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THE ACUTE EFFECTS OF ORAL (—)-TRYPTOPHAN IN HUMAN SUBJECTS

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1 The psychotropic effects of a single oral dose of (−)-tryptophan (5 g) in human volunteers were investigated using a series of physiological and psychological tests.

2 Self-ratings of mood showed increase in drowsiness but no euphoria was detected.

3 Severe initial nausea occurred and headache increased; other bodily symptoms were unaffected.

4 Tryptophan caused increased activity in the slow wavebands of the EEG but did not alter the other physiological measures.

5 The levels of total and free tryptophan in the plasma increased 8 and 20 fold respectively to peak levels 2 h after ingestion.

Introduction

Tryptophan is the naturally occurring precursor of the neurotransmitter 5-hydroxytryptamine, (serotonin, 5HT). Tryptophan hydroxylase, the rate limiting enzyme for 5HT synthesis, is normally unsaturated with tryptophan (Eccleston, Ashcroft & Crawford, 1965). Thus increasing the availability of tryptophan to the brain may conceivably influence brain function. Most tryptophan in plasma is not directly available since it is bound to plasma albumin (McMenamy & Oncley, 1958). Recently, it has been shown that increasing the amount of plasma tryptophan which is unbound i.e., 'free' also increases brain tryptophan (Knott & Curzon, 1972). Administration of tryptophan produces a rise in both total and free tryptophan concentration but as the binding sites– approximately one per albumin molecule (Curzon, Friedel, Kantameneni, Greenwood & Lader, 1974)– approach saturation the concentration of free tryptophan rises more rapidly and to a relatively greater extent than does total tryptophan.

Over the past fifteen years, tryptophan, both alone and with a monoamineoxidase inhibitor (MAOI), has been given by mouth to normal subjects (Oates & Sjoerdsma, 1960; Smith & Prockop, 1962; Hodge, Oates & Sjoerdsma, 1964; Hartman, 1967) and to psychiatric patients (Lauer, Inskip, Bernsohn & Zeller, 1958; Pare, 1963; Pollin, Cardon & Kety, 1961). The main findings are an increase in drowsiness (Alexander, Curtis, Sprince & Crosley, 1963; Pare, 1963; Coppen, Shaw & Farrell, 1963) amounting to a hypnotic effect (Hartman, 1967; Wyatt, Engelmann, Kupfer, Fram, Sjoerdsma & Snyder, 1970; Griffiths, Lester, Coulter & Williams, 1972), such that claims have been made that tryptophan is a 'natural sedative' (Wyatt *et al.*, 1970). However, its soporific effect is not sufficient to make it a practical substitute for patients habituated to conventional hypnotics (Brezinova, Loudon & Oswald, 1972). Another consistent early finding is that tryptophan has a euphoriant action both when given alone (Smith & Prockop, 1962; Oswald, Ashcroft, Berger, Eccleston, Evans & Thacore, 1966) and with MAOIs (Oates & Sjoerdsma, 1960; Pollin *et al.*, 1961; Alexander *et al.*, 1963; Glassman & Platman, 1969). Tryptophan was claimed to augment the antidepressive effect of MAOI's (Coppen *et al.*, 1963) and to have antidepressive actions of its own, being compared favourably with ECT (Coppen, Shaw, Herzberg & Maggs, 1967) and imipramine (Coppen, Whybrow, Noguera, Maggs & Prange, 1972) although other trials have shown tryptophan alone to be ineffective (Carroll, Mowbray & Davies, 1970; Herrington, Bruce, Johnstone & Lader, 1974).

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In an earlier attempt to clarify these conflicting results (Greenwood, Friedel, Bond, Curzon & Lader, 1974) we gave tryptophan by infusion. The effects were assessed on a series of psychological and physiological measures known to be sensitive to psychotropic drug effects (Bond & Lader, 1972; Sakalis, Curry, Mould & Lader, 1972). However, although intravenous infusion of 100 mg/kg (−)-tryptophan increased free tryptophan 40 fold and bound tryptophan 8 fold, there were few objective effects. The EEG showed a significant increase in slow-wave activity with a trend to decreased fast wave activity; some impairment of a motor speed task was also noted. Subjective ratings showed a reduction of arousal and somewhat increased drowsiness but no euphoria. It is possible that the difference between our findings and those of previous workers was related to the route of tryptophan administration. For example, behavioural effects of oral administration could be a consequence of intestinal metabolism of tryptophan. Therefore our previous study has been repeated but using tryptophan given by mouth.

Method

The subjects were 10 healthy, drug-free volunteers, 6 men and 4 women, age range, 21-38 years, mean = 27.8 years, who attended the laboratory on two occasions approximately 10 days apart, and who had agreed to take (−)-tryptophan by mouth. The experiment was performed using a balanced design under double-blind controlled conditions; on one occasion the subjects received (−)-tryptophan (5 g) as 10 (0.5 g) tablets and on the other matching placebo tablets (obtained from Cambrian Chemicals Ltd). Both types of tablet had an extremely unpleasant taste, tryptophan more than the placebo when tasted consecutively, but the subjects were unable to recall the taste sufficiently clearly to be able to distinguish between the two.

The series of tests used was that routinely available in the laboratory and carried out using a PDP-12A computer on-line (Bond & Lader, 1972; Lader & Tyrer, 1972). Several paper and pencil psychological tests which had failed to show significant effect with infused tryptophan were omitted from the test battery.

Test battery

The test battery comprised self-rating scales of bodily symptoms and mood, the physiological measures of EEG, mean evoked response, palmar skin conductance, pulse rate and the psychological tests of reaction time and key tapping.

Self-rating scales. Two sets of visual analogue scales (100 mm lines) were used to measure change of symptoms and mood. The first set of thirteen scales was constructed from the reported side effects of tryptophan, one end being labelled none and the other the most severe experience of the symptom. The second consisted of sixteen scales in which pairs of opposing adjectives were listed at opposite ends of the lines (Norris, 1971). This is a valid test of mood (Folstein & Luria, 1973). Subjects were asked to make a perpendicular line at that point along the line corresponding to how they felt at that particular time.

Physiological measures

Electroencephalogram. The EEG was recorded during a reaction time task in which the subject was instructed to press a key in response to a series of 32 auditory click stimuli presented at random intervals of between 8-12 seconds through a loudspeaker behind his head. The record was taken from bipolar pad electrodes one at the vertex and the other on the left side of the head (C_z to C_3 in the 10-20 system). The electroencephalogram was amplified and monitored on an oscilloscope; the input was then fed into four parallel band-pass filters: (1) 2.4-4.0 Hz; (2) 4.0-7.5 Hz; (3) 7.5-13.5 Hz; (4) 13.5-26 Hz. Each filter output was sampled for five second periods between the clicks and then rectified and averaged to yield the mean voltage in the four frequency bands. The four values were added together to give the total voltage.

Mean evoked response was measured by averaging the 500 msec epochs of EEG following each of the 32 click stimuli. The latencies and amplitudes of the main components of the evoked response were quantified.

Finger tremor was measured for 1 min using an Ether BLA-2 accelerometer strapped to the dorsum of the left middle finger (Marsden, Foley, Owen & McAllister, 1967; Tyrer & Bond, 1974). The subject's left arm was strapped into position such that the wrist projected beyond the arm rest and the subject was requested to hold the left hand with fingers spread and extended as still as he could.

Palmar skin conductance (sweat-gland activity) and the number of fluctuations in conductance were measured during the reaction time task according to a standard procedure (Lader & Wing, 1966).

Radial pulse was recorded from two flat ECG electrodes strapped over the dorsum of each wrist (to reduce muscular interference during the reaction time task).

Psychological tests

Reaction time. The interval between the presentation of each auditory stimulus and the response was measured in milliseconds, and the mean value computed.

Key tapping. The subject tapped a key as quickly as possible for 60 s using the middle finger of the right hand.

All the subjects had starved overnight but were allowed one cup of tea or coffee at least one hour before attending the laboratory at 09.00 hours. After the first testing, which took approximately 20 min, the subjects were given a glass of water and the large tablets either whole or crushed according to preference. Two subjects were unable to swallow all the tablets because of severe nausea and were excluded from the study. Blood samples (20 ml) were withdrawn from a forearm vein at the start of the experiment and hourly until 3 h after ingestion of the drug. A slow intravenous infusion of physiological saline kept the vein open and obviated the need for multiple venepunctures. The samples, in sodium heparin tubes, were immediately spun and the plasma separated and frozen at -20°C prior to the determinations of free and bound tryptophan. Total tryptophan was determined by the method of Denkla & Dewey (1967) using plasma diluted with water (1.5 at highest concentrations). Free tryptophan was determined using ultrafiltrate prepared by centrifuging plasma (3 ml) in a CF50 Diaflo membrane cone (Amicon Ltd.) at 800 g for 30 min at room temperature. The amount of heparin used did not affect the free tryptophan concentrations.

Analysis of data

Parametric analysis of variance was performed on all the variables, the main sources of variance being drugs, times, occasions and subjects; differences between drug effects were obtained from the drugs x times interaction and their significance was estimated against within subject, within days error variance.

Variables showing significant F ratios were subjected to Tukey's t-test to determine the significance between pairs of drug means. Analyses for linear and quadratic trends were also carried out to ascertain the time course of drug effect. Biochemical analyses were made on eight of the ten subjects.

Results

Self ratings

Bodily symptoms. Significant drug effects were found on one of the scales, headache (drug x times, $F = 3.22$; $P < 0.05$). Subjects rated themselves as having somewhat increasing amounts of headache in the second hour after taking placebo, an effect which then levelled off. A much more marked increase in headache occurred at the first hour after ingestion of tryptophan which fell steeply to reach the placebo level by the end of the experiment.

This finding of a curvilinear drug effect was confirmed by trend analysis ($F_{\text{Quad d.f.}1,9} = 6.52$; $P < 0.05$).

Subjects rated themselves as more slowed down on tryptophan than on placebo ($F = 7.37$, $P < 0.05$). No other bodily symptom showed significant drug effects.

Mood self-rating scales. Four of the scales showed an overall drug effect with subjects rating themselves as more drowsy ($F = 9.88$; $P < 0.025$), more clumsy ($F = 6.32$; $P < 0.05$), more muzzy ($F = 8.98$; $P < 0.025$) and more mentally slow ($F = 6.14$; $P < 0.05$) on tryptophan compared to placebo.

In addition there was a significant drugs x times interaction on the alert-drowsy continuum; on placebo subjects rated themselves as becoming more drowsy as the experiment progressed whereas on tryptophan the effect was more marked, maximal 1 h after ingestion ($t = 4.17$; $P < 0.001$) thereafter falling steeply to the level reached on placebo by the end of the experiment (Figure 1).

Trend analysis confirmed the course of the effect of tryptophan on mental slowing ($F_{\text{Quad}} = 8.53$; $P < 0.05$) and on clumsiness ($F_{\text{Quad}} = 11.01$; $P < 0.05$).

Physiological measures

Electroencephalogram. (a) *2.4-4.0 Hz* This slow waveband showed a significant drug effect—tryptophan increasing the amount of electrical activity compared to placebo ($F = 11.47$; $P < 0.01$) (Figure 2). (b) *4.0-7.5 Hz* Tryptophan induced an increase in the amount of voltage in this band significantly greater than the slight increase which occurred with placebo over time (drugs x times $F = 3.8$; $P < 0.05$). The greatest increase occurred between the first and second hours after ingestion ($t = 3.6$; $P < 0.01$) but the effect was still apparent in the third hour. (c) *13.5-26 Hz* On placebo an overall upward trend

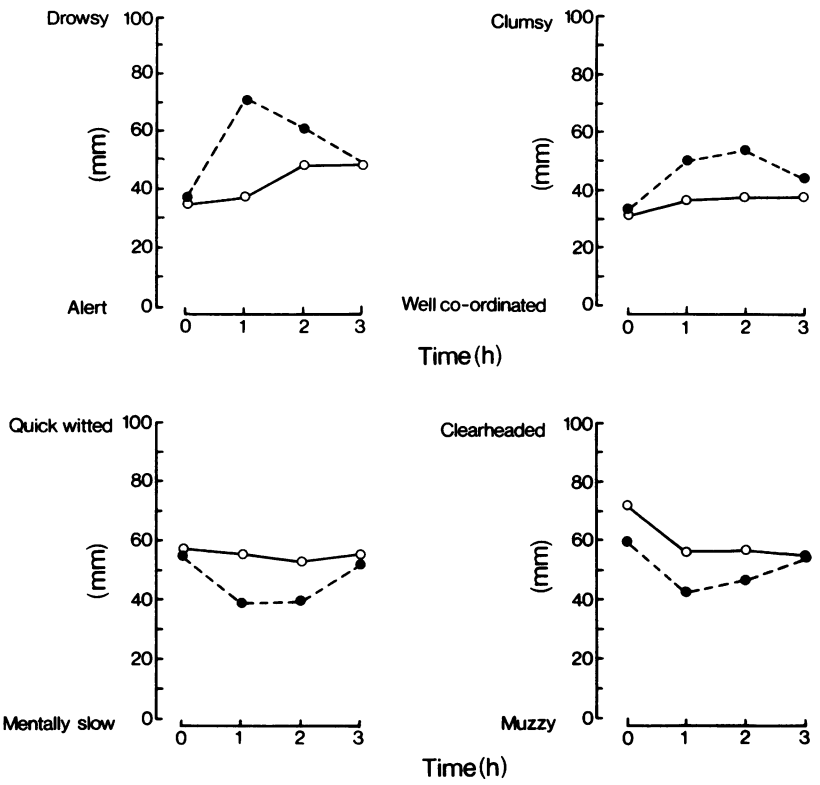

Figure 1 Mean values of analogue mood rating scales for tryptophan (5 mg) (•) and placebo (○).

occurred with time but this was greatly accentuated by tryptophan (drugs x times $F = 3.80$; $P < 0.05$). The increase was maximal at the second hour after ingestion ($t = 3.48$; $P < 0.01$) and then declined. This pattern was confirmed by trend analysis: $F_{Quad} = 5.98$; $P < 0.05$).

Total electroencephalogram. As expected, total EEG voltage showed a significant effect (drugs x times, $F = 5.20$; $P < 0.01$). The amount of electrical activity increased slightly over time on placebo but on tryptophan a much steeper increase occurred maximal at 2 h ($t =$,4.03; $P < 0.001$) remaining steady for the third hour at a significantly greater level ($t = 2.96$; $P < 0.05$) than placebo.

Trend analysis confirmed a linear trend ($F_{Lin} = 6.17$; $P < 0.05$) but the quadratic component was not significant.

Evoked response. The evoked response showed some significant drug effects; the latency of P_1 component tending to be reduced on tryptophan as was the amplitude of P_2-N_2 ($F_{Lin} = 8.78$; $P < 0.05$). However, these findings were inconsistent and will not be considered further.

The other physiological measures, palmar skin conductance, pulse rate and tremor did not show any significant drug effects, neither did the performance tests of reaction time and key tapping.

Total tryptophan

Mean total plasma tryptophan concentration fell slightly but significantly over 3 h after administration of placebo ($t = 3.99$; $P < 0.01$) (Table 1). After giving tryptophan there was a highly significant increase with time ($F = 67.63$; $P < 0.001$), the plasma concentration rising most sharply during the first hour after ingestion to a maximum value at the second and decreasing slightly by the third hour (Figure 3). Trend analysis confirmed both the linear and quadratic elements of the curve ($F_{Lin} = 128.9$; $P < 0.001$; $F_{Quad} = 138.0$; $P < 0.001$).

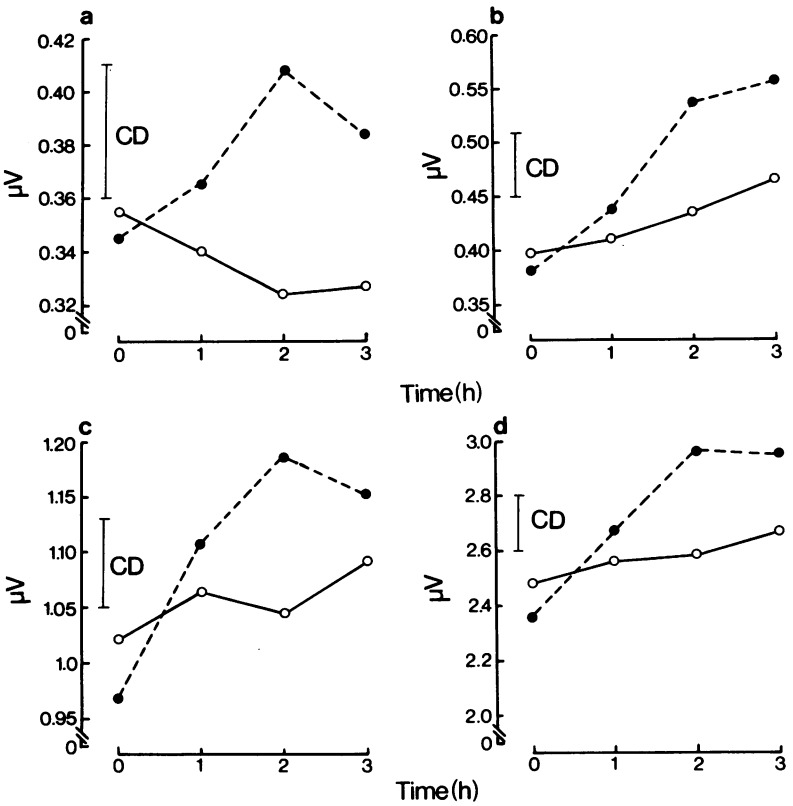

Figure 2 Mean values of electrical activity in EEG wavebands (a 2.4-4.0 Hz; b 4.0-7.5 Hz; c 13.5-26.0 Hz; d total EEG) for tryptophan (5 mg) (•) and placebo (○). The vertical line CD represents 0.05 critical difference i.e. any two means lying further apart than this distance are significantly different by at least the 0.05 level of confidence.

Free tryptophan

A highly significant drugs x times interaction occurred with free tryptophan (drugs x times $F = 17.3$; $P < 0.001$) (Figure 3). Mean plasma free tryptophan concentration did not alter significantly after placebo administration but rose twenty fold after tryptophan. The rise was maximal at the second hour and fell slightly by the third hour (Table 1). This was confirmed by trend analysis showing linear and quadratic components ($F_{Lin} = 45.43$; $P < 0.001$, $F_{Quad} = 12.57$; $P < 0.001$).

Table 1 Plasma total and free tryptophan (TP) levels

		Time (h)			
		0	1	2	3
TP load	TP total	13.43 ± 2.10 (7)	83.61 ± 16.45 (7)	102.27 ± 19.14 (7)	97.50 ± 9.24 (7)
	TP free	1.00 ± 0.26 (7)	15.25 ± 9.15 (7)	23.24 ± 10.04 (7)	19.82 ± 8.11 (7)
Controls	TP total	13.07 ± 1.20 (10)	11.51 ± 1.65 (10)	10.98 ± 1.56 (9)	10.65 ± 1.50 (10)
	TP free	1.05 ± 0.23 (10)	0.97 ± 0.35 (9)	0.83 ± 0.19 (9)	0.91 ± 0.23 (10)

All values are μg/ml ± one s.d. with number of determinations in brackets.

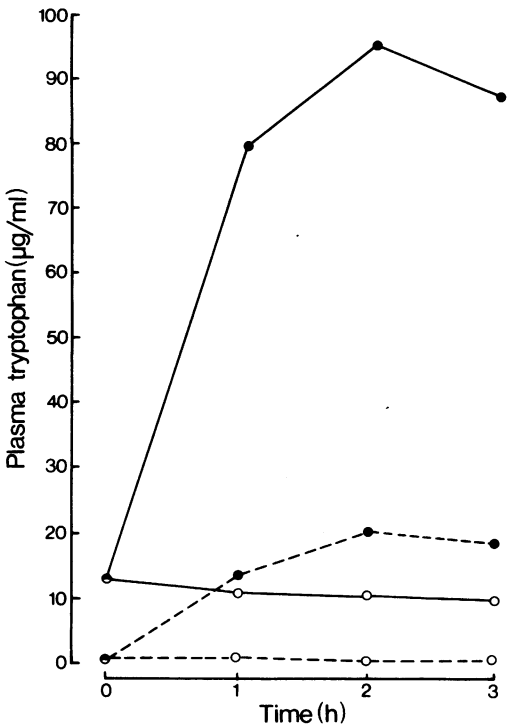

Figure 3 Mean values for free (---) and total (—) tryptophan plasma values after tryptophan (5 g) (•) and placebo (○).

Discussion

By far the most dramatic effect of a single dose of (−)-tryptophan (5 g) given orally was a tendency to nausea. Two subjects actually vomited before they had finished taking the tablets and nausea of a slightly lesser degree occurred in at least half the subjects between 10 and 30 min after ingestion. However, the effect was no longer apparent one hour later when the subjective ratings form was filled in. Nausea did not occur with placebo. From the rapidity of onset of this drug effect it would seem that nausea is produced by a peripheral rather than by a central mechanism. Contributory factors might be the unpleasant taste of tryptophan, or possibly a gastric irritant effect. Other workers have disguised the unpalatable taste of tryptophan using apple sauce, orange juice (when the bitter taste is still very noticeable), bitter chocolate or coffee. The former agents might lead to increased blood glucose which would tend to decrease free fatty acids (Dole, 1966) and hence produce a fall in plasma free tryptophan (Lipsett, Madras, Wurtman & Munro, 1973); the latter contain caffeine which increases free fatty acids (Bellet, Kershbaum & Finck, 1968) and therefore might increase the proportion of tryptophan which is unbound: both were therefore unacceptable in our study. Nausea has been reported in other studies (Pare, 1963; Alexander *et al.*, 1963; Carroll, 1970) and together with our findings emphasizes the need for a more palatable formulation should the substance be given therapeutically (Wyatt *et al.*, 1970; Brezinova *et al.*, 1972).

Headache was the only symptom to be significantly affected by tryptophan, although the increase in headache was slight. This may be of interest since there are indications of altered 5-HT metabolism in migraine (Anthony, Hinterberger & Lance, 1967; Curzon, 1968). Many migraine precipitants tend to increase plasma fatty acid concentration (Curzon & Hilton, 1975) and hence might increase plasma free tryptophan albeit to a lesser extent than in the present study. Headache was greatest one hour after taking tryptophan when plasma tryptophan was rising rapidly but had almost disappeared by the second hour when the concentration was highest.

The changes in the mood self ratings were in the direction expected from the infusion study and from other reports of drowsiness after tryptophan administration (Pare, 1963; Coppen *et al.*, 1963; Griffiths, Lester, Coulter & Williams, 1972), but no subject fell asleep nor were the ratings so high as to indicate any great desire to sleep. Hartman (1967) found that two of his eight subjects fell asleep within 20 min of an oral dose of tryptophan (6 g). However, it was given at bedtime when the subjects were presumably more tired than those in the present study to whom the drug was given at 09 h 30 min after a night's sleep. Smith & Prockop (1962) sent five of their seven patients to sleep during the day with a little over 6 g (−)-tryptophan but their experimental design was not balanced and did not control for temporal effects such as the increasing boredom of the participants. Another mood change that has consistently been found to occur with oral tryptophan is euphoria (Pollin, 1961; Smith & Prockop, 1962; Oswald, Ashcroft, Berger, Eccleston, Evans & Thacore, 1966). We were unable to demonstrate any such an effect with either intravenous or oral tryptophan. This cannot be attributed to lack of sensitivity of the rating scales since factor analysis of the mood scales produced a grouping of happiness which is sensitive to changes in normal subjects (Bond & Lader, 1974).

The four mood scales which did show a significant effect with tryptophan had all returned to the placebo value by the third hour. In contrast the plasma levels of tryptophan, although levelling

off or even falling, were still greater than at the first hour. It may be relevant that Graham-Smith (1971) has noted that in rats given tryptophan with a MAOI drug behavioural changes correlated with the rate at which brain 5-HT increased rather than with the 5-HT concentrations.

Similarly in schizophrenics gross behavioural changes follow abrupt but not gradual alteration in 5-hydroxytryptophan dosage (Wyatt, Kaplan & Vaughan, 1973).

The effect of a single oral dose of tryptophan (5 g) on the EEG was of a similar order but of lower magnitude to that produced by intravenous tryptophan. The main effect after oral tryptophan was an increase in the slow waveband activity, 2.4-4.0 Hz; the 4.0-7.5 Hz band also showed a significant increase but to a lesser degree. Voltage in 4.0-7.5 Hz band increases as sleep develops (Tizard, 1966) and it was this band that showed the most marked effect after infusion. A slight increase in fast wave activity also occurred after oral tryptophan but only a trend in this direction was apparent after infusion. The overall pattern is thus one of increased slow and some increased fast frequencies with little change in alpha activity; this is the pattern typically found with tricyclic antidepressants (Fink, 1968).

Plasma tryptophan levels were rather similar after a 3 h infusion of (−)-tryptophan (5 g) and after the same amount given as a single oral dose. The highest levels reached of free and total tryptophan respectively were 37-50 μg/ml and 137-171 μg/ml in the earlier study and 15-42 μg/ml, 90-124 μg/ml after oral dosage.

Once again we have found that extremely high plasma free tryptophan concentrations have relatively little objective effect on normal subjects in acute studies. We confirm that normal subjects feel somewhat drowsy but there was no evidence of any disinhibition of mood. It is possible that more striking changes might occur in normal subjects in chronic studies and in depressed patients in whom brain tryptophan metabolism may be defective.

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