EXPERIMENTAL VITAMIN B₆ DEFICIENCY AND THE EFFECT OF OESTROGEN-CONTAINING ORAL CONTRACEPTIVES ON TRYPTOPHAN METABOLISM AND VITAMIN B₆ REQUIREMENTS

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SUMMARY

1. A vitamin B₆-deficient diet was fed to an adult male subject to confirm previously described changes in tryptophan metabolism and urinary 4-pyridoxic acid excretion, and erythrocyte alanine and aspartate aminotransferase activities.

2. The results were compared with those obtained in women taking oestrogen-containing oral contraceptives.

3. The development of dietary vitamin B₆ deficiency was indicated by decreased 4-pyridoxic acid excretion, increased urinary concentrations of xanthurenic acid, kynurenine and 3-hydroxykynurenine, an elevated 3-hydroxykynurenine/3-hydroxyanthranilic acid ratio and impaired erythrocyte aminotransferase activities.

4. Tryptophan metabolites and 4-pyridoxic acid excretion were determined in thirty-one women when they had been taking an oral contraceptive for 6–36 months. Of these, twenty-six had abnormal tryptophan metabolism, but the 4-pyridoxic acid was decreased in only seven. In six of these seven a raised 3-hydroxykynurenine/3-hydroxyanthranilic acid ratio supported a diagnosis of subclinical vitamin B₆ deficiency; erythrocyte alanine aminotransferase activity was determined in five of the six, and was decreased in three.

5. Erythrocyte aminotransferases were determined in sixteen women when they had been taking an oral contraceptive for 3–6 months, and in thirty-four women after 6–36 months treatment. Neither group showed any change in alanine aminotransferase activity, but the aspartate aminotransferase was elevated in the group treated for 6 months or longer.

Key words: tryptophan, vitamin B₆, oral contraceptives, oestrogens.

The metabolic pathway by which L-tryptophan is converted into nicotinic acid ribonucleotide, and several of the side reactions along the main pathway, involve pyridoxal phosphate dependent enzyme systems (Fig. 1), and in consequence a disturbance of tryptophan metabol-
ism occurs in vitamin B₆ deficiency. This is characterized by an elevated excretion of xanthurenic acid and several other intermediates in urine collected after an oral dose of the amino acid (Price, Brown & Yess, 1965).

Women who are taking oestrogen-containing oral contraceptives have an abnormality of tryptophan metabolism which is similar in most respects to that seen in vitamin B₆ deficiency, and which is reversed by pyridoxine administration (Rose, 1966). However, although it is generally considered that this metabolic disturbance indicates either an increased requirement

![Pathway Diagram]

FIG. 1. Major pathways of tryptophan metabolism (PLP indicates the known pyridoxal phosphate-dependent enzyme reactions).

for vitamin B₆ or interference with the normal coenzyme functions of pyridoxal phosphate (Rose & McGinty, 1970), no direct evidence has been published to support this interpretation.

In the present study the urinary excretion of tryptophan metabolites and 4-pyridoxic acid have been determined in both experimental vitamin B₆ deficiency and in women using one of the combined oestrogen–progestagen type of oral contraceptives. Particular attention has been paid to the ratio of 3-hydroxykynurenine (HK) to 3-hydroxyanthranilic acid (HA) excreted, since these intermediates of the tryptophan–nicotinic acid ribonucleotide pathway are the substrate and product respectively of the vitamin B₆-dependent kynureninase enzyme system.
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(Fig. 1), and an increase in the HK/HA ratio is considered to be a reliable indication of vitamin deficiency in man (O'Brien & Jensen, 1963; Heeley, 1965).

Further evidence of impaired pyridoxal phosphate function has been sought by the determination of erythrocyte aspartate aminotransferase and alanine aminotransferase activities, as these enzymes have been shown previously to be of value in the assessment of vitamin B₆ nutrition in man (Raica & Sauberlich, 1964; Cinnamon & Beaton, 1970).

MATERIALS AND METHODS

The effect of dietary vitamin B₆ deficiency was studied in a healthy 35-year-old male (one of the authors). The daily composition of the diet used is given in Table 1. It was essentially the high

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Weight (g)</th>
<th>Constituent</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>105</td>
<td>Carrots</td>
<td>15</td>
</tr>
<tr>
<td>Gelatin</td>
<td>46</td>
<td>Celery</td>
<td>15</td>
</tr>
<tr>
<td>Rice</td>
<td>20</td>
<td>Onions</td>
<td>15</td>
</tr>
<tr>
<td>Cream of rice</td>
<td>45</td>
<td>Sucrose</td>
<td>112</td>
</tr>
<tr>
<td>Peaches</td>
<td>100</td>
<td>Fat mixture*</td>
<td>91</td>
</tr>
<tr>
<td>Pears</td>
<td>100</td>
<td>Corn oil</td>
<td>39</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>120</td>
<td>Methionine</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vitamin/mineral supplement †</td>
<td></td>
</tr>
</tbody>
</table>

* Fat mixture (g/100 g): pure beef dripping, 22.1; margarine, 25.6; lard, 40.9; corn oil, 11.4.
† Given as Geriplex (Parke-Davis) one capsule daily containing riboflavin, 5 mg; cyanocobalamin, 0.002 mg; thiamine mononitrate, 5 mg; ascorbic acid, 50 mg; nicotinamide, 15 mg; d-α-tocopheryl acid succinate, 5 i.u.; vitamin A, 4,000 units; choline dihydrogen citrate 20 mg; ferrous sulphate, 30 mg; copper sulphate, 4 mg; manganese sulphate, 4 mg; zinc sulphate, 2 mg; dicalcium phosphate 200 mg, plus folic acid, 2 mg.

protein, vitamin B₆-deficient diet devised by Miller & Linkswiler (1967), and contained approx. 0.16 mg of vitamin B₆/day. The control tests were carried out while the subject was taking his normal self-selected diet. The vitamin B₆-deficient diet was then given for 20 days, at the end of which time normal food intake was recommenced, plus a supplement of pyridoxine hydrochloride (20 mg daily).

The male controls for the study of erythrocyte aminotransferase activities were healthy members of the laboratory staff aged 21–35 years; the females were women investigated before they started taking an oral contraceptive.

The women studied during the administration of an oral contraceptive had all freely given their consent to the investigation. They were taking one of the following combined oestrogen-progestagen preparations: Minovlar (Schering), which contains ethinyl oestradiol (0.05 mg) and norethisterone acetate (1 mg); Gynovlar 21 (Schering), ethinyl oestradiol (0.05 mg) and
norethisterone acetate (3 mg); Ovulen 50 (Searle), ethinyl oestradiol (0·05 mg) and ethynodiol diacetate (1 mg); Minilym (Organon), ethinyl oestradiol (0·05 mg) and lynestrel (2·5 mg); Volidan (British Drug Houses), ethinyl oestradiol (0·05 mg) and megestrol acetate (4 mg); Norinyl-1 (Syntex), mestranol (the 3-methyl ether of ethinyl oestradiol 0·05 mg) and norethisterone (1 mg); or, in one subject only, Orthonovin 1/80 (Ortho), mestranol (0·08 mg) and norethisterone (1 mg). Thus all but one of the oral contraceptive group were receiving a low dose (0·05 mg) of oestrogen preparation.

Urinary tryptophan metabolite excretions were determined in thirty-one women who had been taking an oral contraceptive for 6-36 months. The excretion of tryptophan metabolites and pyridoxic acid (4-P) in the urine of these women has been compared with the tryptophan metabolites in twenty-two, and urinary 4-P concentrations in twenty-eight control women who were studied before commencing oral contraceptive administration.

Erythrocyte aspartate aminotransferase (L-aspartate-2-oxoglutarate transaminase, EC 2.6.1.1) was determined in ten, and alanine aminotransferase (L-alanine-2-oxoglutarate transaminase, EC 2.6.1.2) in sixteen women when they had been taking an oestrogen-containing oral contraceptive for 3-6 months, and in sixteen and thirty-four women respectively after 6-36 months administration.

The subjects were starved overnight for the tryptophan load tests and the next morning a 2 g dose of L-tryptophan (Koch-Light Laboratories Ltd) suspended in a glass of milk was given orally. Urine was then collected for 24 h, and a sample stored at -15° until the analyses were carried out. Kynurenine, 3-hydroxykynurenine (HK) and 3-hydroxyanthranilic acid (HA) were determined by the ion-exchange-column procedures of Brown & Price (1956) as modified by Heeley (1965); xanthurenic acid (XA) was determined by a t.l.c. method (Walsh, 1965) and 4-pyridoxic acid (4-P) by ion-exchange chromatography (Reddy, Reynolds & Price, 1958). The urine samples were not hydrolysed before analysis.

Erythrocyte alanine aminotransferase (Ala-AT) and aspartate aminotransferase (Asp-AT) activities were assayed by the method of Woodring & Storvick (1970), which is based on the phenylhydrazone colorimetric procedure devised by Tonhazy, White & Umbreit (1950). Enzyme activities were also determined with 100 μg of pyridoxal phosphate added to the 3 ml assay medium [stimulation in vitro, and the percentage stimulation was then calculated from the basal activity (without added coenzyme) and the activity after stimulation in vitro]. Preliminary experiments had shown that 100 μg of pyridoxal phosphate was the amount that produces maximal stimulation of Asp-AT; concentrations less than this, although grossly in excess of normal erythrocyte concentrations, are relatively ineffective.

Haemoglobin (Hb) concentration of the haemolysate was determined by a cyanmethaemoglobin method (Dacie, 1958); Ala-AT activity was expressed as μg of sodium pyruvate produced h⁻¹ mg of Hb⁻¹, and Asp-AT activity as μg of oxaloacetic acid produced h⁻¹ mg of Hb⁻¹.

Student's t test was used for the statistical examination of the results, P<0·05 being regarded as significant.

RESULTS

Erythrocyte aminotransferase activities in control subjects

The male controls had a significantly higher basal Ala-AT activity than the females (P<0·001), but there was no difference in the pyridoxal phosphate stimulation in vitro. There was no
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Fig. 2. Changes in the urinary excretion of XA (○), kynurenine (△) and 4-P (●) during vitamin B₆ deficiency study.

Fig. 3. Changes in the urinary excretion of HK (○), HA (●), and HK/HA ratio (□) during vitamin B₆ deficiency study.
sex difference in the basal Asp-AT activity, but the stimulation in vitro was greater for the female controls ($P < 0.02$).

**Effects of experimental dietary vitamin B$_6$ deficiency**

The changes in the urinary excretion of 4-P, XA, and kynurenine during the development of experimental vitamin B$_6$ deficiency are shown in Fig. 2, and the HK and HA excretions, together with the HK/HA ratios, in Fig. 3.

The 4-P excretion decreased steadily during the depletion period and reached a value of 0.73 µmol/24 h on day 20. After 7 days of a normal diet plus 20 mg of pyridoxine daily the excretion of 4-P was 84.2 µmol/24 h. The excretion of both XA and kynurenine increased as vitamin B$_6$ deficiency progressed, but the XA excretion was elevated at least 7 days before there was any change in the kynurenine excretion. On day 20 of depletion a dramatic increase in the excretions of kynurenine and HK occurred, and the excretion of HK then exceeded that of XA by 45 µmol. There was little change in either the excretion of HA or the HK/HA ratio during the early stages of the experiment (Fig. 3), but there was a relatively small, but definite increase in the output of HA on day 20 although, because of the exceedingly high excretion of HK at that time, there was also a marked elevation of the HK/HA ratio. After the period of vitamin B$_6$ deficiency, a normal diet and supplemental pyridoxine brought about a rapid return to normal tryptophan metabolism.

The changes in Ala-AT and Asp-AT activities during the ingestion of a vitamin B$_6$-deficient diet are shown in Figs. 4 and 5. The Ala-AT activity (Fig. 4) fell to 58% of the control value during the depletion period, and returned to pre-deficiency values after 9 days of a normal diet plus a supplement of 20 mg of pyridoxine hydrochloride daily. The decrease in basal enzyme activity was accompanied by an increase in the stimulation in vitro by pyridoxal phosphate, indicating that the low enzyme activities were due to a lack of coenzyme. Although the net trend was for the Asp-AT activity to diminish during the period of vitamin B$_6$ deficiency (Fig. 5), there was considerable fluctuation in both the basal enzyme activity and the pyridoxal phosphate stimulation.

**Effects of administration of oral contraceptive**

Table 2 gives the tryptophan metabolite excretions, the HK/HA ratios, and the excretions of 4-P for the group of thirty-one women who were investigated when they had been taking an oestrogen-containing oral contraceptive for 6-36 months, and compares them with the control data obtained from untreated women. Although the concentrations of tryptophan metabolites in urine and the HK/HA ratio were significantly higher than those of the control group, there was no change in the excretion of 4-P. However, when the individual HK/HA ratios and the 4-P excretions from the group of twenty-two control subjects and thirty-one oral contraceptive-treated women are plotted together (Fig. 6) it is seen that seven of the women taking contraceptive steroids excreted subnormal amounts of 4-P, and that six of these had elevated HK/HA ratios. The 4-P excretion by another woman with a high HK/HA ratio was at the lower limit of the normal range. There was a good negative correlation between the HK/HA ratios and the 4-P excretions for the oral contraceptive-treated women ($r = -0.718; P < 0.001$), but an examination of the results did not show a correlation between the duration of treatment and either of these determinations.

The erythrocyte aminotransferase activities in the two groups of women studied when taking
FIG. 4. Changes in erythrocyte alanine aminotransferase activity [μg of sodium pyruvate h⁻¹ mg Hb⁻¹ (○)] and % stimulation (●) by addition of pyridoxal phosphate in vitro during the development of dietary vitamin B₆ deficiency, and its subsequent correction.

FIG. 5. Changes in erythrocyte aspartate aminotransferase activity [μg of oxaloacetate h⁻¹ mg Hb⁻¹ (○)] and % stimulation (●) by addition of pyridoxal phosphate in vitro during the development of dietary vitamin B₆ deficiency, and its subsequent correction.
oral contraceptives are given in Table 3. There was no significant change in either the basal Ala-AT activity or the pyridoxal phosphate stimulation during administration of these steroids. However, the basal Asp-AT activity was significantly elevated \((P<0.01)\) in the group of women who had been using an oral contraceptive for 6 months or longer.

Table 4 shows the enzyme activities, the HK and HA excretions and the HK/HA ratios for five oral contraceptive-treated women with low 4-P excretions. Although four of the five

Table 2. Excretion of metabolites by control group and by thirty-one women taking an oral contraceptive for 6-36 months; values are given as means ± SD; ns = not significant

<table>
<thead>
<tr>
<th>Metabolite excretion (µmol/24 h)</th>
<th>Kynurenine</th>
<th>HK</th>
<th>HA</th>
<th>HK/HA</th>
<th>XA</th>
<th>4-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (see text)</td>
<td>36±20</td>
<td>33±17</td>
<td>28±15</td>
<td>1.25±0.5</td>
<td>63±40</td>
<td>3.9±0.7</td>
</tr>
<tr>
<td>Oral contraceptive group</td>
<td>122±137</td>
<td>147±164</td>
<td>62±39</td>
<td>2.15±1.2</td>
<td>292±175</td>
<td>3.4±1.2</td>
</tr>
</tbody>
</table>

Significance of difference 0.01 > \(P\) > 0.001 0.01 > \(P\) > 0.001 \(P\) < 0.001 0.01 > \(P\) > 0.001 \(P\) < 0.001 ns

![Fig. 6. HK/HA ratios and 4-P excretions by twenty-two control subjects (●) and thirty-one women treated with an oral contraceptive for 6-36 months (○). The upper limit of normal for the HK/HA ratio has been taken as the mean value + 2 SD for the controls, and is indicated by the horizontal dotted line. The lower limit of normal for the 4-P (mean value – 2 SD for the controls) is indicated by the vertical dotted line.](image)

subjects had elevated HK/HA ratios, only three of them (Nos. 3, 4 and 5) had decreased Ala-AT activity, and one of these (No. 5) showed a considerable variation in enzyme activities. On one occasion her enzyme results were normal, whereas 1 month later there was low basal Ala-AT activity with high stimulation \textit{in vitro}. Subject 1 showed an elevated Asp-AT activity, the other four subjects having normal amounts of this enzyme.
Subjects 1–3 were given 20 mg of pyridoxine hydrochloride, daily for 1 month. This lowered the tryptophan metabolite excretions and the HK/HA ratios, and also resulted in marked increases in the basal activities of both enzymes with corresponding decreases in the stimulatory effect of pyridoxal phosphate added in vitro.

**TABLE 3.** Erythrocyte aminotransferase activities in male and female controls and oral contraceptive-treated women; results are expressed as means ± SD of the number of subjects given in parentheses

<table>
<thead>
<tr>
<th>Group</th>
<th>Alanine aminotransferase</th>
<th>Aspartate aminotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activity (µg of pyruvate h⁻¹ mg of Hb⁻¹)</td>
<td>Stimulation (%)</td>
</tr>
<tr>
<td>Male controls</td>
<td>1.41 ± 0.49*</td>
<td>14 ± 12 (11)</td>
</tr>
<tr>
<td>Female controls</td>
<td>0.88 ± 0.36</td>
<td>17 ± 14 (49)</td>
</tr>
<tr>
<td>Oral contraceptive given for 3–6 months</td>
<td>0.78 ± 0.32</td>
<td>22 ± 20 (16)</td>
</tr>
<tr>
<td>Oral contraceptive given for 6 months or more</td>
<td>0.93 ± 0.41</td>
<td>20 ± 22 (34)</td>
</tr>
</tbody>
</table>

Values significantly different from female controls: * P < 0.001; † P < 0.01; ‡ P < 0.02.

**TABLE 4.** Erythrocyte aminotransferase activities in oral contraceptive-treated women with low urinary 4-pyridoxic acid excretions

<table>
<thead>
<tr>
<th>Subject</th>
<th>Treatment duration</th>
<th>Metabolite excretion (µmol/24 h)</th>
<th>Alanine aminotransferase</th>
<th>Aspartate aminotransferase</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4-P</td>
<td>HK</td>
<td>HA</td>
</tr>
<tr>
<td>1</td>
<td>8 months</td>
<td>2.11</td>
<td>216</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>after vitamin B₆*</td>
<td>27.80</td>
<td>43</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>36 months</td>
<td>1.88</td>
<td>85</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>after vitamin B₆*</td>
<td>20.50</td>
<td>20</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>34 months</td>
<td>2.18</td>
<td>180</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>after vitamin B₆*</td>
<td>108.0</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>15 months</td>
<td>1.17</td>
<td>203</td>
<td>52</td>
</tr>
<tr>
<td>5</td>
<td>16 months</td>
<td>1.17</td>
<td>729</td>
<td>182</td>
</tr>
<tr>
<td></td>
<td>17 months</td>
<td>1.29</td>
<td>310</td>
<td>123</td>
</tr>
</tbody>
</table>

* Pyridoxine hydrochloride (20 mg) administered daily for 1 month.

**DISCUSSION**

The changes in tryptophan metabolism brought about by dietary vitamin B₆ deficiency were essentially those expected from the studies reported by Yess, Price, Brown, Swan & Linkswiler (1964), and Miller & Linkswiler (1967), although these workers did not determine the HA excretion. The 4-P is the major excretory product of vitamin B₆ (Reddy et al., 1958); decreased
concentrations in urine indicate retention of the available vitamin due to low dietary intake or an increase in the body's requirements. The prompt decrease in 4-P excretion during the period of dietary deficiency demonstrates that very little vitamin B₆ is actually stored in the tissues to meet future needs.

The HK/HA ratio is raised in vitamin B₆ deficiency because of the marked increase in HK excretion (Fig. 3). Ogasawara, Hagino & Kotake (1962) have shown that the activity of the supernatant kynureninase responsible for the cleavage of alanine from HK to yield HA is markedly decreased in the vitamin B₆-deficient rat, whereas Ueda (1967) found the mitochondrial kynurenine aminotransferase which produces XA from HK to be little affected. It is this extreme sensitivity of kynureninase to a lack of pyridoxal phosphate, compared with other enzymes of the pathway which also require this coenzyme, which accounts for the elevated urinary excretions of XA, HK and kynurenine, and the consequent rise in the HK/HA ratio, in vitamin B₆ deficiency.

Previous reports have described a decrease in basal erythrocyte Ala-AT and Asp-AT activities, and a significant increase in the stimulation in vitro by pyridoxal phosphate, during the development of experimental vitamin B₆ deficiency (Raica & Sauberlich, 1964; Cinnamon & Beaton, 1970). In the present study, although the trend was for the activities of both enzymes to diminish during a period of dietary deficiency, single determinations of Ala-AT appeared more likely to provide a reliable indication of vitamin B₆ depletion, because this enzyme was less prone to marked day-to-day fluctuations than Asp-AT.

The finding of a sex difference in Ala-AT activity is of note, as it suggests that the activity of this enzyme in erythrocytes may be under the influence of endogeneous sex hormones. There was no difference between the sexes in the stimulation in vitro of enzyme activity by pyridoxal phosphate, indicating that the observed difference in basal activity was due to a lower apo-enzyme concentration in the female controls, rather than a relative lack of cofactor. A sex difference in basal Asp-AT activity was not detected, but in this instance there was greater stimulation in vitro by added pyridoxal phosphate in the female group. It would seem, therefore, that the amount of unsaturated Asp-AT apoenzyme is higher in the erythrocytes of female controls than in the normal male subjects.

The mechanism by which oestrogen-containing oral contraceptives, and oestrogens alone, modify tryptophan metabolism is not fully established, although correction of the abnormal excretion of metabolites by pyridoxine administration implies a disturbance of vitamin B₆ function (Brown, Rose, Price & Wolf, 1969).

Rose (1966) postulated that oestrogens stimulate the activity of one or more enzymes concerned with the biosynthesis of nicotinic acid ribonucleotide from tryptophan, and that the consequently accelerated turnover rate of the metabolic pathway increases the requirement for pyridoxal phosphate. It has now been shown that in rat liver oestrogens stimulate tryptophan oxygenase, and also produce an increase in the activities of two pyridoxal phosphate-requiring enzymes, tyrosine aminotransferase and alanine aminotransferase (Braidman & Rose, 1971). Although it has not been established that oestrogen-containing oral contraceptives produce similar changes in hepatic enzyme activities in man, they do cause a decrease in plasma tyrosine concentrations, and this has been attributed to increased catabolism of this amino acid by tyrosine aminotransferase (Rose & Cramp, 1970).

A report from Ali, Donald & Simpson (1971) indicates decreased plasma concentrations of several amino acids in women treated with oestrogen-progestagen preparations, and so it is
possible that oral contraceptives cause an increased turnover rate of a number of pyridoxal phosphate-dependent amino acid-metabolizing pathways, and that this is responsible for an abnormally high vitamin B₆ requirement.

In the present study seven of thirty-one women who had been taking oral contraceptives excreted subnormal amounts of 4-P, and in six an elevated HK/HA ratio provided supporting evidence of vitamin B₆ deficiency. Although there was no overall change in erythrocyte Ala-AT activity during oral contraceptive administration, the enzyme activities were decreased in three of the five subjects studied with other biochemical evidence of vitamin B₆ deficiency.

However, although the investigation has shown that a subclinical vitamin B₆ deficiency occurs in a minority of women using an oestrogen-containing oral contraceptive, the results do not explain why most women taking these steroids have abnormal tryptophan metabolism that is correctable by pyridoxine administration.

Mason & Gullekson (1960) have shown that sulphate esters of oestrogens interfere in vitro with the activity of pyridoxal phosphate-dependent enzymes by competing with the coenzyme for sites on the apoenzyme molecule. Oestradiol disulphate was effective in inhibiting supernatant kynurenine aminotransferase at the very low concentration of 0.5 μM. It should be emphasized that the product of this enzyme is kynurenic acid, not xanthurenic acid (Fig. 1).

It is likely that the supernatant enzymes are more vulnerable to inhibition by oestrogen conjugates than those contained within the mitochondria, and there is evidence that pyridoxal phosphate is more tightly bound to mitochondrial kynurenine aminotransferase than to the supernatant kynureninase (Ogasawara et al., 1962; Ueda, 1967). Therefore, inhibition of kynureninase may result in an accumulation of HK within the liver, which will then be preferentially metabolized to XA by kynurenine aminotransferase within the protection of the mitochondrial membrane. Treatment with large doses of pyridoxine could reverse this mode of inhibition because a high concentration of pyridoxal phosphate in the liver would displace the oestrogen conjugates from the apoenzyme.

These considerations led to the suggestion that similar derivatives formed from the oestrogen component of the oral contraceptives may be responsible, at least in part, for the abnormal tryptophan metabolism by women using these preparations (Rose, 1966; Price, Thornton & Mueller, 1967; Mason, Ford & Wu, 1969).

There is no direct evidence that low-dose oestrogen oral contraceptives will yield sufficient concentrations of oestrogen conjugates in human liver to produce inhibition of the type described by Mason & Gullekson (1960) in the rat. However, as the administration of these steroids produces metabolic effects not normally observed outside pregnancy, it is clear that at the site of their conjugation in the hepatocyte concentrations of conjugates may occur sufficiently above the physiological range to have this effect.

One objection to the hypothesis is that although oestradiol-treated male rats show a decrease in kynureninase activity towards normal female values, this enzyme is not impaired in female rats receiving oestradiol, and females treated with a mestranol–norethynodrel combination actually show an increase in kynureninase activities (Rose & Brown, 1969). However, these observations may not be applicable to man since the metabolic handling of oestrogens by the liver, the distribution of conjugates between bile and urine and the enterohepatic circulation of these steroid derivatives (all factors which will influence the steady-state hepatic concentration) show considerable species variation and in particular differ in man and rodents (Sandberg, Kirdani, Back, Weyman & Slaunwhite, 1967). It is also relevant to the question of species
differences that whereas human pregnancy is associated with an abnormality of tryptophan metabolism which is identical with that seen in oral contraceptive-treated women (Rose & McGinty, 1970), the pregnant rat has been shown to excrete normal urinary amounts of xanthurenic acid (Mainardi, 1957).

The elevated erythrocyte Asp-AT activity in women who had been taking an oral contraceptive for 6 months or longer was an unexpected finding which has been reported recently by Ali et al. (1971). This could be due to either stimulation of enzyme protein synthesis or stabilization of the enzyme molecule with a decrease in the rate of degradation. It is more likely to be secondary to a metabolic effect of long-term oral contraceptive administration than a direct action of either the oestrogen or progestagen component on the enzyme, since the increase was only seen in the group treated for 6 months or longer.

Increased apoenzyme synthesis can also be induced by the administration of cofactor (Greengard & Gordon, 1963) and this, in addition to activation of pre-existing enzyme, may have been responsible for the elevated basal Ala-AT and Asp-AT activities when pyridoxine was given to subjects 1–3. The long-term administration of pyridoxine results in exceedingly high enzyme activities (D. P. Rose, R. Strong, P. W. Adams & P. E. Harding, unpublished work). In subject 1 both hormonal and cofactor inductions were probably operating to produce the very high basal Asp-AT activity after pyridoxine administration, as the activity was already increased before treatment with the vitamin.

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Vitamin B₆ and oral contraceptives


