CHAPTER VI

GENERAL SUMMARY AND CONCLUSIONS
Vitamin status of women using oral contraceptives:

(Chapter III)

Though the hormonal method of contraception for women has been in use since 1950s, the earliest reports of its adverse effects on vitamin nutrition of women appeared around 1966, when it was shown that women using OC had abnormal tryptophan metabolism, suggesting pyridoxine deficiency. About the same time, conflicting reports regarding the effects of OC on blood folate levels appeared. One of the physicians in the Institute observed higher incidence of oral lesions, such as angular stomatitis and glossitis in women using OC, and these lesions were found to respond to treatment with vitamins such as riboflavin and pyridoxine.

The incidence of vitamin deficiencies amongst low income group Indians is known to be high. Intensive family planning measures are being introduced in India, and all efforts are being made to popularize the use of pills even amongst the women of poor income groups. In view of all this, it became pertinent to see the biochemical and clinical reactions of these already malnourished women to the use of OC, particularly with regard to vitamins.

At the time this study was initiated, no information was available on the effects of OC on vitamins such as thiamin and riboflavin, and all reports regarding pyridoxine, folic acid and vitamin A were in wellnourished women. Also, almost all the
reports dealt with one single vitamin and no attempt had
been made to obtain an overall picture of the nutritional
status with regard to vitamins, whose deficiencies are
known to exist in large sections of populations.

In the studies reported in Chapter III of this thesis, attempt has been made to study the effects of two 50/μg estrogen pill preparations, Ovral (ethinyl estradiol 0.05 mg and dl-norgestrel 0.5 mg) and Ovulen-50 (ethinyl estradiol 0.05 mg and ethinodiol diacetate 1.0 mg) on the nutritional status of women belonging to poor income groups with regard to vitamins such as riboflavin, thiamin, pyridoxine, folic acid and vitamin A. Due to paucity of blood samples, vitamin B₁₂ and ascorbic acid could not be included in this study. Well established biochemical parameters were used.

In a cross-sectional study, a group of women who had used OC for periods between 6 and 12 months was compared with another group of women who had never used OC. Another partial follow-up study was also conducted in which women were examined initially and then at different points of time in the first 6 months. Both these studies showed that the use of OC leads to the following changes:

(1) Increase in urinary excretion of XA and KA following a tryptophan load, suggesting pyridoxine deficiency.
(2) Higher EAspAT activity, but also an increase in the in vitro stimulation of the enzyme with PALP. The latter suggests pyridoxine inadequacy perhaps due to higher requirement brought about by alterations in PALP enzymes.

(3) A marginal fall in ETK activity, but no change in TPP effect. This may indicate slight deterioration of thiamine nutritional status which may be of no physiological or nutritional consequence in a population where thiamin deficiency is rare.

(4) A fall in erythrocyte riboflavin concentration associated with a decrease in EGR activity and increase in FAD effect. All these are clear evidences of deterioration in riboflavin status. It may be pointed out that majority of the women of this community have biochemical riboflavin deficiency. This tends to worsen with the use of OC.

(5) A decrease in erythrocyte folacin levels. Here again, a number of women had erythrocyte folate levels below 100 ng/ml, indicating biochemical deficiency to start with, which becomes worse following treatment.

(6) The PCV and haemoglobin showed an increase in the first two cycles of OC treatment, perhaps, due to lower menstrual loss. Majority of the women included in the study had adequate haemoglobin levels.

(7) Plasma vitamin A levels were on the low side of normal initially, and showed significant increase
after treatment. This increase should not be misunderstood as improvement in vitamin A status, since experiments in animals reported elsewhere and in Chapter V (Annexure) suggest that the increase is probably due to a shift of the vitamin from liver into circulation as a result of an increase in the retinol binding protein.

Most of the above changes were observed with 2 to 3 cycles of OC treatment. Also, the limited data suggest that of the two OC preparations, the severity of the biochemical changes was greater with Ovulen-50 which contains ethinodiol diacetate as the progestogen rather than Ovral which contains norgestrel as the progestogen. These differences seem to be due to the antiestrogenic property of norgestrel.

The results of this study in women led to two logical questions. (1) What is the additional vitamin requirement of women using OC to prevent a slide down in their nutritional status? (2) What is the biochemical basis for the higher vitamin requirement?

Vitamin supplements for women using oral contraceptives: (Chapter IV)

To obtain answers to the first question, the following study was carried out in women:

Thirty young women were examined initially for their vitamin nutritional status and were then started on the OC,
Ovral, along with vitamin supplements as follows:

Group 1: Four women - 20 mg pyridoxine daily.
Group 2: Thirteen women - 10 mg pyridoxine daily.
Group 3: Ten women - 10 mg pyridoxine plus one multivitamin tablet containing riboflavin 2 mg, thiamin 3 mg, nicotinamide 20 mg, vitamin C 30 mg, vitamin A 5000 IU, and vitamin D₂ 1000 IU.

Women in Group 1 were examined at the end of 1 to 2 cycles. Women in Group 2 were examined at different time points in 6 cycles. Two women from this group were examined after 9 and 12 cycles respectively. Women of Group 3 were examined after 3 to 6 cycles. The following results were obtained:

(1) Twenty mg pyridoxine supplement daily could fully mitigate the abnormality in tryptophan metabolism brought about by OC treatment.

(2) The urinary excretion of tryptophan metabolites XA and KA was elevated only by 60 to 70 per cent in women who received 10 mg pyridoxine. This increase seems marginal compared to the 5 to 10 fold rise seen in women who did not receive pyridoxine supplements (Chapter III).

(3) Both 10 and 20 mg pyridoxine supplements produced increase in EASpAT activity and fall in PALP effect,
indicating improvement in pyridoxine nutritional status. This beneficial effect was seen even after 9 to 12 months of treatment with 10 mg pyridoxine.

From these results, the author feels that the minimum requirement of pyridoxine in women using OC can be regarded as 10 mg. While a higher supplement may be essential for the complete prevention of the abnormality in tryptophan metabolism, high doses of pyridoxine may affect amino acid metabolism by raising the levels of amino acid metabolising enzymes. In view of this, a decision has to be taken whether full correction of tryptophan metabolism is essential or whether a slight biochemical abnormality can be tolerated.

(4) Three mg of thiamin, supplemented through multivitamin tablet, could prevent the fall in ETK activity seen in OC treated women not receiving thiamin (Chapter III). This would suggest that the additional thiamin requirement of women using OC is certainly not higher than 3 mg.

(5) Two mg riboflavin, supplemented through multivitamin tablet, could prevent the further deterioration of riboflavin status due to use of OC and also partially correct the existing state of deficiency. This would suggest that, while 2 mg riboflavin is adequate to mitigate the contraceptive effect, a higher dose may be essential for correcting the initial state of deficiency.
(6) While OC treatment produced a significant increase in plasma vitamin A concentration, 5000 IU of vitamin A did not elevate it further. This would suggest that vitamin A supplement will in no way be a health hazard in women taking OC and may in fact help to build up liver reserves.

Biochemical basis for increased requirement of riboflavin following the use of oral contraceptives (Chapter V):

The mechanism by which OC affect pyridoxine and folate acid nutrition has been investigated by other workers. The deterioration of thiamin status observed in the present study was marginal and of doubtful nutritional significance. Riboflavin deficiency is a public health problem in India and hence it was decided to investigate the biochemical basis of the riboflavin deficiency associated with the use of OC. The following possibilities were considered for the observed effects:

1. Defective absorption;
2. Defective conversion to the coenzyme forms, and
3. Higher cellular requirement due to increase in riboflavin binding proteins such as flavoprotein enzymes.

These mechanisms are known to operate in relation to riboflavinosis associated with other drugs and hormones.

To investigate these alternatives, four experiments were carried out in adult female rats fed on high and low riboflavin diets with or without Ovulen-50 treatment. The
following changes were observed in rats treated with Ovulen-50:

(1) A decrease in urinary excretion of riboflavin at a time point after 12 days of treatment,

(2) Higher hepatic riboflavin concentrations and FAD levels, but no change in FAD to total riboflavin ratio.

(3) Increase in the activities of some flavin enzymes such as DAO and GR, no change in the enzymes XO and PAMPO, and a fall in NADH-DH. The fall in NADH-DH, due to Ovulen-50 treatment, was more marked on the low riboflavin diet.

(4) Fall in red cell and plasma riboflavin, but no change in EGR activity or FAD effect.

(5) A tendency for slower turnover of $^{14}$C-riboflavin and higher concentrations of labeled riboflavin in livers of the treated animals.

From these results it was concluded that OC do not affect the absorption of riboflavin or synthesis of flavin coenzymes, but the locus of OC effect may be flavin enzymes. Increase in the levels of some of these enzymes may augment the cellular requirement of the vitamin and a redistribution of the available vitamin between its enzyme systems such that activities of some enzymes increase whereas
that of others decrease. The latter would produce pockets of conditioned deficiency.

Alterations in enzyme activities can be due to modification of existing enzymes or due to increase in enzyme concentration. The latter may be due to synthesis of new enzyme or due to stabilization of the enzyme. To investigate the first alternative, Bamji, Tanaka and Yagi (unpublished results) examined the effects of several steroids including 3-estradiol, ethinyl estradiol and progesterone (singly and in combinations) on purified hog kidney DAO. Most steroids seem to bind to this enzyme and inhibit its activity, but by far the most effective steroid in this respect was progesterone. The inhibition of DAO by progesterone was competitive with regard to the substrate D-alanine. Simultaneous presence of progesterone and ethinyl estradiol had the same effect as that of progesterone alone, norgestrel and ethinodiol diacetate were not studied.

Subsequent experiments by the author showed that similar inhibition of DAO can be demonstrated in rat liver and kidney homogenates in vitro, and in rat liver in vivo following a single injection of progesterone. The inhibitory effect of progesterone was not seen with other flavin enzymes such as GR and NADH-DH. While the physiological significance of these effects remain to be investigated, the results suggest that perhaps the in vivo increase in DAO and GR in the livers of Ovulen-50 treated rats may be due to increase in enzyme
concentration rather than modification of enzyme activity. *In vitro* addition of progesterone to DAO did not improve the stability of the enzyme. It should, however, be pointed out that the progestogen in Ovulen-50 is ethinodiol diacetate and that in the pill both estrogen and progestogen are present in combination. Hence the observed effects of progesterone in isolation may not be comparable to those of ethinyl estradiol and ethinodiol diacetate in combination.