Effects of oestradiol and vitamin B_6 on tryptophan metabolism in the rat: implications for the interpretation of the tryptophan load test for vitamin B_6 nutritional status

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Effects of oestradiol and vitamin B_6 on tryptophan metabolism in the rat: implications for the interpretation of the tryptophan load test for vitamin B_6 nutritional status

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1. The effects of the administration of oestradiol and vitamin B_6 on tryptophan metabolism in the rat have been assessed by measurement of the release of $^{14}CO_2$ from [^{14}C]tryptophan, in vivo, in order to determine whether, and to what extent, the abnormalities of tryptophan metabolism that are associated with oestrogen administration can be attributed to drug-induced vitamin B_6 deficiency or depletion. Two positional isomers of [^{14}C]tryptophan have been used; [*ring*-2-^{14}C]tryptophan as an index of the activity of tryptophan oxygenase (L-tryptophan: oxygen oxidoreductase (decyclizing), *EC* 1.13.11.11) and [*methylene*-^{14}C]trytophan as an index of the activity of kynureninase (L-kynurenine hydrolase, *EC* 3.7.1.3).

2. The administration of 500 μg oestradiol/kg body-weight led to a reduction in the release of $^{14}CO_2$ from both positional isomers of tryptophan, suggesting that the activities of both tryptophan oxygenase and kynureninase are reduced following oestrogen treatment. The kinetics of the release of $^{14}CO_2$ from [*methylene*-^{14}C]tryptophan after the administration of oestradiol were compatible with competitive inhibition of kynureninase by oestradiol or a metabolite.

3. The administration of 10 mg pyridoxine hydrochloride/kg body-weight also reduced the production of $^{14}CO_2$ from both positional isomers of [^{14}C]tryptophan, suggesting some toxicity of such a high dose of the vitamin.

4. In animals which had received the supplementary dose of vitamin B_6, the administration of oestradiol led to further reduction in the production of $^{14}CO_2$ from [*ring*-2-^{14}C]tryptophan, suggesting a further reduction in the activity of tryptophan oxygenase, and an increase in the production of $^{14}CO_2$ from [*methylene*-^{14}C]tryptophan, but with a delay in the peak of production.

5. These results confirm that there is no induction of tryptophan oxygenase by oestradiol, but rather reduced activity of the enzyme after the administration of a relatively high dose of the hormone. They also confirm that the inhibition of kynureninase by oestrogen metabolites that has been reported previously in partially-purified enzyme preparations also occurs in vivo.

6. It is suggested that the abnormal results of the tryptophan load test that have been reported in women receiving oestrogens, and which have been interpreted as indicating some extent of drug-induced vitamin B_6 deficiency, can be accounted for by the inhibition of tryptophan metabolism by oestrogens or their metabolites. Therefore it seems likely that the practice of administering supplements of vitamin B_6 to women receiving oestrogens may not be appropriate, and indeed may exacerbate the changes in tryptophan metabolism that result from the administration of oestrogens. The tryptophan load test would appear to be unreliable as an index of vitamin B_6 nutritional status in women receiving oestrogens.

The tryptophan load test for vitamin B_6 nutritional status depends on the fact that kynureninase (L-kynurenine hydrolase, *EC* 3.7.1.3) is a pyridoxal phosphate-dependent enzyme and, in vitamin B_6 deficiency, the activity of the enzyme falls as its cofactor is depleted (Knox, 1953). This results in a considerable accumulation in the liver of kynurenine and hydroxykynurenine, the substrates of kynureninase, and an increase in the formation and excretion of kynurenic and xanthurenic acids after the administration of a loading dose of tryptophan. The excretion of these metabolites after the administration of a loading dose of tryptophan is considered to reflect the state of the body's reserves of vitamin B_6 (Coursin, 1964; Price *et al.* 1965).

A number of studies have demonstrated abnormalities of tryptophan metabolism which appear to be the result of vitamin B_6 deficiency or depletion, in women receiving oestrogens as oral contraceptives or menopausal hormone-replacement therapy (Rose & Braidman,

1971; Rose *et al.* 1972; Aylward, 1976). As a result of these studies, a number of preparations have been marketed for sale without prescription, providing relatively large amounts of vitamin B_6 (of the order of 20–50 mg/d, some ten to twenty times greater than the US recommended daily allowance for vitamin B_6) to overcome the supposed oestrogen-induced vitamin deficiency, and hence some of the unwanted side-effects of oestrogen administration. It is doubtful whether such supplementation with large amounts of vitamin B_6 is either appropriate or desirable. Doses of the order of 20 mg of the vitamin/d are required to correct the abnormalities of tryptophan metabolism in women receiving oestrogens (Luhby *et al.* 1971) but studies that have assessed vitamin B_6 nutritional status by metabolic criteria other than the ability to metabolize a test dose of tryptophan, have shown no impairment of vitamin B_6-dependent metabolism as a result of oestrogen administration (Brown *et al.* 1975; Leklem *et al.* 1975).

Previous studies from this laboratory have suggested that the effects of oestrogen administration on tryptophan metabolism are not due to vitamin B_6 depletion, but can be accounted for by inhibition of kynureninase by oestrogen metabolites (Bender & Wynick, 1981). Bender *et al.* (1982) showed that after chronic administration of oestrogens to rats there were changes in tryptophan metabolism that would be compatible either with such simple inhibition or with depletion of tissue reserves of the vitamin, but were unable to demonstrate any effect of oestrogen administration on blood or tissue concentrations of pyridoxal phosphate, or the urinary excretion of the principal metabolite of the vitamin, 4-pyridoxic acid. They concluded that oestrogens do not deplete vitamin B_6.

The present study was undertaken in order to determine whether the inhibition of kynureninase by oestrogen metabolites that has been demonstrated using a partially-purified preparation of the enzyme (Bender & Wynick, 1981) is also important in vivo in response to the administration of oestrogens, and to confirm the finding that oestrogens do not induce new synthesis of tryptophan oxygenase (L-tryptophan: oxygen oxidoreductase (decyclizing), *EC* 1.13.11.11) (Bender *et al.* 1983). The activities of tryptophan oxygenase and kynureninase were determined in vivo by measurement of the release of $^{14}CO_2$ after the administration to rats of either [*ring*-2-^{14}C]tryptophan (for tryptophan oxygenase activity) or [*methylene*-^{14}C]tryptophan (for kynureninase activity). The pathway involved is shown in Fig. 1. The results confirm that oestrogens do not induce tryptophan oxygenase, but rather lead to a slight reduction in the activity of this enzyme (Bender *et al.* 1983), and also that there is inhibition of kynureninase in vivo following the administration of oestradiol. It therefore seems unlikely that the abnormalities of tryptophan metabolism that have been observed in women receiving oestrogens are the result of drug-induced vitamin B_6 depletion, and supplementation of such women with the vitamin would appear to be inappropriate.

METHODS

Female Wistar rats weighing 195–210 g, bred in the Courtauld Institute of Biochemistry, were used. They were allowed free access to standard animal house diet (Diet 86; E. Dixon & Sons, Ware, Herts) at all times, apart from the 4 h period during which exhaled $^{14}CO_2$ was collected.

On the first day of each study, each animal received an intraperitoneal injection of 1 ml ethanol–saline (0·15 M-sodium chloride; 50 ml/l) at 11.30 hours and then the radioactive material, dissolved in 1 ml saline, at 12.30 hours. On the second day of each study the same animals received an intraperitoneal injection of 500 μg oestradiol/kg body-weight in 1 ml ethanol–saline (50 ml/l) and the same radioactive tracer as the previous day, again at 12.30 hours. Animals treated with vitamin B_6 received an intraperitoneal injection of 10 mg pyridoxine hydrochloride/kg body-weight, dissolved in 1 ml saline, at 16.30 hours the day before each experiment with radioactive material. For studies with [^{14}C]tryptophan, animals

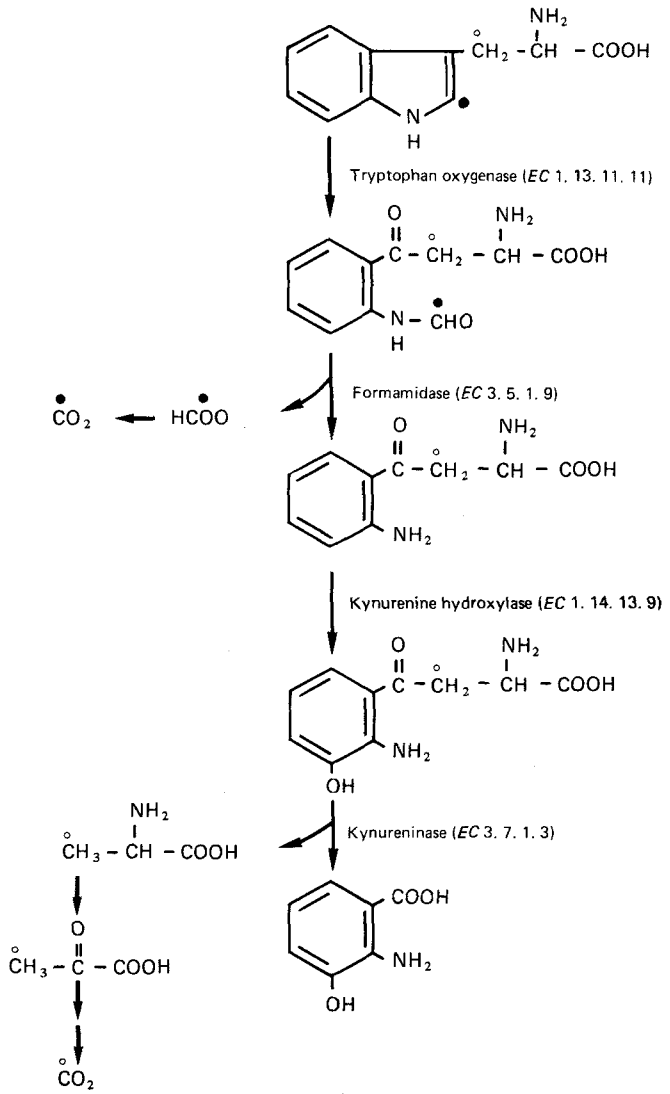

Fig. 1. The oxidative metabolism of tryptophan, showing the production of ^{14}C-labelled carbon dioxide from (●), L-[*ring*-2-^{14}C]tryptophan and (○), L-[*methylene*-^{14}C]tryptophan.

received 50 mg L-tryptophan/kg body-weight, together with a tracer dose of either L-[*ring*-2-^{14}C]tryptophan (Commissariat d'Energie Atomique, Gif-sur-Yvette, France) or L-[*methylene*-^{14}C]tryptophan (Amersham International, Amersham, Bucks) at a specific activity of 0·66 mCi/mmol. For studies with [^{14}C]formate and L-[U-^{14}C]alanine (both from Amersham International) animals received 0·7 μCi of the radioactive material/kg body-weight, undiluted by any additional non-radioactive carrier. Thus, the metabolism of the radioactive material was assessed in the same animal before and after the administration of oestradiol; separate groups of rats were used for each of the four radioactive materials, and separate groups for the experiments in which the animals were pretreated with vitamin B_6.

Immediately after the injection of radioactive material, animals were placed individually

in sealed glass vessels through which moistened air was passed from a small compressor. Air leaving each vessel was bubbled through 2 ml of a solution of Hyamine hydroxide solution (50 g/l methanol–propan-2-ol (1:1, v/v)) to absorb CO_2. Every 10 min the compressor was stopped for about 1 min and the solution of Hyamine hydroxide was replaced. The collection of $^{14}CO_2$ was continued for 4 h after the administration of [^{14}C]tryptophan and for 3 h after [^{14}C]formate or alanine. The solution of Hyamine hydroxide was washed into scintillation-counter vials with a total of 10 ml of a solution of PPO (3 g/l) and POPOP (0·3 g/l) in toluene; radioactivity was measured using a Beckman LS 7500 liquid-scintillation counter equipped with automatic quench correction.

$^{14}CO_2$ production in each 10 min interval (shown in Figs. 2 and 3) was plotted and lines drawn by inspection. Values of the first-order rate-constant for the decay of $^{14}CO_2$ production (k) and the half-time of the decay were calculated from the gradient of the linear plot of log (radioactivity) *v.* time; this gradient was calculated by unweighted least squares regression.

For the studies of urinary excretion of tryptophan metabolites, rats were housed individually in stainless-steel metabolism cages (Acme Metal Co. Inc., Chicago, Ill, USA). On the first day of the experiment, each animal received an intraperitoneal injection of 50 mg L-tryptophan/kg body-weight, dissolved in 5 ml saline, at 09.00 hours. Urine was collected over the next 7 h. During this time the animals had free access to water, but not food. At 16.00 hours, each animal received an intraperitoneal injection of 10 mg pyridoxine hydrochloride/kg body-weight in 1 ml saline; the injection of tryptophan and collection of urine for 7 h from 09.00 hours were repeated the next day. Overnight, all animals had free access to stock diet and water. Urine samples were diluted to 20 ml with water and were stored at −20° until they were required for analysis.

N^1-methyl nicotinamide was measured by the small-scale modification of the alkali-ketone fluorescence method of Carpenter & Kodicek (1950) that has been described previously (Bender, 1980*b*). Xanthurenic and kynurenic acids and kynurenine were separated by ion-exchange chromatography on small columns of Dowex 50 W (H^+) resin (Satoh & Price, 1958); the two acids were determined fluorimetrically (Satoh & Price, 1958) and kynurenine colorimetrically after diazotization and coupling to naphthylene ethylene diamine (Joseph & Risby, 1975). 3-Hydroxykynurenine was measured fluorimetrically by the method of Watanabe *et al.* (1970).

Methyl pyridone carboxamide was measured colorimetrically after conversion to the amine by means of the König reaction, diazotization and coupling to naphthylene ethylene diamine by the following modification of the method of Holman (1954). Potentially-interfering ionized compounds in urine were removed by mixing 5 ml of the diluted urine sample with 500 mg finely-powdered Zerolit DMF mixed-bed ion-exchange resin; after 1 h the resin was removed by centrifugation (2000 *g* for 15 min). This procedure was considerably simpler than the method described by Price (1954) using a column of alternating layers of anion- and cation-exchange resins; the recovery of authentic methyl pyridone carboxamide through the resin treatment was routinely between 98–100%. Portions of the deionized urine (1 ml) were mixed with 250 μl of a freshly-prepared solution of alkaline sodium hypobromite (1 ml of a solution of 125 g bromine and 144 g potassium bromide/l in 10 ml of 0·25 M-sodium hydroxide), and was allowed to stand in the dark at room temperature for 5 min. This was then mixed with 250 μl of a solution of 5 g phenol/l water, and heated to 105° for 20 min in screw top vials. After cooling, samples were mixed with 250 μl 6 M-hydrochloric acid and cooled in ice for 20 min, followed by the addition at 5 min intervals of 200 μl 2·5 g sodium nitrite/l water, 200 μl ammonium sulphamate (100 g/l water) and 200 μl naphthylene ethylene diamine (10 g/l ethanol–water (950 ml/l)). The solutions of $NaNO_2$ and naphthylene ethylene diamine were prepared freshly for each batch of analyses. After standing at

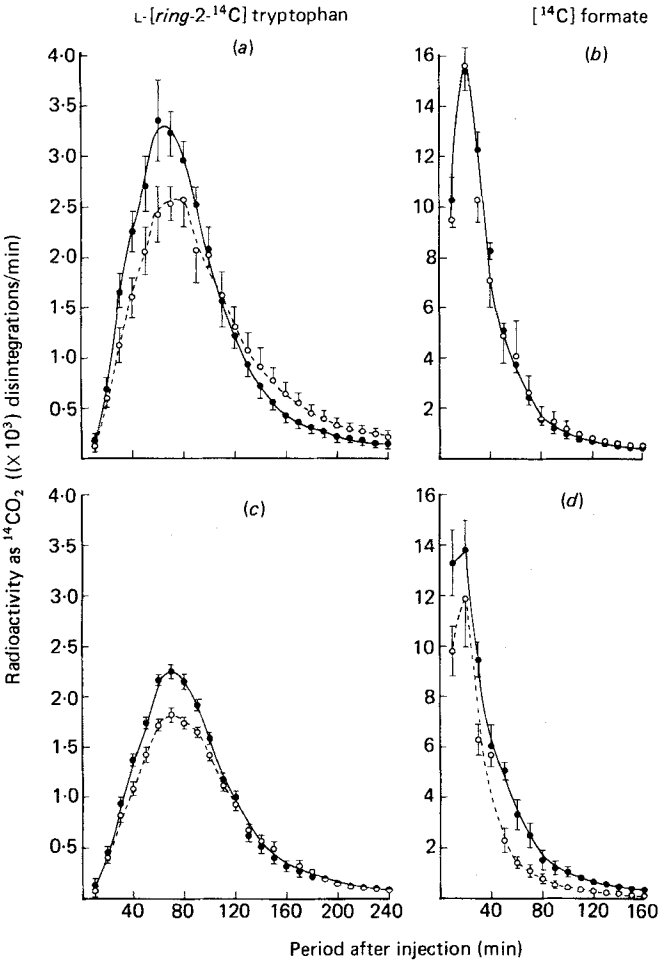

Fig. 2. The effects of oestradiol (500 μg/kg body-weight) on the production of ^{14}C-labelled carbon dioxide from L-[*ring*-2-^{14}C]tryptophan and [^{14}C]formate in (*a*, *b*) control animals and (*c*, *d*) animals pretreated with 10 mg pyridoxine hydrochloride/kg body-weight. (●), Ethanol–saline control; (○), oestradiol in ethanol–saline. Points are mean values with their standard errors, represented by vertical bars, for three or six animals per group, as shown in Table 1.

room temperature for 60 min the absorbance of the product was measured at 590 nm. Authentic methyl pyridone carboxamide for use as a standard was synthesized by the method of Holman & Wiegand (1948).

RESULTS

As can be seen from Fig. 2 and Table 1, the administration of 500 μg oestradiol/kg body-weight led to a reduction in the production of $^{14}CO_2$ from [*ring*-2-^{14}C]tryptophan, and a significant fall in the value of k, the first-order rate-constant for the decay phase of the curve of $^{14}CO_2$ production. A similar effect of oestradiol was observed whether or not the animals had been treated with 10 mg vitamin B_6 the day before the administration of [^{14}C]tryptophan. The administration of vitamin B_6 led to a considerable reduction in the production of $^{14}CO_2$ from [*ring*-2-^{14}C]tryptophan.

Table 1. *The production of ^{14}C-labelled carbon dioxide from [^{14}C]tryptophan, [^{14}C]formate and [^{14}C]alanine in rats treated with oestradiol (500 μg/kg body-weight), pyridoxine hydrochloride (10 mg/kg body-weight; vitamin B_6) or both*

	No. of rats	Radioactivity in $^{14}CO_2$ (($\times 10^3$) disintegrations/min)				Time to peak (min)	Decay of $^{14}CO_2$ production			
		Total		Peak			Rate-constant (($\times 10^{-3}$)/min)		Half-time (min)	
		Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM
L-[*Ring*-2-^{14}C]tryptophan										
Ethanol–saline control	6	28·8	4·1	3·36	0·63	60	18·54	0·52	37·4	1·1
Oestradiol	6	26·6	3·5	2·59	0·26	80	15·9**	0·73	43·6**	2·0
Vitamin B_6	6	19·9*	1·7	2·26	0·09	70	18·36	0·21	37·7	0·4
Vitamin B_6 + oestradiol	6	17·6	1·4	1·83†	0·09	70	17·75†	0·16	39·0†	0·4
[^{14}C]formate										
Ethanol–saline control	6	66·2	3·16	15·42	0·66	20	27·0	1·2	25·7	1·2
Oestradiol	3	64·9	9·5	15·52	0·49	20	24·4	1·1	28·4	1·3
Vitamin B_6	3	62·3	14·1	13·88	2·29	20	24·8	0·4	27·9	0·4
Vitamin B_6 + oestradiol	3	39·9†	3·1	11·98††	1·81	20	27·6††	0·53	25·1††	0·5
L-[*Methylene*-^{14}C]tryptophan										
Ethanol–saline control	6	24·6	1·7	2·18	0·18	80	13·22	0·12	52·4	0·5
Oestradiol	6	27·6	1·6	2·07	0·67	110	12·03***	0·19	57·6***	0·9
Vitamin B_6	6	25·3	2·0	1·81	0·47	110	9·77***	0·14	70·9***	1·0
Vitamin B_6 + oestradiol	6	33·9††	2·8	2·23	0·85	130	7·84†††	0·13	88·4†††	1·5
L-[U-^{14}C]alanine										
Ethanol–saline control	6	57·9	11·4	8·69	1·9	20	15·89	0·57	43·6	1·6
Oestradiol	3	71·3	8·9	9·5	0·9	20	16·45	0·72	42·1	1·9
Vitamin B_6	3	37·6*	5·8	7·85	1·0	20	17·48*	0·51	39·6*	1·2
Vitamin B_6 + oestradiol	3	27·6	3·5	5·06††	0·41	20	14·26†††	0·22	48·6†††	0·8

Significance of differences from ethanol–saline controls (by *t* test): * $0{\cdot}1 \geqslant P \geqslant 0{\cdot}05$, ** $0{\cdot}05 \geqslant P \geqslant 0{\cdot}001$, *** $P \leqslant 0{\cdot}001$.
Significance of differences from vitamin B_6-treated animals (by *t* test): † $0{\cdot}1 \geqslant P \geqslant 0{\cdot}05$, †† $0{\cdot}05 \geqslant P \geqslant 0{\cdot}001$, ††† $P \geqslant 0{\cdot}001$.

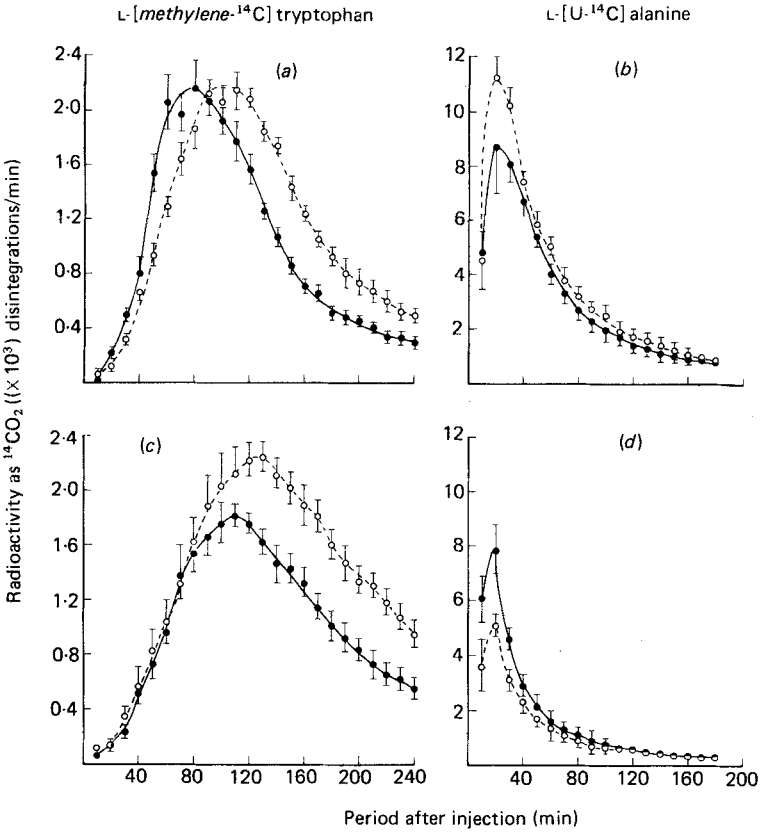

Fig. 3. The effects of oestradiol (500 μg/kg body-weight) on the production of ^{14}C-labelled carbon dioxide from L-[*methylene*-^{14}C]tryptophan and L-[U-^{14}C]alanine in (*a*, *b*) control animals and (*c*, *d*) animals pretreated with 1·0 mg pyridoxine hydrochloride/kg body-weight. (●), Ethanol–saline control; (○), oestradiol in ethanol–saline. Points are mean values with their standard errors, represented by vertical bars, for three or six animals per group, as shown in Table 1.

Neither vitamin B_6 nor oestradiol alone had any effect on the production of $^{14}CO_2$ from [^{14}C]formate. However, in animals which had been pretreated with vitamin B_6, the administration of oestradiol led to a lower total recovery of $^{14}CO_2$ from [^{14}C]formate and a lower peak of $^{14}CO_2$ production, but a higher value of k.

Fig. 3 shows the production of $^{14}CO_2$ from [*methylene*-^{14}C]tryptophan and [U-^{14}C]alanine. There was no significant effect of oestradiol on the total recovery of $^{14}CO_2$ from [*methylene*-^{14}C]tryptophan, or the peak height, but there was a considerable delay in the time of the peak of $^{14}CO_2$ production. This was apparent whether the animals had received vitamin B_6 or not, and was associated with a significant reduction in the value of k. The administration of vitamin B_6 also led to a delay in the production of $^{14}CO_2$ from [*methylene*-^{14}C]tryptophan and a reduction in the value of k.

Oestradiol had no significant effect on the production of $^{14}CO_2$ from [U-^{14}C]alanine in animals that had not received vitamin B_6. Administration of the vitamin led to a reduction in the production of $^{14}CO_2$ and an increase in the value of k; the administration of oestradiol after vitamin B_6 pretreatment led to a further reduction in the total production of $^{14}CO_2$ from [^{14}C]alanine, and a fall in the value of k.

Table 2. *The effects of pyridoxine hydrochloride (vitamin B_6) on the urinary excretion of tryptophan metabolites over 7 h after the intraperitoneal injection of 50 mg L-tryptophan/kg body-weight to female rats weighing 200 ± 5 g*

(Mean values with their standard errors for six animals per group)

Treatment...	Control		After 10 mg pyridoxine HCl/kg body-weight at −17 h	
Metabolite	Mean	SEM	Mean	SEM
Kynurenine (μmol)	130	9	124	11
3-Hydroxykynurenine (μmol)	70	7	88	9
Kynurenic acid (mmol)	1·5	0·2	1·1	0·1
Xanthurenic acid (mmol)	0·67	0·03	0·64	0·04
N^1-Methyl nicotinamide (μmol)	1·52	0·27	0·83**	0·093
Methyl pyridone carboxamide (μmol)	0·45	0·036	0·30**	0·035

Significance of differences from control (by *t* test): ** $0{\cdot}05 \geqslant P \geqslant 0{\cdot}001$.

Table 2 shows the urinary excretion of tryptophan metabolites by rats over a 7 h period after administration of 50 mg L-tryptophan/kg body-weight, before and 17 h after receiving 10 mg pyridoxine hydrochloride/kg body-weight. Vitamin B_6 led to a significant reduction in the excretion of both N^1-methyl nicotamide and methyl pyridone carboxamide, but had no significant effect on the excretion of kynurenine, hydroxykynurenine, kynurenic acid or xanthurenic acid.

DISCUSSION

The production of $^{14}CO_2$ is not a direct consequence of the metabolism of either of the positional isomers of [^{14}C]tryptophan that have been used in this study. With [*ring*-2-^{14}C]tryptophan the result of the activities of tryptophan oxygenase and formylkynurenine formamidase (*EC* 3.5.1.9) is the production of [^{14}C]formate. With [*methylene*-^{14}C]tryptophan the immediate product of kynureninase action is [^{14}C]alanine, which must undergo transamination to pyruvate and then oxidation by way of the citric acid cycle before $^{14}CO_2$ is released. This means that with either substrate the effects of experimental treatments on metabolic reactions occurring later than the enzymes under consideration may affect the results. The effects of oestradiol and vitamin B_6 on the metabolism of [^{14}C]formate and [^{14}C]alanine have therefore been assessed; the results eliminate this possible artefact.

These results show that even the relatively high dose of oestradiol used in the present study (500 μg/kg body-weight) does not cause induction of tryptophan oxygenase, an effect that would be expected to increase the rate of production of $^{14}CO_2$ from [*ring*-2-^{14}C]tryptophan. Induction of tryptophan oxygenase by oestradiol was reported by Patnaik & Sarangai (1980), but Bender *et al.* (1983) were unable to reproduce their findings and suggested that what they had observed was an artifact due to changes in the recovery of kynurenine (the product of tryptophan oxygenase action) as a result of inhibition of kynureninase by oestrogen metabolites. Leonard & Hamburger (1974) suggested that in vivo there might be some increase in the activity of tryptophan oxygenase after oestrogen treatment as a result of increased synthesis of haem, and hence increased saturation of the apo-enzyme with its cofactor. No such effect has been observed in the present study. The present results confirm the previous observation (Bender *et al.* 1983) that after the

administration of oestrogens there is a slight reduction in the activity of tryptophan oxygenase.

Bender & Wynick (1981) showed that oestrone sulphate and glucuronide were competitive inhibitors of kynureninase. They suggested that if such inhibition also occurred in vivo, as a result of the administration of oestrogens, it would explain the abnormalities of tryptophan metabolism that have been observed in women receiving oestrogens (Rose & Braidman, 1971; Rose *et al.* 1972; Aylward, 1976). The present results show that such inhibition of kynureninase does indeed result from the administration of oestrogens and is not ameliorated by the administration of vitamin B_6. The results are compatible with a mechanism of inhibition that is competitive with respect to the substrate; a lower value of the rate constant (k) after the administration of oestradiol and a delay in the time at which the peak of production of $^{14}CO_2$ is achieved, although with eventual attainment of the same peak height as before the administration of oestradiol.

The inhibition of tryptophan metabolism by the administration of a relatively high dose of vitamin B_6 (10 mg/kg body-weight) seems to be the result of an effect at the level of tryptophan oxygenase. No satisfactory explanation can be advanced for this; the enzyme is not known to be pyridoxal phosphate-dependent, although it is inhibited by a number of hydrazine derivatives that form biologically inactive adducts with the vitamin (Young *et al.* 1978; Bender, 1980*a*). The administration of vitamin B_6 does not seem to affect the activity of kynureninase, as determined by the production of $^{14}CO_2$ from [*methylene*-^{14}C]tryptophan, beyond such inhibition of production of $^{14}CO_2$ as can be accounted for by the reduced activity of tryptophan oxygenase. Table 2 shows that there was no change in the urinary excretion of kynurenine, hydroxykynurenine, kynurenic or xanthurenic acids after the administration of vitamin B_6; inhibition of kynureninase would be expected to lead to an increase in the excretion of all of these metabolites. There was, however, a significant reduction in the excretion of two metabolites of the nicotinamide nucleotides, N^1-methyl nicotinamide and methyl pyridone carboxamide, after the administration of vitamin B_6. This presumably reflects a lower rate of synthesis of the nucleotides because of the lower rate of entry of tryptophan into the oxidative pathway. Such an effect may be clinically important in man; Serdaru *et al.* (1981) have reported the development of a pellagra-like neurological condition in two alcoholic patients treated with very large amounts of vitamins B_6 and B_1. In the one patient in whom it was tried, the addition of nicotinamide to the parenteral vitamin mixture led to recovery.

Previous studies from this laboratory (Bender & Wynick, 1981; Bender *et al.* 1982) have suggested that the abnormalities of tryptophan metabolism that have been reported in women receiving oestrogens (Rose & Braidman, 1971; Rose *et al.* 1972; Aylward, 1976) are not the result of oestrogen-induced vitamin B_6 deficiency or depletion, but rather the result of direct inhibition of tryptophan metabolism by oestrogens or their metabolites. The results of the present study confirm this, and suggest that the administration of relatively large supplements of vitamin B_6 may not be an appropriate treatment for the side-effects of oestrogens used as contraceptives or as menopausal hormone-replacement therapy. Indeed, the inhibition of tryptophan metabolism by the administration of large amounts of the vitamin demonstrated here suggests that such supplementation may be undesirable.

Coon & Nagler (1969) showed that in patients suffering from a variety of diseases affecting the secretion of steroid hormones, and even under conditions of increased secretion of glucocorticoids as a result of the stress of severe illness, the tryptophan load test gave misleading results, indicative of vitamin B_6 deficiency, as a result of changes in tryptophan metabolism caused by changes in hormone status, although other indices of vitamin B_6 status were normal. The present study, and previous studies from this laboratory (Bender

& Wynick, 1981; Bender *et al.* 1982) suggest that in women receiving oestrogens as contraceptives or as menopausal hormone-replacement therapy the tryptophan load test may similarly be unreliable as an index of vitamin B_6 nutritional status.

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