in clinical and biochemical remission with some evidence of osteosclerotic healing of the lytic areas of bone. This is usually associated with a very good prognosis.

Of the four agents used in this regimen, BCNU is most effective. It may well be that other nitrosourea compounds are equally effective, and future trials with CCNU (1-(2-chloroethyl)-3-cyclohexyl-l-nitrosourea) and methyl-CCNU, in combination with other alkylating agents, should be undertaken.

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Plasma Tryptophan and Sleep

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Summary

Free, bound, and total plasma tryptophan (F.P.T., B.P.T., and T.P.T.) levels have been measured throughout the night in six young female volunteers. All-night polygraphic sleep recordings were also made. No direct temporal relationship was found between plasma tryptophan levels and specific sleep stages. The mean F.P.T. levels, however, were found to have a positive correlation with rapid-eye-movement (R.E.M.) sleep and a negative correlation with non-R.E.M. sleep. An inverse relationship existed between the F.P.T. and B.P.T. levels. There appeared to be a diurnal variation in F.P.T. levels, with high readings in the first half of the night.

Introduction

Animal experiments by Jouvet *et al.* (1967) have shown that 5-hydroxytryptamine (5-HT) may mediate slow-wave sleep and noradrenaline rapid-eye-movement (R.E.M.) sleep. Human experiments, however, have yielded discrepant findings. An increase in R.E.M. sleep has, in fact, been shown to occur after intravenous injection of 5-hydroxytryptophan (Mandell *et al.*, 1965) and oral doses of tryptophan (Hartmann, 1967) in normal adults. Oswald *et al.* (1964) reported that oral administration of tryptophan reduced R.E.M. latency. When parachlorophenylalanine was used to suppress the synthesis of 5-HT R.E.M. sleep was reduced (Wyatt *et al.*, 1969). Nevertheless, tryptophan was also found to enhance total sleep time, non-R.E.M. sleep, and delta-wave sleep, suggesting that tryptophan and 5-HT may have different effects on sleep (Wyatt *et al.*, 1970). It is difficult to reconcile these findings when attempting to relate specific monoamines to changes in sleep stages.

Normally about 90% of the total plasma tryptophan (T.P.T.) is bound to albumin (B.P.T.) and the remaining plasma tryptophan is present in the free fraction (F.P.T.). It has been shown that the F.P.T. levels may directly affect the brain tryptophan levels, which would lead to an increase in the rate of turnover (Tagliamonte *et al.*, 1971 a; Knott and Curzon, 1972) or in the amount of brain 5-HT (Moir and Eccleston, 1968; Wurtman and Fernstrom, 1972). A technique for estimating F.P.T., B.P.T., and T.P.T. has been developed (Eccleston, 1973). We report here the results of a preliminary investigation on the relationship between plasma tryptophan (F.P.T. and B.P.T.) levels and electroencephalographic sleep in normal young women.

Method

After one adaptation night polygraphic sleep recordings were taken between 11 p.m. and 7 a.m. from six young, healthy female volunteers. Standard sleep electroencephalographic techniques (Rechtschaffen and Kales, 1968) were adopted. All records were scored by an independent observer. Blood samples were collected by means of an indwelling cannula. The procedure for the estimation of F.P.T., B.P.T., and T.P.T. was the same as that described elsewhere (Coppen *et al.*, 1973). The plasma samples were numbered at random and the code was not broken until all the samples had been estimated. Diet was controlled on the day before blood collection.

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Results

PLASMA TRYPTOPHAN AND SLEEP STAGES

Neither the F.P.T. nor B.P.T. levels bore a direct temporal relationship with any specific sleep stage. As shown in tables I and II, however, the mean F.P.T. levels were positively related to the total amount of R.E.M. sleep expressed in minutes ($r=+0{\cdot}952$; $P<0{\cdot}005$) and as a percentage of the total sleep time ($r=+0{\cdot}940$; $P<0{\cdot}01$). They were also negatively related to the duration of non-R.E.M. sleep expressed as a percentage of the total sleep time ($r=-0{\cdot}940$; $P<0{\cdot}01$). The mean B.P.T. levels were found to have a negative correlation with total R.E.M. sleep expressed in minutes ($r=-0{\cdot}874$; $P<0{\cdot}05$) and as a percentage of the total sleep time ($r=-0{\cdot}910$; $P<0{\cdot}05$) and a positive correlation with total non-R.E.M. sleep expressed in minutes ($r=+0{\cdot}837$; $P<0{\cdot}05$) and as a percentage of the total sleep time ($r=+0{\cdot}909$; $P<0{\cdot}05$). It was thus apparent that an inverse relationship existed between the F.P.T. and B.P.T. levels, and this proved to be statistically significant ($r=-0{\cdot}940$; $P<0{\cdot}01$). Though the mean F.P.T. levels were negatively related to R.E.M. latency this failed to reach a statistically significant level ($r=-0{\cdot}63$).

A partial correlation between the mean F.P.T. levels and total R.E.M. sleep expressed in minutes was calculated holding the mean B.P.T. levels constant and a positive correlation was found ($r=+0{\cdot}73$). No correlation was found between the mean F.P.T. levels and the total non-R.E.M. sleep expressed in minutes holding the mean B.P.T. levels constant ($r=-0{\cdot}097$).

TABLE I—*R.E.M. Sleep, Non-R.E.M. Sleep, and Mean Plasma Tryptophan Levels in Six Normal Female Subjects*

Subject No.	R.E.M. Sleep			Non-R.E.M. Sleep		Mean Plasma Tryptophan (μg/ml)		
	Latency (Min.)	Total Amount (Min.)	% Total Sleep	Total Amount (Min.)	% Total Sleep	F.P.T.	B.P.T.	T.P.T.
1	51·0	99·7	23·4	326·6	76·6	1·69	11·93	13·62
2	84·3	126·0	28·4	318·0	71·6	2·48	8·32	10·80
3	23·0	104·7	26·5	290·3	73·5	2·14	8·94	11·08
4	94·7	71·7	17·0	350·6	83·0	1·60	12·23	13·83
5	254·3	60·0	13·7	379·3	86·3	1·18	12·46	13·64
6	111·3	113·3	30·2	261·7	69·8	2·25	8·34	10·59

TABLE II—*Statistical Relationship between Mean Plasma Tryptophan Levels and R.E.M. or Non-R.E.M. Sleep**

	Total R.E.M. Sleep Time	R.E.M. Sleep as % of Total Sleep Time	R.E.M. Latency	Total Non-R.E.M. Sleep Time	Non-R.E.M. Sleep % of Total Sleep Time
F.P.T.	$r=+0{\cdot}952$; $P<0{\cdot}005$	$r=+0{\cdot}940$; $P<0{\cdot}01$	$r=-0{\cdot}630$; N.S.	$r=-0{\cdot}809$; $P<0{\cdot}1$	$r=-0{\cdot}940$; $P<0{\cdot}01$
B.P.T.	$r=-0{\cdot}874$; $P<0{\cdot}05$	$r=-0{\cdot}910$; $P<0{\cdot}05$	$r=+0{\cdot}434$; N.S.	$r=+0{\cdot}837$; $P<0{\cdot}05$	$r=+0{\cdot}909$; $P<0{\cdot}05$
T.P.T.	$r=-0{\cdot}831$; $P<0{\cdot}05$	$r=-0{\cdot}880$; $P<0{\cdot}05$	$r=+0{\cdot}365$; N.S.	$r=+0{\cdot}827$; $P<0{\cdot}05$	$r=+0{\cdot}880$; $P<0{\cdot}05$

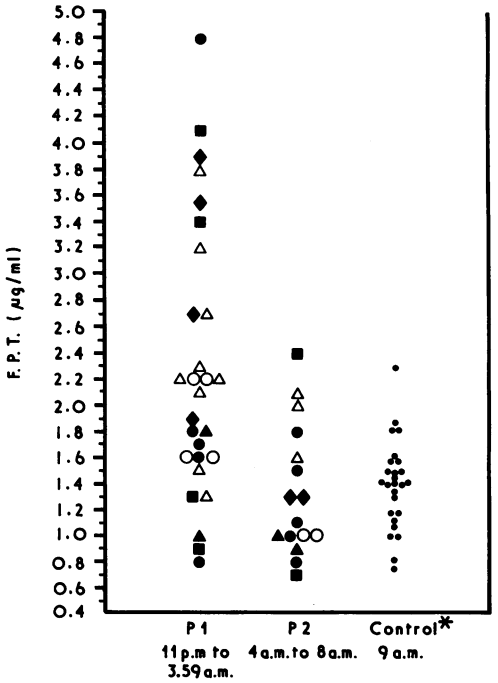
*For all comparisons D.F. = 5, two-tailed.
N.S. = not significant.

TABLE III—*Mean Plasma Tryptophan Levels (μg/ml) in Six Normal Female Subjects during Two Periods of Night (P1 and P2)*

	P1 (11 p.m. to 3.59 a.m.)	P2 (4 to 8 a.m.)	P (two-tailed)
F.P.T.	2·217	1·328	$<0{\cdot}01$ ($t=4{\cdot}46$; D.F. = 5)
T.P.T.	12·859	11·832	N.S. ($t=1{\cdot}5$; D.F. = 5)

POSSIBLE DIURNAL VARIATION IN F.P.T. LEVELS

Both the F.P.T. and T.P.T. levels showed considerable fluctuations but overall the F.P.T. levels appeared to diminish after 4 a.m. Thus when the night was divided into two periods (P1 and P2; table III), using 4 a.m. as the cut-off point, the mean F.P.T. levels but not the mean T.P.T. levels were found to be significantly different ($t=4{\cdot}46$; $P<0{\cdot}01$). The F.P.T. levels in P1 showed a wide range of fluctuations but those in P2 were closely similar to control values at 9 a.m. reported by Coppen *et al.* (1973) (see fig.). The T.P.T. levels in both periods showed little change in the range of fluctuations.



F.P.T. levels in six normal female subjects during two periods of night, 4 a.m. being used as cut-off point. Each symbol represents values for same subjects. ($t5=4{\cdot}46$; $P<0{\cdot}01$).
*Values for normal female subjects at 9 a.m. in another study are used for comparison (Coppen *et al.*, 1973).

When we divided the night into three periods (P'1, P'2, and P'3; table IV) using 2 a.m. and 5 a.m. as the cut-off points the mean decrease in the levels of F.P.T. but not T.P.T. was found to be significant at the 0·5% level by analysis of variance. Scheffe's method of selected comparisons (McNemar, 1962) also showed that the differences in mean F.P.T. levels between periods taken in pairs were statistically significant between P'1 and P'3 ($t5=5{\cdot}8$; $P<0{\cdot}005$) and between P'2 and P'3 ($t5=3{\cdot}681$; $P<0{\cdot}05$) but not between P'1 and P'2 ($t5=2{\cdot}114$; $0{\cdot}05<P<0{\cdot}10$). The differences in mean T.P.T. levels across the three periods were not significant.

TABLE IV—*Mean Plasma Tryptophan Levels (μg/ml) in Five Normal Subjects during Three Periods of Night (P'1, P'2, and P'3) Examined Statistically by Analysis of Variance Technique and by Scheffe's Method*

	Sleep Periods			Analysis of Variance	Scheffe's Method		
	P'1 (11 p.m. to 1.59 a.m.)	P'2 (2 a.m. to 4.59 a.m.)	P'3 (5 a.m. to 7.59 a.m.)		P'1 *v.* P'2	P'2 *b.* P'3	$\overset{+}{P'1}$ [1]. $\overset{+}{P'3}$
F.P.T.	2·40	1·955	1·182	$F_{2,8}=17{\cdot}23$; $P<0{\cdot}005$	$t5=2{\cdot}114$; N.S.	$t5=3{\cdot}681$; $P<0{\cdot}025$	$t5=5{\cdot}8$; $P<0{\cdot}005$
T.P.T.	12·94	12·726	12·295	$F_{2,8}<1$; N.S.	N.S.	N.S.	N.S.

Discussion

Because of the small number of subjects in this study the findings should be interpreted with caution. It is noteworthy, however, that there was a strong positive correlation between the mean F.P.T. levels and the total amount of R.E.M. sleep. As the F.P.T. levels may determine brain tryptophan and 5-HT concentrations our result may lend some support to other observations in man that R.E.M. sleep is closely related to indoleamines. Thus it is also of great interest to note, as recently reported by Bloom *et al.* (1973), that unit neuronal activity of 5-HT-containing cells in the nucleus raphe magnus is highest during R.E.M. sleep and lowest during slow-wave sleep.

Nocturnal F.P.T. levels may be in part controlled by long-term and more immediate dietary factors. Thus it is noteworthy that impaired nutrition can cause disturbed sleep in the second half of the night both in patients with anorexia nervosa (Crisp *et al.*, 1971; Lacey, 1974) and in those with a variety of psychiatric illnesses (Crisp and Stonehill, 1973). Furthermore, Březinová and Oswald (1972) and Southwell *et al.* (1972) have shown that sleep in the second half of the night is especially affected by the ingestion of a milk-cereal drink before going to bed. It has also been shown that subjects who are depressed (a condition sometimes associated with early-morning waking) also have low F.P.T. levels (Coppen *et al.*, 1973) and low tryptophan levels in the cerebrospinal fluid (Coppen *et al.*, 1972). R.E.M. sleep is normally more abundant in the second half of the night and, in anorexia nervosa patients at least, it is the total R.E.M. sleep that is most substantially reduced (Lacey, 1974). F.P.T. levels have also been shown to be increased by non-esterified fatty acids (Curzon *et al.*, 1973) but reduced by a heavy carbohydrate intake or increased plasma insulin level (Fernstrom and Wurtman, 1974). Neutral amino-acids may also hinder the accessibility of plasma tryptophan to the brain (Wurtman and Fernstrom, 1972) or increase its binding by raising the plasma inuslin level (Fajans *et al.*, 1967; Sukkar *et al.*, 1967).

Our study also shows that the mean F.P.T. levels in normal subjects declined during the night and that the mean F.P.T. levels after 4 a.m. were not much different from those found by others to be present at around 9 a.m. It appears that certain changes occurred in the early morning. Adrenocortical hormones are known to induce the liver enzyme tryptophan pyrrolase, which controls another pathway in tryptophan metabolism (Knox and Auerbach, 1955). Thus increased adrenocortical activity may cause more plasma tryptophan to be metabolized by tryptophan pyrrolase rather than to be available for 5-HT synthesis in the brain. Plasma corticosteroids show a diurnal rhythm, with maximum levels between 6 and 9 a.m. and minimum levels at midnight and a few hours afterwards (Loraine and Bell, 1971). It may be that nocturnal F.P.T. levels depend in part on this rhythm of corticosteroids, establishing an inverse relationship between them.

Finally, it has been reported that the F.P.T. levels change in response to a number of factors (Tagliamonte *et al.*, 1971 b; Korf *et al.*, 1972; Aylward and Maddock, 1973; Curzon and Knott, 1973). The use of normal subjects on no medication in this study minimized such possible effects with the exception of that of heparin. In order to ensure a safe and adequate functioning of the indwelling cannula an infusion of about 400 ml of heparinized saline (5,000 U heparin/500 ml saline) was given to each subject per night. Indeed, this may have affected our results, though our dosage of heparin was much less than that given to animals (5,000 U/kg), wherein an effect on F.P.T. has been shown (Curzon and Knott, 1973). Moreover, since heparinized saline was continuously but slowly infused throughout the night each sample was to some extent comparable in this respect (Curzon, 1974).

A series of investigations to explore further the relationship between F.P.T. levels and R.E.M. sleep are now in progress and will be reported shortly.

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