALP effect.

We had seen earlier that OC led to an increase in PALP effect (Chapter III), suggesting development of pyridoxine deficiency. The results of the present investigation show that this could not only be completely prevented, but an improvement could be brought about in EAspAT activity and PALP effect with 10 mg of pyridoxine. Thus, it appears that whereas 10 mg (or perhaps lower supplement) of pyridoxine is enough to improve the EAspAT activity and PALP effect, higher amount of pyridoxine is necessary for complete correction of the abnormality in tryptophan metabolism.

In view of the fact that 10 mg of pyridoxine could not fully prevent the defect in tryptophan metabolism, a positive statement regarding the most appropriate level of pyridoxine supplementation for women using OC cannot be made.
However, since 10 mg pyridoxine corrects most of the lesion in tryptophan metabolism and brings about a marked improvement in EAspAT test, it can be said that women using OC should receive at least 10 mg pyridoxine daily.

Brown et al (1975) studied the effect of pyridoxine depletion and subsequent repletion at various levels (0.8, 2.0 and 20 mg) on plasma PALP, erythrocyte aminotransferase acid activities and urinary 4-pyridoxic acid excretion in control women and women using OC. Plasma PALP and urinary 4-pyridoxic acid were lower in OC treated women during all stages of pyridoxine depletion, and on repletion with pyridoxine, OC treated women showed a slower rate of return to normal compared to the control. The aminotransferase activities showed decreases on pyridoxine depletion in both the groups, the rates of decrease being the same. Leklem et al (1975a, 1975b) in similar studies of pyridoxine depletion and repletion demonstrated that estrogen containing OC selectively alter the PALP function in the tryptophan-niacin pathway, while other PALP dependant systems remain unaltered. They, thus, concluded that use of OC may not increase the pyridoxine requirement in majority of the women and that only a minority of them may develop pyridoxine deficiency.

Rose et al (1973a) suggested that further induction of aminotransferase activities by pyridoxine in OC treated women may worsen the hypoaminoacidemia due to a decrease in the amino acid pools, and this may be particularly undesirable in
populations having protein calorie malnutrition. Miller et al (1975) recently studied the effect of 50 mg pyridoxine supplementation in women using OC on plasma tryptophan and plasma \( \alpha \)-amino nitrogen. They demonstrated that plasma \( \alpha \)-amino nitrogen was slightly lower in women taking OC with or without supplementation of pyridoxine. Plasma tryptophan was not different in OC treated women compared to controls whether the former were supplemented with pyridoxine or not. Though pyridoxine supplementation produced increase in plasma PALP levels, this occurred to a lesser extent in women taking OC. This was postulated to be due to an impairment of conversion of pyridoxine to PALP or due to an increased tissue uptake.

In view of these reports and speculations, one wonders if women using OC should indeed be given large doses (greater than 10 mg) of pyridoxine to correct the abnormality in tryptophan metabolism fully. A lower dose of 10 mg pyridoxine per day may be adequate.

**Riboflavin status:**

Most of the subjects in this investigation (in all the three groups) had subclinical riboflavin deficiency as judged by an FAD effect greater than 20 per cent, before starting on OC. When these subjects were treated with 20 mg (Group 1) or 10 mg (Group 2) pyridoxine along with Ovral for 1 to 2 cycles, there was no further deterioration in the riboflavin status. Ovral given along with pyridoxine for 3 to 6 cycles (Group 2) led to a decrease in red cell riboflavin
concentration and EGR activity with a significant increase in the FAD effect. In the previous Chapter in a small number of subjects, it was shown that Ovral had markedly lesser effect on riboflavin status compared to Ovulen-50. In the present study, Ovral when given along with pyridoxine, led to a deterioration in the riboflavin status to a greater extent. This decline seems to be a combined effect of both Ovral as well as pyridoxine, since pyridoxine alone has been shown to reduce red cell riboflavin concentration (Sharada and Bemji, 1972).

When the subjects were given 2 mg riboflavin along with 10 mg pyridoxine supplementation through the multivitamin tablet (Group 3), there was no further decrease in the red cell riboflavin concentration and EGR activity. FAD effect showed a significant decrease. However, the values in most of the subjects were still greater than 20 per cent, indicating that while 2 mg riboflavin was enough to mitigate the adverse effect of OC on riboflavin status, it could not treat completely the original state of deficiency. Thus, when the initial riboflavin status itself is poor, as observed here, a greater supplement of riboflavin may be required.

Thiamin status:

None of the subjects studied in the three groups had thiamin deficiency as judged by a TPP effect (less than 15 per cent). Treatment with 10 mg or 20 mg pyridoxine for 1 to 2 cycles did not produce any alteration in thiamin status.
Ovral and 10 mg pyridoxine (Group 2) when given for 3 to 6 cycles, led to small but significant fall in the ETK activity like that observed earlier (Chapter III). The mean TPP effect increased in these women, although this was not significant. These results again indicate a marginal deterioration in the thiamin status due to the use of Ovral. Pyridoxine does not seem to further deteriorate this. When women were supplemented with 3 mg thiamin daily (Group 3) along with 10 mg pyridoxine, there was no further decrease in ETK activity and in fact, many of the subjects showed slight improvement in ETK activity, suggesting that a daily supplement of 3 mg thiamin should be more than enough to maintain good thiamin status.

The small decrease observed in the ETK activity on OC treatment may not be of physiological or nutritional significance and additional thiamin supplements may not be essential unless thiamin deficiency is commonly seen in that community.

Prasad et al (1975b) studied the urinary excretion of thiamin and riboflavin in women using OC alone and those supplemented with vitamins. They studied both upper and lower socio-economic group subjects and found that in the upper socio-economic group, thiamin and riboflavin supplementation increased the urinary excretion of these vitamins.
Vitamin A status:

The extent of increase in the plasma vitamin levels was the same with 10 mg pyridoxine or 10 mg pyridoxine plus a multivitamin tablet containing 5000 IU vitamin A. These results suggest that supplementation of vitamin A may not lead to further elevation of plasma levels of vitamin A in women using OC. Prasad et al (1975b), however, observed higher plasma vitamin A levels in women taking OC supplemented with vitamin A than those who were not supplemented.

Gal and Parkinson (1973) suggested that supplementation of vitamin A to women using OC may not be desirable since this was thought to further elevate the already high plasma concentration of vitamin A. However, in populations where vitamin A status is poor (like in the present study), further supplements may not result in increases which would reach toxic levels. In fact, none of the women supplemented with vitamin A had reached the toxic levels of 300/μg/100 ml in this investigation. It is quite possible that additional supplements may help to replenish the depleted liver stores of vitamin A due to OC and prove to be beneficial.

Summary

Additional requirements of pyridoxine, riboflavin and thiamin necessary to prevent a decline in the nutritional status of these vitamins in women using Ovral were studied.
The effect of vitamin A supplementation on plasma vitamin A levels was also investigated.

It was observed that a daily supplement of 20 mg pyridoxine with Ovral from the beginning of OC treatment could completely prevent the abnormality in tryptophan metabolism. Ten mg pyridoxine could prevent this to a marked extent up to 3 to 6 cycles. The results suggest that 10 mg of pyridoxine may not be enough to correct the abnormal tryptophan metabolism in women using OC for prolonged periods of time. Since this dose could improve the EAspAT activity and PALP effect, and to a marked extent the abnormal tryptophan metabolism, it is suggested that women using OC should receive at least 10 mg pyridoxine daily.

Many of the women had subclinical riboflavin deficiency before starting on OC and this deteriorated further on treatment with Ovral and 10 mg pyridoxine. A daily supplement of 2 mg riboflavin could prevent the deterioration in riboflavin status due to Ovral and pyridoxine treatment; however, this could not correct the initial state of deficiency. It is suggested that while 2 mg riboflavin may be enough to prevent the decline in riboflavin status in women using OC, a higher dose may be needed in women who are initially deficient.

A daily supplement of 3 mg thiamin was able to completely prevent the decline in its status due to the use of Ovral. Since the fall in ETK activity due to the use of OC
is very marginal and may not be of physiological or nutritional significance, it looks as if additional supplements of thiamin may be necessary only in populations where thiamin deficiency is known to be prevalent.

Plasma vitamin A levels tended to increase following Ovral treatment and with additional supplements of 5000 IU vitamin A per day. The rise was of the same magnitude as seen earlier with Ovral alone. Plasma concentrations did not reach toxic levels. It is possible that additional supplements of vitamin A to women using OC may help to replenish the depleted liver stores to certain extent. Such supplements may be particularly helpful to women using OC whose vitamin A status is poor.