Oral contraceptives and tryptophan metabolism: Effects of oestrogen in low dose combined with a progestagen and of a low-dose progestagen (megestrol acetate) given alone

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SYNOPSIS The effect upon tryptophan metabolism of the use of combined oestrogen-progestagen oral contraceptives containing a low (0.05 mg) dose of oestrogen, or of the continuous administration of megestrol acetate, has been studied by determining the excretion of tryptophan metabolites in urine collected after a 2 g oral dose of the amino acid.

An investigation of 10 women before being given oral contraceptives and after 21 days and three months of their use showed that xanthurenic acid excretion is increased within 21 days and that by three months the urinary levels of xanthurenic acid, kynurenine, 3-hydroxykynurenine, and 3-hydroxyanthranilic acid may all be elevated.

Ten other women studied after taking a combined type of oral contraceptive for six months or longer excreted significantly higher levels of all four metabolites than did those who had been treated with these steroids for only three months. The abnormal urinary excretion of tryptophan metabolites was completely reversed by treatment with pyridoxine hydrochloride, 20 mg daily for one month.

Studies of four women when they were taking an oestrogen-containing oral contraceptive and at intervals after they had discontinued its use showed that abnormal tryptophan metabolism may persist for three months or longer.

Megestrol acetate, a progestagen used as an oral contraceptive, was found to have no significant effect upon tryptophan metabolism.

The possible clinical significance of the effects of oral contraceptives upon tryptophan metabolism is discussed.

There have been several reports of disturbed tryptophan metabolism in women using oral contraceptives, the abnormality being characterized by an elevated excretion of xanthurenic acid and other metabolites of the tryptophan-nicotinic acid pathway (Fig. 1) in urine collected after an oral dose of the amino acid (Rose, 1965, 1966; Price, Thornton, and Mueller, 1967; Luhby, Davis, Murphy, Gordon, Brin, and Spiegel, 1970). These changes are similar to the altered excretion of tryptophan metabolites which occurs in vitamin B₆ deficiency, they are reversed by treatment with large doses of pyridoxine, and they are also present in patients treated with oestrogens alone (Brown, Rose, Price, and Wolf, 1969).

Received for publication 13 September 1971.

The clinical significance of the disordered tryptophan metabolism produced by oestrogens remains to be established, but, as will be discussed later, it may be related to two recognized complications of the use of oral contraceptives—mental depression and impaired glucose tolerance.

The women studied in previous investigations were using a combined oestrogen-progestagen type of oral contraceptive, and the majority were receiving a high dose of oestrogen (0.075 to 0.1 mg). Rose (1966) did report abnormal tryptophan metabolism by five women taking a preparation containing only 0.05 mg ethinyl oestradiol, but the 5 g dose of L-tryptophan which he used in his original study is equal to approximately five times the normal daily dietary intake of this amino acid, and is therefore a

Fig. 1 The metabolic pathways for the biosynthesis of nicotinic acid ribonucleotide and 5-hydroxytryptamine from tryptophan. (PLP indicates pyridoxal phosphate dependent enzyme reactions.)

grossly unphysiological load. The advantages of using a 2 g tryptophan load have been discussed by Price, Brown, and Yess (1965).

Since the recommendations of the Committee on Safety of Drugs (Scowen, 1969) the dose of ethinyl oestradiol or its 3-methyl ether, mestranol, in the combined oestrogen-progestagen oral contraceptives now in common use in the United Kingdom has been limited to 0.05 mg. Clinical and biochemical investigation of continuous low-dosage progestagen preparations given alone indicate that these steroids provide an alternative form of oral contraception which is moderately efficient and is not associated with significant metabolic abnormalities (Mears, Vessey, Andolsek, and Oken, 1969; Larsson-Cohn, Tengstrom, and Wide, 1969).

Therefore, in the present investigation we have reassessed the effects on tryptophan metabolism of combined oral contraceptives containing a low dose of oestrogen, and, as the effects of continuous low-dosage progestagen administration on tryptophan metabolism have not been reported, we have studied these in women taking megestrol acetate 0.5 mg daily.

Subjects and Methods

Ten women were studied before they started to take an oral contraceptive, and the tests were then repeated after the administration of an oestrogen-progestagen preparation for 21 days, and again after three months. The preparations used and the number taking each one are given in Table I. A further 10 women were investigated when they had been taking a combined type of oral contraceptive for six to 18 months (Table II). Four subjects taking oral contraceptives were studied before and after the administration of pyridoxine hydrochloride, 20 mg daily, for one month.

Tryptophan metabolism by four other subjects (Table II, nos. 3, 4, 8, 9) was assessed when they had been using an oral contraceptive for several months, and then at intervals after they had discontinued its use.

Five women were investigated when they had been taking megestrol acetate, 0.5 mg daily, for three to 23 months (Table V).

The subjects fasted overnight, and the next morning 2 g L-tryptophan was taken orally, sus-

Oral Contraceptive	Oestrogen	Progestagen	No. of Subjects
Minovlar	Ethinyl oestradiol 0.05 mg	Norethisterone acetate 1 mg	6
Gynovlar	Ethinyl oestradiol 0.05 mg	Norethisterone acetate 3 mg	1
Norinyl-1	Mestranol 0.05 mg	Norethisterone acetate 1 mg	2
Minilyn	Ethinyl oestradiol 0.05 mg	Lynestrenol 2.5 mg	1

Table I Combined oestrogen-progestagen oral contraceptives used in the study

Subject No.	Oral Contraceptive	Duration of Use (months)	Excretion of Metabolite ($\mu\text{mole}/24 \text{ hr}$) ^a			
			K	HK	HA	XA
1	Ovulen 50 ¹	6	139	216	45	365
2	Minovlar	8	120	158	46	418
3	{ Ovulen 50	5 }	322	630	150	950
4	{ Minovlar	5 }				
5	Ovulen 50	11	115	101	71	312
6	Ovulen 50	11	48	45	40	312
7	Minovlar	13	91	176	56	514
8	Norinyl-1	13	187	194	130	422
9	Volidan ³	13	341	693	60	715
10	Gynovlar	15	110	135	110	360
	Norinyl-1	18	134	220	84	346
Upper limit normal range ⁴			66	67	56	137

Table II Tryptophan metabolite excretions by women taking a combined type oral contraceptive for at least six months

¹Ethinyl oestradiol 0.05 mg, ethynodiol diacetate 1 mg

²K = kynurenine; HK = 3-hydroxykynurenine; HA = 3-hydroxyanthranilic acid; XA = xanthurenic acid

³Ethinyl oestradiol 0.05 mg, mestranol acetate 4 mg

⁴Mean value plus twicestandard deviation for 10 women studied before treatment with an oral contraceptive

pended in a glass of milk. Urine was then collected for 24 hours and an aliquot stored at -15°C until the analyses were carried out. Xanthurenic acid was determined by thin-layer chromatography on cellulose (Walsh, 1965), and kynurenine, 3-hydroxykynurenine, and 3-hydroxyanthranilic acid by the ion-exchange chromatography method of Heeley (1965).

Statistical examination of the results was by Student's *t* test, differences giving $P = 0.05$ being regarded as significant.

Results

After 21 days' treatment with a combined type of oral contraceptive there was a significant increase in the urinary xanthurenic acid excretion ($P < 0.01$; Table III), only one of the 10 subjects not having an elevated excretion of this metabolite. However, the excretions of kynurenine, 3-hydroxykynurenine, and 3-hydroxyanthranilic acid were unchanged (Table III). By three months the xanthurenic acid excretions were considerably higher than they had been at 21 days, and there were also increases in the mean values

for the excretion of the other three metabolites, although, owing to the considerable degree of variance within the groups, these changes were not statistically significant.

The tryptophan metabolite excretions by the 10 patients who had been taking an oestrogen-containing preparation for six months or more are given in Table II. They all excreted grossly elevated levels of xanthurenic acid, all but one (no. 5) excreted markedly increased quantities of kynurenine and 3-hydroxykynurenine, and in six there was a raised excretion of 3-hydroxyanthranilic acid. Comparison of the excretion of metabolites after three months' treatment with the urinary levels of the women who had been taking an oral contraceptive for six months or longer (Table III) showed that prolonged administration of a preparation containing 0.05 mg of oestrogen results in an accentuation of the metabolic disturbance, significant further increases occurring in the excretions of xanthurenic acid ($P < 0.01$), kynurenine, and 3-hydroxykynurenine ($P < 0.05$).

Table IV shows that treatment with 20 mg pyridoxine hydrochloride daily for one month

Duration of Treatment	Excretion of Metabolites ($\mu\text{mole}/24 \text{ hr}$)			
	K	HK	HA	XA
Before administration	32 $\pm$ 17	33 $\pm$ 17	28 $\pm$ 14	61 $\pm$ 38
Twenty-one days	37 $\pm$ 27	47 $\pm$ 36	31 $\pm$ 16	184 $\pm$ 91 ¹
Three months	74 $\pm$ 80	80 $\pm$ 72	49 $\pm$ 35	232 $\pm$ 96 ²
Six to 18 months	161 $\pm$ 97 ²	287 $\pm$ 228 ²	79 $\pm$ 39 ²	470 $\pm$ 210 ²

Table III Changes in urinary excretion of tryptophan metabolites during administration of a low dose oestrogen-containing oral contraceptive

Differences from pretreatment value: ¹ $P < 0.01$; ² $P < 0.001$.

Subject No.	Duration of Contraceptive Use (months)		Excretion of Metabolites ($\mu\text{mole}/24 \text{ hr}$)			
			K	HK	HA	XA
1	6	(a) ¹	139	216	45	365
		(b) ²	34	43	65	82
11	3	(a)	134	144	147	360
		(b)	29	32	32	96
12	4	(a)	432	310	123	370
		(b)	43	50	41	50
13	5	(a)	36	45	29	216
		(b)	24	20	26	38

Table IV Effect of pyridoxine administration on tryptophan metabolism by women taking a combined type of oral contraceptive

¹(a) Metabolite excretions when taking oral contraceptives alone

²(b) Metabolite excretions when taking oral contraceptive plus pyridoxine HCl 20 mg daily for 1 month.

Fig. 2 Reduction in the levels of metabolites excreted by subjects nos. 3 (Δ), 4 ($\bullet$), 8 ($\blacksquare$), and 9 ($\circ$) after discontinuing administration of a combined type oral contraceptive. The dotted lines indicate the upper limit of normal range for metabolite excretion.

completely reverses the abnormal excretion of tryptophan metabolites by women receiving a combined type of oral contraceptive.

The rate of return of tryptophan metabolism to normal after discontinuing oral contraceptives is illustrated in Fig. 2, and it is apparent that there is an association between the severity of the initial abnormal excretion of metabolites and the time required for the levels to reach pretreatment values. One patient (no. 3) continued to excrete a high level

of 3-hydroxykynurenine 15 weeks after stopping the use of an oral contraceptive.

The levels of tryptophan metabolites in the urine of five women taking megestrol acetate are given in Table V. Subject no. 14 had been using an oestrogen-containing oral contraceptive until three months before the metabolic studies, and it seems likely that the elevated excretions of kynurenine, 3-hydroxykynurenine, and 3-hydroxyanthranilic acid reflect a residual oestrogen effect. The xanthurenic acid

Subject No.	Duration of Use (mth)	Excretion of Metabolites ($\mu\text{mole}/24 \text{ hr}$)			
		K	HK	HA	XA
14	3	206	117	78	67
15	14	58	36	69	58
16	16	48	41	26	110
17	18	62	45	55	62
18	25	57	50	36	77
Upper limit normal range		64	67	56	137

Table V Tryptophan metabolism by women taking megestrol acetate

excretion was normal. Subject no. 15 appeared to excrete a slightly increased quantity of 3-hydroxyanthranilic acid, but the levels of the other metabolites were normal.

Discussion

The present study has shown that a reduction of the oestrogen component of the combined types of oral contraceptive to 0.05 mg does not eliminate the abnormal metabolism of a 2 g tryptophan load, which has previously been noted with Enavid-E, a preparation containing 0.1 mg mestranol and 2.5 mg norethynodrel (Price *et al*, 1967). An elevated urinary excretion of xanthurenic acid becomes apparent after only 21 days of treatment with a low-dose oestrogen oral contraceptive, although at this

Fig. 3 Comparison of the excretion of kynurenine (K), 3-hydroxykynurenine (HK), and xanthurenic acid (XA) by a group of women taking a low-dose oestrogen oral contraceptive (0.05 mg ethinyl oestradiol or mestranol) with the group taking Enavid-E (mestranol 0.1 mg) reported by Price *et al* (1967).

Values indicated are the means $\pm$ SD.

Open blocks: low oestrogen preparation for three months; cross-hatched blocks: low oestrogen preparation for six to 18 months; solid black blocks: Enavid-E for several months.

time the levels of the other metabolites are unchanged.

Prolonged administration of an oestrogen-containing oral contraceptive produces increased excretions of kynurenine, 3-hydroxykynurenine, and 3-hydroxyanthranilic acid, as well as of xanthurenic acid. Figure 3 compares the output of metabolites by women taking a 0.05 mg dose of oestrogen for only three months or for six months or longer with the levels reported by Price *et al* (1967) in their study of women who had been receiving Enavid-E for several cycles. There is a higher excretion of xanthurenic acid by the Enavid-E-treated group compared with the subjects who had received the lower dose of oestrogen for six months or more, but the levels of kynurenine and 3-hydroxykynurenine are similar. The group of women treated with 0.05 mg oestrogen for only three months excreted lower levels of these metabolites than the Enavid-E-treated women. These results suggest that the severity of the disturbance of tryptophan metabolism is influenced by both the dose of oestrogen in the oral contraceptive and by the duration of its administration.

Recently, Rose and McGinty (1970) have reviewed the possible mechanisms by which oestrogens may influence metabolism along the tryptophan-nicotinic acid pathway. It is suggested that these steroids both stimulate the activity of tryptophan oxygenase, the first and rate-limiting enzyme, and also inhibit the supernatant pyridoxal phosphate-dependent enzymes of the pathway, but not the mitochondrial aminotransferase which yields xanthurenic acid (Fig. 1). There is evidence from animal studies in support of both these possibilities (Braidman and Rose, 1971; Mason, Ford, and Wu, 1969).

Regardless of the precise biochemical mechanisms involved in the production of the metabolic disturbance, its correction by treatment with pyridoxine has been interpreted as indicating an increased requirement for this vitamin in women using oestrogen-containing oral contraceptives (Toseland and Price, 1969; Luhby *et al*, 1970).

The effect of oestrogens on tryptophan metabolism may be relevant to some of the other side effects of oral contraceptives, including mental depression, the appearance of rheumatic symptoms, and impaired glucose tolerance. There is considerable evidence that failure of normal brain 5-hydroxytryptamine synthesis from L-tryptophan may be important in the aetiology of depression (Curzon, 1969). Winston (1969) has suggested that in women who develop depression while taking an oral contraceptive, production of this amine is low due to inhibition of the pyridoxal phosphate-dependent decarboxylation step (Fig. 1), and Rose (1971) has recently reviewed the biochemical evidence in support of both this hypo-

thesis, and the possibility that increased turnover of the tryptophan-nicotinic acid ribonucleotide pathway, due to oestrogen action, results in a lack of substrate for 5-hydroxytryptamine biosynthesis.

Abnormal tryptophan metabolism occurs in rheumatoid arthritis (McMillan, 1960; Bett, 1966), and rheumatic symptoms may develop during the use of oral contraceptives (Bole, Friedlander, and Smith, 1969; Spiera and Plotz, 1969). However, the precise mechanism for the elevated excretion of tryptophan metabolites in rheumatoid arthritis remains to be established, and so for the present the clinical significance of any association between abnormal tryptophan metabolism and rheumatic complaints in oral contraceptive-treated women is uncertain.

Wynn and Doar (1966, 1970) observed impaired oral glucose tolerance in over 75% of women using a combined type of oral contraceptive, and in 13% there was chemical diabetes mellitus. They drew attention to the elevated plasma cortisol levels which occur during oestrogen administration, and postulated that the changes in glucose tolerance in women receiving oral contraceptives result from exposure of the liver to high glucocorticoid concentrations. In a further paper, Doar and Wynn (1970) pointed out the similarity between the changes in glucose tolerance and pyruvate metabolism observed in non-obese subjects taking oestrogen-containing oral contraceptives and those seen in non-obese patients treated with glucocorticoids and obese non-diabetic patients not receiving steroids, and they suggested that the metabolic abnormality in all three situations results from excessive glucocorticoid activity. The high levels of corticosteroid-inducible enzymes in the livers of oestrogen-treated rats, and the changes in tryptophan metabolism in oestrogen-treated human subjects, women using oestrogen-containing oral contraceptives, and subjects treated with hydrocortisone (Rose and McGinty, 1968) provide strong indirect support for this hypothesis.

Impaired glucose tolerance may also be directly related to the abnormal tryptophan metabolism by women taking oral contraceptives. The elevated urinary levels of xanthurenic acid which were observed in the present study are presumably accompanied by high plasma and liver concentrations of this metabolite, and experimental work by Kotake *et al* (1968) has shown that xanthurenic acid is capable of forming a complex with insulin which reduces the effect of this hormone on the blood glucose level by about 50%. The clinical significance of this interesting observation remains to be established.

Impaired glucose tolerance may persist for six weeks or more after withdrawal of an oral contra-

ceptive (Wynn and Doar, 1970) and so it is especially noteworthy that abnormal tryptophan metabolism has been demonstrated eight to 15 weeks after discontinuing the administration of these preparations.

Studies of individual steroids indicate that the abnormalities of both carbohydrate and tryptophan metabolism in women using the combined types of oral contraceptives are produced by the oestrogen component alone (Di Paola, Robin, and Nicholson, 1970; Rose, 1966). Consistent with this is the present observation that the continuous administration of the progestagen megestrol acetate in low dosage for contraceptive purposes does not produce any significant change in tryptophan metabolism, and also that in a recent study of this steroid it has been shown to have no effect upon glucose tolerance (Adams and Wynn, 1971). In addition, we have found that another 17 α -hydroxyprogesterone derivative, medroxyprogesterone acetate, does not alter tryptophan metabolism (unpublished data).

We are indebted to Professor Victor Wynn for his interest, and for allowing us to study his patients. This work was supported by a grant from the Medical Research Council and by contract no. Ph-43-67-1344 from the United States National Institutes of Health.

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