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METABOLISM OF AN ORAL TRYPTOPHAN LOAD. I: EFFECTS OF DOSE AND PRETREATMENT WITH TRYPTOPHAN

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1 The metabolism of three oral doses of L-tryptophan (50, 25 and 10 mg/kg) in healthy young males has been investigated.

2 There was a linear relationship between both peak height and area under curve of the total plasma tryptophan concentrations whilst the relationship between these parameters and plasma free tryptophan was hyperbolic.

3 Before the tryptophan load about 85% of plasma tryptophan was bound to albumin. As plasma tryptophan concentrations increased there was a hyperbolic increase in free tryptophan. Scatchard analysis revealed 1.4 binding sites/molecule albumin with a dissociation constant (K_d) of 57.9 μM. Following administration of L-tryptophan (50 mg/kg) twice daily for 7 days there was no alteration in the number of binding sites but the dissociation constant (K_d) had decreased to 30.9 μM.

4 L-Tryptophan (50 mg/kg twice daily for 7 days) markedly increased both basal plasma total and free tryptophan. However following a further load the total tryptophan curve was comparable to that seen after acute administration. The plasma free tryptophan curve was lowered relative to that seen after an acute dose.

5 Increasing the tryptophan dose shortened the plasma half-life and decreased the volume of distribution and the rate of clearance. Longer term tryptophan administration had no significant effect on plasma half-life or volume of distribution but did decrease the rate of plasma clearance.

6 The plasma kynurenine concentration increased with increasing tryptophan dose and basal concentrations increased markedly after longer term tryptophan administration.

7 Tryptophan administration either acutely or chronically produced little change in urinary tryptophan or 5-hydroxyindole acetic acid excretion. Urinary kynurenine and indole acetic acid excretion increased with increasing doses of tryptophan.

8 Data are discussed in relation to the administration of L-tryptophan for the treatment of depression.

Introduction

Tryptophan is the aromatic amino acid precursor of the neurotransmitter 5-hydroxytryptamine (5-HT or serotonin). Since the rate limiting enzyme for 5-HT synthesis, tryptophan hydroxylase, is not normally saturated with tryptophan, increasing its availability to the brain increases brain 5-HT synthesis (Green & Sawyer, 1966; Moir & Eccleston, 1968; Grahame-Smith, 1971). Tryptophan exists in plasma in two forms, that bound to albumin and the 'free' or non-albumin bound. The degree to which brain tryptophan concentration depends on plasma free tryptophan has been the subject of some controversy (reviewed by Green, 1978; Curzon, 1979). However, there is good evidence that rat plasma free tryptophan is a better predictor of brain tryptophan concentration than plasma total tryptophan, under both physiological and pathological circumstances (Bloxam & Curzon, 1978; Bloxam, Tricklebank,

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Patel & Curzon, 1980; Mans, Biebuyck, Saunders, Kirsch & Hawkins, 1979) and also after tryptophan administration (Gessa & Tagliamonte, 1974). Evidence in man is consistent, albeit less direct (Curzon, 1979).

Tryptophan has been used for several years in the treatment of depression, but its efficacy is, however, still controversial. It has been suggested to be as effective as ECT (Coppen, Shaw, Herzberg & Maggs, 1967) and no better than placebo (Carroll, Mowbray & Davies, 1970; Murphy, Baker, Goodwin, Miller, Kotin & Bunney, 1974). Whilst one recent study has indicated that the efficacy of tryptophan is similar to that of imipramine (Jensen, Fruensgaard, Ahlfors, Pihkanan, Tuomikoski, Ose, Dencker, Lindberg & Nagy, 1975) another has suggested that its value alone is small, but that it potentiates other antidepressant drugs such as chlorimipramine or monoamine oxidase inhibitors (d'Elia, Hanson & Raotma, 1978).

A major problem in administering tryptophan involves the optimum dose to be given. The major route of tryptophan metabolism is the kynurenine pathway, which accounts for around 98% of tryptophan metabolism (Hagen & Cohen, 1966) and which is initiated by tryptophan pyrrolase, an enzyme which is induced by its substrate, tryptophan (Knox & Auerbach, 1955). The object of therapeutic tryptophan administration, however, is to increase metabolism down another pathway leading to 5-HT. It has been suggested that high doses of tryptophan may be self-defeating because pyrrolase induction might divert so much tryptophan on the kynurenine pathway, producing a relative deficit of precursor, that the amount available for 5-HT synthesis would be less than at lower tryptophan doses (Young & Sourkes, 1977). Certainly, it may be relevant that when pyrrolase is induced by injecting rats with hydrocortisone brain 5-HT synthesis is decreased (Green & Curzon, 1968; Green, Sourkes & Young, 1975; Green, Woods, Knott & Curzon, 1975).

Despite awareness of these problems there has been almost no investigation of the effect of dose and duration of tryptophan treatment on its peripheral metabolism in humans. In this investigation we have examined tryptophan metabolism following 3 different single doses of tryptophan and following longer term administration of the amino acid.

Methods

Subjects

The subjects investigated were healthy young male members of staff (mean age 28 years; range 23–35), none of whom was taking any drugs or alcohol during the study. The investigation had been approved by the hospital ethics committee.

Protocol

A 24 h urine collection (over 10 ml glacial acetic acid) was taken from 10.00 h–10.00 h, the bladder having been emptied immediately prior to the start of the collection. At the end of this collection period (10.00 h) a 10 ml blood sample was taken from the antecubital vein into a lithium heparin tube. A second 24 h urine collection was started and the subject was given an oral L-tryptophan load of either 50, 25 or 10 mg/kg. This was given as Optimax tablets (without vitamin supplement; E. Merck, Alton, Hants) which were powdered, and the powder suspended in an orange drink, in an attempt to minimise individual variation in drug absorption from the gut. Further venous blood samples (10 ml) were taken at 11.00 h, 12.00 h, 13.00 h, 15.00 h and 16.00 h. Subjects were not fasted before administration of the load and were allowed coffee, tea and lunch during the experiment.

In the study on the effect of longer term tryptophan administration on the metabolism of the amino acid, subjects took L-tryptophan tablets (Optimax, without vitamin supplement) at a dose of 50 mg/kg twice daily (at 10.00 h and 22.00 h) for 7 days. On Day 8 they were given L-tryptophan (50 mg/kg) at 10.00 h as crushed Optimax tablets in an orange drink and the protocol continued as described above.

Analytical procedures

Blood was centrifuged at 2500 *g* for 5 min, plasma removed and kept at −20°C until analysis of tryptophan and kynurenine. Total urine volumes were measured and aliquots kept at −20°C for subsequent analysis of tryptophan, kynurenine, 5-hydroxyindole acetic acid (5-HIAA) and indole acetic acid (IAA).

Total plasma and urine tryptophan concentrations were measured by the method of Denckla & Dewey (1967). Plasma free tryptophan was measured as described by Bloxam, Hutson & Curzon (1977). Plasma and urine kynurenine were measured by the method of Joseph & Risby (1975) but scaled down by 50% and with the perchloric acid/tiron step used for both the plasma and urine measurement. Urinary 5-HIAA was determined by the method of Udenfriend, Weissbach & Brodie (1958) and urinary IAA by the method of Coppen, Shaw, Malleson, Eccleston & Grundy (1965) except that all volumes were reduced by 50%.

Mathematical methods

The half-times of the terminal exponentials of tryptophan elimination from the plasma were calculated by least-squares linear regression using a Hewlett-Packard Model 9810A desk-top computer. The areas under the curves (AUC) of the plasma tryptophan concentration *v* time curve were calculated using the

trapezoidal rule for the first part of the curve above basal concentrations, and integration of the terminal exponential curve for the time to basal concentrations. The use of these calculations assumes that basal concentrations would remain constant during the period of study. In the case of the load following longer term administration, it was assumed that the raised basal concentration would normally be constant and the AUC was calculated until the expected time of the next dose 12 h later.

Plasma clearance was calculated using the equation:

$$\text{Clearance} = \text{Dose/AUC}$$

and apparent volume of distribution (V_d) using the equation:

$$V_d = \text{Dose/AUC} \times \beta$$

where $\beta = \ln 2/T_{1/2}$.

Due to a printing error previous values (Green, Bloomfield, Woods & Seed, 1978) for clearance read 0.716 and 0.792 and should have read 2.716 and 2.792. Results obtained in the current investigation for clearance, V_d and $T_{1/2}$ are similar to this corrected result and the other values previously obtained.

Comparison between the groups were made by analysis of variance.

Results

Total and free plasma tryptophan concentrations following various oral doses of tryptophan

The change in total plasma tryptophan concentration in male subjects following a dose of 50 mg/kg was essentially identical to that seen in young females previously (Green *et al.*, 1978). Lower doses caused a correspondingly smaller plasma tryptophan peak (Figure 1a). A similar pattern was seen in the free plasma tryptophan concentration (Figure 1b). However, when a plot of dose vs peak height was made, it was seen that whilst the relationship between the peak total tryptophan concentration and dose was linear, that of the peak free tryptophan and dose was hyperbolic (Figure 2b). There were similar relationships between total tryptophan and AUC and free tryptophan and AUC (Figure 2a).

Using mean, free and total tryptophan values at different times following the 50 mg/kg dose of tryptophan a plot was constructed of the relationship between free and bound tryptophan. At basal plasma tryptophan concentrations it was found that about 85% of the tryptophan was albumin-bound. As the concentration of tryptophan increased, there was a hyperbolic increase in free tryptophan (Figure 3). A Scatchard binding plot of this data reveals a straight

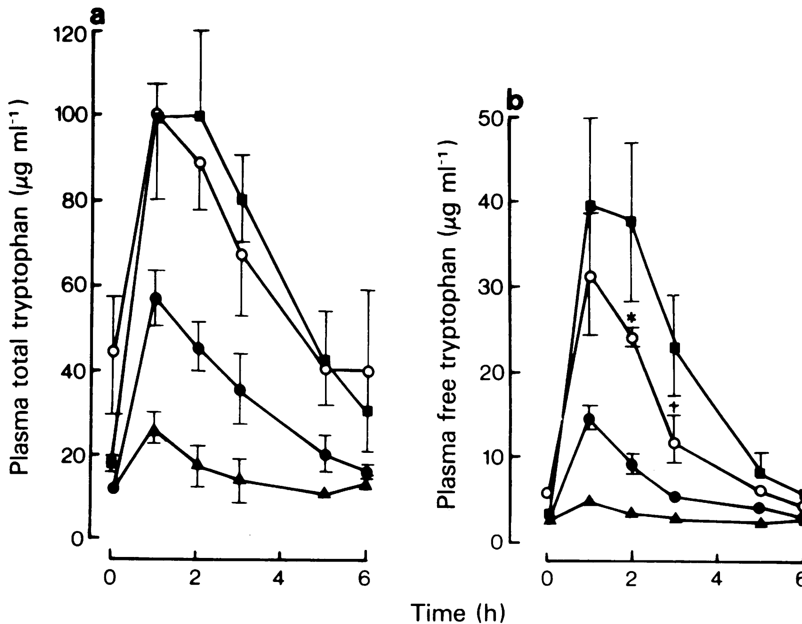

Figure 1 Plasma tryptophan concentration following various doses of L-tryptophan. Results show (a) the total tryptophan and (b) free tryptophan concentration following an acute L-tryptophan dose of 10 mg/kg (▲, n = 4), 25 mg/kg (●, n = 5), 50 mg/kg (■, n = 7) and following a dose of 50 mg/kg after 7 days pretreatment with L-tryptophan (50 mg/kg twice daily) (○, n = 4). Results reported as mean ± s.d. Response following chronic L-tryptophan different from acute dose of 50 mg/kg * $P < 0.05$, † $P < 0.02$.

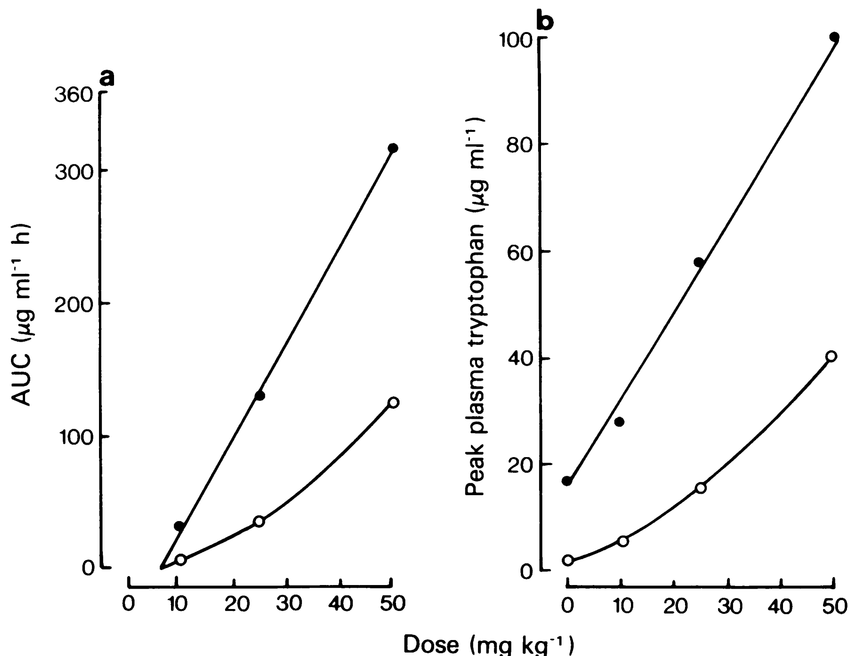

Figure 2 Relationship between (a) the area under the plasma tryptophan concentration curve and dose of tryptophan and (b) peak plasma tryptophan concentration and dose of tryptophan for total (●) and free (○) tryptophan. Data calculated for values shown in Figure 1.

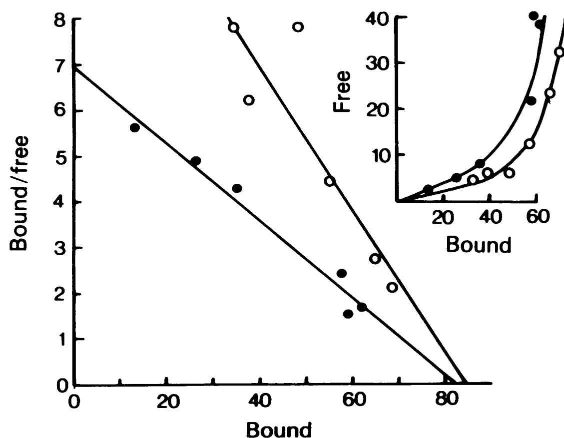

Figure 3 Relationship between free and bound tryptophan concentration at increasing concentrations of total tryptophan in the plasma, following acute (●) and chronic (○) L-tryptophan (50 mg/kg) administration; shown in inset graph. Scatchard analysis of these data shown in major figure. The maximum tryptophan bound is between 82–85 μg/ml plasma, giving 1.34 tryptophan binding sites per mol albumin (see **Results**). The dissociation constant (K_d) of tryptophan for the binding sites is 57.9 μM in the acute study and 30.9 μM after chronic L-tryptophan administration. Acute administration $r = 0.91$, chronic administration $r = 0.94$, lines significantly different $P < 0.001$.

line with a maximum binding capacity of 83 μg tryptophan/ml plasma (0.41 μmol tryptophan/ml plasma) and a dissociation constant (K_d) of 57.9 μM and 1.4 binding sites for tryptophan/molecule albumin (assuming 34 g albumin/litre plasma (Biology Data Book, 1974)).

When subjects had been given tryptophan (50 mg/kg twice daily) for 7 days the basal total plasma tryptophan and free tryptophan concentrations were considerably raised. However following a further loading dose of 50 mg/kg tryptophan, subsequent plasma total tryptophan values were comparable to those found after a single dose (Figure 1a). Free tryptophan values, on the other hand, were lower than found after a single dose with significant differences at 2 h and 3 h (Figure 1b). Scatchard analysis of the data obtained for plasma free and total tryptophan following chronic tryptophan administration revealed that there was no alteration of the total number of binding sites but that the Kd of the site had decreased markedly to 30.9 μM (Figure 3). Parameters are shown in Table 1. It was found that increasing the dose of oral tryptophan shortens the plasma half-life of total tryptophan and decreases both the volume of distribution and the rate of clearance. As shown in Figure 1b the AUC increases with the dose. Longer term tryptophan administration has no significant effect on $T_{1/2}$ or volume of distribution but does decrease the rate of plasma clearance.

Table 1 Plasma half-life ($T_{1/2}$), plasma clearance, area under the curve of the plasma concentration versus time (AUC) and apparent volume of distribution (V_d) of tryptophan following an oral tryptophan load

	Dose (mg kg⁻¹)	n	$T_{1/2}$ *(h)*	*AUC (μg ml⁻¹ h)*	V_d *(l kg⁻¹)*	*Clearance (ml min⁻¹ kg⁻¹)*
Total						
	10	4	4.34±1.84*	31± 17§	2.27±1.01§	7.57±0.65§
	25	4	2.83±0.07*	130± 30†	0.80±0.17**	3.30±0.65
	50	7	2.31±0.42	318± 48	0.54±0.07	2.67±0.43
	50 (following 50 twice daily) for 7 days	4	2.83±0.58	501±123	0.54±0.18	1.74±0.47†
Free						
	10	4	4.43±1.21*	7± 2§	7.33±0.55§	21.35±4.70§
	25	4	2.51±0.53*	35± 14*	2.77±0.85**	13.22±4.38*
	50	7	1.40±0.14	113± 29	0.90±0.25	7.80±2.27
	50 (following 50 twice daily) for 7 days	4	1.72±0.13	99± 14	1.26±0.21	8.52±1.29

Values calculated from total tryptophan measurements and free (non-albumin bound) tryptophan. Details of mathematical methods given in **Methods** section. Results shown mean ± s.d. of the observations (n = number of subjects).
Different from acute tryptophan dose (50 mg/kg⁻¹) * $P<0.05$, ** $P<0.01$, † $P<0.02$, § $P<0.001$.

In general the data calculated from the free tryptophan measurements are similar except that the apparent rate of clearance in subjects given tryptophan for a week was not statistically different from subjects taking tryptophan acutely (Table 1).

Plasma kynurenine concentrations following various oral doses of L-tryptophan

Tryptophan administration increases the plasma concentration of kynurenine (Figure 4) and this increase is approximately proportional to the dose given. Following longer term tryptophan administration basal and peak kynurenine concentrations are markedly elevated (Figure 4).

Urinary tryptophan and kynurenine following various oral doses of tryptophan

Administration of oral tryptophan produces a small increase in urinary tryptophan excretion in the 24 h following the load. However this increase is small (about 5 mg) and apparently not dose-related (Table 2). Pretreatment with tryptophan (50 mg/kg twice daily) for 1 week does not increase this excretion (Table 2).

Low oral doses of tryptophan (10 or 25 mg/kg) have little effect on kynurenine excretion, whereas an oral dose of 50 mg/kg results in nearly a ten-fold rise in kynurenine excretion (Table 2). This excretion is increased further in subjects taking tryptophan for the previous week (Table 2).

Table 2 The urinary excretion of tryptophan and some tryptophan metabolites following various doses of L-tryptophan

Dose (mg kg⁻¹)	n	*Tryptophan*	*Kynurenine*	*5-HIAA*	*IAA*
—	6	7.6±2.2	1.55± 0.39	4.9±2.1	4.88±0.99
10	4	10.4±1.3	4.1 ± 3.1	2.4±0.7	3.76±2.54
25	4	14.4±6.1	3.9 ± 1.6	2.4±0.4	9.6 ±6.24
50	6	12.3±3.1	13.6 ± 6.2	6.3±4.5	15.09±2.19
50 (following 50 twice daily) for 7 days	4	13.7±7.6	55 ±26	3.6±2.2	4.31±0.92

All values reported as mg compound excreted/24 h and shown as mean ± s.d. of the number of observations shown in column 2. 5-HIAA: 5-hydroxyindole acetic acid, IAA: indole acetic acid.

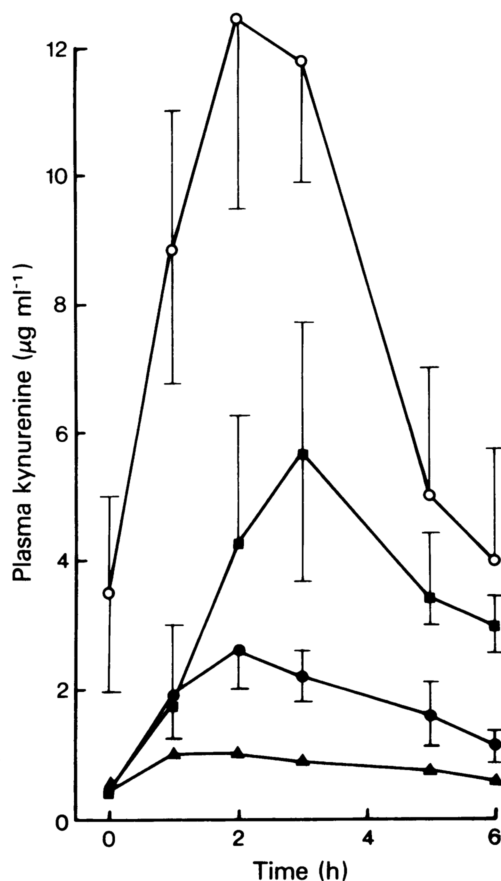

Figure 4 Plasma kynurenine concentration following oral L-tryptophan. Results show plasma kynurenine during the 6 h following an acute L-tryptophan dose of 10 mg/kg (▲), 25 mg/kg (●), 50 mg/kg (■) and following a dose of 50 mg/kg after 7 days pretreatment with L-tryptophan (50 mg/kg twice daily) (○). Number of subjects as shown in Figure 1. Results reported as mean ± s.d. Plasma kynurenine following chronic L-tryptophan administration different from acute L-tryptophan dose (50 mg/kg) different at a level of statistical significance of $P < 0.02$ or better at all time points.

Urinary 5-HIAA and IAA excretion following various oral doses of tryptophan

In agreement with previous results (Green *et al.*, 1978), oral administration of tryptophan did not significantly alter urinary 5-HIAA excretion (Table 2).

Urinary IAA excretion did not increase after giving L-tryptophan (10 mg/kg), but increased in proportion to dose after giving 25 mg/kg and 50 mg/kg (Table 2). However when tryptophan had been taken for the previous week a subsequent oral tryptophan load failed to increase urinary IAA excretion above the original basal levels (Table 2).

Discussion

Increasing doses of L-tryptophan lower both the volume of distribution and the plasma clearance rate of the amino acid. The decrease in volume of distribution suggests a saturation of the compartments into which tryptophan is normally transported, presumably various body tissues. Whilst animal data have not suggested that the brain compartment saturates even at higher doses of tryptophan than used here (Grahame-Smith, 1971) it is certainly possible that other tissues, such as muscle, would saturate and thereby alter the volume of distribution. The fact that the plasma clearance rate also decreases with increasing dose would indicate that metabolism is also saturable, which argues against any considerable induction of the main degradative enzyme tryptophan pyrrolase as a result of administering doses of up to 50 mg/kg of its substrate. While plasma half-life is shorter at higher concentrations, this results from a greater fall in V_d than clearance with increasing dose and does therefore not reflect faster catabolism. It should be pointed out that our value for $T_{1/2}$ following 50 mg/kg and calculated from total tryptophan data was very similar to that found previously (Green *et al.*, 1978; Domino & Krause, 1974).

Pretreatment with L-tryptophan (50 mg/kg) twice daily for a week did not increase the rate of metabolism of a subsequent tryptophan load; indeed the total tryptophan clearance rate decreased further following pretreatment although this was not seen in the analysis of the free tryptophan data. Whatever the relative contributions of plasma free and total tryptophan concentrations are to central tryptophan metabolism, it is useful to have values for both as the bound component must at the least be of importance as a plasma tryptophan store. In this regard it is interesting that the binding affinity for tryptophan to plasma albumin increased after chronic treatment, although the number of binding sites was unchanged. McMenamy & Oncley (1958) demonstrated the specificity of this site for tryptophan binding. Why its dissociation constant should change following repeated tryptophan administration is impossible to answer at present. The binding of tryptophan to the site is saturable in agreement with previous reports (Curzon, Friedel, Kantamanini, Greenwood & Lader, 1974; Curzon & Greenwood, 1975) and the data suggest around 1 binding site/molecule albumin, in good agreement with previous estimates obtained *in vitro* (McMenamy & Oncley, 1958) and *in vivo* (Curzon *et al.*, 1974).

The V_d following pretreatment with tryptophan was the same as that seen after a single acute dose of L-tryptophan (50 mg/kg). It appears, therefore, that a single administration of this dose saturates the other compartments.

A small increase in urinary tryptophan excretion

was seen following acute or chronic tryptophan administration but this was not dose dependent. Kynurenine excretion increased only after the highest tryptophan dose studied (50 mg/kg) and was much higher when this was given following longer term tryptophan treatment (Table 2). This may well reflect a relative saturation of the kynurenine pathway following longer term treatment or a relative vitamin B_6 deficiency, since many of the degradative enzymes are pyridoxal dependent (see Green *et al.*, 1978). Such an interpretation is supported by the failure of a tryptophan load to increase IAA excretion following longer term tryptophan administration since the decarboxylase enzyme which forms tryptamine (the IAA precursor) is also a B_6 dependent enzyme. The increase in IAA following higher doses of tryptophan has been observed previously (Green *et al.*, 1978).

The failure of tryptophan loads to increase urinary 5-HIAA was also observed in our previous study (Green *et al.*, 1978) and may suggest that human peripheral 5-HT metabolism (unlike brain metabolism) cannot be accelerated by precursor loading. In the rat L-tryptophan administration (50 mg/kg i.p.) does increase plasma 5-HT and 5-HIAA (Gal, Young & Sherman, 1978) and high protein intake has been shown to increase urinary 5-HIAA (Nomura, Colmenares & Wurtman, 1977).

The high plasma kynurenine concentration attained during chronic tryptophan treatment is of particular interest as Green & Curzon (1970) and Kiely & Sourkes (1972) both found that kynurenine interfered with tryptophan transport into rat brain. The experiments of Gal *et al.* (1978) in the rat suggest that the plasma concentrations observed in the current study might well cause a lowering of the amount of tryptophan transported into the brain. Recent findings by Singleton & Marsden (1979) are also consistent with less effective tryptophan transport to the brain when the amino acid is given chronically. They found that the rise of brain tryptophan in mice after 3 days on a high tryptophan diet largely disappeared after 18 days on the diet.

Young & Sourkes (1977) explained conflicting reports on the anti-depressant properties of tryptophan in terms of a 'therapeutic window', suggesting that higher doses are ineffective. Various mechanisms by which this might occur have been suggested (Chouinard, Young, Annable & Sourkes, 1979). It is possible that high plasma kynurenine concentrations occurring during chronic tryptophan treatment may be at least partly responsible. If this is so it might be advantageous to give the drug together with either a pyridoxal supplement to facilitate destruction of kynurenine or with tryptophan pyrrolase inhibitors. In the case of the latter proposition, however, it is uncertain whether increased therapeutic efficacy is achieved (see Green, Aronson, Curzon & Woods, 1980).

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References

BIOLOGY DATA BOOK (1974). Chemical composition and physical properties of lymph: mammals. Biology Data Book **3**, *Fed. Am. Soc. exp. Biol.*, Bethesda, Md.

BLOXAM, D.L. & CURZON, G. (1978). A study of proposed determinants of brain tryptophan concentration in rats after portocaval anastomosis or sham operation. *J. Neurochem.*, **31**, 1255–1263.

BLOXAM, D.L. HUTSON, P.H. & CURZON, G. (1977). A simple apparatus for ultrafiltration of small volumes: application to the measurement of free and albumin-bound tryptophan in plasma. *Analyt. Biochem.*, **83**, 130–142.

BLOXAM, D.L., TRICKLEBANK, M.D., PATEL, A.J. & CURZON, G. (1980). Effects of albumin, amino acids and clofibrate on the uptake of tryptophan by the rat brain. *J. Neurochem.*, **34**, 43–49.

CARROLL, B.J., MOWBRAY, R.M. & DAVIES, B. (1970). Sequential comparison of L-tryptophan with ECT in severe depression. *Lancet*, **i**, 967–969.

CHOUINARD, G., YOUNG, S.N., ANNABLE, L. & SOURKES, T.L. (1979). Tryptophan-nicotinamide, imipramine and their combination in depression. A controlled study. *Acta Psychiat. Scand.*, **59**, 395–414.

COPPEN, A., SHAW, D.M., HERZBERG, B. & MAGGS, R. (1967). Tryptophan in the treatment of depression. *Lancet*, **ii**, 1178–1180.

COPPEN, A., SHAW, D.M., MALLESON, A., ECCLESTON, E. & GRUNDY, E.G. (1965). Tryptamine metabolism in depression. *Br. J. Psychiat.*, **111**, 993–998.

CURZON, G. (1979). Relationship between plasma, CSF and brain tryptophan. *J. Neural Transm., Suppl. 15*, 81–92.

CURZON, G., FRIEDEL, J., KANTAMANINI, B.D., GREENWOOD, M.H. & LADER, M.H. (1974). Unesterified fatty acids and the binding of tryptophan in human plasma. *Clin. Sci. mol. Med.*, **47**, 415–424.

CURZON, G. & GREENWOOD, M.H. (1975). Plasma protein binding: free and bound tryptophan. In *Neuropsychopharmacology*. Excerpta Medica Int. Congr. Series **359**, 108–116. Amsterdam: Elsevier.

d'ELIA, G., HANSON, L. & RAOTMA, H. (1978). L-tryptophan and 5-hydroxytryptophan in the treatment of depression. A review. *Acta Psychiat. Scand.*, **57**, 239–252.

DENCKLA, W.D. & DEWEY, H.K. (1967). The determination of tryptophan in plasma, liver and urine. *J. lab. clin. Med.*, **69**, 160–169.

DOMINO, E.F. & KRAUSE, R.R. (1974). Plasma tryptophan tolerance curves in drug free normal controls, schizophrenic patients and prisoner volunteers. *J. Psychiat. Res.*, **10**, 247–261.

GAL, E.M., YOUNG, R.B. & SHERMAN, A.D. (1978). Tryptophan loading: consequent effects on the synthesis of kynurenine and 5-hydroxyindoles in rat brain. *J. Neurochem.*, **31**, 237–244.

GESSA, G.L. & TAGLIAMONTE, A. (1974). Serum free tryptophan: Control of brain concentration of tryptophan and of synthesis of 5-hydroxytryptamine. In *Aromatic amino acids in the brain.* Ciba Foundation Series, **22**, 207–216. Amsterdam: Elsevier.

GRAHAME-SMITH, D.G. (1971). Studies *in vivo* on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with a monoamine oxidase inhibitor and L-tryptophan. *J. Neurochem.*, **18**, 1053–1066.

GREEN, A.R. (1978). The effects of dietary tryptophan and its peripheral metabolism on brain 5-hydroxytryptamine synthesis and function. *Essays in Neurochem. Neuropharmac.*, **3**, 103–127. Chichester: J. Wiley & Sons.

GREEN, A.R., ARONSON, J.K., CURZON, G. & WOODS, H.F. (1980). Metabolism of an oral tryptophan load. II. Effect of pretreatment with the putative tryptophan pyrrolase inhibitors nicotinamide or allopurinol. *Br. J. clin. Pharmac.* **10**, 611–615.

GREEN, A.R., BLOOMFIELD, M.R., WOODS, H.F. & SEED, M. (1978). Metabolism of an oral tryptophan load by women and evidence against the induction of tryptophan pyrrolase by oral contraceptives. *Br. J. clin. Pharmac.*, **5**, 233–241.

GREEN, A.R. & CURZON, G. (1968). Decrease of 5-hydroxytryptamine in the brain provoked by hydrocortisone and its prevention by allopurinol. *Nature, Lond.*, **220**, 1095–1097.

GREEN, A.R. & CURZON, G. (1970). The effect of tryptophan metabolites on brain 5-hydroxytryptamine metabolism. *Biochem. Pharmac.*, **19**, 2061–2028.

GREEN, H. & SAWYER, J.L. (1966). Demonstration, characterisation and assay procedure of tryptophan hydroxylase in rat brain. *Analyt. Biochem.*, **15**, 53–64.

GREEN, A.R., SOURKES, T.L. & YOUNG, S.N. (1975). Liver and brain tryptophan metabolism following hydrocortisone administration to rats and gerbils. *Br. J. Pharmac.*, **53**, 287–292.

GREEN, A.R., WOODS, H.F., KNOTT, P.J. & CURZON, G. (1975). Factors influencing the effect of hydrocortisone on rat brain tryptophan metabolism. *Nature, Lond.*, **225**, 170.

HAGEN, P.B. & COHEN, L.H. (1966). Biosynthesis of indolealkylamines: phsyiological release and transport of 5-hydroxytryptamine. In *Handbook of Experimental Pharmacology,* ed. Erspamer, V. **19**, 182–201. Berlin: Springer-Verlag.

JENSEN, K., FRUENSGAARD, K., AHLFORS, U.G., PIHKANAN, T.A., TUOMIKOSKI, S., OSE, E., DENCKER, S.J., LINDBERG, D. & NAGY, A. (1975). Tryptophan/imipramine in depression. *Lancet*, **ii**, 920.

JOSEPH, M. & RISBY, D. (1975). The determination of kynurenine in plasma. *Clinica Chim. Acta*, **63**, 197–204.

KIELY, M.E. & SOURKES, T.L. (1972). Transport of L-tryptophan into slices of rat cerebral cortex. *J. Neurochem.*, **19**, 2863–2872.

KNOX, W.E. & AUERBACH, V.H. (1955). The hormonal control of tryptophan peroxidase in the rat. *J. biol. Chem.*, **214**, 307–313.

MANS, A.M., BIEBUYCK, J.F., SAUNDERS, S.J., KIRSCH, R.E. & HAWKINS, R.A. (1979). Tryptophan transport across the blood-brain barrier during acute hepatic failure. *J. Neurochem.*, **33**, 409–418.

McMENAMY, R.H. & ONCLEY, J.L. (1958). The specific binding of L-tryptophan to serum albumin. *J. biol. Chem.*, **233**, 1436–1447.

MOIR, A.T.B. & ECCLESTON, D. (1968). The effects of precursor loading in the cerebral metabolism of 5-hydroxyindoles. *J. Neurochem.*, **15**, 1093–1108.

MURPHY, D.L., BAKER, M., GOODWIN, F.K., MILLER, H., KOTIN, J. & BUNNEY, W.E. (1974). L-Tryptophan in affective disorders: indoleamine changes and differential clinical effects. *Psychopharmacology*, **34**, 11–20.

NOMURA, M., COLMENARES, J.L. & WURTMAN, R.J. (1977). Effect of dietary protein on urinary 5-hydroxyindoleacetic acid levels. *J. Neurochem.*, **29**, 267–271.

SINGLETON, L. & MARSDEN, C.A. (1979). Increased responsiveness to 5-methoxy, N,N-dimethyl tryptamine in mice on a high tryptophan diet. *Neuropharmacology*, **18**, 569–572.

UDENFRIEND, S. WEISSBACH, H. & BRODIE, B.B. (1958). Assay of serotonin and related metabolites, enzymes and drugs. *Methods Biochem. Anal.*, **6**, 95–130.

YOUNG, S.N. & SOURKES, T.L. (1977). Tryptophan in the central nervous system: regulation and significance. *Adv. Neurochem.*, **2**, 133–191.

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