# THE STUDY OF SOME BIOLOGICAL FACTORS OF WAKEFULNESS AND THEIR INFLUENCE UPON SLEEP

by

JONATHAN MARK PORTER

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For my parents

CONTENTS

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ABSTRACT

The thesis discusses the significance of some aspects of wakefulness, namely exercise and diet, upon human sleep using the electroencephalogram (EEG).

Part One of this thesis examines the relationship between exercise and sleep. An initial literature review produced equivocal findings, although it appeared that several factors might be involved in this relationship such as the time of day of exercising, the fitness of the subject and the amount of stress associated with the exercise prescribed.

In the first exercise study (Study I) the amount of exercise taken by the subjects was standardised at 40% of the individual work capacities as assessed by a submaximal estimation of maximum aerobic power. The results indicate that the ensuing wakefulness following morning exercise was sufficient for recovery but the proximity of the late afternoon exercise period to the sleep period may result in an intrusion of recovery into initial sleep, as shown by a small increase in delta activity during the first few hours of sleep. This study suggested that sleep, as assessed by the EEG, is not necessary for recovery from muscular fatigue.

The second exercise study (Study II) extended the earlier study by obtaining general data from questionnaires on tiredness and sleep behaviour following day-to-day variations in normal physical activity. The findings do not suggest that the variations encountered in daily activity levels or in the time of activity have any major effect upon the variables assessed, although it appeared that fit subjects either need less sleep or sleep more efficiently than unfit subjects.

The various factors thought to influence the effect of exercise upon sleep are discussed at the end of Part One.

Part Two of this thesis examines the relationship between the diet and sleep. An initial literature review provided good evidence for the existence of this relationship although there was little evidence to suggest by which mechanism the diet influenced sleep.

The first diet study (Study III) assessed the influence of carbohydrate supplements taken as a late evening supper upon sleep. The results showed that the progressive increase in carbohydrate content of the supplementary diet was associated with a progressive increase in REM sleep during the first half of the night and a progressive decrease in wakefulness and light sleep throughout the night. These changes appear to be as a result of the increase in the carbohydrate content rather than the calorie intake as work published following this experiment provided clear evidence that an isocaloric increase in the carbohydrate content of a diet was associated with similar changes in sleep as described for Study III.

The remainder of the thesis is devoted to the development of a theoretical model which attempts to explain one of the mechanisms by which the diet might influence sleep. Two further reviews were undertaken. The first review assessed the influence of the diet upon neurotransmitter metabolism and it was concluded that changes in brain serotonin metabolism could be mediated by changes in the diet. The second review assessed the relationship between changes in brain serotonin metabolism and sleep. A theoretical model was developed by the integration of these two reviews, which suggested that the duration of REM sleep may be influenced by changes in brain serotonin metabolism following variations in the carbohydrate and fat content of the diet.

The last study of the thesis (Study IV) provided experimental evidence to test the theoretical model. Two non-isocaloric experimental diets were designed: one was expected

to provide a high ratio of free plasma tryptophan to the other large, neutral amino acids whereas the other experimental diet was expected to produce a low ratio. According to the model, it was predicted that the consumption of the first diet (high carbohydrate and fat content, low protein content) would be associated with a greater amount of REM sleep than the latter diet (low carbohydrate and fat content, high protein content). The results were found to be consistent with this prediction.

A good degree of agreement was also found from a comparison of the findings in the literature and the predictions from the model.

Part Two concludes with a brief discussion of some avenues for research in the diet and sleep relationship.

# CHAPTER 1

## INTRODUCTION

## 1. THE SCOPE OF THE THESIS

This thesis is concerned with the relationship between sleep and some of the biological factors of wakefulness. It does not attempt to determine the function of sleep because decades of research have failed to isolate any specific unquestionable functions other than the alleviation of tiredness.

There have been, and are, many theories which attempt to explain the functions of sleep and it is very likely that sleep fulfills many functions. However, the quality of the theory or model depends to a large extent upon the quality of the original research upon which it was based. Thus, a prerequisite to the understanding of the functions of sleep requires the study of the relationships between wakefulness and sleep and the identification of those factors which have an influence upon sleep. These factors may include psychological factors such as arousal, mood and perceived stress, physiological factors such as exercise, diet, injury, disease or environmental factors such as temperature, humidity and altitude.

Additionally, the various factors should be studied within a normal range encountered in everyday life as the relevance of findings based on abnormal states upon the functions of sleep are debatable. For example, extreme amounts of exercise can be very stressful and severe depression is often associated with an altered dietary intake. Thus, any changes in sleep may be due to changes in other associated factors apart from the main factor of interest.

This thesis commences with an investigation of the exercise and sleep relationship. This area was chosen because of the theoretical implications for one of the current theories of sleep (see Section 1, Chapter 2). The existing literature at that time was equivocal and it was hoped that a more systematic approach would provide clearer information concerning the effects of exercise upon sleep. From the findings of

the two experimental studies and more recent literature it appears that normal or unstressful variations in physical activity have little influence upon sleep in subjects of average fitness. The fact that large amounts of activity taken by very fit subjects can influence sleep is very interesting and it possibly reflects the long term influence of activity upon sleep.

Although continued study of the exercise and sleep relationship would be warranted, it was decided to investigate the effects of the diet upon sleep as the existing literature indicated that sleep was responsive to acute dietary changes. The remainder of the thesis was concerned with the confirmation of the above and the study of the mechanisms involved in the diet and sleep relationship.

Another reason for the decision to study the influence of exercise and the diet upon sleep stems from the number of quotations that can be found referring to such relationships. For example:

*"To tired limbs and over-busy thoughts,*
*Inviting sleep and soft forgetfulness."*

WORDSWORTH The Excursion Book (iv) line 1323.

*"Tir'd Nature's sweet restorer, balmy sleep!"*

YOUNG Night Thoughts, Night (i), line 1.

*"The sleep of a labouring man is sweet"*

OLD TESTAMENT: Ecclesiastes, V, 12.

*"Sleep is the nourice (nurse) of digestion"*

CHAUCER Canterbury Tales.

Also, one often hears that people 'sleep like a log' after a hard day of physical work or play or report feelings of tiredness following large meals. Similarly,

children are often urged to play hard so that they will sleep quieter or informed that they cannot have a bedtime snack containing cheese or chocolate as they will have nightmares.

Whilst the above 'folklore' is not based upon scientific fact it is clear that exercise and the diet are prime candidates by which to account for day-to-day variations in sleep.

## 2. THE STRUCTURE OF THE THESIS

For clarity, the thesis has been divided into two parts; Part I discusses the exercise and sleep relationship while Part II discusses the diet and sleep relationship.

A summary of contents is provided at the beginning of the thesis which lists the individual chapters and a detailed list is presented on the first page of each chapter. The chapters describing the experimental studies all contain a section entitled "Summary and Conclusions", which may be useful to the reader requiring a rapid, but detailed, account of the experiment.

Chapter 2 is the first chapter of Part I and it briefly discusses the theoretical significance of the exercise and sleep relationship. It also contains a review of the existing literature up to 1974, when the first exercise study commenced. Chapter 3 discusses the general considerations for the study of exercise upon sleep and the first study (Study I) is described in Chapter 4. This EEG study is followed by the second exercise study (Study II) which investigated sleep behaviour by questionnaires and is described in Chapter 5. Chapter 6 contains a review of published studies from 1974 to the present and concludes Part One of the thesis with a general discussion concerning the effects of exercise upon sleep.

Part Two of the thesis has been subdivided into three sections. Section A includes Chapters 7 and 8 which contain a literature review of the diet and sleep literature up to

1977 and a description of the first diet study (Study III), respectively.

Section B includes Chapters 9 to 11 and describes the development of the theoretical model for the diet and sleep relationship. The approach adopted for this development is described in the preface to this section. Chapter 9 assesses the influence of the diet upon neurotransmitter metabolism and Chapter 10 assesses the expected effect of such changes upon sleep (the studies discussed in this chapter are reviewed in Appendix IV). Chapter II integrates the findings from the previous two chapters to form a theoretical model from which it is possible to predict the effects of certain changes in the diet upon sleep. The validity of the model is initially assessed with reference to the studies reviewed in Chapter 7 and to Study III.

Section C includes Chapters 12 and 13 and investigates the validity of the proposed model. Chapter 12 describes the second diet study (Study IV) which was designed to test the theoretical model. Chapter 13 concludes Part Two of the thesis with a review of recent evidence pertaining to the model and a brief discussion of the avenues for future research in the diet and sleep relationship.

The data used for the statistical analyses are presented in the Appendices.

PART ONE: EXERCISE AND SLEEP

CHAPTER 2

EXERCISE AND SLEEP - LITERATURE REVIEW I

# 1. INTRODUCTION

The study of the effects of exercise upon sleep has been carried out using the electroencephalogram since the early 1960's. However, it was felt that the available studies did not provide a coherent picture of the sleep response to daytime exercise. Further research into the effects of exercise upon sleep is required because of the theoretical implications. Several researchers have developed theories concerning the functions of sleep and there appears to be general support for the view that deep sleep (SWS) is specifically concerned with general body restitution (Oswald, 1969, 1970, 1973, 1974, 1976; Hartmann, 1973).

## 1.1 General body restitution and SWS

### 1.1.1 Evidence cited in favour of this theory

Briefly, the main evidence to support this view is described below:-

a) Sleep deprivation

The first recovery night following sleep deprivation typically contains large amounts of SWS (Berger and Oswald, 1962). Other evidence indicates strongly that the duration of wakefulness preceding the sleep period is a strong determinant of stage 4 sleep (Webb and Agnew, 1972). Also, the imposition of one hour of wakefulness in the middle of the night has been reported to increase SWS during the remainder of the night (Beck, Brezinova, Hunter and Oswald, 1975).

b) Human growth hormone (HGH)

HGH is a sleep-dependent hormone which typically shows its largest secretion peaks during SWS (Sassin, Parker, Mace, Gotlin, Johnson and Rossman, 1969; Schnure, Raskin and Lipman, 1971). This hormone is thought to increase

the rate of synthesis of protein and RNA (Korner, 1965).

c) Cell division

Peaks of mitotic activity have been reported to occur in human bone marrow and skin soon after the usual sleep onset time (Cooper, 1939; Killman, Cronkite, Fliedner and Bond, 1962; Mauer, 1965; Fisher, 1968). Similar findings can also be found in the animal literature (Clark and Korst, 1969; Halberg and Barnum, 1961).

d) Starvation

Acute fasting, which increases the metabolism of tissue reserves, has been found to increase SWS in the human (MacFadyen, Oswald and Lewis, 1973; Karacan, Rosenbloom, London, Salis, Thornby and Williams, 1973).

e) Diseases of the thyroid gland

Hyperthyroidism is a condition in which increased demands are made on the tissue reserves of the body. Patients suffering from hyperthyroidism have been reported to exhibit large amounts of SWS compared to normal subjects (Dunleavy, Oswald, Brown and Strong, 1974). Moreover, as the patients responded to treatment the amount of SWS taken nightly progressively declined towards normal levels.

Conversely, hypothroidism has been associated with low amounts of SWS which increase during treatment (Kales, Heuser, Jacobson, Kales, Hanley, Zweizig and Polson, 1967).

f) Exercise

Some of the early studies reported that an increase in daytime exercise levels was associated with an increase in SWS in both animals (Hobson, 1968) and humans (Baekland and Lasky, 1966).

### 1.1.2 Arguments against this theory

Whilst the evidence summarised above appears to be in accord with the general body restitution theory of SWS, it must be noted that other interpretations are possible. Horne (1979) questions whether the evidence summarised above is sufficient to assign the role of body restitution to SWS. For example, the link between SWS and the sleep release of HGH may not be casual as SWS depriviation does not abolish the sleep HGH peak (Sassin et al, 1969). There is evidence that the sleep release of HGH may be under neural rather than metabolic control, as glucose infusions were unexpectedly found to be ineffective in suppressing the sleep HGH peak (Parker and Rossman, 1971). However, the nocturnal peak can still be suppressed by elevating the level of plasma free fatty acids (Lipman, Taylor, Schenk and Mintz, 1972). This is in agreement with recent reviews (Kostyo and Nutting, 1974; Hunter, 1972) that HGH secretion is closely involved in the regulation of fat metabolism.

The role of HGH is still far from clear but it is apparent that its stimulating effect upon protein synthesis is only one of several functions. Possibly, of equal importance is its protein sparing action effected mainly by the redistribution of fat.

If the sleep peak of HGH is taken to indicate increased rates of protein synthesis during the first part of the night then it is surprising that the oxygen consumption rate and carbon dioxide excretion rate both fall early in the night (Brebbia and Altshuler, 1965; Webb and Hiestand, 1975). Both of these variables were reported to be at a minimum during SWS in the study by Brebbia and Altshuler (1965) although this was not confirmed by the later study of Webb and Hiestand (1975). The decline of both these variables indicates that energy expenditure is reduced during the period when the secretion of HGH is at its peak.

Recent work by Young, Steffee, Pencharz, Winterer and Scrimshaw, 1975, has reported that the differences in total body protein synthesis found between various age groups are related to their differences in energy expenditure. If this factor is controlled for, then protein synthesis, measured as grams per calorie of energy expenditure, shows no variation between the age groups. This finding, together with the similar observations of Munro (1969) and Waterlow (1968), strongly suggests that protein synthesis accounts for a significant portion of the resting metabolic rate. Therefore, the studies of Brebbia and Altshuler (1965) and Webb and Hiestand (1975) do not support the theory that protein synthesis is greatly increased during SWS, compared to levels found during relaxed wakefulness.

The findings of increased mitotic activity may also be re-interpreted. Horne (1979) points out that mitosis is greatly influenced by the circulating levels of corticosteroids and adrenaline. These hormones have sleep-independent circadian rhythms which peak in the early morning (approximately 7 a.m.) and trough in the late evening (Weitzman, 1976). Therefore, the findings of increased mitotic activity following sleep onset may be only temporally associated with SWS. In support of this, Fisher (1968) has reported peaks of epidermal mitotic activity occurring before sleep onset in some human subjects. Fisher also reported that the elevation of corticosteroids and adrenaline by exercise delayed the peak in mitotic activity to later in the night.

Regarding the increases in SWS associated with starvation, it should be noted that the increases occur only after at least three days of fasting (MacFadyen et. al., 1973; Karacan et. al., 1973). From this fact it would appear that SWS levels do not change appreciably, on a day-to-day basis, in response to variations in general body metabolism.

Further evidence indicating a lack of responsiveness of SWS to changes in varying metabolic conditions can be found in hyperthyroid patients (Dunleavy et. al., 1974). Whilst these patients became clinically euthyroid within six weeks to eight months following treatment, the decrease in SWS did not parallel the clinical state but took place slowly during fifteen months.

The finding that increases in daytime exercise are associated with increases in SWS in the subsequent sleep period is obviously crucial to those theorists who argue that SWS is intimately linked with general body restitution However, many of the more recent exercise studies have not found such increases in SWS (Hauri, 1968; Baekeland, 1970; Zir, Smith and Parker, 1971; Adamson, Hunter, Ogunremi, Oswald and Percy-Robb, 1974).

This lack of consistency in the findings of the exercise studies may well be a result of the fitness of the subjects used, the type of exercise programme imposed and the time at which it is taken.

## 1.2 Proposals for research

From the above brief discussion it is evident that the validity of the general body restitution theory of SWS is suspect. If SWS has a "physically restorative function" (Hartmann, 1973), then it would be expected that this stage of sleep would be enhanced following days of great wear and tear to the body. Thus, the effect of exercise upon SWS is of great interest and theoretical significance to the study of sleep.

At present, the literature concerning the effects of exercise upon sleep has produced equivocal findings. Considering the significance of this area of research it was decided that further work was warranted.

The rest of this chapter will be devoted to a review of the existing exercise and sleep literature. Following this review it is intended to assess which factors are involved in the exercise and sleep relationship. A more systematic approach to the study of exercise and sleep can then be carried out by controlling for these factors.

## 2. REVIEW OF THE EXERCISE AND SLEEP LITERATURE

### 2.1 Animal studies

Matsumoto, Nishisho, Suto, Sadahiro and Miyoshi, (1968) studied the effect in adult rats of four hours of continuous exercise on a treadmill upon sleep in the subsequent 24 hour period. Electroencephalographic recordings were made via chronically implanted electrodes. Each rat underwent a control recording taken one week prior to the experimental recording. This control period consisted of placing the rats on treadmills for four hours without enforcing exercise or allowing them to sleep, eat or drink.

Sleep following the control period was compared to that following the exercise period and the authors noted that the occurrence of REM sleep was inhibited following the exercise. However, the percentage of total sleep time occupied by REM sleep was not found to be reduced following the exercise. NREM sleep was found to appear earlier following exercise in these rats, the difference from the control period being statistically significant ($p<0.01$). Unfortunately, no data was provided concerning the total time spent in NREM sleep during the 24 hour sleep period, and it is therefore difficult to conclude that exercise enhances NREM sleep as the reduction in its onset time following the exercise period was only an average of 9 minutes.

Hobson (1968) conducted an extensive and careful study of the effects of exercise upon sleep in cats. Several pilot-studies were carried out to determine the exercise

load to enforce in the main experiment. The cats were exercised on a power-driven treadmill. The load chosen was described as moderate (4-8 rev/minute for two hours) and this load consistently produced hyperventilation and resistance to work. Higher speeds of rotation could not be maintained for the two hour period by the cats and resulted in exhaustion with subsequent hypervigilance.

The author realised that any observed change in sleep following the exercise period may have been due to the effect of sleep deprivation alone and proceeded to compare the sleep behaviour of cats following

i) no treatment

ii) sleep deprivation by the flower-pot method (this selectively deprives the cat of REM sleep only)

iii) subtotal sleep deprivation under EEG control (this eliminated REM sleep and all but a small amount of SWS).

Hobson found very little difference in subsequent sleep between these various conditions when their duration was for two hours or less. The subtotal sleep deprivation was chosen as the control for the main experiment as he considered that it approximated most closely to the sleep deprivation incurred during exercise.

In the main experiment the sleep of four cats was continuously monitored, except between 2 to 4 p.m. each day for 6 days. During this period, exercise and subtotal sleep deprivation were alternated on a daily basis.

The results showed that exercise was followed by an earlier onset of NREM sleep (an average of approximately 20 minutes) and a later onset of REM sleep (an average of

approximately 65 minutes) when compared to subtotal sleep deprivation. The decrease in wakefulness and the increase in SWS in the first 5.5 hours following exercise were both significant ($p<0.01$). Although the total duration of REM sleep during the first 5.5 hours was unaffected by the exercise, it was found that the relative amount of sleep time spent in REM sleep was significantly reduced ($p<0.5$).

The above findings would appear to indicate that NREM sleep is increased following the rigours of exercise. However, as the author noted, the total quantity of sleep, and of its component stages, during the entire recording period did not show any significant changes following the two conditions even though there were marked differences in its distribution within that time; the exercised cats sleeping first and awakening later, whereas the controls slept later.

Hobson remarked that "this would seem to mean that environmental effects contribute relatively little to the total quantity of sleep". Whilst this appears to be true for the cat, it does not follow that the same is true for other animals and man. The cat typically enjoys a large daily amount of sleep and it is quite possible that a large portion of this is a luxury form of sleep. By the term "luxury" it is implied that the cat could, if necessary, remain awake for longer periods without undue discomfort or physical deterioration. Thus, any change in a cat's sleep requirement could be masked by this luxury sleep. However, over-sleeping generally consists of light sleep interspersed with REM periods and, therefore, the lack of any significant change in NREM sleep during the entire recording period may not be due to the masking effect of luxury sleep.

## 2.2 Human studies

This literature review describes the exercise and

sleep studies in historical order and contains only those studies available prior to the first exercise and sleep study of this thesis. The more recent studies are described in Chapter 6 and all of the human studies reviewed are summarised in Table 1 of this later chapter.

Baekeland and Lasky (1966) studied the effects of exercise upon sleep in ten very fit male students. These subjects attended the sleep laboratory on four non-consecutive nights, generally a week or more apart. No recordings were analysed for the first night to allow for possible "first night" effects (Agnew, Webb and Williams, 1966) to disperse. On the three remaining experimental days the subjects were instructed to take their normal sporting activities in the afternoon, evening or not at all. The sequence of exercise conditions was randomised for each subject and the EEG records were analysed for the first six hours of sleep only.

The results showed that the duration of SWS was highest on nights following the afternoon-exercise (40.1%), intermediate on nights with evening-exercise (35.4%) and lowest on nights following no-exercise (32.5%). The difference between afternoon-exercise and no-exercise was found to be significant ($p<0.01$); this effect being evident throughout the night.

Stage 1 sleep was significantly decreased following afternoon-exercise compared to evening-exercise ($p<0.05$) and no-exercise ($p<0.01$).

This study strongly suggested that SWS is positively related to the amount of exercise taken during the daytime. The increase in SWS following exercise was compensated for by a decrease in light sleep (stages 0 and 1), whilst REM sleep time remained unaffected. No significant differences were found between sleep following the evening-exercise and no-exercise conditions suggesting that the

time-of-day when the exercise is taken is an important factor.

The authors noted that sleep following evening-exercise was more disturbed by brief periods of wakefulness and suggested that the recent exercise had an arousing influence on the brain which opposed the SWS-enhancing effect of exercise. However, although wakefulness was significantly greater on the evening-exercise night compared to the afternoon-exercise night ($p<0.02$), the difference was only about two minutes; that between the evening-exercise and the no-exercise night being even less. Of special interest is the fact that the amount of SWS taken in the second half of the night was still greater following afternoon-exercise than evening-exercise, by which time the arousing influence of the late exercise would probably have disappeared. Unfortunately, the amount of exercise taken during the afternoon and evening was not stated so it may have been possible that the subjects did not exercise quite so enthusiastically during the evening.

Hauri (1968, 1969) provided more information concerning the effects of evening-exercise upon sleep. Fifteen young, male subjects spent four non-consecutive nights in the laboratory. No recordings were analysed from the first night because of possible "first-night" effects. In the following three nights the subjects engaged in three different pre-sleep activities, each lasting for six hours, in a counter-balanced order. The three activities studies were relaxation, exercise and studying. The exercise period involved, on average, an equivalent of 50 miles on a bicycle and 90 minutes lifting 15 pound weights. The subjects retired immediately after the evening activity and EEG data was recorded for the first 3½ hours of sleep.

The results showed that the subjects took similar times to fall asleep (either to stage 1 or stage 2 sleep) after exercise and after relaxation, although studying delayed

sleep onset by an average of about 6 minutes. No significant differences in any sleep stage duration or cycle length were found between the three experimental nights. Also, no changes were found concerning the number and duration of body movements during sleep.

Hauri concluded that these findings provided "no support to the hypothesis that specific kinds of sleep represent extensions of or recovery from specific waking activities". It is possible that this uniformity of sleep following the various pre-sleep activities was a consequence of the short duration of EEG recording. However, it is well known that the majority of SWS occurs in the first half of the night and the possibility of an increase during the second half of the night following evening exercise was not indicated by the previous study of Baekland and Lasky (1966).

It seems unlikely that the lack of influence of the activities studied upon the sleep parameters was as a result of too little experimental variation. The six hour exercise period, taken by subjects not selected specifically for their fitness, can be classed as strenuous. Hauri points out that the subjects felt that the level of exercise could not have been maintained over a significantly longer time than the six hours imposed.

This inability of strenuous exercise taken immediately prior to sleep to influence sleep is in accord with the study by Baekeland and Lasky (1966), and it may be argued that this strengthens the belief that the arousing effect of exercise on the brain opposes the exercise-mediated increase in SWS. However, in Hauri's study it appears that there is no difference in sleep following studying and exercise (apart from sleep onset time). Both of these conditions have an arousing influence on the brain whilst only one, according to the body restitution theorists, enhances SWS. Thus, it may be argued that SWS should have been found to be reduced following the

studying period as a consequence of the arousal to the CNS in the absence of an increased need for SWS.

Baekeland (1970) continued his study of the effects of exercise upon sleep by investigating the effects of exercise deprivation in fourteen students who were used to regular exercise (three to four days a week). The subjects' sleep was recorded on a total of 6 nights: nights 1 and 2 followed two non-consecutive days when they took their normal exercise, whilst nights 3 to 4 were taken at intervals of about 2, 7, 14 and 30 days after the subjects had last exercised. No adaption or re-adaption nights were used in this study.

Although the results showed that SWS tended to be greater on night 2 than on night 3, the difference was not significant. Also, no significant changes in SWS were noted during the no-exercise period. Baekeland suggested that this lack of change in SWS from a period of regular exercise to a period of no-exercise might be due to two factors. Firstly, the subjects in this experiment exercised less frequently and strenuously than those in the previous study (Baekeland and Lasky, 1966). Secondly, it appeared (according to stage REM parameters) that the subjects' adaptation to the laboratory was not complete until night 3 (the first no-exercise night).

The complete lack of control for "first night" effects is the main criticism of this study. Furthermore, it is possible that re-adaptation would be necessary on the subsequent recording nights made at one and two week intervals. Also, by ignoring the use of adaptation nights, the author has little knowledge of the subjects' sleep on the nights prior to the recording nights. Obviously, a poor night's sleep or a late awakening can have substantial influence upon the subsequent night"s sleep.

Both wakefulness and the number of body movements in the second three hours of sleep showed significant increases ($p<0.05$) as the period of no-exercise continued, although these changes were small (an average increase of 7½ minutes of wakefulness on night 6 compared to night 3). Baekeland attributed these changes to the increased anxiety of the subjects during this period of relative inactivity.

Unfortunately, this study did not investigate the effects of a return to normal physical activity upon sleep. It would have been very interesting to know if SWS had increased during this period.

Zir, Smith and Parker (1971) studied the effect of light and moderate exercise upon SWS and HGH release in 10 young Navy corpsmen in good physical condition. The experiment lasted for 5 or 6 consecutive days allowing for 2 adaptation days, 2 control days, an experimental exercise day and, in the moderate exercise group, a recovery day. During all days except the exercise day, the subjects remained completely at chair rest except for a short walk to the canteen.

Five subjects completed a 2 hour period of light exercise starting at 2.00 p.m. This consisted of an equivalent of 10 miles cycling up a moderate slope on a bicycle ergometer, 20 round trips of the buildings, 4 flights of stairs, 20 push-ups, 20 chin-ups and 40 overhead pushes of a 20 pound barbell. The remaining five subjects completed a 6 hour period of exercises identical to those above but increased three-fold. This moderate exercise group exercised from 8.00 - 1.00 a.m. and from 1.00 - 4.00 p.m.

Percentage SWS during the first 3 hours of sleep, and total sleep and the peak HGH (ng/ml) were determined for all subjects on each night. The results showed that mean sleep length was increased following both the exercise conditions (no data provided). However, SWS was found to be

increased above control values on the exercise night in only 2 subjects when considering the whole night data and in 3 subjects for the first 3 hours only. Interestingly, only one subject from the moderate exercise group was found to exhibit an increase in SWS and that was during the first three hours of sleep. Only 2 of the subjects had a greater peak of HGH concentration on the exercise night and neither of these subjects were from the moderate exercise group. However, both of these subjects exhibited an increase in SWS during the first 3 hours of sleep on the exercise night.

The authors concluded that these findings "... do not support our hypothesis that SW sleep and HGH release would be adaptively responsive to daytime exercise, at least as tested by our present light and moderate exercise protocols". Admittedly, the light exercise condition was not particularly trying for Navy corpsmen and most of this group experienced no fatigue prior to retiring.

This was not true for the moderate exercise group who all reported feelings of fatigue at bedtime. Thus, the fact that most of the predicted changes in SWS and HGH release were found only in the light exercise group further strengthened the authors' conclusion.

Ryback and Lewis (1971) (see also Ryback, Lewis and Lessard, 1971) reported on the effects of prolonged bed rest upon sleep in eight young trainee airmen. The study was divided into control, bed-rest and recovery periods of 5, 5 and 6 weeks respectively. During the bed-rest period, 4 subjects (exercise group) continued to exercise on a total-body ergometer while confined to bed, while the other subjects (no-exercise group) remained inactive. All subjects performed 600 kcal of exercise (in three periods) daily except for those in the no-exercise group during the bed-rest.

The authors went to great lengths to control the physical activity of each subject during the bed-rest period. To this end the subjects remained supine in bed aided by special prism glasses which enabled them to read and eat in this position. Furthermore, they were carried to the ergometer and lavatory. The subjects were continuously monitored by staff and could not sleep or nap during the day. During the entire study the subjects all received a 3,334 kcal daily diet.

EEG sleep recordings were taken a total of 5 times during the study: 1 during the control period, 2 during the bed-rest period and a further 2 during the recovery period. These recording nights occurred between the 5th and 18th day of the control period, between the 11th and 27th day of the bed-rest period and between the 3rd and 33rd day of the recovery period. One adaptation night was given prior to the first recording night but no further adaptation was given before subsequent recordings.

The results showed that the total sleep time did not vary significantly during the different periods of the study or between the exercise and no-exercise groups during the bed-rest period. SWS was increased by 44% ($p<0.01$) and light sleep (stages 1 and 2) was decreased by 10% ($p<0.005$) during the bed-rest period compared to the control period when the two groups were combined. However, when analysed separately, only the no-exercise group showed a significant increase in SWS (+53% $p<0.05$) although the exercise group did show a progressive increase over the bed-rest period. During the recovery period, SWS and light sleep showed decreases and increases respectively but, although they were no longer significantly different to control values, they had not returned to control values by the second recovery night (a minimum of 25 days after the completion of the bed-rest period). Stage 4 sleep was found to be

significantly elevated ($p<0.05$) during both the bed-rest (+123%) and recovery (+85%) periods compared to the control values when the two groups were combined. No significant changes were reported for REM sleep.

The findings of increased SWS during prolonged bed-rest does not appear to be in accord with the body restitution theory of SWS function. However, examination of the SWS data shows that the control values are much lower than found in a study of normal males within this age range (Kales et. al., 1966). This is especially true for the stage 4 values. It is possible that these low values were as a result of the subjects' insufficient adaptation to the EEG recording equipment, although the length of sleep and duration of stage REM and stage 1 sleep do not appear to be atypical.

The authors attempted to explain their findings within the restitution theory by suggesting that the decrease in physical activity during bed-rest would cause atrophy of the subjects' muscular system and thus induce an increase in SWS to help repair this system. They note that the no-exercise group would suffer greater muscular atrophy than the exercise group and therefore require a quicker and greater increase in SWS than the exercise group. Furthermore, they suggested that SWS remained elevated during the recovery period because of the effect of exercise upon the atrophied muscular system resulting in a greater demand on the "physiological reparative process" than normally encountered.

Whilst the above suggestions may be plausible, it is possible that these changes in SWS were as a result of the sensory deprivation incurred during the bed-rest period. Ryback et. al (1971) suggested that the decrease in sensory input from the muscular system was the primary factor underlying the EEG changes in sensory deprivation studies rather than a decrease in environmental stimulation on the five senses.

This exercise deprivation study differs markedly from that of Baekeland (1970) as regards the normal life style of the subjects. The subjects in Baekeland's study were asked to refrain from taking their accustomed exercises for one month, during which time they spent four nights in the sleep laboratory. Thus, it appears that the life-style of these subjects was not significantly changed beyond the change in activity. However, the subjects in Ryback and Lewis' study underwent a major change in their life-style due to the enforced experimental procedure. It is possible that the differences in the findings of these two studies may be due, in part, to the gross changes in life-style of the later study.

A further variable which might have some bearing upon the findings of this study is the subjects' diet. As this remained constant during the entire study it seems very likely that the subjects, especially those in the no-exercise group, were effectively over-eating during the bed-rest period. This may influence sleep as the sleep-inducing effects of large meals are well-known. Also, the "over-eating" probably led to the subjects gaining weight during the bed-rest period which may have an influence upon sleep.

Considering the time and effort spent on this study it is unfortunate that only one recording was taken during the control period. The findings would be far stronger if at least two recordings were taken with, preferably, two adaptation nights preceding them.

Zloty, Burdick and Adamson (1973) noted that the college athletes studied by Baekeland and Lasky (1966) showed considerably more SWS, even on the no-exercise night, compared to levels recorded with non-athletes in the same age range (see Williams, Agnew and Webb, 1964). They suggested that people who partake in regular, heavy exercise may characteristically show large amounts of SWS. To test this hypothesis

they studied the sleep of 16 long distance runners on the second of three consecutive nights. Night one was not used in the analysis because of the possible first night effects and the authors considered that sleep on night 3 might have been affected by the fact that it was the last experimental night. The minimum period of serious running by these subjects was 4 years.

The results showed that the average duration of SWS was 23.1% of the subjects' total sleep time (an average of 7½ hours). Stages 3 and 4 occupied 11.9% and 11.2% of total sleep time, respectively.

The authors compared these values with those from a study of "normals" (i.e. not selected for their physical fitness) who spent an average of 10.0% and 3.5% of their total sleep time in stages 3 and 4 respectively. They concluded from this comparison that their long distance runners did have more SWS than normative groups and that this elevation was due mainly to an increase in Stage 4 sleep.

In addition, the authors compared their subjects with those used by Baekeland (1970). These subjects exhibited an average of 7.6% and 8.9% of stages 3 and 4 respectively on the second control night following their normal daily exercises. Admittedly, Baekeland used a 6 hour period of analysis whereas Zloty et. al. used a 7½ hour period but, as Zloty et. al. noted, this comparison is "conservative" because most SWS occurs during the first half of the night. The authors concluded that their findings were "consistent with the hypothesis that the amount of SWS is related to the amount of daytime energy expenditure".

Considering the implications of their findings, I believe that the above evidence is not at all convincing and should not be taken as evidence in favour of the

body-restitution theory of SWS. Levels of SWS reaching and exceeding 23% of total sleep time are not uncommon during the control periods of several sleep studies. For example, MacFadyen et. al. (1973) reported an average of 25% of total sleep time was spent in SWS over the four consecutive nights of their baseline period. The total sleep time during this period averaged 7 hours 53 minutes which is comparable to the 7 hours 30 minutes of sleep analysed by Zloty et. al. The subjects used in the MacFadyen et. al. study were "10 healthy, non-obese volunteers aged 20 to 23 years...". In another study using young men (Phillips, Chen, Crisp, Koval, McGuiness, Kalucy, Kalucy and Lacey, 1975) it was reported that 25.4% of their total sleep time (average of 7 hours 27 minutes) was spent in SWS during the control period.

Further evidence of control levels of SWS exceeding those reported by Zloty et. al. can be found during the control periods of the more recent exercise and sleep studies (Horne and Porter, 1975: 8 healthy subjects of average fitness, mean % SWS = 27.7, period of analysis = 7½ hours sleep time; Moses, Lubin, Naitoh and Johnson, 1977: 18 Navy men of average fitness (bed-rest group), mean of SWS = 26.6, period of analysis not specified but subjects were allowed 8 hours in bed).

Adamson, Hunter, Ogunremi, Oswald and Percy-Robb, (1974) studied the effects of strenuous daytime physical exercise upon sleep and plasma growth hormone levels in 12 healthy males. Five of these subjects regularly enjoyed some type of sporting activity whereas the remaining seven took little exercise. Each subject had one or more adaptation nights before the study began. On the exercise day, the subjects were instructed to take "considerably more than their usual amount of exercise, to take it before 14.00 hours, but not to the point of severe exhaustion."

Control data was taken 2-12 days after the exercise day. Eight subjects were recorded on 2 exercise-recovery nights and 2 control nights, whereas the other 4 subjects were studied on only one night following exercise and control activities.

The EEG data showed that the subjects fell asleep slightly more quickly after exercise. However, both SWS and REM sleep were slightly decreased and the sleep was generally more disturbed than on the control nights. None of these small changes were found to be significant. Interestingly, the nocturnal plasma growth hormone levels (areas under the curve between 24.00 and 06.30 hours) were found to be significantly elevated in 10 of the subjects even though SWS was not increased. This does not necessarily contradict the negative findings of Zir et. al. (1971) as these latter authors referred only to peak values of growth hormone.

## 3. DISCUSSION

The findings from the two animal studies suggest that NREM sleep appears earlier than usual during the sleep period if an appreciable amount of exercise has been taken prior to sleep. However, neither of these studies have shown that SWS levels were increased during a 24 hour period in response to the exercise loads given.

The findings from the human exercise studies appear to be equivocal. It was felt that the categorisation of the studies might highlight any consistent findings. To this end the human studies were divided into the following categories depending upon the type of exercise conditions imposed or prevalent at the time of study:-

a) acute exercise

b) chronic exercise

c) acute exercise deprivation

d) chronic exercise deprivation

## 3.1 Acute exercise

The term "acute exercise" is used here to describe exercise in excess of that normally encountered and of short duration (i.e. measured in hours as opposed to days or years), regardless of the fitness of the subjects.

Hauri (1968), Zir et. al. (1971) and Adamson et. al. (1974) have not found acute exercise to be associated with increased levels of SWS.

## 3.2 Chronic exercise

The term "chronic exercise" is used here to describe the regular heavy exercise which is taken as a way of life. It is not clear whether chronic exercise endows the athlete with increased amounts of SWS as several studies using subjects of average fitness have reported levels of SWS in excess of those reported by Baekeland (1970) and Zloty et. al. (1973). However, very high amounts of SWS were reported in the study by Baekeland and Lasky (1966).

## 3.3 Acute exercise deprivation

The term "acute exercise deprivation" is used here to describe an appreciable decrease in physical activity over a short period of time (i.e. up to a few days), regardless of the fitness of the subjects.

No studies reviewed have specifically studied the effects of acute exercise deprivation upon sleep. It might be argued that the no-exercise night in the study by Baekeland and Lasky (1966) recorded the effect of acute exercise deprivation as these subjects exercised regularly on five to six days a week. This would suggest that SWS is decreased

following a short period of relative inactivity. The subsequent study by Baekeland (1970) provides more evidence concerning the effects of acute exercise deprivation. No significant changes in SWS were found between nights 1 and 2 (normal exercise) and night 3 (no exercise for between 1 and 3 days) of this study. It is not possible to assess the effects of acute exercise deprivation from the study by Ryback and Lewis (1971) as the first recording night during the bed-rest period occurred between the 11th and 17th nights.

### 3.4 Chronic exercise deprivation

The term "chronic exercise deprivation" is used here to describe an appreciable decrease in physical activity over a prolonged period of time (i.e. several weeks), regardless of the fitness of the subjects.

Chronic exercise deprivation lasting for 4 to 5 weeks has been reported to either have no effect on SWS (Baekeland, 1970) or to increase SWS (Ryback and Lewis, 1971). This latter study reduced physical activity to a bare minimum in the no-exercise group during bed-rest whereas the subjects in the earlier study only refrained from taking their accustomed exercise (normally on 3 to 4 days a week). However, because of the drastic change in the life-style of the subjects during the bed-rest period and the very low baseline values of SWS reported for these subjects, there is not sufficient evidence to conclude that chronic exercise deprivation, per se, is associated with changes in SWS.

It is apparent that the above categorisation of the studies has not revealed a clear relationship between the various forms of exercise and sleep. It is possible that any influence of exercise upon sleep is dependent upon other factors.

## 3.5 Other possible factors

The only study that has reported significant increases of SWS following exercise is that of Baekeland and Lasky (1966). The other studies reviewed did not confirm this report possibly because the exercise conditions imposed were less strenuous and/or the subjects were not at such a peak of physical fitness.

Another factor in the exercise and sleep relationship may be the time of day when the exercise is taken. This is evident in the Baekeland and Lasky (1966) study and this may explain why Hauri (1968) found no effect of evening exercise upon sleep.

The general lack of significant changes in SWS following either an increase or decrease in physical activity may be due to the arbitary system adopted for sleep stage scoring. The current system (Rechtschaffen and Kales, 1968) divides SWS into stage 3, containing between 20% and 50% by time of delta activity, and stage 4, containing greater than 50% by time of delta activity. No assessment of the extent of delta activity outside of these two stages is allowed by this system as the duration of delta activity can vary from 0 to 20% by time in stage 2 epochs. Thus, it is possible that a subtle increase in delta activity may occur as a result of daytime exercise and not be noticed. As the duration of stage 2 sleep often occupies 50% or more of the total sleep time, it is apparent that the change of delta activity from an average of, say, 5% to 15%, by time, constitutes a significant increase in this type of brain activity. For example, if stage 2 duration for one night is 225 minutes, then the difference in the duration of delta activity would be 22.5 minutes. Furthermore, this represents the amount of continuous delta activity which has escaped detection and this is equivalent to up to 45 minutes of stage 4 sleep or up to 112.5 minutes of stage 3 sleep.

## 4. CONCLUSIONS

The review of the exercise and sleep literature up to 1974 (when the first study of this thesis was undertaken) has produced equivocal findings. Sleep following both exercise and bed-rest has been found to exhibit increases in SWS, whereas the majority of the studies have not found any change in SWS. The sub-division of the studies into those of exercise or exercise deprivation of chronic or acute duration has not clarified the issue. It is possible that other factors, such as the fitness of the subject or the time of exercising, influence the effects of exercise upon sleep and these should be controlled for in future studies. Also, some account should be taken for the variation in delta activity during stage 2 sleep.

CHAPTER 3

CONSIDERATIONS FOR FUTURE RESEARCH

## 1. GENERAL CONSIDERATIONS

From the literature review it is apparent that further study of the exercise and sleep relationship is merited. The existing studies suffer from a general lack of appreciation of factors which may be involved in such a relationship, and any subsequent work should take care to control for these factors. Specifically, a new study should make provisions for the following:-

1. the selection of subjects who are healthy, both mentally and physically, do not take any medications and have no sleep problems,

2. the use of at least two adaptation nights in the laboratory before the first recording period and a further adaptation night before subsequent periods of recording,

3. no recordings should be taken during the weekends as life-styles may change considerably,

4. the amount of delta activity occurring within stage 2 sleep should be assessed on each recording night,

5. at least two nights should be used to record control data,

6. the night following the exercise recovery night should be recorded in case there are any carryover effects,

7. the amount of work done by the subjects during the exercise period should be quantifiable,

8. the subjects should perform identical types of activities during the exercise period,

9. the exercise should be taken at a consistent time of day (i.e. morning, afternoon, early evening, late evening) by all subjects,

10. the duration of the exercise should be constant for all subjects.

Points 1-6 are concerned with removing or decreasing the effects of extraneous variables upon the main variable, namely exercise, which is to be studied. The remaining points are concerned with the design of the experimental exercise load and they are discussed in more detail in the following section.

## 2. CONSIDERATIONS FOR THE DESIGN AND ASSESSMENT OF EXERCISE

### 2.1 Quantification of work done

It should be appreciated that the amount of work done during the exercise period is one of the most important variables to be studied. The effects of light exercise upon sleep, if they exist, are probably very small whereas the effects of very heavy exercise may include non-specific effects arising from psychological stress if the subjects are not accustomed to such strenuous activity. Thus, the use of moderate, but not stressful, exercise loads is to be recommended when assessing the influence of exercise alone upon sleep.

These loads should be quantifiable to allow for comparisons between studies. Unfortunately, the majority of studies reviewed in the previous chapter did not state the workload imposed in units of work (i.e. watts, kilopond-metres) but rather in terms of duration, with a brief description of the activities.

#### 2.1.1 Exercise load in relation to maximum work capacity

However, more important than stating the absolute amount of work done by a subject is the knowledge of the

amount of work done relative to this subject's maximum work capacity. As the maximum work capacity of an athlete trained at a specific activity can be several times larger than that of an unfit untrained person at the same activity, it would be poor practice to assign an identical experimental workload to both of these subjects. The superior performance of the athlete's cardiac and respiratory systems would be able to cope with the workload far more efficiently than the unfit subject and it is possible that a workload which the athlete would regard as light to moderate could severely strain an unfit subject, both physiologically and pyschologically.

With regard to the physiological response, the athlete would derive energy from almost exclusively aerobic processes (i.e. those requiring the presence of oxygen) to sustain the same workload. This is because the athletes' cardiac and respiratory system would be more efficient than an unfit subject, and would enable a faster flow of oxygen to reach the exercising muscles, and a faster excretion rate of carbon-dioxide from the energy-liberating processes. These improvements are mediated by increases in the blood supply to the exercising muscles as a result of:-

a) reduced blood flow to the skin (during initial exercise only) and splanchnic area

b) increased venous return to the heart due to the pumping action of the working muscles and the vigorous breathing movements

c) increased force and frequency of the cardiac muscle contractions to cope with increased venous return

d) dilation of arterioles and capillaries in the working muscles due to nervous control and the effect of increased metabolism upon the composition and pH of the interstitial fluid.

As the workload increases, the anaerobic contribution becomes more important. One of the products of anaerobic respiration is lactic acid and the subjective feeling of fatigue during exercise is closely related with the levels of this acid in the blood.

The individual's capacity for heavy, prolonged physical work is therefore dependent upon the oxygen supply to the active muscles. In types of work which use large groups of muscles, the limiting factor for the aerobic work capacity will be the capacity and regulation of the oxygen-transporting system. This knowledge provides the basis for the physiological tests of maximum work capacity.

### 2.1.2 Evaluation of maximum work capacity

In many types of muscular exercise the steady-state oxygen uptake increases roughly linearly with an increase in work load (Åstrand and Saltin, 1961). A steady-state condition describes the situation where the oxygen uptake equals the oxygen requirement of the tissues. Heart rate, cardiac output and pulmonary ventilation should have attained fairly constant levels and there should be no accumulation of lactic acid in the blood. The knowledge of a subject's maximal oxygen uptake (maximal aerobic power) will confer information regarding that subject's maximum work capacity for aerobic muscular exercise. The maximal aerobic power is defined as the highest oxygen uptake the individual can attain during physical work breathing air at sea level.

A direct measurement of the maximal aerobic power can be made, but it demands a high degree of co-operation from the subjects since they have to exercise very close to the point of exhaustion. For this reason an estimation or prediction of a subject's maximal aerobic power is often made from submaximal exercise tests using the nomogram developed by Åstrand and Rhyming (1954). The error of this

nomogram has been assessed as not greater than ±10% (Shephard et. al., mult., 1968) and between 10 and 15% (Åstrand and Rodahl, 1970), even when the tests are carried out during strictly standardised conditions (i.e. time since last meal, temperature and humidity of the room).

Thus, the prediction of an individual's maximum work capacity during aerobic exercise can only be approximate but, as pointed out by Åstrand and Rodahl (1970), it is sufficiently accurate to select the best, worst and average from a group.

A detailed description of the submaximal test can be found in the following chapter. The bicycle ergometer is often chosen as the instrument of exercise as the oxygen uptake can be predicted with greater accuracy than for any other type of exercise (Åstrand and Rodahl, 1970, page 362).

### 2.1.3 Standardised exercise load

With a knowledge of the subjects' approximate maximum aerobic work capacity, it is possible to prescribe a standardised exercise load to each subject; this standardised load being simply a fixed percentage of each subjects' personal maximum aerobic work capacity. Thus, if a very fit subject has a maximal aerobic work capacity which is $1\frac{1}{4}$ times that of a subject of below average fitness, then the more athletic subject will be given an experimental work load $1\frac{1}{4}$ times that given to the less fit subject.

In order to decide what this fixed percentage of the maximum aerobic work capacity should be, one has to consider that the experimental load should be rated as moderate, as opposed to stressful, by the subjects. Since the feelings of greater exertion and fatigue can lead to psychological stress, it is apparent that the experimental load should not

induce an accumulation of lactic acid in the body as the levels of this acid are known to be related to such feelings. When work periods are extended to 1 hour, it has been found that the level of arterial lactic acid is not significantly elevated provided the oxygen uptake is not higher than about 50% of the maximum (Åstrand et. al., 1959; Åstrand, 1960; Shephard et. al., 1968).

As the steady-state oxygen uptake is linearly related to work load it was decided to prescribe a work load equivalent to 45% of a subject's maximum work capacity.

The type of activity chosen and its duration and time of onset are all important variables and are discussed in the subsequent sections.

## 2.2 Type of exercise

In order to prescribe the various standardised work loads it would be possible to sample the subjects' oxygen consumption (using a Douglas bag) at regular intervals during exercise to ensure that the subject was working at the required load. However, this is a tedious method and rather uncomfortable for the subject. It is far simpler to ensure that each subject exercises at the correct work load by using a bicycle ergometer, as the task load can be pre-set (the bicycle ergometer is described in the next chapter).

## 2.3 Duration of exercise

The duration of the exercise must be carefully assessed to ensure that, whilst the subjects are not stressed by prolonged work periods, they do perform significant amounts of work. It has been reported that the introduction of a rest period into a work programme can have considerable beneficial effects upon the total duration of activity that a subject can tolerate (Åstrand et. al., 1960 a, b;

Christensen et. al., 1960). The use of split exercise periods is thus a good method to study the effects of large amounts of work upon sleep without causing any undue stress to the subjects.

## 2.4 Time of exercise

There is some evidence to suggest that the time of day of exercising is an important factor in the exercise and sleep relationship. The influence of this factor can be assessed either by inter-study comparisons or, preferably, by a suitable experimental design which accommodates the study of this factor. This latter method will be more sensitive than inter-study comparisons because of the differences in subjects, experimental design and exercise variables between studies.

The exercise could be given either during the morning, afternoon or evening. If the exercise is taken during the evening, then it will be difficult to determine whether any observed changes in sleep are as a direct result of an increased need for body recovery following the exercise or as an indirect result of the increased heart rate, blood pressure and alertness associated with exercise.

## 2.5 Control for day-to-day variations in physical activity

Obviously, the daily physical activity levels of the subjects must show little variation over the entire study except for the experimental exercise days. Therefore, if a subject walked to town twice a week then he or she should either omit this journey or undergo its equivalent at roughly the same time-of-day during every day of the study including the exercise days. The same applies to sporting activities.

## 3. SUMMARY AND PROPOSALS FOR RESEARCH

This chapter has described the general considerations that should be taken when assessing the influence of exercise upon sleep. Many of the previous studies did not pay respect to all of these considerations and, consequently, the findings were constrained due to the lack of valid control data. In addition, the various exercise loads imposed were often poorly described, thereby hindering inter-study comparisons.

The most important variables in the description of an experimental exercise load have been discussed and these include the type, the time of onset, the duration and the ratio between a subject's prescribed experimental load and his maximum work capacity. The knowledge of this last variable can be used to prescribe standardised work loads to groups of subjects.

The first study in this thesis was designed to assess the time-of-day effects of standardised exercise upon subsequent sleep in humans. More specifically, moderate morning and afternoon exercise were studied and stage 2 sleep was additionally quantified in respect of delta activity.

CHAPTER 4

STUDY I

CHAPTER 4 - CONTINUED

## 1. AIMS OF THE STUDY

There is a theoretical argument, backed by a degree of evidence, that SWS is specifically concerned with body restitution (see Chapter 2, Section 1.1 for discussion). However, the predicted increase in SWS following daytime exercise has not been reported on several occasions.

The duration of SWS during the night is often regarded as an accurate indicator of delta activity, but up to 20% of this activity is included in the non-SWS stage 2. To improve the assessment of delta activity, this study will subdivide stage 2 according to whether the delta activity is 10% or less (stage 2(i)) or greater than 10% but less than 20% (stage 2(ii)).

Other factors which may be of significance in the exercise and sleep relationship are the fitness of the subjects, the work load prescribed, the type of activity and the time of onset and duration of the exercise. There has been a lack of control of these factors in the previous exercise studies which may contribute to the equivocality of findings. This present study has been designed with these considerations in mind (see Chapter 3 for discussion).

The aim of this first study is to assess the effects of standardised moderate exercise, taken in the morning or afternoon, upon subsequent sleep in humans. The results from this study will indicate whether exercise does influence sleep and, if so, whether the extent of this influence is sensitive to the time-of-day at which the exercise was taken.

## 2. SUBJECT SELECTION

The experimenter advertised for subjects within the student community on the campus. Prospective subjects were requested to complete a screening questionnaire (see Appendix I). Only those subjects who were young (17 - 25

years), healthy, free from medication and sleep problems and did not report daytime naps were considered for this study.

Suitable subjects were then informed that it was important that they should display little variation in their daily physical activities throughout the study (except for the imposed exercises). Prospective subjects who typically enjoyed a variety of sporting commitments were not chosen for this study because of the difficulties that would be encountered in regulating the daily activity levels. Even if these subjects promised to refrain from such regular activities they were not chosen because their control data would reflect exercise deprivation rather than normal activity.

## 3. PILOT-STUDY TO DETERMINE INDIVIDUAL STANDARDISED WORK LOADS

### 3.1 Aims of pilot-study

This pilot-study predicted the maximum aerobic power, and thus an estimate of the maximum work capacity, for each subject. The standardised work load given in the main exercise and sleep experiment was 45% of this maximum work capacity for each subject.

### 3.2 Prediction of $\dot{V}O_2$ max.

Maximal aerobic power ($\dot{V}O_2$ max) was predicted using the sub-maximal exercise nomogram method of Åstrand (1960).

#### 3.2.1 Type of exercise

The bicycle ergometer was chosen in preference to the step method and the treadmill as the instrument of exercise, for the following reasons:-

a) the oxygen uptake can be predicted with greater accuracy than for any other type of exercise (Åstrand and Rodahl, 1970),

b) within limits, the mechanical efficiency of the bicycle ergometer is independent of body weight- Wahlund (1948). Tests have also shown that the mechanical efficiency does not vary with the height of the handlebars and saddle, provided that this variation is kept within reasonable limits (Åstrand, 1954),

c) variations in pulse rate due to habituation and learning are slight,

d) the subject exercises in a sitting position with arms and chest relatively immobile. Good ECG tracings can therefore be obtained,

e) the work load can easily be adjusted and recorded, and the instrument is reasonably mobile.

The bicycle ergometer used incorporated a whirling current braking system. Attached to the wheel was a copper disc which passed between the poles of two strong permanent magnets. The position of these magnets could be adjusted by operating a crank handle and this varied the braking force (i.e. the work load).

### 3.2.2 Choice of load

When testing circulatory-respiratory fitness, the work must engage large groups of muscles and the load must be relatively high. The duration of the work must be sufficient to allow for circulatory and respiratory adjustments to that work load (i.e. steady-state conditions).

The nomogram of Astrand and Ryhming was developed from the results of 5 - 6 minute exercises on the bicycle ergometer, step test and treadmill. The greatest accuracy of the nomogram was obtained when the load imposed a steady heart rate somewhere between the 125 and 170 region (Åstrand and Ryhming, 1954). Within these limits there was an almost linear increase in metabolism with heart rate.

It was decided to run a succession of 6 minute exercises starting at a load designed to produce a heart rate at the lower end of this region. The subject was then allowed to rest until his heart rate was back to normal. Several other increasing work loads were carried out, providing heart rates in the middle and upper regions.

3.2.3 Precautions

a) Subjects were screened for any circulatory or respiratory handicap; they were also required to be infection-free.

b) Subjects refrained from energetic activities prior to the work tests.

c) The tests were not performed within 2 hours of the last meal, because heart rate and respiration would be increased for approximately an hour after a heavy meal.

d) No smoking was allowed during the last 30 minutes prior to the tests and until their completion. Tobacco contains up to 4% carbon monoxide by volume. This combines with haemoglobin thereby decreasing oxygen transport to the tissues. Smoking also increases the resistance of the air passages due to the swelling of the mucous membranes.

e) Subjects refrained from taking alcohol prior to the tests. Alcohol causes an increase in respiration and heart rate (Blomquist et. al., 1969).

f) The subjects were tested at approximately the same time, but on different days, to reduce the effects of circadian variation between the subjects.

g) The environmental temperature was between $18^{o}$ to $20^{o}C$ and the relative humidity was within the range of 40 to 60%. This was in accordance with Åstrand and Ryhming's studies. At high temperatures, blood is diverted to the sub-cutaneous vessels during rest and sub-maximal exercise and predictions of maximal aerobic power ($\dot{V}O_2$ max) based on pulse rate fall into error.

h) The saddle and handle bars of the ergometer were adjusted to suit individual subjects. The most comfortable position, and in the case of heavy loads the most effective one, is a saddle height producing a slight bend of the knee when the ball of the foot rests on the pedal and the leg is stretched.

i) A pedalling rate of 60 revs./minute was used since it is the most comfortable rate for people of average fitness.

j) Subjects were requested always to remain seated, and to sit upright whilst exercising. This was to avoid changes in the mechanical efficiency of the ergometer and to prevent changes in the heart rate due to changes in the body position.

### 3.2.4 Measurement of heart rate

The heart rates of the subjects were recorded on an electrocardiogram (ECG) during the last minute of exercise. A three-electrode system was used as only the R wave component was of interest.

The grid I electrode was placed on the left chest in the anterior axillary line of the 5th interspace; the grid II electrode over the manubrium and the earth on the right chest, symmetrically opposite the positive electrode.

### 3.2.5 Calculation of predicted $\dot{V}O_2$ max and maximum work capacity

The knowledge of each subject's heart rate at specified sub-maximal work loads enabled the prediction of $\dot{V}O_2$ max from the nomogram. The average $\dot{V}O_2$ max was calculated from those predictions using a heart rate between 125 and 170 beats per minute. The maximum work capacity of each subject was estimated from this average $\dot{V}O_2$ max using the appropriate scales on the nomogram. These values are shown in table 1.

### 3.2.6 Calculation of individual standardised work loads

The work load chosen for the main study was 45% of the subject's maximum work capacity. This was in accordance with the results of prolonged work loads on untrained subjects, whose maximum was around the 50% mark (Åstrand, 1970). The individual and average loads are described in Table 1. The range of standardised work loads was from 90 to 150 watts with an average of 121 watts, at a pedal rate of 60 revs./minute.

Table 1: Summary of results for pilot-study

| SUBJECT | Estimated $\dot{V}O_2$ (max) | Estimated Max. Work Rate | Standardised work load (45% max.) |
|---|---|---|---|
| | l/mins | watts | watts |
| 1 | 2.9 | 216 | 97 |
| 2 | 3.9 | 288 | 130 |
| 3 | 3.6 | 266 | 120 |
| 4 | 3.5 | 255 | 115 |
| 5 | 2.8 | 200 | 90 |
| 6 | 3.8 | 282 | 127 |
| 7 | 4.7 | 333 | 150 |
| 8 | 4.4 | 313 | 141 |

### 3.2.7 Limitations of this method

The error of the nomogram method for predicting $\dot{V}O_2$ max has been assessed as not greater than ±10% (Shephard et. al., 1968) and between 10 and 15% (Åstrand, 1970).

As $\dot{V}O_2$ max is predicted from the knowledge of heart rate at various sub-maximal work loads, it is apparent that any non-metabolic changes in the heart rate will induce unquantifiable errors in the prediction. For example, fear, excitement and stress may also cause marked elevation of the heart rate at a sub-maximal work load without either maximal oxygen uptake or performance capacity being affected. However, the heavier the work load, the less pronounced is this effect and it is usually recommended that the test load should bring the pulse rate up to or above 150 beats/minute for young subjects.

The effect of stress was also reduced by the informal attitude of the experimenter. Any anxiety typically decreases upon repetition of the test and this was used to

advantage as the latter tests, which used the heavier work loads, were the ones used in the calculations. The corrections for the age factor (heart rate declines with age after about twenty) were used.

Thus, the prediction of an individual's maximum work capacity during aerobic exercise can only be considered approximate. However, as discussed in the previous chapter, it is sufficiently accurate to distinguish between unfit, fit and very fit people.

## 4. PILOT STUDY TO DETERMINE THE DURATION OF EXERCISE

### 4.1 Aims of pilot-study

To determine the length of time the subjects could exercise at their standardised work loads without undue exertion and stress. One rest period was allowed in order to increase the duration of exercise.

### 4.2 Method

The two subjects with the highest and lowest maximum work capacity were used in this pilot-study. They exercised at their prescribed standardised workloads on the bicycle ergometer until they felt that a break was urgently required. The duration of this break was kept to a minimum as judged by the subjects, and the subjects continued their exercise. Liquid refreshment, in the form of water or sweetened orange juice, were available at all times.

### 4.3 Results

After 42 minutes of continuous exercise, both subjects demanded a rest. The subjects either sat or laid down during the break and were ready to restart after quarter of an hour. Approximately half-an-hour later, the subjects reported that it was time to stop although they continued to exercise for an identical length of time as in the pre-rest session at the suggestion of the experimenter. The subjects did not find this extra ten minutes required undue

exertion on their part although, if the decision had been theirs, they would have stopped after 30 minutes.

From the findings it was decided to split the exercise period into two halves, each of 42 minutes duration, with a break of 15 minutes in between.

The reports of fatigue and the desire to finish exercising were comparable with these two subjects, even though one was exercising at 90 watts whilst the other exercised at 150 watts. This highlighted the value of prescribing individual standardised work-loads.

## 5. CONTROL OF EXTRANEOUS VARIABLES

Variables such as age, general health, taking of medicines and sleeping quality were controlled for via subject selection (see Section 2). Subjects were instructed not to change their diet during the study or to drink tea, coffee or alcohol in the evenings. Also, daytime naps were not permitted.

One of the most important variables to be controlled for was the daily physical activity of the subjects (see Chapter 3, Section 2.5).

No recordings were made during the weekend as the changes in life style of the subjects may influence sleep.

## 6. EXPERIMENTAL PROCEDURE

The experimental timetable is described in Table 2. The main experiment lasted for two consecutive weeks although no recordings were made during the weekends. The subjects were recorded in pairs and the experiment always started on a Monday. Monday and Tuesday night of the first week were used as adaptation nights with full electrode attachment but no recording. The following night (Wednesday)

Table 2. Experimental Timetable

| Day | Subject | |
|---|---|---|
| | 1,3,5 & 7 | 2,4,6 & 8 |
| 1 (Monday) | Adaptation<br>NA, NR | Adaptation<br>NA, NR |
| 2 (Tuesday | Adaptation<br>NR, NR | Adaptation<br>NA, NR |
| 3 (Wednesday) | Control 1<br>NA, R | Control 1<br>NA, R |
| 4 (Thursday) | AM exercise<br>R | PM exercise<br>R |
| 5 (Friday) | AM carryover<br>NA, R | PM carryover<br>NA, R |
| 6 (Saturday) | FREE | FREE |
| 7 (Sunday) | FREE | FREE |
| 8 (Monday) | Adaptation<br>NA, R | Adaptation<br>NA, R |
| 9 (Tuesday) | Control 2<br>NA, R | Control 2<br>NA, R |
| 10 (Wednesday) | PM exercise<br>R | AM exercise<br>R |
| 11 (Thursday) | PM carryover<br>NA, R | AM carryover<br>NA, R |

Key:-

| | |
|---|---|
| AM exercise | morning exercise |
| PM exercise | afternoon exercise |
| NA | normal activities |
| R | EEG recordings taken |
| NR | No EEG recordings taken |

was used to record the first control night and the first exercise period was taken on the next day (Thursday). EEG recordings were also taken on this night (exercise recovery night) and on the Friday night (exercise carry-over night). The subjects returned to the lab on the next Monday night when the electrodes were fitted but no recordings were taken. The Tuesday night of the second week was used to record the second control night and the Wednesday and Thursday nights were used to record the second exercise recovery and carryover nights, respectively.

On the first exercise day, one subject of each pair performed morning exercise whilst the other performed afternoon exercise. These conditions were reversed for the second exercise day. The subjects performed their prescribed exercise load (for two 42 minute periods of exercise with a 15 minute rest period in between) either between 10.00 and 12.00 (morning) or 16.00 and 18.00 (afternoon).

The subjects arrived at the sleep laboratory at approximately 10.30 p.m. when the daily activity log was completed and the electrodes were attached. The montage recommended by Rechtschaffen and Kales (1968) was used, namely: C3-A2; C4-A1; left-eye A1; right-eye A1; EMG electrodes were located on either side of the chin. Lights out was at 12.00 p.m. and all-night recordings were made with a Grass model 78 electroencelphalogram at a paper speed of 10 mm/second. The subjects were allowed to sleep until they felt refreshed and ready to get up.

## 7. DATA ANALYSIS

The sleep records were divided into one minute epochs and classified into sleep stages according to the criteria described by Rechtschaffen and Kales (1968) by two experienced scorers. In addition, stage 2 was subdivided into

stage 2(i), containing less than 10% by time of delta activity, and stage 2(ii), containing between 10% and 20% by time of delta activity.

Only the first 7½ hours of sleep, as measured from the onset of stage 2, were used for analysis; this being the shortest sleep length for the group of subjects over the experimental period.

For each subject, the percentage of time spent in each stage of sleep were calculated for each of the control, exercise recovery and exercise carryover nights during:-

a) the whole night (i.e. 450 minutes)
b) the first half (i.e. first 225 minutes) of the night
c) the second half (i.e. last 225 minutes) of the night

In addition, various other parameters concerned with REM sleep were calculated, including the amount of stages 0+1, 2(i), 2(ii), 3 and 4 taken before the first REM period.

Related t tests were used to compare the data for the exercise recovery and exercise carryover nights with the average control values. No predictions were made concerning SWS as the literature review had failed to find any consistent effects of exercise upon SWS.

## 8. RESULTS

The group data is summarised in tables 3 and 4 and the changes in delta activity are illustrated in figures 1 - 4. The standard deviations for the group means are provided in Appendix I.

The whole night percentages of sleep stages remained within baseline values on all experimental nights. Also,

Table 3. Group means (%) for sleep stages

| | Baseline | Morning exercise | | Afternoon exercise | |
|---|---|---|---|---|---|
| | | Recovery night | Carryover night | Recovery night | Carryover night |
| **Whole night** | | | | | |
| Stages 0+1 | 5.2 | 6.2 | 5.7 | 5.4 | 4.8 |
| 2ii | 9.9 | 8.8 | 8.9 | 11.7 | 9.6 |
| 3 | 9.5 | 9.7 | 10.8 | 10.8 | 8.5 |
| 4 | 18.2 | 19.1 | 17.2 | 17.2 | 16.8 |
| 3+4 | 27.7 | 28.8 | 27.9 | 27.9 | 25.3 |
| 2ii+3+4 | 37.6 | 37.6 | 36.8 | 39.6 | 34.9 |
| REM | 23.5 | 25.2 | 24.1 | 23.7 | 24.4 |
| **First half** | | | | | |
| Stages 0+1 | 2.8 | 3.2 | 3.8 | 2.7 | 2.6 |
| 2ii | 11.6 | 8.0†(4.4) | 10.0 | 14.6 | 12.2 |
| 3 | 13.2 | 11.5 | 13.6 | 16.2†(4.0) | 11.0 |
| 4 | 31.6 | 35.3 | 26.1 | 29.3 | 30.8 |
| 3+4 | 45.1 | 46.7 | 39.7 | 45.3 | 41.9 |
| 2ii+3+4 | 56.7 | 54.7 | 49.7*(3.0) | 59.9 | 54.1 |
| REM | 13.7 | 15.9 | 16.3 | 13.2 | 14.5 |
| **Second half** | | | | | |
| Stages 0+1 | 7.8 | 9.2 | 7.6 | 8.2 | 7.1 |
| 2ii | 8.2 | 9.5 | 8.1 | 8.8 | 7.1 |
| 3 | 5.7 | 8.0 | 7.9 | 5.3 | 6.1 |
| 4 | 4.9 | 2.8 | 8.2 | 5.1 | 2.8 |
| 3+4 | 10.4 | 10.9 | 16.1 | 10.8 | 8.7 |
| 2ii+3+4 | 18.6 | 20.4 | 24.3*(2.5) | 19.6 | 15.8 |
| REM | 33.3 | 34.4 | 31.8 | 34.7 | 34.4 |

*Significant at 0.05 level (two tailed)
†Significant at 0.01 level (two-tailed)
t values in brackets, df=7

Table 4. Group means for REM parameters

| | Baseline | Morning exercise | | Afternoon exercise | |
|---|---|---|---|---|---|
| | | Recovery night | Carryover night | Recovery night | Carryover night |
| Time (min) before first REM period | | | | | |
| Stages 0+1 | 1.0 | 0.7 | 1.4 | 2.1 | 1.7 |
| 2ii | 10.6 | 5.4*(3.1) | 7.8 | 16.6 | 9.3 |
| 3 | 14.4 | 10.7 | 12.5 | 19.6*(3.6) | 11.6 |
| 4 | 47.9 | 50.2 | 42.0 | 56.2 | 40.0 |
| 3+4 | 62.1 | 60.9 | 54.5 | 75.7 | 51.6 |
| 2ii+3+4 | 72.7 | 66.3 | 62.3 | 92.3 | 60.9 |
| REM latency | 91.9 | 79.4 | 79.4 | 112.9 | 89.5 |
| First REM period length | 14.4 | 11.9 | 9.0 | 15.8 | 10.9 |
| REM periodicity | | | | | |
| First period | 88.4 | 87.9 | 77.2 | 77.1 | 81.7 |
| Mean of first and second periods | 92.3 | 92.0 | 90.4 | 89.9 | 89.6 |

*Significant at 0.05 level (two-tailed)
t values in brackets, df=7

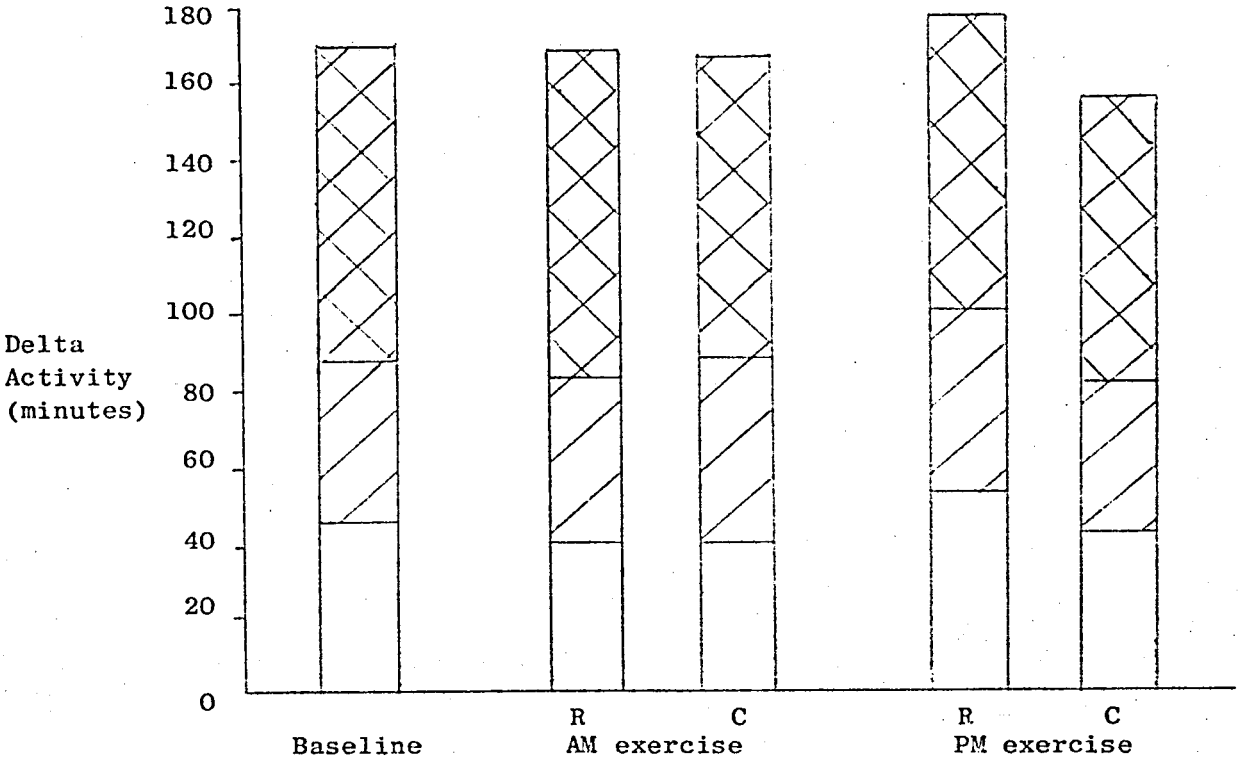



FIGURE 1: Whole night duration of delta activity

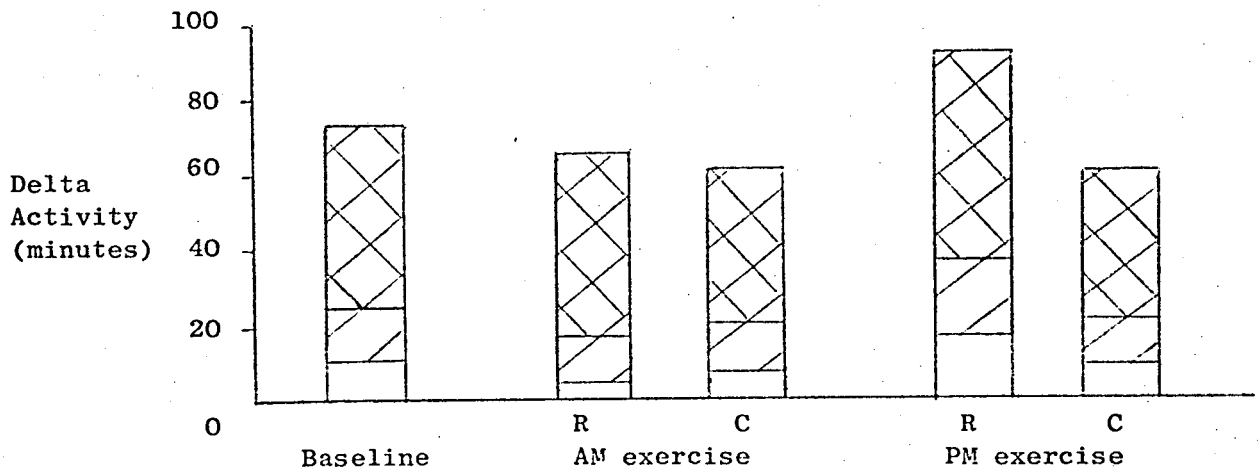
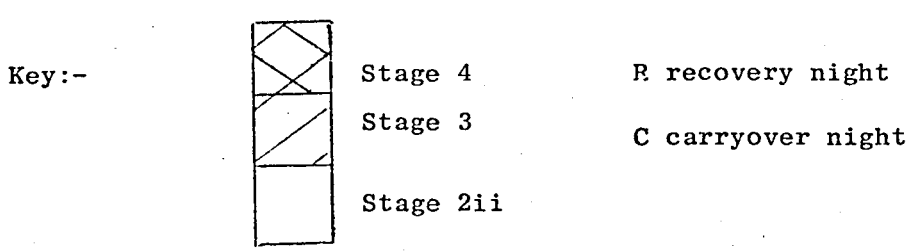



FIGURE 2: Delta activity prior to first REM period



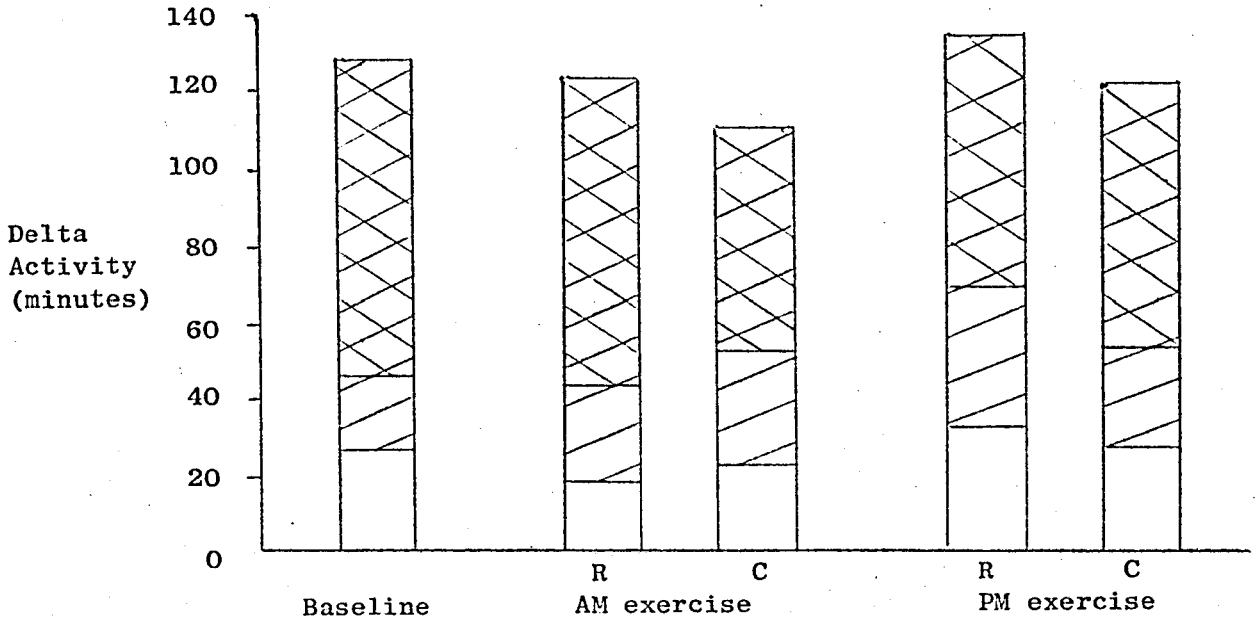



FIGURE 3: Delta activity during the first 225 minutes of sleep.

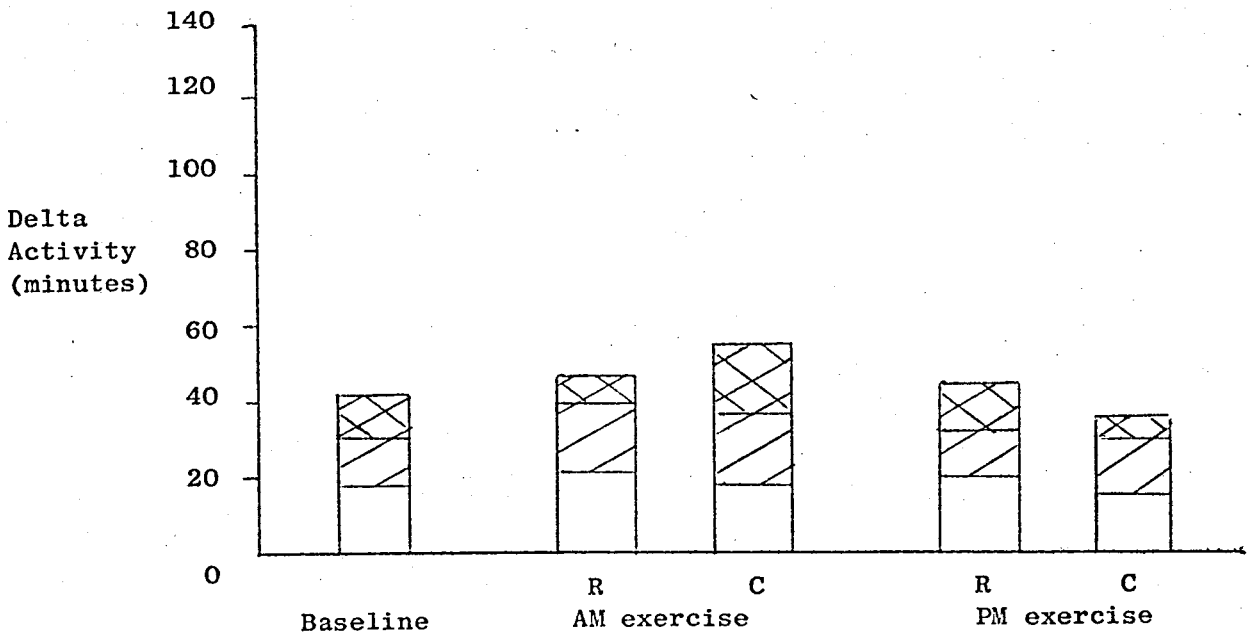
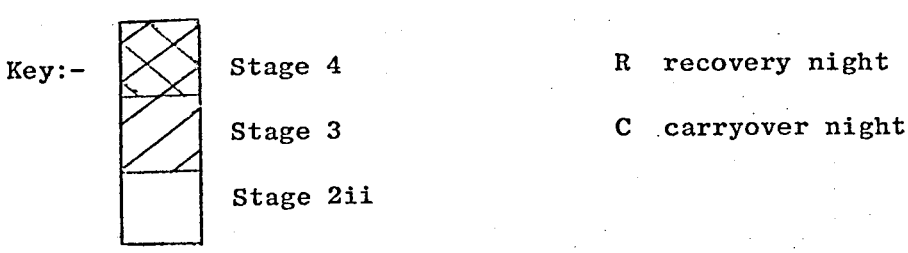



FIGURE 4: Delta activity during the second 225 minutes of sleep



REM latency and periodicity were unaffected. However, subtle changes in delta activity occurred following PM exercise with stage 3 showing a significant increase during the first half of the night (6.7 minutes, $p<0.01$) and, related to this, prior to the first stage REM period (5.2 minutes, $p<0.05$). Although the mean duration of stage 2(ii) and stage 4 prior to the first stage REM period also showed increases, (6.0 and 8.3 minutes, respectively), these changes did not reach significance. No significant changes in the various sleep parameters were found on the PM exercise carryover night.

The only significant change found to follow AM exercise was a decrease in stage 2(ii) both prior to the first stage REM period (5.2 minutes, $p<0.05$) and during the first half of the night (8.1 minutes, $p<0.01$). On the AM exercise carryover night summated stages 2(ii)+3+4 showed a significant decrease during the first half of the night (15.7 minutes, $p<0.05$) followed by a significant increase during the second half of the night (12.8 minutes, $p<0.05$).

## 9. DISCUSSION OF RESULTS

The results of this study suggest that moderate exercise does have a small effect upon subsequent sleep and that this effect is influenced by the time of day when the exercise is taken.

The effects of AM exercise upon subsequent sleep appear to be minimal as the decrease in stage 2(ii) during the first half of the night was the only significant change. The overall measure of delta activity (stages 2(ii)+3+4) did not show a significant decrease during this half of the night due to the non-significant increase in stage 4. The only significant findings on the AM exercise carryover night were a significant decrease of stage 2(ii)+3+4 during the first half, followed by a significant increase during

the second half. This temporal shift of delta activity appears to be mainly as a consequence of the non-significant changes in stage 4 sleep and may be due to the arbitary decision to divide total sleep into the first and second periods of 225 minutes.

The same amount of exercise taken during the afternoon does appear to influence subsequent sleep. Specifically, the PM exercise recovery night shows a significant increase in stage 3, both prior to the first stage REM period and during the first 225 minutes of sleep.

However, these increases do not extend into the second half of the night and no significant increases in any of these stages, either individually or collectively, were found for the whole night data.

With regard to stage REM sleep, no significant changes were found on any night, either for whole night data or during the first and second halves. REM latency, length of the first stage REM period and REM periodicity remained at baseline values on all nights except for the non-significant increase in REM latency on the PM exercise recovery night. Interestingly, three subjects actually missed their normally expected first stage REM period. This increase in REM latency for these subjects following the PM exercise would allow more NREM sleep to occur which may account for the significant increase in stages 2(ii)+3+4 found prior to the first stage REM period on this night.

The results of this study have shown that moderate daytime exercise appears to have a time-of-day effect upon subsequent sleep. If the exercise is taken in the morning, then the influence upon sleep is minimal. Afternoon exercise appears to induce small increases in delta activity during the first few hours of sleep.

It is possible that a greater increase in delta activity may have occurred if more strenuous exercise had been prescribed. However, as previously discussed, the effects of exercising at higher levels than voluntarily contemplated would be physiologically and psychologically stressful. Apart from the hormonal changes induced (e.g. stress-increased activity of the cortex and medulla of the adrenals), it is possible that the subjects may suffer from body aches and pains which would influence subsequent sleep. Other psychological factors such as resentment at being bullied to do more work or frustration at not achieving the prescribed work levels could also affect sleep.

The work done by the subjects in this study was not sufficient to cause undue discomfort, either physiologically or psychologically, after rest and refreshment. At the same time, however, the work done was appreciably in excess of normal daily activity.

If SWS does fulfill a bodily recovery function then it is surprising that the high work load incurred by the subjects did not show a more conclusive increase in SWS. The fact that AM exercise did not show any increase in SWS or delta activity prompts the conclusion that the remaining hours of wakefulness were sufficient to allow for full recovery. If exercise is taken later during the day, then recovery may not be completed before the sleep period, resulting in an intrusion of recovery into the first few hours of sleep.

Whilst the t test is a correct method of analysis for the data, it must be appreciated that the risk of making Type 1 errors (i.e. rejecting the null hypothesis when, in fact, it is true) is increased when the analysis involves many comparisons. Thus, the error rate (probability of

making a Type 1 error) has to be considered in terms of the experiment (experimentwise error rate) and not merely in terms of each individual comparison.

The simplest solution to this problem is to use a smaller significance level (i.e. alpha value - thereby reducing the probability of making a Type 1 error) based on the number of comparisons made. The required alpha value for any given number of comparisons can be calculated from the following formula (see Jacobs, 1976):-

$$\text{alpha}_{EW} = 1 - (1 - \text{alpha})^{c}$$

where $\text{alpha}_{EW}$ is the experimentwise error rate, alpha is the per comparison error rate and c is the number of independent comparisons.

The analysis involved a total of 124 comparisons and the alpha value used for assessing significance was 0.05. Using the table provided by Jacobs (1976) it is possible to determine the per comparison error rate which must be set to achieve a nominal experimentwise error rate. Although this table only includes values of experimentwise error rate for up to 100 comparisons this is suitably accurate for the present study. From the table it is apparent that a per comparison error rate of 0.0005 must be set to achieve an experimentwise error rate less than 0.05.

Obviously, such an alpha value would be very difficult to attain, especially with only 8 subjects. As the power of this method increases as the number of comparisons decrease, the alpha value could be corrected for the comparisons made concerning delta activity on the exercise recovery nights only, as this was the central point of interest in this study. The number of comparisons made between stages 2(ii), 3, 4, 3+4, and 2(ii)+3+4 amount to

40 and this requires a per comparison error rate of 0.001 to achieve an experimentwise error rate less than 0.05. As none of the comparisons made using the t test reached significance at $p<0.001$, this can be taken as further evidence concerning the lack of effect of moderate exercise levels upon subsequent sleep.

The findings from this study, and from those reviewed, are discussed in Chapter 6.

## 10. SUMMARY AND CONCLUSIONS

The time-of-day effects of standardised exercise upon sleep was studied with eight young males. Pilot-studies were carried out on a bicycle ergometer to assess individual maximum work capacity and the duration for which the subjects could tolerate exercise at 45% of their maximum work capacity.

In the sleep study, each subject performed the prescribed exercise, once in the morning (10.00 - 12.00 hours) and once in the afternoon (16.00 - 18.00 hours), on separate days. Sleep was scored according to the criteria recommended by Rechtschaffen and Kales (1968) and, in addition, stage 2 was sub-divided depending on whether the epoch contained more or less than 10%, by time, of delta activity.

No significant changes were found with whole night data on either of the exercise recovery nights when compared to baseline values. Following the afternoon exercise it appeared that delta activity was increased during the first few hours of sleep.

In conclusion, it appears from the findings of this study that sleep is not necessary for recovery from physical fatigue. Thus, if moderate exercise is taken in the morning, the ensuing wakefulness may be sufficient for recovery whereas later exercise may result in an intrusion of recovery into the first few hours of sleep.

## 11. PROPOSALS FOR FURTHER RESEARCH

The conclusion from this experiment may surprise the layman to the study of exercise and sleep. As mentioned in the introduction to this thesis, it is commonly believed that an increase in daytime physical activity is beneficial to sleep. Exactly how sleep is improved appears to vary from person to person, but the following responses to increased physical activity are often reported:-

a) feel tired earlier in the evening

b) fall asleep quicker than usual

c) "sleep like a log"

These factors are rarely assessed in the exercise and sleep studies, with the exception of sleep onset times. In a laboratory study, the subjects are usually asked to arrive at specified times which may be either too early or too late on some nights, depending upon how tired the individual subject feels. Also, subjects who feel tired at home during the evening may be stimulated into greater arousal by the journey to the laboratory, the laboratory environment and the personnel. Thus, any small effect of exercise upon sleep may not be detected in a laboratory study.

It is proposed that the next experiment will study the effects of exercise upon sleep by a less obtrusive method than that typically employed. More specifically, it is intended to supply sleep questionnaires to subjects of various fitness and of various activity regimes. No EEG recordings will be taken, thereby allowing the subjects to enjoy their normal routine. The subjects will be asked to fill in both a pre-sleep and post-sleep questionnaire for several nights. These will be designed to provide information concerning the fitness of the subjects, the time and

amount of physical activity taken, subjective assessment of tiredness, time to bed, time to rise, sleep quality and other variables of interest. By using this method, it is thought that the detection of any changes in sleep behaviour following exercise will be highlighted. Admittedly, these, if found, will be subjective in nature but it is important that any such changes are known as they can then be investigated more objectively using existing or revised laboratory methods.

CHAPTER 5

STUDY II

## 1. AIM OF THE STUDY

The aim of this study is to investigate the effects of exercise upon sleep behaviour using questionnaires. The information obtained from these questionnaires will naturally be mainly subjective (i.e. feelings of tiredness, sleep quality) although some objective data will be available (i.e. time spent in bed). The justification for this method of study has been discussed in section 11 of the previous chapter. The main advantage of this method is that the subjects can go about their normal routine, without performing potentially stressful experimental exercise programmes or being required to sleep away from home for several nights. It may be considered that the lack of objective sleep measures is a disadvantage of the questionnaire method but this is not felt to be so in this case. Many studies have been carried out in the last decade which have assessed the effects of exercise upon sleep using objective sleep measures, but there is still little evidence for any major change in these objective measures following exercise. Thus, there is little that sleep research can offer to the general public to explain why it is a common belief that physical activity is beneficial to sleep. Presumably physical activity is beneficial to sleep otherwise such beliefs would not exist today. These beliefs can only have been derived from subjective criteria such as the degree of tiredness before and after sleep and some basic objective measures such as the time of retiring or the duration of sleep. As yet, these beliefs have not yet been validated using these criteria, as research has been focussed on the intricasies of sleep behaviour (i.e. sleep stage duration and periodicity) following specified activities.

It was felt that this study was of great interest as it would present evidence for or against the belief that physical activity is beneficial to sleep. If evidence was found to support this belief, then laboratory methods could

be orientated towards the more detailed study of this evidence, be it pre-sleep tiredness, daytime napping, time of retiring or some other variable identified from the questionnaire analysis.

## 2. QUESTIONNAIRE DESIGN

### 2.1 General description

Three questionnaires were prepared for this study, namely a subject questionnaire, a pre-sleep questionnaire and a post-sleep questionnaire. These questionnaires are included in Appendix II.

The subject questionnaire was given to prospective subjects and used to record the subjects personal details such as age, sex, health, fitness and any sleeping problems. Subject selection was made at this stage.

The pre-sleep questionnaire was designed to be completed immediately before the subjects retired to bed and was used to obtain information concerning the amount of physical activity taken during the day and to assess pre-sleep tiredness. It was stressed to the subjects that the pre-sleep questionnaire should not be completed until the subject was about to retire. Some thought was given to this decision because this questionnaire contained the Stanford sleepiness scale (Hoddes, Zarcone, Smythe, Phillips and Dement, 1973). This scale is used to subjectively quantify sleepiness and it was included to see whether the subjects felt more tired and sleepy following high levels of physical activity compared to normal or below normal levels. Obviously, the response given by a subject will be influenced by the time of day when the scale is completed. It can be argued that this time should remain constant to control for this influence. However, if this was specified, then there would be the risk that the subject would forget to record his or her feelings of sleepiness at the specified

time. This might result in the subject failing to return the questionnaire. If the questionnaire is completed immediately before retiring then there is less chance of the subject forgetting. It was appreciated that the time of day of completion may not be consistent from night to night. Thus, it was necessary to record the time of retiring every night as this presumably related to the sleepiness of the subject. An early time of retiring in conjunction with great feelings of tiredness will obviously indicate increased sleepiness. Similarly, a late time of retiring in conjunction with no feelings of tiredness will indicate decreased sleepiness. Early retiring times with no tiredness or late retiring times with great tiredness will be difficult to interpret. As the time of retiring is dependent upon other factors than tiredness (i.e. social commitments, travelling, boredom), it was decided that the time when the subject felt ready for bed should also be recorded.

The post-sleep questionnaire was designed to obtain information concerning the quantity and quality of sleep as well as post-sleep tiredness. It was stressed to the subjects that they should complete this questionnaire only after they had been awake for approximately 15 minutes. This period allowed the subjects time to assess their level of tiredness.

The design of the three questionnaires is discussed in more detail below. The left-hand column lists the information required from the subjects whilst the right-hand column comments upon the use of such information.

2.2 Subject Questionnaire

| Information Required: | Comments |
|---|---|
| 1. Subject details. Name, age, sex, married/single. | This information might be useful for detailed analysis of the results or for comparison with any future work. |
| 2. Subject screening. Determine whether the subject has any health problems. | If so, ascertain the nature of the disablement and medication taken if any. A physical handicap might influence the subject's response to activity, whereas a mental handicap might be associated with disturbed sleep. In either case, the subject's data should not be analysed. |
| Determine whether the subject has any sleeping problems. | If so, find out more details and if any remedies or medications are taken. If the problem is severe or variable then the subject's data should not be analysed. |
| 3. Subject fitness. Determine how fit the subject considers himself or herself to be. | It is possible that fit subjects will show different changes in sleep behaviour than unfit subjects following high activity levels.<br><br>Thus, an indication of subject fitness is required for this analysis. It is not practicable to assess each subject's fitness by objective criteria as this would discourage many potential |

subjects and be extremely time consuming. Therefore, each subject was asked to rate his, or her, perceived fitness on a 4-point rankable scale from "very fit" to "very much out of condition".

2.3 Pre-Sleep Questionnaire

| Information Required: | Comments |
|---|---|
| 1. Determine subject's pre-sleep tiredness. | Use Stanford Sleepiness Scale (Hoddes et. al., 1973). This scale is used to quantify subjective changes in sleepiness for any time period of the day or night. |
| 2. Determine the subject's level of activity during the day. | 5-point rankable scale from "greatly above normal" to "greatly below normal". |
| 3. If physical activity levels are greater than normal then determine at what time(s) of day the activity was taken. | Choice of morning, afternoon, early evening, late evening. Determine the nature of the activity. |
| 4. Determine when the subject felt ready for bed. | This time is an indicator of how tired the subject felt. It is possible that the subject retired to bed early or late due to factors other than tiredness. |

| | Information Required: | Comments |
|---|---|---|
| 5. | Determine whether the subject felt that he or she had retired to bed too early, at the right time, or too late. | This gives another indication of the subject's pre-sleep tiredness. A early or late bedtime is often imposed because of factors other than tiredness. Therefore, bedtime on its own cannot always be used as an indication of tiredness. |
| 6. | Determine whether the subject has had a disturbing day. | Anxiety and stress would be reflected in disturbed sleep behaviour. The data for such days should not be analysed. |
| 7. | Determine bedtime. | This allows for the assessment of other variables such as time spent in bed and sleep onset times. |
| 8. | Determine whether the subject has had any daytime naps. | Can be used as an indication of tiredness. |

2.4 Post-Sleep Questionnaire

| | Information Required: | Comments |
|---|---|---|
| 1. | Determine the subject's post-sleep tiredness. | Use Stanford Sleepiness Scale. |
| 2. | Determine subjective sleep onset time, sleep length and time spent in bed. | Useful parameters for assessing the need for sleep. |

| | Information Required: | Comments |
|---|---|---|
| 3. | Determine subjective sleep quality. | 5 point rankable scale from "much better than normal" to "much worse than normal". The subject was invited to give reasons for not sleeping well as this could be due to many factors (i.e. noisy neighbours, not tired, cramp). If it was due to extraneous variables then the data for that night was not analysed. |
| 4. | Determine whether the subject felt in need of more sleep. | Indicates whether the subject's need for sleep has increased given that no changes are reported for sleep length or sleep quality. The time of awakening is often pre-determined by commitments and the length of sleep cannot always be used to assess the sleep requirement. |
| 5. | Determine whether the subject used an alarm clock. | The use of an alarm clock could invalidate the use of sleep time as an indicator of sleep requirements. Care should be taken to ensure that the frequency of use of alarm clocks is comparable following the various physical activity levels otherwise the use of an alarm clock might become a main factor. |

## 3. SUBJECT SELECTION AND EXPERIMENTAL PROCEDURE

In order to investigate the effects of physical activity upon sleep behaviour it was necessary to obtain information concerning the subject's sleep following a variety of activity levels. As a 5-point rankable scale was used to assess daily physical activity levels, it would be desirable to have the subjects complete one or more sets of pre-sleep and post-sleep questionnaires following each of the five levels of activity. However, this was considered to be impracticable because it would have extended the duration of the study which could have resulted in a poor return of completed questionnaires. Also, a subject may go for several days without a change in activity level and then forget to complete the set of questionnaires when the activity level changes. This could result in the subject 'remembering' his or her level of tiredness, sleep times and other details which is not desirable.

To minimise the possibility of subjects forgetting to complete the questionnaires it was decided that the questionnaires would be completed every night and day for seven consecutive days, commencing on the day when the subject received them.

To maximise the likelihood of obtaining data following a wide range of activity levels, it was decided that careful selection of the subjects would be necessary. In addition to being healthy and free from medication and sleep problems, it was important that the subjects took part in a physically demanding pastime at least once or twice a week. It was hoped that this would ensure that the majority of the subjects would report some measurable degree of variation in their daily activity levels during the course of the week.

In order to select such subjects, the experimenter visited several sports centres which offered facilities

for squash, badminton, swimming and other activities. Prospective subjects were informally interviewed to determine if they had any health or sleep problems and to assess whether their daily activity levels showed much variation during the course of a typical week. Suitable subjects were shown the questionnaires and asked if they would like to take part in the study. If the subject appeared willing, then each question on the questionnaire was discussed to make sure that the subjects would have no difficulty in completing the questionnaire. The subjects were asked to complete the first questionnaire that evening and to continue for seven consecutive days. A stamped, addressed envelope was provided with the questionnaires and the subjects were asked to post them the following week upon their completion. A total of 80 packs of questionnaires were distributed.

It was appreciated that the information from the questionnaires may be influenced by the individual's expectations of the effects of activity upon sleep. To minimise such an effect, the experimenter made no reference to any experimental or folk-lore evidence of such a relationship.

## 4. DATA ANALYSIS

Fifty-one subjects returned their questionnaires, giving a good response rate of approximately 64%. A total of 332 days of data were used for analysis after the questionnaire had been inspected for omissions or reports of anxiety or sleep disturbance by extraneous factors.

The factors and variables of interest in this study are described below.

### 4.1 Factors

Daily activity levels (5 point scale), time of day when activity taken (4 point scale) and subject fitness

were the three factors of interest in this study.

### 4.1.1 Activity

The effect of activity upon sleep behaviour was the main factor of interest in this study because of the varied findings reported in the EEG studies of exercise and sleep. Each subject's daily activity level was ascertained from the 5 point scale in the pre-sleep questionnaires which varied from 'greatly below normal', 'below normal', 'normal', 'above normal' to 'greatly above normal'.

However, as only three subjects reported daily activity levels of 'greatly below normal' (giving a total of 3 days data) and only twelve subjects reported levels of 'greatly above normal' (giving a total of 18 days data), it was decided to class the activity levels into three groups namely, 'less than normal', 'normal', and 'greater than normal'. This gave 46, 147 and 139 days of data for these classes of activity, respectively (see table 1).

### 4.1.2 Time of day

The time of day of activity was considered because the first study of this thesis (study I) had indicated that this factor may modify the effects of activity upon sleep. Whenever a subject reported activity levels greater than normal, he or she was then asked to specify what time(s) of day the activity was taken, given a choice of morning, afternoon, early evening and late evening (see pre-sleep questionnaire). If a subject marked two or more time periods then the later period was used in the analysis.

### 4.1.3 Fitness

It was not possible to implement standardised activity levels in this study because its objective was to

assess sleep behaviour following various activity levels encountered normally, and without the impositions usually encountered in laboratory studies. It was not considered practicable to assess fitness by objective criteria because this would discourage many potential subjects and would be very time consuming. Subjects described their level of fitness in the subject questionnaire by choosing from a four point scale comprising 'very fit, 'fit', 'a bit out of condition' and 'very much out of condition'.

Out of the fifty-one subjects, only 2 described themselves as 'very fit' (giving a total of 11 days data) and only 2 as 'very much out of condition' (giving a total of 14 days data). The four point fitness classification was therefore reduced to 2 classes, namely, fit and unfit giving 179 and 153 days of data for these classes, respectively (see table 1).

Table 1: Number of days data for Activity and Fitness Factors

| Subject Fitness | Activity | | | TOTAL |
|---|---|---|---|---|
| | Less than normal | Normal | Greater than normal | |
| Fit | 21 | 77 | 81 | 179 |
| Unfit | 25 | 70 | 58 | 153 |
| TOTAL | 46 | 147 | 139 | 332 |

## 4.2 Variables

The variables of interest, their extraction from the questionnaires and their units of measurement are detailed below.

| Question | Variable | Data |
|---|---|---|
| At what time did you start thinking about going to bed tonight? | Thinking of bed | Decimal clocktime (choice of 30 min. periods) |
| At what time are you going to bed tonight? | Actual bedtime | Decimal clocktime |
| If you have fallen asleep during the day please state when and for how long. | Daytime napping | Minutes |
| At what time did you<br>a) fall asleep last night?<br>b) wake up this morning?<br>c) get up this morning? | Sleep onset | Minutes (calculated as the difference between the reported times of going to bed and falling asleep) |
| | Sleep length | Minutes (calculated as the difference between the reported time of falling asleep and waking) |
| | Time taken to rise | Minutes (calculated as the difference between the reported times of waking and getting out of bed) |
| Do you feel that you have gone to bed<br>a) too early?<br>b) at the right time?<br>c) too late? | Bedtime suitable | 3 point scale |

| Question | Variable | Data |
|---|---|---|
| Which one of the following statements do you consider to best describe your feeling during the last quarter of an hour? (Stamford Sleepiness Scale) | Pre-sleep tiredness | Examined on 7 point scale (1 - alert, 7 - sleep onset soon). Statistical analysis performed on 3 point scale (original points 1, 2 and 3 - not tired, points 4 and 5 - slightly tired, points 6 and 7 - very tired) |
| Which of the following do you consider to best describe the quality of your sleep last night?<br>a) Much better than normal<br>b) Better than normal<br>c) Normal<br>d) Worse than normal<br>e) Much worse than normal | Sleep Quality | Examined as a 3 point scale due to the low frequencies of 'much better' or 'much worse' reports.<br>a) Poor<br>b) Normal<br>c) Improved |
| If you could, would you have liked to have slept longer this morning<br>a) Yes<br>b) No | More sleep required | 2 point scale |

## 4.3 Statistical Analysis

The statistical analysis was carried out using Glim - a software package for the analysis of general linear models. The analysis investigated the main effects of activity, time of day of activity and fitness as well as the activity and fitness interaction effect. The questionnaire data was analysed separately for the time data (clocktime or minutes duration) and the subjective ratings (tiredness, yes/no etc.). As time data is ratio order, the analysis was carried out assuming a normal (gaussian) error structure. The subjective ratings were analysed with respect to the frequency with which each rating was made and, therefore, a Poisson error structure was appropriate.

It was appreciated that the use of an alarm clock was a factor to be considered regarding sleep length and, consequently, other variables such as post-sleep tiredness. To assess whether any of the significant correlations between activity or fitness and the variables were due, in part, to the varied use of an alarm (i.e. maybe unfit subjects use alarm clocks more than fit subjects, or alarm clocks are used more often following late evening exercise), the frequency of use was determined across these factors.

## 5. RESULTS

The statistical analysis is summarised in Table 2 which shows the mean values for the time data and the frequencies for the subjective ratings. The significant findings are displayed in figures 1-7 where the frequencies are expressed as percentages for each fitness group or activity level where appropriate.

Activity was significantly related ($p<0.05$) with the suitability of bedtime. From figure 1 it appears that the

Table 2. Summary of Questionnaire Data

| VARIABLE | FACTOR | | | | | | | | | SIGNIFICANCE |
|---|---|---|---|---|---|---|---|---|---|---|
| | ACTIVITY | | | | | | | FITNESS | | |
| | Less than normal | Normal | Greater than normal | Time of day exercise completed (for '+' activity days only) | | | | Fit | Unfit | A activity<br>F fitness<br>T time of day<br>* $p<0.05$<br>** $p<0.01$ |
| | | | | Morning | Afternoon | Early evening | Late evening | | | |
| Time thinking about bed (decimal clock-time) | 11.0 | 11.1 | 11.1 | 11.0 | 11.1 | 11.0 | 11.7 | 11.2 | 10.9 | F** F=9.3, df = 1,326<br>T* F=3.3, df = 3,135 |
| Actual bedtime (decimal clocktime) | 11.9 | 11.8 | 11.9 | 11.7 | 11.8 | 11.8 | 12.5 | 12.00 | 11.7 | F* F=5.3, df = 1,326<br>T* F=3.8, df = 3,135 |
| Daytime naps (mins) | 7.6 | 6.9 | 4.2 | 8.3 | 1.7 | 4.5 | 4.6 | 6.5 | 5.1 | |
| Sleep onset (mins) | 31.2 | 28.0 | 31.4 | 37.7 | 37.9 | 27.7 | 21.3 | 24.4 | 36.2 | F** F=17.9, df = 1,326 |
| Sleep length (mins) | 465.0 | 453.6 | 445.4 | 430.5 | 444.0 | 460.9 | 426.5 | 442.6 | 462.4 | F* F=5.2, df = 1,326 |
| Time taken to rise (mins) | 28.3 | 39.5 | 34.2 | 23.0 | 42.1 | 31.1 | 35.4 | 38.9 | 32.1 | |
| Bedtime suitable? (frequency) | | | | | | | | | | |
| too early | 4 | 8 | 1 | 0 | 0 | 1 | 0 | 4 | 9 | A* $\chi^2$=10.7, df = 4 |
| suitable | 28 | 85 | 96 | 13 | 32 | 37 | 14 | 114 | 95 | |
| too late | 14 | 54 | 42 | 7 | 13 | 13 | 9 | 61 | 49 | |
| Pre-sleep tiredness (frequency) | | | | | | | | | | |
| not tired | 27 | 89 | 79 | 10 | 31 | 26 | 12 | 100 | 95 | |
| slightly tired | 10 | 45 | 42 | 8 | 13 | 13 | 8 | 54 | 43 | |
| very tired | 9 | 13 | 18 | 2 | 1 | 12 | 3 | 25 | 15 | |
| Post-sleep tiredness (frequency) | | | | | | | | | | |
| not tired | 35 | 106 | 91 | 15 | 30 | 32 | 14 | 129 | 103 | F* $\chi^2$ = 7.0, df = 2 |
| slightly tired | 8 | 32 | 38 | 3 | 12 | 15 | 7 | 44 | 34 | |
| very tired | 3 | 9 | 10 | 2 | 2 | 4 | 2 | 6 | 16 | |
| Sleep quality (frequency) | | | | | | | | | | |
| poor | 10 | 14 | 25 | 4 | 6 | 9 | 6 | 17 | 32 | F** $\chi^2$=10.1, df = 2 |
| normal | 27 | 111 | 85 | 13 | 30 | 31 | 11 | 131 | 92 | A* $\chi^2$=9.6, df = 4 |
| improved | 9 | 22 | 29 | 3 | 9 | 11 | 6 | 31 | 29 | |
| More sleep required? (frequency) No | 23 | 77 | 64 | 9 | 22 | 18 | 15 | 101 | 63 | F** $\chi^2$=8.2, df = 1 |
| Yes | 23 | 70 | 75 | 11 | 23 | 33 | 8 | 78 | 90 | |

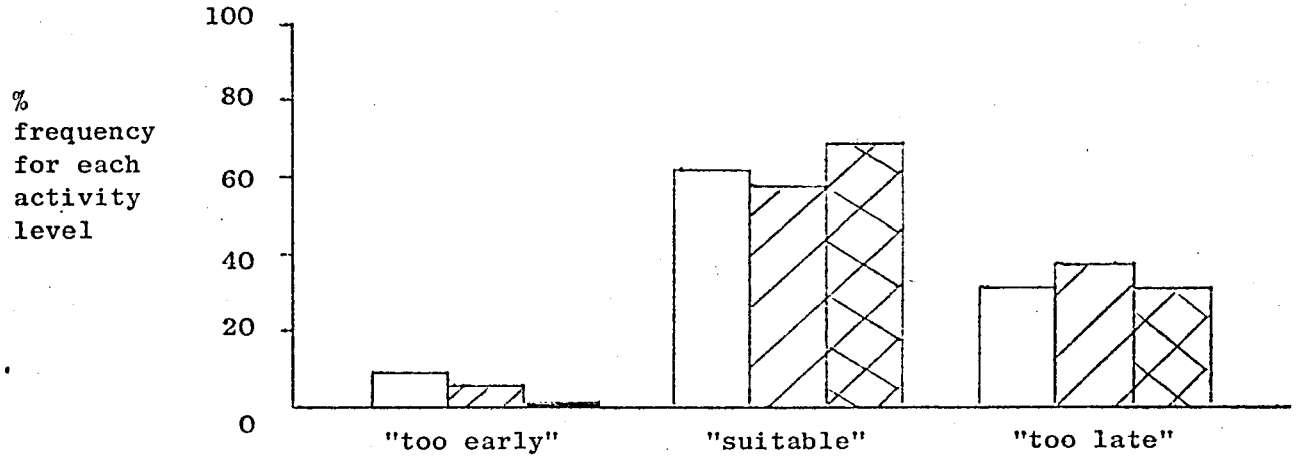



FIGURE 1: Relationship between daily activity level and assessment of bedtime suitability.

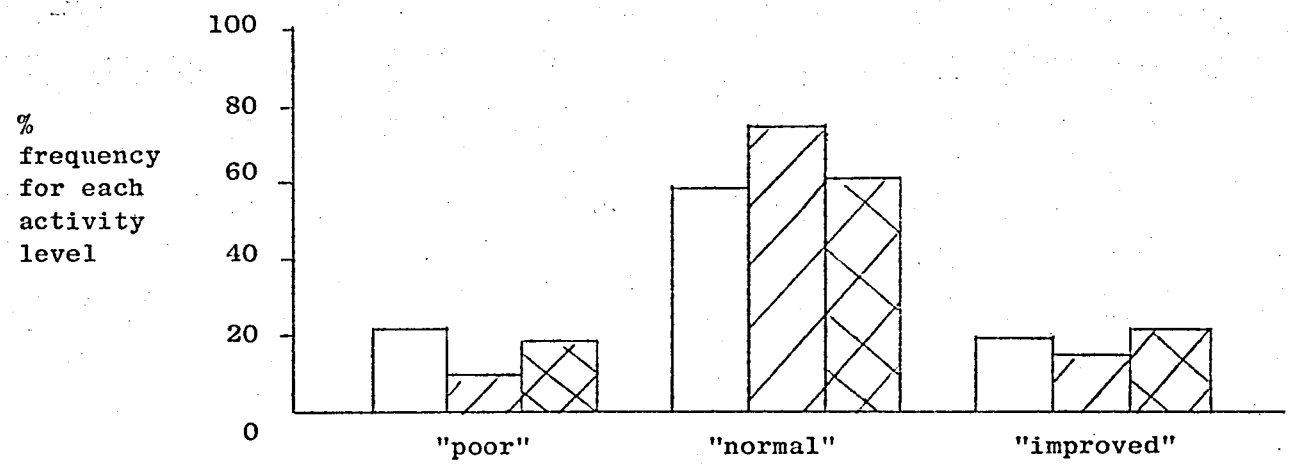
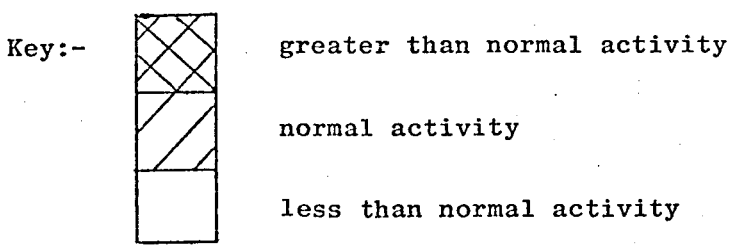



FIGURE 2: Relationship between daily activity level and subsequent sleep quality.



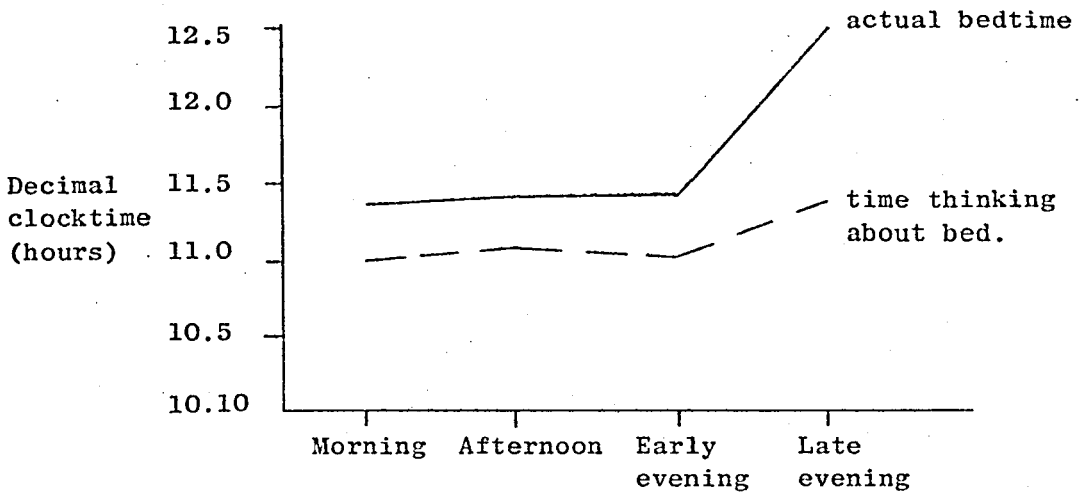



FIGURE 3: Relationship between daily activity and bedtime.

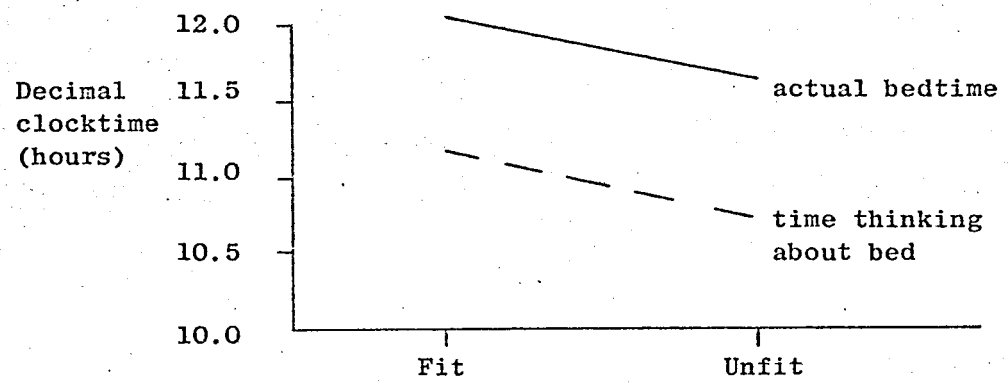



FIGURE 4: Relationship between fitness and bedtime

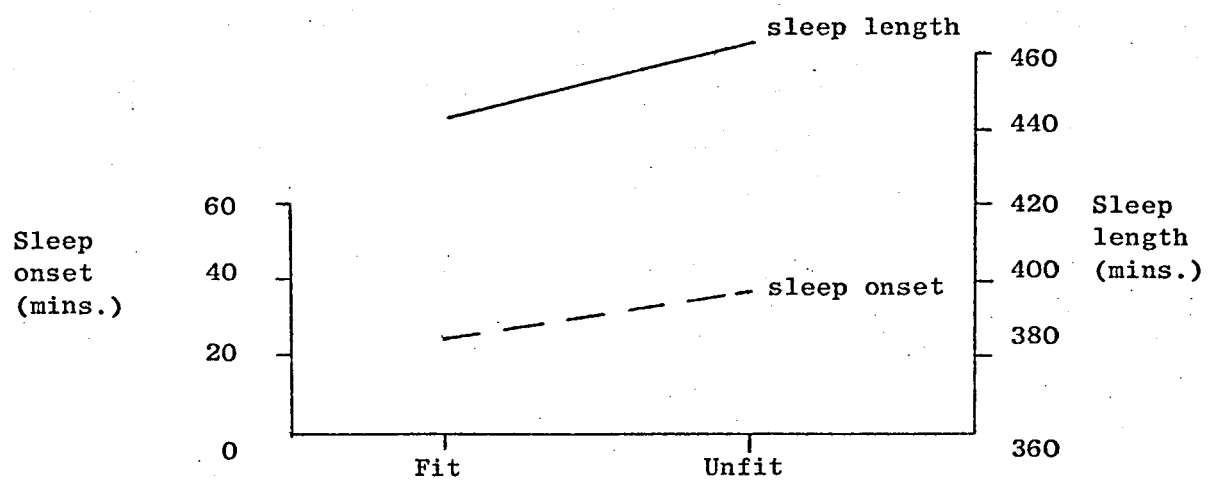



FIGURE 5: Relationship between fitness and the onset and duration of sleep.

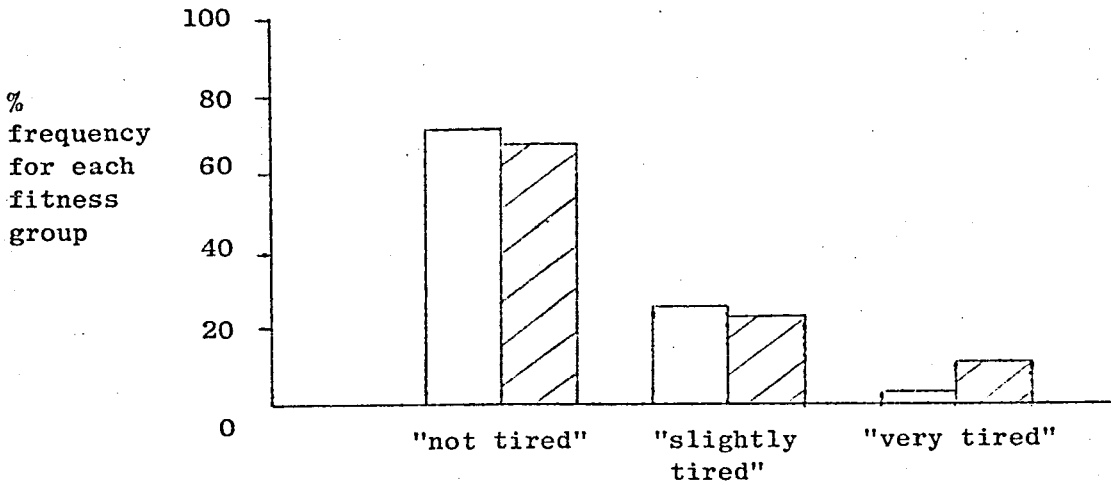



FIGURE 6: Relationship between fitness and post-sleep tiredness.

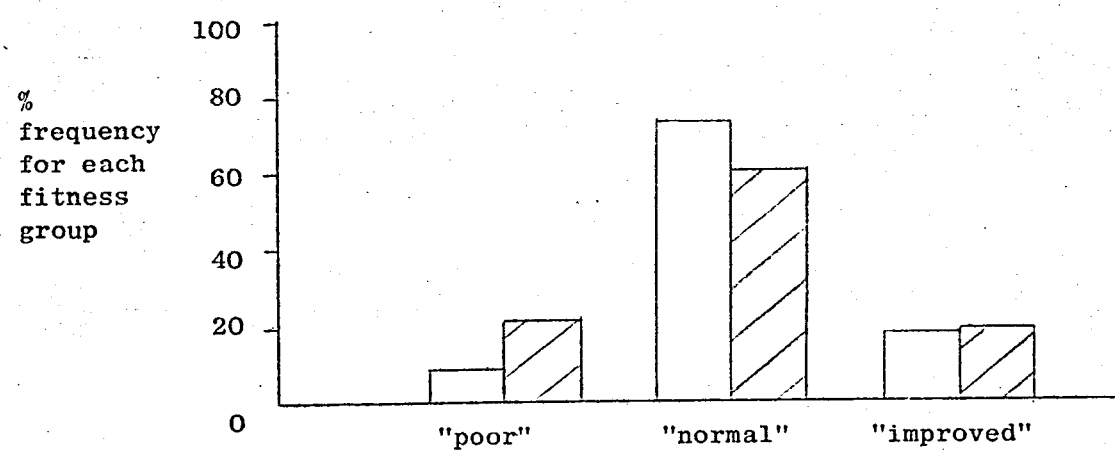
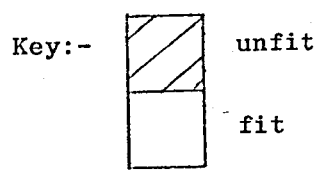



FIGURE 7: Relationship between fitness and sleep quality.



incidence of going to bed 'too early' is very low and the frequency of this response may be inversely related to the daily activity levels. The judgement that the bedtime was 'too early' was made on 9%, 5% and 1% of occasions for "less than normal", "normal" and "greater than normal" activity levels, respectively.

Activity was also found to be significantly related ($p<0.05$) with subsequent sleep quality. However, from figure 2 it is apparent that this relationship is not simple. Compared to reports concerning sleep quality following "normal" levels of activity, both an increase or decrease in daily activity level were found to be associated with increases in reports of "poor" sleep quality (approximately 22, 10 and 18% of reports following "less than", "normal" and "greater than normal" activity levels, respectively). Similarly, both an increase or decrease in daily activity level were found to be associated with small increases in reports of "improved" sleep quality (approximately 20, 15 and 21% of reports following "less than", "normal" and "greater than normal" activity levels, respectively).

Although activity was not significantly related with the two bedtime variables (time thinking of bed and actual bedtime), the time of activity was found to significantly related ($p<0.05$) with both of these variables. From figure 3 it can be seen that the time of thinking about bed and the actual bedtime are not influenced by the time of activity unless it is taken in the late evening, when both of these variables are delayed by approximately 40 minutes.

The remainder of the significant relationships were found to be with the fitness of the subjects. Fitness was significantly related with both the time of thinking about bed ($p<0.01$) and the actual bedtime ($p<0.05$); both of these variables occurring later (22 and 16 minutes

respectively) with the fit subjects (see figure 4).

Both sleep onset ($p<0.01$) and sleep length ($p<0.05$) were found to be significantly shorter for the fit subjects, by an average of approximately 12 minutes and 20 minutes, respectively (see figure 5).

Post-sleep tiredness was found to be related ($p<0.05$) to the fitness of the subject. Figure 6 shows that approximately 3% of the fit subjects reported feeling "very tired" compared to approximately 10% of the unfit subjects.

Fitness was found to be significantly related ($p<0.01$) with sleep quality. From figure 7 it appears that the unfit subjects reported a higher incidence of 'poor' sleep quality than the fit subjects (approximately 20% of reports by unfit subject compared to 10% by fit subjects), although the reports of 'improved' sleep quality were very similar between these two groups.

Fitness was also found to be significantly related ($p<0.01$) with the desire to sleep longer, with 59% of the unfit group expressing the desire to sleep longer compared to approximately 44% of the fit group.

No significant interaction effects of fitness and activity were found and the use of an alarm clock was not found to be significantly related to any of the factors under scrutiny.

## 6. DISCUSSION

The results of the questionnaire analysis provide little support for the notion that people 'sleep like a log' following days of increased activity. For example, although daily activity levels were found to be significantly related to subsequent sleep quality, it is interesting to note that "improved" sleep quality was reported

on 20% of occasions following "less than normal" activity levels compared to 21% of occasions following "greater than normal" activity levels. Furthermore, both an increase or decrease in daily activity levels were associated with more reports of "poor" sleep quality compared to reports following "normal" activity levels. Thus, the association between daily activity levels and subsequent sleep quality is rather confusing and it is possible that this association may not have been evident if the sample size had been larger. It should be noted that sleep quality was also found to be related to fitness but this finding was consistent with several other significant findings indicating a general difference in sleep behaviour between fit and unfit subjects. The activity and fitness interaction effect was not found to be significant.

No significant relationship was found between activity and daytime naps, bedtime (thinking of bedtime or actual bedtime), sleep onset, sleep length, time taken to rise, tiredness or the desire to sleep longer. In fact, the only other apparent influence of activity was regarding the suitability of the bedtime, even though the time thinking of bed and the actual bedtime showed no change across the three activity levels. The finding that the incidence of going to bed 'too early' may be inversely related to the daily activity levels is the only measure found to suggest that the subjects felt more tired following high levels of activity. It must be remembered, though, that the variations in activity are 'normal' and not experimentally imposed when the subjects often take more exercise than they would normally undertake.

The time of day when the activity was taken was found to have little effect on the variables except both the time of thinking about bed and the actual bedtime were delayed following late evening activity.

Interestingly, the analysis has revealed that subject fitness is related to several of the sleep variables. More specifically, it appears that the fit subjects thought about bed and went to bed later, took less time to fall asleep, spent less time asleep, awoke feeling less tired and reported poor sleep quality and the desire for more sleep on fewer occasions compared to the unfit subjects. Taken together, these findings could suggest that fit subjects need less sleep or sleep more efficiently than unfit subjects. It would be difficult to comment further on these possibilities due to the nature of the study and the data. For example, the classification between fit and unfit subjects was made by each individual according to his or her own understanding of the terms and these will vary from individual to individual. However, the fact that several differences were found concerning subjective assessments of various sleep variables between these two groups of subjects clearly indicates that the relationship between physical fitness and sleep is an avenue for future research.

The absence of any significant activity and fitness interaction effects also argues against the importance of variations in daily activity levels to influence the sleep variables used in this study. Furthermore, the frequency of use of an alarm clock was not found to be significantly different between the activity levels removing the possibility that some of the variables, for example sleep length, were artificially constrained.

Obviously, there is a link between activity and fitness in that to become and remain fit one must partake in regular physical activity. Thus, the findings concerning fitness might also refer to long term activity levels.

The assessment of daily activity levels and sleep in this study were rather coarse and this may have hidden any

real relationship between these variables. Hopefully, this shortcoming is counter-balanced by the advantages of this study described earlier (see Section 1). Furthermore, the findings of this study are intended to compliment the laboratory studies of the exercise and sleep relationship.

The findings and ramifications of this study are included in the general discussion of Chapter 6.

## 7. SUMMARY AND CONCLUSIONS

This study investigated the effects of daily activity levels, including the time of day when the activity was taken, and subject fitness upon sleep behaviour using questionnaires. Three questionnaires were designed, namely a subject screening questionnaire and pre-sleep and post-sleep questionnaires. The pre-sleep questionnaire was completed immediately before the subject retired to bed and was used to record the daily activity levels and to assess pre-sleep tiredness. The post-sleep questionnaire was designed to obtain information concerning the quantity and quality of sleep as well as post-sleep tiredness.

Eighty subjects were selected following screening for ill-health and sleeping problems and were asked to complete the sleep questionnaires for seven consecutive days. Subjects were enlisted from sports centres to ensure that the majority of the subjects would report some measurable degree of variation in their daily activity levels during the course of the week. Fifty-one subjects returned their questionnaires and a total of 332 days of data were used for the statistical analysis.

The findings do not suggest that variations in the daily activity level have any major effect upon the tiredness and sleep variables assessed. Also, the time of day

when the activity was taken was found to have little influence upon the variables except that the bedtime was delayed by late evening activity. The findings do suggest, however, that fit subjects either need less sleep or sleep more efficiently than unfit subjects.

In conclusion, it would appear that the tiredness and sleep variables assessed in this study are not greatly influenced by normal variations in daily activity levels. The finding that many of the sleep variables vary according to the fitness of the subject suggests that long term activity levels do have an influence upon sleep.

CHAPTER 6

EXERCISE AND SLEEP - LITERATURE REVIEW II AND GENERAL DISCUSSION

## 1. INTRODUCTION

This chapter concludes Part One of this Thesis. It contains a literature review of exercise studies from 1974 to the present, and thereby completes the review of Chapter 2. All of the human studies reviewed are summarised in Table 1. The last section of this chapter is devoted to a general discussion concerning the effects of exercise upon sleep and will include all of the studies reviewed as well as the two studies carried out for this thesis.

## 2. REVIEW OF STUDIES PUBLISHED AFTER 1974

As in the earlier review (see Chapter 2), the studies described in this section are presented in historical order.

Desjardins, Healey and Broughton (1974) studied the effects of early evening exercise upon the sleep of six young males (23-28 years). These subjects exercised on a motorized treadmill at high and low intensities in a counter balanced order. The low intensity exercise lasted 2 hours (approximately 19.30 to 21.30 hours) whereas the high intensity exercise was divided by three 15 minute rest periods. Both exercises ended 2 hours before the subjects retired to bed.

No significant differences were reported between the control and exercise recovery nights or between the high and low intensity recovery nights. This is consistent with the findings of Baekeland and Lasky (1966) and Hauri (1968), who also reported no major changes in sleep following evening exercise.

Shapiro, Griesel, Bartel and Jooste (1975) systematically investigated the effects of graded exercises on the sleep of two highly trained young male subjects (24-26 years). Following an adaptation night, EEG recordings were taken after various daytime activities.

On the first day the subjects did no specific exercise

whilst on days 2 to 4 they exercised on a bicycle ergometer at 50% of their individual $\dot{V}O_2$ max for 2, 4 and 6 hours respectively. On day 5 the subjects cycled at 75% of $\dot{V}O_2$ max for a total of 160 minutes with a 60 minute rest period taken after 80 minutes (this load was designed to be equivalent, in terms of total work done, to day 3). On day 6 the subjects exercised for 3 hours at 50% of $\dot{V}O_2$ max in a hot-box with the wet and dry bulb temperatures at 32 and 33°C, respectively. This exercise consisted of stepping up and down on a block at a set rate. It was intended that this exercise should last for 4 hours to be comparable with day 3 but the exercise was cut short when the subjects' body temperatures rose above 39°C. The experimental days were non-consecutive and separated by 1 to 4 non-experimental days, during which the subjects performed light exercise (40% $\dot{V}O_2$ max) for 2 hours. The exercises were all completed by 1300 to 1400 hours, except on day 6 when the exercise and thermal stress ended at 10.00 hours.

The authors reported that SWS showed a progressive increase with increasing physical fatigue over nights 1 to 6. The average duration of SWS was 20.5% on the no-exercise night and 30.0% on the night following the thermal stress and exercise. The SWS level showed a peak of 38.5% on night 5. This progressive increase in SWS was associated with a progressive increase in total sleep time and a progressive decrease in REM sleep.

As the total sleep time showed a considerable increase as the experiment progressed, it is worthwhile studying whether the changes reported in % SWS and % REM were as a direct consequence of the increased sleep time. Inspection of the data for REM sleep expressed as duration in minutes still reveals a considerable decrease from night 1 to night 6 (a maximum decrease of approximately 40 minutes between nights 1 and 5 even though total sleep time showed an

increase of approximately 30 minutes between these two nights). This finding is surprising because an extended sleep period typically contains a large proportion of REM sleep.

The extension of total sleep time appears to reflect the increase in SWS during the first three nights although the increases in SWS on the subsequent nights are greatly above the increases in total sleep time (a maximum increase in SWS of approximately 90 minutes between nights 1 and 5 whilst total sleep time is increased by only approximately 30 minutes between these two nights). This shows that the changes in SWS are not merely a consequence of the changes in sleep length.

These findings were taken to support the body restitution theory of SWS. It should be noted that these findings do not necessarily conflict with those from Study I of this thesis as the lowest amount of experimental exercise taken by the subjects in the study by Shapiro et. al. (50% $\dot{V}O_2$ max for 120 minutes) was greater than that prescribed to my subjects (45% maximum work capacity for 84 minutes). Furthermore, the increase in SWS reported by Shapiro et. al. was not particularly evident, as only 2 subjects were used, until night 3 which followed 240 minutes of continuous exercise at 50% $\dot{V}O_2$ max. This latter exercise was approximately 3-fold that prescribed in Study I.

However, these increases in SWS reported by Shapiro et. al. do not appear to be solely as a consequence of the increased work done by the subjects during the exercises. For example, the exercises completed on days 3 and 5 were designed to be equivalent with respect to the amount of work done by the subjects, but one of the subjects displayed an increase of 109 minutes of SWS on night 5 compared to night 3. This dramatic increase in SWS cannot be associated

with an increased amount of work done as this was not the case. Thus, it appears that some other factor(s) associated with the exercises must account for this large increase. One possibility to be considered is that the high work-rate imposed on day 5 (75% $\dot{V}O_2$ max) resulted in an increased demand upon anaerobic energy resources as the oxygen uptake could not meet the oxygen demands of the body.

However, increased anaerobic respiration does not appear to account for the increase in SWS between nights 3 and 5 as the subject who displayed this increase did not exhibit an increase in lactic acid concentration in the blood at the end of the exercise at 75% $\dot{V}O_2$ max. Moreover, the other subject showed only a 3% increase (15 minutes) in SWS on night 5 compared to night 3 although the lactic acid concentration reached 21.3 mg/100 ml following exercise at 75% $\dot{V}O_2$ max; this was more than double the value recorded after exercising at 50% $\dot{V}O_2$ max.

Similarly, the greatly increased levels of SWS on night 6 following the 3 hours of exercise at 50% $\dot{V}O_2$ max were not associated with an increase in work done by the subjects as this was equivalent to three-quarters of the work done on day 3. However, the day 6 exercise was reported to be the most tiring for the subjects, probably as a result of the thermal stress induced by the use of a hot-box. Although the levels of SWS were similar on nights 5 and 6 for both subjects, the lactic acid concentration and oxygen consumption measured at the end of the exercise period on day 6 were approximately a third and a half, respectively, compared to values on day 5.

Thus, neither the amount of work done nor the extent of anaerobic respiration appear to account for the dramatic increase in SWS on nights 5 and 6.

It is interesting that subject B, the fitter subject (i.e. the one who could exercise at 75% $\dot{V}O_2$ max without elevated levels of lactic acid), showed the largest increase in SWS. If SWS does fulfill a recovery function after exercise then it might be expected that the less fit subject (subject A) would display the largest increase in SWS as he was objectively more fatigued than subject B. Although both subjects exercised at fixed percentage of their $\dot{V}O_2$ max, the physiological recordings showed that subject A was pushed closer to his limit of endurance than subject B. For example, the heart rate, lactic acid concentration and oxygen consumption at the end of exercising on day 5 were 188 beats/minute, 21.3 mg/100 ml and 2.62 l/minute for subject A and 130 beats/minute, 13.4 mg/100 ml and 1.98 l/minute for subject B.

The authors attribute this difference to individual variation. However, it could be that extremely fit people, who typically exercise at high levels several times a week, develop the ability to extend the duration of SWS when required. This could be regarded as an extension of the physiological changes that take place as a person of average fitness trains to become an athlete. The well-known changes that occur in such individuals include the development of muscle groups and the increase of $\dot{V}O_2$ max due to the increased efficiency of the cardiac-pulmonary system and the increased endurance to high levels of lactic acid. These changes allow the athlete to perform more work than previously possible and, also, enable him to recover from the effects of exercise more quickly. If SWS does aid body recovery processes, then a dramatic increase in SWS in response to high levels of exercise could represent a training effect. The absence of such large increases in SWS following exercise in the majority of studies so far received suggests that this putative training effect takes

considerably longer to develop than the more obvious physiological changes described earlier, and is characteristic of only extremely fit subjects.

From a methodological viewpoint it is unfortunate that the authors did not record sleep on the nights between the experimental nights, as this would show whether SWS was increased after high exercise days only or if it showed a general increase on all nights as the study continued. Also, the provision for more subjects would have been advisable, allowing for statistical treatment of the data.

Browman and Tepas (1976) studied the effects of pre-sleep activity upon the sleep of nine young males (average age age 18.9 years) of average fitness. The activities studied were progressive relaxation, an auditory vigilance task and light dynamic exercise. Each activity lasted for 45 minutes and the subjects retired to bed within 5 minutes of completion. The order of activity was counterbalanced on nights 2, 3 and 4 and the first night was used for adaptation purposes. During the exercise task the subjects rested for 3 minutes after every 5 minutes of exercise and rode an equivalent of 19.0 to 21.5 Km on a bicycle ergometer.

The latency of sleep onset was found to be shortest after relaxation and longest after exercise. No relationship was found between sleep latency and pre-sleep heart rate and EMG activity, although heart rate was significantly higher following exercise than the other two conditions. REM latency was found to be significantly longest after exercise (106 minutes) and shortest after vigilance (80 minutes) although no significant changes in sleep stages were found prior to the first REM period during the whole night or during the first, second or third period (150 minutes) of the night.

The authors concluded that those pre-sleep activities used in their study, whilst influencing sleep onset times, did not have any substantial effect upon the normal sleep pattern. They point out that other activities or increased levels of the activities studied might well have produced different results. This conclusion is consistent with the findings of other studies which have assessed the effects of evening exercise upon sleep (Baekeland and Lasky, 1966, Hauri, 1968, Desjardins et. al., 1974).

Moses, Lubin, Naitoh and Johnson (1977) studied the effects of exercise and sleep loss on recovery sleep in 30 young male (18 - 22 years) naval volunteers of average fitness. This study was an extension of an earlier study (Moses, Hord, Lubin, Johnson and Naitoh, 1975) where they compared the recovery sleep of subjects who napped with those who exercised during a 40 hour period. One of the findings was an increase in SWS displayed by the exercise group. However, it is well-known that an increase in SWS is a typical characteristic of post-deprivation recovery sleep (e.g. Berger and Oswald, 1962; Moses et. al., 1975a,b), so the changes in the exercise group's recovery sleep may have been due to sleep loss alone. This later study therefore examined the effects of exercise and sleep loss on recovery sleep following 40 hours sleep deprivation.

The experimental programme consisted of one adaptation and one baseline night followed by the sleep deprivation period. This period was divided into 10 epochs of approximately 4 hours duration. For one hour during each epoch, one group of 20 subjects remained awake in bed (bedrest group) whilst the other 10 subjects exercised on a bicycle ergometer (exercise group). This exercise was controlled so that the subjects maintained a heart rate which was 50% above baseline. The subjects were allowed 8 hours of total bedtime on both the baseline and recovery nights.

The EEG records of 3 subjects were unscorable so only 18 and 9 subjects were used for analysis of the bedrest and exercise groups respectively. The analysis was carefully designed to reduce the likelihood of Type I errors occurring as a result of the multiple comparisons between groups.

Using the conservative Dunn-Bonnferroni criterion, only the percentage total sleep time (total time in stages 2, 3, 4 and REM divided by total bed time) showed a significant difference between the bedrest and exercise groups, with the exercise group showing a 9.8% increase on recovery compared to the 2.1% increase of the bedrest group. No significant changes were found for total SWS duration even if a 1-tail t test was applied.

Thus, 10 hours of moderate exercise, spaced over a 2 day period with no sleep permitted on the intervening night, did not increase SWS levels compared to a group which did not partake in such exercises.

Walker, Floyd, Fein, Cavness, Lualhati and Feinberg (1978) tested the hypothesis that SWS would be increased as a function of either acute or chronic exercise. They examined the sleep of 10 distance runners and 10 non-runners during typical conditions and reversed conditions (i.e. runners not running and non-runners running).

The study lasted for four consecutive days in which the two groups carried out their normal routine for the first three days. For the runners this consisted of a mean daily running distance of 10.2 km completed in an average time of 35.3 minutes. This routine was performed between 13.00 and 16.00 hours. The non-runners were not sedentary and engaged in pastimes such as softball and swimming but with no sustained exertion or training. On the fourth day, the runners abstained from running whilst

the non-runners jogged 2.4 km at a self-regulated pace (mean time of 13.6 minutes). This exercise was taken between 13.00 and 16.00 hours so that time-of-day effects would not confound the comparison between the 2 groups. Sleep was recorded on all four nights, with the first night acting as the adaptation night.

The results showed that the absolute amount of SWS was virtually identical for both groups in both conditions. When the non-runners engaged in the modest exercise there was a trend for stage 3 sleep to decline whilst stage 4 sleep was unaffected. However, the runners showed significantly more NREM sleep in the non-exercise condition than the non-runners in either condition. This finding was also true for runners in the exercise condition compared to non-runners in the non-exercise condition. Analysis of covariance revealed that NREM sleep was greater in the runners independently of age. This increase in NREM-sleep was due, in part, to the longer total sleep times of the runners. Computer analysis of total 0.5 - 2 Hz activity per night showed that delta activity varied little between the two groups even though the runners exhibited larger amounts of NREM sleep. REM sleep was not found to differ significantly between the 2 groups although the runners appeared to exhibit smaller amounts of eye movement activity than the non-runners.

The findings from this study do not support the restitution theory of sleep as both visual scoring and computer analysis failed to detect any changes in SWS between the groups or conditions.

Griffin and Trinder (1978) studied the effect of both physical fitness and exercise upon sleep. They hypothesized that exercise affects SWS in two ways. One being that fit subjects characteristically show high levels of SWS

compared to unfit subjects. The second being that daytime exercise causes an increase in SWS in fit subjects, but has no effect, and even a negative effect, on unfit subjects.

In order to test their hypothesis, the authors studied two groups of subjects, namely a fit and unfit group each containing four female and four male subjects. Subjects were assigned to either the fit or unfit groups on the basis of their physical work capacities as assessed by a sub-maximal method (PWC 170) with a correction for weight. The study lasted for three non-consecutive days, with an adaptation night preceding the two experimental nights. On the exercise day, both groups were required to complete a 7.3 km run over a hilly course although the unfit subjects were allowed to walk when necessary. This exercise was taken between 16.00 and 18.00 hours. On non-exercise days the subjects engaged only in sufficient activity to carry out their normal daily routine, including training sessions. The authors do not state that the order of the experimental conditions was balanced and it appears that the exercise was taken on the first experimental day.

The analysis of stage 3 and stage 4 data revealed that the fit subjects had significantly more SWS than the unfit subjects. This effect was found to be due almost entirely to variations in stage 3 sleep. This finding supported the first hypothesis described above.

The main effect of exercise was not significant for either SWS, stage 3 or stage 4 sleep. However, the interaction between level of fitness and amount of exercise was significant for stage 3 data but not for SWS or stage 4 sleep. The fit subjects showed an increase in stage 3 following the exercise whereas the unfit subjects showed a decrease. These changes in stage 3 sleep were reflected in the onset latencies to stage 3 sleep; these being decreased for fit and increased for unfit subjects following

exercise. The authors took these results to support their second hypothesis even though the interaction effect was not found to be significant with SWS data.

In order to determine whether the sleep of the unfit subjects was disturbed by the exercise, thereby preventing an increase in SWS, the combined duration of wakefulness during the sleep period, stage 1 sleep and movement time were analysed for both groups under both conditions. Again, the interaction effect was found to be significant, using either absolute duration or percentage of sleep period time, with this variable being increased for the unfit and decreased for the fit as a function of exercise.

These findings are extremely interesting as they indicate why the effects of exercise upon sleep have been varied from study to study. It would follow that those studies using fit subjects only would find an increase in SWS following exercise whereas those using unfit subjects only would not find such a relationship. Furthermore, that the baseline values of SWS would be higher in studies using fit subjects only compared to studies using unfit subjects only. The validity of these suggestions can be assessed with reference to the studies reviewed in this section and the earlier literature review (see Chapter 2, Section 2.2, see also Table 1 of this Chapter).

Those studies using fit subjects only or a group of fit subjects are Baekeland and Lasky (1966), Baekeland (1970), Shapiro et. al. (1975) and Walker et. al. (1977). Of these four studies, only two (Baekeland and Lasky, 1966 and Shapiro et. al., 1975) have reported an increase in SWS (or stage 3 or stage 4 sleep) following exercise. As regards the suggestion concerning the possibility of characteristically high baseline values of SWS in fit subjects, it is apparent that more evidence is required (see review

of Zloty et. al., 1973; page 24 ). Thus, the findings of Griffin and Trinder (1978) remain to be validated in future studies.

When reviewing the other studies it has been usually apparent whether or not the findings support the body restitution theory of SWS. In the study by Griffin and Trinder, however, the results appear to be partially consistent and partially in disagreement with this theory. Their finding that SWS increased following exercise in the fit subjects is clearly in agreement with the theory. Also, the finding that the fit subjects showed more SWS than the unfit subjects, even during the no-exercise night, is in accordance with the theory as it suggests that habitual exercise facilitates the appearance of SWS. Whether this increase in SWS represents a chronic increase in SWS as an adaptation to habitual exercise or represents a residual effect of exercise taken over the previous days is yet to be determined. However, the finding that unfit subjects show a decrease in SWS following exercise is clearly inconsistent with the restitution theory.

Griffin and Trinder offer two explanations to reconcile this latter finding with the theory. The first suggests that the unfit subjects were stressed by the unaccustomed exercise and that this had a disruptive effect upon their sleep (as evidenced by the data on percentage wakefulness + stage 1 + movement time) which counteracted with the exercise-induced increase in SWS.

The second argues that the amount of exercise habitual to the unfit subjects is less than that provided for by their chronic level of SWS and that the single exercise session was not demanding enough to deplete the 'reserve' of SWS.

The first explanation appears to be a good working hypothesis. However, REM sleep did not show any significant changes in the experiment when a reduction might have been expected (according to Hartmann, 1973) as a consequence of the stress displayed by the unfit subjects during and after their exercise session. It is possible that this putative stress effect had disappeared by the second half of the night and therefore had little effect on REM sleep.

The second explanation appears to be rather weak and poses more questions than it answers. For example, if SWS is related to body restitution, then why should unfit have a 'reserve' of SWS not normally called upon when fit subjects do not? As the experimental exercise was identical for both fit and unfit subjects (with the exception that the unfit subjects were allowed to walk when necessary) then the relative increase in activity above habitual levels was greater for the unfit subjects than the fit subjects. So, if SWS is related to body restitution, then it is not clear why the greater relative need of the unfit subjects for SWS can be met by their 'reserve' whereas the smaller relative need of the fit subjects cannot be met by their 'reserve'.

## 3. GENERAL DISCUSSION

The main details of all the studies reviewed and study 1 of this thesis (Horne and Porter, 1975, 1976) are summarised in Table 1.

### 3.1 SWS and the body restitution theory

From the first literature review of the exercise and sleep studies it was concluded that the effects of exercise upon SWS were equivocal. The majority of studies did not report any change in SWS although increases in SWS were reported during bed-rest and in one exercise

| STUDY | SUBJECTS | EXERCISE | | SLEEP VARIABLES A - no. adaptation nights R - no. recording nights<br>Recovery Night | | | | | Carryover Night | Significance |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Experimental exercise | Time of day | A R | SWS | REM | Other | | | |
| Baekeland & Lasky (1966) | 10 young male athletes accustomed to regular, strenuous exercise | normal sporting activities | afternoon | 1 1 | Analysis of 1st 6 hrs sleep:- 40.1%(1) | NSD | Stage 1 4.1%(2) | Stage W 0.1 %(3) | Not recorded | S.D. to:<br>(1) low p<0.01<br>(2) evening p<0.05 low p<0.01<br>(3) evening p<0.02 |
| | | normal sporting activities | evening | 1 | 35.4% | | 6.1% | 0.8% | | |
| | | low | | 1 | 32.5% | | 7.3% | 0.4% | | |
| Hauri (1968) | 15 young males (professional) | 6 hours of: strenuous exercise | prior to retiring | 1 1 | NSD | NSD | sleep latency S1 8.9 | S2 12.3 | Not recorded | S.D. to:<br>(1) exercise relaxation p<0.05<br>(2) exercise relaxation p<0.001 |
| | | studying | | 1 | | | 14.2(1) | 20.8(2) | | |
| | | relaxation | | 1 | | | 8.9 | 13.2 | | |
| Baekeland (1970) | 14 young males accustomed to regular exercise | normal sporting activites | various | 0 2 | Deprivation period NSD | NSD | NSD | | Not recorded | |
| | | 1 month of exercise deprivation | | 4 | | | | | | |
| Zir et. al. (1971) | 10 young males (17-23 yrs.) in "good physical condition" | control (exercise deprivation) | | 2 2 | NSD | NSD | NSD | | Not recorded | |
| | | light (5 $S^S$) (2 hours) | afternoon | 1 | | | | | | |
| | | moderate (5 $S^S$) (6 hours) | morning & afternoon | 1 | | | | | | |
| Ryback & Lewis (1971) | 8 young males (18-24 yrs.) of average fitness | control (5 weeks) | | 1 1 | 16.4% | NSD | Stages 1 & 2 59.0% | | Stage 4 still increased compared to control p<0.05 | (1) SD to control p<0.01<br>(2) control p<0.005 |
| | | bed-rest (5 weeks) | | 2 | 23.7%(1) | | 53.7%(2) | | | |
| Zloty et. al. (1973) | 16 male long-distance runners (19-32 yrs) | normal sporting activites | various | 1 1 | 23.0% Authors compared this value with Backeland (1970) & concluded that their runners showed more SWS as they exercised harder. | | NSD | | - | |

Table 1. The effects of changes in physical activity upon human sleep.

| STUDY | SUBJECTS | EXERCISE | | SLEEP VARIABLES A - no. adaptation nights R - no. recording nights<br>Recovery Night | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Experimental exercise | Time of day | A-R | SWS | REM | Other | Carryover Night | Significance |
| Adamson et. al. (1974) | 12 young males (19-31 yrs.) of varied fitness | control | before 14.00 hrs. | 1-2 | NSD | | | NSD | |
| | | considerably above normal | | 1+ 1-2 | | | | | |
| Desjardins et. al (1974) | 6 males (23-28 yrs.) | control | | ? 1 | NSD | 24.5% | NSD | Not recorded | S.D. to (1) control $p<0.05$ |
| | | low (0.109 cal/ kg/min) | evening | 1 | | 20.6%(1) | | | |
| | | high (0.317 cal/ kg/min | evening | 1 | | 19.8%(1) | | | |
| Horne & Porter (1975, 1976) | 8 young males (18-22 yrs.) of varied fitness | control | | 2+1 2 | Stage 3 in 1st 225 mins. of sleep 13.2% | NSD | NSD | NSD | S.D. to (1) control $p<0.05$ |
| | | moderate (45% estimated max. work capacity for 85 mins.) | morning | 1 | 11.5% | | | | |
| | | | afternoon | 1 | 16.2%(1) | | | | |
| Shapiro et. al. (1975) | 2 very fit young males (24-26 yrs.) | no specific exercise | | 1 1 | 20.5% | 19.0% | | Not recorded | No statistical analysis as only 2 subjects were recorded. |
| | | 50% $VO_2$ max. for: 2 hrs. | exercises completed by 13.00 to 14.00 hrs. | 1 | 24.5% | 16.5% | | | |
| | | 4 hrs. | | 1 | 27.5% | 13.5% | | | |
| | | 6 hrs. | | 1 | 32.5% | 11.0% | | | |
| | | 75% $VO_2$ max. for 2 hrs. 40 min. (1 hr. rest after 1 hr. 20 min) | afternoon | 1 | 38.5% | 9.5% | | | |
| | | 50% $VO_2$ max. in hot-box for 3 hrs. | morning | 1 | 30.0% | 12.0% | | | |

Table 1. Continued.

| STUDY | SUBJECTS | EXERCISE | | SLEEP VARIABLES A - no. adaptation nights R - no. recording nights<br>Recovery Night | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Experimental exercise | Time of day | A-R | SWS | REM | Other | Carryover Night | Significance |
| Browman & Tepas (1976) | 9 young males (av. age 18.9 yrs.) of average fitness | relaxation (45 mins,) | prior to retiring | 1<br>1 | NSD | NSD | REM latency / Sleep latency S1 S2<br>87.3 / (2) 7.3 (3) 11.6<br>106.1(1) / 12.4 / 17.2<br>80.4 / 11.6 / 15.5 | not recorded | S.D. to:<br>(1) relaxation vigilance $p<P.05$<br>(2) exercise vigilance $p<0.05$<br>(3) exercise vigilance $p<0.05$ |
| | | light exercise (45 mins) | | 1 | | | | | |
| | | vigilance task (45 mins) | | 1 | | | | | |
| Moses et. al (1977) | 27 young males (18-22 yrs.) of average fitness | baseline | | 1<br>1 | NSD | NSD | % total sleep time (TST)<br>bed rest gp: 92.5<br>exercise gp: 86.3<br>sleep dep. with bedrest 94.6<br>sleep dep. with exercise 96.1 | not recorded<br>not recorded | Sig. increase in TST following sleep dep. with exercise than sleep dep. with bed rest $p<0.05$ |
| | | 40 hr. sleep deprivation For 1 in every 4 hrs: $18S^S$ rested in bed. | | 1 | | | | | |
| | | $95^S$ exercised (moderate) | | 1 | | | | | |
| Walker et. al. (1978) | 10 distance runners (18-20 yrs) | runners running | afternoon | 1<br>2 | NSD | NSD | NREM sleep (mins)<br>347.0(1) | not recorded | S.D. to:<br>(1) non-runners: not running $p<0.01$<br>(2) non-runners: running $p<0.01$ |
| | | runners not running | | 1 | | | 341.7(1)(2) | | |
| | 10 non-runners (18-22 yrs) | non-runners running | afternoon | 1 | | | 313.4 | | |
| | | non runners not running | | 2 | | | 313.0 | | |
| Griffin & Trinder (1978) | 2 groups:-<br>8 fit (4 males, 4 females mean age 23 yrs.) | no exercise | | 1<br>1 | SWS: (mins) 114.2 / Stage 3: (mins) 53.9 | | %(stage W & stage 1 + movement time):<br>11.1 | not recorded | SWS: Fit. S.D. to Unfit<br>Stage 3: Fit S.D.to Unfit.<br>Fitness x exercise S.D.<br>% W+1+MT: Fitness x exercise S.D.<br>$p<0.05$ |
| | | exercise (7.3 km run) | afternoon | 1 | 125.1 / 66.8 | | 7.9 | | |
| | 8 unfit (4 males, 4 females mean age 23 yrs.) | no exercise | | 1 | 97.2 / 42.6 | | 9.2 | | |
| | | exercise (7.3km run & walk) | afternoon | 1 | 90.7 / 32.9 | | 12.6 | | |

Table 1. Continued.

study (Baekeland and Lasky, 1966). The two animal studies reviewed (Matsumoto et. al., 1968; Hobson, 1968) are often cited in favour of the body restitution theory of SWS but neither study has shown that SWS levels were increased during a 24 hour period following the exercise.

The studies reviewed in this chapter and the main exercise study (study 1) of this thesis allow a somewhat clearer picture to be made from the available evidence, although a clear-cut link between exercise and subsequent sleep, in particular SWS, has still not been established. To date, Hauri (1968), Baekeland (1970), Zir et. al. (1974), Adamson et. al. (1974), Horne and Porter (1975), Browman and Tepas (1976), Moses et. al. (1977) and Walker et. al. (1977) have not found high levels of physical activity to be associated with increased levels of SWS compared to normal or low levels of physical activity. The experimental evidence from the exercise studies in favour of the body restitution theory is supplied by Baekeland and Lasky (1966), Zloty et. al. (1973), Shapiro et. al. (1975) and, to some extent, by Griffin and Trinder (1978). The evidence provided by Zloty et. al. (1973) must be viewed with caution for the reasons described when reviewing this study. As the findings of Griffin and Trinder (1978) were only partly in agreement with the restitution theory it appears that only two studies to date have provided sound evidence which links exercise and SWS. The reason why these two studies report such a relationship when the other studies have reported negative findings is not clear. The majority of these latter studies have been well designed and competently executed and this argues against a general relationship between exercise and SWS. It seems likely, therefore, that these two studies elicited a positive finding because some experimental factor or factors were different in these studies.

### 3.1.1 Factors influencing the effect of exercise upon sleep

From the first literature review it was suggested that the following factors might be involved in the exercise and sleep relationship:-

i) Fitness of the subjects.

ii) Work load.

iii) Time of day when the exercise was taken.

These factors are discussed in the following sub-sections.

#### 3.1.1.1 Subject Fitness

Baekeland and Lasky (1966) and Shapiro et. al. (1975) both used very fit subjects who habitually exercised hard on several days a week and this might explain why these two studies reported an increase in SWS following exercise. However, Baekeland (1970) and Walker et. al. (1977), also used subjects who were accustomed to regular exercise, but they did not report an increase in SWS following exercise. It is probable that the subjects in the latter two studies were not quite as fit as those used by Baekeland and Lasky (1966) or Shapiro et. al. (1975) and certainly the remaining studies all used subjects of average or varied fitness with the exception of Griffin and Trinder (1978). Griffin and Trinder reported that their fit subjects showed an increase in SWS following exercise whereas their unfit subjects actually showed a decrease in SWS. Furthermore, the fit subjects displayed larger amounts of SWS on days of inactivity compared to the unfit subjects. Whether this latter finding is due to a residual effect of exercise taken on previous days or to the adaptation to habitual exercise is open to debate. However, this study does highlight the importance of subject fitness when

discussing the effects of exercise upon sleep.

None of the subjects used in Study I of this thesis could be described as very fit and so the data from this experiment can not be used to provide evidence for or against the effect of subject fitness. From Study II of this thesis it was apparent that the subjects who classified themselves as fit appeared to require less sleep or to sleep more efficiently than subjects who described themselves as unfit. The fit subjects also fell asleep quicker and reported poor sleep quality and the desire to sleep longer on fewer occasions. However, as activity was not found to have any major influence on any of the sleep variables studied, either as a main effect or combined with fitness as an interaction effect, it is not possible to assess whether the fitness of a subject modifies the effects of activity upon sleep. Possibly if the activity levels had been more extreme, as encountered in some laboratory studies, then such an effect may have been visible. Why the effect of exercise may be varied according to the fitness of the subject is not clear. It is possible that the effects of anxiety, resulting from the exercise, may have balanced an exercise-induced increase in SWS in unfit subjects. Evidence for this suggestion is provided by Griffin and Trinder (1978) who report an increase in wakefulness and light sleep following exercise in the unfit subjects. A similar change in sleep quality was not reported by Walker et. al. (1977) for their non-runners after running although they noted a strong trend for heart rate to remain elevated during sleep following exercise in this group.

Another possibility, which has already been described in the review of Shapiro et. al. (1975), is that this exercise-mediated increase in SWS found in very fit subjects is an extension of the well-known improvements in the

efficiency of some organ and body processes following regular exercises. Thus, an improvement in sleep efficiency may follow improvements in cardiac and pulmonary efficiency in order to allow the body to react to and recover from exercise quickly.

A third possibility is that the effects of exercise upon SWS may vary from individual to individual and be genetic in origin. If an exercise-induced increase in SWS does in fact allow the body to recover more quickly from exercise, then it is possible that those individuals who display such an increase are more suited to athletic endeavours than those who do not. In other words, their natural ability to spend a high proportion of their sleep in SWS might allow them to partake in more rigorous training procedures than other people who display relatively small amounts of SWS.

3.1.1.2 Work Load

Intuitively, one could expect that a high work load would be more likely to elicit an increase in SWS than a low work load. The work load undertaken by the subjects of Baekeland and Lasky (1966) was presumably fairly high, although no objective assessment was taken. The work loads endured by the two subjects of Shapiro et. al. (1975) were objectively assessed and graded and the authors reported that SWS was progressively increased as the subjects became increasingly fatigued during the experiment. The increase in SWS was not particularly evident until night 3 following 4 hours of exercise at 50% $\dot{V}O_2$ max. This work load is very high and probably could not be tolerated by an unfit subject. It is difficult to compare this work load with most of the other studies due to the lack of objective measurement. However, the work loads prescribed on days 3 to 6 by Shapiro et. al. (1975) were clearly above

those undertaken in other studies, with the possible exception of Baekeland and Lasky (1976). For example, the work load on day 3 was approximately 3-fold that prescribed to the subjects in Study I of this thesis.

Both Zir et. al. (1971) and Desjardins et. al. (1974) have conducted studies involving graded exercise, although no changes in SWS were observed with increasing work load. Thus, it appears that an increase in SWS may not occur until the work load is very high. It is clear from the study by Griffin and Trinder (1978) that work load is not the sole factor underlying the exercise and sleep relationship. In this study both the fit and unfit subjects performed the same exercise, although the fit group performed it at a faster rate, but only the fit group showed a subsequent increase in SWS. Furthermore, the fact that six hours of strenuous evening exercise prescribed by Hauri (1968) did not elicit any changes in SWS indicates that the effects of exercise upon sleep are subject to several factors.

Interestingly, there is some evidence that SWS is also increased when the work load is drastically reduced for prolonged periods (Ryback and Lewis, 1971). As discussed when reviewing this paper, this finding may be due to the very low baseline values reported for SWS. The lifestyle of these subjects was also drastically changed during the course of the experiment and this may account for the changes in SWS. Also, Baekeland (1970) reported no changes in SWS following a month of exercise deprivation (as opposed to the complete bed-rest prescribed by Ryback and Lewis, 1971).

In Study I it was recognised that the work load was possibly a major factor underlying the exercise and sleep relationship and precautions were taken for its control.

It was considered more appropriate to prescribe individually standardised work loads resulting in similar degrees of subjective fatigue across the subjects, rather than identical absolute work loads which would result in varied reports of fatigue. Thus, this study does not provide any information concerning the effects of graded exercise upon sleep.

Study II reported upon the effects of various subjective levels of physical activity upon sleep behaviour, although no changes were found for any of the variables studied except for the suitability of the bedtime. As neither the work loads or the subsequent sleep quality were objectively assessed, it might not seem surprising that the findings were negative. However, it is commonly believed that a hard day's work (physical) is rewarded by a good night's sleep, so it could be considered surprising that none of the subjective variables, except the judgements concerning the suitability of the bedtime, were responsive to the daytime activity levels. The subjects used in this questionnaire study were all volunteers and they reported upon their normal daily activities. As they played various sports for enjoyment only and were not paid to unduly exert themselves, it is highly unlikely that even the reported activity level of "greatly above normal" corresponded to the work load on day 3 of the study by Shapiro et. al. (1975).

3.1.1.3 Time of Day of Exercise

In both the studies by Baekeland and Lasky (1966) and Shapiro et. al. (1975) the exercises were completed during the afternoon. Interestingly, when the subjects in the former study exercised in the evening, they did not display an increase in SWS. Other studies have also investigated the effects of evening exercise upon sleep (i.e. Hauri, 1968; Desjardins et. al., 1974 and Browman and Tepas, 1976) and these have all failed to report

changes in SWS. Even six hours of strenuous exercise taken by subjects of varied fitness did not produce an increase in SWS (Hauri, 1968). This suggests that the effects of exercise upon sleep may be variable according to the time of day when the exercise is taken.

Further support for this suggestion was provided by Study I of this thesis. In fact, the assessment of this variable was one of the objectives of this study. Neither exercise taken in the morning or afternoon was found to affect whole night sleep data, although it appeared that SWS was increased during the first few hours of sleep following afternoon exercise.

Thus, from the available evidence it appears that exercise taken during the afternoon is more likely to increase SWS than exercise taken earlier in the morning or later during the evening. The reasons for this time of day effect are not clear but it has been suggested that evening exercise has an arousing influence upon the brain which opposes any SWS-enhancing effect of the exercise. (Baekeland and Lasky, 1966).

The effects of this putative arousal resulting from evening exercise appears to either increase wakefulness (Baekeland and Lasky, 1966), to reduce REM sleep (Desjardins et. al., 1974), to increase REM latency (Browman and Tepas, 1976) or to increase sleep latency (Browman and Tepas, 1976) compared to relaxation. These changes all appear to be in accord with the notion that evening exercise has a general arousing influence upon the brain. Unfortunately, none of these evening exercise studies recorded sleep on the following night to see if a SWS debt existed. If SWS was necessary for recovery from physical fatigue then such a debt would be expected to be displayed at the first convenient occasion.

However, from the findings of Study I of this thesis, it appears that such a debt does not exist. In this study, afternoon exercise was found to have a subtle influence upon SWS which was not apparent following morning exercise. Clearly, the failure of morning exercise to influence sleep cannot be ascribed to its arousing influence as this will either by non-existent by late evening or it will be less than that resulting from the afternoon exercise. Furthermore, this study did record sleep on the night following the exercise recovery nights and it was apparent that no debt was carried over from the recovery night. These findings led to the conclusion that recovery from physical fatigue may occur during wakefulness if the exercise is taken early in the day. However, if the exercise is taken late in the day, recovery may not be completed and it will continue during sleep; the existence of such recovery taking place during sleep being characterised by an increase in SWS. This stage of sleep is, therefore, not seen to be necessary for recovery to take place but rather beneficial to recovery. Exactly what benefits are available during this stage of sleep are still debatable although the relationship between growth hormone release and SWS is often cited as evidence of the reparative properties of this stage of sleep. If this stage of sleep is disrupted, for example by evening exercise, then it is suggested that recovery will take place without the benefits of increased amounts of SWS.

Study II of this thesis did not find any significant relationship between the time of activity and any of the tiredness or sleep variables examined, except that the time of thinking of bed and the actual bedtime were delayed following late evening activity. This finding also indicates that late evening activity has an arousing influence on the brain. No increases in sleep onset times were

found, as reported by Browman and Tepas (1976), and the data suggests that sleep onset was actually quicker following late evening exercise than morning or evening exercise. This finding is probably a consequence of the fact that the subjects could retire when they wished and not when instructed to by an experimenter. The lack of any other significant effects concerning the time of activity is not surprising as this study failed to find any effect of activity, taken at any time, upon the tiredness and sleep variables studied, except for the suitability of the bedtime.

## 3.2 Effects of exercise upon other sleep variables

Because of the theoretical implications, much attention has been paid to assessing the effect of exercise upon SWS. However, there seems to be little evidence for a major effect of exercise upon other sleep stages. As discussed in the previous section, evening exercise has been reported to delay sleep onset, decrease REM sleep or delay its appearance and to increase wakefulness. These changes appear to result from the increased arousal induced by the late exercise. A decrease in REM sleep following afternoon exercise has been reported by Shapiro et. al. (1975) and this is possibly as a consequence of the increases in SWS. An increase in sleep disturbance, as assessed by % stage W + stage 1 + movement time, has been reported following afternoon exercise by unfit subjects although the fit subjects reported a decrease in sleep disturbance following exercise (Griffin and Trinder, 1978). The unfit subjects may have been still aroused by evening following their exertions although it is a possibility that the sleep disturbance was due to cramps or aches or pains resulting from the unaccustomed exercise. Percent total sleep time has been reported to be increased following sleep deprivation with exercise compared to sleep deprivation with bed-rest although no simple, direct effect upon specific sleep stages was found (Moses et. al., 1971). From Study II

of this thesis it is apparent that normally encountered levels of physical activity have little effect upon general sleep behaviour as assessed by subjective reports of pre- and post-sleep tiredness and sleep quality.

## 3.3 Overview

From the above discussion it is clear that the exercise and sleep relationship is very complex. Three major factors, namely subject fitness, work load and time of day of exercise, have been identified as being of importance in this relationship.

It appears that exercise is most likely to increase SWS when high work loads are performed by very fit individuals during the afternoon. If the work load is too low or the exercise taken too early then recovery may have taken place during the subsequent wakefulness. If the exercise is taken in the evening then this may prevent the expected increase in SWS from occurring because of the disruption of sleep as a result of the increased arousal of the subjects.

It is not clear why only very fit subjects should demonstrate an increase in SWS following exercise. For example, the unfit subjects used by Griffin and Trinder (1978) completed the same exercise (4½ mile run) as the fit subjects but they actually displayed a decrease in SWS. Furthermore, it seems surprising that a 4½ mile run by fit subjects should elicit an increase in SWS whereas six hours of moderate exercise (Zir et. al., 1971) or 85 minutes of moderate exercise (Study I), taken by subjects of good to variable physical condition respectively, failed to show an increase. As the exercises were all carried out during the afternoon this cannot be explained by time of day effects. It could be argued that the less fit subjects

were still aroused upon retiring and that this counter-balanced the exercise-induced increase in SWS. However, although Griffin and Trinder (1978) reported an increase in sleep disturbance in the unfit subjects, neither Zir et. al. (1971) or Study I reported any changes in wakefulness, light sleep or REM sleep to suggest that the subjects were still aroused by the exercise.

Possibly the best explanation at present is that those individuals who are at the peak of physical fitness develop the ability to increase their quota of SWS when necessary. This increase in sleep efficiency would complement the well-known increases in cardiac and pulmonary efficiency and would enable the athlete to cope with extremely high work loads. It is suggested that this improvement of sleep efficiency is characteristic of only extremely fit individuals.

The main purpose of this first part of the thesis has been to investigate the validity of the body restitution theory of SWS by examining the effects of exercise upon sleep. The other evidence in support of this theory, arising from studies of sleep deprivation, human growth hormone, cell division and diseases of the thyroid gland are outside the sphere of this thesis although they have been briefly mentioned in Chapter 2. With regard to the evidence arising from the exercise studies it would appear that SWS is, in certain cases, increased after exercise and therefore may have some part to play in the recovery processes. However, it is quite clear that in the majority of situations exercise is not associated with an increase in SWS. This indicates that either the usual quota of SWS is sufficient for recovery or that SWS is not necessary for recovery. From the findings of Study I it would appear that SWS is not necessary for recovery from physical fatigue but that the recovery processes may

continue into early sleep. As SWS occurs mainly during the first half of the night it is quite probable that this stage of sleep aids recovery, both by means of enforced rest and the presence of growth hormone. Thus, whilst SWS probably facilitates recovery from physical fatigue, it does not appear to be necessary for the recovery of physical fatigue.

## PART TWO: DIET AND SLEEP

SECTION A: Literature Review and Preliminary Study

SECTION B: Development of a Theoretical Model

SECTION C: Experimental Investigation of the Theoretical Model

# PART TWO: DIET AND SLEEP

## SECTION A: Literature Review and Preliminary Study

CHAPTER 7 Literature Review

CHAPTER 8 Study III

CHAPTER 7

LITERATURE REVIEW

## 1. INTRODUCTION

It is a widespread belief that a good meal can promote feelings of drowsiness, which may possibly culminate in a post-prandial nap. It is also thought that the restlessness and alertness which is often associated with hunger can have a disruptive influence upon sleep. However, it is not clear whether these changes in sleep behaviour are mediated directly by the diet, and consequently the nutritional status of the animal, or indirectly through other factors. For example, in the human, nutritional status might influence sleep by at least three such indirect factors:-

a) A full stomach makes physical activity very unpleasant and, consequently, the well-fed human typically enjoys the relaxation of a comfortable chair or bed.

b) An empty stomach is not usually considered desirable and, therefore, usually occurs in association with the lack of food supplies. This may be due to a lack of financial resources, a bad harvest resulting from natural disasters or even to an imposed limited feeding regime as found in some prisoner-of-war camps. All of these possibilities are stressful and this resultant agitation may well produce the sleep disruption.

c) An empty stomach might disrupt sleep because of the increase in hunger pangs.

This chapter will review the available evidence concerning the influence of the diet upon sleep and attempt to assess the current knowledge with regard to two questions:

a) Is there a valid relationship between the diet and sleep behaviour of an animal or human, and if so then

b) By what mechanisms is this relationship mediated?

When this review was initially undertaken, prior to the first diet and sleep study, only some of the studies published during 1975 were available. However, this present review describes studies published prior to the Summer of 1977 when the second diet and sleep study was completed. It was decided that this procedure was warranted in order to present and discuss the available literature in a comprehensive form. Studies published after the second study are described in the last chapter of the thesis.

## 2. ANIMAL STUDIES

### 2.1 Food Deprivation

There is a general agreement from the animal studies that food deprivation is associated with restlessness and increased activity (Wald and Jackson, 1944; Siegel and Steinberg, 1949; Bolles, 1963), although prolonged deprivation evenutally leads to physical weakness and activity therefore declines (Wald and Jackson, 1944).

Jacobs and McGinty (1971) observed the sleep of rats during four days of ad libitum feeding followed by a variable number of days (6-11 days) of total food deprivation. The food deprivation was terminated when starvation was imminent and EEG sleep was also recorded on this recovery day. The sleep was recorded for only one three-hour period a day, although the authors checked the validity of this sample with several 24-hour samples.

The results showed that the amount of wakefulness increased as a function of the duration of food depreviation. NREM sleep was found to decline gradually during the first few days of fasting and thereafter to decline rapidly toward zero. On the other hand, REM sleep was actually increased (approximately 20%) to a significant extent on the first day of fasting. During the subsequent days REM sleep remained within baseline values until the last two days prior to its

complete disappearance. On the recovery day, REM sleep showed a large rebound whereas NREM sleep remained below baseline values. However, some of the rats had reached terminal starvation by this time and soon died.

The general decrease in NREM and REM sleep is to be expected as the amount of wakefulness increases. However, it is very interesting that REM sleep actually increased on the first day of fasting. Unfortunately, it is not stated how many hours the rats had been food deprived before the sleep recordings were taken on this first day of fasting.

Ruckebusch and Gaujoux (1976) found that 3 days of total food deprivation did not significantly affect total sleep time, NREM or REM sleep in the cat. However, if pig fat was provided during a three-day fasting period then both NREM and total sleep time were significantly decreased whereas REM sleep was only slightly decreased.

This effect of the fat was not considered to be mediated by metabolic changes as the cats found this food unpleasant and, consequently, ate only small amounts. The authors considered that the effect of the fat might have been due to the increased stress resulting from the fasting in the presence of food, even though unpalatable, than in the absence of food. Thus, the changes in rat sleep during total food deprivation as reported by Jacobs and McGinty (1971) may have been due to the stress of fasting rather than by any metabolic factors. Also, the cats studied by Ruckebusch and Gaujoux were not fasted for as long as the rats in the study by Jacobs and McGinty. It is highly likely that the cat can survive on its body fat stores for longer than the rat because of its larger size, so the metabolic changes in the rat following food deprivation would be expected to be swifter and larger than those in the cat. This factor may explain the difference in the extent of sleep disruption reported by these

two studies.

Recently a study by Borbély (1977) used continuous telemetric EEG recording to determine the vigilance states of the rat during control, food deprivation and refeeding periods. He found that 80 hours of food deprivation did not induce a significant change in the daily amounts of the vigilance states, the diurnal rhythm of sleep and waking or REM sleep periodicity. However, REM sleep was close to being abolished in the dark phase and increased during the light phase of the diurnal cycle, possibly as a rebound, especially during the first hour of light. Also the duration of the sleep episodes were shortened throughout the period of food deprivation whereas REM sleep and SWS episodes were decreased only in the dark phase.

Borbély suggested that sleep may be more disrupted by food deprivation (and the subsequent lipolytic phase when the fat stores are metabolised) during the dark period because the rat's metabolism is geared to receiving external energy sources at this time of day. During the light period, however, the rat is accustomed to metabolising its energy stores and therefore sleep would not be so disturbed by the fasting.

Upon refeeding, Borbély found that the sleep episodes increased in duration and actually exceeded control values. Also, episodes of activity, which were shortened during food deprivation, were markedly lengthened during the refeeding period. The actual amount of activity did not show any significant differences over the three different conditions as would be expected from the studies of Wald and Jackson (1944), Siegel and Steinberg (1949) and Bolles (1963).

Borbély suggested that the shortened length of these behaviourial episodes represented a major adaptive mechanism for the rat; the frequent changes in the vigilance states

and activity levels serving to increase the likelihood of finding food with a minimum energy expenditure.

## 2.2 Feeding

Anokhin (1961) fed cats via a stomach tube and, in addition, administered glucose intravenously. He observed that their EEG promptly changed to a synchronised pattern, similar to SWS.

Fara, Rubinstein and Sonnenschein (1969) reported that the administration of milk into the cat duodenum had a significant sedating effect characteristed by the animal curling up and EEG spindling if the animal was active or a transition to sleep if it was already drowsy. Furthermore, it was reported that the frequency or duration of REM periods was markedly increased in the hour following the administration of milk. The administration of milk into the stomach, however, produced no detectable changes in arousal. The authors proceeded to identify the sleep-promoting component of milk. Equal volumes of water, saline or solution of glucose, lactose or casein were administered into the duodenum but had no effect upon sleep whereas corn oil was found to enhance sleepiness, even at a dose of only 0.5 ml. The authors suggested that this increased sleepiness might be mediated by the effect of a gastrointestinal hormone on the CNS.

This hypothesis was further examined in a study by Rubinstein and Sonnenschein (1971). They confirmed the observation of Fara et. al. (1969) by finding that total sleep time was increased in a three hour period following the introduction of fat into the cat duodenum. This increase in sleep (from 33 to 48 per cent of the 3 hour period) was associated with an increase in the number of REM periods (from 2 to 6). In another group of cats it

was found that a single large meal of Purina cat pellets increased total sleep time in the 3 hour period following the meal compared to the 3 hour period immediately prior to the meal (from 46 per cent to 55 per cent of the 3 hour period). Both the number and duration (from 8 per cent to 14 per cent of the 3 hour period) of REM periods were also increased. In a third group of cats, the denervation of the gut was associated with reduced total sleep time although an increase in number and duration of REM periods was still elicited following a single large meal. In other animals the intravenous infusion of the gastrointestinal hormone secretin and cholecystokinin induced NREM sleep and long lasting REM periods after a latency of 10-25 minutes.

The authors took these findings to be consistent with the hypothesis that the gastrointestinal hormones released by the presence of food intothe gut may be involved in the regulation of sleep. However, each experiment involved only three cats and further studies with a larger number of cats is warranted. For example it would be interesting to see if a single, large meal still elicited the sleep response if it contained no fat.

Dallaire and Ruckebusch (1974) investigated the sleep of three housed ponies over a period of several weeks during which their diet was changed from hay to oats. The oats replaced the hay on a 1:2 weight basis so that the energy yields of the 2 diets were approximately the same. The authors noted that this change was associated with an increase in total sleep time (30-150 minutes) due to an increase in both SWS and REM sleep.

The authors proposed that this reduced feeding time on the oat diet might have increased sleep because the total afferent impulses over the olfactive and trigeminal nerves were diminished. This would decrease the total

sensory input and thereby the ascending recticular activating system would be less active resulting in reduced arousal and increased sleep. However, this change in sleep length may have been partly due to the fact that the oat diet could be eaten in a relatively short time compared to the bulkier hay diet. This would leave more "leisure" time for the pony and this might explain the increase in sleep length following the oat diet.

Ruckebusch and Gaujoux (1976) investigated the effect of a high protein diet upon sleep in two sheep. EEG recordings were taken from 10.00 p.m. to 8.00 a.m. during baseline periods when the sheep were fed on hay, either in natural form or pelleted. During the first high protein period the sheep were administered with a dose of 0.1 g/1.0 Kg of urea into the Lumen. This dose was increased by 0.1 g/Kg daily for two weeks. This non-protein nitrogen source was made available for protein metabolism by the micro-organisms present in the sheeps' digestive system. The results showed that both NREM and REM sleep were increased by the high-protein diet. NREM sleep was greatly increased, by an average of 1 hour, whereas REM sleep was increased by an average of only four minutes.

The second high protein diet consisted of a dose of 0.4 g/Kg urea, increasing by 0.49 g/kg daily over a four day period. The amount of hay eaten was restricted to that eaten by the sheep during the first high protein diet (an average of 800 g hay as compared to the 1200 g consumed during the baseline period). Again NREM sleep was greatly increased (by nearly 2 hours) whereas REM sleep was only slightly increased (by approximately 5 minutes). This minimal increase in REM sleep is very surprising considering the large increase in NREM sleep. It may be construed that the high protein diet actually decreased REM sleep as REM sleep, expressed as a percentage of total sleep, was decreased.

It is worth noting that sleep was unchanged when the sheep fed on either long hay or pelleted cubes, even though the time spent feeding on the long hay was greater than for the cubes. This finding is not consistent with the proposal of Dallaire and Ruckebusch (1974) that the changes in sleep length are due to changes in the total sensory input. Also, it appears that the increased "leisure" time made available by the pelleted hay does not necessarily induce greater sleep length. Thus, these changes in sleep appear to be due to the content of the diet as opposed to other non-dietary factors. However, as the effects of the high protein diet may have been influenced by hyperammonemia resulting from the urea administration, it is difficult to extrapolate this finding with urea administration to the effects of protein upon sleep in mammals other than ruminants.

Ruckebusch and Gaujoux (1976) also studied the effect of "overfeeding" upon sleep in three cats. During this three day period of feeding ad libitum both total sleep time and REM sleep were increased, although NREM sleep remained unchanged, as compared to the "control" cats who were limited to approximately 250 g of commercial cat food to maintain constant body weight. However no data is presented to state how much the "overfed" cats ate and the use of the terms "overfed" and "control" can be criticised. Presumably the authors regarded the feeding ad libitum of commercial food to be less representative of the cats natural food intake than the limited diet which was designed to maintain a constant body weight.

### 2.2.1 Feeding and REM sleep

Siegel (1974) found that the amount of time spent in REM sleep during a 12 hour period was an accurate predictor of the food intake in the subsequent 12 hour period in undisturbed cats fed ad libitum. EEG sleep data were calculated

separately for the lights-on, day period (9.00 a.m. - 9.00 p.m.) and the lights-off, night period (9.00 p.m. - 9.00 a.m.) over 5 to 9 days and nights. The total numbers of 10 second periods of REM sleep, NREM sleep and wakefulness in each 12 hour period were determined for each cat and were then correlated (using Pearson's product-moment correlation) with the number of grammes of food eaten (Purina cat chow) in the preceding, same and subsequent 12 hour periods.

In all but one of the cats, a significant negative correlation was found between REM sleep and subsequent food intake. The period used for prediction did not overlap the subsequent predicted period and therefore increased REM sleep was not correlated with decreased food intake merely because the cat was asleep for more of the time. There were no significant correlations between NREM sleep and food intake and only one with wakefulness, and that was with subsequent food intake. However, the correlation between REM sleep and subsequent food intake was larger than the correlation with wakefulness in this cat. In no case was the correlation between food intake and subsequent REM sleep significant. The data showed, therefore, that REM sleep is a better predictor of subsequent food intake than previous wakefulness, NREM sleep or food intake.

This relationship appears to be valid only in cats fed ad libitum. Thus, when the cats were fasted during the night period it was found that REM sleep during this period was not significantly correlated with subsequent food intake.

Siegel, by integrating his finding with those of Rubinstein and Sonnenschein, proposed the existence of a two-way relationship between REM sleep and food intake. For example, if REM sleep was decreased during a 12 hour period then food consumption would be increased in the subsequent 12 hour period. The increased food consumption

would cause an increase in REM sleep (Rubinstein and Sonnenschein, 1971) in this same period. (Siegel suggested that the relatively short time course of the experiment by Rubinstein and Sonnenschein, only 3 hours, probably accounts for the fact that he failed to find a significant correlation between food intake and subsequent REM sleep as his analysis was carried out on 12 hour blocks of data). This increase in REM sleep would, in turn, decrease the food intake in the next 12 hour period and REM sleep in this same period would, therefore, also be decreased. Siegel argued that this feedback relationship would serve to stabilize both REM sleep and food intake over a 24 hour period. However, this interesting rationale requires further experimental confirmation before it can be established with confidence.

It is worth noting that other links have been found between REM sleep and food intake. Dement (1969) observed that REM sleep deprivation produced hyperphagia. This deprivation has been found to alter self-stimulation thresholds in the lateral hypothalamus, an important centre for food regulation (Steiner and Ellman, 1972). The lateral hypothalamus is also a major area of passage for anteriorly projecting noradrenergic fibres and ascending serotonergic fibres and both of these neurotransmitter pathways are thought to be involved in the regulation of sleep (Jouvet, 1969). Thus, whilst these links do not provide cohesive evidence for Siegel's rationale, they do suggest that changes in sleep may be associated with changes in subsequent food intake.

## 2.3 Summary

It can be generalised from the animal literature that food deprivation is associated with increased activity and disrupted sleep. Conversely, feeding appears to enhance sleepiness and increase total sleep time, especially REM sleep. There is some evidence to suggest that food intake is, in part, influenced by REM sleep.

The relationship between REM sleep and food intake does not appear to be solely due to the bulk of the food. For example, the change of diet from hay to half the weight of oats was associated with an increased sleep time in the pony. Similarly, the administration of small amounts, by weight, of urea to a hay diet was found to increase sleep time in the sheep. The important factor may be the calorific yield of the diet, although the finding of increased sleep in ponies changing from a diet of hay to one of oats, both providing similar energy yields, suggests that other factors are also involved.

From these animal studies it would appear that food intake does affect sleep directly (i.e. by changes in some aspects of metabolism) and not only by indirect factors such as the amount of "leisure" time.

## 3. HUMAN STUDIES

### 3.1 Food Deprivation

#### 3.1.1 Acute food deprivation

MacFadyen, Oswald and Lewis (1973) carried out a well-designed study to investigate the effects of acute starvation upon the sleep of ten young subjects. The experimental programme lasted for 14 consecutive days, comprising 2 adaption, 4 base-line, 4 fasting and 4 refeeding nights. In the fasting periods, the subjects were allowed to drink water and other non-calorific beverages and they received potassium supplements.

Analysis of the EEG data showed that this acute starvation period was associated with a significant increase in SWS (an average of 22 minutes) and a significant decrease (an average of 26 minutes) in REM sleep. The examination of the individual nights showed that SWS was not increased until the subjects had been food deprived for three days whereas REM sleep showed a progressive decline over the four-day

period. Total sleep time did not show a significant reduction during the starvation period, although the fourth night's sleep was nearly one hour shorter than the base-line mean. Sleep during the refeeding period was not found to be significantly different to the base-line nights.

Karacan, Rosenbloom, London, Salis, Thornby and Williams (1973) also examined the effects of acute fasting upon sleep. Although similar findings were reported, the experimental design was poor. The authors used 11 healthy young male subjects who had been adapted to EEG monitoring during sleep prior to the study. Originally the study was designed to last for three consecutive days, providing data for baseline, 30-37th hour and 60-67th hour of fasting. However, the authors were also interested in the response of growth hormone during sleep to fasting and it was found that their blood sampling procedure had a major influence upon the subjects' sleep. Thus, the experiment was repeated without blood sampling after a 6 month interval and only the data from the second experiment was used to assess the effect of fasting upon sleep.

It was concluded that 60-67 hours of fasting produced a significant decrease in the number of REM periods (an average of one per night) and a significant increase (14 minutes) in the percentage stage 4 sleep compared to the base-line night. Thirty-seven hours of fasting did not produce any significant changes in sleep compared to the base-line night.

Unfortunately, it appears that the subjects were not re-adapted to the sleep laboratory before this second experimental period. As the experiment did not record sleep during re-feeding it is possible that the data were subject to experimental bias resulting from the subjects' insufficient adaption to the laboratory environment. However, inspection of the data for the base-line night did

not reveal any such influence.

Thus, it appears that acute starvation is associated with increased SWS and probably decreased REM sleep. Although the study of Karacan and his colleagues did not find a significant reduction in REM sleep, it did report a reduction in the number of REM periods. It is possible that a reduction in REM sleep would have occurred if the period of fasting had been increased to four days as in the MacFadyen et. al. study. In this latter study, REM sleep was maximally depressed on the fourth starvation night although a large decrease was evident on the first night.

It could be argued that REM sleep is reduced during acute starvation because it is a "fragile" state (Hartmann, 1968) which is susceptible to disruption by non-specific stress. Thus, the decrease in REM sleep reported by MacFadyen et. al. might have been due to the psychological stress of acute starvation. Decreases in REM sleep have been reported to occur following pre-examination stress and pre-operative stress in humans (Darwaj, Smyk and Czochra, 1977) although the intensity of the phasic events are apparently increased. Unfortunately, REM density data was not provided in the above starvation studies, so it is not possible to assess if starvation increases the quality whilst decreasing the quantity of REM sleep. If this was found to be so, it could be that these changes in REM sleep following food deprivation were brought about to accommodate the increased time spent in SWS without any loss in the efficiency of REM sleep function. However, REM sleep showed a substantial drop on the first starvation night when SWS was not increased, suggesting that the changes in REM sleep do not appear to be mediated by the changes in SWS. The reported decrease in REM sleep might well have been as a direct consequence of the reduced total sleep time if this reduction occurred mainly at the end of the sleep period. At this time REM

sleep occurs more frequently, and for a longer duration, than in the first half of the night, and so the disturbance of sleep would result in a decrease in REM sleep without affecting SWS.

Thus, the effects of starvation upon human sleep may be mediated by two factors. One factor being the stress and discomfort, which might explain the decreases in sleep length and REM sleep, whereas the increases in SWS may have been due to a second factor possibly related to the increased catabolism during starvation. There is always the problem of "cause or effect" when interpreting decreases of REM sleep within decreases of total sleep time. For example, it is possible that REM sleep was decreased because total sleep time was decreased or it is possible that total sleep time was decreased because the need for REM sleep was reduced.

### 3.1.2 Chronic food deprivation

The data concerning food deprivation and chronic starvation in humans arises mainly from descriptions of famine and concentration camp victims. It is quite clear that these situations are extremely stressful and the consequent changes reported in activity and sleep may well have been greatly influenced by emotional factors. The findings are consistent with those described previously for animals.

Davis (1951) reported restlessness, irritability and insomnia, especially in the latter half of the night, as a result of under-nutrition in post-war Germany. If the food deprivation continued, leading to terminal starvation, then the typical features included immobility, apathy and somnolence (Hassin, 1924; Lipscomb, 1945; Leyton, 1946; Hottiger, Gsell, Vehinger, Salzmann and Labhardt, 1948).

For obvious reasons it was not possible to obtain more objective measures of sleep in the studies above. The only

study found to provide detailed sleep assessment following chronic food deprivation reports upon the recovery of obese patents to, or towards, normal weight (Crisp and Stonehill, 1970). It must be appreciated that this study involved food-limitation as opposed to total food deprivation.

Crisp and Stonehill (1970) reported a tentative relationship between sleep length, as assessed by a questionnaire twice weekly, and weight loss in obese patients over several months of dieting. A later study (Crisp, Stonehill, Fenton and Fenwick, 1973) recorded nocturnal motility and psychoneurotic status, as measured by the Middlesex Hospital Questionnaire (MHQ), in addition to sleep reports, in a group of five female, obese patients during their first four months of hospitalisation. Nocturnal motility was measured in only four of these patients for five consecutive nights at approximately monthly intervals. The motility scores were corrected for body weight and only the latter three nights were used in the analysis.

Interestingly, the authors found that the self-reported duration of sleep showed a month-by-month reduction (from 7 hours 20 minutes to 6 hours 15 minutes) as the patients' weight dropped over the four month period (from 110 kg to 87 kg). The average MHQ scores varied little during this period and, consequently, they were not considered to be closely related to the changes in weight or sleep. The patterns of motility and reported broken sleep were found to be very similar to one another. However, when the authors computed a correlational matrix on the data, no significant relationships were found between weight, sleep, motility and the MHQ scores.

One of the obese patients had her sleep polygraphically monitored on 2 consecutive nights at four intervals during her 8 months hospital treatment. Over this period her weight dropped from 109 kg. to 76 kg. The EEG data did not reveal any consistent changes in sleep length but it did indicate

a shift towards lighter slow-wave sleep (i.e. reduced stage 4 compensated by increased stage 2) as weight reduction continued.

This observation is not consistent with the reported increase in SWS following starvation. However, as stated earlier, 4 days total food deprivation and 8 months food limitation are two distinct conditions. The chronic dieting regime involved only relatively small changes in food intake compared to the acute studies of total food deprivation. Furthermore, chronic dieting allows for habituation, both psychological and physiological to take place.

It is of interest that the reduction in SWS reported by Crisp et. al. (1973) was only really apparent on the last EEG recording period which was 8 months after the commencement of the regime. Whilst the patient lost 8 kg and 22 kg in weight between the first 3 EEG recordings, only 2kg were lost in the 2 month period between the 3rd and 4th recordings. Thus, the reduction in SWS does not appear to be necessarily a consequence of weight loss, otherwise the major change in SWS would have been evident on the 3rd EEG recording.

## 3.2 Feeding

### 3.2.1 Short-term effects of diet upon sleep

#### 3.2.1.1 Non-isocaloric dietary changes

The great majority of studies reporting on the effects of certain foods upon sleep have administered the food, often as a beverage, immediately prior to bed-time.

Giddings (1934) examined the effects of cold water, cold milk, warm water, warm milk, a cold beverage containing 0.6 g caffeine and 20 g sucrose, and cold orange juice

sweetened with 20 g sucrose upon the "sleep patterns" of 12 children (9 - 14 years). These "sleep patterns" were assessed with a hypnograph which recorded the number of body movements made during the night when the children were in bed (nocturnal motility). The various beverages were each given for five consecutive nights and compared to the control "sleep pattern", which was based upon fifteen consecutive nights when the children presumably did not receive a bedtime beverage. The only beverage found to reduce nocturnal motility in a large proportion of children (42%) was the warm milk. Interestingly, both the cold milk and warm water did not elicit similar changes in motility and it was concluded that the warm milk effect was due to a combination of an easily digestible and assimilable food given at a temperature near that of the body. However, the reduction in motility was only small; the total number of minutes wherein activity occurred was approximately 75 without a beverage and approximately 65 after the warm milk.

Giddings also studied the effects of various types of evening meals upon night motility in 24 children. Three types of meal were studied, each being eaten two hours before retiring. The author concluded that the eating of a heavy meal (3 course meal with sweets to follow) by children produced marked restlessness, which often continued throughout the night, as compared to a normal meal (fruit, cereal or eggs, bread and butter, milk). The number of minutes during which activity occurred was approximately 95 following the heavy meal as compared to approximately 65 after the normal meal. The eating of a very light evening meal (1 slice of bread and butter, milk) was not found to affect nocturnal motility compared to motility levels observed following the normal evening meal.

Stanley and Teschner (1932) reported that a more restful sleep may be obtained by adult men if nothing is

eaten before retiring. The quality of sleep was assessed by recording the number of body movements made during the sleep period by seven men, and calculating the average number of movements made per hour. Compared to the motility levels following the normal diet with no pre-sleep supper, an average of 7.9 movements per hour, it was found that a protein ($\frac{1}{4}$lb eggs or meat; 9.0 movements per hour) fat (1 ounce butter on toast; 8.8 movements per hour) or carbohydrate ($\frac{1}{4}$lb cake; 8.6 movements per hour) supper all increased nocturnal motility. These various suppers were each given on ten consecutive evenings. However, the extent of these motility changes were not great and the largest average difference between the protein supper and no supper was only 1.1 movements per hour.

Laird and Drexel (1934) reported that the quality of sleep after a bedtime snack was dependent upon the digestibility of the food. These authors suspected that hunger pangs might be a variable which influenced sleep quality. They rationalised that the consumption of food prior to bedtime would delay the onset of hunger pangs during sleep and thereby improve sleep quality. However, they considered that "hard to digest foods" might induce gastric discomfort which would offset the possible improvement resulting from a reduction in hunger pangs due to the presence of food. Accordingly, two types of meals were studied; an easily digestible meal consisting mainly of cornflakes and a hard to digest meal rich in hemicellulose-containing foods. The effects of these pre-sleep meals upon nocturnal motility were observed in 8 adult men and 8 children. The first 30 minutes of the motility record were not assessed since the subjects were awake for an unknown length of time during this period.

The results showed that nocturnal motility, compared to that following the childrens' normal supper (presumably a moderately digestible meal) or the adults' abstinence of

a supper, was increased by the hard-to-digest supper and decreased by the easily digestible supper. These motility changes were greater for children than the adults; the range of average movement frequency from the easy-to-digest to hard-to-digest suppers were 68-91 and 75-85 for the children and adults respectively.

Thus, it appears from the studies reviewed that the consumption of easily digestible foods prior to retiring will improve sleep quality, as assessed by body motility, compared to the consumption of more difficult to digest foods or no food at all. Although Stanley and Teschner reported that sleep is more restful if nothing is eaten before retiring it is apparent that this study did not assess the influence of a light, easily digestible supper upon sleep. However, Hamilton et. al. (1966) reported that a light bedtime supper of cereal, sugar and milk did not have any effect upon nocturnal motility or subjective sleep quality. This study observed motility in 36 subjects during alternating periods when the subjects either ate a bedtime supper or had no supper at all.

One difficulty which arises in assessing these studies is deciding to what extent the effects may be due to suggestion. It is very unlikely that the subjects were not aware of the nature of the experiment and, unlike EEG recordings, it could be very easy for the subjects to manipulate their levels of nocturnal motility in the perceived desired direction. Thus, the results may be influenced not only by the effect of the diet but also by the expected effect, as perceived by the subjects, of the diet. Also, it would appear that the levels of motility reported in the studies reviewed so far do not differentiate between small and large body movements, but just reflect the number of novements (as in Stanley and Teschner (1932) and Laird and Drexel (1934)) or the number of "active" minutes wherein movement(s) took place

(as in Giddings (1934)). This gross measurement may well explain why Hamilton et. al. (1966) did not find any difference in nocturnal motility following a light supper.

More recently, several studies have reported upon the effects of a hot, bedtime milk-cereal drink (Horlicks) upon sleep quality.

Southwell, Evans and Hunt (1972) used time-lapse photography to monitor body movements every 15 seconds throughout the night. Data was recorded from four young subjects under 3 experimental conditions, including a control night (no bedtime beverage), a warm water drink and a warm Horlicks drink made with milk and 5 large teaspoons of Horlicks powder. The body movements were divided into 2 categories; namely large changes, in which the trunk was turned or translocated without turning, and small changes, involving the hands, feet, or head, or more than one of these. Although the number of large body movements did not differ in the 3 experimental conditions, there was a significant reduction in the number of small movements after the Horlicks drink in the latter half of the night. This finding showed that a detailed assessment of nocturnal motility is warranted in these studies. Unfortunately, this policy has not been adopted in other studies.

Brezinova and Oswald (1972) compared the effect of an inert capsule with a hot, bedtime milk-cereal drink (Horlicks) upon sleep. All-night EEG recordings were taken and body movements were estimated using the submental EMG record. The subjects were divided into 2 age groups with 10 subjects aged 20-30 years (mean 22 years) and 8 subjects aged 42-66 years (mean 55 years).

The results showed that restlessness during sleep was diminished after Horlicks in the young age group, especially in the last 3 hours of sleep. However, these

changes were small. The mean number of movements recorded in the last 3 hours of sleep being 38.3 after Horlicks and 42.9 after placebo. In the older group, sleep after Horlicks was of longer total duration (by approximately 10 minutes) and was less broken by periods of wakefulness; this again being most apparent in the second half of the night. During this period the mean intervening wakefulness was 15.5 minutes after placebo and only 3.6 minutes after Horlicks. This improvement in sleep quality was found to be increased with repeated administration.

Obviously these latter two studies were not conducted blind; both the experimenters and the subjects were fully aware when the Horlicks and the yellow capsule were presented. It is possible, therefore, that pre-conceived ideas concerning the benefits of a hot bedtime beverage may have had some effect upon subsequent sleep quality. However, the major changes in sleep quality were found to occur in the latter half of the sleep period by which time suggestion effects would, it can be argued, be of little influence. Brezinova and Oswald attempted to control for this possible source of experimental bias by intimating to their subjects that the inert capsule contained a folk remedy of doubtful efficacy.

The third study (Adam, Adamson and Oswald, 1976) compared subjective ratings of sleep quality and anxiety after a placebo, drug (nitrazepam, 5 mg) and Horlicks. This experiment specifically investigated the power of suggestion upon sleep quality by informing the subjects that the placebo pill would make their sleep more restful without causing any hangover. The study incorporated 6 "base-line" nights, on half of which, and in balanced order, each of the 10 subjects received a pink placebo pill. The effects of Horlicks and nitrazepam were studied over a 10 week administration period and during the first week of withdrawal.

Analysis of treatment effects (Horlicks and nitrazepam) were made for the early treatment period (first week), the late treatment period (after 6-7 weeks administration) and the early withdrawal period (first week). The subjective ratings showed no significant differences after the placebo whereas both the late drug and early food drink administration were found to improve subjective sleep quality compared to the baseline nights. These results do not support the notion that suggestion plays a major part in subjective estimations of sleep quality.

These Horlicks studies indicate that the consumption of food prior to sleeping will improve sleep quality, as assessed by either nocturnal motility, intervening wakefulness or subjective ratings. So far, only the study by Brezinova and Oswald (1972) has recorded EEG data after changes in the diet. Although no significant changes in REM sleep or SWS were found, this does not exclude the possibility that these changes would occur, as in animals, following larger changes in the diet.

The work of Parker and Rossman (1971) and Parker, Rossman and Vanderlaan (1972) provided data suggesting that major changes in diet do have a pronounced effect upon EEG sleep. Unfortunately, very little EEG data was provided in these studies as the authors were primarily interested in the sleep release of growth hormone after carbohydrate ingestion. Presumably, EEG sleep was monitored to ensure that the experimental procedures did not disturb sleep. The authors hypothesised that the release of growth hormone during sleep is a primary neural rhythm relatively independent of substate concentration and, therefore, would not be suppressed by hyperglycemia as it is during wakefulness.

The first study (Parker and Rossman 1971) monitored blood levels of growth hormone and glucose, in addition to recording EEG data, in 6 young subjects over 3 consecutive

nights. The first 2 nights were baseline nights and on the third night a 25 g bolus of 50% glucose was inserted into the forearm vein, immediately prior to bedtime. This was followed by a constant infusion of 20% buffered glucose at 6 mg/kg/minute throughout the first 2 sleep cycles. As the authors predicted, the sleep release of HGH was not suppressed on this infusion night. Unfortunately, the EEG data was not provided although sleep profiles were provided for one of the subjects over the 3 nights. From these it appeared that the infusion night displayed less SWS and stage 1 and more REM sleep compared to the baseline nights. These differences were found, yet again, mainly in the second half of the night.

It must be noted that no adaption nights were used and thus one would expect the first 2 base-line nights to be more disturbed than the third night. This, however, does not explain the reduced amount of SWS on the infusion night. Admittedly, the above changes in sleep are based only on one subject's data and these findings require confirmation from other studies.

The second study, (Parker et. al., 1972), extended the first and examined the effects of fasting upon the sleep release of HGH. Three subjects were studied over 2 consecutive baseline nights, on the second and third nights of an 80 hour fast and on the third and sixth nights of a 600 g carbohydrate 4000 calorie diet. Again the HGH release was not suppressed during the 6 days of high calorific intake when the plasma glucose levels were significantly increased throughout the sleep period. Fasting was found to enhance peak HGH concentration during sleep without greatly changing the typical pattern of release. These findings strengthened the authors belief that HGH release is under neural control. Unfortunately, detailed sleep data were omitted; only data for SWS being presented.

All 3 subjects showed an increase in SWS during the fasting period compared to both the baseline and feeding periods. In 2 subjects the re-feeding produced a decrease in SWS compared to baseline values. The average percentage SWS in the baseline, fasting and feeding periods were 14.4, 16.8 and 13.5 respectively. These averages were fairly low because one of the subjects exhibited very small amounts of SWS (less than 5%) over all 3 conditions. These observations suggest that carbohydrate intake, in the short-term, may be negatively associated with SWS.

Bell, Blair, Owens, Guilleminault and Dement (1976) studied the effects of various foods upon lunchtime sleepiness in 15 subjects. The subjects were all requested to eat a high carbohydrate breakfast before 8.30 a.m. and then to refrain from napping, eating and drinking (except water) until 12.25 p.m. when the test meal was provided. The subjects were asked to sit quietly until 2.00 p.m. Sleepiness was assessed by the Stanford Sleepiness Questionnaire both before and at 15 minute intervals after the test meals. The difference between the average of the post-meal sleepiness scores and the pre-meal scores was calculated for all of the test meals, each meal being studied on separate days. The results indicated that glucose, potato, warm milk, large composite (hamburgers and fried potatoes), corn oil ice cream, cheese and chocolate cookies induced greater feeling of sleepiness than the other foods studied (namely beef, wheat, breakfast square, fruit drink, turkey, cane sugar, cold milk, small composite, lettuce and sugarless gum) or fasting.

A subsequent study (Bell, Rosekind, Hargrave, Guilleminault and Dement, 1977) investigated this finding further by recording objective sleep variables during naps taken after various meals as well as assessing sleepiness. Twelve subjects received four isovolumic meals (2 fluid ounces

corn oil, 100 g dextrose, 5 g tryptophan and water-only control), each on three separate occasions, over a six week period. On one of these occasions the subjects were asked to rate their sleepiness using the Stanford Sleepiness Scale whilst on the other two occasions the subjects nap sleep was recorded during a 2 hour period of darkness. Each session lasted for 3 hours. Only tryptophan was found to produce a greater increase in sleepiness than the control meal. From the average of the two nap recordings following tryptophan it appeared that sleep latency was significantly decreased (by 7 minutes) and total sleep time significantly increased (by 20 minutes) compared to the control values (12 minutes and 68 minutes respectively). Dextrose and corn oil both showed a trend towards a reduced sleep latency but neither were significant. However, both these meals did significantly increase total sleep time (corn oil by 11 minutes and dextrose by 18 minutes) although the number of body movements were also increased (control - 6.4, corn oil - 9.7, dextrose - 9.8).

The authors concluded that the three foods did induce greater sleepiness taken at lunchtime compared to water only and that the relative influence of the foods ranked in descending order were tryptophan, dextrose and corn oil.

Both of these studies by Bell and his colleagues suggest that carbohydrates, especially the easily assimilated glucose, and fats have more of an effect upon sleepiness, both subjective and objective, than protein or fasting. The finding that tryptophan, an essential amino acid, induces sleepiness is in agreement with several studies which are discussed in Chapter 10 of this thesis.

### 3.2.1.2 Iso-caloric Dietary Changes

Recent evidence (Phillips, Chen, Crisp, Koval, McGuiness, Kalucy, Kalucy and Lacey, 1975) has shown that

isocaloric dietary changes, in which the amount of protein was held constant, can modify subsequent sleep. Eight young, male subjects of normal weight and stable dietary habits, underwent two courses of dietary manipulation, each lasting 4 days and separated by a 2 week interval. On the first 2 days of each 4 day period the subjects ate a normal, balanced diet (350 g carbohydrate, 140 g fat, 75 g protein), whilst on the remaining 2 days they ate a high carbohydrate/low fat iso-caloric diet (600 g carbohydrate, 33 g fat, 75 g protein) during 1 week and a low carbohydrate/high fat iso-caloric diet (100 g carbohydrate, 255 g fat, 75 g protein) during the other experimental week. EEG recordings were taken on all nights except the first of each 4 day period.

The results showed that the high carbohydrate/low fat diet significantly increased REM sleep by an average of approximately 33 and 15 minutes compared to values observed on the balanced diet and the low carbohydrate/high fat diet, respectively. The difference in REM sleep amounts was also significant between these latter 2 diets. The high carbohydrate/high fat diet significantly decreased SWS by approximately 18 minutes and 19 minutes compared to the values observed on the balanced diet and the low carbohydrate/high fat diet, respectively. The amounts of SWS were very similar between balanced and low carbohydrate/high fat diets. Stage 1 was significantly reduced by both experimental diets and sleep latency and sleep length were decreased and increased respectively, although not to a significant extent.

The authors were aware of the inherent weakness of the experimental design and suggested that the reduction in light sleep and sleep latency and the increase in REM sleep and total sleep by the experimental diets might have been due, in part, to the adaptation effects arising within each

half of the experiment. However, as the amount of REM sleep was significantly different between the 2 experimental diets, it seems very likely that REM sleep is sensitive to changes in the carbohydrate and fat content of a diet, and that these increases were not solely the result of adaptation to the sleep laboratory. It would appear that a high carbohydrate intake and, to a lesser extent, a high fat intake are both associated with an increase in REM sleep.

This latter finding may explain why REM sleep has been reported to be positively correlated with body weight (Adam, 1977a). It could be argued that overweight people typically enjoy a diet rich in carbohydrates and fat whereas underweight people are careful to avoid these fattening foods. Thus, these correlations between body weight and REM sleep may be due to the diet and not body weight per se. However, in the Adam study, REM sleep was not found to be significantly correlated with the percentage deviation from ideal body weight, as would be predicted from this rationale. (This study is reviewed in section 3.3.)

### 3.2.2 Long-term effects of diet upon sleep

In addition to studying the effects of sustained weight loss upon the sleep of obese patients, Crisp and his colleagues have examined the effects of weight gain during the treatment of patients suffering from anorexia nervosa. Anorexia nervosa is a state of starvation and emaciation associated with an avoidance of eating carbohydrate-rich foods. The anoretic displays many of the features of starvation (e.g. reduced metabolic rate, increased body hair etc.) but is characteristically energetic and alert.

In 1965, Crisp reported that patients with anorexia nervosa tended to have disturbed sleep during the latter half of the night. Subsequently, in a study of 60 patients,

Crisp (1967) found that their total sleep time was much lower than the general population norm. While these patients generally had little trouble in going to sleep, they suffered from early morning waking and restlessness which did not appear related to any affective disturbance. Crisp further noticed that the degree of sleep disturbance was proportional to the severity of the nutritional deficit and that sleep problems were generally more common in those patients who were highly aroused during the day. Crisp viewed these sleep disturbances as a regression to an infantile sleep rhythm brought about by the effects of starvation.

Crisp and his colleagues continued the investigation into the sleep disorders of anoretics by recording both subjective and objective sleep data during their treatment. Crisp and Stonehill (1971) studied a group of 10 young female patients before and after a period (8-11 weeks) of treatment involving the restoration of their body weight to the general population mean for their height and age. The treatment consisted of bed rest, refeeding on a 3000 caloric diet, psychotherapy and chlorpromazine. Chlopromazine was not administered during the 2 weeks before retesting.

The results showed that the weight gain (39.9 kg - 54.4 kg) was correlated with an increase of 1 hour in the average self-reported total sleep time (6½ hours - 7½ hours). Nocturnal motility was found to be approximately halved after treatment. This reduction was apparent throughout the night but greatest during the first 2 hours. Both these variables (sleep length and motility) were not found to be significantly correlated with either MHQ or EPI scores.

Crisp, Stonehill and Fenton (1971) recorded the EEG

sleep profiles of 5 patients (4 female, 1 male) suffering from anorexia nervosa for 4 consecutive nights, both before and after treatment. Only the latter 3 nights of the recording period were used for analysis, thereby allowing for the patients to adapt to the laboratory environment. However, none of the patients reached their target weights and 1 female gained no weight at all. This latter patient was therefore regarded as a control.

The results showed that refeeding was associated with a reduction in wakefulness and, therefore, an increase in total sleep time compared to the pre-treatment levels (6 hours 20 minutes - 7 hours), incorporating increases in both SWS and REM sleep. REM latency was also reduced in the latter recording period. The control patient's sleep data revealed no major changes, thereby strengthening the probability that weight change was a major factor in the other patients sleep improvement. Mood ratings carried out before and after treatment revealed no consistent changes.

This study was extended by Lacey, Crisp, Kalucy, Hartmann and Chen (1975), who recorded data from a larger sample of 10 patients, all of whom reached their target weights. The patients again received a diet of 3000 calories per day and it was envisaged that body weight increases of 2-3½ lb/week would take place. In addition to this refeeding, the patients were restricted to virtually complete bed rest. EEG recordings were taken before and after treatment. Each recording period lasted for 3 days but only the third night was used for analysis; the other 2 nights allowed the patients to adapt to the experimental situation.

As before, the increase in weight (37.5 - 52.0 Kg) was associated with a decrease in wakefulness and, concomitantly,

an increase in total sleep time compared to the pre-treatment values (from 6 hours 27 minutes to 7 hours 7 minutes). The data clearly showed that wakefulness recorded during the low body weight period was concentrated in the latter half of the night.

REM sleep was found to be increased by 17% after refeeding. However, as this stage of sleep tends to become increasingly predominate as the night proceeds, it is possible that this increase was due mainly to the increased sleep length. When this was corrected for there still remained a significant increase of 13.6% in REM sleep.

SWS showed a tendency to be increased after refeeding, but this was not significant as in the earlier study. Lacey and his colleagues suggested that this difference between the 2 studies might result from the fact that the patients of the Crisp et. al. (1971) study did not reach their target weights. Therefore, at the time of the second recording of sleep, these patients may well have been in a phase of "active refeeding with weight gain". The authors claimed that an increase in SWS would be expected during this period of increased anabolism and they suggested that once the target weight had been reached the amount of SWS may have declined to pre-treatment levels. The authors also noted that the patients in the Crips et. al. study gained weight faster than the patients in the Lacey et. al study. Again, it was suggested that the SWS increase observed by Crisp et. al. may have been as a consequence of this increased anabolism.

Foster and Kupfer (1976) and Foster, Kupfer, Spiker, Grau, Coble and McPartland (1976) have also reported that anoretics have low amounts of REM sleep, both before and during refeeding.

## 3.3 Weight Changes and Sleep

The studies by Crisp and his colleagues culminated in the formulation of a hypothesis concerning changes in nutrition and the associated changes in sleep. The hypothesis predicted that weight loss would be associated with reduced sleep and early waking whereas weight gain would be associated with increased sleep and later waking. It was also predicted that this relationship between nutrition and sleep would be independent of changes in mood.

To test this hypothesis, Crisp and Stonehill (1973) recorded data from 375 psychiatric out-patients with respect to their psychiatric and mood states, levels of nutrition and changes in weight and sleep during the illness. Information was recorded via questionnaires and the enquiry into weight and sleep characteristics centred mainly around the periods "just before the illness started" and "the last few weeks". Analysis of this enormous amount of data illuminated some very complicated, but significant, associations between nutrition, sleep, mood and psychiatric diagnosis.

The findings provided evidence consistent with the experimental hypothesis stated above. Furthermore, weight loss was found to be associated with increased interruptions of sleep whereas the converse was true for those patients who had gained weight. Weight changes bore no direct relationship to the time of getting off to sleep, which was found to be more closely related to mood states. It is of interest to find that early waking was also significantly associated with falling asleep early - a fact which as the authors note, is rarely taken into account in clinical practice.

Further evidence for the relationship between body weight and sleep has been recently provided by Adam (1977a,b) who recorded the EEG sleep of 16 volunteers

(6 males and 10 females aged 52-67 years) on 6 consecutive nights every 4 weeks during a 16-week period. Body weight was recorded at the beginning and end of each 6 day session. Significant positive correlations were found between the following variables:-

a) Body weight and REM sleep expressed as a percentage.

b) Log body weight and REM sleep in minutes.

c) Percentage deviation from the ideal body weight (as assessed by height) and the mean length of the first 3 NREM-REM cycles. The cycle length being the number of minutes between the beginning of one period of NREM sleep to the end of the following period of REM sleep. Cycles containing more than 1 minute of wakefulness were excluded from the analysis.

d) Percentage deviation from ideal body weight and the total duration of sleep.

e) Mean sleep cycle length and total duration of sleep.

These correlations between body weight and REM sleep are consistent with the findings of studies reviewed earlier. For example, correlation (a) is in agreement with the findings of increased REM sleep when anoretic patients gain weight, (Lacey et. al., 1975) and decreased REM sleep during starvation (MacFadyen et. al., 1973). Correlation (d) agrees with the report of shortened sleep in obese patients when they reduce weight (Crisp et. al., 1973) and the lengthened sleep in anoretic patients upon refeeding (Lacey et. al., 1975). The variable "log body weight" has

been shown to be highly correlated with the daily metabolic rate (Kleiber, 1961) and, therefore, correlation (b) suggests that REM sleep is closely linked to metabolism. (Zepelin and Rechtschaffen (1974) have reported, using data from animal studies, that total sleep time rather than REM sleep is the most highly correlated factor with metabolic rate). However, a significant correlation between body weight and REM sleep is difficult to interpret as other factors such as height are obviously involved. For example, it could be construed that tall people enjoy more REM sleep than short people.

It would have been more illuminating if REM sleep had been found to be correlated to the difference between actual body weight and the ideal body weight as assessed by height. However, this was not the case although the duration of sleep was found to be positively correlated to the percentage deviation from ideal weight. In other words, it appears that overweight people may have a tendency to sleep longer than underweight people. If it can be assumed that overweight people are examples of normal weight people on a chronically increased food intake regime whereas underweight people are examples of normal weight people on a chronically decreased food intake regime, then these sleep changes may have been mediated by the differences in food intake rather than body weight. It is possible that psychological factors may be involved as overweight people are often regarded as jolly and carefree compared to underweight people who may worry a lot. The sleep change might well be explained by these psychological variables alone. However, Crisp and Stonehill (1973) have reported that changes in sleep following body weight changes appear to be independent of changes in mood. Whilst mood may not be a consistent factor in the sleep changes, it may be an important factor for some individuals.

## 3.5 Discussion

Acute starvation has been found to increase SWS in humans, whereas REM sleep appears to be decreased. These changes in REM sleep do not seem to be a consequence of the increased amounts of SWS, as REM sleep was found to be decreased on the first night of fasting when SWS was still within baseline values. However, the changes in REM sleep may have been related to the decreases in total sleep time. Chronic food limitation is also associated by a reduction in sleep length but SWS and REM sleep do not appear to be greatly affected. Chronic starvation is typically associated with restlessness and disrupted sleep although somnolence is a feature of terminal starvation.

Evidence has been provided that indicates that sleep quality, as assessed by either nocturnal motility, intervening wakefulness or subjective ratings, is improved by the consumption of an easy-to-digest meal prior to sleep. A heavy or hard-to-digest meal, on the other hand has been found to increase nocturnal motility which is considered to be detrimental to sleep quality.

The effects of food intake upon EEG sleep in humans are summarised in Table 1. In an attempt to determine which components of a diet are responsible for the observed changes in sleep, the various studies were initially ordered according to whether they improved or disrupted sleep. A good index of the quality of sleep is obviously the amount of wakefulness or the length of sleep, and these variables were used to divide the studies into 2 groups. The studies were then further ordered, within groups, according to the extent of the changes in sleep. Thus a high carbohydrate diet was assessed as inducing a large sleep change as both REM sleep, SWS and Stage 1 sleep were affected. This does not imply that sleep was more improved following a high carbohydrate diet than, for example, after a bedtime beverage; it only implies that sleep was more

Table 1. Effects of Food Intake upon Human Sleep

| Dietary Factor | Calorie Intake | Sleep Variables | | |
|---|---|---|---|---|
| | | REM | SWS | Wakefulness &/or Stage 1 |
| High Carbohydrate Diet (Phillips et. al., 1975. Similar indications from Parker & Rossman 1971 and Parker et. al. 1972) | Unchanged | ↑ (32%) | (15%) ↓ | ↓ |
| High Fat Diet (Phillips et. al., 1975) | Unchanged | ↑ (18%) | | ↓ |
| Refeeding of anoretics (Lacey et. al., 1975) | Increased | ↑ (13%) | | ↓ |
| Body Weight (High) (Adam, 1977) | Increased | ↑ | | |
| Bedtime Horlicks (Brezinova & Oswald, 1972) | Increased | | | Old People ↓ |
| Weight Gain (Crisp & Stonehill, 1973) | Increased | | | |
| Overweight (Adam, 1977) | Increased | | | Increased Sleep Length ↓ |
| Underweight (Adam, 1977) | Decreased | | | Decreased Sleep Length ↑ |
| Weight Loss (Crisp & Stonehill, 1973) | Decreased | | | |
| Body Weight (Low) (Adam, 1977) | Decreased | ↓ | | |
| Acute Starvation (MacFadyen et. al., 1973; Karacan et. al., 1973) | Decreased | ↓ | ↑ | (Sleep Length Reduced) ↑ |

influenced following the high carbohydrate diet.

Whilst the rankings of the high carbohydrate diet, high fat diet, refeeding of anoretics and starvation can be easily assessed by the direction and extent of the sleep changes, it is not possible to meaningfully rank the other studies because many of the findings were reported as correlations. Therefore, sleep following a bedtime beverage, weight gain or associated with being overweight should be regarded as being improved mainly due to a decrease in wakefulness, with no major sleep stage changes. Conversely, sleep after weight loss or in underweight people appears to be worsened mainly due to increases in wakefulness. Now, by examining Table 1, it may be possible to find some characteristics of the diets that vary with the direction and extent of the changes in sleep. One possible characteristic may be the amount of food consumed or the calorie intake. Thus, weight gain, being overweight, refeeding of anoretics and the consumption of a bedtime beverage all represent an increased food and calorie intake and all have been reported to be associated with improved sleep. Similarly, weight loss, being underweight and starvation all represent a reduced food and calorie intake and all have been found to be associated with disturbed sleep. Therefore, the amount of food eaten might be one factor in the diet and sleep relationship.

However, it is of great importance that sleep has been reported to be greatly influenced by isocaloric dietary changes. This strongly implies that other factors, apart from food or calorie intake per se, are involved in the diet and sleep relationship. As these isocaloric dietary changes involved major changes in the amount of carbohydrate or fat consumed (the protein content was held constant) it would be of interest to assess the changes in these constituents in the other studies. For example,

the refeeding of anoretics would be associated with increases in both of these constituents as these "fattening" foods are typically avoided, or voided by vomiting or purgatives, in untreated anoretics. One could speculate that the diet of overweight people usually contains more carbohydrate and fat compared to underweight people or people losing weight and it is clear that the consumption of these foods is terminated during starvation.

Thus, with reference to Table 1, it superficially appears that the direction and extent of the changes in sleep may be related to the changes in the amount of carbohydrate and fat consumed. If this is correct then it would appear that REM sleep is more sensitive to changes in carbohydrate and fat intake than SWS; SWS being affected only following major changes in their intakes. Furthermore, it is probable that these changes in sleep are more sensitive to carbohydrate intake than fat intake as a high carbohydrate diet is associated with large changes in both REM sleep and SWS whereas an isocaloric high fat diet only moderately increases REM sleep.

The changes in wakefulness do not seem to be proportional to the changes in carbohydrate and fat content as the amount of wakefulness or the total sleep time were not significantly changed following the isocaloric dietary changes, although Stage 1 sleep was greatly reduced by the increased intake of carbohydrate or fat. The changes observed in wakefulness or sleep length may be, therefore, more closely related to food intake or calorie intake than food content.

From the starvation studies it is clear that changes in food intake can have major effects upon sleep although it must be remembered that these changes were abrupt and dramatic in extent. The changes in REM sleep may have been

the result of the stress of the experiment, whilst the increase in SWS was possibly mediated by the increased metabolic demand incurred during starvation.

Psychological factors may also be involved in the diet and sleep relationship. For example, people losing weight, being underweight or starved may all be associated with worry and distress compared to those gaining weight, enjoying warm milky bedtime drinks or receiving medical treatment (i.e. refeeding of anoretics). However, Crisp and his colleagues have not found changes of mood to be consistently linked to the sleep changes resulting from weight changes and the short-term consumption of various isocaloric diets would not be expected to produce any significant changes in psychological variables.

Although the mechanisms by which the diet influences sleep are not yet known, it is clear that sleep is sensitive to the general nutritional status of the individual and, furthermore, to short term changes in the content of the diet. The available evidence argues that sleep is directly influenced by the diet, rather than mediated indirectly by feelings of hunger or fullness only. If sleep is directly influenced by the diet then it might be expected that the changes in sleep are effected by changes in substate availability for the production of energy or some other substance(s) necessary for the maintenance of body function. Thus, it may be possible that sleepiness occurs following a good meal because this is possibly one of the most efficient times for sleep to occur. For example, food gathering and food consumption are two of the prime objectives of the waking state and, once completed, the sleep state would allow for energy conservation and efficient digestion, both via immobility. Conversely, if food was scarce, then it might be biologically viable to prolong the period of wakefulness until sufficient food has been obtained. In the laboratory situation,

this increased wakefulness would be seen as general restlessness and not organised food gathering activity as this is not possible.

## 4. CONCLUSIONS

It is apparent from both the animal and human studies that decreased food intake is associated with disrupted and shortened total sleep whereas increased food intake or feeding appears to enhance sleep. However, it is possible that a hard-to-digest meal, taken prior to retiring, might not enhance sleep as it is known to increase nocturnal motility in humans. Thus, there is good evidence in favour of a relationship between the diet and the sleep behaviour of an animal or human.

The mechanisms by which the diet affects sleep are not yet clear. The digestibility of the food may be an important factor when a meal is eaten prior to sleep, but if sufficient time has been allowed for the digestion of the meal then the digestibility of the food would not be of great consequence.

The changes in sleep reported following changes in the amount of food eaten may be mediated by the changes in the total calorie intake. It is suggested that this may be a factor which influences the duration of sleep or the amount of intervening wakefulness. Obviously, if the calorie intake is reduced drastically, then the induced sleep disturbance will prevent the normal quota of SWS and REM sleep to be displayed. However, isocaloric dietary changes have been found to have a major effect upon sleep in the human. It is possible, therefore, that sleep is affected by changes in the protein, carbohydrate and/or fat content of the diet.

## 5. PROPOSALS FOR RESEARCH

Whilst there is strong evidence that sleep is influenced by the diet and nutritional status, our understanding of the underlying factors is sorely lacking. A more detailed knowledge of these factors may have great value in clinical applications such as insomnia and depression. For example, the sleep disorders commonly associated with mental illness may be due, in part, to an impaired action of the digestive process resulting in abnormal concentrations of some plasma or tissue constituents. Furthermore, the investigation of the influence of the diet upon sleep will provide information pertaining to natural or "physiological" changes in sleep as opposed to the non-physiological changes resulting from the use of drugs. Thus, the subsequent chapters will be devoted to investigating the factors underlying the diet-mediated changes in sleep.

When this review was initially undertaken, the study by Phillips et. al. (1975) was not published and there was only the incidental data from Parker and Rossman (1971) and Parker et. al. (1972) to suggest that the administration of glucose or a high carbohydrate diet could affect sleep. For this reason, it was decided that a study should be carried out specifically to assess the influence of a high carbohydrate diet upon sleep. If this proposed study showed that a high carbohydrate diet affected sleep then it was envisaged that a subsequent study would be necessary to investigate whether the effects upon sleep where a function of the calorie intake or the carbohydrate nature of the diet. This would be carried out using isocaloric diets but, as Phillips et. al. (1975) carried out a suitable study, the subsequent line of investigation was tailored to include their findings.

CHAPTER 8

STUDY III

## 1. AIM OF THE STUDY

The aim of this study was to assess the influence of a high carbohydrate diet upon human sleep.

## 2. EXPERIMENTAL RATIONALE AND DESIGN

When assessing the effect of a compound upon sleep, it is good practice to administer the substance in various amounts or concentrations. The advantage of this method lies in the greater strength of the findings if a significant dose-response relationship is elicited. Consequently, it was decided that this study should employ several levels of carbohydrate intake varying from zero, through low, to high. In order to assess the influence of an increased carbohydrate intake upon sleep it was necessary to keep the amounts of protein and fat in the diet constant, otherwise it is possible that any observed changes in sleep were mediated by changes in these other dietary constituents. It was decided that the simplest method to achieve this was to provide a carbohydrate supplement to the subjects' normal diets.

### 2.1 Control of basic diet and other variables

#### 2.1.1 Basic diet

Obviously, if various carbohydrate supplements are to be added to a subjects' normal diet then the normal diet must be fairly stable so that any effects found upon sleep can be ascribed to the supplement only and not also to the day-to-day variations in the basic diet.

These variations were minimised by two means:-

a) The selection of subjects who enjoyed a good appetite and had a stable dietary intake and body weight. This was assessed by both an informal interview with the prospective subjects and by examination of diet logs which detailed the content of their daily diet over a period of

seven days.

It was decided that only those prospective subjects whose body weight was within ± 1 stone of their ideal body weight, as assessed by height (Metropolitan Life Insurance Company Statistical Bulletin, 1959), would be selected for this study. It is possible that obese people are habituated to the effects of a high carbohydrate intake and thus their sleep may not be as sensitive to increases in carbohydrate intake as people of normal weight. Underweight people who actively regulate their diet to remain thin might be psychologically stressed during the administration of a high carbohydrate diet and this would be difficult to control for.

Furthermore, prospective subjects who typically consumed a large bedtime supper were not selected for this study as it was envisaged that the combined consumption of the normal supper plus the high carbohydrate supplementary diet might be rather distressing to the subject. If these subjects were asked not to partake of their habitual supper, then the zero carbohydrate supplementary diet would be, in effect, equivalent to food deprivation.

b) The subjects selected for the study were asked to keep a diet log during the first part of the experiment, during which one of the three supplementary diets were administered. The subjects were then instructed to repeat those meals, day for day, when the other two supplementary diets were administered. This regime ensured that the subjects ate an identical basic diet with each of the three supplementary diets. Thus, the only dietary change would be as a consequence of eating the supplementary diets.

Subjects were not allowed to drink alcohol during the study and were requested to refrain from drinking tea or coffee, after 6 p.m. This was to minimise the influence of components of the diet known to influence the central

nervous system, such as alcohol and caffeine.

### 2.1.2 Activity

It was envisaged that the activity of the subjects, both physical and mental, would be fairly consistent during the weekdays Monday to Friday. However, the weekend is often associated with mental relaxation and/or increased physical exercise so it was decided to use only the weekdays for experimental purposes. The subjects were asked to maintain consistent levels of physical and mental activity during this period, although they were free to do as they chose over the weekend.

## 2.2 Administration of supplementary diets

### 2.2.1 Duration of administration

In order that activity levels could be controlled for, it was decided to study each supplementary diet for a period from Monday to Friday, inclusive. No recordings were made on Monday nights so that the effects of strenuous exercise, alcohol, late-nights and so forth taken during the weekend would not influence the EEG data. It was decided that it would be of interest to record EEG data following the withdrawal of the supplementary diets and this left three nights for the study of the supplementary diets. This was considered to be adequate as the work of Parker and Rossman (1971) suggested that sleep was affected during the first night of glucose administration.

### 2.2.2 Time of administration

The majority of studies have used the evening supper, taken prior to retiring, to investigate the effects of the diet upon sleep as assessed by the EEG or body motility. So that the results of this present study should be comparable with the previous studies it was decided to administer the supplementary diets before the subjects retired to bed. More specially they were given 45 minutes before

actual bedtime so that they had ample time to eat the meal and get prepared for bed.

## 2.3 Content of the diet

In designing the supplementary diets, some problems were encountered in deciding:-

a) how much carbohydrate to administer in each of the three supplementary diets,

b) in what form should the carbohydrate be given

c) how to control for bulk so that all three supplementary diets gave similar feelings of satiety.

### 2.3.1 Carbohydrate content

The answer to this first problem was based upon the work of Parker and Rossman (1971 - reviewed in Section 3.2.1.1, Chapter 7); their study being the only one wherein sleep has been monitored after a high carbohydrate intake immediately prior to, and during, early sleep in humans. The authors' interests were primarily orientated towards the effects of acute hyperglycemia upon the sleep release of growth hormone and the EEG recordings were taken, presumably, to ensure that sleep was not disturbed by the experimental procedures. Although no changes were reported in the subjects' sleep after the high carbohydrate night, it is likely that no statistical tests were applied. The sleep profile provided for one subject did, however, suggest that sleep was affected by the high carbohydrate intake. On the high carbohydrate intake night the subjects received a 25 g bolus of 50% glucose into the forearm vein, 2 to 3 minutes before retiring to bed, whereupon 20% buffered glucose was infused at 6 mg/kg/min. during the first two sleep cycles.

Therefore, the approximate average glucose intake

(assuming an average body weight of 64 kg and an average length of the first two sleep cycles of 200 minutes) was 90 g. Data from the same subject whose sleep profiles were presented showed that the plasma glucose levels were greatly increased at the beginning of the sleep period and approached 300 mg/100 ml. After about 30 minutes the level rapidly declined although it was maintained at a high level (approximately 175 mg/100 ml) over the first two sleep periods. Thus, the carbohydrate content of the high carbohydrate supplementary diet of this present study should be over 90 g and preferably be capable of augmenting the plasma glucose levels throughout the first half of the night.

### 2.3.2 Choice of foods and the control of protein, fat and bulk.

It was decided not to use the glucose infusion technique to vary the carbohydrate intake because:-

a) the facilities were not readily available and

b) the study was concerned with the effect of diet, as encountered in everyday life, upon sleep.

Whilst the glucose infusion technique would provide easily quantifiable data on the effect of various levels of carbohydrate upon sleep, the results could not be directly related to the effects of dietary changes. Therefore, the carbohydrate intake was varied by administering different diets. The supplementary diets chosen were specifically designed to provide three levels of carbohydrate intake but with comparable amounts of protein and fat and of a similar bulk, so that any observed sleep changes could be ascribed to change in the carbohydrate content only. The high carbohydrate diet was designed to meet the criteria described above (i.e. greater than 90 g carbohydrate intake which elevates plasma glucose levels for half the night) and then the other two diets were adjusted to control for protein, fat and bulk values.

It was apparent that a glucose meal alone would not serve to maintain high plasma glucose levels during the first half of the night. The glucose would cause a dramatic, but not sustained, increase in plasma glucose levels. In order to provide a sustained supply of glucose to the blood it was necessary that the high carbohydrate supplementary diet also contained a food which required several hours to be completely digested and broken down to glucose. A good candidate for this was the potato. Furthermore, as it is well known that the presence of fat in the stomach decreases the emptying of the stomach, it was decided that the potatoes should be deep-fried in oil so that the release of glucose from this food should be further delayed. Thus, the high carbohydrate supplementary diet was comprised of glucose and fried potatoes.

The next problem was to decide how much of these foods to administer. It was important that the bulk of the food should not be too great as this might invoke gastric discomfort which would be likely to influence sleep. Therefore, the amount of fried potatoes was limited to a quantity that was considered to be enjoyable and easily consumable prior to retiring. The amount was ascertained with the help of several volunteers who were given a large portion of fried potatoes late in the evening and asked to eat only as much as was comfortable. From this exercise it was decided that a 7 oz. portion of fried potatoes was easily consumed at this time of day. From the relevant table of food composition it was determined that this amount would contain approximately 74 g of available carbohydrate. The amount of glucose administered within the high carbohydrate supplementary diet was, therefore, to be over 20 g and to be sufficient to raise the plasma glucose levels quickly and to a fairly considerable extent. It was decided to use 2 oz. of glucose, dissolved in half a pint of water for ease of consumption, as this is approximately the amount used in the standard glucose tolerance test which is well known to increase plasma glucose levels to well over 200 mg/100 ml in normal, non-diabetic humans. Therefore, the

high carbohydrate supplementary diet was composed of 7 oz. of fried potatoes and 2 oz. of glucose. Having ascertained this, the other two supplementary diets were designed to provide comparable amounts of protein and fat and to be of a similar bulk, as roughly assessed by their dry weight, and yet to contain either comparatively low or zero amounts of carbohydrate.

The low carbohydrate supplementary diet was designed to provide approximately half the carbohydrate intake of the high carbohydrate supplementary diet so that the effect of a carbohydrate intake comparable to a usual bedtime supper could be investigated. The content of this diet was decided upon by a trial and error method. The protein, fat, dry weight and available carbohydrate values of the high carbohydrate supplementary diet were ascertained and the content of the low calorie supplementary diet was varied until it contained a suitable amount of carbohydrate and comparable levels of the other variables. The calculations are summarised in Table 1. This diet was composed of 5 slices of Ryvita (a slimming biscuit), 2 oz. each of cucumber and tomatoes and 5/6ths oz. of butter. Half a pint of water was also given as Ryvita is a dry biscuit.

The design of the zero carbohydrate supplementary diet posed problems. This diet had to be of a similar bulk to the other two diets and contain similar amounts of protein and fat but contain no carbohydrate. No common food appeared to fulfill these criteria so it was decided to use capsules of "Tri-Hex-Tin", a slimming aid consisting primarily of methylcellulose. Because this compound is undigestible in the human, great care had to be taken when deciding how many capsules to administer to the subjects.

Although the dry weight of a meal might be a satisfactory index of "fullness" immediately following the ingestion of a meal, its relevance thereafter depends to a great extent upon the digestability of the meal. Whereas

Table 1. Contents of the Supplementary Diets

| Diet | Foods | Dry Weight | Protein | Fat | Available Carbohydrate | Energy Yield from Carbohydrate (4 kcals/g) |
|---|---|---|---|---|---|---|
| HIGH CHO | 2 oz Glucose | 56g* | - | - | 56 g | 224 kcals |
| | 7 oz fried potatoes | 103.9 g | 7.7 g | 18.2 g | 74.2 g | 296.8 kcals |
| | TOTALS | 103.9 g | 7.7 g | 18.2 g | 130.2 g | 520.8 kcals |
| LOW CHO | 5 slices Ryvita (2 oz) | 51.8 g | 5.2 g | 1.2 g | 43.8 g | 175.2 kcals |
| | 2 oz cucumber | 2.0 g | 0.4 g | - | 1 g | 4 kcals |
| | 2 oz tomatoes | 3.7 g | 0.6 g | - | 1.6 g | 6.4 kcals |
| | 5/6 oz butter | 20.1 g | 0.08 g | 19.9 g | 46.4 g | |
| | TOTALS | 77.6 g | 6.3 g | 21.1 g | 0 g | 185.6 kcals |
| ZERO CHO | 6 capsules of Tri-Hex-Tin | - | - | - | | 0 kcals |
| | TOTALS | - | - | - | 0 g | 0 kcals |

*Not included in the dry weight estimate of the high carbohydrate as it is administered in solution 15 minutes before the meal and is quickly absorbed in the stomach.

These values were estimated from tables in "The Composition of Foods" R.A. McCance and E.H. Widdowson, London: HMSO 1960

the digestability of the high and low carbohydrate supplementary diets are fairly comparable, it is evident that a similar dry weight of methylcellulose capsules would be rather distressing to the digestive system. Therefore it was decided that subjective estimates of "fullness" taken at least 30 minutes following the consumption of the various supplementary diets would be a better method to ascertain how many capsules to administer for the zero carbohydrate supplementary diet.

To this end, two volunteers consumed the high and low carbohydrate supplementary diets on separate nights, approximately 45 minutes before retiring to bed. Upon retiring, they were asked to rate their feelings of "fullness" on a 5-point scale varying from "very hungry", "hungry", "not concerned", "full" to "very full". The volunteers then consumed an increasing number of cellulose capsules and half a pint of water on separate nights (four on the first night, increasing by a further 2 on subsequent nights up to 8 capsules), approximately 45 mins. before retiring. Again they rated their feelings of "fullness".

Both volunteers agreed that these supplementary diets made them feel "full" upon retiring although they were capable of eating more. With respect to the cellulose capsules it was found that one volunteer rated the 8 capsules dose as giving rise to feelings of being "very full" upon retiring whereas doses of 6 or 4 gave rise to feelings of being "full". The other volunteer reported feeling "full" after 4, 6 or 8 capsules.

It was therefore decided that 6 capsules would be a suitable dose to control for bulk. It would have been possible to have also controlled for the protein and fat content by adding some boiled meat and fatty cheese to the capsules. However, as the low and high carbohydrate supplementary diets contained similar amounts of these constituents, it was of interest to compare the effects of these two supplementary diets upon sleep in comparison with sleep

following the inert cellulose meal. Any progressive change in sleep as the carbohydrate content increased could not be ascribed to the changes in fat or protein content as these were similar in two of the supplementary diets.

Having completed the design of the three supplementary diets, it was decided that an assessment of the actual changes in plasma glucose levels throughout the night following these supplementary diets would be of great value. This would check that the high carbohydrate supplementary diet did fulfill its task of maintaining highly elevated plasma glucose levels well into the sleep period. Also, it would provide evidence concerning the effect of the low and zero carbohydrate supplementary diets upon plasma glucose levels. Obviously, the zero carbohydrate supplementary diet would not be expected to elevate the plasma glucose levels, but the knowledge of these levels was required to assess the extent of the changes following the other two supplementary diets.

The influence of the supplementary diets upon plasma glucose levels was investigated in a small pilot-study which is described below.

## 3. PILOT STUDY

### 3.1 Aim

To assess the effects of various supplementary diets upon plasma glucose levels.

### 3.2 Method

The supplementary diets were eaten by two young subjects (one male, one female) between 11.15 and 11.30 p.m.: lights out was at 12.00 p.m. The plasma glucose levels were assessed at 12.00, 2.00, 4.00 and 8.00 a.m. by the Glu-cinet method which involved taking a small volume of

capillary blood from the fingertip. As this procedure required awakening the subjects it was decided that only one recording would be taken during sleep per night. Thus, two nights were required for each diet and blood samples were taken whilst the subjects were in bed at 12.00 and 4.00 a.m. during the first night and at 2.00 and 8.00 a.m. during the second night. Both subjects received each supplementary diet on two consecutive evenings with a period of 2-3 days between each diet. The diets were tested in the order: high carbohydrate, low carbohydrate, zero carbohydrate.

## 3.3 Results and Discussion

The results are summarised below (see Tables 2 and 3).

Table 2. Plasma glucose levels at various times during the night following the consumption of various supplementary diets prior to retiring.

| Diet | Plasma Glucose Levels (mg/100 ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 12.00 p.m. | | 2.00 a.m. | | 4.00 a.m. | | 8.00 a.m. | |
| | $S^1$ | $S^2$ | $S^1$ | $S^2$ | $S^1$ | $S^2$ | $S^1$ | $S^2$ |
| High CHO | 260.4 | 220.0 | 153.9 | 164.1 | 115.1 | 98.7 | 85.2 | 95.8 |
| Low CHO | 95.7 | 101.4 | 103.4 | 115.6 | 91.9 | 95.2 | 83.7 | 88.6 |
| Zero CHO | 88.6 | 98.1 | 89.0 | 90.5 | 84.2 | 93.2 | 80.5 | 89.1 |

Table 3. Mean plasma glucose levels during the night following the consumption of various supplementary diets prior to retiring.

| Diet | Mean Plasma Glucose Levels (mg/100 ml) | |
|---|---|---|
| | $S^1$ | $S^2$ |
| High CHO | 153.6 | 144.6 |
| Low CHO | 93.7 | 100.1 |
| Zero CHO | 85.6 | 92.7 |

As expected, the plasma glucose levels showed little change during the night when the zero carbohydrate supplementary diet has been consumed. However, it is apparent that the high carbohydrate supplementary diet greatly increased the mean plasma glucose levels during the night compared to both the other two supplementary diets. This increase was greatest at the beginning of the night, reaching an average maximum of 240 mg/100 ml, at 12.00 p.m. as compared to 93 mg/100 ml for the zero carbohydrate supplementary diet. Although this high level dropped as the night proceeded, the levels were still elevated at 4.00 a.m., but by 8.00 a.m. they were comparable with those levels following the consumption of the other two diets. The low carbohydrate diet produced a moderate rise in plasma glucose levels, with a average maximum of 109 mg/100 ml at 2.00 a.m. although by 4.00 a.m. the levels were comparable to those following the zero carbohydrate supplementary diet.

## 3.4 Conclusions

This pilot-study has shown that the high carbohydrate supplementary diet has fulfilled one of its basic design criteria, which was to produce a large and extended elevation of plasma glucose levels throughout the first half

of the night. The low carbohydrate supplementary diet was found to elevate plasma glucose levels only moderately; the effect lasting for only about 3 hours or so. The zero carbohydrate supplementary diet was not found to have any major influence upon plasma glucose levels.

4. SELECTION OF SUBJECTS

The experimenter advertised for subjects within the student community on the campus. Prospective subjects were informed that the study was designed to investigate the effects of various bedtime suppers upon the quality of sleep. They were told the exact nature of these suppers, but not what the expected changes would be upon sleep.

All prospective subjects were requested to fill in a screening questionnaire. Only those subjects who were young (17-25 years), healthy, free from medication and sleep problems and did not report daytime napping were considered for the next selection process. This involved the subjects completing a diet log, detailing their food intake for one week, and having their body weight measured. Those subjects who were greater than ± 1 stone from their ideal body weight, as assessed by height (Metropolitan Life Insurance Company Statistical Bulletin, 1959), were not eligible for this study. Furthermore, those subjects who typically consumed a large supper before retiring, or did not have a stable diet, were also not eligible. The rationale behind these decisions has been explained earlier (see section 2.1.1).

5. EXPERIMENTAL PROCEDURE

The experimental programme lasted for three consecutive weeks, with one supplementary diet administered per week. Each supplementary diet was given to six male subjects (18-21 years) for three consecutive days from Tuesday to Thursday. EEG recordings were made on all nights except the weekends, when the subjects did not come to the

laboratory, and Monday nights which were used for adaption purposes. On the first week an extra night's adaption was given. All six subjects received the supplementary diets in a different order according to the balanced design described in Table 4.

Table 4. Order of administration of supplementary diets.

| Subject | Week 1 | Week 2 | Week 3 |
|---|---|---|---|
| 1 | High | Low | Zero |
| 2 | High | Zero | Low |
| 3 | Low | High | Zero |
| 4 | Low | Zero | High |
| 5 | Zero | High | Low |
| 6 | Zero | Low | High |

The timetable for one of the subjects (subject 1) is tabulated below (Table 5).

Table 5. Timetable for subject 1

| Week | Sunday | Monday | Tuesday/ Wednesday/ Thursday | Friday | Saturday |
|---|---|---|---|---|---|
| 1 | Adapt-ation | Adapt-ation | High carbo-hydrate sup-plementary diet | Post-diet | Free |
| 2 | Free | Adapt-ation | Low carbo-hydrate sup-plementary diet | Post-diet | Free |
| 3 | Free | Adapt-ation | Zero carbo-hydrate sup-plementary diet | Post-diet | Free |

The subjects were recorded in pairs. They arrived at the sleep laboratory at 11.00 p.m. when the electrodes

were attached. The montage recommended by Rechtschaffen and Kales (1968) was used, namely: C3-A2; C4-A1; left eye-A1; right eye-A1; EMG electrodes were located on either side of the chin.

The diets were eaten between 11.20 and 11.30 p.m. Lights-out was at 12.00 p.m. and all night recordings were made with a Grass model 78, 12-channel electroencephalogram at a paper speed of 10 mm/sec . Immediately before lights-out the subjects' heart rates were recorded to ensure that the high carbohydrate diet did not have a general arousing influence on the body. The subjects were allowed to sleep until they felt refreshed and ready to get up.

## 6. DATA ANALYSIS

The sleep records were divided into one minute epochs and classified into sleep stages according to the criteria described by Rechtschaffen and Kales (1968) by two experienced scorers. Only the first 7½ hours of sleep, as measured from the onset of stage 2, were used for analysis; this being the shortest sleep length for the group of subjects over the experimental period. For each subject, the number of minutes spent in a specific stage of sleep were calculated for each of the diet and post-diet nights during:-

a) the whole night (i.e. 450 minutes)

b) the first half (i.e. first 225 minutes) of the night

c) the second half (i.e. last 225 minutes) of the night.

In addition, various other parameters (i.e. REM latency, REM periodicity) were calculated.

### 6.1 Analysis of supplementary diet periods

Statistical analysis was performed using Analysis of Variance techniques. A suitable computer programme

(Genstat V Mark 3.07, Lawes Agricultural Trust - Rothamsted Experimental Station) was used because of the complex and time consuming method of analysis. This programme generated F values for the main effects of diet and night as well as the diet x night interaction effect. As the main effect of subjects and those interaction effects involving subjects were not considered relevant to this analysis, the subjects were used as a blocking variable.

When the analysis of variance indicated that a significant effect existed (i.e. $p<0.05$) then the Studentized Range Test (see Snedecor and Cochran, 1967) was used to determine which diets or nights were significantly different to one another. The upper 5% levels of Q were used to calculate the minimum differences between the means (three for the main effects and nine for the interaction effect) ensuring that the probability of any erroneous claim of signifiance is $\leqslant 0.05$.

The Genstat programme was also used to generate standard errors of differences between diet means.

### 6.2 Analysis of Post-Diet Nights

The post-diet nights were analysed with respect to one another using the related t test method. In addition, each post-diet night was compared to the averaged data for the period when that diet was consumed. This latter analysis allows for the assessment of carry-over effects of the various diets.

## 7. RESULTS

The results of the statistical analysis are summarised in Tables 6 and 7 and Figures 1 - 3. As the diet and night interaction effect was not significant for any of the sleep variables the group means for the individual nights of each diet are included in Appendix III. The significant findings are described below:-

Table 6. Group means for the various supplementary diet periods

| Means for Dietary Periods | ZERO | LOW | HIGH | S.E. of differences between diet means | Significance |
|---|---|---|---|---|---|
| Whole night data (mins) | | | | | |
| Stage 3+4 | 89.6a | 104.3 | 92.1 | 4.46 | Diet $F_{2,40}=6.68$, $p<0.005$ |
| 4 | 46.5 | 53.6b | 40.8 | 3.90 | Diet $F_{2,40}=5.55$, $p<0.025$ |
| 3 | 43.1 | 50.7 | 51.3 | 3.73 | |
| REM | 101.8 | 103.9 | 113.1 | 4.92 | Diet $F_{2,40}=3.04$, $0.1>p>0.05$ |
| 2 | 221.6 | 211.1 | 220.4 | 5.50 | |
| 0+1 | 37.0 | 30.7 | 24.4c | 4.50 | Diet $F_{2,40}=4.01$, $p<0.05$ |
| S.4 latency | 20.2 | 14.8 | 19.0 | 3.92 | |
| REM latency | 81.2 | 91.0 | 69,8 | 10.78 | |
| REM periodicity (mean 1st 2 inter REM periods) | 94.9 | 96.2 | 101.5 | 16.11 | |
| Number of REM periods | 4.2 | 4.3 | 4.8c | 0.93 | Diet $F_{2,40}=4.375$ $p<0.05$ |
| First half data (mins) | | | | | |
| Stage 3+4 | 74.6 | 81.6 | 77.0 | 4.12 | |
| 4 | 43.6 | 48.2b | 37.6 | 3.65 | Diet $F_{2,40}=4.27$, $p<0.025$ |
| 3 | 31.0 | 33.4 | 39.4c | 3.30 | Diet $F_{2,40}=3.41$, $p<0.05$ |
| REM | 33.1 | 32.7b | 42.0c | 3.40 | Diet $F_{2,40}=4.68$, $p<0.025$ |
| 2 | 105.2 | 100.5 | 99.7 | 3.97 | |
| 0+1 | 12.1 | 10.2 | 6.3 | 2.22 | |
| Second half data (mins) | | | | | |
| Stage 3+4 | 15.0 | 22.7 | 15.1 | 3.35 | Diet $F_{2,40}=3.87$, $p<0.05$ |
| 4 | 2.9 | 5.4 | 3.2 | 1.89 | |
| 3 | 12.1 | 17.3 | 11.9 | 2.42 | Diet $F_{2,40}=3.47$, $p<0.05$ |
| REM | 68.7 | 71.2 | 71.1 | 3.87 | Night $F_{2,40}=4.28$, $p<0.025$ (night 1 & 3) |
| 2 | 116.4 | 110.6 | 120.7 | 5.19 | |
| 0+1 | 24.9 | 20.5 | 18.1 | 4.26 | |

Key:- a...significant difference between zero & low carbohydrate diets
b...significant difference between low & high carbohydrate diets
c...significant difference between zero & low carbohydrate diets

Table 7. Group means for Post-Diet Nights

| Means for Post-Diet Nights | ZERO | LOW | HIGH | Significance (df=5) |
|---|---|---|---|---|
| Whole night data (mins) | | | | |
| Stage 3+4 | 95.4 | 96.8 | 99.9 | |
| 4 | 54.5 | 48.2b | 37.8c | t(b)13.53, t(c)13.49, p<0.025 |
| 3 | 40.9 | 48.6 | 62.1c | t=4.63 p<0.01 |
| REM | 101.7 | 103.0 | 108.4 | |
| 2 | 218.7 | 217.8 | 209.7 | |
| 0+1 | 34.2 | 32.4 | 32.0 | |
| S.4 latency | 12.8 | 16.3 | 20.8 | |
| REM latency | 94.3 | 88.0b | 66.7 | t=3.00 p<0.05 |
| REM periodicity (mean 1st 2 inter REM periods) | 98.7 | 96.6 | 101.2 | |
| Number of REM periods | 3.8 | 3.8b | 4.5c | t(b)=3.16, t(c)=3.16, p<0.05 |
| First half data (mins) | | | | |
| Stage 3+4 | 82.6 | 82.1 | 79.2 | |
| 4 | 51.3 | 44.5 | 34.0c | t=2.92 p<0.05 |
| 3 | 31.3 | 37.6 | 45.2 | |
| REM | 27.7 | 31.5 | 39.4 | |
| 2 | 103.2 | 101.5 | 95.4 | |
| 0+1 | 11.5 | 9.9 | 11.0 | |
| Second half data (mins) | | | | |
| Stage 3+4 | 12.8 | 14.7 | 20.7 | |
| 4 | 3.2 | 3.7 | 3.8 | |
| 3 | 9.6 | 11.0 | 16.9 | |
| REM | 74.0 | 71.5 | 69.0 | |
| 2 | 115.5 | 116.3 | 114.3 | |
| 0+1 | 22.7 | 22.5 | 21.0 | |

Key:- a...significant difference between post-zero and post-low carbohydrate diets

b...significant difference between post-low and post-high carbohydrate diets

c...significant difference between post-zero and post-high carbohydrate diets

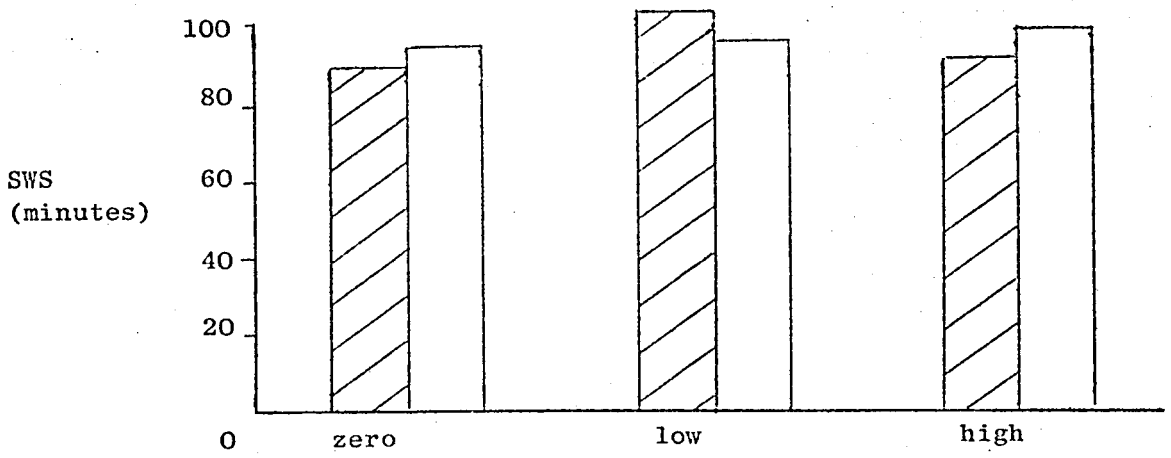



FIGURE 1: Effects of the supplementary diets upon SWS.

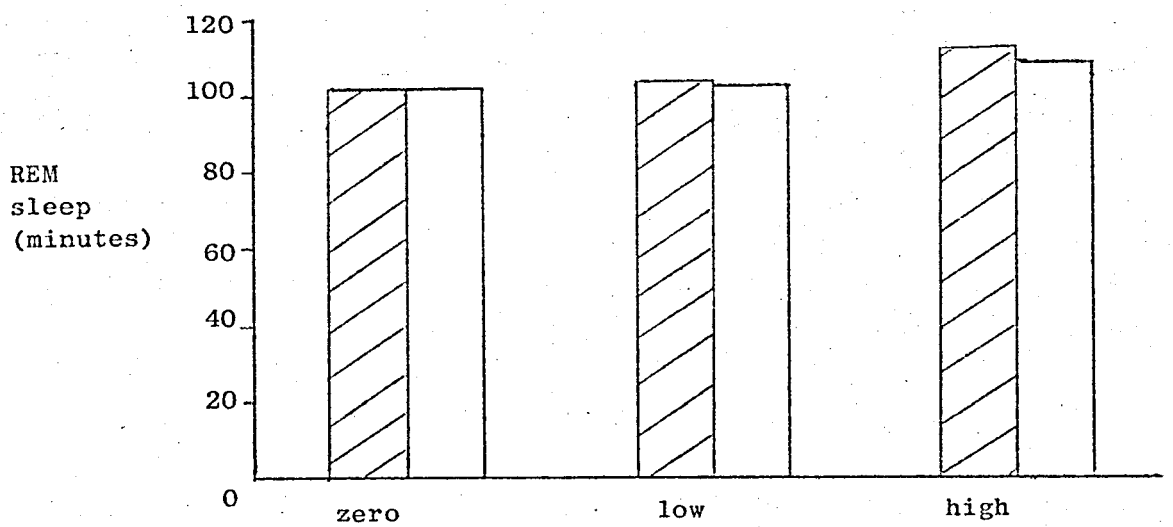



FIGURE 2: Effects of the supplementary diets upon REM sleep.

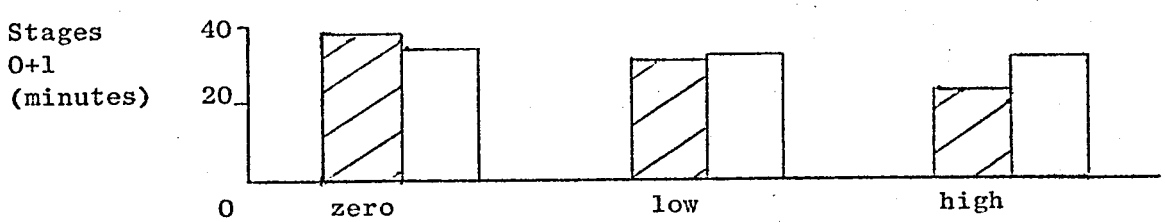
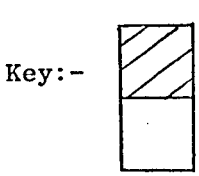



FIGURE 3: Effects of the supplementary diets upon stages 0+1.



## 7.1 Supplementary diet period

1. SWS. Sleep, following the consumption of both the zero and high carbohydrate supplementary diets, contained lower amounts of SWS than following the low carbohydrate diet, although the difference was only significant between the zero and low carbohydrate diets ($p<0.005$). Stage 4 was found to be significantly lower ($p<0.025$) during the high carbohydrate dietary period compared to the low carbohydrate period, especially during the first half of the night ($p<0.025$). The amount of Stage 3 during the first half of the night was found to be significantly higher during the high carbohydrate period compared to the zero carbohydrate period ($p<0.05$).

2. Stage REM. REM sleep was enhanced by the high carbohydrate diet, compared to both of the other diets, during the first half of the night ($p<0.025$). This increase nearly reached significance for the whole night data ($0.1>p>0.05$).

REM sleep during the second half of the night was found to be higher on night 1 than for night 3 ($p<0.025$) across all diets.

3. Stages 0 + 1. The average amounts of Stages 0 + 1 were significantly lower ($p<0.05$) following the high carbohydrate diet compared to the zero carbohydrate diet.

4. Number of REM periods. The average number of REM periods showed a small, but significant ($p<0.05$), increase following the high carbohydrate diet as compared to the zero carbohydrate diet.

## 7.2 Post-diet night

1. SWS. No significant changes were found for SWS in general. Stage 4 was found to be significantly lower

($p<0.025$) on the post-high carbohydrate diet night compared to both the other post-diet nights. This effect was evident during the first half of the night but only the post-zero and post-high carbohydrate diets were significantly different ($p<0.05$).

Stage 3 was significantly higher ($p<0.01$) on the post-high carbohydrate night compared to the post-zero carbohydrate diet night.

2. REM latency. REM latency was decreased during the post-high carbohydrate diet night, although only to a significant extent ($p<0.05$) when compared to the post-low carbohydrate diet night.

3. Number of REM periods. The number of REM periods was significantly higher ($p<0.05$) on the post-high carbohydrate diet night compared to both the other post-diet nights.

4. Carryover Effects. No significant differences were found for any of the variables studied between the post-diet nights and their respective diet nights (using averaged data for all three diet nights). The extended duration of stage 3 sleep on the post-high carbohydrate night was nearly significantly different to the averaged data for the high carbohydrate dietary period ($0.1>p>0.05$).

## 8. DISCUSSION OF RESULTS

The fact that significant differences were found for SWS during the various dietary periods does not necessarily infer that these changes were solely as a result of the changes in carbohydrate intake, as a dose-response relationship was not evident. Compared to the zero carbohydrate diet, both the low and high carbohydrate diets showed an increase in stage 3 sleep, although this was significant only for the high carbohydrate diet during the first half

of the night. However, whilst stage 4 sleep also showed a non-significant increase during the low carbohydrate diet, the high CHO diet showed a non-significant decrease; the difference between the low and high diets being significantly different during the first half and for the whole night. This general increase in both stage 3 and stage 4 during the low carbohydrate diet period resulted in a significant increase in SWS compared to the zero carbohydrate diet. The increase of stage 3 during the high carbohydrate diet period was counter-balanced by the decrease in stage 4 and the duration of SWS was similar to that during the zero carbohydrate diet.

From the analysis of the post-diet data it appears that the duration of SWS showed little variation on the post-diet nights. However, stage 3 sleep was still increased on the post-low and post-high carbohydrate diets compared to the post-zero carbohydrate diet night. This stage was greatly increased on the post-high carbohydrate night and the difference reached significance. Again, this increase in stage 3 associated with the high carbohydrate diet was counter-balanced by a decrease in stage 4 on the post-diet night. This decrease was significantly different over the whole night to both the post-zero and post-low carbohydrate diet nights and occurred during the first half of the night. The post-low carbohydrate diet night also showed a small decrease in stage 4 compared to the post-zero carbohydrate diet night which did not reach significance.

These changes in SWS are difficult to assess. As stated above, if the change in carbohydrate intake was the only factor influencing SWS then one would expect that the changes in SWS would display a trend with respect to the changes in carbohydrate intake. Whilst it is possible that the increase in stage 3 sleep is associated with an increased carbohydrate intake, the changes in stage 4 sleep do not appear to be.

The duration of REM sleep was found to be elevated as the carbohydrate content of the supplementary diets increased, although this change was evident only during the high carbohydrate diet period. This extension to REM sleep reached significance during the first half of the night compared to both the zero and low carbohydrate diet periods and this was reflected in the whole night data with which the increase approached significance. The duration of REM sleep was very similar during the zero and low carbohydrate diet periods suggesting that a small increase in carbohydrate intake (46g) has little effect upon REM sleep. The carbohydrate content of the high carbohydrate diet was nearly three times that of the low carbohydrate diet.

The number of REM periods showed an increase as the carbohydrate content increased, with the high carbohydrate diet being significantly different to the zero carbohydrate diet. Interestingly, the REM periodicity showed a non-significant increase during the high carbohydrate diet. This change in both the number of REM periods and REM periodicity may seem incongruous within a fixed period of analysis, but it should be noted that REM latency was also reduced, although not to a significant extent, during the high carbohydrate diet period. Also, the measure of REM periodicity used was the mean of the first two inter-REM periods and not all of the periods for each night. This approach was taken as all of the subjects displayed a minimum of three REM periods on every night.

These changes in REM sleep and the other REM parameters were still evident on the post-diet nights. The increase in REM sleep again occurred during the first half of the night, although the difference was not significant. The number of REM periods was significantly increased on the post-high carbohydrate diet night compared to both the post-zero and post-low carbohydrate diet nights and REM

latency was significantly decreased compared to the post-low carbohydrate diet night.

Stages 0+1 were found to be decreased as the carbohydrate content of the supplementary diets increased with the high carbohydrate diet period being significantly different to the zero carbohydrate diet period. The decrease in stages 0+1 was apparent throughout the night. This relationship between stages 0+1 and carbohydrate intake was abolished on the post-diet nights.

Thus, it appears that an increase in the carbohydrate intake in the late evening is associated with an increase in REM sleep during the first half of the night and a decrease in wakefulness and light sleep throughout the night. This suggests that the administration of a high carbohydrate supper serves to maintain the integrity of sleep. As the combined duration of Stage REM, Stage 1 and wakefulness during the sleep period are very similar following the administration of all three supplementary diets (mean duration of 138.8, 134.6 and 137.5 minutes during the zero, low and high carbohydrate diet periods respectively), it is possible that the reduction in light sleep and wakefulness was a direct result of the enhancement of REM sleep during the high carbohydrate diet period.

However, it could be argued that the decrease in light sleep and wakefulness during this high carbohydrate diet period allowed for the extended duration of REM sleep. This latter course is a possibility which must not be ignored, although it appears that the former suggestion may be more appropriate in this study. This is because the increase in REM sleep was evident only during the first half of the night, whilst neither SWS or Stage 2 sleep showed similar increases as would be expected following a general reduction in light sleep and wakefulness during the first few hours of sleep. Furthermore, it would be expected that a reduction in light

sleep and wakefulness at the end of the night would allow for the extended duration of both REM sleep and Stage 2 sleep, as these stages are predominate at this time. As REM sleep showed little variation in the second half of the night during the high carbohydrate diet period, it is unlikely that the observed increase in REM sleep during the first half of the night was a direct consequence of the reduction in light sleep and wakefulness at this time.

SWS may be responsive to the carbohydrate intake but the relationship does not appear to be simple. It is possible that other dietary factors such as the digestibility of the food may have influenced the changes in SWS. For example, the cellulose in the zero CHO diet is not digestible by humans and, consequently, it might be possible that this caused some discomfort to the subjects which resulted in a more restless sleep. This could be evidenced by a decrease in SWS and REM sleep and an increase in wakefulness and light sleep. However, this does not appear to be the case as the changes in REM sleep and stages 0+1 appear to be progressive across the three levels of carbohydrate intake. Also, REM sleep does not show a rebound on the post-zero carbohydrate diet night. However, the non-significant increase in stage 4 sleep on the post-zero carbohydrate diet compared to the averaged data during the zero carbohydrate diet period may represent a rebound as a consequence to the possible 'gastric distress' following the high cellulose intake.

It is interesting to note that if the levels of stage 4 were slightly higher during the zero carbohydrate diet period, then the effects of increasing carbohydrate intake upon SWS would be more simple; stage 4 sleep progressively decreasing whilst stage 3 sleep increases. It is possible that this effect might have been elicited if the subjects had received only water instead of cellulose and water for

zero carbohydrate diet. Thus, whilst the cellulose was used to control for the influence of bulk, it is possible that the effects of digestibility may have been just as important. It should be noted that none of the subjects reported any adverse effects such as diarrhoea or stomach pains resulting from the cellulose diet.

The post-diet night data indicated that the changes observed in SWS during the administration of the various supplementary diets were still evident. A non-significant increase in REM sleep was still found following the termination of the high carbohydrate diet, although the decrease in stages 0+1 with increasing intake was not apparent. This suggests that the diet may have both short and long term influence upon sleep.

The findings of this study are related to the conclusions from the literature review in the next section of this chapter.

## 9. OVERVIEW

The results of Study III are consistent with the general findings from the literature review (see Table 1, Chapter 7) as the consumption of the high carbohydrate supplementary diet was found to be associated with an increase in REM sleep and a decrease in wakefulness and light sleep. The effects upon SWS are not so clear although it is suggested that the nature of the zero carbohydrate diet may have influenced SWS more by its indigestibility than its carbohydrate content. It is possible, therefore, that the duration of SWS remains unchanged in response to carbohydrate intake although there is evidence to show that there is a shift towards stage 3 sleep as the carbohydrate content of the supplementary diet increases.

These findings strongly suggest that the evening diet

has an influence upon subsequent sleep. The possible mechanisms by which this influence is effected have already been discussed in the previous chapter (see Section 3.5) and include the digestibility of the food, the amount of food consumed, the calorie intake and the protein, carbohydrate and fat content of the diet. Whilst the indigestible nature of the zero carbohydrate diet may have influenced sleep it is important to note that the changes in REM sleep and stages 0+1 were progressive across the diets as the carbohydrate content increased, indicating that these changes were not mediated by variations in the digestibility of the supplementary diets. As all of the supplementary diets were designed to be of a similar bulk and the low and high carbohydrate diets contained similar amounts of protein and fat, it would appear that the changes in sleep were probably mediated by either the increased calorie intake or the increased CHO intake.

As stated in the introduction to the literature review, the review contains studies which were not published before Study III was completed. Until the study by Phillips et. al. (1975) was published it was not clear whether the findings of Study III were mediated by the increase in calorie intake or the increase in CHO content. Thus, it was intended that a subsequent study would be necessary to identify which of these two factors, or to what extent each of them, were responsible for the observed sleep changes. However, Phillips et. al. (1975) provided clear evidence that sleep can be greatly influenced by iso-caloric dietary changes. This study has been reviewed previously (see Chapter 7, section 3.2.1.2) but to recapitulate they reported that a high carbohydrate/low fat diet significantly increased REM sleep and significantly decreased stage 1 sleep compared to values observed during a normal balanced dietary period. These findings are consistent with those from Study III and it seems that the increase in the carbohydrate content per

se, and not the concomitant increase in calorie intake, was responsible for these changes in sleep. The increase in REM sleep reported by Phillips et. al. (1975) was larger than found in Study III presumably because their high carbohydrate diet contained more carbohydrate than that used in Study I. Whilst Study III used a maximum carbohydrate supplement of 130g to the normal diet, the study of Phillips et. al. (1975) gave a supplement of 250g.

Phillips et. al. (1975) also reported that SWS was decreased by the high carbohydrate diet compared to the balanced diet. This finding was not observed in Study III, although such a trend may have been masked by the possible 'gastric distress' following the zero carbohydrate supplementary diet. Examining the data summary table for Study III, it is apparent that stage 4 sleep was significantly lower following the high carbohydrate supplementary diet than following the low carbohydrate supplementary diet. Similar changes were found for SWS although these did not reach significance.

Whilst there is now a large body of evidence to show that the diet can influence sleep, there has been little attempt to determine the mechanisms by which this influence is mediated. Therefore, the remainder of this thesis is devoted to the investigation of a possible mechanism by which the diet influences sleep. The proposals for further research are detailed in the preface to Section B, Part II of this thesis.

## 10. SUMMARY AND CONCLUSIONS

This study investigated the effects of increased carbohydrate intake, in the form of a supplementary meal taken approximately 40 minutes before retiring, upon the sleep of six young males of normal weight. Three levels of carbohydrate supplement were administered; the zero

carbohydrate supplement consisted of methyl cellulose and water (available carbohydrate - 0 g), the low carbohydrate supplement consisted of slimming biscuits, butter and raw vegetables (available carbohydrate - 46 g) and the high carbohydrate supplement consisted of glucose solution and fried potatoes (available carbohydrate - 130 g). These supplementary diets were designed to give similar feelings of 'fullness' after ingestion and the low and high carbohydrate supplementary diets contained similar amounts of protein and fat. The effect of these supplementary diets upon plasma glucose levels was assessed by a pilot-study. The high carbohydrate supplementary diet was found to greatly enhance these levels during the first half of the night whereas only a small increase was found following the low carbohydrate supplementary diet.

The experimental programme lasted for three consecutive weeks, with one supplementary diet administered for three consecutive days, Tuesday to Thursday, each week. All night sleep records were recorded on all nights following the administration of the supplementary diets and, in addition, on the first night when the supplementary diets were withdrawn. The supplementary diets were administered in a balanced order. The daily variations in the subject's normal diet were standardised by asking the subject to fill in a diet log for every day during the first week of the study and then to repeat those meals, day for day, during the subsequent two weeks.

The results showed that the progressive increase in the carbohydrate content of the supplementary diet was associated with a progressive increase in REM sleep during the first half of the night and a progressive decrease in wakefulness and light sleep throughout the night. The changes in SWS were not so clear, possibly due to the indigestible nature of the zero carbohydrate supplementary

diet. It is suggested that SWS shows more of a change in quality than quantity as evidenced by a shift towards stage 3 sleep as the carbohydrate intake increases.

Similar changes in SWS and REM sleep were still evident on the post-diet nights although the decrease in wakefulness and light sleep was not evident. This suggests that the diet may have both immediate and extended influences upon sleep.

The mechanisms by which diet influences sleep are not known, although it appears that the carbohydrate content, and not necessarily the concomitant increase in calorie intake, is an important factor.

PART TWO: DIET AND SLEEP

SECTION B: Development of a Theoretical Model

Preface: Proposals for Further Research

CHAPTER 9: Diet - Its Influence upon Neurotransmitter Metabolism.

CHAPTER 10: The Influence of Brain Serotonin Metabolism Upon Sleep.

CHAPTER 11: A Theoretical Basis to the Diet and Sleep Relationship.

PROPOSALS FOR FURTHER RESEARCH

From the literature review and the first diet and sleep study it is apparent that changes in the diet can have an influence upon sleep. At present the research lacks a theoretical basis to explain the experimental findings, although it would seem probable that this influence is mediated by changes in the transport of certain substances to the brain which are required for the normal functioning of sleep.

From the work of Jouvet and many other researchers it seems likely that some, if not all, of the neurotransmitters are involved in the sleep mechanism. Thus, an appraisal of the influence of the diet upon neurotransmitter metabolism may provide some clues concerning the nature of the diet's influence upon sleep. This is undertaken in Chapter 10.

If there appears to be good experimental evidence in favour of a link between changes in the diet and neurotransmitter metabolism, then a subsequent chapter will attempt to assess the effects of such changes in neurotransmitter metabolism upon sleep.

From these two literature reviews it may be possible to construct a rationale or theoretical model from which the effects of various diets upon sleep can be predicted. This will be undertaken in Chapter 11. The validity of this rationale will be initially scrutinised by comparing the predicted changes in sleep with the observed changes after the various diets reviewed in Chapter 7. If the validity of the rationale is still intact, it will be subjected to a closer examination by using it to design diets which should influence sleep in a pre-determined manner.

It is appreciated that if the predicted and observed changes in sleep are consistent, then this does not necessarily infer that the rationale is correct. However, it is

very useful to have a theoretical model as future studies can be designed to test the model and thereby systematically increase our knowledge in the field of diet and sleep. The investigation of the model will also contribute to the neurochemical literature, as many of the studies have only reported on the effects of changes in neurotransmitter metabolism which are outside the physiological range (e.g. from the use of enzyme-inhibiting drugs or massive doses of precursors).

CHAPTER 9

DIET - ITS INFLUENCE UPON NEUROTRANSMITTER METABOLISM

## 1. INTRODUCTION

This chapter will assess the influence of the diet upon the metabolism of serotonin, the catecholamines and acetycholine in the brain as these neurotransmitters have all been reported to be involved in the sleep mechanism.

The major part of this chapter is devoted to the dietary regulation of brain serotonin synthesis. This is not because of the author's bias but reflects the orientation of existing literature in this field of research.

## 2. SEROTONIN AND THE DIET

The influence of the diet upon brain serotonin metabolism is very complex, and it has taken many years of research to discover some of the factors involved. This section will describe our present understanding of the dietary influence upon brain serotonin metabolism from a historical perspective.

### 2.1 Tryptophan content

The amino acid tryptophan is the dietary precursor of serotonin and there exists abundant experimental evidence that manipulators of brain tryptophan can produce parallel alterations in brain serotonin synthesis of the rat. The techniques utilised in the earlier experiments made use of either high tryptophan diets, via the parenteral administration of the amino acid, or synthetic diets deficient in tryptophan.

There is strong evidence that the reduced dietary intake of tryptophan results in a decreased synthesis of brain serotonin. (Zbinden, Pletscher and Studer, 1958; Gál, Drewes and Barraclough, 1961; Gál and Drewes, 1962; Culley, Saunders, Mertz and Jolly, 1963; Boullin, 1963; Biggio, Fadda, Fanni, Tagliamonte and Gessa, 1974). Unfortunately, most of the studies did not record brain tryptophan levels. The evidence

shows that the decrease in brain serotonin synthesis is enhanced as the duration of the experiment lengthens. However, it is difficult to assess the latency of this decrease in synthesis as the majority of studies used experimental periods of days or weeks. The more recent study by Biggio et. al. (1974) showed that it is possible to produce a marked depletion of serum and brain tryptophan and also brain serotonin and brain 5-hydroxtryptamine (5-HIAA), the end-product of serotonin metabolism in the CNS, within two hours after feeding rats a diet deficient in tryptophan.

The effects of the tryptophan supplemented diets upon brain serotonin metabolism are also in close agreement with one another. The evidence shows that an increase in plasma tryptophan, either by the means of the diet or by intra-peritoneal (i.p.) injections, is associated with increases in brain tryptophan, serotonin and 5-HIAA levels. (Wang, Harwalker and Waisman, 1962; Green, Greenberg, Erickson, Sawyer and Ellizon, 1962; Ashcroft, Eccleston and Crawford, 1965; Eccleston, Ashcroft and Crawford, 1965; Eccleston, Ashcroft, Moir, Parker-Rhodes, Lutz and O'Mahoney, 1968; Moir and Eccleston, 1968; Horita and Carino, 1970; Meek, Fuxe and Anden, 1970; Fernstrom and Wurtman, 1971; Grahaeme-Smith, 1971; Tagliamonte, Biggio, Vargio and Gessa, 1973).

The relative changes between plasma and brain tryptophan levels after the intra-peritoneal injection of 800 mg/kg l-tryptophan (Ashcroft, et. al., 1965) was described as follows:

"The blood/brain ratio is seen to increase rapidly during the first half-hour after loading, as a result of the failure of brain uptake processes to keep pace with the increase in blood tryptophan levels. Between one and two hours, the ratio decreases as brain levels continue to rise whilst blood levels fall. At two and four hours, the ratio is lower than in the unloaded animal, as the fall in brain levels is slower than that in blood. By eight hours, the ratio is again returning to normal."

It is of great interest that an intra-peritoneal dose of only 12.5 mg/kg of l-tryptophan, given to rats, caused a 20-25% increase in brain serotonin after one hour (Fernstrom and Wurtman, 1971). This dose is less than 1/20th of their normal dietary intake and it was administered at a time when the tryptophan concentrations in the plasma and the brain were at a minimum. The elevation of brain tryptophan levels after this dose were within the normal daily range. This last point is very important as it provides evidence that the relationship between plasma tryptophan and brain serotonin applies to doses of a physiological order and not only for the comparatively massive doses.

Fernstrom and Wurtman's data provided evidence of a dose-related response between tryptophan and brain serotonin levels. Although the maximum dose given was only 125 mg/kg, they found that the brain serotonin concentrations levelled off between the 50 and 125 mg/kg doses, even though the brain tryptophan levels still increased. This is in accord with the earlier work of Moir and Eccleston (1968) who found that both the rate of increase of brain serotonin and its maximal level are very similar in response to doses of 50 to 1600 mg/kg of l-tryptophan. This larger dose does, however, prolong the increase (Eccleston et.al., 1965).

These facts, together with the finding that brain 5-hydroxytryptophan (5-HTP) levels did not increase even with very large doses of tryptophan, (Ashcroft et. al., 1965; Eccleston et. al., 1965) indicated that brain serotonin metabolism is limited by tryptophan hydroxylation (Ashcroft et. al. 1965; Moir and Eccleston, 1968). Brain serotonin is synthesised from the dietary precursor tryptophan in a two-step pathway involving hydroxylation to 5-HTP by tryptophan hydroxylase and the subsequent decarboxylation to serotonin by aromatic acid decarboyxlase. Confirmation of this rate-limiting effect of tryptophan hydroxylase was provided by Jéquier, Robinson, Lovenberg and Sjoerdsma (1969).

who found that the Michaelis constant of tryptophan hydroxylase for its substrate tryptophan is much higher than the concentration of tryptophan typically found in the brain. As the brain tryptophan levels rise, this enzyme becomes increasingly saturated and, consequently, the rate of serotonin synthesis increases until the enzyme becomes fully saturated when the rate of serotonin synthesis will plateau. This suggests that a dose of 50 mg/kg is sufficient to saturate tryptophan hydroxylase in the brain and instigate a maximal rise of brain serotonin synthesis. The duration of this maximum rate of synthesis will be dependent upon the length of time that tryptophan hydroxylase is saturated which, in turn, depends upon the levels of brain tryptophan.

## 2.2 Carbohydrate content

The previous section has shown that brain serotonin levels are influenced by plasma tryptophan levels. However, work by Fernstrom, Wurtman and others has opened up a new area concerned with diet and the regulation of brain serotonin levels. Fernstrom and Wurtman (1971) reported that the injection of insulin (2 units i.p.) or the consumption of a carbohydrate diet caused an increase in the plasma tryptophan levels of rats (30-50% and 25-35% respectively). This finding was surprising as insulin has a general depressing effect on most amino-acids in the plasma (Munro, 1964). Within two hours of receiving the insulin both brain tryptophan and brain serotonin levels were also increased by 36% and 28%, respectively.

In order to determine whether a similar effect would occur when insulin was physiologically stimulated, the experimenters fasted a group of rats for 15 hours before allowing them 1 to 3 hours access to a carbohydrate diet. This diet would be expected to stimulate insulin secretion by natural means and also, a very important point, avoid an increase of dietary tryptophan.

In a typical experiment the rats ate an average of 5 grams/hour during the first hour and 2 grams/hour during the second and third hours. The plasma tryptophan levels were significantly elevated by 25% during the first hour and remained at this level for the remaining two hours. Brain tryptophan levels rose 22% in the first hour and reached a maximum increase of 66% by the end of the second hour. Brain serotonin levels were increased 19% by the first hour and remained at that level during the next two hours.

Thus, it was apparent that brain serotonin levels are greatly and rapidly influenced by a large carbohydrate intake. This is of great interest because this diet does not provide a source of tryptophan to explain the observed increase in plasma tryptophan levels. This suggests that tryptophan is, in some way, an "odd man out" compared to the other amino acids. The plasma levels of tryptophan appear to be affected by some factor which does not modify the levels of the other amino acids. This point will be discussed later in this review.

Whilst the above study shows that plasma tryptophan levels can be increased in rats by both a carbohydrate diet and an insulin injection, it cannot be assumed that this will also occur in humans. In fact, it has been reported that the oral administration of glucose (Lipsett, Madras, Wurtman and Munro, 1973) or the injection of insulin (Fernstrom, Larin, Schonfield and Wurtman, 1971) to humans is associated with a slight increase in plasma tryptophan levels. However, the intravenous administration of glucose appears to increase plasma tryptophan levels in normal subjects,although decreases were observed in some patients with carcinoid tumors (Feldman and Plonk, 1976).

## 2.3 Protein content

On the basis of their own findings and those of others (see Section 2.1), Fernstrom and Wurtman predicted that any

increase in plasma tryptophan would be followed by an increase in brain serotonin levels. Since dietary protein should elevate plasma tryptophan levels, both by stimulating insulin secretion and by increasing the dietary intake of this amino acid, they anticipated that the consumption of a protein meal would also increase brain serotonin levels (Fernstrom and Wurtman, 1971).

However, the issue was not found to be that simple. Other researchers, using brain slices (Blasberg and Lajtha, 1965) or animals treated with doses of individual amino acids (Guroff and Udenfriend, 1962), have shown that groups of amino acids are transported into the brain via different active carrier systems. It was found that there existed different carrier systems for the neutral, acidic and basic amino acid groups and that the member amino acids competed with one another for transport within each of these groups. Tryptophan, being a neutral amino acid, has to compete with five other neutral amino acids, namely leucine, isoleucine, valine, phenylalanine and tyrosine. As tryptophan is one of the least abundant amino acids found in dietary protein, the rise in plasma tryptophan levels after a protein meal will be relatively small compared to the other amino acids. Therefore, a protein meal might not increase brain serotonin levels even though the intake of tryptophan is increased. This is because the ratio of tryptophan to the other five similarly neutral amino acids will be decreased after a protein meal, resulting in tryptophan's decreased transport to the brain.

This theory agrees well with the findings of Fernstrom and Wurtman (1972) who fed groups of rats a carbohydrate diet diet supplemented with either:-

a) 18% dry-weight casein

b) 18% dry-weight synthetic amino acids similar to casein

c) the same as (b) minus those amino acids that should theoretically compete with tryptophan for uptake into the brain.

The rats were deprived of food for 15 hours before being fed and were killed 1 or 2 hours after the diet had been presented. Control rats were similarly fasted but were not fed during the experiment. These control rats were killed at 0, 1 or 2 hours after the experiment had commenced.

No increases in brain tryptophan, serotonin or 5-HIAA were found for those rats fed diets (a) or (b), even though the plasma tryptophan levels were elevated by 75% after one hour. However, those rats fed with diet (c) showed increases of 250% for brain tryptophan, 35% for brain serotinin and 60% for brain 5-HIAA after two hours, clearly showing that serotonin synthesis was enhanced.

To test that these results were not influenced by non-specific effects resulting from the lack of any group of amino acids, the investigators fed a further group of rats with diet (b) minus the amino acids aspartate and glutamate. These two amino acids are charged at blood pH values and are transported to the brain via a different carrier system to that of tryptophan. As with diets (a) and (b), no changes were observed in brain tryptophan, serotonin or 5-HIAA levels even though the plasma tryptophan levels had risen.

Fernstrom and Wurtman concluded that brain serotonin levels are not simply a reflection of plasma tryptophan levels per se, but of the ratio of tryptophan to the other neutral amino acids in the plasma. To illustrate this relationship, a correlation analysis was carried out between brain tryptophan and the ratio of plasma tryptophan to its competitors after diets (a), (b) and (c). A high degree of correlation ($r=0.95$; $p<0.001$ that $r=0$) was observed. When brain tryptophan was correlated to plasma tryptophan only, the result was still significant but far less impressive ($r=0.66$; $p<0.001$ that $r=0$).

Earlier studies, in which large doses of l-phenylalanine, (Yuwiler and Louttit, 1961; Wang et. al., 1962; Green et. al., 1962; McKean, Boggs and Peterson, 1968; Aoki and Siegel, 1970), l-leucine (Yuwiler and Geller, 1965; Ramanamurthy and Srikantia, 1970), or l-tyrosine (Fernstrom and Wurtman, 1972), were administered to rats, have all been reported to decrease brain tryptophan or brain serotonin levels. These findings are consistent with the proposal that the transport of tryptophan to the brain is dependant upon the ratio of tryptophan to the other large, neutral amino acids in the plasma.

To recapitulate, evidence has been provided to show that brain serotonin synthesis is influenced by the content of the diet. Specifically, a diet which is rich in carbohydrate would be expected to increase brain serotonin synthesis above fasting levels. However, if the protein content was substantial ($\geq$18% and maybe even smaller) then the brain serotonin level would not be expected to be altered.

This discovery that brain serotonin levels were influenced by the diet stimulated research into the effects of fasting and feeding upon these levels.

## 2.4 Fasting and Feeding

Pérez-Cruet, Tagliamonte, Tagliamonte and Gessa, (1972) provided more extensive data regarding the effects of fasting and feeding upon brain serotonin levels. These experimenters trained several groups of rats to eat their normal daily meal within two hours by presenting the food once a day during the period 10.00 a.m. to 12.00 noon. After one week of training, groups of rats were killed at various intervals of time (0, 6, 12, 24 and 48 hours) after the food had been removed. The rats' diet contained $\geq$23% protein, $\geq$4.5% fat, $\geq$6% crude fibre, $\geq$9% ash; the remainder being carbohydrate.

The analysis showed that the plasma tryptophan levels decreased as the duration of fasting increased, whereas the brain tryptophan and 5-HIAA levels actually increased. Brain serotonin levels did not change to a statistically significant amount throughout the experiment.

In an associated experiment, Pérez-Cruet et. al. showed that the synthesis rate of brain serotonin was about 30% higher in rats fasted for 24 hours compared to rats fed for two hours (according to the method of Tozer, Neff, and Brodie, 1966). This difference was not due to time-of-day effects as the rats were all killed at noon. Moreover, rats killed at midnight showed comparable results.

To compete this particularly thorough study, similar experiments were carried out with hypophysectonized rats to see if the variations of serotonin synthesis were secondary to stress-induced changes of plasma corticosterone levels. The results showed again that serotonin synthesis was higher in rats fasted for 24 hours than in rats fed for 2 hours, implying that these changes were not stress-induced.

The experimentors concluded that 2 hours food intake actually decreased brain tryptophan and 5-HIAA levels by approximately 30% and 22%, respectively, compared to rats fasted for 24 hours. This reduction was associated with a 30% decrease in serotonin synthesis even though the plasma tryptophan levels rose by 37%.

### 2.4.1. Free and bound plasma tryptophan

The majority of subsequent studies investigating the effects of fasting and feeding upon brain serotonin metabolism paid attention to the changes not only in plasma tryptophan levels, but also to the changes in the free and bound portions. McMenamy and Oncley (1958) reported that tryptophan is the only amino acid present in plasma which is bound

to albumin. Thus, total plasma tryptophan (TPT) can be subdivided into:-

a) bound plasma tryptophan (BPT) which consistutes approximately 90% of the total in humans, and

b) free plasma tryptophan (FPT) constituting the remainder.

As it has been shown that the rate of certain biologically active compounds are retarded when they are bound to plasma proteins (Martin, 1965), it was of interest to see if the changes in brain serotonin metabolism following fasting or feeding were linked to changes in free plasma tryptophan levels.

Knott and Curzon (1972) investigated the relationship between free plasma tryptophan levels and brain tryptophan levels in the rat. They found that increases of brain tryptophan, due to either food deprivation (24 hours) or immobilization stress (3 hours), were associated with increases in free plasma tryptophan levels.

The effects of immobilization stress were associated with a 47% increase in the free plasma tryptophan levels and a non-significant rise of 18% for both brain tryptophan and 5-HIAA levels, whereas the total plasma tryptophan levels showed a non-significant decrease of 15%.

The changes observed after 24 hours food deprivation followed the same trend but were quantitatively much larger. Although the total plasma tryptophan levels were unchanged, the free fraction showed an increase of 150% compared to control rats. Brain tryptophan and 5-HIAA were increased by 74% and 48%, respectively, indicating that serotonin synthesis was elevated even though the serotonin levels did not change. Knott and Curzon suggested that serotonin levels did not show an increase because either:-

a) the procedures were stressful, which possibly increased the firing rate of the serotonin neurons thereby lowering the serotonin levels, or

b) the intra-cellular storage capacity of serotonin had reached its maximum and the excess serotonin had been metabolically degraded by monamine oxidase to 5-HIAA.

Tagliamonte, Biggio, Vargio and Gessa (1973) monitored levels of total plasma tryptophan, free plasma tryptophan and brain serotonin metabolites in response to two conditions known to elevate brain tryptophan levels, namely the intra-peritoneal administration of l-tryptophan and 24 hours fasting.

Rats were fasted for 12 hours prior to the injection of l-tryptophan (50 mg/kg i.p.). The results showed that total plasma tryptophan, free plasma tryptophan, brain tryptophan, serotonin and 5-HIAA were all significantly elevated for over an hour, although base line levels were observed after 3 hours. It was apparent that the changes in free plasma tryptophan were more pronounced than those in total plasma tryptophan (536% increase versus 156% increase after 30 minutes). Also, the increases in free plasma tryptophan were of a similar degree to those of brain tryptophan (525% increase after 30 minutes) and of a similar duration. Although brain tryptophan levels were greatly increased, the changes in brain serotonin and 5-HIAA were comparatively small (18% and 59% increases, respectively, after 30 minutes).

The effects of fasting were studied in two groups of rats. Both groups were exposed to light from 9.00 to 21.00 hours and both were allowed food for two hours a day prior to the experiment. However, to exclude possible time-of-day effects of serotonin metabolism, one group was fed at 10.00 a.m. whilst the other group was fed at 10.00 p.m. The animals were trained to this regime for two weeks before the experiment began. Rats from each group were killed either after 24 hours fasting (noon to midnight) or at the end of the two hour feeding period.

The results showed that the total plasma tryptophan levels were lowered in those rats which had been fasted for 24 hours. However, the free plasma tryptophan levels were actually elevated in the fasted rats compared to the fed rats. This was true whether the rats were fed at 10.00 a.m. or 10.00 p.m. As noted before (Pérez-Cruet et. al., 1972), the brain tryptophan levels were higher in the fasted rats than in the fed rats. Whilst this change was associated with a decline in total plasma tryptophan levels, it was both parallel and proportional to the changes in free plasma tryptophan levels.

From the above findings Tagliamonte and his colleagues concluded that brain tryptophan and serotonin turnover are controlled by free plasma tryptophan levels.

It is interesting that serotonin synthesis has been shown to increase even though the total plasma tryptophan levels remain unchanged (Knott and Curzon, 1972) or even decreased (Pérez-Cruet et. al., 1972; Tagliamonte et. al., 1973) by fasting. However, these findings do not necessarily conflict with the proposal by Fernstrom and Wurtman (1972) that it is the ratio of total plasma tryptophan to its competitor amino acids that regulates the transport of tryptophan to the brain. As the levels of the competitor amino acids were not recorded in these subsequent studies, it is not possible to examine this proposal. It is possible that these competitor amino acids could have shown larger decreases (not being bound to albumin) than tryptophan and the ratio would thus have been increased.

Greenwood, Friede, Bond, Cruzon and Lader (1974) showed that the administration of l-tryptophan in humans also increased levels of free plasma tryptophan. The tryptophan was administered by intravenous infusion lasting for 3 hours and both the doses of 75 mg and 100 mg/kg body weight were found to increase total plasma tryptophan levels; the 100 mg/kg infusion produced a 40-fold increase in free tryptophan although the total tryptophan levels were increased only 8-fold.

From the studies reviewed so far, it is apparent that brain serotonin synthesis is influenced by either the ratio of total plasma tryptophan to the other neutral amino acids or the free plasma tryptophan levels. Whilst both of these variables may be good indicators of synthesis rate, it is quite possible that a more sensitive indicator may be found by integrating these two approaches. Although only the free fraction of plasma tryptophan can gain entry to the brain (the blood-brain barrier does not allow albumin to leave the circulation), its rate of transport into the brain is most likely governed by the levels of other neutral amino acids in the plasma. Thus, the ratio of free plasma tryptophan to the other neutral amino acids may be a valuable indicator of serotonin synthesis rate.

Pérez-Cruet et. al. (1974) examined the relationship between changes in this, and other, proposed indicators to changes in brain serotonin synthesis in humans. This experiment involved taking CSF samples from lumbar punctures in 29 subjects who had neurological disorders (Parkinson's disease, Huntington's chorea etc.). From these samples, the levels of tryptophan and 5-HIAA were recorded after 12 hours fasting (10.00 a.m.) and approximately 4 hours following feeding (4.00 p.m. - their normal diet consisted of 25 g protein, 25 g fats and 55 g carbohydrates). Blood samples were also taken at these times and the levels of total, free and bound plasma tryptophan were recorded.

Nine of the patients fasted throughout the day and no significant changes in CSF 5-HIAA were noted. However, those patients who received a balanced diet at midday were found to have significantly decreased levels of CSF tryptophan and CSF 5-HIAA in the postprandial sample. The postprandial blood samples showed that both total and bound plasma tryptophan levels were increased whereas free plasma tryptophan levels were consistently decreased.

Significant negative correlations between total or bound plasma tryptophan and CSF tryptophan or CSF 5-HIAA were found, whereas a non-significant positive correlation was obtained between free plasma tryptophan and these CSF metabolites. The plasma levels of valine, isoleucine, leucine, tyrosine and phenylalanine were also recorded in six of the patients who received identical diets. It was found that the ratio of total plasma tryptophan to these other neutral amino acids was not significantly correlated to these CSF metabolites, whereas the ratio using free plasma tryptophan was significantly correlated.

As discussed later in this review (see Section 2.6), it is likely that changes in the level of tryptophan in the CSF may reflect changes in the transport rate of tryptophan into the brain from the plasma. If this is true, then the above study provides further evidence consistent with the idea that brain serotonin synthesis is decreased after a balanced meal containing approximately 25% protein by weight, as compared to fasting levels. This decrease in serotonin synthesis appears to be mediated via a decrease in the transport of tryptophan into the brain as depicted by the reduced ratio of free plasma tryptophan to the other neutral amino acids.

Young, Lal, Feldmuller, Sourkes, Ford, Kiely, and Martin, (1976), however, did not find that the ratio of either free or total plasma tryptophan to the other neutral amino acids were significantly correlated to levels of CSF tryptophan in two patients with venticular drains. In both these patients, CSF tryptophan was found to be significantly correlated to free plasma tryptophan levels, but not total plasma levels. It should be noted that both of these patients were fed intravenously because of their comatose conditions, although one patient was also fed at intervals through a nastrogastric tube. This study does not necessarily detract from the finding of Pérez-Cruet et. al. (1974) because the findings refer to only 2 subjects, but it does show that the choice of a suitable

indicator for assessing the rate of serotonin synthesis is still controversial. Data from normal subjects would be very desirable although the obvious difficulties render this an unlikely source of information.

## 2.5 Fat content

There is also evidence to show that the fat content of the diet may influence brain serotonin synthesis. For example, Knott and Curzon (1972) noticed that the increase in free fatty acid levels in fasted rats was associated with a comparable percentage increase in free plasma tryptophan levels (159% increase in free fatty acids versus 150% increase in free plasma tryptophan).

These authors further investigated this effect by injecting heparin, which is known to increase free fatty acid levels (Robinson and French, 1960), into another group of rats. Compared with control rats, the free fatty acid levels were increased by 88% and the free plasma tryptophan levels by 89%, whereas the total plasma tryptophan levels showed a non-significant decrease of 18%. Thus, it appeared that the changes in free plasma tryptophan and free fatty acids were parallel and proportional.

The remaining issue was to determine whether the increase in plasma free fatty acids caused the rise in free plasma tryptophan levels or were just associated with them. Yet again another clue was found in the work of McMenamy and Oncley (1958), who showed that the addition of oleate to bovine plasma albumin reduced the number of sites binding tryptophan. The oleate was therefore shown to have a greater affinity for albumin than tryptophan, resulting in the displacement of bound tryptophan. However, this work involved high concentrations of oleate and its relevance to a physiological situation was debatable.

Thus, Curzon, Friedel and Knott (1973) studied the effects, in vitro, of physiologically occurring free fatty acids (linoleic, oleic and palmitic acids) within the range of physiological concentrations upon free plasma tryptophan in rat and human plasma. The results showed that the increase of free fatty acid levels was correlated significantly with the increase of free plasma tryptophan for all three of the acids and for both rat and human plasma. For example, an increase of the fatty acid concentration from 0.5 to 1.0 meq/litre gave 4-fold and nearly 2-fold increases of free tryptophan in rat and human plasma, respectively.

Completmentary evidence was provided by Lipsett, Madras, Wurtman and Munro, (1973) who studied the effects of decreased free fatty acid concentrations upon free plasma tryptophan in human subjects. Seven adult males fasted overnight from 8 p.m. to 8 a.m. when they were given 75 g of glucose to drink in solution. Blood samples were taken immediately before the subjects were given the drink and subsequently at 30, 60, 90, 120 and 180 minutes after drinking. The maximal elevation of blood glucose levels ($\sim$48% increase) was obtained with the 30 minutes sample and by 60 minutes the levels showed a decline. The plasma free fatty acid levels progressively declined after drinking the glucose solution, reaching a minimum ($\sim$50% decrease) after 90 minutes and remaining at this level for the 120 and 180 minute samples. The total plasma tryptophan levels decreased gradually throughout the experiment, reaching a minimum level (17% decrease) at 180 minutes. This was not reflected in the bound plasma tryptophan levels, which showed little change throughout the 3 hours, but by the free tryptophan levels which had fallen 35% by 90 minutes and remained at this minimum for the rest of the samples. Thus, the decline of free tryptophan levels was associated with a decline of free fatty acids in the plasma.

This finding was to be expected from a logical extension to the findings of McMenamy and Oncley (1958). As the free fatty acids left the blood, the number of available binding sites for free tryptophan to albumin increased thereby reducing the free plasma tryptophan levels. However, the major component of this reduction was most likely due to the general influence of insulin upon free plasma amino acids. It is worthwhile noting that the total plasma tryptophan levels were not substantially elevated after glucose administration with these human subjects whereas the earlier work by Fernstrom and Wurtman with rats did show an increase.

In a parallel study, (Lipsett et. al., 1973), plasma was taken from a human subject at 0, 90 and 120 minute intervals after glucose administration. These samples were divided into two; to one of which oleic acid was added in a sufficient quantity to increase the free fatty acid content by 0.4 meq/litre. As before, the untreated samples showed a decline of free fatty acid and free plasma tryptophan levels after the glucose administration. However, the addition of oleic acid was found to greatly increase the free plasma tryptophan levels at the expense of the bound fraction. For example, the free plasma tryptophan levels were 5.1 and 8.1 µg/ml for the untreated and oleic acid treated samples respectively, at 0 minutes and 3.1 and 4.8 µg/ml at 120 minutes after the glucose administration.

This study shows that the levels of free plasma tryptophan can be increased, in vitro, by the addition of a free fatty acid within the physiological range. However, the findings from this 'in vitro' study required confirmation from 'in vivo' experiments.

A suitable experiment was conducted by Madras, Cohen, Messing, Munro and Wurtman (1974) to determine whether dietary fat modified the binding of tryptophan to albumin.

Groups of rats were fasted prior to feeding upon complete diets containing 20% casein, various amounts of fat (0%, 15%, 30 or 45%) and carbohydrates (presumably 71%, 56%, 41% or 26%, respectively). The rats were killed two hours after the food was presented. The post-prandial free fatty acid levels varied from 0.26 meq/litre to 1.8 meq/litre in those rats consuming the 0% and 45% fat meal respectively.

Related to this increase of the free fatty acid levels were increases of 75%, 185% and 192% of free plasma tryptophan for the 15%, 30% and 45% fat diet, respectively, compared to the 0% fat diet. The total plasma tryptophan levels showed little change although they were slightly decreased by the 45% fat diet.

These findings are in agreement with those of McMenamy and Oncley (1958), Curzon et. al. (1973) and Lipsett et. al. (1973), showing that the free fatty acid levels are a major physiologic regulator of the extent to which plasma tryptophan binds to albumin. The changes are, in general, parallel and proportional. Therefore, it appears that the changes in free plasma tryptophan levels during short-term fasting (24 hours) and feeding are due largely to changes in the levels of plasma free fatty acids.

To explain why total plasma tryptophan levels rose in rats after eating a carbohydrate meal, Fernstrom and Wurtman (1974) suggested that molecules of tryptophan diffused out of the surrounding tissues, especially skeletal muscle, due to the free-fatty acid-mediated decline of free tryptophan in the plasma. The carbohydrate meal would decrease plasma free fatty acids because of the increased insulin secretion which inhibits the mobilization of free fatty acids from adipose tissue. Therefore, the number of available albumin binding sites for tryptophan would be increased, resulting in an increase of bound tryptophan at the expense of the free fraction. As the free plasma tryptophan levels dropped, more

tryptophan would enter the blood by diffusion and the total plasma tryptophan levels would show an increase.

These authors pointed out that humans do not exhibit this increase and suggested that the affinity of tryptophan for albumin is lower in humans than in rats.

## 2.6 Serotonin and the Human Diet

Compared to fasted controls, evidence has been presented to show that brain serotonin synthesis in the rat is increased by the administration of either l-tryptophan or a pure carbohydrate meal, whereas synthesis is reduced by the consumption of a balanced meal. The influence of the diet upon brain serotonin synthesis is therefore complex; its effects being mediated by the three major dietary constituents, namely carbohydrate, protein and fat.

However, the backbone of this evidence is based upon studies with rats and it is important to determine whether there is sufficient evidence available from human studies to indicate that brain serotonin synthesis is similarly influenced by the diet in humans. This is obviously a problem area as the techniques used to record brain serotonin synthesis in rats require the animal to be sacrificed. There are only a few techniques by which brain metabolism can be studied 'in vivo' with humans, and the conclusions thus formed must be made with great caution.

The nearest approach that can be made to the brain is by post-mortem examination. However, studies often suffer from the lack of adequate controls for variables such as time of death, time of chemical estimation, previous ingestion of food and/or drugs and the general bodily and mental health of the deceased.

For these reasons, the majority of studies concerned with the metabolism of the biogenic amines in the human brain use

samples of cerebrospinal fluid (CSF) as an estimator. Moir, Ashcroft, Crawford, Eccleston and Guldberg (1970) critically appraised the justification of the above assumption. From the results of animal studies they concluded that the measurement of cerebral metabolites in the CSF could provide information regarding cerebral metabolism itself. However, the relationships were shown to be complex and the interpretations made from the CSF samples should be made with care. For instance, there is good evidence that peripheral sources of 5-HIAA contribute little to the 5-HIAA concentration in the CSF, but there are data that suggest that a substantial portion of brain 5-HIAA is removed directly by the blood without entering the CSF. Also, anatomical studies have shown that serotonin nerve terminals are present in the spinal cord, especially in the lumber and sacral regions, and approximately half of the 5-HIAA in lumbar CSF may represent metabolic changes of the spinal tissue itself (Bulat and Zivkovic, 1971; Garelis, Young, Lal and Sourkes, 1974; Post, Goodwin, Gordon and Watkin , 1973). Thus, these techniques are not necessarily very accurate in assessing brain serotonin metabolism, but the levels of CSF tryptophan should be a good indicator of the transport rate of plasma tryptophan into the brain.

Using such an indicator, Pérez-Cruet et. al. (1974) have provided evidence suggesting that brain serotonin synthesis is reduced in humans, as found with animals, following the consumption of a balanced meal compared to fasting levels. Unfortunately, similar experiments have not been carried out with humans following l-tryptophan injections or the consumption of carbohydrate meals. An indication as to whether brain serotonin synthesis is increased after these two treatments in humans, as has been reported with rats, can be obtained by comparing the effects of these treatments upon the plasma constituents of both humans and rats. In order that this comparison may be made, it is necessary to list the major changes found from the rat studies. These are:-

i) Injections of l-tryptophan increase both total and free plasma tryptophan levels (Tagliamonte et. al., 1973).

ii) Compared to fasted control rats, the consumption of a pure carbohydrate diet increases total plasma tryptophan levels (Fernstrom and Wurtman, 1972; Madras et. al., 1974 - see Section 2.7) whereas free fatty acids, free plasma tryptophan (Madras et. al., 1974) and other amino acids decline (Munro, 1964).

iii) Compared to fasted control rats, the consumption of a balanced diet increases total plasma tryptophan levels (Pérez-Cruet et al., 1972; Tagliamonte et. al., 1973) whereas free fatty acids and free plasma tryptophan levels decline (Knott and Cruzon, 1972). However, the levels of other amino acids are increased.

It is apparent that, apart from the increase in total plasma tryptophan levels after a carbohydrate meal, all these changes in the plasma constituents have been reported to occur in humans undergoing the same treatments.

For example, Greenwood et. al. (1974) observed that free plasma tryptophan levels were markedly increased following an infusion of l-tryptophan. Lipsett et. al. (1973) reported that both free fatty acids and free plasma tryptophan levels declined following glucose administration. The fact that total plasma tryptophan levels do not increase in humans after glucose administration has already been discussed in the previous section. However, Feldman and Plonk (1976) have reported that total plasma tryptophan levels were increased, whilst plasma tyrosine levels were decreased, following the intravenous administration of glucose to normal subjects. Similarly, recent evidence from Hartmann, Crisp, Evans, Gaitonde and Kirkwood (1979), which was not

available when this review was undertaken, has shown that the ratio of total plasma tryptophan to plasma tyrosine levels is increased in humans following the consumption of a high carbohydrate supplement (see Section 2, Chapter 13 for a review of this study). Pérez-Cruet et. al. (1974) reported that blood samples following a balanced meal contained increased levels of total and bound plasma tryptophan, whereas free plasma tryptophan levels were decreased.

As the above dietary changes have been reported to induce similar changes in the plasma constituents of rats and humans, this would suggest that brain serotonin synthesis is also similarly influenced in rats and humans. This assumes that tryptophan is transported from the plasma into the brain by similar systems. Rats have been found to transport tryptphan into the brain by a active carrier system which also transports the other large, neutral amino acids. It has been found that the rate of transport of tryptophan to the brain (as measured by brain tryptophan levels) is increased in rats when the ratio of total plasma tryptophan to the other competitor neutral amino acids is increased (Fernstrom and Wurtman, 1972). Evidence of a similar carrier system in humans has been provided by the finding that CSF tryptophan levels are positively correlated with the ratio of free plasma tryptophan levels to other neutral amino acids (Pérez-Cruet et. al., 1974). It is interesting that a significant correlation was not found with total plasma tryptophan as the numerator.

Thus, it would appear that the available evidence does suggest that the administration of various diets will induce similar changes in brain serotonin synthesis rate for both rats and humans.

## 2.7 Indirect assessment of brain serotonin synthesis rate in humans

The direct assessment of brain serotonin synthesis rate is impracticable in humans and the indirect assessment from the CSF is probably not suited for use with normal subjects. For these reasons, it would be a great advantage if changes

in brain serotonin synthesis rate could be estimated from the changes in plasma constituents following various diets. There is already experimental evidence that these diet-induced changes in brain serotonin synthesis are significantly correlated to various plasma variables. To recapitulate, changes in the following variables have been positively correlated with changes in serotonin synthesis in the brain or CSF:-

a) In rats - the ratio of total plasma tryptophan to the other neutral amino acids (Fernstrom and Wurtman, 1972).

b) In rats - levels of free plasma tryptophan (Knott and Curzon, 1972; Tagliamonte et. al., 1973).

c) In humans - the ratio of free plasma tryptophan to the other neutral amino acids. (Pérez-Cruet et. al., 1974).

However, it should be noted that the studies of Knott and Curzon (1972) and Tagliamonte et. al. (1973) did not record the levels of the other neutral amino acids. Therefore, the evidence in favour of variable (b) may also be eligible for variable (c). As the free plasma tryptophan levels in these two studies were found to be increased during the fasting period as compared to the post-prandial period, it is apparent that the levels of the other neutral amino acids would have shown opposite changes as their levels would be increased by the ingestion of a balanced meal as compared to fasting levels. Therefore, the ratio of free plasma tryptophan to the other neutral amino acids would be expected to be increased during a fasting period as compared to the period following the consumption of a balanced meal, and the changes in the transport rate of tryptophan into the brain and the associated changes in brain serotonin synthesis rate may well have been more highly correlated to variable (c) than variable (b).

Similarly, the study of Fernstrom and Wurtman (1972) did not record free plasma tryptophan levels and the evidence for variable (a) may also have been eligible for variable (c).

However, Madras et. al. (1974) extended the earlier study of Fernstrom and Wurtman by recording free plasma tryptophan levels after the administration of a carbohydrate meal. These authors found that, compared to 20 hour fasted rats, brain tryptophan was significantly increased within two hours of consuming the meal. Although this was again associated with increased levels of total plasma tryptophan, it was found that the free plasma tryptophan levels actually decreased (as noted in humans also after glucose administration by Lipsett et. al., 1973).

In another experiment, Madras et. al. (1974) found that the large increases in free plasma tryptophan levels resulting from the administration of a high fat diet were not associated with increases in brain serotonin synthesis rates when compared to rats fed on a fat-free diet. In fact, neither brain tryptophan, serotonin or 5-HIAA levels were found to change and the authors pointed out that the total plasma tryptophan levels also showed little variation.

From these two experiments, Madras et. al. concluded that brain tryptophan levels, and therefore brain serotonin synthesis rates, are not necessarily determined by free plasma tryptophan levels. This conclusion strongly suggests that variable (b) is not a suitable indicator of brain serotonin synthesis. It is important to determine if these findings also cast suspicion upon variable (c).

In their first experiment, brain tryptophan levels were found to be increased even though free plasma tryptophan levels were decreased. As the plasma levels of the other neutral amino acids were not recorded it is not possible to assess the validity of variable (c). However, these levels are known to fall rapidly after carbohydrate administration and this experiment does provide further support for variable (a).

As this increase in variable (a) was due to an increase

in the bound plasma tryptophan levels, it may seem surprising that this was associated with the increased transport of tryptophan into the brain, since it is known that albumin-bound compounds do not readily pass the blood-brain barrier. To interpret their data, Madras et. al. stated that the free and bound pools of tryptophan are in an equilibrium that obeys the law of mass action and that the brain could withdraw tryptophan not only from the free pool but also, by mass action, from the bound pool when the affinity for brain transport sites was greater than its affinity for albumin. Thus, when the ratio of total plasma tryptophan to the other neutral amino acids is increased, more brain transport sites will be available causing tryptophan to dissociate from its binding to albumin and enter the brain. This may well be true in rats as the ratio is greatly increased following carbohydrate ingestion. However, total plasma tryptophan levels have actually been reported to be slightly decreased in humans following carbohydrate ingestion, although it is likely that the other amino acids are decreased to a greater extent.

In the second experiment it can be argued that an increase in brain tryptophan, serotonin or 5-HIAA levels should not be expected, even though free plasma tryptophan levels were increased following the high fat diet, given that:-

i) the rise of these variables in the first experiment became significant only after two hours had elapsed following the consumption of the carbohydrate meal. As the food was available for two hours before being removed it is apparent that the changes in these brain variables became significant four hours after food presentation. In the second experiment, the rats were killed two hours after the presentation of the high-fat diets and it is possible that the increased free plasma tryptophan levels were not associated with significantly elevated levels of brain tryptophan, serotonin or 5-HIAA because of the short observation period.

ii) all of the fat diets contained 20% casein dry-weight. It has been shown that the administration of 18% casein dry-weight to a carbohydrate diet prevented the expected rise of brain tryptophan, even though the total plasma tryptophan levels were elevated 75% after one hour in the rat (Fernstrom and Wurtman, 1972). This protein intake may explain why brain tryptophan levels were not increased after the high-fat diets even though the free plasma tryptophan levels were increased.

From the above qualifications it appears that these findings of Madras et. al. (1974) do not seriously detract from the suitability of variable (c) as an indicator of brain serotonin synthesis.

Thus, we are left with two possible indicators, namely variables (a) and (c). As we hope to assess changes in brain serotonin synthesis in humans it is perhaps justified that more attention is paid to variable (c) as it was derived from a human study. It is of great interest to note that non-significant correlations with CSF tryptophan were found in this study for variables (a) and (b). The 21% and 35% decreases in CSF tryptophan and CSF 5-HIAA respectively following the ingestion of a balanced meal were associated with a 50% decrease in variable (c). Variable (a) was, in fact, increased by 1%.

Therefore, it would appear that the ratio of free plasma tryptophan to the other neutral amino acids is the most suitable of the three proposed indicators of brain serotonin synthesis in the human. Those diets which serve to increase this ratio would be expected to also increase brain serotonin synthesis. These diets would be rich in carbohydrate and/or fat but fairly low in protein. The addition of fat to a diet has been shown to greatly increase the levels of free plasma tryptophan even in the presence of insulin (Madras et. al., 1974). Whilst it is known that a pure carbohydrate meal can increase brain serotonin synthesis in the rat, it can only be inferred that this is so in the human. In both the rat and human, the ratio of free plasma tryptophan levels to the other

neutral amino acids is likely to be increased, although not necessarily to a large extent. Therefore, these reported increases in brain serotonin synthesis in the rat may be due to their greatly increased total plasma tryptophan levels from which free tryptophan molecules are extracted by mass action as previously described. As total plasma tryptophan levels have been reported to show a slight decrease following oral glucose administration in humans (Lipsett et. al. 1973), although intravenous administration appears to increase these levels (Feldman and Plonk, 1976), it is possible that brain serotonin is only slightly increased in humans following the consumption of a carbohydrate meal. However, if the carbohydrate meal is supplemented with fat, then this may serve to further increase the ratio of free plasma tryptophan to the other amino acids resulting in a greater synthesis rate of serotonin.

These diet-induced changes in brain serotonin synthesis are described in Figure 1. The change in the rate of tryptophan transport into the brain is depicted by a balance between the effects of the protein content of the diet versus those of the carbohydrate and fat contents.

## 2.8 Summary

Evidence has been provided to show that dietary changes have a large effect upon brain serotonin synthesis rate. For example, Pérez-Cruet et. al. (1972) have shown that brain serotonin synthesis was approximately 30% higher in rats fasted for 24 hours compared to rats fed a balanced meal (containing approximately 25% protein). Furthermore, the ingestion of a carbohydrate meal has been found to increase brain serotonin synthesis above that of fasted control rats (Fernstrom and Wurtman, 1971; Madras et. al., 1974). Thus, it would appear that the synthesis rate of brain serotonin would be greatly altered by changing from a balanced diet to a carbohydrate diet.

Such changes in synthesis rate of brain serotonin would be in response to the altered transport rate of plasma tryptophan into the brain, as illustrated by the levels of brain tryptophan. It has been proposed that this transport rate is dependent upon the ratio of the free plasma tryptophan levels to the other neutral amino acids namely valine, tyrosine, phenylalanine, leucine and isoleucine; an increase in this ratio being associated with an increased transport rate and vice versa. However, in the rat it may be that the transport rate is dependant also upon the ratio of total plasma tryptophan levels to these other neutral amino acids following a high carbohydrate diet. An increase in the levels of brain tryptophan will increase the saturation of tryptophan hydroxylase and consequently increase the rate of brain serotonin synthesis. This is because the hydroxylation of tryptophan to 5-HTP is the rate-limiting step in brain serotonin synthesis. Similarly, a decrease in brain tryptophan levels will lead to a decrease in brain serotonin synthesis.

Although the bulk of literature arises from animal studies, it is apparent that sufficient information is available from human studies to warrant the assumption that similar changes would be found in brain serotonin synthesis rates following various dietary changes.

From experimental observation and the rationale depicted in Figure 1, it is possible to rank some diets according to the effect they would have upon brain serotonin synthesis rate, compared to the consumption of a balanced diet. (See Table 4).

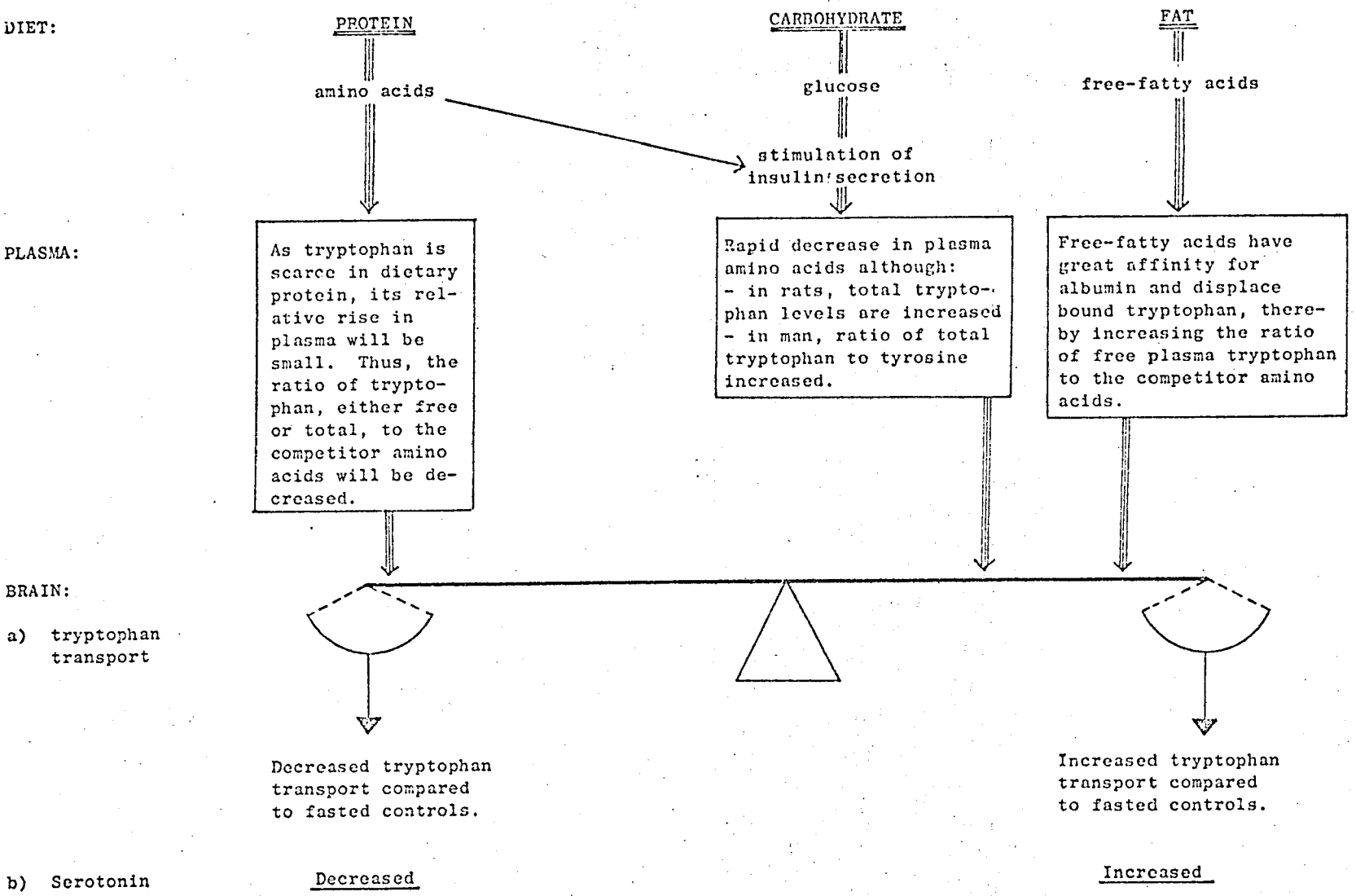



FIGURE 1: The influence of the diet upon brain serotonin synthesis.

Table 4. Various diets ranked according to their expected effect upon brain serotonin synthesis rate compared to the consumption of a balanced diet.

| Diet | Effect upon Brain Serotonin Synthesis Rate |
|---|---|
| 1. High carbohydrate and/or high fat diet, low in protein<br>2. Fasting (short duration i.e. 24 hours)<br>3. Balanced diet ( 25% protein) | ⇧ high rate of brain serotonin synthesis |
| 4. High protein diet, low in carbohydrate and fat | ⇩ low rate of brain serotonin synthesis |

## 3. CATECHOLAMINES AND THE DIET

The literature concerning the influence of the diet upon brain catecholamine metabolism is very small compared to that available for brain serotonin metabolism.

### 3.1 Tyrosine and phenylalanine content

The catecholamines are synthesised intra-neuronally from the dietary precursor amino acids l-tyrosine and l-phenylalanine. If phenylalanine is used as the substrate, it is first hydroxylated to tyrosine. Tyrosine is then hydroxylated to l-dihydroxyphenylalanine (l-DOPA) by tyrosine hydroxylase enzyme. However, it has been estimated that the concentration of tyrosine in the brain is at least ten times the michaelis constant for tyrosine hydroxylase (Fernstrom and Wurtman, 1972). Therefore, although large doses of tyrosine or phenylalanine might increase the hydrozylation rate, it is debatable whether smaller changes in brain tyrosine would greatly influence this hydroxylation rate and, subsequently, catecholamine synthesis.

For example, large intra-peritoneal injections of l-tyrosine 2 g/kg) have been reported to increase brain tyrosine levels in rats by a factor of more than 40 within 2 hours (McKean et. al.,

1968). Similarly, the administration of a 7% tyrosine fortified diet for three weeks was found to increase brain tyrosine levels by a factor of nearly 60 (McKean et. al., 1968). However, only a 2-fold increase in brain tyrosine levels has been reported with smaller doses of l-tyrosine after one hour (375 mg/kg - Fernstrom and Wurtman, 1972; 100 mg/kg - Fernstrom and Wurtman, 1975). This order of change in brain tyrosine levels has been found to increase l-DOPA levels in rats pre-treated with a l-amino acid decarboxylase inhibitor, which blocks the formation of dopamine from l-DOPA. Compared to control rats, who did not receive the l-tyrosine injection, the l-DOPA level was increased by approximately 16% after 45 minutes. Therefore, the increase in brain dopamine and noradrenaline levels would be expected to be fairly small as both of these neurotransmitters are dependant upon l-DOPA levels. This small change may be compared to the 68% increase in brain serotonin levels reported 30 minutes following an injection of 100 mg/kg l-tryptophan in rats (Moir and Eccleston, 1968). Thus, it would appear that serotonin synthesis is more sensitive than catecholamine synthesis to the administration of small doses of their respective amino acid precursors.

### 3.2 Other dietary factors

It would seem likely that the changes in brain catecholamine synthesis rate following various diets would be opposite to those of brain serotonin. This is because those diets that serve to increase the transport of tyrosine and phenylalanine into the brain would decrease the transport of tryptophan and vice versa. However, these changes in catecholamine synthesis rate would be expected to be of a smaller extent compared to those changes in serotonin synthesis rate. This is because the levels of brain tyrosine are normally well above the saturation point for the enzyme tyrosine hydroxylase whereas the enzyme tryptophan hydroxylase is not normally saturated and is thus a rate-limiting step in the synthesis of serotonin. Also, it is thought that a negative feedback system controls the rate of catecholamine synthesis to meet the moment to moment demands for neurotransmitter release. Thus, although brain tyrosine levels may be altered following

dietary changes, it does not necessarily follow that brain catecholamine synthesis will also be altered to any appreciable extent.

No studies have been found which report upon the effects of carbohydrate or fat diets upon brain tyrosine levels or brain catecholamine synthesis. Preliminary studies by Fernstrom and Wurtman (1975) have shown that high protein meals (40%) serve to increase both brain tyrosine and l-DOPA levels in rats compared to fasted controls.

## 4. ACETYLCHOLINE AND THE DIET

The literature concerning the influence of the diet upon brain acetycholine metabolism is sparse compared to that available for brain serotonin metabolism.

### 4.1 Choline content

Acetylcholine is synthesised from choline and acetyl coenzyme A in cholinergic nerve terminals. This reaction is catalysed by the enzyme choline acetyl transferase. As acetyl coenzyme A is formed by the metabolism of carbohydrates and fats in all living cells, it appears that the supply of acetyl coenzyme A is not a major factor controlling the metabolic rate of acetycholine. However, choline has to be actively supplied to the neuron and it is likely that the availability of blood choline is an important factor controlling the metabolic rate of acetycholine.

Choline is an indispensable constituent of the body but choline deficiency is rare in humans because acetyl choline can be synthesised in the liver and it is readily available from the diet. Choline is a constituent of several phospholipids (e.g. lecithin, sphingomyelin) which are present in most cellular membranes. Unlike the liver, the brain cannot synthesise choline and is thus dependent upon the supply of choline from the blood.

Although the specific pathways whereby choline is supplied to the cholinergic neurons are not yet clear, there is some evidence to show that changes in choline intake have been associated with changes in brain acetylcholine levels. Nagler et. al. (1968) have reported significant decreases in brain acetycholine content of weanling rats deprived of choline for 5 days. Cohen and Wurtman (1975) have shown that a single intraperitoneal administration of choline can cause sequential rises in brain choline and acetycholine in rats. However, these findings do not necessarily infer that changes in the diet can influence brain acetylcholine metabolism as the above studies did not observe the effects of changes in choline intake within the normal physiological range.

A later study by Cohen and Wurtman (1976) provided evidence to show that physiological variations of choline intake are associated with parallel changes in brain acetylcholine levels. Groups of rats were fed either on a choline deficient diet or with a choline supplemented diet (choline content 0.18 or 1.83 percent) for 11 days. Approximately half the rats were then killed by microwave irradiation and their brain acetycholine levels recorded, whilst the remaining animals were decapitated and their plasma choline levels recorded. (The use of a focussed microwave beam directed at the brain was used to prevent the rapid rise in free brain choline which occurs after death (Stavindha, 1973). This technique inactivates cholinesterase which would otherwise liberate choline from acetycholine).

Plasma choline levels were found to be proportional to the mean dietary choline intake and the levels of brain acetylcholine were similarly increased as a result of larger intakes of choline. The increase in brain acetylcholine levels was apparently due to an increase in acetylcholine synthesis, rather than a decrease in its breakdown, as the increases found following an increased choline intake or after treatment with physostigmine (which inhibits the action of cholinesterase) were additive.

Cohen and Wurtman (1976) suggested that the variations in choline intake used in their study may be comparable to day-to-day or long term variations in choline intake encountered in human diets.

## 4.2 Other dietary factors

No studies have been found which report upon the effects of dietary changes, other than changes in the choline intake, upon brain acetycholine metabolism. Thus, whilst diets which contain large amounts of meat (meat has a relatively high choline content compared to most other foods) will contain more choline than diets composed mainly of fruits and soft vegetables, it is not known whether other dietary factors such as the protein, fat and carbohydrate content might have an influence upon the transport of choline to the brain.

## 5. LIMITATIONS TO THE ASSESSMENT OF NEUROTRANSMITTER METABOLISM BY PRECURSOR AVAILABILITY

Whilst this chapter has been orientated towards the effects of the diet upon neurotransmitter metabolism, it has only considered the changes in metabolism as a consequence of changes in precursor availability to the brain. It is very likely that their metabolism is influenced by other dietary factors. For example, psychological distress occurring during fasting or following the consumption of indigestible or noxious foods may well influence neurotransmitter metabolism by routes other than precursor availability. Thus, the assessment of neurotransmitter metabolism in these cases with respect to precursor availability may be suspect.

Some foods contain psychologically active constituents; for example, coffee contains caffeine, tea contains caffeine and theophylline, and cocoa contains caffeine and theobromine. All these drugs produce a stimulating effect on the nervous system. The dose of caffeine required to produce stimulation is of the order of 100 - 300 mg, which is equivalent to one or two cups of coffee, tea or cocoa. The effects of caffeine

vary from person to person, but a dose of 1000 mg or more can produce undesirable effects such as insomnia, excitement, increased heart rate and breathing, increased urination, ringing in the ears and flashes of light in front of the eyes. The drinking of alcoholic beverages and tobacco smoking are often associated with eating and both of these practices can lead to changes in behaviour. Thus, it is clear that the ingestion or inhalation of psychologically active drugs must be avoided or controlled for when assessing the influence of the diet upon neurotransmitter metabolism with reference to precursor availability to the brain. As most of the population are reliant on some, if not all, of the drugs described above, it would be unwise to ban them during any experimental study because of the complicating factor of withdrawal symptoms.

Another limitation to the assessment of neurotransmitter metabolism by precursor availability is that it does not take the current behaviour of the animal or human into account. It is probable that different behaviours, (for example, sexual behaviour, fear, sleep) are associated with varied demands upon the neurotransmitter systems, either in general or for specific systems only, resulting in changes in their metabolism. Thus, the assessment of changes in neurotransmitter metabolism following various diets is unlikely to be accurate if behaviour patterns are markedly different during the dietary periods. For experimental purposes, this means that care should be taken to ensure that the subjects are not subject to periods of great stress or depression during any study where the assessment of neurotransmitter metabolism is assessed by precursor availability only.

## 6. OVERVIEW

The effects of the diet upon neurotransmitter synthesis are by no means clear. Whilst the great majority of studies have reported upon the effects of the diet upon brain serotonin metabolism, it should be noted that most of these findings refer to rats and it is possible that the diet has different

effects on brain serotonin metabolism in humans. However, the findings from the human studies suggest that this is not so.

The available literature suggests that the effects of various dietary changes upon brain serotonin synthesis rates can be indirectly assessed with reference to various components of the blood. Of the three such indicators examined (the ratio of total plasma tryptophan to the other neutral amino acids; the levels of free plasma tryptophan; the ratio of free plasma tryptophan to the other neutral amino acids), it was decided that the ratio of free plasma tryptophan to the other neutral amino acids was the most suitable indicator of brain serotonin synthesis rate in humans.

Accordingly, it is proposed that diets can be ranked according to the effect they would have upon brain serotonin synthesis in humans. For example, a high carbohydrate and high fat diet, which was low in protein, would be expected to increase brain serotonin synthesis compared to rates following the consumption of a high protein diet which was low in carbohydrate and fat.

This prediction does not allow for the possibility that brain serotonin metabolism is sensitive to behavioural changes and it is important that the psychological disposition of the subject does not vary greatly between the two dietary periods.

The effects of dietary changes upon brain catecholamine metabolism are not clear, although it is suggested that the changes would be opposite and of a smaller extent to those regarding brain serotonin metabolism.

The influence of the diet upon brain acetylcholine is even less understood and no clear statement can be made concerning the natural dietary changes as opposed to the addition of choline supplements.

CHAPTER 10

# THE INFLUENCE OF BRAIN SEROTONIN METABOLISM UPON SLEEP

## 1. INTRODUCTION

From the previous chapter, it would appear the changes in the diet can influence neurotransmitter metabolism, especially brain serotonin metabolism. This chapter will attempt to identify the relationship, if any, between brain serotonin metabolism and sleep, with special reference to humans.

It is appreciated that the mechanisms whereby the diet can influence sleep are probably complex and the likelihood of such an influence being mediated only by changes in brain serotonin metabolism is very remote.

The literature is reviewed in Appendix IV and this chapter discusses the general findings from the neurophysiological, pharmacological and correlational approaches to the study of brain serotonin metabolism and sleep.

## 2. SUMMARY OF THE NEUROPHYSIOLOGICAL STUDIES

### 2.1 Problems of This Approach

This approach to the study of sleep has several drawbacks. Although the targets for destruction are usually small areas, carefully chosen to affect only specific neurotransmitter systems, there is still the problem of other, as yet unknown, systems being affected. This is a very real possibility because it is known that the destruction of areas of the locus coeruleus and substantia nigra will not only affect the functioning of the catecholaminergic system, but also the cholinergic and serotonergic systems. As the techniques for detecting putative neurotransmitters becomes more sophisticated, it is quite likely that new systems will be discovered which may be intimately involved with sleep.

The inevitable damage caused to the brain during the neurophysiological studies may affect local blood supply and other variables which could influence sleep behaviour via a

non-specific mode of action. Another drawback of these studies is that they do not observe the effect of physiological changes in the neurotransmitter systems.

In the early stimulation experiments the animals had to be restrained and kept immobile due to the sensitivity of the equipment. This is obviously stressful and it is very important to use satisfactory control methods. If the electrical stimulation of a certain area is found to consistently induce sleep, there is still the problem that this change in behaviour is probably not as a consequence of a change of a physiological magnitude in the function of the target site. For example, the current and frequency used may not be of a physiological magnitude.

Due to the nature of this approach, our knowledge is based almost entirely on animal studies and it can only be assumed that this knowledge is applicable to the human brain.

## 2.2 General Findings

In spite of these drawbacks, a lot of very useful data has come from the neurophysiological approach. These studies have been reviewed in Section 5, Appendix IV. The main findings are described below.

a) Jouvet (1969) reported that the destruction of the 5-HT containing perikarya located in the raphe system induced insomnia in the cat. It was demonstrated that a significant correlation existed between the extent of the lesion, the intensity of the resulting insomnia and the decrease of brain 5-HT.

The large scale destruction of the raphe nuclei greatly reduced NREM sleep and abolished REM sleep.

b) Kostowski and Giacalone (1969) and Gumulka, Samanin, Valzelli and Consolo (1971) have reported that behavioural

and EEG sleep can be induced by low frequency electrical stimulation of the raphe system.

c) Jouvet (1972) suggested that the anterior raphe neurons may be more involved with the regulation of NREM sleep whereas the caudal raphe neurons may be involved in the "priming" of REM sleep.

d) Jouvet (1974) suggested that the arousal following the destruction of the raphe system is probably mediated by catecholaminergic mechanisms.

These findings present evidence that brain serotonin is involved in the sleep mechanism. However, as this evidence is solely from animal studies and its relevance to brain serotonin function in humans can only be assumed.

There is evidence to indicate that brain serotonin may be involved in the regulation of both NREM and REM sleep, although the effects of the destruction of the raphe neurons probably influences other neurotransmitter systems which may also be involved in the regulation of these states of sleep.

## 3. PHARMACOLOGICAL STUDIES

### 3.1 Problems of This Approach

The difficulty in finding drugs that act exclusively upon the metabolism of a certain neurotransmitter is the crucial limitation to pharmacologically based studies of sleep mechanisms. For example, the monoamine oxidase inhibitors, (MAOI), cocaine, tricyclic anti-depressants and reserpine affect the CNS levels and turnover of all the monoamine neurotransmitters. It would appear, therefore, that the precursor loading studies should provide the clearest data on the function of a specific neurotransmitter in the sleep mechanism. However, whilst the administration of tryptophan selectively influences the metabolism of brain serotonin,

because the enzyme tryptophan hydroxylase is present only in serotonergic neurons, this is not true for the administration of 5-HTP. This is because exogenous 5-HTP can be synthesised to serotonin in catecholaminergic neurons as the enzyme l-amino acid decarboxylase is present in both serotonergic and catecholaminergic neurons.

Another variable to be assessed is the drug dosage. For any given drug this varies from study to study, but there is usually a large difference between human and animal dosages. For example, in the PCPA studies, the animal doses ranged from 50-1000 mg/kg whereas the human dosages were rarely above 50 mg/kg. The reported effects of PCPA upon sleep are far greater for animals than humans and this difference in dosage might well be a major factor.

Comparisons between animals and humans with regard to drug response are always extremely difficult. The dosages can be roughly equated by adjusting for body weight, brain weight or metabolic rate differences. The first method has been used to achieve approximately equal drug concentration in the body fluids of large and small mammals, unless the drug is concentrated in the brain when the second method is of more value. Scaling by metabolic rate allows for the equivalent excretion rate of the drug and its metabolites. Thus, it is often difficult to compare animal studies with human studies and, therefore, only the human studies will be summarised. This does not infer that the animal studies have little value in sleep research; in fact the majority of discoveries have been made in animal studies. However, this review is primarily concerned with the investigation of those neurotransmitters that are closely involved in the regulation of sleep in man.

A further problem with the pharmacological studies is the fact that the drug doses studied often induce major

changes in neurotransmitter metabolism which are outside the normal physiological range. Thus, the relevance of these studies to the understanding of the sleep mechanisms in natural drug-free sleep is unclear.

## 3.2 General Findings

The literature from the pharmacological studies is reviewed in Section 6, Appendix IV.

From these studies, it appears that the number of drugs that consistently influence SWS in humans is very limited. Only reserpine has been consistently reported to decrease SWS (see Section 6.5, Appendix IV), although SWS has been reported to be either unchanged or decreased following the administration of methysergide (see Section 6.6, Appendix IV) or large doses of 5-HTP (see Section 6.3.2, Appendix IV). Significant increases in SWS have been reported in some tryptophan studies, but the majority report an unchanged duration of SWS throughout the dose range studied (see Section 5.3.1, Appendix IV).

Significant changes in REM sleep in humans have been reported with all of the drugs reviewed, although the associated changes in brain serotonin metabolism (assessed by the levels, synthesis or turnover rate of brain serotonin) do not appear to exhibit a consistent relationship to these changes in REM sleep.

There is, however, some strong evidence of a positive relationship between brain serotonin metabolism and REM sleep. For example, the administration of p-chlorophenylalanine (PCPA), which decreases brain serotonin synthesis, also decreases REM sleep without appreciably affecting other stages of sleep or sleep length (see Section 5.4, Appendix IV). The lack of a REM rebound following the withdrawal of this drug indicates that PCPA interferes with a major pathway for

the appearance of REM sleep, rather than just suppressing its occurence. As PCPA blocks the formation of 5-HTP from tryptophan, it is of interest that the administration of 5-HTP reverses the depression of REM sleep in chronically PCPA-treated patients.

Methysergide, which blocks the action of serotonin at the post-synaptic receptor sites, has also been reported to decrease REM sleep (see Section 5.6, Appendix IV). Whilst the administration of this drug appears to have little influence upon brain serotonin metabolism, the effect it induces is similar to that following a decrease in brain serotonin synthesis where the availability of serotonin to the synapse is reduced.

Conversely, the administration of small doses of 5-HTP have been reported to increase REM sleep (see Section 5.3.2., Appendix IV). As the administration of 5-HTP is known to increase brain serotonin synthesis, this evidence also indicates a positive relationship between brain serotonin metabolism and REM sleep. Interestingly, low doses of MAOI have also been reported to increase REM sleep (see Section 5.8, Appendix IV). This drug is known to decrease the turnover of brain serotonin, thereby increasing the levels of brain serotonin.

From the above, there appears to be a putative link between the availability or efficiency of serotonin at the synapse and REM sleep in humans. Thus, a decrease in the availability of serotonin following PCPA administration, or a decrease in the efficiency of serotonin to stimulate the post-synaptic receptor sites following the administration of methysergide have both been reported to decrease REM sleep. An increase in the availability of serotonin following the administration of small does of 5-HTP or MAOI have both been reported to increase REM sleep.

Whilst such a putative relationship is attractive, it appears that the findings from the other studies are not consistent with this relationship. For example, the administration of large doses of 5-HTP or MAOI both decrease REM sleep. The administration of the re-uptake blocking drugs, such as cocaine or the tricyclic anti-depressants, have been reported to decrease REM sleep even though they would be expected to prolong the duration of the stimulation of the post-synaptic membrane (see Section 6.7., Appendix IV). Reserpine, which blocks the intra-neuronal storage of serotonin and induces a decrease in the levels of brain serotonin, has been reported to increase REM sleep (see Section 6.5., Appendix IV). The relationship between the administration of tryptophan, which would be expected to increase brain serotonin synthesis, and REM sleep is not clear as this stage of sleep has been reported to be increased, unchanged or decreased (see Section 5.3.1., Appendix IV).

The findings from the studies administering reserpine, cocaine, tricyclic antidepressants and large doses of 5-HTP and MAOI may all influence the metabolism of brain catecholamines. This non-specific action of these drugs could explain why their effects upon REM sleep are not consistent with the putative relationship described above.

Interestingly, the intraperitoneal administration of large doses of MOAI (100mg/kg pargyline, 20mg/kg tranylcypromine, 20mg/kg phenelzine; Aghajanian, Graham and Sheard (1970), large doses of 5-HTP and tryptophan (>25mg/kg; Trulson and Jacobs, 1975, 1976) and the intravenous administration of anti-depressant tricyclics (Sheard, Zolovick and Aghajanian, 1972) have all been found to decrease the firing rate of the raphe neurons in the rat. (Most of the perikarya containing serotonin are located in the nuclei of the raphe system). These authors have also studied the effects of these drugs upon the firing rate of neighbouring non-raphe neurons and

have reported that they are either unaffected or increased, although a few neurons showed a slight reduction in rate. All these drugs are known to increase the availability of serotonin in the brain, either by increased synthesis or decreased breakdown, and are thus thought to enhance synaptic transmission in the serotonergic system. Therefore, the finding that they actually decrease the firing rate of the raphe neurons is somewhat surprising.

It has been proposed (Aghajanian et. al., 1970) that the depression of raphe neuron firing may be a negative feedback mechanism which compensates for the drug-induced enhancement of synaptic transmission. Taking this one step further, it has been suggested (McGinty, Harper, and Fairbanks, 1973) that when these drugs are administered, the negative feedback control may be stimulated more than the remote receptor sites by the increased availability of serotonin. If this is true, then the net result would be a depression of synaptic transmission in the serotonergic system.

This rationale may explain why the administration of the anti-depressant tricyclics and large doses of 5-HTP (13 mg/kg) and MAOI (60 mg/kg phenelzine) have all been reported to decrease REM sleep in humans, even though the availability of serotonin at the synapse is increased.

However, it has been reported that small doses of MAOI (5-15 mg/kg phenelzine) or 5-HTP (8 mg/kg) can increase REM sleep in humans. If the above rationale is correct then it may be suggested that the intraneuronal levels of serotonin were not increased to a sufficient degree to stimulate the negative feedback control. This possibility is supported by the fact that raphe neuron firing has only been observed to be decreased in animals with doses of MAOI and 5-HTP larger than those found to increase REM sleep in

humans. As stated before, there are problems with equating dose levels between animals and humans, but it is of interest that the effects of 5-HTP in animals are also dependant upon the dose administered (see Section 5.3.2., Appendix IV). Low doses (1-5mg/kg) appear to enhance cortical synchronisation, although REM sleep also shows a tendency to be increased, whereas large doses (20-60mg/kg) definitely induce prolonged synchronisation and REM sleep is completely suppressed. This 2-fold effect may be a result of a drug-induced decrease in the firing rate of serotonergic neurons at high doses of 5-HTP as Trulson and Jacobs (1975) have reported that the decrease in firing rate of the raphe neurons is dependant upon the dose of 5-HTP administered. For example, 150 mg/kg 5-HTP reduced the firing rate by ∿70% whereas 25mg/kg only effected a 30% decrease and 10mg/kg did not significantly affect the rate.

Thus, it appears that the changes in REM sleep reported by drugs which affect brain serotonin metabolism may be related to the influence of these drugs upon synaptic transmission in the serotonergic system. For example, low doses of 5-HTP and MAOI would be expected to increase the availability of serotonin to the synapse, and thereby enhance synaptic transmission. Conversely, PCPA, methysergide, antidepressant tricyclics and large doses of 5-HTP and MAOI may depress synaptic transmission. This depression would be mediated by a decrease in post-synaptic receptor stimulation following methysergide administration or by a decrease in neuronal firing rate following the administration of large doses of 5-HTP and MAOI.

The fact that the administration of tryptophan has a similar effect to 5-HTP upon raphe neuron firing in rats (Trulson and Jacobs, 1975) would suggest that the effect of tryptophan upon REM sleep might also be dose dependent. There is some evidence for this from the study by Hartmann, Cravens and List,(1974) who reported that REM sleep was not

influenced by doses of 5g or less of tryptophan, whereas 10g and 15g doses produced a decrease. However, Griffiths, Lester, Coulter and Williams (1972) reported an increase in REM sleep following the administration of 12g of tryptophan, and Hartmann (1967) reported an increase with a dose between 5-10g tryptophan. Clearly, further study is required concerning the effect of tryptophan upon REM sleep in humans.

One factor which may be of relevance to the study of tryptophan upon sleep is the subject's diet or the nature of the vehicle with which the tryptophan dose is administered. From Chapter 9 it is clear that the diet has an influence upon the transport of tryptophan to the brain. It may be more than coincidence that the study by Griffiths et. al. (1972), which reported a large increase in REM sleep, used a high carbohydrate vehicle.

## 4. SUMMARY OF THE CORRELATIONAL STUDIES

### 4.1 Problems of This Approach

The correlational approach to the study of sleep has, as yet, contributed little compared to the pharmacological and neurophysiological approaches to our understanding of the roles of the neurotransmitters during sleep. This does not infer that the correlational approach is inferior to the other approaches, but that the findings from such an approach are inherently complex. This approach is extremely useful for identifying associations between physiological and psychological events, but its main drawback lies in the fact that it is often difficult to decide whether the observed changes in physiological variables are caused by changes in psychological variables or vice versa. For example, when we go to sleep our heart-rate slowly declines and remains fairly steady, although during REM sleep the heart-rate is very variable and it can often be considerably increased above levels during relaxed wakefulness.

This association cannot be taken to infer that a slow heart-rate causes NREM sleep whilst a variable heart-rate causes REM sleep. In addition to this problem of "cause or effect", there is also the possibility that the observed association is purely temporal with no underlying functional interaction.

For these reasons, the findings from the correlational approach need to be complemented by findings from the other approaches.

4.2 General Findings

A review of the correlational studies is presented in Section 7, Appendix IV. The main findings are described below.

a) Chen, Kalucy, Hartmann, Lacey, Crisp, Bailey, Eccleston and Coppen (1974) have reported that mean free plasma tryptophan levels during sleep are positively correlated with REM sleep and negatively correlated with NREM sleep in man.

b) McGinty, Harper and Fairbanks (1973) reported that the firing rate of the raphe neurons was greatly decreased during REM sleep in the cat.

c) Wyatt, Neff, Vaughan, Franz and Ommaya (1974) observed that the concentration of ventricular 5-HIAA was highest during NREM sleep and lowest during REM sleep in patients with presenile dementia. The concentration of 5-HIAA during wakefulness was found to be between the levels noted during NREM and REM sleep.

d) Kuhn, Rysanek, Brodan and Spankova (1976) found that prolonged sleep deprivation was associated with an increase in free plasma tryptophan levels in humans. Such an increase may induce an increase in brain serotonin synthesis as has been reported by Hery, Pujol, Lopez, Macon and Glowinski (1970)

with REM deprived cats. However, sleep deprivation is stressful and many studies have reported increases in brain serotonin synthesis following various stressors (see Section 7.2 for references). The increase in free plasma tryptophan observed by Kuhn et. al. (1976) was possibly mediated by an increase in plasma free fatty acids which have also been reported to increase during sleep deprivation (Kuhn, Braun, Brodan, Valek and Vojtechovsky, 1965). Knott and Curzon (1972) have observed increases in free plasma tryptophan following immobilisation stress in rats and this suggests that the changes observed by Kuhn et. al. (1976) were stress-induced.

The finding of a positive correlation between mean free plasma tryptophan levels and REM sleep in humans was taken by the authors to suggest that brain serotonin synthesis was closely linked with the REM sleep mechanism. This was because increased free plasma tryptophan levels have been associated with increased brain serotonin synthesis in animals (see Section 2.4.1., Chapter 9).

Such a conclusion is consistent with the proposed putative relationship between changes in REM sleep and changes in the synaptic transmission of the serotonergic system developed in the previous section. However, both the finding of a large decrease in the firing rate of the raphe neurons during REM sleep in cats and the reported decrease in 5-HIAA levels during this stage of sleep in humans argue that synaptic transmission in the serotonergic system is depressed during REM sleep.

It is important to note that the above findings are not inconsistent with the proposed relationship, although it needs to be qualified to incorporate these findings. As the proposed relationship was derived from the drug studies, it relates to gross changes in REM sleep and synaptic

transmission over periods of several hours or days. Thus, it does not necessarily follow that an enhancement of synaptic transmission would immediately induce REM sleep. However, it is possible that a prolonged enhancement or depression of the serotonergic system may be associated with an increase or decrease in REM sleep, respectively, over a representive time period.

## 5. OVERVIEW

From the experimental evidence summarised in this chapter, it is apparent that brain serotonin metabolism is involved in the sleep mechanism. Apart from the neurophysiological studies, the discussion has been concerned with the influence of brain serotonin metabolism upon sleep in the human. It is suggested that the prolonged enhancement or depression of synaptic transmission in the serotonergic system may be associated with an increase or decrease in REM sleep, respectively, over a representative time period.

It is appreciated that other neurotransmitters are involved in the sleep mechanism and, also, that sleep can be influenced by many factors other than changes in neurotransmitter metabolism.

CHAPTER 11

THE THEORETICAL MODEL

## 1. INTRODUCTION

In this chapter it is intended to integrate the literature reviews of the last two chapters to develop a theoretical model from which it is possible to predict the effects of certain changes in the diet upon human sleep. These predictions will be based upon the expected changes in neurotransmitter metabolism following the dietary changes.

The initial validity of this theoretical model will be examined by comparing the predicted changes in sleep following dietary changes with those reported in the diet and sleep studies reviewed in Chapter 7 and from Study III of this thesis. If these comparisons show a good degree of agreement then it is proposed to design an experiment to further test the model.

## 2. THE THEORETICAL MODEL

The purpose of this section is to summarise the findings from the previous two chapters in the form of a model which describes one possible mechanism by which the diet can influence human sleep.

Of the neurotransmitters studied, it is clear that there is a substantial amount of evidence linking changes in the carbohydrate, fat and protein content of the diet with changes in brain serotonin metabolism (see Section 2.8, Chapter 9). There is little direct experimental evidence concerning the effects of such changes upon brain catecholamine metabolism, although it is likely that any changes in metabolism will be small compared to those in brain serotonin (see Section 3.5, Chapter 10). The effects of dietary changes upon brain acetylcholine metabolism are not yet known, unless they involve large changes in choline intake. Thus, the influence of the diet upon sleep will be assessed by changes in brain serotonin metabolism only.

From the previous chapter, it is apparent that brain serotonin metabolism is involved in the sleep mechanism. More specifically, it was suggested that the "prolonged

enhancement or depression of synaptic transmission in the serotonergic system may be associated with an increase or decrease in REM sleep, respectively, over a representative time period".

Thus, one possible mechanism by which the diet can influence human sleep is via changes in the functional activity of the serotonergic system. Moderately large changes in brain serotonin synthesis rate would be predicted following a change of diet from a high protein to a high carbohydrate and/or fat diet. This change of diet would be predicted to enhance synaptic transmission in the serotonergic system and, consequently, increase REM sleep. The increase in the availability of serotonin to the synapse would not be as radical as following large doses of 5-HTP or MAOI, and the putative negative feedback mechanism would not be expected to come into operation. (See Section 3.2, Chapter 10). Similar changes would be expected following a change from a balanced diet or fasting to a high carbohydrate and/or fat diet, although to a lesser extent. The predicted changes in REM sleep following various dietary changes are summarised in Table 1.

Table 1: The predicted effects of various diets upon some aspects of brain serotonin metabolism and REM sleep, compared to the consumption of a balanced diet.

| DIET | Predicted Effects Upon | | |
|---|---|---|---|
| | Brain serotonin synthesis | Synaptic transmission in the serotonergic system | Duration of REM sleep |
| 1. High carbohydrate and/or high fat diet, low in protein.<br>2. Fasting (24 hrs.)<br>3. Balanced diet (∿25% protein).<br>4. High protein diet, low in carbohydrate and fat. | ↑ increased<br>↓ decreased | ↑ enhanced<br>↓ depressed | ↑ increased<br>↓ decreased |

## 3. COMPARISON OF PREDICTED AND OBSERVED CHANGES IN REM SLEEP.

The comparison between predicted and observed changes in sleep will be a difficult task. Whilst it is proposed that the content of the diet is a major factor influencing sleep, it is also accepted that sleep is influenced by many other factors which may, or may not, be associated with the diet. Fasting, for example, induces both psychological and physiological stress and it is not yet clear to what extent each of these stresses influences sleep.

Another problem is the fact that many of the diet and sleep studies did not report on the amounts of the various dietary contents. These studies cannot, therefore, be used for comparison purposes. Comparisons will be made with both the animal and human literature.

### 3.1 Animal Studies

It has been reported that feeding in animals is associated with increased sleep, especially REM sleep, compared to fasted controls (Fara, Rubinstein and Sonnenschein, 1969; Rubinstein and Sonnenschein, 1971; Ruckebusch and Gaujoux, 1976). Furthermore, it has been reported that food intake during a 12 hour period, day or night, shows a significant negative correlation to the amount of REM sleep taken during the preceding 12 hour period (Siegel, 1974).

Thus, there appears to be a link between food intake and REM sleep in animals. However, the contents of protein, fat and carbohydrate were rarely specified in the literature and it is therefore difficult to predict what changes would occur in REM sleep.

The administration of fat into the duodenum of cats has been reported to increase the frequency and/or duration of REM periods in the subsequent 3 hour period compared to fasted

controls (Rubinstein and Sonnenschein, 1971). This is consistent with the predicted changes in REM sleep. However, both NREM and REM sleep have been reported to be increased in sheep fed on a urea-fortified diet (Ruckebusch and Gaujoux, 1976). This diet is not, strictly speaking, a high protein diet as urea is not a protein. However, it can be made available for protein synthesis by the action of the micro-organisms in the sheep's digestive system. This finding is not consistent with the predicted changes in REM sleep but this discrepancy may be due to the effects of urea administration, such as hyperammonemia.

### 3.2 Human Studies

The effects of food intake upon human sleep have already been summarised in Table 1 of Chapter 7. To recapitulate, these various studies were ordered according to the direction and the extent of the sleep changes. From this ordering it appeared that changes in food intake or calorie intake might account, in part, for the changes in sleep. However, the finding that isocaloric dietary changes are associated with large sleep changes strongly suggests that the content of the diet is also an important factor. It was proposed that the direction and extent of sleep changes observed in the studies may have been related to the changes in the amount of carbohydrate and/or fat consumed as progressive increases in the amount of carbohydrate and/or fat consumed were associated with progressive changes in sleep.

Small increases in carbohydrate and/or fat intake, for example after a bedtime beverage (Brezinova and Oswald, 1972) or possibly associated with periods of weight gain (Crisp and Stonehill, 1973), have been associated with increased sleep length or decreased wakefulness. A more substantial increase in carbohydrate and fat intake, for example during the refeeding of anoretics (Crisp, Stonehill, and Fenton, 1971;

Lacey, Crisp, Kalucy, Hartmann and Chen, 1975), the administration of a high fat diet (Phillips, Chen, Crisp, Koval, McGuiness, Kalucy, Kalucy and Lacey, 1975), or the consumption of a high carbohydrate supper (Study III), were associated with decreased wakefulness or stage 1 sleep and moderately increased REM sleep. The administration of a high carbohydrate diet was associated with decreased stage 1 sleep, greatly increased REM sleep and moderately decreased SWS (Phillips et. al., 1975).

There is evidence, therefore, that as the carbohydrate and/or fat content of a diet increases, then REM sleep also increases. This is consistent with the predicted changes in REM sleep as determined from Table 1 of this chapter.

According to the prediction, short-term fasting (i.e. 24 hours) would be expected to increase REM sleep above values observed following the consumption of a balanced meal. This predicted change was not observed in the two starvation studies. One study (MacFadyen, Oswald and Lewis, 1973) reported progressive decreases in REM sleep as the duration of fasting continued, although the other study (Karacan, Rosenbloom, London, Salis, Thornby and Williams, 1973) did not observe this decrease but noted a decreased in the number of REM periods after 60-70 hours of fasting. However, as stated when reviewing these studies, this decrease in REM sleep may have been a consequence of the psychological stress of fasting and the discomfort arising from hunger pangs. These factors may well have balanced or exceeded the opposite effects which were predicted on the basis of dietary-induced changes in brain serotonin synthesis alone.

This lack of agreement between the predicted and observed changes in REM sleep during fasting highlights the importance of viewing sleep as a behavioural state which can be influenced by a great variety of factors. For

example, psychological stress probably influences the synaptic activity of all neurotransmitters, known and unknown, and predictions concerning changes in REM sleep as a result of changes in serotonergic synaptic activity alone following stressful dietary changes, such as fasting, must be viewed with great caution.

## 3.3 Summary

The animal literature was not detailed enough to allow for accurate predictions concerning changes in REM sleep following various dietary changes. Fortunately, comparisons were possible with some of the human studies and, with the exception of fasting, it would appear that there exists good agreement between the predicted and observed changes in REM sleep in humans following various dietary changes. This is taken to suggest that, under normal unstressful conditions, the duration of REM sleep may be influced by dietary-induced changes in brain serotonin metabolism and, more specifically, by the enhancement or depression of synaptic transmission in serotonergic system.

PART TWO: DIET AND SLEEP

SECTION C: Experimental Investigation of the Theoretical Model

CHAPTER 12 Study IV

CHAPTER 13 General Discussion

CHAPTER 12

STUDY IV

CHAPTER 12 - CONTINUED

## 1. AIM OF THE STUDY

The aim of this study was to design various diets, the consumption of which would be expected to produce predictable changes in REM sleep in accordance with the theoretical model described in the previous chapter.

## 2. DESIGN OF THE EXPERIMENTAL DIETS

It was apparent from the previous chapter that the experimental investigation of this model must be carried out under non-stressful conditions. Thus, the investigation should not involve massive dietary changes. Whilst fasting is obviously a stressful procedure, it is likely that gross over-feeding may also be stressful due to feelings of nausea, stomach pains or the worry of putting on weight. If these excessive dietary changes were found to affect REM sleep, then the degree of influence of stress itself will be an unknown factor. Therefore, it was decided not to use experimental diets which involved major changes in food intake, but to limit the investigation to the effects of non-stressful changes in the content of the diet.

According to the theory outlined in Chapter 11, it would be expected that a dietary change from a total protein diet to a total carbohydrate and fat diet would effect a significant increase in brain serotonin synthesis. As a consequence of this, it would also be expected that REM sleep would be enhanced. However, both these diets are unnatural to the human, and any observed changes in REM sleep following the consumption of these two diets may well have been induced to an unknown extent by more basic means, such as a more disturbed sleep due to stomach upsets and nausea. Therefore, a compromise must be made when deciding which diets to administer. Specifically, these diets should maximise the changes in brain serotonin synthesis whilst being appetising and readily eaten by the subjects.

It was decided that two experimental diets would be designed; one expected to provide a high ratio of free plasma tryptophan to the other large, neutral amino acids and the other expected to provide a low ratio. According to the theoretical model, it would be expected that the consumption of the first diet would be associated with a greater amount of REM sleep than the latter diet.

There are two possible methods to design such experimental diets:-

a) provide various balanced diets, containing constant amounts of protein, carbohydrate and fat, but vary the total tryptophan intake by selecting tryptophan-rich or tryptophan-poor foods. Furthermore, the high tryptophan diet must be designed so that the ratio of the tryptophan intake to the other large neutral amino acids is increased, otherwise the transport of tryptophan to the brain will not be increased by this diet. Conversely, the low tryptophan diet must provide a low ratio of tryptophan to the other large neutral amino acids.

b) provide various diets with differing amounts of protein, carbohydrate and fat. To avoid any possible anxiety or stress effects, the extent of these experimental dietary changes should not greatly exceed the extremes found in day-to-day normal dietary changes.

The choice of suitable experimental diets is discussed in the next sections. It was also decided to observe sleep during a control period during which the subjects ate their normal diet. This control data would allow for an assessment of the direction of change, if any, in REM sleep (i.e. increased or decreased) during the experimental dietary periods.

## 2.1 Tryptophan Content

In order to ascertain whether it was possible to design

the two experimental diets according to the criteria described in (a) above, it was necessary to assess the individual large, neutral amino acid content of common foods and then calculate the ratio, for a given food, of its tryptophan content to that of the other large, neutral amino acids. If certain foods were found to have a high ratio of tryptophan content to that of the other neutral amino acids, then these foods would be used to constitute a major part of the high tryptophan diet. Similarly, those foods with a low ratio would form the low tryptophan diet.

However, it is apparent that the great majority of foods contain a similar ratio of tryptophan to the other large amino acids. For example, mutton, lamb, pork, beef, chicken, eggs, apple, banana, fig, grape, soya bean, flour, cereal, rice, cabbage, turnip, celery, cucumber and tomato were all found to provide a ratio between 0.040 and 0.053. Potatoes (0.071) and brazil nuts (0.923) and mushrooms (0.082) were the only foods studied that had comparatively higher tryptophan ratios and onion (0.138) was the only food found with a comparatively low tryptophan ratio.

Thus, the above method would not appear to be suitable for the purpose of this study. It would be very difficult to design presentable diets, of a similar bulk, which would provide major changes in the ratio of tryptophan intake to that of the other large, neutral amino acids. One other possibility considered was to administer tryptophan, in powder form, with one of the experimental diets, thereby increasing the free plasma tryptophan ratio. However, this method would not provide suitable information on the influence of the diet alone upon sleep and it was decided that changes in the carbohydrate, fat and protein content of the two diets would be a more suitable method.

## 2.2 Carbohydrate, fat and protein content

From the proposed theory, it would be expected that a

change in diet from one rich in protein, but low in carbohydrate and fat, to one rich in carbohydrate and fat, but low in protein, would be associated with an increase in REM sleep. As stated earlier, care must be taken when designing these diets so that they are palatable and present no problems to the digestive system.

The first task in designing suitable diets was to assess the carbohydrate, fat and protein content of common foods and then make separate lists of foods which were rich in carbohydrate and fat, but low in protein, and those which were rich in protein, but low in carbohydrate and fat.

Before deciding upon the menus for these two experimental diets it was necessary to decide:-

a) how many days they would be administered,

b) whether they would supplement or replace the subjects' normal meal(s), and

c) whether they should be designed to be isocaloric.

### 2.2.1 Duration of administration of the experimental diets.

It appears from Study III of this thesis and the work by Phillips, Chen, Crisp, Koval, McGuiness, Kalucy, Kalucy and Lacey (1975) that the effects of dietary changes upon sleep can be observed during the first night following the change. Therefore, it was not considered necessary that the experimental diets should be administered for extended periods of time. The three day period as used in Study III has several advantages allowing for a simple experimental design with each diet being studied in one week. Whilst it would be interesting to see the effects of long-term dietary changes upon sleep, it was decided to use only a three day period as it would be necessary to observe an effect during

this acute change before the examination of a chronic change is justified.

2.2.2. Complete versus partial dietary change

From Study III it was found that the administration of a high carbohydrate supplementary diet was associated with small increases in REM sleep compared to low and zero carbohydrate supplementary diets. The changes in REM sleep observed by Phillips et. al. (1975) following the administration of a high carbohydrate/low fat diet were comparatively large, possibly as a consequence of two factors:-

a) the daily dietary changes were greater compared to those of Study III

b) the experimental diets used by Phillips et. al. (1975) replaced the normal diet and, thus, the subjects ate high carbohydrate/low fat meals throughout the day.

As it appears that the transport of tryptophan into the brain may be a function of the ratio of free plasma tryptophan to the other large neutral amino acids, it would follow that the duration of the changes in this ratio is another important factor to be considered when assessing changes in brain serotonin synthesis. Thus it would be expected that the high carbohydrate/low fat diet used by Phillips et. al. (1975) would increase brain serotonin synthesis to a greater extent than the high carbohydrate supplementary diet used in Study III. It was decided, therefore, that this study (Study IV) would implement a complete change of diet during the experimental periods, as this would be expected to maximise the effects of the dietary change upon brain serotonin synthesis and sleep.

2.2.3 Isocaloric versus variable intake

The study by Phillips et. al. (1975) used two experimental

diets and a normal balanced diet, all of which were isocaloric to one another. This study was important as it allowed for the distinction between the effects of changes in calorie intake and the changes in the intake of carbohydrate and fat upon sleep. It was found that, for a normal calorie intake, the amounts of carbohydrate and fat in the diet do have an influence upon sleep.

However, there can be some problems when administering isocaloric diets. One such problem is to design diets that are acceptable to all the subjects. Another problem is that, although daily calorie intake may be controlled for, there is the possibility that on some days the subjects may prefer less food or more food and, because the calorie intake was fixed, feel either full or hungry at night. These daily changes in appetite are fairly common and can often result from changes in activity levels or environment.

For the purposes of this study it was decided that the subjects would not be fed on strict isocaloric diets but, instead, be allowed to choose what and how much they ate from a menu. The menu would be different for the two experimental diets and no control would be exerted during the control period when the subjects ate their normal diet. By this method, it was envisaged that the subjects would eat sufficient at each meal to be content (i.e. not too full or still hungry). Whilst the calorie intake was not controlled by the experimenter, it was hoped that the variations in calorie intake between the three conditions (i.e. 2 experimental diets and the control diet) would not be very large. The subjects were informed of this problem and were requested not to consciously "over" or "under" eat on any day during the three conditions.

### 2.2.4 Experimental diets

From the previous sections it was decided that the experimental diets should contain various amounts of carbohydrate, fat and protein and that they would be administered

for three days as a complete replacement of the normal diet. The selection of foods during each of the experimental diets would be made by the subjects from menus which are described below. These menus are based upon foods which are either rich in carbohydrate and/or fat or rich in protein, but low in carbohydrate and fat content.

Menu for the high carbohydrate and fat, low protein diet

| | |
|---|---|
| Breakfast: | Cereals with sugar and milk, toast with butter or margarine and assorted jams, honey etc. |
| Lunch and other breaks: | Pastries, sausage roll, biscuits, potato crisps, jam sandwiches (buttered), chocolate, sweets, dried fruit, bananas. |
| Evening meal: | Fried meat or fish, meat pie, fried or saute potatoes, spaghetti in tomato sauce, apple pie and custard. |
| Beverages: | Tea, coffee, chocolate, Ribena, orange squash. |

Menu for the low carbohydrate and fat, high protein diet

| | |
|---|---|
| Breakfast: | Fresh grapefruit (no sugar), low-fat unsweetened yogurt, slimming biscuits (no butter or margarine), marmite. |
| Lunch and other breaks: | Slimming biscuits (no butter or margarine), boiled ham, chicken or beef (fat removed), assorted vegetables, raw fruits (except bananas). |
| Evening meal: | Boiled meat or fish (except herring or salmon), boiled potatoes, boiled or raw vegetables (except lentils or split peas), raw fruit (except bananas), low-fat unsweetened yogurt. |
| Beverages: | Tea and coffee, marmite, bovril. |

Throughout both the experimental periods and the control period the subjects completed a diet-log so that the content and calorie intake of each diet could be assessed.

To ensure that the subjects had suitable food available, the experimenter provided for breakfast and the evening meal at the sleep laboratory during the administration of the experimental diets. In addition, the subjects received a lunch-box containing a selection of foods described under "lunch and other breaks" in the above menus.

## 3. CONTROL OF EXTRANEOUS VARIABLES AND SUBJECT SELECTION

In order that any changes observed in sleep during the experimental periods can be ascribed to the dietary changes, it was necessary to control for other variables which were likely to also influence sleep, such as:-

a) activity levels - both physical and mental

b) sleeping problems

c) medication and health

d) age

e) body weight, appetite and meal-times

### 3.1 Activity levels

Activity levels, both physical and mental, were controlled for as in Study III (see Chapter 8 Section 2.1.2).

### 3.2 Sleeping problems

Prospective subjects suffering from any sleeping problems or who often took daytime naps were excluded from the study. Subjects who typically slept soundly between 12.00 p.m. and 8.30 a.m. were preferred for this study so that several subjects could be studied in the sleep laboratory at one time.

### 3.3 Medication and Health

Prospective subjects taking medicines or whose state of health was not conducive to normal sleep were excluded from the study.

### 3.4 Age

Sleep is known to show subtle changes as the age of the subjects increases. To obtain a homogenous group of subjects it was decided that prospective subjects outside the age range 17-25 years would not be considered for selection.

### 3.5 Body weight

Subjects who were greater than ±1 stone from their ideal weight, as assessed by height (see Metropolitan Life Insurance Company Statistical Bulletin, 1959), were not eligible for this study so that a fairly homogenous group of subjects could be selected. It is possible that obese or underweight people might show subtle changes in their response, as regards sleep, to the dietary changes. For example, overweight people may typically eat foods rich in carbohydrates and fat and thus, according to the theoretical model, REM sleep might not be substantially affected by the administration of the high carbohydrate and fat diet.

### 3.6 Appetite and Meal Times

Prospective subjects were also asked to fill in a detailed diet-log for one week. Only those subjects who had stable diets, as assessed by total calorie intake, and took their meals (at least three a day) at regular times were considered suitable for this study. This restriction would minimise the possibility that the average calorie intakes for each of the three conditions (two experimental and one control diets) would show a large variation due to spasmodic

fluctuations in appetite. The eating of at least three meals per day at regular times was considered important because the changes in the diet would be effective throughout most of the day.

From the review of the diet and sleep literature it was noted that the digestability of a snack, eaten prior to retiring, appears to have an influence upon subsequent sleep quality. So that this factor may be controlled for, it was decided to select those subjects who:-

i) ate their evening meal at a regular time. This would ensure that there were no gross changes in the amount of time available for digestion of the food before retiring.

ii) did not usually desire or take a late evening supper. It was decided that the subjects would not be allowed to eat a meal or a substantial snack after 8.00 p.m.

3.7 Sex

The sex of the subject was not considered to be a major variable in the diet and sleep relationship. Of the eight subjects selected, two were women.

## 4. EXPERIMENTAL PROCEDURE

The experimental programme lasted for three consecutive weeks, with one diet (either control or experimental) investigated per week. EEG recordings were taken on Monday, Tuesday and Wednesday night of each week during which period the diets were consumed. Recordings were also taken on Thursday nights following the termination of the two experimental diets. Two adaption nights were allowed at the beginning of the study and a further night preceding the administration of the experimental diets as the subjects were allowed to sleep at home on the Friday and Saturday nights.

Table 1. Order of administration of the diets

| Subject | Week 1 | Week 2 | Week 4 |
|---|---|---|---|
| 1<br>2<br>3<br>4 | Control diet | Low carbohydrate and fat, high protein diet | High carbohydrate and fat, low protein diet |
| 5<br>6<br>7<br>8 | Control diet | High carbohydrate and fat, low protein diet | Low carbohydrate and fat, high protein diet |

The timetables for one of the subjects (subject 1) is tabulated below (Table 2):-

Table 2. Timetable for subject 1

| Week | Saturday | Sunday | Monday, Tuesday and Wednesday | Thursday |
|---|---|---|---|---|
| 1 | Adaptation | Adaptation | Control diet | Free |
| 2 | Free | Adaptation | Low carbohydrate and fat, high protein diet | Post-diet |
| 3 | Free | Adaptation | High carbohydrate and fat, low protein diet | Post-diet |

On Monday to Wednesday of each week, each subject completed a diet-log following each meal. During the administration of the experimental diets, the experimenter provided the breakfast, evening meal and a lunch-box (see section 2.2.4).

The subjects arrived at the sleep laboratory at 11.00 p.m. when the electrodes were attached. The montage recommended by Rechtschaffen and Kales (1968) was used, namely: C3-A2; C4-A1; left-eye-A1; right-eye-A1; EMG electrodes were located on

either side of the chin. Lights out was at 12.00 p.m. and all-night recordings were made with a Grass model 78 electroencephalogram at a paper speed of 10 mm/sec. The subjects were allowed to sleep until they felt refreshed and ready to get up.

## 5. ANALYSIS OF DIETARY CONTENTS

The average carbohydrate, fat and protein intake for each subject was assessed for each dietary period from the subjects' diet-logs by reference to the appropriate food tables (In McCance and Widdowson, 1960). In addition, the approximate total energy intake was estimated for each diet using the approximate conversion factor for grammes weight to caloric value (kcal) of 4, 9 and 4 for available carbohydrate, fat and protein, respectively (see Table 3). Thus, the carbohydrate intake in the "unavailable form", such as hemicellulose and fibre, is not represented.

Table 3. Mean daily intake (grams) of protein, fat and carbohydrate during the control and experimental diet periods.
Standard deviation in brackets.

| | Control diet | High Carbohydrate and fat, low protein diet | Low Carbohydrate and fat, high protein diet |
|---|---|---|---|
| Protein | 101.8 (±15.6) | 80.1 (±9.0) | 132.4 (±30.3) |
| Fat | 99.4 (±15.8) | 118.5 (±16.3) | 83.5 (±8.5) |
| Carbohydrate | 364.4 (±66.5) | 439.4 (±61.6) | 318.9 (±50.1) |
| Approximate total energy intake (kcals) | 2759.4 (±369.6) | 3144.5 (±391.4) | 2556.4 (±287.8) |

From table 3, it can be seen that the actual intake of carbohydrate, fat and protein during the two experimental diets were as planned. That is, there were large differences in the proportions of these constituents although the total calorific yields were not greatly dissimilar.

## 6. PREDICTIONS

As the two experimental diets were designed to produce predictable changes in REM sleep, according to the proposed theoretical model, it is necessary to state precisely what changes were to be predicted.

Specifically, the consumption of the high carbohydrate and fat, low protein (HCF) diet was predicted to be associated with an increase in REM sleep compared to the amounts observed during the consumption of the low carbohydrate and fat, high protein (LCF) diet.

Although the difference in REM sleep between the experimental diets and the control diet might be predicted using the theoretical model, it is debatable whether these smaller dietary changes, and thereby reduced changes in brain serotonin synthesis, would be sufficiently large enough to influence REM sleep. From table 3 it is apparent that the HCF diet contained more carbohydrate and fat, but less protein, than the control diet and therefore an observed decrease in REM sleep during the HCF diet period compared to the control diet period would not be compatible with the theoretical model. Similarly, if REM sleep was found to be increased during the LCF diet period compared to the control diet period, then this finding would not be compatible with the theoretical model.

## 7. DATA ANALYSIS

The sleep records were divided into one minute epochs and classified into sleep stages according to the criteria described by Rechtschaffen and Kales (1968) by two experienced scorers.

The shortest sleep length, as measured from the onset of stage 2 sleep, was noted for each subject over the experimental period and this individual time period was used for analysis on all the recorded nights. Six subjects had a time period of 450 minutes, one subject a period of 425 minutes and the remaining subject a period of 400 minutes. This gave an average time period for anlaysis of 440.6 minutes.

For each subject, the number of minutes spent in a specific stage of sleep were calculated for each night during:-

a) The whole night

b) The first half of the night

c) The second half of the night.

In addition, various other parameters (i.e. REM latency, REM periodicity) were calculated.

## 7.1 Analysis of experimental dietary periods

Statistical analysis was performed using Analysis of Variance techniques. A suitable computer programme (Genstat V Mark 3.07, Lawes Agricultural Trust - Rothamsted Experimental Station) was used because of the complex and time consuming method of analysis. This programme generated F values for the main effects of diet and night as well as the diet x night interaction effect. As the main effect of subjects and those interaction effects involving subjects were not considered relevant to this analysis, the subjects were used as a blocking variable.

When the analysis of variance indicated that a significant effect existed (i.e. $p<0.05$), then the Studentized Range Test (see Snedecor and Cochran, 1967) was used to determine which diets or nights were significantly different to one another. The upper 5% levels of Q were used to calculate the minimum differences between the means (three for

the main effects and nine for the interaction effect) ensuring that the probability of any erroneous claim of significance was $\leqslant 0.05$.

The Genstat programme was also used to generate standard errors of differences between diet means.

7.2 Analysis of post-diet nights

In order to identify any carryover effects, the post-diet nights were compared with the averaged data for the preceding three days when the respective experimental diets were consumed. The post-diet nights were also compared to the averaged control data.

Unfortunately, the post-diet data was complete for only five subjects as unforeseen circumstances prevented three of the subjects attending the sleep laboratory on one of these post-diet nights. Thus, the post-diet data was compared with the averaged data for these five subjects only from the control and experimental diet periods.

8. RESULTS

The individual sleep data is presented in Appendix V. The results of the statistical analysis are summarised in Tables 4-8 and Figures 1-2. The following letters were used to signify which diets were significantly different to one another:-

a ..... significant difference between experimental diets

b ..... significantly different to control diet

c ..... significant difference between post-diet night and respective dietary period.

Table 4. Group means for the various dietary periods

| | CONTROL | HCF | LCF | S.E. of difference between diet means | Significance |
|---|---|---|---|---|---|
| Whole night data (mins | | | | | |
| Stage 3+4 | 114.3 | 107.9 | 108.0 | 4.22 | |
| 4 | 66.8 | 64.2 | 67.3 | 3.32 | DxN $F_{4,56}$=3.24, p<0.025 |
| 3 | 47.5 | 43.7 | 40.7 | 3.61 | |
| REM | 83.9 | 103.5a,b | 90.0 | 2.80 | Diet $F_{2,56}$=24.96, p<0.001 |
| 2 | 202.0 | 203.6 | 208.9 | 4.49 | |
| 1 | 37.1 | 24.5a,b | 32.8 | 3.32 | Diet $F_{2,56}$=7.41, p<0.005 |
| 0 | 3.3 | 1.1 | 0.9 | 1.03 | Night $F_{2,56}$=5.62, p<0.01 DxN $F_{4,56}$=2.61, p<0.05 |
| S.4 latency | 15.3 | 13.6 | 14.5 | 1.58 | |
| REM latency | 89.8 | 72.2 | 84.4 | 7.83 | |
| REM periodicity (mean 1st 2 REM cycles) | 96.1 | 90.5 | 97.4 | 3.55 | |
| Number of REM periods | 4.2 | 4.6 | 4.3 | 0.18 | |
| First half data (mins) | | | | | |
| Stage 3+4 | 87.0 | 83.5 | 86.9 | 3.57 | |
| 4 | 55.5 | 53.0 | 57.9 | 2.66 | DxN $F_{4,56}$=4.19, p<0.005 |
| 3 | 31.5 | 30.5 | 29.0 | 2.81 | |
| REM | 28.7 | 33.6 | 31.3 | 3.56 | DxN $F_{4,56}$=2.74, p<0.05 |
| 2 | 91.8 | 94.1 | 91.5 | 4.43 | |
| 1 | 11.6 | 8.8 | 10.5 | 1.86 | |
| 0 | 1.2 | 0.3 | 0.1 | 0.65 | |
| Second half data (mins) | | | | | |
| Stage 3+4 | 27.3 | 24.4 | 21.1 | 3.10 | DxN $F_{4,56}$=3.38, p<0.025 |
| 4 | 11.3 | 11.2 | 9.4 | 2.22 | DxN $F_{4,56}$=2.96, p<0.05 |
| 3 | 16.0 | 13.2 | 11.7 | 3.30 | |
| REM | 55.2 | 69.9 | 58.7 | 4.38 | (Diet $F_{2,}$ 5616.83, p<0.005 DxN $F_{4,56}$=3.81, p<0.01 |
| 2 | 110.2 | 109.5 | 117.4 | 4.25 | DxN $F_{4,56}$=3.33, p<0.025 |
| 1 | 25.5 | 15.7a,b | 22.3 | 2.38 | Diet $F_{2,56}$=8.80, p<0.005 |
| 0 | 2.1 | 0.8 | 0.8 | 0.70 | Night $F_{2,56}$=5.76, p<0.01 (night 1 significantly higher than nights 2 & 3). |

Table 5. Group means for night 1 of the dietary periods

| | CONTROL | HCF | LCF |
|---|---|---|---|
| WHOLE NIGHT DATA (MINS) | | | |
| Stage 3+4 | 119.4 | 99.7 | 108.2 |
| 4 | 78.3 | 60.7b | 66.0b |
| 3 | 41.1 | 39.0 | 42.2 |
| REM | 87.2 | 106.7 | 85.6 |
| 2 | 191.9 | 201.2 | 212.0 |
| 1 | 34.1 | 31.5 | 32.9 |
| 0 | 8.0 | 1.5b | 1.9b |
| S.4 latency | 12.0 | 14.6 | 15.5 |
| REM latency | 72.0 | 72.2 | 83.0 |
| REM periodicity (mean 1st 2 inter-REM periods) | 100.4 | 88.7 | 104.9 |
| Number of REM periods | 4.1 | 4.6 | 4.2 |
| FIRST HALF DATA (MINS) | | | |
| Stage 3+4 | 91.5 | 82.6 | 88.5 |
| 4 | 63.7 | 53.4 | 58.6 |
| 3 | 27.8 | 29.2 | 29.9 |
| REM | 30.7 | 34.5 | 37.4 |
| 2 | 83.5 | 92.1 | 85.3 |
| 1 | 11.5 | 10.7 | 9.1 |
| 0 | 3.1 | 0.4 | 0.0 |
| SECOND HALF DATA (MINS) | | | |
| Stage 3+4 | 27.9 | 17.1 | 19.7 |
| 4 | 14.6 | 7.3 | 7.4 |
| 3 | 13.3 | 9.8 | 12.3 |
| REM | 56.5 | 72.2a,b | 48.2 |
| 2 | 108.4 | 109.1a | 126.7b |
| 1 | 22.6 | 20.8 | 23.8 |
| 0 | 4.9 | 1.1 | 1.9 |

Table 6. Group means for night 2 of the dietary periods

| | CONTROL | HCF | LCF |
|---|---|---|---|
| WHOLE NIGHT DATA (MINS) | | | |
| Stage 3+4 | 111.2 | 111.6 | 105.1 |
| 4 | 60.7 | 68.8 | 66.0 |
| 3 | 50.5 | 42.8 | 38.5 |
| REM | 77.9 | 100.7 | 91.6 |
| 2 | 212.0 | 206.1 | 206.3 |
| 1 | 38.5 | 21.7 | 37.0 |
| 0 | 1.0 | 0.5 | 0.6 |
| S.4 latency | 17.9 | 12.9 | 13.5 |
| REM latency | 98.8 | 68.2 | 69.6 |
| REM periodicity (mean 1st 2 inter-REM periods) | 99.1 | 94.6 | 91.7 |
| Number of REM periods | 4.0 | 4.3 | 4.6 |
| FIRST HALF DATA (MINS) | | | |
| Stage 3+4 | 79.1 | 89.5 | 85.9 |
| 4 | 48.3 | 60.2b | 56.9 |
| 3 | 30.8 | 29.3 | 29.0 |
| REM | 34.4 | 30.7 | 21.6b |
| 2 | 94.0 | 92.4 | 101.8 |
| 1 | 12.4 | 7.6 | 10.9 |
| 0 | 0.4 | 0.1 | 0.1 |
| SECOND HALF DATA (MINS) | | | |
| Stage 3+4 | 32.1 | 22.1b | 19.2b |
| 4 | 12.4 | 8.6 | 9.7 |
| 3 | 19.7 | 13.5 | 9.5 |
| REM | 43.5 | 70.0b | 70.0b |
| 2 | 118.0 | 113.7 | 104.5 |
| 1 | 26.1 | 14.1 | 26.1 |
| 0 | 0.6 | 0.4 | 0.5 |

Table 7. Group means for night 3 of the dietary periods

| | CONTROL | HCF | LCF |
|---|---|---|---|
| WHOLE NIGHT DATA (MINS) | | | |
| Stage 3+4 | 112.2 | 112.4 | 110.7 |
| 4 | 61.5 | 62.9 | 69.2 |
| 3 | 50.7 | 49.5 | 41.5 |
| REM | 87.0 | 102.9 | 92.6 |
| 2 | 202.0 | 203.7 | 208.6 |
| 1 | 38.6 | 20.2 | 28.5 |
| 0 | 0.8 | 1.4 | 0.2 |
| S.4 latency | 15.9 | 13.4 | 14.4 |
| REM latency | 98.5 | 76.1 | 100.5 |
| REM periodicity (Mean 1st 2 inter-REM periods) | 88.9 | 88.1 | 95.6 |
| Number of REM periods | 4.4 | 4.6 | 4.0 |
| FIRST HALF DATA (MINS) | | | |
| Stage 3+4 | 90.5 | 78.2 | 86.2 |
| 4 | 54.4 | 45.5a | 58.2 |
| 3 | 36.1 | 32.7 | 28.0 |
| REM | 21.0 | 35.6b | 35.0b |
| 2 | 97.6 | 98.0 | 87.4 |
| 1 | 11.0 | 8.1 | 11.6 |
| 0 | 0.2 | 0.4 | 0.1 |
| SECOND HALF DATA (MINS) | | | |
| Stage 3+4 | 21.7 | 34.2b | 24.5 |
| 4 | 7.1 | 17.4b | 11.0 |
| 3 | 14.6 | 16.8 | 13.5 |
| REM | 66.0 | 67.3 | 57.6 |
| 2 | 104.4 | 105.7 | 121.2 |
| 1 | 27.6 | 12.1 | 16.9 |
| 0 | 0.6 | 1.0 | 0.1 |

Table 8. Group means for the dietary periods and the post-diet nights (N=5).

| | Control | HCF | Post HCF | LCF | Post LCF | Significance (df=4) |
|---|---|---|---|---|---|---|
| Whole night data (mins) | | | | | | |
| Stage 3+4 | 121.0 | 117.7 | 116.6 | 113.7 | 105.6 | |
| 4 | 66.5 | 65.9 | 74.6 | 66.2 | 65.4 | |
| 3 | 54.5 | 51.8 | 42.0c | 47.5 | 40.2 | t=3.86, p<0.025 |
| REM | 84.1 | 101.0 | 84.0c | 89.3 | 93.8 | t=3.22, p<0.05 |
| 2 | 211.6 | 209.5 | 208.0 | 214.7 | 222.6 | |
| 1 | 31.0 | 21.1 | 26.2 | 31.4 | 23.8 | |
| 0 | 2.3 | 0.7 | 14.4 | 0.9 | 4.2 | |
| S4 latency | 14.9 | 13.7 | 12.2 | 14.6 | 18.2 | |
| REM latency | 90.9 | 74.9 | 87.8 | 84.1 | 60.6b | t=3.81, p<0.025 |
| REM periodicity | 101.0 | 97.9 | 115.9 | 98.6 | 97.6 | |
| No. REM periods | 4.1 | 4.5 | 3.8 | 4.4 | 4.6 | |
| First half data (mins) | | | | | | |
| Stage 3+4 | 92.9 | 88.5 | 95.6 | 91.5 | 82.4 | |
| 4 | 55.8 | 51.4 | 67.4 | 57.4 | 49.2 | |
| 3 | 37.1 | 37.1 | 28.2b | 34.1 | 33.2 | t=3.01, p<0.05 |
| REM | 27.5 | 34.2 | 28.0 | 34.1 | 25.2 | |
| 2 | 94.6 | 95.2 | 93.6 | 89.6 | 114.0 | |
| 1 | 9.7 | 7.1 | 7.8 | 9.7 | 7.4 | |
| 0 | 0.3 | 0.0 | 0.0 | 0.1 | 1.0 | |
| Second half data (mins) | | | | | | |
| Stage 3+4 | 28.2 | 29.2 | 21.0 | 22.2 | 23.2 | |
| 4 | 10.7 | 14.5 | 7.2 | 8.8 | 16.2 | |
| 3 | 17.5 | 14.7 | 13.8 | 13.4 | 7.0b | t=3.12, p<0.05 |
| REM | 56.5 | 66.8 | 56.0 | 55.2 | 68.6 | |
| 2 | 117.0 | 114.3 | 115.2 | 125.1 | 108.6 | |
| 1 | 21.3 | 14.0 | 18.4 | 21.7 | 16.4 | |
| 0 | 2.0 | 0.7 | 14.4 | 0.8 | 3.2 | |

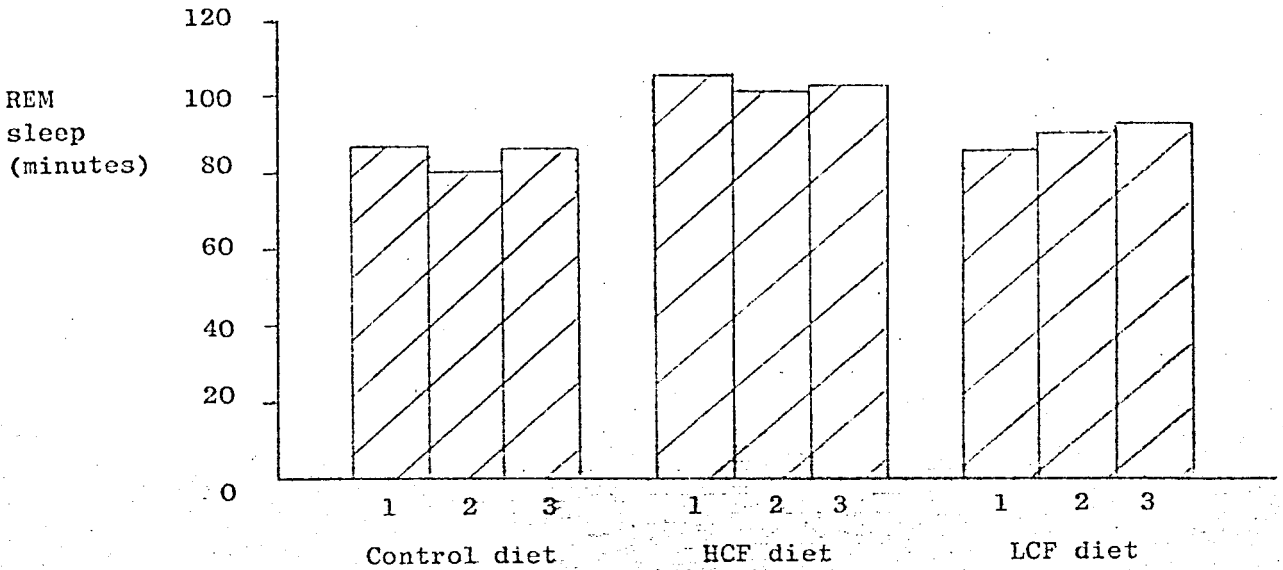



FIGURE 1: REM sleep duration on individual nights of the dietary periods

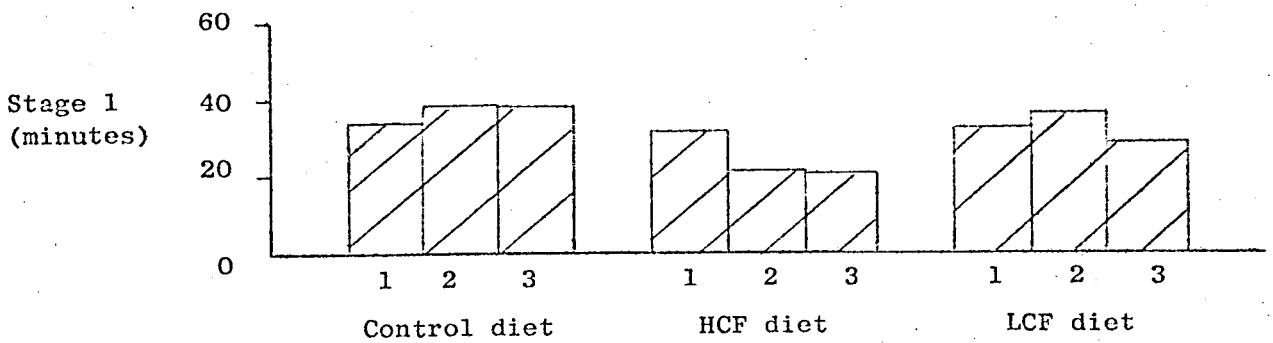



FIGURE 2: Stage 1 duration on individual nights of the dietary periods.

## 8.1 Dietary Periods

1. SWS - No significant differences were found for Stages 3+4, Stage 4 or Stage 3 between the dietary periods as a whole, either for whole night data or half night data. However, several significant diet and night interaction effects were found. A significant interaction effect ($p<0.025$) was found for stage 4 during the whole night. This was due to high levels on the first night of the control diet which were significantly different to the first night of the experimental diets and also to the subsequent nights of the control diet.

   A significant interaction effect ($p<0.005$) was also found for stage 4 during the first half of the night. The amount of stage 4 during the first half of the second diet night was significantly increased following the HCF diet compared to the control diet. On the third diet night, the HCF and LCF diets were significantly different during the first half of the night due to a decrease on the HCF diet.

   Significant interaction effects were found for both stage 3+4 ($p<0.025$) and stage 4 ($p<0.05$) during the second half of the night. On the second diet night, the amount of stage 3+4 was significantly lower on both the HCF and LCF diets compared to the control diet. On the third diet night, both the amount of stage 3+4 and stage 4 were significantly higher on the HCF diet than on the control diet during this period of the night.

2. Stage REM - REM sleep over the whole night was significantly increased ($p<0.001$) during the HCF diet period (see Figure 1).

This increase occurred during the second half of the night for the HCF period ($p<0.005$), although a significant diet and night interaction effect ($p<0.01$) indicated that only the first diet night showed an increase in REM sleep compared to both the other diets. Also, the second HCF and LCF diet nights both contained significantly more REM sleep than the second control diet night.

A significant diet and night interaction effect ($p<0.05$) was also found for REM sleep during the first half of the night. This showed that the second night of the LCF diet contained less REM sleep than the second night on the control diet and the third night on the HCF and LCF diets both contained significantly more REM sleep than the third control night.

3. Stage 2 - No significant differences were found for Stage 2 sleep between the dietary periods as a whole, although a significant diet and night interaction effect ($p<0.025$) was found for Stage 2 sleep during the second half of the night. This was due to a significant increase in Stage 2 sleep during this period of the night on the first night of the LCF diet compared to the first night on the other two diets.

4. Stage 1 - Stage 1 was significantly decreased ($p<0.005$) during the HCF diet period compared to both the LCF diet and control diet periods (see Figure 2). This decrease occurred mainly during the second half of the night ($p<0.001$).

5. Stage 0 - Significant night effects were found for wakefulness, both for the whole night ($p<0.01$) and for the second half of the night ($p<0.025$), with the amount of wakefulness being higher on the first diet

night compared to nights 2 and 3. A significant diet and night interaction ($p<0.05$) was found for wakefulness during the whole night, which showed that the first night of the control diet contained more wakefulness than the first nights of the experimental diets and also more than the subsequent control nights.

## 8.2 Post-diet nights

As mentioned earlier, the post-diet data was incomplete for 3 subjects, for reasons beyond the experimenter's control, and the post-diet analysis was limited to the data from the remaining 5 subjects only.

### 8.2.1 Post-HCF diet night

1. SWS - No significant differences were found for Stages 3+4 on the post-HCF diet night compared to the averaged data for the HCF diet period or the control diet period. However, Stage 3 was significantly decreased for the whole night data compared to the HCF diet period ($p<0.05$) and during the first half of the night compared to the control diet period ($p<0.05$).

2. Stage REM - REM sleep over the whole night was significantly lower ($p<0.05$) on the post-HCF diet night compared to the HCF diet period.

### 8.2.2 Post-LCF diet night

1. SWS - No significant differences were found for Stages 3+4 on the post-LCF diet night compared to the averaged data for the LCF diet period or the control period. However, Stage 3 was significantly decreased ($p<0.05$) during the second half of the night when compared to the control period.

2. REM latency - REM latency was found to be significantly decreased ($p<0.05$) on the post-LCF diet night compared to the control period. This decrease approached significance when compared with the LCF diet period.

## 9. DISCUSSION OF RESULTS

The SWS data does not appear to be greatly influenced by the experimental diets as no significant differences were found between the three dietary periods. Although several diet x night interactions were found, they do not seem to be consistent. For example, the duration of Stage 4 on the first night of both of the two experimental diets was found to be significantly lower than on the first control night. However, it does not appear that the experimental diets both decreased Stage 4 on the first night of their administration, as the duration of this stage can be seen to be similar to that on nights 2 and 3 of the experimental diets. The fact that the duration of Stage 4 on the first control night is significantly higher than on the subsequent control nights would strongly suggest that this interaction effect was not due to any influence of the experimental diets, but rather to control variation.

The other significant interaction effects referred to Stage 4 during the first half of the night and Stage 3+4 and Stage 4 data during the second half of the night. This would indicate that the temporal distribution of these stages were influenced by the experimental diets but, again, the effect was not consistent. On the second night, Stage 4 was significantly increased during the first half of the night following the HCF diet compared to the control diet, although Stage 3+4 were significantly decreased during the second half of the night by both experimental diets compared to the control diet. However, on the third HCF diet night, this shift in SWS was reversed with a significant decrease

in Stage 4 during the first half of the night compared to the LCF diet and a significant increase in Stage 3+4 and Stage 4 during the second half of the night compared to the control night. The reason for these temporal shifts is not clear but the fact that they are not consistent from night to night would strongly suggest that they are a consequence of the arbitary decision to analyse the data in two equal time periods. It is possible that some other method of dividing the night which was linked to the sleep stage cycling (e.g. the duration of SWS prior to the first or second REM period) might have failed to find the shifts described above.

The analysis of the Stage REM data has revealed a significant, and consistent, effect of the HCF diet to increase REM sleep compared to both the LCF diet and the control diet periods. From Figure 1 it can be seen that REM sleep was increased throughout the HCF diet period with a mean increase of 19.6 and 13.5 minutes compared wtih the control and LCF diet periods, respectively. The increase in REM sleep occurred mainly during the second half of the night, although significant interaction effects were found for REM sleep during both the first and second halves of the night. On the first diet night, REM sleep was significantly increased during the second half of the night following the HCF diet compared to both the LCF and control diets. On the second night, REM sleep was still increased during the second half of the night following the HCF diet compared to the control diet, but not compared to the LCF diet. This was due to the fact that the REM sleep on the second LCF diet night was significantly decreased compared to the control diet during the first half of the night, followed by a significant increase compared to the control diet in the second half of the night. This shift in the appearance of REM sleep did not appear on the other nights and it may well have been due to the arbitrary nature of the analysis, as discussed above. A similar shift occurred on night 3 of the control diet with REM sleep being

significantly shorter during the first half of the night compared to the experimental diets.

Stage 2 showed no consistent change in response to the experimental diets with the only significant finding being an increase during the second half of the first LCF diet night compared to the other two diets.

Stage 1 was found to be significantly, and consistently, decreased during the HCF diet period compared to the LCF diet and control diet periods, with a mean decrease of 12.6 and 8.3 minutes, respectively. From Figure 2 it appears that this effect was greatest on nights 2 and 3 of the HCF diet. The decrease occurred, again significantly and consistently, during the second half of the night.

The amount of wakefulness did not show much variation between the diets, except for the large amount of wakefulness observed throughout the first control night. This occurred especially during the second half of the night and led to the finding of a significant night effect whereby night 1 of all the diets was found to contain significantly more wakefulness than nights 2 and 3.

As the control diet was always the first diet to be studied, it is possible that this comparatively large amount of wakefulness on the first control diet night reflected incomplete adaptation to the sleep laboratory. Inspection of the sleep stage means for this night do not show any other signs of incomplete adaptation and it appears that the wakefulness was increased at the expense of Stage 2.

To summarise the findings discussed so far, it appears that the LCF diet did not influence sleep to any appreciable extent compared to sleep during the control diet period. However, the HCF diet was found to consistently increase REM sleep and decrease Stage 1 compared to the LCF diet period and the control diet period. Both of these changes occurred

mainly during the second half of the night. No consistent changes were found for any of the other sleep stages.

As the observed increase in REM sleep during the HCF diet period compared with the LCF diet period was predicted (see Section 7 of this chapter), this finding offers experimental support for the theoretical model detailed in Chapter 11. Whilst the increase in REM sleep was only an average of 13.5 minutes compared to the LCF diet, it must be remembered that the analysis period was fixed for each subject according to his or her shortest sleep period over the experiment. However, when the duration of REM sleep was calculated over the entire night, the difference between the two experimental diets was only slightly increased to 14.3 minutes.

Interestingly, REM sleep showed the greatest difference between the control diet and the HCF diet periods. Whilst the direction of this change is consistent with the theoretical model, it was expected that the greatest difference in REM sleep would be found between the two experimental diet periods (see Section 7). As the duration of REM sleep on the second control diet night may have been unusually low (see figure 1) this could explain the comparatively low mean duration of this stage for the control period as a whole.

The extended duration of REM sleep during the HCF dietary period appears to be related to the decreases in Stage 1 sleep and wakefulness during the sleep period, as the combined duration of these stages of sleep are 124.3, 129.1 and 123.7 minutes for the control, HCF and LCF diet periods, respectively. A similar relationship was found in the previous study (Study III - see Section 8, Chapter 8) when it was suggested that the increase in REM sleep was not a direct consequence of a general reduction in Stage 1 sleep and wakefulness following the high carbohydrate supplementary diet.

The possibility that the increase in REM sleep observed in this present study was a result of decreases in Stage 1 sleep and wakefulness must again be considered, although it appears unlikely for the reasons described in Study III. That is, assuming that the HCF diet did directly decrease Stage 1 sleep and wakefulness during the sleep period, it would be expected that the other stages of sleep would all show an increase during the HCF diet period. However, neither SWS or Stage 2 sleep showed such an increase. Also, even though the reduction in Stage 1 sleep and wakefulness occurred to the greatest extent in the second half of the night during the HCF diet period, there is no evidence of a compensatory increase in Stage 2 sleep at this time when this stage of sleep is predominate. Thus, it seems more likely that the increase in REM sleep during the HCF diet period was associated with a compensatory reduction in Stage 1 sleep and wakefulness rather than the converse being true.

The increase in REM sleep observed in the present study was more substantial than that reported in Study III. In the previous study, the increase in REM sleep was significant only during the first half of the night during the high carbohydrate supplementary diet period, although the whole night data approached significance. The increased carbohydrate intake as a result of this supplementary diet amounted to 130 g (see Table 1, Chapter 7), whereas the increased carbohydrate intake during the HCF diet period compared to the LCF diet period was an average of 120 g (see Table 3, this Chapter). Thus, the greater enhancement of REM sleep during the HCF diet period compared to the high carbohydrate supplementary diet period was not due to a larger increase in carbohydrate intake.

This difference in the enhancement of REM sleep during the above two diet periods may be due to the associated increase in fat and decrease in protein intake during the HCF diet period, as these changes would be expected to complement the effect of the increased carbohydrate intake to increase the

ratio of plasma tryptophan to the other large, neutral amino acids (see Section 2.7, Chapter 9). Furthermore, the present study investigated the effects of dietary changes throughout the day rather than immediately prior to sleep as in Study III, so any increase in this ratio would be more sustained in the present study than in Study III. According to the theoretical model, brain serotonin synthesis would be expected to be increased to a larger extent during the HCF diet period compared to the high carbohydrate supplementary diet, and this may explain why the increase in REM sleep in Study IV reached significance over the whole night whereas it only approached significance in Study III.

The increase in REM sleep of 33 minutes reported by Phillips et. al. (1975 - reviewed in Section 3.2.1.2, Chapter 7) during the high carbohydrate/low fat diet period compared to the iso-caloric balanced diet period was large compared to the increases observed in Studies III and IV. However, the difference in carbohydrate intake between these two diet periods amounted to 250 g, which is double that investigated in Studies III and IV.

No predictions were made concerning the post-diet data when the subjects returned to their normal diet because the dietary changes were small compared to the differences between the HCF and LCF diets, although if any changes were observed it was expected that they should be consistent with the theoretical model. The post-diet analysis was based on data from only 5 subjects but, even so, REM sleep was found to be significantly shorter (by an average of 17 minutes) on the post-HCF diet night compared to the averaged data for the HCF diet period. This is consistent with the model as the dietary change was from a high carbohydrate and fat, low protein content to a balanced diet, containing lower quantities of carbohydrate and fat with additional protein. No significant changes in REM sleep were found on the post-LCF diet night, although REM

latency was significantly decreased compared to the averaged data for the control diet period. The reason for this latter finding is not clear, especially as the REM periodicity, calculated as the mean of the first two REM cycles (a cycle being measured from the end of one Stage REM to the beginning of the next Stage REM period), was not affected on this night.

The only other significant findings concerning the post-diet night were a decrease in Stage 3 occurring during the first half of the night on the post-HCF diet night and during the second half of the night on the post-LCF diet night. As the duration of Stage 3+4 did not decrease on the post-HCF diet night, it seems that there was a shift in SWS towards an increase in Stage 4. On the post-LCF night such a shift was not evident and the duration of Stage 3+4 showed a non-significant decrease. The significance of these changes in SWS is not clear, but it must be remembered that the data is limited to only 5 subjects and the comparisons were made using the t test method with averaged data for the control and experimental dietary periods. Thus, no allowance was made for the night-to-night variability within each dietary period and it is possible that these findings were a consequence of the comparatively coarse method of analysis.

The observed changes in REM sleep in this study are consistent with the predictions made from the theoretical model. As the study also observed sleep during a control (normal, balanced diet) period, it appears that the administration of the HCF diet increased REM sleep above control values whilst the LCF diet had lttle influence upon REM sleep derivation. This distinction would not be otherwise obvious as the duration of REM sleep during the HCF diet period was not unusually high. For example, the duration of REM sleep during the HCF diet period (103.5 minutes) was similar to the duration observed during the baseline period of Study I (105.7 minutes), during the administration of the zero carbohydrate supplement

in Study III (101.8 minutes) and the normal, balanced diet in the study by Phillips et. al. (1975, 103.6 minutes). From a comparison with these baseline data only, it would have appeared that the LCF diet had actually decreased REM sleep whilst the HCF diet had little influence. These differences between the duration of REM sleep in the control periods of this present study and the others described above are due, in part, to the shorter sleep period used for analysis (440.6 minutes as opposed to 450 minutes in Studies I and III) and to individual differences in REM sleep duration.

Although the observed changes in REM sleep were in agreement with the predictions, it must be stressed that this does not infer that the theoretical model is correct. The actual validation of the model would involve the assessment of changes in amino acid content of the plasma and of changes in brain serotonin synthesis rate in normal humans. Thus, the present study has only investigated the predictions from the model, and not the validity or detail of the model.

The study would have benefitted from a knowledge of the actual plasma changes during the various dietary periods, although there appears to be sufficient experimental evidence to suggest that the expected changes did occur. The lack of any objective information concerning changes in the rate of brain serotonin synthesis is another shortcoming of the study. However, there is no acceptable technique, at present, that could have been used in the sleep laboratory with normal subjects to provide this information.

## 10. SUMMARY AND CONCLUSIONS

This study was designed to provide experimental evidence to test the predictions from the theoretical model, developed in Chapters 9-11, concerning the effect of the diet upon REM sleep. Two non-isocaloric experimental diets were designed; one providing a high ratio of free plasma tryptophan to the

other large, neutral amino acids (valine, leucine, isoleucine, tyrosine and phenylalanine) and the other providing a low ratio. According to the proposed theory, it was predicted that the consumption of the first diet (high carbohydrate and fat content, low protein content) would be associated with a greater amount of REM sleep than the latter diet (low carbohydrate and fat content, high protein content).

Eight, healthy, young subjects (6 males, 2 females) of normal weight took part in the study. The experimental programme lasted for three consecutive weeks with the subjects receiving their normal 'control' diet during the first week and the experimental diets, in a balanced order, during the second and third weeks. Each of the experimental diets were consumed for three consecutive days, Monday to Wednesday, and EEG recordings were taken on these nights throughout the three week period. Recordings were also taken on the Thursday night following the return from the experimental diets to a normal diet. Subjects were allowed two nights for adaptation to the sleep laboratory at the beginning of the study and a further night immediately before the administration of the experimental diets.

The results showed that the high carbohydrate and fat, low protein diet consistently increased REM sleep and decreased Stage 1 throughout its administration compared to the low carbohydrate and fat, high protein diet and the control diet. Both of these changes occurred mainly during the second half of the night. When this experimental diet was terminated, both REM sleep and Stage 1 returned to normal values.

Thus, the observed changes in REM sleep were in agreement with the predictions made from the theoretical model.

CHAPTER 13

GENERAL DISCUSSION

## 1. INTRODUCTION

This chapter concludes Part Two of this Thesis. It contains a review of recent experimental evidence relevant to the theoretical model, followed by a brief discussion of future avenues for research of the diet and sleep relationship.

## 2. RECENT EVIDENCE AND THE THEORETICAL MODEL

Lacey, Stanley, Hartmann, Koval and Crisp (1978) have recently extended the work of Phillips, Chen, Crisp, Koval, McGuiness, Kalucy, Kalucy and Lacey (1975) by studying the effect of nocturnal infusions of amino acids and glucose upon human sleep. This method of administration was employed for two reasons. Firstly, the oral ingestion of pure amino acids is very unpleasant and, secondly, intravenous feeding is a common practice in hospitals and the effects upon sleep are of interest.

The nine female subjects were divided into three equal groups and each group sent four consecutive nights in the sleep laboratory. The first night was used for adaptation purposes, whilst on the remaining three nights the groups received 1000 ml infusions of saline, amino acids (aminosol) or 5% glucose solution in a balanced order. In order that the calorific intake should be identical for both the aminosol and glucose infusions, the subjects drank 20 ml of saccharin water before the saline or aminosol infusion and 20 ml of a concentrated glucose water before the glucose infusion, thereby supplying 310 kcal. EEG recordings were taken on the infusion nights and, in addition, the subjects completed questionnaires concerning the quality of their sleep.

The results showed that SWS, expressed as a percentage of total sleep time, was an average of 31.6, 28.8 and 23.9 percent during the aminosol, glucose and saline infusion nights, respectively. The difference in SWS between the aminosol and

saline nights reached significance.

REM sleep, again expressed as a percentage of total sleep time, was an average of 18.4, 20.8 and 22.8 percent during the aminosol, glucose and saline nights, respectively. The difference in REM sleep was significant between the aminosol and saline nights.

From the questionnaire analysis it appeared that the subjects reported that their sleep was more restless during the aminosol infusion than the other infusions and that the depth of sleep tended to be reduced during the aminosol infusion and increased during the glucose infusion.

This study is very interesting as it has confined itself to the study of the immediate effect of changes in the intake of amino acids and glucose. As such, it would have been predicted from the theoretical model that REM sleep would be higher during the glucose infusion compared to the aminosol infusion. This was found to be so, although the difference was not significant. However, the finding that percent REM sleep was lower during the glucose infusion night compared to the saline night would not be expected according to the model.

From the examination of the total sleep time data, it is apparent that one of the subjects (subject 2) had a grossly disturbed night during the glucose infusion (the total sleep time of this subject was reduced by half on this night). If the data from this subject is removed, the recalculated duration of REM sleep during the glucose night is 22.3 percent, which is very close to that observed during the saline night.

It is possible that REM sleep was not actually increased during the glucose infusion night because the intake of glucose was not sufficiently large. The glucose infusion and glucose water taken prior to sleep contained 310 kcals and this is considerably less than that administered in the high carbohydrate supplementary diet used in Study III of this thesis.

This latter supplementary diet included 20 g of glucose dissolved in half a pint of water and 7 oz. of fried potatoes, which amounted to 520 kcals whereas the low carbohydrate supplementary diet contained 186 kcals. The results of Study III showed only a trend ($p<0.1$, number of subjects = 6) towards increased REM sleep over the whole night as the carbohydrate content of the diet increased, with a mean increase of 2 minutes and 11 minutes for the low and high carbohydrate supplementary diets, respectively, compared to the zero carbohydrate supplementary diet. This may explain why the glucose infusion was not found to significantly increase REM sleep. REM sleep was found to be significantly increased during the first half of the night following the high carbohydrate supplementary diet but this finding cannot be compared with the study by Lacey et. al. (1978) because they did not subdivide the data.

Al-Marachi and Freeman (1977) have studied the effects of dietary tryptophan upon sleep. The authors chose this amino acid in particular because previous work had indicated that it produced sleepiness. The effects of tryptophan administration upon human sleep have been reviewed in Section 5.1.3 of Chapter 9. This study is very interesting because the tryptophan was administered in a natural form as a component of the food rather than as a laboratory refined supplement.

The authors designed 2 diets: the high tryptophan diet, which included large amounts of beef and milk (2.031 g tryptophan in 2145 calories; 130 g protein, 113 g fat, 173 g carbohydrate), and the low tryptophan diet, which contained large amounts of fruit and turkey (0.518 g tryptophan in 1943 calories; 47 g protein, 77 g fat, 343 g carbohydrate).

Each diet was eaten by 4 young, female subjects for a 5 day period from Monday to Friday, with 1 week interposed between the 2 diets. During this intervening week the subjects ate their normal diet. In order to determine whether the

diets were influencing blood tryptophan levels as anticipated, blood samples were taken on each Thursday. The mean serum levels were found to be 1.75, 2.05 and 2.47 mg/100 ml during the low, normal and high tryptophan diets respectively. Sleep was monitored during the last 3 nights of both dietary periods, the first night of which was used for adaption purposes in each period.

Analysis of the sleep records showed few significant findings. During the high tryptophan diet the subjects spent less time awake (1.2 minutes versus 9.2 minutes) and enjoyed fewer awakenings (1.8 versus 3.9) per night. In addition to the polygraphic data, the subjects rated their sleep from 1 (poor) to 7 (excellent) each morning upon arising. This score averaged 6.6 for the high tryptophan diet compared to 5.4 on the low tryptophan diet, and the difference was found to be statistically significant.

These findings suggest that sleep quality can be improved during an increased intake of dietary tryptophan. However, the subjects were well informed of the nature of this study; the authors pointed out that they even helped to design the diets. Thus, there was potential for suggestion effects in this study which may have been an important factor.

According to the theoretical model, it would have been expected that an increase in the tryptophan content of the diet would have increased REM sleep. Although the mean durations of REM sleep were 90 and 84 minutes during the high and low tryptophan dietary periods, respectively, this difference was not found to be significant. The lack of a significant increase in REM sleep could be explained by one or both of the following points.

Firstly, whilst there is an abundance of evidence in the animal literature that an increased intake of tryptophan is associated with increased levels of brain serotonin (see Section 2.1 Chapter 10), those studies using a tryptophan-

supplemented diet (Wang, Harwalker and Waisman, 1962; Green, Greenberg, Erickson, Sawyer and Ellizon, 1962), as opposed to intra-venous or intra-peritoneal injection, incorporated at the minimum a 10-fold increase in tryptophan above normal values. The high tryptophan diet in the study by Al-Marachi and Freeman supplied only a 2 and 4 fold increase compared with normal and low tryptophan diets, respectively. It is possible, therefore, that a larger increase in dietary tryptophan would be required before brain serotonin metabolism was increased to an extent whereby, according to the theoretical model, REM sleep would also be increased.

Secondly, this study altered the dietary proportions of protein, fat and carbohydrate to accommodate the high and low levels of tryptophan intake, whereas the animal studies used normal diets supplemented with tryptophan. It is clear that the addition of a tryptophan supplement will increase the transport of tryptophan to the brain in the animal studies because the ratio of plasma tryptophan (either free or total) to the other large, neutral amino acids will be increased. However, this study varied dietary tryptophan and it is not so clear that the ratio of plasma free tryptophan to the other large, neutral amino acids was increased during the high tryptophan compared to the low tryptophan diet because of the nature of the diets used. (It is the ratio of free, and not total, plasma tryptophan that is of interest because there is evidence to suggest that this measure is more suitable for assessing tryptophan transport to the brain in humans - see Section 2.7, Chapter 10).

Indirect assessments, based upon the proportions of protein, carbohydrate and fat content of the two diets as well as the tryptophan intake, would suggest that the low and high tryptophan diets would be associated with similar ratios of free plasma tryptophan to the competitor amino acids.

This is because the low tryptophan diet contained nearly a third less protein and nearly half as much again of carbohydrate and fat, by weight. (Although the low tryptophan diet contained less fat than the high tryptophan diet, it contained almost double the amount of carbohydrate). These dietary changes in the protein and carbohydrate content between the two diets would be expected to have an opposite influence upon the free tryptophan ratio than that expected from a consideration of the changes in tryptophan intake only.

The relevance of the protein, carbohydrate and fat content of the diet upon tryptophan transport to the brain is summarised in Section 2.8 of Chapter 10.

Very recent work by Hartmann, Crisp, Evans, Gaitonde and Kirkwood (1979 - draft paper for publication) has investigated the effects of three supplementary diets upon both sleep and plasma amino acid levels in 12 normal males.

Each subject spent two periods of five consecutive nights in the laboratory and received the various supplements with their evening meal according to a rather complex design, including at least one day without a supplement. The supplements were administered as drinks and included a high protein supplement (Nefranutrin), a high fat supplement (Prosperol) and a high carbohydrate supplement (Hycal). The amount of supplement taken was standardised for each subject by administering 100% of the individual daily intake of fat and carbohydrate, and 50% of the individual daily intake of protein, as determined from diet logs completed prior to the experiment. Blood samples were taken before the evening meal and at hourly intervals thereafter, including during the sleep period 'where feasible'. No specific times of sampling were presented which is unfortunate as the time course of the changes in plasma amino acids following the various supplements would be of great interest.

The results reported only the initial analysis of the data which was confined to the effect of the supplements on REM sleep and the relative amount of tryptophan to tyrosine in the plasma (as this index has been suggested by Fernstrom and Wurtman (1971) to be a good approximation to the ratio of plasma tryptophan to the other neutral amino acids). The log $\frac{\text{tryptophan}}{\text{tyrosine}}$ was used to normalise the data and stabilise the variances.

Whilst REM sleep was not found to be significantly related to the average nightly values of log $\frac{\text{tryptophan}}{\text{tyrosine}}$, it was found that this latter variable was significantly influenced by the supplements. The ratio for the fat, carbohydrate and protein supplements were 0.76, 0.88 and 0.99, respectively, compared to 0.77 following the evening meal without a supplement. Presumably, the protein, carbohydrate and fat content of the daily diet, excluding supplements, was consistent throughout the study although this was not stated. The fat supplement appeared to have no influence upon the ratio whilst the carbohydrate and protein supplemented diets increased the ratio by 14 per cent and 29 per cent, respectively.

The finding that the carbohydrate supplement increased the ratio is consistent with Fernstrom and Wurtman's work with rats (see Section 2.2, Chapter 10), the significance of which has been included in the theoretical model. However, the consumption of a protein supplement would not be expected to increase the ratio as tryptophan is a rare amino acid compared to the other neutral amino acids. This apparent inconsistency with Fernstrom and Wurtman's work, and thereby with the theoretical model, was due to the fact that the protein supplement contained the essential amino acids only, for reasons of palatability. Thus, all of the large, neutral amino acids were present in the protein supplement except for tyrosine, and this would account for the large increase in the ratio of tryptophan to tyrosine in the plasma. The authors point out that the ratio will be re-calculated

using phenylalanine values for the denominator.

The fat supplement did not appear to influence the ratio. This finding is consistent with the theoretical model because the increased intake of fat in humans would only be expected to increase the free fraction of plasma tryptophan, at the expense of the bound fraction, with the total levels remaining fairly constant (see Section 2.5, Chapter 10).

The fact that a significant relationship between the ratios and REM sleep was not found is not necessarily inconsistent with the theoretical model because no account was taken of the free plasma tryptophan levels. As mentioned above, the ratio of the free plasma tryptophan to the other neutral, amino acids is possibly a better indicator of tryptophan transport to the brain in humans.

Also, the relationship between REM sleep and these ratios should be examined both for values of the ratios during the day as well as, or maybe instead of, during the night. From a comparison of studies III and IV of this thesis, it is apparent that the changes in REM sleep were greater following a complete change of diet than following a late evening supplement. Thus, the duration of the changes in the free plasma tryptophan to the other neutral amino acids is probably an important factor when relating this ratio to changes in REM sleep.

From the review of the recent evidence pertaining to the theoretical model it appears that there are no findings inconsistent with the model. Of special interest is the finding that a carbohydrate supplement increases the ratio of tryptophan to tyrosine in the plasma of human subjects. This point had hitherto been assumed from the work of Fernstrom and Wurtman with rats.

## 3. AVENUES FOR RESEARCH

Part Two of this thesis has examined the influence of the diet upon sleep in some detail and it appears that there is strong evidence in favour of such a relationship. One possible mechanism whereby the diet could influence sleep has been suggested and it seems that this theoretical model has a good degree of "face validity". This does not mean that the model is correct and it is expected that future findings will necessitate additions, qualifications or the abandonment of the present model. In any field of research, it can be advantageous to be able to test a theoretical model as this provides a focal point for research and stimulates a systematic approach to the investigations. Thus, one possibly fruitful avenue of research would be to investigate the proposed model more thoroughly. For example, more attention could be paid to studying the changes in plasma constituents in humans during various dietary periods. The extent and time course of such changes could then be compared to the changes in sleep, if observed.

The model developed in this thesis has focussed upon the influence of changes in the carbohydrate, fat and protein content of the diet. Whilst these dietary changes were not designed to be iso-caloric, the variation in daily calorie intake was small. It would be of interest to study the influence of progressive changes in daily calorie intake whilst the carbohydrate, fat and protein ratio remained constant. For example, the consumption of $\frac{1}{4}$, $\frac{1}{2}$ or $1\frac{1}{2}$ times the normal daily calorie intake could be studied in healthy subjects over time periods of a few days to a few weeks. Such studies would compliment the starvation studies which represent an extreme change in daily calorie intake.

Other interesting experiments could include the study of time-of-day effects of the diet upon sleep, the study of whether bad sleepers are more sensitive to dietary changes than good sleepers or the study of the interactions between the diet and other factors such as illness. This latter proposal could be an extremely rewarding avenue of research as brain serotonin

metabolism appears to be abnormal in patients suffering from various affective disorders (see Coppen, Eccleston and Peet, 1973; Jimerson, Post and Goodwin, 1976). It is possible that the diet may have a therapeutic value in the treatment of these disorders, either in conjunction with standard drug treatments or by itself.

From the work reviewed and undertaken in this thesis, it is clear that the importance of the diet and sleep relationship should not be underestimated. It is recommended that future sleep studies investigating the influence of other variables or drugs should control or, at least, monitor the subject's diet during the experimental periods. This may help to remove some of the night-to-night variation in sleep stage duration as a consequence of some dietary changes. Furthermore, the study of the diet and sleep relationship provides evidence pertaining to the normal physiological mechanisms controlling sleep, whereas the pharmacological and neurophysiological studies rarely examine the influence of changes in neurotransmitter metabolism or function within the physiological range.

## APPENDIX I: ADDITIONAL INFORMATION AND DATA FOR STUDY I

### 1. Subject Screening Questionnaire

NAME: ADDRESS:

AGE:

SEX:

Please answer the following questions by placing a tick in the relevant box.

1. Have you received medial treatment during the last four weeks?

| | |
|---|---|
| YES | |
| NO | |

If so, please briefly describe the reason for treatment and any drugs administered (if known).

2. Have you any long-term health problems, such as asthma, migraine, diabetes, etc.?

| | |
|---|---|
| YES | |
| NO | |

If so, please describe the problem and any treatments that you use.

3. Do you have any sleep problems?

| | |
|---|---|
| YES | |
| NO | |

If so, please describe the problem(s), stating how often it occurs and when you last suffered.

4. Do you regularly take naps during the day?

| | |
|---|---|
| YES | |
| NO | |

If so, how long do these naps usually last?

5. Please list your regular sporting activities, stating how often you partake in each activity.

2. Individual data.

The data is presented for the whole night, for the first and second halves of the night and prior to the first REM period. The duration of each stage of sleep or sleep variable is presented in the following format:

| | | | | | |
|---|---|---|---|---|---|
| $S_1B_1$ | $S_1PM$ | $S_1C(PM)$ | $S_1B_2$ | $S_1AM$ | $S_1C(AM)$ |
| $S_2$ | | | | | |
| $S_3$ | | | | | |
| $S_4$ | | | | | |
| $S_5$ | | | | | |
| $S_6$ | | | | | |
| $S_7$ | | | | | |
| $S_8$ | | | | | |

where

$S_{1-8}$ denotes the subject

$B_1$,$B_2$ denote the first and second control nights, respectively

PM, AM denote the afternoon and morning exercise recovery nights, respectively.

C(PM), C(AM) denote the afternoon and morning exercise carryover nights, respectively.

| STAGES 2ii+3+4 | | WHOLE NIGHT DATA (%) | | | |
|---|---|---|---|---|---|
| 35.7 | 48.5 | 41.1 | 40.4 | 43.7 | 45.3 |
| 37.9 | 42.0 | 34.3 | 28.3 | 42.0 | 33.6 |
| 36.6 | 32.6 | 31.7 | 46.2 | 39.9 | 41.3 |
| 42.5 | 47.9 | 37.4 | 47.4 | 42.7 | 44.6 |
| 50.9 | 43.2 | 40.0 | 40.9 | 40.4 | 39.2 |
| 40.2 | 42.0 | 39.7 | 37.7 | 38.7 | 32.1 |
| 28.2 | 30.3 | 26.7 | 24.3 | 20.9 | 28.4 |
| 34.6 | 30.4 | 28.7 | 31.6 | 32.4 | 30.7 |
| STAGES 3+4 | | | | | |
| 26.8 | 34.7 | 31.2 | 24.9 | 33.6 | 37.3 |
| 27.3 | 33.1 | 27.3 | 24.8 | 33.3 | 30.3 |
| 28.2 | 22.5 | 20.4 | 30.5 | 32.4 | 31.0 |
| 32.9 | 39.0 | 32.2 | 40.4 | 37.1 | 37.6 |
| 38.0 | 27.9 | 25.6 | 29.8 | 26.0 | 26.5 |
| 28.6 | 26.7 | 27.5 | 25.3 | 28.9 | 22.5 |
| 20.2 | 18.3 | 21.1 | 21.6 | 17.3 | 17.3 |
| 24.2 | 21.3 | 17.4 | 20.7 | 22.0 | 21.1 |
| STAGE 4 | | | | | |
| 18.4 | 22.1 | 19.2 | 19.2 | 17.4 | 20.2 |
| 16.7 | 18.9 | 19.4 | 12.6 | 22.7 | 21.0 |
| 18.1 | 12.0 | 13.6 | 13.8 | 22.3 | 19.2 |
| 25.6 | 29.6 | 25.1 | 29.1 | 28.6 | 29.3 |
| 27.1 | 20.6 | 16.8 | 22.9 | 15.6 | 14.9 |
| 19.3 | 15.8 | 17.7 | 14.6 | 16.7 | 11.2 |
| 10.0 | 6.7 | 12.9 | 12.7 | 14.0 | 7.3 |
| 15.5 | 11.7 | 9.6 | 16.5 | 15.3 | 14.2 |
| STAGE 3 | | | | | |
| 8.4 | 12.7 | 12.0 | 5.6 | 16.2 | 17.1 |
| 10.6 | 14.1 | 7.8 | 12.4 | 10.6 | 9.3 |
| 10.1 | 10.6 | 6.8 | 16.7 | 10.1 | 11.7 |
| 7.3 | 9.4 | 7.0 | 11.3 | 8.4 | 8.2 |
| 10.9 | 7.3 | 8.8 | 6.9 | 10.4 | 11.6 |
| 9.3 | 10.9 | 9.8 | 10.7 | 12.2 | 11.3 |
| 10.2 | 11.6 | 8.2 | 8.9 | 3.3 | 10.0 |
| 8.7 | 9.6 | 7.8 | 4.2 | 6.7 | 6.9 |
| STAGE REM | | | | | |
| 27.0 | 25.8 | 22.8 | 28.4 | 25.4 | 29.8 |
| 19.7 | 22.0 | 24.0 | 23.0 | 23.0 | 25.3 |
| 26.3 | 18.6 | 28.2 | 18.3 | 21.6 | 21.1 |
| 22.0 | 25.6 | 24.4 | 22.3 | 22.5 | 22.8 |
| 24.4 | 25.1 | 21.8 | 22.9 | 25.8 | 22.9 |
| 18.4 | 21.8 | 25.1 | 20.0 | 23.8 | 20.4 |
| 29.1 | 24.9 | 25.6 | 29.8 | 34.9 | 23.6 |
| 24.0 | 25.6 | 23.6 | 20.4 | 24.7 | 26.7 |
| STAGE 2ii | | | | | |
| 8.9 | 13.8 | 9.9 | 15.5 | 10.1 | 8.0 |
| 10.6 | 8.9 | 7.0 | 3.5 | 8.7 | 3.3 |
| 8.4 | 10.1 | 11.3 | 15.7 | 7.5 | 10.3 |
| 9.6 | 8.9 | 5.2 | 7.0 | 5.6 | 7.0 |
| 12.9 | 15.3 | 14.4 | 11.1 | 14.4 | 12.7 |
| 11.6 | 15.3 | 12.2 | 12.4 | 9.8 | 9.6 |
| 8.0 | 12.0 | 5.6 | 2.7 | 3.6 | 11.1 |
| 10.4 | 9.1 | 11.3 | 10.9 | 10.4 | 9.6 |
| STAGES 0+1 | | | | | |
| 1.2 | 1.9 | 2.6 | 0.9 | 2.1 | 1.4 |
| 5.2 | 2.7 | 5.8 | 6.6 | 4.5 | 6.0 |
| 6.3 | 10.3 | 2.6 | 3.8 | 6.6 | 4.4 |
| 6.1 | 5.4 | 5.6 | 5.4 | 7.5 | 3.1 |
| 7.3 | 3.7 | 4.0 | 1.3 | 2.0 | 2.5 |
| 6.3 | 4.5 | 5.8 | 5.1 | 7.2 | 9.3 |
| 2.9 | 10.4 | 6.2 | 10.6 | 10.9 | 12.9 |
| 7.2 | 5.5 | 7.1 | 4.7 | 8.2 | 5.5 |

REM LATENCY (MINUTES)

| | | | | | |
|---|---|---|---|---|---|
| 73 | 77 | 59 | 115 | 90 | 56 |
| 81 | 87 | 105 | 109 | 71 | 69 |
| 51 | 54 | 49 | 59 | 73 | 62 |
| 71 | 145 | 87 | 66 | 79 | 80 |
| 89 | 131 | 80 | 74 | 68 | 82 |
| 122 | 145 | 144 | 132 | 74 | 70 |
| 141 | 128 | 125 | 130 | 116 | 138 |
| 78 | 136 | 67 | 79 | 64 | 78 |

REM PERIODICITY (MINUTES)

| | | | | | |
|---|---|---|---|---|---|
| 82 | 85 | 88 | 80 | 91 | 88 |
| 115 | 62 | 100 | 118 | 110 | 86 |
| 80 | 92 | 80 | 108 | 93 | 89 |
| 78 | 83 | 105 | 90 | 87 | 84 |
| 92 | 101 | 78 | 68 | 89 | 96 |
| 89 | 95 | 83 | 99 | 87 | 87 |
| 105 | 118 | 107 | 103 | 93 | 95 |
| 83 | 81 | 73 | 85 | 84 | 95 |

| STAGE 2ii+3+4 | | PRIOR TO FIRST REM PERIOD (MINUTES) | | | |
|---|---|---|---|---|---|
| 57 | 69 | 50 | 105 | 76 | 46 |
| 55 | 81 | 60 | 56 | 55 | 52 |
| 46 | 36 | 40 | 56 | 61 | 45 |
| 60 | 119 | 78 | 58 | 73 | 75 |
| 82 | 116 | 73 | 69 | 60 | 78 |
| 104 | 120 | 56 | 113 | 63 | 55 |
| 88 | 99 | 85 | 87 | 87 | 82 |
| 68 | 98 | 45 | 59 | 55 | 65 |
| STAGES 3+4 | | | | | |
| 55 | 56 | 45 | 82 | 69 | 45 |
| 40 | 75 | 43 | 49 | 49 | 50 |
| 42 | 31 | 36 | 51 | 59 | 43 |
| 56 | 109 | 71 | 54 | 69 | 70 |
| 76 | 85 | 69 | 60 | 55 | 71 |
| 85 | 103 | 37 | 89 | 57 | 46 |
| 65 | 68 | 73 | 79 | 78 | 53 |
| 63 | 79 | 38 | 48 | 52 | 58 |
| STAGE 4 | | | | | |
| 47 | 51 | 35 | 59 | 53 | 39 |
| 31 | 58 | 26 | 11 | 32 | 39 |
| 41 | 22 | 31 | 38 | 57 | 27 |
| 55 | 94 | 57 | 49 | 63 | 63 |
| 67 | 66 | 60 | 56 | 47 | 55 |
| 69 | 71 | 25 | 53 | 43 | 31 |
| 42 | 39 | 58 | 55 | 63 | 32 |
| 55 | 49 | 28 | 40 | 44 | 49 |
| STAGE 3 | | | | | |
| 7 | 5 | 11 | 21 | 16 | 6 |
| 14 | 18 | 17 | 38 | 16 | 11 |
| 1 | 8 | 5 | 13 | 2 | 16 |
| 3 | 15 | 14 | 5 | 5 | 6 |
| 9 | 19 | 9 | 4 | 8 | 16 |
| 16 | 32 | 12 | 36 | 14 | 15 |
| 23 | 29 | 15 | 24 | 15 | 21 |
| 8 | 30 | 10 | 8 | 8 | 9 |
| STAGE 2ii | | | | | |
| 2 | 13 | 4 | 22 | 7 | 1 |
| 15 | 6 | 17 | 7 | 6 | 2 |
| 4 | 5 | 4 | 5 | 2 | 2 |
| 4 | 11 | 7 | 4 | 4 | 5 |
| 6 | 31 | 4 | 9 | 5 | 7 |
| 19 | 17 | 19 | 24 | 6 | 9 |
| 23 | 31 | 12 | 8 | 9 | 29 |
| 5 | 19 | 7 | 11 | 3 | 7 |
| STAGE 0+1 | | | | | |
| 0 | 1 | 0 | 0 | 0 | 0 |
| 2 | 0 | 3 | 13 | 0 | 1 |
| 2 | 7 | 0 | 1 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 2 | 4 | 0 | 0 | 3 |
| 0 | 7 | 7 | 0 | 6 | 7 |
| 0 | 0 | 0 | 0 | 0 | 0 |

STAGES 2ii+3+4 FIRST HALF DATA (%)

| | | | | | |
|---|---|---|---|---|---|
| 56.3 | 66.2 | 67.6 | 68.1 | 54.9 | 54.0 |
| 51.8 | 65.3 | 53.0 | 45.1 | 60.5 | 40.4 |
| 57.8 | 53.0 | 52.1 | 63.9 | 57.7 | 59.1 |
| 66.6 | 64.8 | 58.7 | 59.6 | 61.0 | 62.0 |
| 69.3 | 72.7 | 56.0 | 60.5 | 54.3 | 48.4 |
| 56.9 | 66.2 | 63.1 | 63.1 | 58.3 | 42.2 |
| 41.3 | 47.5 | 44.5 | 39.1 | 38.1 | 41.8 |
| 56.9 | 53.7 | 43.5 | 52.0 | 52.5 | 39.6 |

STAGES 3+4

| | | | | | |
|---|---|---|---|---|---|
| 46.9 | 53.5 | 53.5 | 49.8 | 47.9 | 44.1 |
| 35.4 | 55.9 | 42.7 | 38.5 | 53.0 | 37.1 |
| 45.1 | 39.4 | 35.7 | 51.2 | 53.0 | 52.1 |
| 58.2 | 52.6 | 50.7 | 53.5 | 55.4 | 55.9 |
| 54.2 | 40.9 | 38.2 | 45.8 | 39.6 | 35.1 |
| 42.7 | 49.8 | 46.2 | 49.3 | 47.6 | 29.8 |
| 28.9 | 29.3 | 32.9 | 35.1 | 34.7 | 31.6 |
| 45.3 | 41.3 | 31.1 | 40.9 | 42.7 | 29.8 |

STAGE 4

| | | | | | |
|---|---|---|---|---|---|
| 32.6 | 38.5 | 34.3 | 38.5 | 31.5 | 26.3 |
| 23.0 | 35.2 | 30.5 | 16.4 | 41.3 | 24.9 |
| 30.0 | 20.2 | 27.2 | 27.2 | 44.6 | 32.4 |
| 47.4 | 41.8 | 42.2 | 43.7 | 43.2 | 45.5 |
| 40.0 | 30.2 | 29.3 | 40.4 | 29.8 | 24.4 |
| 32.9 | 31.6 | 35.6 | 29.3 | 33.3 | 16.9 |
| 18.7 | 13.3 | 25.8 | 24.4 | 28.0 | 14.7 |
| 31.1 | 23.6 | 19.1 | 32.9 | 30.7 | 21.8 |

STAGE 3

| | | | | | |
|---|---|---|---|---|---|
| 14.4 | 15.0 | 19.2 | 11.3 | 16.4 | 17.8 |
| 12.4 | 20.7 | 12.2 | 22.1 | 11.7 | 12.2 |
| 15.0 | 19.2 | 8.4 | 23.9 | 8.4 | 19.7 |
| 10.8 | 10.8 | 8.4 | 9.9 | 12.2 | 10.3 |
| 14.2 | 10.7 | 8.9 | 5.3 | 9.8 | 10.7 |
| 9.8 | 18.2 | 10.7 | 20.0 | 14.2 | 12.9 |
| 10.2 | 16.0 | 7.1 | 10.7 | 6.7 | 16.9 |
| 14.2 | 17.8 | 12.0 | 8.0 | 12.0 | 8.0 |

STAGE REM

| | | | | | |
|---|---|---|---|---|---|
| 12.9 | 20.7 | 13.6 | 17.8 | 13.1 | 23.0 |
| 14.4 | 8.4 | 18.8 | 8.4 | 21.6 | 18.3 |
| 11.3 | 17.4 | 15.0 | 22.1 | 14.5 | 11.7 |
| 9.4 | 9.4 | 22.1 | 13.6 | 12.7 | 8.4 |
| 15.1 | 15.1 | 8.9 | 12.4 | 12.4 | 14.2 |
| 15.1 | 9.8 | 8.0 | 14.2 | 17.3 | 21.3 |
| 10.2 | 13.3 | 13.3 | 16.0 | 21.3 | 8.9 |
| 16.4 | 11.6 | 16.0 | 11.1 | 14.7 | 24.4 |

STAGE 2ii

| | | | | | |
|---|---|---|---|---|---|
| 9.4 | 12.7 | 14.1 | 18.3 | 7.0 | 9.9 |
| 16.4 | 9.4 | 10.3 | 6.6 | 7.5 | 3.3 |
| 12.7 | 13.6 | 16.4 | 12.7 | 4.7 | 7.0 |
| 8.4 | 12.2 | 8.0 | 6.1 | 5.6 | 6.1 |
| 15.1 | 21.8 | 13.3 | 14.7 | 14.7 | 13.3 |
| 12.9 | 16.4 | 16.9 | 13.8 | 10.7 | 12.4 |
| 12.4 | 18.2 | 5.8 | 4.0 | 4.4 | 17.8 |
| 11.6 | 12.4 | 12.4 | 11.1 | 9.8 | 9.8 |

STAGES 0+1

| | | | | | |
|---|---|---|---|---|---|
| 1.8 | 1.9 | 1.4 | 1.4 | 2.8 | 2.8 |
| 3.5 | 3.0 | 2.5 | 9.1 | 4.0 | 4.0 |
| 3.8 | 7.0 | 3.3 | 3.3 | 5.2 | 2.8 |
| 1.9 | 2.3 | 2.3 | 1.9 | 6.6 | 1.9 |
| 0.0 | 0.4 | 1.8 | 0.4 | 0.0 | 0.9 |
| 1.8 | 1.8 | 3.6 | 0.4 | 2.7 | 6.2 |
| 0.4 | 3.6 | 4.4 | 0.0 | 3.1 | 8.9 |
| 3.6 | 1.3 | 1.8 | 3.1 | 0.0 | 1.8 |

STAGES 2ii+3+4 SECOND HALF DATA (%)

| | | | | | |
|---|---|---|---|---|---|
| 15.1 | 33.0 | 14.5 | 12.7 | 32.4 | 36.6 |
| 23.8 | 18.7 | 15.6 | 11.6 | 23.5 | 26.8 |
| 15.5 | 12.2 | 11.3 | 28.7 | 22.0 | 23.5 |
| 18.3 | 30.9 | 16.0 | 35.2 | 24.4 | 27.2 |
| 32.5 | 23.8 | 24.1 | 21.4 | 26.6 | 29.9 |
| 23.4 | 17.4 | 16.4 | 12.4 | 19.1 | 21.9 |
| 15.1 | 13.1 | 14.6 | 9.4 | 2.7 | 15.0 |
| 12.4 | 7.1 | 13.7 | 11.2 | 12.4 | 21.7 |

STAGES 3+4

| | | | | | |
|---|---|---|---|---|---|
| 6.7 | 15.9 | 8.9 | 0.0 | 19.2 | 30.5 |
| 19.1 | 10.3 | 11.9 | 11.1 | 13.6 | 23.5 |
| 11.3 | 5.6 | 5.2 | 9.9 | 11.7 | 9.9 |
| 7.5 | 25.3 | 13.6 | 27.2 | 18.8 | 19.2 |
| 21.8 | 14.9 | 8.5 | 13.8 | 12.4 | 17.9 |
| 14.6 | 3.6 | 8.9 | 1.3 | 10.2 | 15.2 |
| 11.5 | 7.3 | 9.3 | 8.1 | 0.0 | 3.1 |
| 3.2 | 1.3 | 3.4 | 0.4 | 1.3 | 12.5 |

STAGE 4

| | | | | | |
|---|---|---|---|---|---|
| 4.3 | 5.6 | 4.2 | 0.0 | 3.3 | 14.1 |
| 10.4 | 2.6 | 8.3 | 8.9 | 4.1 | 17.1 |
| 6.1 | 3.8 | 0.0 | 0.5 | 0.0 | 6.1 |
| 3.8 | 17.4 | 8.0 | 14.6 | 14.1 | 13.1 |
| 14.2 | 11.1 | 4.4 | 5.3 | 1.3 | 5.3 |
| 5.8 | 0.0 | 0.0 | 0.0 | 0.0 | 5.3 |
| 1.3 | 0.0 | 0.0 | 0.9 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.7 |

STAGE 3

| | | | | | |
|---|---|---|---|---|---|
| 2.4 | 10.3 | 4.7 | 0.0 | 16.0 | 16.4 |
| 8.8 | 7.5 | 3.4 | 2.7 | 9.5 | 6.4 |
| 5.2 | 1.9 | 5.2 | 9.4 | 11.7 | 3.8 |
| 3.8 | 8.0 | 5.6 | 12.7 | 4.7 | 6.1 |
| 7.6 | 4.0 | 8.9 | 8.4 | 11.1 | 12.4 |
| 8.8 | 3.6 | 8.9 | 1.3 | 10.2 | 9.8 |
| 10.2 | 7.3 | 9.3 | 7.1 | 0.0 | 3.1 |
| 3.2 | 1.3 | 3.4 | 0.4 | 1.3 | 5.8 |

STAGE REM

| | | | | | |
|---|---|---|---|---|---|
| 41.2 | 31.0 | 31.9 | 39.0 | 37.6 | 36.6 |
| 25.0 | 35.5 | 29.2 | 37.6 | 24.4 | 32.2 |
| 32.9 | 33.8 | 33.8 | 22.5 | 30.5 | 33.8 |
| 43.2 | 27.7 | 34.3 | 23.0 | 30.5 | 33.8 |
| 33.7 | 39.5 | 34.7 | 33.4 | 39.1 | 31.6 |
| 21.7 | 33.8 | 42.2 | 25.8 | 30.3 | 19.5 |
| 48.0 | 36.5 | 37.9 | 43.6 | 48.5 | 38.3 |
| 31.6 | 39.6 | 31.2 | 29.7 | 34.7 | 29.0 |

STAGE 2ii

| | | | | | |
|---|---|---|---|---|---|
| 8.4 | 15.0 | 5.6 | 12.7 | 13.2 | 6.1 |
| 4.7 | 8.4 | 3.7 | 0.5 | 9.9 | 3.3 |
| 4.2 | 6.6 | 6.1 | 18.8 | 10.3 | 13.6 |
| 10.8 | 5.6 | 2.4 | 8.0 | 5.6 | 8.0 |
| 10.7 | 8.9 | 15.6 | 7.6 | 11.1 | 8.9 |
| 3.6 | 5.8 | 5.3 | 1.3 | 2.7 | 4.4 |
| 9.3 | 5.8 | 10.2 | 10.7 | 11.1 | 9.3 |

STAGE 0+1

| | | | | | |
|---|---|---|---|---|---|
| 0.5 | 1.9 | 3.8 | 0.5 | 1.4 | 0.0 |
| 7.6 | 2.5 | 9.1 | 8.1 | 5.0 | 8.1 |
| 8.9 | 13.6 | 1.9 | 4.2 | 8.0 | 6.1 |
| 10.3 | 8.0 | 8.9 | 8.9 | 8.4 | 4.2 |
| 14.7 | 7.1 | 6.2 | 2.2 | 4.0 | 4.0 |
| 10.7 | 7.1 | 8.0 | 9.8 | 11.6 | 12.4 |
| 5.3 | 17.3 | 8.0 | 21.3 | 18.7 | 16.9 |
| 10.7 | 9.8 | 12.4 | 6.2 | 16.4 | 9.3 |

3. Standard Deviations for group means in Study I

NOTE This table should be read in conjunction with tables 3 and 4 of Chapter 4

| | Baseline 1 | Baseline 2 | Morning exercise Recovery night | Morning exercise Carryover night | Afternoon exercise Recovery night | Afternoon exercise Carryover night |
|---|---|---|---|---|---|---|
| Whole night | | | | | | |
| Stages 2ii | 1.8 | 4.8 | 3.3 | 4.3 | 9.3 | 3.4 |
| 3 | 1.2 | 4.1 | 3.8 | 3.1 | 2.1 | 1.7 |
| 4 | 5.5 | 7.5 | 5.0 | 6.8 | 7.2 | 4.7 |
| 3+4 | 5.4 | 6.3 | 6.7 | 7.4 | 7.2 | 5.3 |
| 2ii+3+4 | 6.6 | 8.1 | 7.4 | 6.4 | 7.4 | 5.4 |
| REM | 3.7 | 4.0 | 4.2 | 3.1 | 8.3 | 6.8 |
| First half | | | | | | |
| Stages 2ii | 2.6 | 4.8 | 3.6 | 4.7 | 4.2 | 3.8 |
| 3+4 | 9.4 | 6.6 | 7.2 | 13.6 | 9.1 | 8.2 |
| 2ii+3+4 | 8.6 | 9.7 | 6.2 | 7.8 | 6.8 | 8.9 |
| REM | 2.6 | 13.4 | 11.9 | 6.3 | 13.6 | 14.9 |
| Second half | | | | | | |
| Stages 2ii | 3.1 | 5.7 | 3.7 | 3.5 | 3.6 | 4.2 |
| 3+4 | 6.3 | 9.0 | 7.1 | 7.0 | 8.1 | 3.2 |
| 2ii+3+4 | 6.8 | 9.3 | 8.7 | 6.0 | 8.4 | 4.1 |
| REM | 9.0 | 11.9 | 9.3 | 10.3 | 4.0 | 10.2 |
| Time (min) before first REM period | | | | | | |
| Stages 2ii | 7.9 | 7.4 | 2.2 | 9.1 | 10.2 | 5.9 |
| 3 | 7.1 | 13.0 | 5.2 | 5.3 | 10.2 | 3.5 |
| 4 | 13.8 | 15.7 | 10.9 | 12.3 | 20.8 | 15.5 |
| 3+4 | 15.2 | 17.0 | 9.8 | 11.3 | 22.0 | 16.4 |
| 2ii+3+4 | 20.2 | 22.9 | 11.4 | 15.3 | 29.7 | 24.8 |
| REM latency | 30.6 | 29.4 | 16.9 | 25.1 | 35.8 | 33.8 |
| First REM period length | 10.4 | 11.4 | 9.7 | 6.7 | 12.8 | 8.8 |
| REM periodicity First period | 17.3 | 27.3 | 19.0 | 30.8 | 27.1 | 26.8 |
| Mean of first and second periods | 13.1 | 16.2 | 8.0 | 14.7 | 16.4 | 13.1 |

## APPENDIX II: DATA AND QUESTIONNAIRES FOR STUDY II

### 1. Data

The data used in the analysis is presented in the following format (as used in the computer analysis):

| Column(s) | Variable | Response |
|---|---|---|
| 1-2 | Subject number | |
| 4-5 | Age | Years |
| 7 | Sex | 1 male, 2 female |
| 9 | Marital status | 1 single, 2 married |
| 11 | Fitness | 1 very fit, 2 fit, 3 a bit out of condition, 4 very much out of condition. |
| 13-14 | Morning/eveningness score | |
| 18-19 | Activity level | 2 greatly above normal 1 above normal, 0 normal, -1 below normal, -2 greatly below normal. |
| 21 | Time of day of activity | 1 morning, 2 afternoon, 3 early evening, 4 late evening. |
| 26-29 | Time when thinking of bed | decimal clock-time. |
| 31-32 | Bedtime suitable? | 1 too late, 0 at the right time, -1 too early. |
| 35-38 | Time of retiring | decimal clock-time |
| 41-43 | Daytime naps | duration in minutes |
| 46 | Pre-sleep tiredness | 1 feeling active, 7 sleep onset soon. |
| 52 | Post-sleep tiredness | as above. |
| 54-56 | Sleep onset | minutes |
| 59-61 | Sleep length | minutes |
| 64-66 | Time taken to rise | minutes |
| 68-69 | Sleep quality | +2 much better than normal -2 much worse than normal |
| 72 | More sleep required? | 0 no, 1 yes. |
| 75 | Used alarm clock? | 0 no, 1 yes. |
| 77-78 | Mood | 1 very enjoyable, 0 normal, -1 upsetting. |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 12345678 |
| 1 31 1 2 1 66 | 1 4 | 11.5 0 | 12.5 30 3 | 3 30 | 450 | 15 0 0 1 1 |
| 1 31 1 2 1 66 | 1 4 | 14.0 1 | 14.0 30 5 | 4 0 | 320 | 120 0 1 0 1 |
| 1 31 1 2 1 66 | 0 | 11.5 1 | 12.0 0 3 | 1 0 | 525 | 0 1 0 0 0 |
| 1 31 1 2 1 66 | 0 | 11.5 0 | 11.5 30 3 | 3 30 | 525 | 0 0 0 0 0 |
| 1 31 1 2 1 66 | 0 | 11.5 0 | 11.5 15 3 | 2 30 | 495 | 0 1 0 0 0 |
| 1 31 1 2 1 66 | 1 3 | 11.5 1 | 12.2 0 5 | 5 15 | 450 | 15 1 1 1 0 |
| 1 31 1 2 1 66 | 1 3 | 12.5 1 | 14.0 30 6 | 6 0 | 420 | 60 0 1 1 0 |
| 2 25 1 2 4 50 | 1 2 | 11.5 0 | 11.8 30 3 | 2 45 | 465 | 5 0 1 0 0 |
| 2 25 1 2 4 50 | 0 | 11.5 1 | 12.0 0 3 | 3 30 | 540 | 0 0 1 0 0 |
| 2 25 1 2 4 50 | 0 | 11.5 1 | 12.5 30 3 | 3 15 | 525 | 15 0 0 0 0 |
| 2 25 1 2 4 50 | 0 | 11.5 1 | 12.5 0 3 | 3 30 | 420 | 45 0 1 1 0 |
| 2 25 1 2 4 50 | 1 2 | 9.5 0 | 10.2 0 3 | 3 135 | 390 | 60 0 1 1 0 |
| 2 25 1 2 4 50 | 1 2 | 10.5 0 | 11.0 0 3 | 4 120 | 360 | 60 -1 1 1 0 |
| 2 25 1 2 4 50 | 1 2 | 11.5 1 | 12.0 0 3 | 4 60 | 300 | 120 -1 1 1 0 |
| 3 18 2 1 3 49 | 1 2 | 10.5 0 | 11.0 0 2 | 3 30 | 600 | 30 0 1 0 1 |
| 3 18 2 1 3 49 | 0 | 9.5 0 | 11.0 0 1 | 2 45 | 585 | 30 0 0 0 0 |
| 3 18 2 1 3 49 | 0 | 11.5 1 | 11.7 30 3 | 2 30 | 540 | 15 -1 1 0 1 |
| 3 18 2 1 3 49 | 0 | 10.5 0 | 11.0 60 1 | 3 30 | 600 | 60 1 1 0 0 |
| 3 18 2 1 3 49 | -1 | 12.5 0 | 13.0 0 1 | 2 15 | 600 | 45 1 0 0 1 |
| 3 18 2 1 3 49 | 0 | 9.5 0 | 10.2 0 1 | 2 45 | 525 | 15 0 1 0 -1 |
| 3 18 2 1 3 49 | 0 | 10.5 0 | 11.5 0 2 | 2 15 | 495 | 15 0 1 0 0 |
| 4 25 1 2 2 70 | 1 1 | 10.5 0 | 11.0 0 3 | 2 15 | 435 | 15 0 0 0 0 |
| 4 25 1 2 2 70 | 1 1 | 11.5 1 | 11.5 0 5 | 2 75 | 435 | 0 0 0 0 0 |
| 4 25 1 2 2 70 | -1 | 11.5 0 | 12.0 0 3 | 4 30 | 510 | 0 0 0 0 0 |
| 4 25 1 2 2 70 | -1 | 9.5 0 | 10.0 60 2 | 4 30 | 540 | 15 0 1 1 0 |
| 4 25 1 2 2 70 | 1 2 | 11.5 0 | 12.2 0 2 | 2 15 | 390 | 45 0 1 1 0 |
| 4 25 1 2 2 70 | 1 3 | 10.5 0 | 11.2 0 3 | 3 30 | 450 | 15 0 0 0 0 |
| 4 25 1 2 2 70 | 1 2 | 10.5 0 | 11.5 0 2 | 2 30 | 420 | 15 0 0 0 0 |
| 5 43 1 2 3 51 | 0 | 11.5 1 | 12.0 15 5 | 3 30 | 420 | 60 0 1 0 0 |
| 5 43 1 2 3 51 | -1 | 12.5 0 | 12.5 0 3 | 3 15 | 420 | 60 0 0 0 0 |
| 5 43 1 2 3 51 | -1 | 12.5 0 | 12.5 0 3 | 3 45 | 345 | 15 -1 0 1 0 |
| 5 43 1 2 3 51 | 0 | 12.0 0 | 12.3 0 3 | 2 15 | 390 | 15 0 0 0 0 |
| 5 43 1 2 3 51 | 1 4 | 12.0 0 | 12.2 0 5 | 4 30 | 375 | 30 -1 1 1 0 |
| 5 43 1 2 3 51 | 0 | 12.0 0 | 12.2 0 5 | 3 30 | 390 | 15 0 0 1 0 |
| 5 43 1 2 3 51 | 0 | 12.7 1 | 12.8 0 3 | 3 45 | 345 | 30 0 1 1 0 |
| 6 37 1 2 2 68 | -1 | 10.5 0 | 10.7 0 5 | 3 15 | 420 | 10 0 0 0 1 |
| 6 37 1 2 2 68 | 0 | 10.5 0 | 11.2 0 5 | 3 10 | 390 | 15 0 0 0 0 |
| 6 37 1 2 2 68 | 0 | 10.5 0 | 11.2 0 5 | 2 10 | 430 | 30 0 0 0 0 |
| 6 37 1 2 2 68 | 1 3 | 10.5 0 | 11.0 0 3 | 3 30 | 450 | 10 0 0 0 0 |
| 6 37 1 2 2 68 | 0 | 10.5 0 | 11.1 0 5 | 3 10 | 420 | 15 0 0 0 0 |
| 6 37 1 2 2 68 | 1 2 | 10.5 0 | 11.0 0 5 | 3 10 | 455 | 5 0 0 0 0 |
| 6 37 1 2 2 68 | 1 1 | 10.5 0 | 10.7 0 5 | 3 10 | 485 | 30 0 0 0 1 |
| 7 34 2 2 2 67 | -1 | 10.5 0 | 10.7 0 6 | 3 10 | 430 | 5 0 0 0 1 |
| 7 34 2 2 2 67 | 1 2 | 10.5 0 | 11.0 0 5 | 3 20 | 465 | 5 0 0 0 0 |
| 7 34 2 2 2 67 | 0 | 10.5 0 | 10.2 0 6 | 3 10 | 510 | 5 0 0 0 0 |
| 7 34 2 2 2 67 | 1 2 | 10.5 1 | 11.2 0 7 | 3 5 | 455 | 5 0 0 0 1 |
| 7 34 2 2 2 67 | 1 1 | 9.5 0 | 10.2 0 6 | 3 5 | 525 | 10 0 0 0 1 |
| 7 34 2 2 2 67 | 1 3 | 9.5 0 | 10.2 0 7 | 3 10 | 535 | 5 0 0 0 0 |
| 7 34 2 2 2 67 | 0 | 10.5 0 | 10.5 0 5 | 3 10 | 510 | 20 0 0 0 1 |
| 8 23 1 1 3 60 | 1 4 | 11.5 0 | 12.3 0 3 | 1 10 | 360 | 15 0 0 0 0 |
| 8 23 1 1 3 60 | -1 | 9.5 -1 | 12.0 0 2 | 2 60 | 320 | 25 0 0 0 0 |

| 1 | | | | | | 2 | | 3 | | | 4 | | | 5 | | | 6 | | | 7 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
| 8 | 23 | 1 | 1 | 3 | 60 | 1 | 1 | 9.5 | 0 | 11.0 | 0 | 5 | 2 | 120 | 270 | 30 | -2 | 0 | 0 | -1 | | | | | | | |
| 8 | 23 | 1 | 1 | 3 | 60 | -1 | | 14.5 | 1 | 15.0 | 0 | 7 | 4 | 15 | 165 | 45 | -1 | 1 | 1 | -1 | | | | | | | |
| 8 | 23 | 1 | 1 | 3 | 60 | 1 | 4 | 10.5 | 0 | 11.5 | 15 | 3 | 5 | 60 | 360 | 75 | -1 | 1 | 0 | -1 | | | | | | | |
| 9 | 23 | 1 | 2 | 3 | 63 | 1 | 4 | 10.5 | 1 | 12.2 | 0 | 5 | 3 | 45 | 390 | 15 | 1 | 1 | 1 | 0 | | | | | | | |
| 9 | 23 | 1 | 2 | 3 | 63 | 0 | | 9.5 | -1 | 10.2 | 120 | 3 | 4 | 30 | 375 | 150 | 1 | 1 | 1 | 0 | | | | | | | |
| 10 | 30 | 2 | 2 | 3 | 58 | 1 | 2 | 9.5 | 0 | 10.5 | 0 | 3 | 2 | 60 | 465 | 45 | -1 | 0 | 1 | 1 | | | | | | | |
| 10 | 30 | 2 | 2 | 3 | 58 | 0 | | 8.5 | 1 | 11.5 | 0 | 4 | 3 | 45 | 390 | 15 | 0 | 1 | 1 | 0 | | | | | | | |
| 10 | 30 | 2 | 2 | 3 | 58 | 0 | | 10.5 | 1 | 13.0 | 0 | 7 | 3 | 15 | 375 | 30 | 0 | 0 | 0 | 1 | | | | | | | |
| 10 | 30 | 2 | 2 | 3 | 58 | -1 | | 9.5 | 0 | 10.5 | 60 | 3 | 2 | 30 | 570 | 10 | -1 | 0 | 0 | 1 | | | | | | | |
| 10 | 30 | 2 | 2 | 3 | 58 | 0 | | 9.5 | 0 | 10.0 | 0 | 2 | 2 | 60 | 555 | 15 | 0 | 0 | 0 | 1 | | | | | | | |
| 10 | 30 | 2 | 2 | 3 | 58 | 1 | 3 | 9.5 | 0 | 10.5 | 15 | 3 | 2 | 60 | 585 | 15 | 0 | 0 | 0 | 1 | | | | | | | |
| 10 | 30 | 2 | 2 | 3 | 58 | 1 | 3 | 9.5 | 0 | 10.5 | 0 | 4 | 3 | 30 | 585 | 15 | 0 | 1 | 0 | 1 | | | | | | | |
| 11 | 27 | 1 | 2 | 2 | 59 | 1 | 2 | 10.5 | 0 | 11.7 | 0 | 3 | 2 | 15 | 480 | 60 | 0 | 0 | 0 | 1 | | | | | | | |
| 11 | 27 | 1 | 2 | 2 | 59 | 0 | | 11.5 | 0 | 12.0 | 0 | 4 | 3 | 30 | 430 | 5 | 0 | 0 | 0 | 0 | | | | | | | |
| 11 | 27 | 1 | 2 | 2 | 59 | 0 | | 11.5 | 1 | 12.2 | 0 | 4 | 3 | 15 | 435 | 0 | 0 | 0 | 0 | 0 | | | | | | | |
| 11 | 27 | 1 | 2 | 2 | 59 | 1 | 2 | 11.5 | 1 | 11.7 | 15 | 4 | 3 | 45 | 390 | 50 | 0 | 1 | 0 | 0 | | | | | | | |
| 11 | 27 | 1 | 2 | 2 | 59 | 1 | 4 | 11.5 | 0 | 12.5 | 0 | 3 | 3 | 10 | 420 | 5 | 0 | 0 | 0 | 0 | | | | | | | |
| 11 | 27 | 1 | 2 | 2 | 59 | 0 | | 11.5 | 1 | 12.0 | 0 | 5 | 3 | 10 | 445 | 10 | 0 | 1 | 0 | 1 | | | | | | | |
| 11 | 27 | 1 | 2 | 2 | 59 | 1 | 2 | 11.5 | 1 | 12.2 | 0 | 5 | 3 | 5 | 445 | 15 | 0 | 1 | 0 | 0 | | | | | | | |
| 12 | 27 | 1 | 2 | 2 | 52 | 1 | 1 | 11.5 | 0 | 11.6 | 0 | 3 | 3 | 10 | 465 | 15 | 0 | 0 | 0 | 0 | | | | | | | |
| 12 | 27 | 1 | 2 | 2 | 52 | 0 | | 12.5 | 0 | 12.5 | 0 | 3 | 4 | 30 | 450 | 20 | 0 | 1 | 1 | 1 | | | | | | | |
| 12 | 27 | 1 | 2 | 2 | 52 | 1 | 3 | 10.5 | 1 | 12.2 | 0 | 5 | 5 | 15 | 405 | 30 | 0 | 1 | 1 | 0 | | | | | | | |
| 12 | 27 | 1 | 2 | 2 | 52 | 0 | | 11.5 | -1 | 11.6 | 0 | 2 | 2 | 60 | 510 | 70 | 0 | 0 | 0 | 0 | | | | | | | |
| 12 | 27 | 1 | 2 | 2 | 52 | 0 | | 11.5 | 0 | 12.2 | 0 | 3 | 5 | 60 | 435 | 60 | -1 | 1 | 0 | 0 | | | | | | | |
| 12 | 27 | 1 | 2 | 2 | 52 | 0 | | 11.5 | 0 | 12.3 | 0 | 3 | 4 | 25 | 360 | 15 | 0 | 1 | 1 | 0 | | | | | | | |
| 12 | 27 | 1 | 2 | 2 | 52 | 0 | | 11.5 | 1 | 12.3 | 0 | 3 | 2 | 30 | 360 | 60 | 0 | 0 | 1 | 0 | | | | | | | |
| 13 | 17 | 1 | 1 | 3 | 62 | 1 | 2 | 10.5 | 1 | 12.0 | 0 | 5 | 4 | 30 | 510 | 15 | 0 | 0 | 0 | 0 | | | | | | | |
| 13 | 17 | 1 | 1 | 3 | 62 | 0 | | 11.5 | 0 | 12.0 | 0 | 4 | 3 | 60 | 510 | 30 | 0 | 0 | 0 | 0 | | | | | | | |
| 13 | 17 | 1 | 1 | 3 | 62 | -1 | | 9.5 | 0 | 10.5 | 0 | 6 | 3 | 30 | 690 | 30 | 1 | 0 | 0 | -1 | | | | | | | |
| 13 | 17 | 1 | 1 | 3 | 62 | 1 | 2 | 11.5 | 0 | 12.0 | 0 | 5 | 3 | 60 | 660 | 30 | 1 | 0 | 0 | 1 | | | | | | | |
| 13 | 17 | 1 | 1 | 3 | 62 | 0 | | 11.5 | 1 | 13.5 | 0 | 5 | 3 | 30 | 420 | 15 | 1 | 1 | 0 | 0 | | | | | | | |
| 13 | 17 | 1 | 1 | 3 | 62 | -1 | | 10.5 | 1 | 11.5 | 0 | 4 | 4 | 30 | 450 | 5 | 0 | 1 | 0 | 0 | | | | | | | |
| 13 | 17 | 1 | 1 | 3 | 62 | 0 | | 10.5 | 1 | 12.2 | 0 | 5 | 4 | 20 | 435 | 10 | 0 | 1 | 0 | 0 | | | | | | | |
| 14 | 19 | 2 | 1 | 3 | 42 | 1 | 2 | 9.5 | 0 | 10.5 | 0 | 3 | 2 | 30 | 720 | 30 | 0 | 1 | 0 | 1 | | | | | | | |
| 14 | 19 | 2 | 1 | 3 | 42 | 0 | | 9.5 | 0 | 10.5 | 0 | 4 | 4 | 5 | 685 | 75 | -1 | 1 | 0 | 0 | | | | | | | |
| 14 | 19 | 2 | 1 | 3 | 42 | -1 | | 11.5 | 0 | 12.0 | 0 | 1 | 2 | 15 | 495 | 60 | -1 | 1 | 0 | 1 | | | | | | | |
| 14 | 19 | 2 | 1 | 3 | 42 | 1 | 2 | 12.5 | 0 | 13.4 | 0 | 2 | 3 | 0 | 560 | 0 | -1 | 1 | 0 | -1 | | | | | | | |
| 14 | 19 | 2 | 1 | 3 | 42 | -1 | | 9.5 | 0 | 10.6 | 0 | 2 | 3 | 60 | 500 | 0 | -1 | 1 | 1 | 0 | | | | | | | |
| 14 | 19 | 2 | 1 | 3 | 42 | 0 | | 9.5 | 0 | 10.6 | 0 | 3 | 3 | 5 | 540 | 10 | -1 | 1 | 1 | -1 | | | | | | | |
| 15 | 25 | 2 | 2 | 3 | 45 | 2 | 4 | 11.5 | 1 | 12.5 | 0 | 5 | 7 | 20 | 370 | 15 | 1 | 1 | 1 | -1 | | | | | | | |
| 15 | 25 | 2 | 2 | 3 | 45 | 0 | | 10.5 | 0 | 11.3 | 0 | 2 | 7 | 20 | 395 | 5 | 0 | 1 | 1 | 0 | | | | | | | |
| 15 | 25 | 2 | 2 | 3 | 45 | 0 | | 11.5 | 1 | 12.2 | 0 | 3 | 4 | 5 | 380 | 0 | 0 | 1 | 1 | 0 | | | | | | | |
| 15 | 25 | 2 | 2 | 3 | 45 | 1 | 1 | 11.5 | 1 | 12.2 | 0 | 5 | 6 | 10 | 415 | 0 | 1 | 1 | 1 | 0 | | | | | | | |
| 15 | 25 | 2 | 2 | 3 | 45 | 1 | 3 | 11.5 | 0 | 12.0 | 0 | 3 | 7 | 5 | 490 | 25 | 1 | 1 | 0 | 0 | | | | | | | |
| 15 | 25 | 2 | 2 | 3 | 45 | 2 | 4 | 11.5 | 0 | 12.0 | 0 | 4 | 4 | 10 | 500 | 50 | -1 | 0 | 0 | 1 | | | | | | | |
| 15 | 25 | 2 | 2 | 3 | 45 | 1 | 1 | 12.5 | 1 | 13.0 | 0 | 3 | 5 | 5 | 290 | 20 | 1 | 1 | 0 | 1 | | | | | | | |
| 16 | 24 | 1 | 2 | 3 | 58 | 2 | 4 | 11.5 | 0 | 12.5 | 0 | 4 | 6 | 10 | 405 | 60 | -1 | 1 | 1 | 0 | | | | | | | |
| 16 | 24 | 1 | 2 | 3 | 58 | 0 | | 10.5 | -1 | 11.3 | 0 | 3 | 3 | 40 | 450 | 30 | 0 | 0 | 0 | 0 | | | | | | | |
| 16 | 24 | 1 | 2 | 3 | 58 | 2 | 1 | 11.5 | 1 | 12.2 | 0 | 6 | 4 | 10 | 425 | 45 | 1 | 0 | 1 | 0 | | | | | | | |
| 16 | 24 | 1 | 2 | 3 | 58 | 0 | | 11.5 | 0 | 12.2 | 0 | 4 | 4 | 5 | 450 | 60 | 1 | 0 | 1 | 0 | | | | | | | |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 12345678 |
| 16 24 1 2 3 58 | 1 4 | 11.5 0 12.0 | 0 3 | 4 15 480 | 15 0 0 | 0 0 |
| 16 24 1 2 3 58 | 2 4 | 11.5 0 12.0 | 0 4 | 4 15 495 | 45 -1 0 | 0 0 |
| 16 24 1 2 3 58 | 1 1 | 12.5 0 13.0 | 0 3 | 3 10 470 | 30 0 1 | 1 0 |
| 17 24 1 2 2 48 | 1 3 | 10.5 0 10.5 | 0 2 | 2 45 495 | 20 1 0 | 1 0 |
| 17 24 1 2 2 48 | 0 | 10.5 0 10.7 | 0 2 | 3 15 410 | 130 0 0 | 1 0 |
| 17 24 1 2 2 48 | 0 | 9.5 0 10.2 | 0 3 | 4 35 510 | 45 1 1 | 1 0 |
| 17 24 1 2 2 48 | 1 4 | 10.5 0 11.0 | 0 1 | 1 20 470 | 50 0 0 | 0 1 |
| 17 24 1 2 2 48 | 2 3 | 10.5 0 11.0 | 30 6 | 4 10 595 | 65 0 1 | 0 1 |
| 17 24 1 2 2 48 | 0 | 10.5 0 11.0 | 0 3 | 3 25 455 | 60 0 1 | 1 1 |
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| 31 23 1 1 3 52 | 1 1 | 9.5 1 12.6 | 75 4 | 1 20 | 465 55 | 0 1 1 0 |
| 31 23 1 1 3 52 | 0 | 14.0 1 14.3 | 0 7 | 5 0 | 530 120 | 0 1 0 1 |
| 31 23 1 1 3 52 | -2 | 12.5 0 12.7 | 0 2 | 1 20 | 405 20 | 0 1 1 0 |

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| 32 25 1 2 3 57 | 0 | 10.5 1 | 11.0 0 3 | 4 0 | 480 15 | 0 1 1 0 |
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| 32 25 1 2 3 57 | 0 | 11.5 1 | 12.4 0 6 | 4 35 | 510 30 | 0 1 0 0 |
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| 33 35 1 2 2 48 | 0 | 10.5 0 | 11.1 0 4 | 3 70 | 430 5 | -1 1 0 0 |
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| 33 35 1 2 2 48 | 1 3 | 10.5 0 | 10.7 0 4 | 5 15 | 480 105 | 1 1 0 0 |
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| 34 27 2 2 3 49 | -1 | 9.5 1 | 11.0 0 3 | 6 60 | 405 15 | -1 1 0 -1 |
| 34 27 2 2 3 49 | 1 2 | 9.5 0 | 10.5 0 3 | 7 90 | 420 30 | 1 1 0 1 |
| 34 27 2 2 3 49 | 0 | 9.5 0 | 10.5 0 4 | 6 60 | 465 30 | 0 1 0 0 |
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| 36 30 1 2 3 56 | 0 | 9.5 0 | 10.4 0 2 | 4 45 | 580 15 | -1 0 0 -1 |
| 36 30 1 2 3 56 | 1 1 | 12.5 1 | 13.2 0 5 | 3 15 | 465 15 | 0 0 0 1 |
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| 36 30 1 2 3 56 | 1 1 | 9.5 0 | 10.2 0 3 | 3 75 | 435 0 | -1 1 1 0 |
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| 37 24 1 1 2 67 | -2 | 14.0 0 | 14.5 0 3 | 3 0 | 360 15 | 1 0 0 1 |
| 37 24 1 1 2 67 | 1 2 | 12.5 1 | 13.0 0 5 | 5 0 | 450 30 | 0 1 0 0 |
| 37 24 1 1 2 67 | 0 | 10.5 1 | 11.7 0 5 | 5 40 | 425 15 | 0 1 1 0 |
| 37 24 1 1 2 67 | 0 | 9.5 0 | 10.7 0 5 | 3 60 | 455 30 | 1 0 0 0 |
| 37 24 1 1 2 67 | 2 3 | 10.5 0 | 11.1 0 3 | 4 10 | 475 20 | 0 1 0 0 |
| 37 24 1 1 2 67 | 1 3 | 11.5 0 | 12.1 0 2 | 5 15 | 435 10 | 0 1 0 0 |
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| 38 26 1 1 2 50 | -1 | 10.5 1 | 12.7 0 5 | 5 5 | 420 25 | 0 1 1 0 |

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| 39 32 2 2 3 69 | 0 | 9.5 0 10.7 | 0 3 | 2 105 430 | 30 0 | 0 0 0 |
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| 39 32 2 2 3 69 | -1 | 10.5 0 11.0 | 0 3 | 2 30 480 | 20 1 | 0 0 0 |
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| 40 22 1 1 3 47 | 1 4 | 14.0 1 14.5 | 0 6 | 3 10 470 | 0 0 | 0 0 1 |
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| 40 22 1 1 3 47 | 0 | 11.5 1 11.6 | 0 3 | 4 20 430 | 0 0 | 1 1 0 |
| 40 22 1 1 3 47 | 0 | 11.5 1 12.0 | 0 4 | 4 40 390 | 1 0 | 1 1 0 |
| 40 22 1 1 3 47 | 0 | 10.5 0 11.1 | 0 3 | 3 25 460 | 0 0 | 1 1 0 |
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| 41 21 1 2 3 46 | 0 | 10.5 -1 11.2 | 0 1 | 1 80 540 | 15 0 | 0 0 0 |
| 41 21 1 2 3 46 | 0 | 11.5 1 12.3 | 0 3 | 6 40 420 | 45 0 | 1 0 0 |
| 41 21 1 2 3 46 | -1 | 11.5 -1 12.2 | 0 1 | 3 60 525 | 20 1 | 1 0 1 |
| 41 21 1 2 3 46 | -1 | 10.5 -1 11.0 | 0 3 | 3 60 525 | 30 0 | 1 1 1 |
| 42 20 1 2 2 58 | 1 3 | 10.5 0 11.0 | 0 2 | 3 30 540 | 0 0 | 1 0 1 |
| 42 20 1 2 2 58 | 1 3 | 10.5 1 12.7 | 0 5 | 4 5 490 | 30 1 | 1 0 1 |
| 42 20 1 2 2 58 | 0 | 12.5 1 13.2 | 0 2 | 3 15 525 | 45 1 | 0 0 0 |
| 42 20 1 2 2 58 | 1 4 | 12.5 1 13.2 | 0 1 | 4 15 420 | 60 2 | 0 0 1 |
| 42 20 1 2 2 58 | -1 | 11.5 0 12.0 | 0 2 | 3 15 495 | 60 1 | 0 0 0 |
| 42 20 1 2 2 58 | 0 | 11.5 0 12.5 | 0 2 | 4 15 495 | 45 1 | 0 0 0 |
| 43 21 1 1 2 68 | -1 | 11.5 1 12.0 | 120 3 | 3 0 430 | 20 0 | 1 0 0 |
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| 43 21 1 1 2 68 | 0 | 11.5 0 11.5 | 0 3 | 2 0 570 | 10 0 | 0 0 0 |
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| 44 40 1 2 2 49 | 1 2 | 10.5 0 11.0 | 0 3 | 3 90 390 | 30 1 | 0 0 1 |
| 44 40 1 2 2 49 | 1 3 | 11.5 1 12.2 | 0 5 | 3 45 360 | 30 0 | 1 0 0 |
| 44 40 1 2 2 49 | 1 1 | 10.5 0 11.2 | 0 3 | 4 135 330 | 15 -1 | 1 0 1 |
| 44 40 1 2 2 49 | 0 | 10.5 0 10.7 | 30 3 | 3 30 470 | 25 0 | 0 0 0 |
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| 45 24 1 1 2 52 | -1 | 13.5 0 13.6 | 0 2 | 3 5 440 | 50 0 | 1 1 1 |
| 45 24 1 1 2 52 | -1 | 13.5 1 13.8 | 20 5 | 3 10 540 | 20 0 | 0 1 0 |
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| 45 24 1 1 2 52 | 1 3 | 11.5 0 11.6 | 0 2 | 3 5 540 | 20 0 | 1 0 1 |
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| 46 24 2 2 3 58 | -1 | 9.5 0 10.7 | 0 4 | 3 75 600 | 0 -1 | 0 0 0 |
| 46 24 2 2 3 58 | 0 | 9.5 0 10.5 | 0 3 | 4 30 480 | 0 0 | 1 1 0 |
| 46 24 2 2 3 58 | 1 3 | 10.5 0 12.6 | 0 5 | 7 10 505 | 50 -1 | 1 0 0 |

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| 47 24 2 1 3 56 | 0 | 10.5 0 | 11.2 0 | 5 | 3 30 | 465 30 0 0 0 0 |
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| 47 24 2 1 3 56 | 0 | 10.5 0 | 11.0 0 | 2 | 3 45 | 470 5 0 0 1 0 |
| 47 24 2 1 3 56 | 2 3 | 11.5 0 | 12.0 0 | 2 | 2 15 | 435 10 0 0 0 0 |
| 47 24 2 1 3 56 | 0 | 12.5 1 | 13.0 0 | 5 | 3 20 | 510 10 0 0 0 1 |
| 47 24 2 1 3 56 | 1 2 | 10.5 0 | 10.6 0 | 4 | 3 50 | 470 15 0 0 0 1 |
| 48 23 1 1 2 57 | 0 | 12.5 0 | 12.7 0 | 2 | 2 0 | 345 60 0 0 1 0 |
| 48 23 1 1 2 57 | 0 | 10.5 0 | 11.0 0 | 3 | 3 30 | 450 210 0 0 0 0 |
| 48 23 1 1 2 57 | 0 | 12.5 1 | 13.5 0 | 2 | 2 30 | 300 30 0 0 0 0 |
| 48 23 1 1 2 57 | 0 | 10.5 0 | 11.0 0 | 2 | 2 15 | 345 180 0 0 0 0 |
| 48 23 1 1 2 57 | 0 | 14.7 1 | 15.0 0 | 2 | 2 30 | 240 30 0 0 0 0 |
| 48 23 1 1 2 57 | 1 3 | 10.5 0 | 12.0 0 | 2 | 2 30 | 360 90 0 0 0 0 |
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| 49 28 1 1 2 44 | 0 | 10.5 -1 | 11.3 0 | 3 | 6 50 | 390 60 -1 1 0 -1 |
| 49 28 1 1 2 44 | 1 4 | 12.5 0 | 13.0 30 | 2 | 3 15 | 495 30 1 0 0 0 |
| 49 28 1 1 2 44 | -1 | 12.5 0 | 12.7 0 | 2 | 3 45 | 540 30 1 0 0 0 |
| 49 28 1 1 2 44 | 0 | 14.0 1 | 14.5 0 | 3 | 1 0 | 450 60 1 0 0 1 |
| 49 28 1 1 2 44 | 1 2 | 11.5 0 | 12.0 20 | 4 | 4 60 | 465 15 1 1 0 0 |
| 49 28 1 1 2 44 | 1 4 | 12.5 1 | 14.2 0 | 6 | 3 15 | 420 30 0 0 0 1 |
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| 50 25 1 1 2 48 | 1 3 | 10.5 1 | 11.9 0 | 5 | 3 65 | 370 0 0 1 1 0 |
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| 51 32 1 1 1 48 | 1 3 | 11.0 0 | 11.0 0 | 7 | 4 60 | 480 10 1 0 1 0 |
| 51 32 1 1 1 48 | 1 3 | 11.0 0 | 11.0 0 | 6 | 4 60 | 480 10 0 0 1 0 |
| 51 32 1 1 1 48 | 1 2 | 11.5 0 | 12.0 0 | 4 | 4 0 | 480 10 0 0 1 0 |

## 2. Questionnaires

The subject, pre-sleep and post-sleep questionnaires are presented in the following pages.

SUBJECT QUESTIONNAIRE

THE QUESTIONNAIRE IS STRICTLY CONFIDENTIAL. YOU DO NOT NEED TO STATE YOUR NAME.

NAME:

AGE: SEX:

MARRIED/SINGLE:

Have you any physical or mental disability?

If so, please briefly describe its nature and state any medications that you are taking.

Which of the following describe your present level of physical fitness? Please tick the relevant box.

| Very Fit | Fit | A bit out of condition | Very much out of condition |
|---|---|---|---|
| | | | |

Do you have any difficulties in sleeping?

| | |
|---|---|
| YES | |
| NO | |

If so, please briefly describe your problem, including its duration and the remedies you use, if any.

PRE-SLEEP QUESTIONNAIRE Date:

IMPORTANT: PLEASE DO NOT COMPLETE THIS FORM UNTIL YOU ARE READY TO RETIRE.
PLEASE TICK THE APPROPRIATE BOX WHEN ANSWERING THE QUESTIONS.

1. Which one of the following statements do you consider to best describe your feelings during the last quarter of an hour?

| | | | |
|---|---|---|---|
| 1. | Feeling active and vital, alert, wide awake. | | 1 |
| 2. | Functioning at a high level, but not at peak; able to concentrate. | | 2 |
| 3. | Relaxed; awake; not at full alertness, responsive. | | 3 |
| 4. | A little foggy; not at peak; let down. | | 4 |
| 5. | Fogginess; beginning to lose interest in remaining awake; slowed down. | | 5 |
| 6. | Sleepiness; prefer to be lying down; fighting sleep; woosy. | | 6 |
| 7. | Sleep onset soon; struggling to remain awake. | | 7 |

2. Do you consider that your general level of physical activity today was:

| Greatly above normal | Above normal | Normal | Below normal | Greatly below normal |
|---|---|---|---|---|
| | | | | |

3. If your physical activity level was greater than normal:
a) At what time(s) of day was the activity taken?

| Morning | Afternoon | Early evening | Late evening |
|---|---|---|---|
| | | | |

b) What specific activities were involved? i.e. squash, walking, tennis etc.

4. At what time did you start thinking about going to bed tonight?

| 7.00 | 7.30 | 8.00 | 8.30 | 9.00 | 9.30 | 10.00 | 10.30 | 11.00 | 11.30 | 12.00 | 12.30 | 1.0 | 1.30 | 2.00 | 2.30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | |

5. Do you feel that you have gone to bed

| Too late | At the right time | Too early |
|---|---|---|
| | | |

6. Has your day been

| Very enjoyable | Normal | Upsetting |
|---|---|---|
| | | |

7. At what time are you going to bed tonight?

8. If you have fallen asleep during the day please state when and for how long.

POST-SLEEP QUESTIONNAIRE Date:

IMPORTANT: PLEASE DO NOT COMPLETE THIS FORM UNTIL YOU HAVE BEEN AWAKE FOR APPROXIMATELY 15 MINUTES.
PLEASE TICK THE APPROPRIATE BOX WHEN ANSWERING THE QUESTIONS

1. Which one of the following statements do you consider to best describe your feelings during the last quarter of an hour?

| | | | |
|---|---|---|---|
| 1. | Feeling active and vital; alert; wide awake. | | 1 |
| 2. | Functioning at a high level, but not at peak; able to concentrate. | | 2 |
| 3. | Relaxed; awake; not at full alertness, responsive. | | 3 |
| 4. | A little foggy; not at peak; let down. | | 4 |
| 5. | Fogginess; beginning to lose interest in remaining awake; slowed down. | | 5 |
| 6. | Sleepiness; prefer to be lying down; fighting sleep; woosy. | | 6 |
| 7. | Sleep onset soon; struggling to remain awake. | | 7 |

2. Please fill in the appropriate times as accurately as possible for the following questions.

| | | |
|---|---|---|
| At what time did you | a) Fall asleep last night? | |
| | b) Wake up this morning? | |
| | c) Get up this morning? | |

3. Which of the following do you consider to best describe the quality of your sleep last night?

| Much better than normal | Better than normal | Normal | Worse than normal | Much worse than normal |
|---|---|---|---|---|
| | | | | |

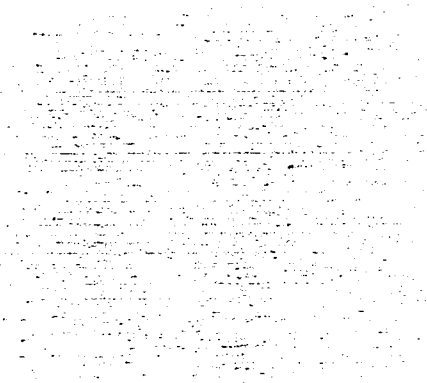
If you did not sleep very well, please could you specify the reasons, if any. i.e. cramp, not tired etc.

4. If you could, would you have liked to have slept longer this morning?

| | |
|---|---|
| YES | |
| NO | |

5. Did you use an alarm clock this morning?

| | |
|---|---|
| YES | |
| NO | |



## APPENDIX III: DATA FOR STUDY III

### 1. Experimental details

| | |
|---|---|
| Number of subjects: | 6 |
| Supplementary diets studied: | zero, low and high carbohydrate |
| Number of nights each diet studied: | 3 consecutive nights, plus one post-diet night. |
| Sleep period: | 450 minutes from onset of stage 2 sleep |

### 2. Individual data

#### 2.1 Data for supplementary diet periods

The data is presented for the whole night and for the first and second halves of the night. The duration of each stage of sleep or sleep variable is presented in the following format (as used in the computer analysis):

$S_1D_1N_1$ $S_1D_1N_2$ $S_1D_1N_3$ $S_1D_2N_1$ $S_1D_2N_2$ $S_1D_2N_3$ $S_1D_3N_1$ $S_1D_3N_2$ $S_1D_3N_3$

$S_2D_1N_1$

$S_3D_1N_1$

$S_4D_1N_1$

$S_5D_1N_1$

$S_6D_1N_1$

where

$S_{1-6}$ denotes the subject

$D_1, D_2, D_3$ denote the zero, low and high carbohydrate supplementary diets, respectively

$N_{1-3}$ denotes the night of administration

## 2.2 Post-diet data

The post-diet data for sleep stage duration is presented in the following format:

$S_1Z$ $S_1L$ $S_1H$ $S_1Z_1$ $S_1L_1$ $S_1H_1$ $S_1Z_2$ $S_1L_2$ $S_1H_2$

$S_2Z$

$S_3Z$

$S_4Z$

$S_5Z$

$S_6Z$

where

| | |
|---|---|
| $S_{1-6}$ | denotes the subjects |
| Z,L,H | denote the whole night data from the post-zero, post-low and post-hight carbohydrate nights, respectively. |
| $Z_1,L_1,H_1$ | denote the first half of the night data |
| $Z_2,L_2,H_2$ | denote the second half of the night data |

STAGES 3+4 WHOLE NIGHT DATA (%)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 22.0 | 26.0 | 23.8 | 25.1 | 22.9 | 24.0 | 21.8 | 22.7 | 26.3 |
| 16.0 | 20.4 | 16.8 | 20.7 | 22.5 | 21.6 | 18.5 | 12.6 | 16.0 |
| 23.1 | 22.7 | 26.4 | 28.0 | 33.6 | 24.9 | 23.1 | 26.0 | 21.6 |
| 18.1 | 13.6 | 21.1 | 17.8 | 22.0 | 19.8 | 17.8 | 23.6 | 23.8 |
| 17.8 | 19.1 | 14.9 | 20.2 | 19.6 | 21.1 | 15.1 | 20.0 | 15.8 |
| 20.0 | 19.7 | 16.9 | 23.1 | 28.0 | 24.2 | 15.1 | 29.6 | 18.7 |

STAGE 4

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 14.4 | 12.7 | 12.4 | 14.9 | 10.0 | 14.0 | 12.2 | 13.4 | 12.7 |
| 6.0 | 12.0 | 8.4 | 9.8 | 8.9 | 9.4 | 8.7 | 4.2 | 8.0 |
| 13.8 | 12.2 | 18.2 | 19.8 | 22.2 | 16.0 | 17.8 | 11.6 | 17.6 |
| 7.5 | 3.3 | 7.3 | 5.8 | 6.0 | 5.6 | 2.2 | 5.1 | 3.8 |
| 10.4 | 8.0 | 6.7 | 9.6 | 12.0 | 8.4 | 6.9 | 4.7 | 3.3 |
| 12.0 | 11.3 | 9.4 | 10.4 | 20.2 | 11.8 | 6.0 | 14.7 | 10.0 |

STAGE 3

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 7.6 | 13.3 | 11.3 | 10.2 | 12.9 | 10.0 | 9.3 | 9.6 | 13.6 |
| 10.0 | 8.4 | 8.4 | 10.9 | 13.6 | 12.2 | 9.8 | 8.4 | 8.0 |
| 9.3 | 10.4 | 8.2 | 8.2 | 11.3 | 8.9 | 5.3 | 14.4 | 4.0 |
| 10.6 | 10.3 | 13.8 | 12.0 | 16.0 | 14.2 | 15.6 | 18.4 | 20.0 |
| 7.3 | 11.1 | 8.2 | 10.7 | 7.6 | 12.7 | 8.2 | 15.3 | 12.4 |
| 8.0 | 8.4 | 7.5 | 12.7 | 7.8 | 10.2 | 9.1 | 14.9 | 8.7 |

STAGE REM

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 26.2 | 28.7 | 23.8 | 28.0 | 24.2 | 26.1 | 25.8 | 28.9 | 27.3 |
| 17.1 | 21.1 | 26.0 | 24.0 | 25.8 | 24.9 | 26.0 | 23.8 | 24.0 |
| 22.0 | 18.4 | 19.8 | 23.8 | 23.3 | 30.0 | 26.2 | 25.6 | 28.3 |
| 20.4 | 24.6 | 20.2 | 24.0 | 23.8 | 20.0 | 30.9 | 23.3 | 19.1 |
| 24.4 | 20.7 | 23.7 | 17.3 | 11.6 | 16.4 | 23.3 | 20.4 | 23.8 |
| 25.3 | 24.9 | 19.5 | 26.4 | 23.8 | 22.4 | 30.4 | 21.1 | 24.4 |

STAGE 2

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 49.1 | 42.7 | 49.3 | 45.4 | 50.2 | 47.8 | 52.0 | 46.8 | 45.1 |
| 56.2 | 49.8 | 50.2 | 51.6 | 46.8 | 49.2 | 52.7 | 59.1 | 56.9 |
| 46.4 | 4.6 | 43.3 | 43.3 | 40.9 | 41.8 | 47.1 | 42.7 | 45.2 |
| 47.4 | 49.8 | 50.5 | 50.4 | 45.3 | 45.8 | 42.4 | 47.8 | 49.1 |
| 49.6 | 42.7 | 50.0 | 42.4 | 50.0 | 57.3 | 48.0 | 50.4 | 50.2 |
| 51.6 | 50.2 | 59.4 | 44.7 | 42.9 | 48.0 | 48.7 | 44.9 | 50.2 |

STAGES 0+1

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 2.7 | 2.7 | 3.1 | 1.6 | 2.4 | 2.0 | 0.4 | 1.3 | 1.3 |
| 10.7 | 8.6 | 6.9 | 3.8 | 4.7 | 4.3 | 2.7 | 3.5 | 2.9 |
| 8.4 | 11.3 | 10.4 | 4.9 | 2.2 | 3.3 | 3.6 | 6.0 | 4.9 |
| 14.1 | 12.0 | 8.2 | 7.8 | 8.9 | 14.5 | 8.9 | 5.3 | 8.0 |
| 8.2 | 17.5 | 11.3 | 20.0 | 18.9 | 5.1 | 13.6 | 9.1 | 10.2 |
| 3.0 | 5.2 | 4.2 | 5.6 | 5.3 | 7.3 | 5.4 | 4.0 | 6.4 |

REM LATENCY (MINS)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 83 | 64 | 61 | 52 | 58 | 55 | 68 | 52 | 68 |
| 127 | 140 | 78 | 71 | 69 | 70 | 97 | 60 | 95 |
| 65 | 70 | 69 | 58 | 66 | 69 | 63 | 59 | 51 |
| 172 | 51 | 61 | 81 | 155 | 56 | 53 | 68 | 81 |
| 86 | 69 | 69 | 153 | 242 | 157 | 74 | 67 | 71 |
| 79 | 59 | 58 | 67 | 97 | 63 | 61 | 86 | 82 |

STAGE 4 LATENCY (MINS)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 13 | 9 | 12 | 11 | 15 | 13 | 13 | 13 | 12 |
| 23 | 18 | 13 | 20 | 16 | 18 | 29 | 22 | 29 |
| 15 | 15 | 11 | 5 | 11 | 9 | 16 | 11 | 10 |
| 14 | 101 | 18 | 25 | 16 | 20 | 40 | 24 | 22 |
| 19 | 13 | 29 | 19 | 19 | 15 | 24 | 13 | 18 |
| 9 | 14 | 17 | 13 | 7 | 14 | 21 | 11 | 14 |

REM PERIODICITY (MINS)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 73.5 | 90.5 | 82.0 | 86.5 | 81.0 | 84.0 | 89.0 | 76.5 | 76.0 |
| 111.0 | 103.0 | 90.0 | 108.5 | 57.5 | 83.0 | 113.5 | 121.5 | 97.0 |
| 86.0 | 100.0 | 73.5 | 85.0 | 94.0 | 89.5 | 105.0 | 95.0 | 85.0 |
| 101.0 | 105.0 | 88.5 | 93.5 | 106.0 | 120.0 | 102.0 | 103.5 | 105.0 |
| 89.5 | 96.5 | 91.5 | 99.5 | 96.0 | 106.5 | 84.5 | 115.0 | 96.5 |
| 96.0 | 112.0 | 114.5 | 117.0 | 101.5 | 123.0 | 132.5 | 120.5 | 110.0 |

NUMBER OF REM PERIODS

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 6.0 | 6.0 | 5.0 |
| 3.0 | 3.0 | 5.0 | 4.0 | 5.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| 4.0 | 4.0 | 5.0 | 5.0 | 5.0 | 5.0 | 4.0 | 5.0 | 6.0 |
| 3.0 | 4.0 | 5.0 | 4.0 | 3.0 | 5.0 | 5.0 | 5.0 | 4.0 |
| 4.0 | 4.0 | 4.0 | 3.0 | 3.0 | 4.0 | 5.0 | 5.0 | 5.0 |
| 5.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 5.0 | 4.0 | 4.0 |

STAGES 3+4 FIRST HALF DATA (%)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 36.8 | 44.4 | 41.8 | 42.7 | 40.4 | 41.5 | 40.0 | 38.7 | 41.3 |
| 30.7 | 28.4 | 32.0 | 28.9 | 36.4 | 32.6 | 32.9 | 22.7 | 31.6 |
| 39.2 | 31.6 | 37.8 | 47.1 | 45.4 | 43.5 | 28.0 | 40.0 | 42.7 |
| 27.2 | 20.2 | 40.4 | 31.2 | 35.1 | 29.3 | 34.2 | 43.1 | 40.5 |
| 32.5 | 29.7 | 27.1 | 36.0 | 31.6 | 30.7 | 29.8 | 33.7 | 28.0 |
| 37.0 | 30.0 | 30.5 | 30.3 | 44.5 | 26.7 | 22.7 | 44.4 | 21.8 |

STAGE 4

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 28.4 | 25.3 | 23.6 | 29.8 | 20.0 | 24.9 | 24.4 | 26.2 | 23.1 |
| 12.0 | 19.1 | 16.9 | 15.6 | 17.8 | 16.7 | 17.3 | 8.4 | 16.0 |
| 27.6 | 18.7 | 31.1 | 37.3 | 34.7 | 30.2 | 22.2 | 23.1 | 35.1 |
| 15.0 | 4.7 | 14.6 | 11.6 | 12.0 | 8.9 | 4.4 | 10.2 | 7.6 |
| 20.9 | 16.0 | 13.3 | 19.1 | 24.0 | 16.9 | 13.8 | 9.3 | 6.7 |
| 23.9 | 21.6 | 16.4 | 15.6 | 32.9 | 17.8 | 11.6 | 28.4 | 12.9 |

STAGE 3

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 8.4 | 19.1 | 182 | 12.9 | 20.4 | 16.6 | 15.6 | 12.5 | 18.2 |
| 18.7 | 9.3 | 15.1 | 13.3 | 18.6 | 15.9 | 15.6 | 14.3 | 15.6 |
| 11.6 | 12.9 | 6.7 | 9.8 | 10.7 | 13.3 | 5.8 | 16.9 | 7.6 |
| 12.2 | 15.5 | 25.8 | 19.6 | 23.1 | 20.4 | 29.8 | 32.9 | 32.9 |
| 11.6 | 13.7 | 13.8 | 16.9 | 7.6 | 13.8 | 16.0 | 24.4 | 21.3 |
| 13.1 | 8.4 | 14.1 | 14.7 | 11.6 | 8.9 | 11.1 | 16.0 | 8.9 |

STAGE REM

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 13.3 | 17.3 | 12.4 | 16.4 | 15.1 | 15.7 | 15.1 | 17.8 | 14.2 |
| 5.8 | 17.8 | 18.2 | 20.4 | 13.8 | 17.1 | 16.9 | 26.7 | 18.2 |
| 10.2 | 10.7 | 14.7 | 12.4 | 14.2 | 25.8 | 19.6 | 12.4 | 20.9 |
| 10.3 | 13.1 | 12.2 | 14.2 | 8.9 | 11.1 | 18.7 | 17.3 | 16.4 |
| 9.3 | 25.3 | 17.3 | 8.4 | 0.0 | 11.6 | 11.6 | 21.8 | 19.1 |
| 15.5 | 23.9 | 18.3 | 20.4 | 16.9 | 19.1 | 29.8 | 14.2 | 24.9 |

STAGE 2

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 49.0 | 37.4 | 43.6 | 39.1 | 42.7 | 40.9 | 44.9 | 43.5 | 44.5 |
| 56.4 | 47.1 | 44.5 | 49.8 | 47.1 | 48.4 | 47.5 | 48.8 | 49.8 |
| 48.4 | 50.1 | 42.2 | 36.9 | 39.1 | 26.3 | 50.2 | 42.7 | 34.6 |
| 55.0 | 50.3 | 42.7 | 46.6 | 49.8 | 51.2 | 42.2 | 38.7 | 41.3 |
| 48.4 | 40.6 | 47.2 | 49.7 | 58.6 | 53.3 | 44.4 | 40.5 | 43.6 |
| 46.6 | 44.7 | 46.0 | 43.5 | 33.3 | 45.8 | 47.1 | 37.8 | 47.5 |

STAGES 0+1

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0.9 | 0.9 | 2.2 | 1.8 | 1.8 | 1.8 | 0.0 | 0.0 | 0.0 |
| 7.1 | 6.7 | 5.3 | 0.9 | 2.7 | 1.8 | 2.7 | 1.8 | 0.4 |
| 2.2 | 7.6 | 5.3 | 3.6 | 1.3 | 4.4 | 2.2 | 4.9 | 1.8 |
| 7.5 | 16.4 | 4.7 | 8.0 | 6.2 | 8.4 | 4.9 | 0.9 | 1.8 |
| 9.8 | 4.4 | 8.4 | 5.3 | 9.8 | 4.4 | 14.2 | 4.0 | 9.3 |
| 0.9 | 1.4 | 5.2 | 5.8 | 5.3 | 8.4 | 0.4 | 3.6 | 5.8 |

STAGES 3+4 SECOND HALF DATA (%)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 7.2 | 7.6 | 5.8 | 7.5 | 5.4 | 6.5 | 3.6 | 6.7 | 11.3 |
| 1.3 | 12.4 | 1.6 | 12.5 | 8.6 | 10.6 | 4.1 | 2.5 | 0.4 |
| 7.0 | 13.8 | 15.0 | 8.9 | 21.8 | 6.3 | 18.2 | 12.0 | 0.5 |
| 9.0 | 7.0 | 1.8 | 4.4 | 8.9 | 10.3 | 1.4 | 4.1 | 7.1 |
| 3.1 | 8.5 | 2.7 | 4.4 | 7.6 | 11.5 | 0.4 | 6.3 | 3.6 |
| 3.0 | 9.4 | 3.3 | 15.9 | 11.5 | 21.7 | 7.5 | 14.8 | 15.6 |

STAGE 4

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0.4 | 0.0 | 1.2 | 0.0 | 0.0 | 3.1 | 0.0 | 0.0 | 2.3 |
| 0.0 | 4.9 | 0.0 | 4.0 | 0.0 | 2.1 | 0.0 | 0.0 | 0.0 |
| 0.0 | 5.7 | 5.3 | 2.3 | 9.7 | 1.8 | 13.4 | 0.0 | 0.0 |
| 0.0 | 1.9 | 0.0 | 0.0 | 0.0 | 2.3 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 1.0 | 2.4 | 5.2 | 7.5 | 5.8 | 0.4 | 1.0 | 7.1 |

STAGE 3

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 6.8 | 7.6 | 4.6 | 7.5 | 5.4 | 3.4 | 3.6 | 6.7 | 9.0 |
| 1.3 | 7.5 | 1.6 | 8.5 | 8.6 | 8.5 | 4.1 | 2.5 | 0.4 |
| 7.0 | 8.1 | 9.7 | 6.6 | 12.1 | 4.5 | 4.8 | 12.0 | 0.5 |
| 9.0 | 5.1 | 1.8 | 4.4 | 8.9 | 8.0 | 1.4 | 4.1 | 7.1 |
| 3.1 | 8.5 | 2.7 | 4.4 | 7.6 | 11.5 | 0.4 | 6.3 | 3.6 |
| 3.0 | 8.4 | 0.9 | 10.7 | 4.0 | 15.9 | 7.1 | 13.8 | 8.5 |

STAGE REM

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 39.1 | 40.1 | 35.2 | 39.6 | 33.3 | 36.5 | 36.5 | 40.0 | 40.5 |
| 28.4 | 24.4 | 33.8 | 27.6 | 37.8 | 32.7 | 35.1 | 20.9 | 29.8 |
| 33.8 | 26.1 | 24.9 | 35.2 | 32.4 | 34.2 | 32.8 | 38.8 | 35.7 |
| 30.5 | 36.1 | 28.2 | 33.8 | 38.7 | 28.9 | 43.1 | 29.3 | 21.8 |
| 39.5 | 16.1 | 30.1 | 26.2 | 23.2 | 21.2 | 35.0 | 19.0 | 28.5 |
| 35.1 | 25.9 | 20.7 | 32.4 | 30.7 | 25.7 | 31.0 | 28.0 | 23.9 |

STAGE 2

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 49.2 | 47.8 | 55.0 | 51.5 | 58.3 | 54.8 | 59.1 | 50.7 | 45.7 |
| 56.0 | 52.7 | 56.1 | 53.2 | 46.9 | 49.9 | 58.1 | 71.4 | 64.4 |
| 44.6 | 45.1 | 44.6 | 49.7 | 42.7 | 57.3 | 44.0 | 42.1 | 55.8 |
| 39.8 | 49.3 | 58.3 | 54.2 | 40.8 | 40.2 | 42.6 | 56.9 | 56.9 |
| 50.8 | 44.8 | 53.0 | 34.7 | 41.2 | 61.5 | 51.6 | 60.5 | 56.8 |
| 56.8 | 55.7 | 72.8 | 46.3 | 52.5 | 46.4 | 51.1 | 52.8 | 53.5 |

STAGES 0+1

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 4.5 | 4.5 | 4.0 | 1.4 | 3.0 | 2.2 | 0.8 | 2.6 | 2.6 |
| 14.3 | 10.5 | 8.5 | 6.7 | 6.7 | 6.8 | 2.7 | 5.2 | 5.4 |
| 14.6 | 15.0 | 15.5 | 6.2 | 3.1 | 2.2 | 5.0 | 7.1 | 8.0 |
| 20.7 | 7.6 | 11.7 | 7.6 | 11.6 | 20.6 | 12.9 | 9.7 | 14.2 |
| 6.6 | 30.6 | 14.2 | 34.7 | 28.0 | 5.8 | 13.0 | 14.2 | 11.1 |

STAGES 3+4 POST-DIET DATA (%)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 22.9 | 24.0 | 24.6 | 43.1 | 38.7 | 42.2 | 2.7 | 9.3 | 7.0 |
| 23.3 | 21.6 | 16.2 | 40.8 | 38.7 | 27.1 | 5.8 | 4.5 | 5.3 |
| 24.9 | 21.8 | 26.7 | 42.6 | 31.1 | 36.0 | 7.2 | 12.5 | 17.4 |
| 18.5 | 21.1 | 19.3 | 30.5 | 37.8 | 25.8 | 6.5 | 4.4 | 12.8 |
| 16.2 | 17.1 | 18.9 | 27.1 | 25.7 | 37.8 | 5.3 | 21.5 | 0.0 |
| 21.8 | 23.6 | 27.1 | 36.1 | 47.1 | 42.3 | 7.5 | 0.0 | 11.9 |

STAGE 4

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 17.3 | 14.7 | 14.4 | 34.7 | 23.1 | 24.9 | 0.0 | 6.3 | 3.9 |
| 14.2 | 8.9 | 6.4 | 24.4 | 17.8 | 12.9 | 4.0 | 0.0 | 0.0 |
| 17.1 | 14.7 | 13.6 | 34.2 | 25.3 | 21.3 | 0.0 | 4.1 | 5.9 |
| 4.9 | 4.4 | 2.0 | 9.4 | 8.9 | 4.0 | 0.4 | 0.0 | 0.0 |
| 7.3 | 4.7 | 1.8 | 14.7 | 9.3 | 3.6 | 0.0 | 0.0 | 0.0 |
| 12.0 | 17.1 | 12.0 | 19.2 | 34.2 | 23.6 | 4.8 | 0.0 | 0.4 |

STAGE 3

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5.6 | 9.3 | 10.2 | 8.4 | 15.6 | 17.3 | 2.7 | 3.0 | 3.1 |
| 9.1 | 12.7 | 9.8 | 16.4 | 20.9 | 14.2 | 1.8 | 4.5 | 5.3 |
| 7.8 | 7.1 | 13.1 | 8.4 | 5.8 | 14.7 | 7.2 | 8.4 | 11.5 |
| 13.6 | 16.7 | 17.3 | 21.1 | 28.9 | 21.8 | 6.1 | 4.4 | 12.8 |
| 8.9 | 12.4 | 17.1 | 12.4 | 16.4 | 34.2 | 5.3 | 21.5 | 0.0 |
| 9.9 | 6.4 | 15.1 | 16.9 | 12.9 | 18.7 | 2.7 | 0.0 | 11.5 |

STAGE REM

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 26.4 | 26.4 | 28.5 | 16.9 | 13.3 | 13.8 | 35.9 | 39.5 | 43.2 |
| 21.1 | 20.9 | 27.6 | 15.6 | 10.2 | 13.8 | 26.6 | 31.6 | 41.4 |
| 24.9 | 20.2 | 25.8 | 12.4 | 13.3 | 22.7 | 37.4 | 27.1 | 28.9 |
| 17.4 | 31.8 | 21.8 | 3.8 | 20.9 | 20.0 | 31.0 | 42.7 | 23.6 |
| 22.4 | 18.0 | 20.7 | 12.4 | 10.2 | 22.7 | 32.4 | 25.8 | 18.7 |
| 23.7 | 20.4 | 20.4 | 12.7 | 16.0 | 12.0 | 34.7 | 24.8 | 28.8 |

STAGE 2

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 47.1 | 49.1 | 45.1 | 36.9 | 48.0 | 42.7 | 57.3 | 50.4 | 47.5 |
| 41.8 | 50.4 | 590.2 | 38.3 | 43.5 | 56.0 | 45.5 | 57.43 | 44.8 |
| 47.1 | 47.3 | 38.4 | 42.8 | 48.5 | 36.0 | 51.4 | 46.1 | 40.8 |
| 52.3 | 42.4 | 49.1 | 56.3 | 38.2 | 45.8 | 48.5 | 45.1 | 52.4 |
| 52.9 | 51.6 | 51.5 | 53.8 | 57.4 | 34.2 | 52.2 | 32.8 | 68.8 |
| 50.5 | 48.4 | 45.1 | 47.0 | 50.7 | 39.5 | 54.0 | 62.8 | 50.9 |

STAGES 0+1

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 3.6 | 0.4 | 1.8 | 3.1 | 0.0 | 1.3 | 4.1 | 0.8 | 2.3 |
| 13.7 | 7.1 | 5.8 | 5.3 | 7.6 | 3.1 | 22.1 | 6.6 | 8.5 |
| 3.1 | 10.7 | 9.1 | 2.2 | 7.1 | 5.3 | 4.0 | 14.3 | 12.9 |
| 11.7 | 4.7 | 9.8 | 9.4 | 3.1 | 8.4 | 14.0 | 6.3 | 11.2 |
| 8.4 | 13.3 | 8.9 | 6.7 | 6.7 | 5.3 | 10.1 | 19.9 | 12.5 |
| 4.0 | 7.3 | 7.3 | 4.2 | 2.2 | 6.2 | 3.8 | 12.4 | 8.4 |

REM LATENCY (MINS)

| | | |
|---|---|---|
| 109 | 66 | 64 |
| 146 | 77 | 61 |
| 63 | 66 | 60 |
| 126 | 90 | 66 |
| 45 | 148 | 74 |
| 77 | 81 | 75 |

STAGE 4 LATENCY (MINS)

| | | |
|---|---|---|
| 12 | 12 | 12 |
| 16 | 17 | 22 |
| 12 | 11 | 13 |
| 12 | 23 | 38 |
| 15 | 17 | 28 |
| 10 | 18 | 12 |

REM PERIODICITY (MINS)

| | | |
|---|---|---|
| 89.5 | 93.5 | 81.0 |
| 93.5 | 100.0 | 100.0 |
| 83.5 | 73.0 | 99.5 |
| 111.0 | 96.5 | 114.0 |
| 91.5 | 95.0 | 104.0 |
| 123.0 | 121.5 | 108.5 |

NUMBER OF REM PERIODS (MINS)

| | | |
|---|---|---|
| 4 | 4 | 5 |
| 3 | 3 | 4 |
| 5 | 5 | 5 |
| 3 | 4 | 4 |
| 4 | 3 | 4 |
| 4 | 4 | 5 |

3. Group data summary tables.

| Night 1 | ZERO | LOW | HIGH |
|---|---|---|---|
| Whole night data (mins) | | | |
| Stage 3+4 | 87.8 | 101.2 | 83.7 |
| 4 | 48.2 | 52.6 | 40.5 |
| 3 | 39.6 | 48.6 | 43.2 |
| REM | 101.7 | 107.5 | 121.9 |
| 2 | 225.4 | 208.5 | 218.3 |
| 0+1 | 35.1 | 32.8 | 26.1 |
| S4 latency | 14.5 | 15.5 | 23.8 |
| REM latency | 102.0 | 80.3 | 69.3 |
| REM periodicity (mean 1st 2 inter-REM periods) | 92.8 | 98.3 | 104.4 |
| Number of REM periods | 4.0 | 4.2 | 4.8 |
| First half data (mins) | | | |
| Stage 3+4 | 76.3 | 81.0 | 70.2 |
| 4 | 47.9 | 48.4 | 35.1 |
| 3 | 28.4 | 32.6 | 35.1 |
| REM | 24.1 | 34.6 | 41.8 |
| 2 | 114.0 | 100.0 | 103.5 |
| 0+1 | 10.6 | 9.4 | 9.5 |
| Second half data (mins) | | | |
| Stage 3+4 | 11.5 | 20.2 | 13.5 |
| 4 | 0.3 | 4.2 | 5.4 |
| 3 | 11.2 | 16.0 | 8.1 |
| REM | 77.6 | 72.9 | 80.1 |
| 2 | 111.4 | 108.5 | 114.8 |
| 0+1 | 24.5 | 23.4 | 16.6 |

| Night 2 | ZERO | LOW | HIGH |
|---|---|---|---|
| Whole night data (mins) | | | |
| Stage 3+4 | 90.9 | 111.6 | 100.8 |
| 4 | 44.5 | 59.3 | 40.5 |
| 3 | 46.4 | 52.3 | 60.3 |
| REM | 103.9 | 99.4 | 107.1 |
| 2 | 212.0 | 207.1 | 220.1 |
| 0+1 | 43.2 | 31.9 | 22.0 |
| S4 latency | 29.3 | 14.0 | 15.7 |
| REM latency | 75.5 | 114.5 | 65.3 |
| REM periodicity (mean 1st 2 inter-REM periods) | 101.8 | 89.3 | 105.3 |
| Number of REM periods | 4.0 | 4.3 | 4.8 |
| First half data (mins) | | | |
| Stage 3+4 | 69.1 | 87.5 | 83.5 |
| 4 | 39.6 | 53.1 | 39.6 |
| 3 | 29.5 | 34.4 | 43.9 |
| REM | 40.5 | 25.8 | 41.4 |
| 2 | 101.5 | 101.6 | 94.5 |
| 0+1 | 13.9 | 10.1 | 5.6 |
| Second half data (mins) | | | |
| Stage 3+4 | 21.8 | 24.1 | 17.3 |
| 4 | 4.9 | 6.2 | 0.9 |
| 3 | 16.9 | 17.9 | 16.4 |
| REM | 63.4 | 73.6 | 65.7 |
| 2 | 110.5 | 105.5 | 125.6 |
| 0+1 | 29.3 | 21.8 | 16.4 |

| Night 3 | ZERO | LOW | HIGH |
|---|---|---|---|
| Whole night data (mins) | | | |
| Stage 3+4 | 90.0 | 100.3 | 91.8 |
| 4 | 46.8 | 49.0 | 41.4 |
| 3 | 43.2 | 51.3 | 50.4 |
| REM | 99.9 | 104.8 | 110.2 |
| 2 | 227.3 | 217.5 | 222.8 |
| 0+1 | 32.8 | 27.4 | 25.2 |
| S4 latency | 16.7 | 14.8 | 17.5 |
| REM latency | 66.0 | 78.3 | 74.7 |
| REM periodicity (mean 1st 2 inter-REM periods) | 90.0 | 101.0 | 94.9 |
| Number of REM periods | 4.7 | 4.5 | 4.7 |
| First half data (mins) | | | |
| Stage 3+4 | 78.5 | 76.5 | 77.2 |
| 4 | 43.4 | 43.2 | 38.0 |
| 3 | 35.1 | 33.3 | 39.2 |
| REM | 34.8 | 37.6 | 42.7 |
| 2 | 99.9 | 99.9 | 101.3 |
| 0+1 | 11.8 | 11.0 | 3.8 |
| Second half data (mins) | | | |
| Stage 3+4 | 11.5 | 23.8 | 14.6 |
| 4 | 3.4 | 5.8 | 3.4 |
| 3 | 8.1 | 18.0 | 11.2 |
| REM | 65.1 | 67.2 | 67.5 |
| 2 | 127.4 | 117.6 | 121.5 |
| 0+1 | 21.0 | 16.4 | 21.4 |

APPENDIX IV

SEROTONIN AND SLEEP - A BRIEF REVIEW OF THE LITERATURE

APPENDIX IV - CONTINUED

## 1. INTRODUCTION

This appendix contains a review of the neurochemical studies of sleep so that the influence of the various neurotransmitters upon the regulation of sleep can be assessed. The findings described in this appendix are discussed in Chapter 10.

Evidence for the complexity and specificity of the neural mechanisms controlling sleep has been provided by both the traditional methods of neurophysiology (lesion and stimulation experiments) and the modern methods of neurochemistry (fluorescence microscopy, precursor loading and chemical interference).

One of the first issues in sleep research was to decide whether the induction and maintenance of sleep is under active or passive control. Many of the early researchers regarded sleep as a passive state with associated neural quiescence. For example, Moruzzi and Magoun (1949) and Bremer (1954) argued that sleep is a consequence of a passive shutdown in the sensory systems mediated by changes in the level of functioning of the reticular activating system.

However, Hess (1944) and Hess, Koella and Akert (1953) demonstrated that sleeping behaviour could be induced in cats by actually stimulating various parts of the diencephalon. Recordings from individual neurons within the brain have shown that the firing rates of some neurons are increased during sleep (Huttenlocher, 1961; Evarts, 1962; Evarts, 1964; Bizzie, et. al., 1964). Also neurons within the same brain site may respond differentially between the transistion from waking to NREM and REM states (Walsh and Cordeau, 1965).

Perhaps the discovery of REM sleep by Aserinsky and Kleitman (1953) was the strongest support for an "active" view of sleep. The finding that sleep was not a unitary phenomenon but was composed of, at least, two distinct states implied that sleep was not merely the result of a massive neuronal shutdown.

NREM sleep is now seen primarily as a cortical phenomen resulting from cortical inhibitions of the recticular activating system, whereas REM sleep appears to be under the control of pontine mechanisms. For

these reasons these two states are sometimes referred to as telencephalic sleep and rhombencephalic sleep, respectively.

As the "active" concept of sleep gained strength it was realised that many aspects of sleep, such as the NREM-REM cycling and the REM rebound, which can last for several days, were difficult to explain in terms of neuronal firing per se, as this is measured in milliseconds as opposed to minutes and days. Thus, attention was turned to the neurochemical analysis of sleep and it is this approach that has been the most fruitful. From these studies it is apparent that some of the neurotransmitters play important roles in the regulation of sleep.

## 2. NEUROTRANSMITTERS AND SLEEP

Our knowledge of chemical transmission in the central nervous system is far from complete, and it is certain that the present list of neurotransmitters is also incomplete. The criteria that have to be met before a substance can be regarded as a transmitter can be briefly summarised as follows:-

1. The substance is present in presynaptic nerve terminals in the appropriate neuronal pathways.

2. Special mechanisms exist in the presynaptic terminal for the storage of the substance, usually in synaptic vesicles, and for its release in response to the arrival of action potentials at the presynaptic nerve terminals.

3. There are specific receptors or postsynaptic membranes with which the substance interacts to cause inhibition or excitation of neuronal activity, usually through specific ionic permeability changes in the postsynaptic membrane.

To date, the list of neurotransmitters contains the following:-

Noradrenaline (NA)
Dopamine (DA)
5-Hydroxytryptamine (5-HT, serotonin)
Acetylcholine (ACL)
Gamma-Aminobutyric acid (GABA)
Glycine (Spinal cord only)

Noradrenaline and dopamine are known collectively as the catecholamines (CA), and the catecholamines and serotonin are known collectively as the biogenic amines or monoamines. The discovery that the monamine neurotransmitters fluoresce when exposed to formaldehyde vapour (Falck et. al., 1962) has allowed the mapping of these chemicals in particular neuronal pathways in the brain and spinal cord. Early experiments involving the lesioning of these pathways has shown that the monoamine neurotransmitters are closely linked to sleep behaviour in animals.

## 3. EXPERIMENTAL APPROACHES TO THE STUDY OF THE ROLE OF NEUROTRANSMITTERS IN SLEEP

### 3.1 Administration of the Neurotransmitter

#### 3.1.1 Problem of the "Blood-brain Barrier"

The administration of the neurotransmitter itself would appear to be an obvious method by which to study the function of a particular neurotransmitter. Unfortunately, the neurotransmitters cannot pass directly from the blood to the brain, so that systemic administrations are ineffective in increasing brain concentrations. This is true for many other drugs and substances and has led to the concept of the "blood-brain barrier". This term is somewhat misleading as there is probably no single anatomical feature that can be classified as a barrier. In addition, this "barrier" does not necessarily prevent certain substances entering the brain, although it greatly reduces their rate of penetration.

The reduced rate of penetration of substances from the bloodstream to the brain is partly due to the lack of fenestrations in the brain capillaries. Substances passing out of the brain capillaries have to pass through the endothelial cells of the capillary wall, as opposed to passing through the fenestrations as in peripheral blood vessels. Furthermore, the brain capillaries are surrounded in sheaths of glial tissue which cover more than 80% of the external surface area of the capillary wall. An additional factor pertinent to the reduced transport of substances to the brain and within the brain is that the

extracellular fluid occupies approximately half of the volume found in most other tissues.

However, some regions of the central nervous system (CNS) appear to lack such a blood-brain barrier, for example, the pineal gland, the posterior lobe of the pituitary gland and the area postrema. Substances can pass from the blood into the cerebrospinal fluid (CSF) at the choroid plexus, the highly vascular structure in the ventricular system from which the CSF is formed. However, this passage into the CSF occurs no more readily than into the brain tissue.

The complete lack of penetration of the monoamine neurotransmitters into the brain is a consequence of their ionization at a physiological pH, since the permeability of cell membranes to the electrically charged ionized form of a molecule is usually very low compared to its electrically neutral form.

In some animals the blood-brain barrier is not fully developed at birth. For example, most substances penetrate easily into the CNS from the blood in chicks for the first week after hatching and in rats during the first few days after birth. Thus, the behavioural effects resulting from the systemic administeration of neurotransmitters can be recorded in young animals. This approach is limited by the fact that the brains of these young animals are very immature and, in addition, the animals do not have the behavioural repertoire of the adult. Therefore, the observed behavioural responses to the neurotransmitter may not reflect the normal responses of the mature animal.

### 3.1.2 Intra-cerebral administration

One way of by-passing the blood-brain barrier is to administer the neurotransmitter directly into the brain. For example, microcannulae can be implanted, using stereotaxic co-ordinates, into a particular area of the brain where the neurotransmitter can then be injected. This method has the advantage that chosen areas can be stimulated by the neurotransmitter. The disadvantage is that tissue damage inevitably occurs at the site of administration and along the length of the cannula.

Also, unless the injection is very small, the neurotransmitter will spread an indeterminate distance from the chosen site.

If extremely localised administration is required, then this can be carried out through micro-electrodes using the technique of expelling drugs by iontophoresis. The disadvantage of this method is that the animal has to be fully anesthetized or immobilised, for technical reasons, and it is therefore unsuitable for behavioural studies.

### 3.2 Administration of Precursors

Because of the technical and behavioural difficulties involved with the above methods, many researchers administer large doses of the natural precursors of the specific neurotransmitter they are studying. These substances are able to pass the blood-brain barrier and are subsequently metabolised to their respective neurotransmitters. Unfortunately, this procedure is only applicable to the biogenic amines (serotonin, noradrenaline and dopamine). In humans, the levorotatory (l) precursors to biogenic amines are thought to be preferentially metabolised to the neurotransmitters, although the d, l racemate has often been used.

The use of the precursors 5-HTP and l-DOPA should be viewed with caution as they can be metabolised to their respective amines in inappropriate cellular locations. This is because both serotonergic and catecholaminergic neurons appear to share a common decarboxylase enzyme which is capable of using either precursor as a substrate. This mix-up normally does not occur naturally because tryptophan and tyrosine hydroxylase enzymes are present only in serotonergic or catecholaminergic neurons, respectively. These two enzymes are involved in the first step of the synthesis of serotonin and the catecholamines from the natural dietary precursors tryptophan and tyrosine (and phenylalanine), respectively. Thus, it is possible, for instance, that 5-HTP may act in a non-physiological manner as a false transmitter or by displacing catecholamines.

### 3.3 Administration of other drugs which influence neurotransmitter metabolism

A wide variety of drugs are known to influence neurotransmitter metabolism and function. For example, brain serotonin metabolism is known to be influenced by the following drugs, in addition to the dietary precursor tryptophan:

| | |
|---|---|
| 5-hydroxytryptophan | - the immediate precursor |
| p-chlorophenylalanine | - a synthesis inhibitor |
| reserpine | - a storage inhibitor |
| methysergide | - a post-synaptic receptor blocker |
| cocaine, tricyclic antidepressants | - re-uptake blockers |
| monoamine oxidase inhibitors | - a catabolism inhibitor |

However, several of the drug-induced changes in brain serotonin metabolism are not specific to this particular neurotransmitter and the assessment of the role of brain serotonin in sleep from these studies is controversial. Examples of these drugs include reserpine, cocaine, tricyclic anti-depressants and monoamine oxidase inhibitors.

### 3.4 Selective lesions of neurotransmitter pathways

As our knowledge of the anatomical distribution of neurotransmitters and their pathways has increased, it is now possible to make selective lesions and, by observing the subsequent change in behaviour, further our understanding of the function of these pathways. Lesions can be effected by traditional surgical techniques or by the administration of selective neurotoxic agents. For example, the compound 5-6-HT can be used to destroy 5-HT neurons.

### 3.5 Stimulation of neurons

The function of neurotransmitters may be assessed by stimulating specific neurons, via implanted microelectrodes, and observing the resultant behaviour.

### 3.6 Assessment of neurotrotransmitter turnover in various behavioural states

It is often assumed that the turnover rate of a neurotransmitter is directly related to the activity of the neurons within which it is contained. If this rate can be assessed, then this technique can be used to improve our understanding of the activity of various neurotransmitter-specific pathways during different behavioural states such as alertness, sleepiness, sleep and sleep deprivation. Direct electrical recordings of these neurons is, at present, very complex and sometimes impossible.

Early investigators measured the excretion of neurotransmitter end-products in the urine, but this method only reflected their turnover rate in the body as a whole and not just the CNS. Thus, in many experiments the animal is immediately sacrificed and the brain frozen following the observation or inducement of various behavioural states. Assays of the neurotransmitter and its metabolites are then taken as soon as possible.

Obviously, this method is not suitable for experiments with humans, apart from post-mortem cases, and samples of the CSF are taken typically from either ventricular or lumber drains. More sophisticated techniques employ radio-actively labelled amines. These are injected into the CSF or the brain, where they are taken up by their appropriate neurons. The rate of turnover of these labelled transmitters can be assessed by following the rate of disappearance of the labelled amines from the brain, either by sacrificing animals at various times after the injection or by analysing the radioactive content of the expired air. The rate of release of the labelled amines from the nerve terminals can also be estimated by perfusing the venticular system of the brain and measuring the amounts of radio-activity.

## 4. THE ROLE OF SEROTONIN IN SLEEP

The number of studies in this field of research is extremely large, but the roles of the various neurotransmitters in the sleep mechanisms are still far from clear. The subsequent sections will review the evidence linking brain serotonin metabolism to sleep. The experimental evidence will be summarised in three categories namely,

a) the neurophysiological studies - the effects of lesion and stimulation of serotonergic pathways

b) the pharmacological studies - the effects of drugs upon brain serotonin metabolism and sleep

c) the correlational studies - the observation of changes in brain serotonin metabolism during various behavioural stages such as wakefulness, SWS and REM sleep.

## 5. NEUROPHYSIOLOGICAL STUDIES

This section will review the effects of lesion and stimulation of the serotonergic system upon sleep. Since one of the main strategies of neurophysiologists is to destroy or stimulate these systems as selectively as possible, the simplist tactic is to interfere with these systems at the place where most of the cell bodies (perikarya) or ascending pathways are concentrated. Since the 5-HT-containing perikarya are concentrated in the midline raphe, this area is often chosen to investigate the effects of 5-HT upon sleep.

There is very little experimental evidence from human studies except for some behavioural observations following brain tumour operations. These lesions are not restricted to one particular neurotransmitter system and the associated effects are very difficult to interpret. This review will therefore be restricted to the findings from animal studies.

### 5.1 Lesion experiments

Most of the perikarya containing 5-HT are located in the nuclei of the raphe system in the rat (Dahlström and Fuxe, 1964) and the cat (Pin et. al., 1968). The destruction of the raphe nuclei has been found to affect sleep both in the cat (Jouvet, 1969) and the rat (Adrien, et. al., 1977).

In the study by Jouvet (1969), sleep was polygraphically monitored from 10-13 days following the operation and the cats were sacrificed

on the 13th day, at the same time of day to avoid the influence of circadian variation upon brain MA. It was demonstrated that a significant correlation existed between the extent of the lesion, the intensity of the resulting insomnia and the decrease of brain 5-HT.

The cats who suffered a large scale destruction of the raphe nuclei (80-90%) became permanently aroused for 3-4 days. Following this period, the amount of NREM sleep did not exceed 10-15 per cent of the control cat values even three weeks after the lesion and REM sleep was never observed. However, a continuous discharge of PGO spikes appeared immediately following the destruction of the raphe nuclei. Brain 5HIAA was also found to be decreased after the coagulation of the raphe system, but no changes in NA or DA concentration were found in the telencephalon or diencephalon compared to normal cats sacrificed under the same conditions.

The animal lesion studies provide evidence that 5-HT neurons are involved in the maintenance of sleep, although it is difficult to assess whether their influence is mediated via a general inhibitory effect upon arousal or by involvement in the sleep mechanism itself. The available evidence favours the latter inference as it has been found that the effect of lesions in the anterior raphe differ from those in the caudal raphe.

The destruction of the anterior raphe was associated with permanent arousal during the first 2-3 days but REM sleep, although decreased in amount, appeared directly without previous NREM sleep (Renault, 1967). On the other hand, the destruction of the caudal raphe is followed by the near complete disappearance of REM sleep whereas NREM sleep is only decreased by about 60%. From these findings, Jouvet (1972) suggested that the anterior raphe neurons are probably more involved with the regulation of NREM sleep whereas the caudal raphe neurons are more involved with the "priming" of REM sleep via its projections to the locus coeruleus complex, where the "executive mechanisms" are located. Jouvet thus believes that the catecholaminergic neurons, which are plentiful in the locus coeruleus complex, are responsible for the production of REM sleep. Jouvet (1974) further suggested that the increased waking which follows the destruction of the raphe system (mainly in its rostral part) is most probably mediated by catecholaminergic mechanisms. This was concluded from the finding that AMPT (alpha-methyl-paratyrosine, an

inhibitor of catecholamine synthesis) induced behavioural sedation and cortical synchronisation in cats whose raphe system had been destroyed. Also, it has been observed that NA turnover, at the level of the dorsal NA bundle terminals, is increased following the destruction of the raphe system (Pujol et. al., 1973; Blondaux, 1974).

### 5.2 Stimulation Experiments

Several studies have reported that behavioural and EEG sleep can be induced in the rat by low frequency electrical stimulation of the medial or dorsal raphe (Kostowski and Giacalone, 1969; Gumulka, et. al., 1971). This stimulation was associated with a long-lasting increase of 5-HIAA in the forebrain, although 5-HT concentration was not affected. This can be interpreted as an increase in turnover of brain 5-HT.

## 6. PHARMACOLOGICAL STUDIES

### 6.1 Brain serotonin metabolism

Brain serotonin (5-HT) is synthesized intra-neuronally from the dietary amino acid precursor tryptophan in a two-step pathway involving hydroxylation to 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase and the subsequent decarboxylation to 5-HT by aromatic acid decarboxylase. This latter enzyme is also present in catecholaminergic neurons. The rate of synthesis of 5-HT is sensitive to the intra-neuronal levels of tryptophan, since the normal concentration of the precursor is not sufficient to completely saturate the enzyme tryptophan hydroxylase.

After 5-HT has been released, it is removed from the synapse via a re-uptake mechanism located in the membrane of the pre-synaptic nerve terminal. 5-HT is degraded metabolically by monoamine oxidase (MAO), an enzyme which is found in both 5-HT and catecholanure neurons, to 5-hydroxyindoleacetic acid (5-HIAA). This appears to be the only major metabolic route for 5-HT breakdown in the CNS.

The metabolism of 5-HT can be pharmacologically altered by the methods described in the following sections.

## 6.2 Cerebral administration of serotonin

From the studies with animals it appears that the administration of 5-HT, whether by intravenous injection in chicks whose blood-brain barrier is permeable (Spooner and Winters, 1965, 1966; Spooner et. al., 1968) or by microinjection into the brain of mature animals (Yamaguchi et. al., 1963; Kostowski et. al., 1975), is associated with cortical synchronization. However, one cannot be sure that exogenous 5-HT mimics the effect of endogenous 5-HT at the receptor site. In addition, the administered 5-HT may be taken up by other monoamine neurons. A further problem is to decide whether the cortical synchronization is an indicator of normal sleep or non-physiological sleep.

## 6.3 Administration of 5-HT precursors: Tryptophan, 5-HTP

### 6.3.1 Tryptophan - dietary precursor

A detailed review of the tryptophan studies is presented because it is known that the diet can influence the transport of this amino acid to the brain (see Chapter 9).

Animal Studies. The effects of a single administration of l-tryptophan, in doses of 150, 300, 450 and 600 mg/kg, have been studied in the rat (Hartmann and Chung, 1972). The two highest doses significantly reduced sleep latency compared to placebo control values, whilst the other doses only showed a trend in this direction. No differences were recorded in NREM sleep, REM sleep or number of awakenings over the 8-hour recording period.

The administration of tryptophan free diets have been reported to either decrease REM sleep (Mendelson et. al., 1977) or to have no effect (Clancy et. al., 1978). It may be significant that the earlier study investigated the effects of an acute administration of a tryptophan-deficient diet, whereas the later study investigated the effect of a chronic deficiency of tryptophan.

Human Studies. Even before sleep studies were conducted it was known that the oral administration of l-tryptophan (30-90 mg/kg) to

normal subjects was associated with drowsiness and an inability to concentrate (Smith and Prockop, 1962). Unfortunately, the early sleep studies did not provide detailed reports of the EEG changes after l-tryptophan. Oswald et. al. (1964, 1966) found that five out of sixteen normal males had reduced REM latencies after 5-10 g l-tryptophan had been ingested upon retiring to bed. Evans and Oswald (1966) studied the effects of a 5 g dose in 7 narcoleptics and reported only an increased duration of the first REM period.

Subsequent studies, providing more detailed data, have generally reported either an increase or no change in total sleep time and SWS. The effect of oral l-tryptophan upon REM sleep is not so consistent and both increases and decreases have been reported. The effects of l-tryptophan upon human sleep are summarised in Table 1.

Cazzullo et. al. (1969) observed that both sleep length and SWS were increased whilst REM sleep was decreased after an unreported dosage of l-tryptophan to six severe depressives.

Williams et. al. (1969) studied the sleep of eleven normal males on two occasions, separated by two weeks, after an oral dose of 7.5 g l-tryptophan in a portion of apple sauce approximately half an hour before bedtime. Baseline data was recorded on three nights; one before, one night between, and one night after the experimental nights. On these baseline nights the subjects received apple sauce, but this was not considered to be suitable for classification as a placebo due to the bitter taste of the tryptophan dose. The results showed that SWS was increased from a baseline average of 21.2 per cent to 28 per cent after the tryptophan doses. Both sleep onset (time to spindle activity) and per cent time awake were reduced, but no consistent changes were found in REM sleep although REM periodicity was decreased.

The authors do not state whether an adaptation period was allowed before the first three baseline nights were recorded. This study also incorporated two nights with a 7.5 g dose of phenylalanine and it is interesting to note that, compared with the average of the first three baseline nights, all the tryptophan, phenylalanine and other baseline nights showed decreases in per cent awake and increases

| Study | Subjects | Dose | Total Sleep Length | SWS | REM | Wake-fulness | Sleep Latency | REM Latency | REM Periodicity |
|---|---|---|---|---|---|---|---|---|---|
| Oswald et. al (1964,1966) | 16 normal males | 5-10g | = | NA | NA | NA | NA | ↓ in 5 s[9] | NA |
| Evans & Oswald (1966) | 7 narcoleptics | 5g | NA | NA | ↑ length of first REM period | NA | NA | NA | NA |
| Cazzulo et. al (1969) | 6 severe depressives | NA | ↑ | ↑ | ↓ | NA | NA | NA | NA |
| Williams et. al (1969) | 11 normal males | 7.5g | NA | ↑ | = | ↓ | ↓ | = | ↓ |
| Wyatt et. al. (1970) | 5 normal females | 7.5g | ↑ | ↑ | ↓ | NA | = | ↑ | NA |
| | 7 insommiacs (3 with affective disorders) | 7.5g | ↑ | = | = | ↓ | = | = | NA |
| Hartmann et. al. (1967, 1971) | 10 normals | 5-10g (120 mg/kg) | ↑ | = | slight ↑ | = | ↓ | = | NA |
| Murri et. al. (1971) | 7 chronic schizophrenics | ~7g (100 mg/kg) | = | = | = | NA | NA | = | NA |
| Griffiths et. al. (1972) | 8 normals | 12g | NA | = | ↑ | ↓ | ↓ | ↓ | = |
| Mendels & Chernick (1972) | 4 normals 4 psychiatric patients | 7.5g | ↑ | NA | NA | ↓ | NA | NA | NA |
| Hartmann et. al. (1974) | 10 normals | 1,2,3,4,5, 10, 15g | = | ↑ 10g only | >10g ↓ | = | ↓ | NA | NA |
| Davis et. al. (1975) | 24 mild insommiacs (12 male, 12 female) | 1g | = | = | = | = | ↓ | ↓ | NA |
| Hartmann & Cravens (1975) | 8 normals:- 4 1g 4 4g | 1,4g | = | = | = | = | = | = | NA |
| Elion & Hartmann (1976) | 42 normals:- 14 placebo 14 1g 14 3g | 1,3g | = | = | = | = | ↓ | = | NA |

Table 1. The Effects of l-Tryptophan upon Sleep in Humans

Key:- NA - information not available
↑ - significantly increased
↓ - significantly decreased
= no significant change

in per cent SWS. In addition, all these nights also showed an increase in percentage REM sleep, except for the second dose of tryptophan. This trend, of special importance because it is also exhibited by the extra baseline nights, suggests that the first three baseline nights do not reflect the normal sleep of the subjects, possibly because of a lack of adaptation nights in the laboratory. If this was true, then the reported effects may have been an experimental artefact. However, the reported sleep changes after the amino acid administration were still exhibited when compared to the latter two baseline nights, between and after the amino acid nights, although the differences may not still be significant.

The findings of the above study were consistent with those of Wyatt et. al. (1970) who studied the effects of tryptophan upon the sleep of normal and insomniac subjects. The sleep of five young, normal females was observed for twenty-two consecutive nights. The first two nights were used for adaptation purposes. Three of the subjects then received the tryptophan dose (7.5 g) during the next ten nights and a placebo during the last ten nights whereas this procedure was reversed for the other two subjects. The l-tryptophan and placebo powder were mixed in a milk-shake to disguise the bitter flavour of the amino-acid and the milk shake was given to the subjects half-an-hour before bed-time.

The results showed that sleep length had been increased by an average of just over an hour during the tryptophan administration. Although SWS was also increased, REM sleep was actually decreased. Also, REM latency was increased and REM density decreased by the tryptophan dose.

Wyatt and his colleagues repeated the experiment with seven insomnia patients. The procedure consisted of four to five adaptation nights followed by three equal and consecutive periods of placebo, l-tryptophan (7.5 g) and placebo. These periods lasted 5 to 10 days, depending upon individual circumstances. Again, the placebo powder and l-tryptophan were given in a milk-shake thirty minutes before bed-time. Surprisingly, the results of this study were not consistent with their previous study.

Although total sleep time was increased, no changes were found in SWS, REM sleep or REM latency. The amount of wakefulness during the sleep period was also found to be decreased. These findings suggest that tryptophan may be a valuable treatment for the problems of insomnia. It is not clear why the insomniac patients should not show similar changes to the normal subjects in their amounts of SWS and REM sleep, but it is possible that a higher dose of l-tryptophan may have elicited these changes.

The finding that REM sleep was decreased after tryptophan administration to normal subjects was not consistent with their finding of an increase in REM sleep after the administration of the precursor 5-HTP, or their earlier finding of a reduction in REM sleep by PCPA administration (Wyatt et. al., 1969), which reduces the synthesis of 5-HT. Also, l-tryptophan has been found to increase SWS whereas PCPA did not affect NREM sleep. The authors therefore suggested that the decrease in REM sleep during tryptophan administration was not mediated by an increase in 5-HT but by some other metabolite of tryptophan.

To test this hypothesis, l-tryptophan (5-14 g) was administered orally, 30 minutes before bedtime, for 3-6 nights in three patients with carcinoid tumours who were receiving PCPA treatment (Wyatt et. al., 1970). The l-tryptophan was given after three weeks of PCPA therapy, by which time REM sleep was maximally depressed. One of the patients received two periods of l-tryptophan administration. During each of these four l-tryptophan plus PCPA periods, both total and NREM sleep increased and REM sleep decreased compared to PCPA only nights. SWS was not affected by the addition of tryptophan. This finding was taken to support their hypothesis that the effect of l-tryptophan upon sleep is not due to changes in 5-HT metabolism.

Two other experimental findings also support this hypothesis. Firstly, PCPA does not prevent the l-tryptophan-induced enhancement of raphe cell fluorescence (Aghajanian and Asher, 1971), (The raphe system is rich in serotonergic neurons). Secondly, PCPA fails to block the usual depression of raphe unit firing by l-tryptophan (Aghajanian, 1972). These findings raise serious questions concerning the assumption that 5-HT is the only functional substance within raphe neurons.

However, the data of Pujol (1970) and Pujol et. al. (1969, 1971) have shown that, in the cat, the inhibition of tryptophan-hydroxylase by PCPA is not total but about 80 per cent. This leaves the possibility that a small amount of 5-HT may still be synthesized and become functional. Also, the doses of PCPA given to humans are generally much lower than those administered in animal studies and thus the tryptophan-hydroxylase inhibition may be even less than 80 per cent in human studies. Tryptophan can also be metabolised by the tryptamine and kynurenine pathways but the significance of these metabolites in the regulation of sleep is, at present, unknown.

Hartmann (1967) studied the effect of l-tryptophan (120 mg/kg, taken 20 minutes before bedtime) in eight normal subjects. It was found that total sleep time was slightly increased and that REM sleep was increased by an average of 16 minutes.

This study was later expanded by the addition of two extra subjects (Hartmann et. al., 1971), whereupon the only significant finding was a reduction in sleep latency on the tryptophan nights. There was a trend, not quite reaching significance towards increased sleep, decreased number of awakenings and increased REM sleep.

Murri et. al. (1972) reported no effects upon the sleep of seven chronic schizophrenics following the administration of 100 mg/kg l-tryptophan. It is possible that the small effect of tryptophan was masked by the disturbed sleep often characteristic of these patients.

Griffiths et. al. (1972) administered 12 g l-tryptophan to eight subjects. This study used a bitter tasting mixture of dutch chocolate and cocoa to mask the taste of the amino acid, and this mixture was also administered by itself as a placebo. This well-designed study incorporated an adaptation night and four base-line placebo nights prior to the first tryptophan night which was followed by a recovery night. Tryptophan was administered again one week later, being preceeded by a baseline night and followed by a recovery night. The placebo and tryptophan were taken 20 minutes before bedtime and the subjects reported extreme drowsiness within this period.

The objective data showed a large increase (an average of 31 minutes) in REM sleep on the tryptophan nights compared to the placebo nights. This increase was associated with decreases in stages 1 and 2 and REM latency and an increase in the duration of the REM periods. REM sleep values were back to baseline values on the recovery nights. SWS was not significantly altered by the tryptophan doses. The amount of wakefulness was decreased on the first tryptophan night but not on the second, or for the average of the two nights. This trend was due primarily to an early time of sleep onset. No significant differences were found between placebo and post-tryptophan recovery nights.

This study puts forward sound experimental evidence that tryptophan increases REM sleep, as had been previously suggested by Hartmann (1967). The changes reported were fairly large and it is surprising that they were not evident in the studies by Williams et. al. (1969) and Wyatt et. al. (1970). This difference between the findings may be a function of the dosage. The potential of the high dose of tryptophan in increasing REM sleep is highlighted by the fact that four out of the eight subjects entered REM sleep, on at least one of the two tryptophan nights, less than six minutes after sleep onset.

Mendels and Chernick (1972) studied the effects of 7.5 g l-tryptophan, with and without pyridoxine (vitamin B6), upon sleep in 4 psychiatric patients and 4 normal subjects. The effect of tryptophan with pyridoxine was investigated because it has been reported that pyridoxine may increase the conversion of l-tryptophan to 5-HT (Berman et. al., 1969). The normal subjects were studied for 19 consecutive nights; 5 baseline nights, 5 tryptophan nights after which two subjects received tryptophan with pyridoxine for 5 nights whilst the other two continued on tryptophan alone and 4 recovery nights. The psychiatric patients were studied for 13 nights: 4 baseline nights, 3 tryptophan nights, 3 tryptophan with pyridoxine nights (for all 4 patients) and 3 recovery nights. The results showed that tryptophan increased sleep length and decreased wakefulness in all the subjects. Pyridoxine was not found to have any major effect when administered with the tryptophan.

The authors suggested that the effects of tryptophan upon SWS may be,

in part, a function of baseline values. They reported that the normal subjects with relatively large amounts of SWS showed either no change or a slight decrease in SWS with tryptophan whereas those subjects with lower levels of SWS had some increase with tryptophan administration. Whilst this point is interesting, it can only be viewed as conjecture because only four normal subjects were recorded. Also three of the psychiatric patients showed no delta sleep at all during both the baseline and tryptophan nights.

Hartmann et. al. (1974) examined the effects of a wide dose range of l-tryptophan (l-15 g) upon ten normal males who reported taking 15 minutes or more to fall asleep. Each subject slept in the laboratory for 12 nights spaced approximately one week apart. The first two nights were used for adaptation purposes and the subsequent ten nights included, in random order, 3 placebo nights and one night on each of seven doses of l-tryptophan (1, 2, 3, 4, 5, 10 and 15 g). The l-tryptophan and a similar tasting placebo were mixed in identical milk shake drinks which were consumed 20 minutes before bedtime. The results showed that tryptophan reduced sleep latency, even with the 1 g dose. This dose reduced sleep latency by 50 per cent but no further decreases were elicited with the larger doses. The amount of waking time was reduced on average but only significantly at intermediate doses. Both REM sleep and SWS were unaffected by the lower doses. However, REM sleep was reduced approximately 30 per cent by the 10 and 15 g doses whereas SWS was increased approximately 18 per cent by the 10 g, but not by the 15 g dose. The authors noted that doses of 1-5 g l-tryptophan produced decreased sleep latency and decreased waking without affecting other aspects of sleep and concluded, therefore, that l-tryptophan may be a natural hypnotic and that sleepiness after a large meal may be related to the tryptophan content of the meal.

The hypnotic qualities of the 1 g dose were further investigated in 24 mild insomniacs (12 males and 12 females), defined as reporting sleep latencies of greater than 30 minutes (Davis et. al., 1975). After adaptation, the subjects received 1 g l-tryptophan for two non-consecutive nights and a placebo (as used by Hartmann, 1974) also for two nights in a balanced order. Again, the 1 g dose was found to reduce sleep latency

without affecting SWS or REM sleep, although REM latency was also reduced.

Elion and Hartmann (1976) studied the effect of l-tryptophan upon the mild insomnia produced by "sleeping in a strange place". Forty-two normal subjects were studied for one night in the laboratory: 14 were given placebo, 14 were given 1 g l-tryptophan and 14 were given 3 g l-tryptophan. Those subjects receiving l-tryptophan were found to have reduced sleep latencies compared to those receiving the placebo. No other sleep changes were reported and the effects of lg and 3 g l-tryptophan were nearly identical.

Hartmann and Cravens (1975) carried out a long-term study to assess the chronic effects of l-tryptophan administration. This study was not designed to investigate sleep latency so normal subjects were used who suffered no trouble in getting to sleep. Four subjects received 1 g and 4 subjects received 4 g l-tryptophan for 28 consecutive nights. The effects of withdrawal were also observed during the next 32 nights. EEG recordings were taken on nights 1-5 and then once a week for both the tryptophan administration period and the withdrawal period. No significant changes in sleep stages or cycling were found during either period.

Summary of the effects of tryptophan upon human sleep

The effects of l-tryptophan upon sleep have been extensively studied and it appears that l-tryptophan is a suitable candidate for a "natural hypnotic" as it has been repeatedly shown to decrease sleep onset and increase total sleep time with no associated side effects, even with chronic usage. Interestingly, these effects are not greatly augmented by increasing the dose above 1 g. The daily diet normally contains between 1 and 2 g l-tryptophan and thus the effect of a 1 g dose can be considered to be within the physiological range. This is a very important point when assessing the pharmacological data on sleep. However, the effects of l-tryptophan upon SWS and REM sleep are unclear. It should be noted that, although the doses are within the daily intake range, the method of administration as a concentrated supplement rather than as a natural dietary constituent may invoke non-physiological changes in the metabolism of tryptophan.

The studies reporting major changes in sleep stages have all incorporated doses of at least 7.5 g. As no studies have reported a decrease in SWS, whilst two studies using normal subjects have reported an increase in SWS, it would appear that l-tryptophan enhances deep sleep, although this may well be a non-specific effect arising from the reduced sleep latency and increased sleep time.

In those studies presenting REM sleep data for normal subjects taking doses of 7.5 g or greater, two show a decrease, two show an increase (although only one is statistically significant) and the other study shows no change.

It is possible that the vehicle in which the l-tryptophan was mixed may have been responsible for the varied findings. The vehicles used were either apple sauce, milk shake or a mixture of chocolate and cocoa. It is very interesting that this latter vehicle was used in the study reporting the large increase in REM sleep and the associated radical reduction in REM latency. As this vehicle was used as a placebo control, these effects cannot be ascribed solely to the vehicle but it appears that this carbohydrate and fat based vehicle greatly enhanced the effects of l-tryptophan. From the review of the diet and sleep literature (Chapter 7) and the findings of Study III it appears that the consumption of a high carbohydrate diet is associated with increased REM sleep and, therefore, it is possible that this relationship is mediated via changes in the metabolism of tryptophan due to the increased blood glucose levels. Fernstrom and Wurtman (1971) have reported that the consumption of a carbohydrate diet was associated with an increase in brain 5-HT levels. (See Section 2.2, Chapter 9).

It is possible that the effects of tryptophan upon sleep are not mediated via 5-HT. Tryptophan is metabolised to tryptamine and kynurenine and its associated metabolites, as well as being available for protein synthesis. Unfortunately, little is known concerning the hypnotic qualities of these metabolites.

### 6.3.2 5-HTP

Animal studies. The effect of d, l 5-HTP is dependent upon the dose injected. Low doses (1-5 mg/kg) appear to enhance cortical synchronisation in cats, although REM sleep also shows a tendency to be increased (Delormé, 1966). Large doses (20-60 mg/kg) definitely induce synchronization which lasts for several hours (Monnier and Tissot, 1958; Jouvet, 1967; Jacoby et. al., 1974; Buckingham and Radulovacki, 1976). Similar effects have been found in the monkey (Macchitelli et. al., 1966). However, although the behaviour of the animal resembles sleep, it appears from the EEG that this cortical synchronisation is different from physiological sleep. For example, PGO activity and REM sleep have been observed to be completely suppressed for 5-6 hours, after which time REM sleep occurs with some rebound (Delormé, 1966).

At even higher doses the effect of 5-HTP is two-fold; firstly a state of sedation occurs which is followed by excitation (Costa et. al., 1960; Green and Sawyer, 1964). This two-fold effect is difficult to interpret. It must be remembered that exogenous 5-HTP may be decarboxylated to 5-HT in brain capillaries and also in catecholamine neurons Lichtensteiger and Langemann, 1966; Ng et. al., 1972). The physiological significance of serotonin formed in this manner is unknown at present.

Human Studies. Although there are many reports on the effects of d, l 5-HTP upon sleep in the literature, only three studies have used normal subjects. The remaining studies reported upon schizophrenics, narcoleptics, depressives, mongols and alcoholics and the generalisations to be made from these can only be tenuous.

From the data on normal subjects, it appears that the effect of 5-HTP upon sleep is dose dependent. Low doses (0.7 mg/kg) do not appear to influence sleep (Hartmann, 1970) whereas a larger dose (8.6 mg/kg) produces an increase in REM sleep and REM density (Wyatt, et. al., 1971). In a third study (Autret et. al., 1976), a dose of 900 mg (approximately 13 mg/kg) 5-HTP was administered to three males and REM sleep was subsequently decreased. Two of the subjects who had low levels of SWS during the base-line periods showed an increase in this sleep stage.

It is clear that these doses are small by comparison to those given to animals and this may explain the differences in the effects of 5-HTP administration between animals and humans.

From the other human studies, doses up to 7.1 mg/kg produced either no change (Bazelon et. al., 1968; Gillin et. al., 1972) or an increase (Mandell et. al., 1964; Murri et. al., 1972; Zarcone et. al., 1973; Zarcone and Hoddes, 1975) in REM sleep whereas doses above 21 mg/kg produced decreases in REM sleep (Guilleminault et. al., 1973; Zarcone et. al., 1973; Dawson et. al., 1974). SWS was found to be either unaffected or reduced (Murri et. al., 1972; Dawson et. al., 1974).

As with the animal studies, a two-fold effect is visible; REM sleep being increased at a medium dose of 5-HTP (8.6 mg/kg) and decreased at higher doses (13 mg/kg). This effect highlights our present lack of knowledge in neuropharmacology. It is possible that the larger doses of 5-HTP produces functionally active 5-HT in catecholaminergic neurons, or the storage of 5-HT in the synaptic vesicles of these neurons displaces the catecholamines which themselves become active.

6.4 Administration of synthesis inhibitors - paracholorphenylalanine (PCPA)

The most potent and selective inhibitor of serotonin synthesis is, at present, p-chlorophenylalanine (PCPA). This drug has been found to decrease the level of 5-HT in the rat's brain without significantly altering the levels of the catecholamines (Koe and Weissman, 1966; Gál et. al., 1970). The levels of 5-HT are decreased after PCPA administration because this drug inhibits the action of the enzyme tryptophan hydroxylase. Thus, although normal amounts of tryptophan may be available, the intra-neuronal conversion of tryptophan to 5-HTP is blocked. However, later work (Pujol, 1970; Pujol et. al., 1971; Deguchi et. al., 1973) suggests that this block is not total.

Animal studies. The effect of PCPA upon sleep has been extensively studied in animals and has been consistently associated with prolonged wakefulness. (In the rat: Mouret et. al., 1968 - 500 mg/kg; Florio et. al.,

1968 - 300 mg/kg; Rechtshaffen et. al., 1969 - 100 mg/kg. In the cat: Delormé et. al., 1966 - 100-300 mg/kg; Koella et. al., 1968 - 50-200 mg/kg; Florio et. al., 1968 - 300 mg/kg; Dement, 1969 - 73-300 mg/kg/24 hours. In the rabbit: Florio et. al., 1968 - 300 mg/kg. In the monkey: Weitzman et. al., 1968 - 600-1000 mg/kg).

It has been assumed that PCPA itself has no direct pharmacological action upon the brain because of the lengthy interval between the injection and the resultant behaviour changes. For example, the injection of 400 mg/kg of PCPA in the cat does not appear to alter behaviour or EEG recordings for about 18-24 hours. Insomnia develops after this time and becomes complete after about 30-40 hours. From this time on, sleep gradually returns to normal, although this takes 7 or 8 days. This recovery of sleep is accompanied by continuous monophasic sharp waves (PGO spikes) in the pons, lateral geniculate body and occipital cortex which normally occur just before and during REM sleep. However, PCPA administration to rhesus monkeys does not decrease REM sleep although NREM is decreased.

From their studies, Mouret et. al. (1968) and Koella et. al. (1968) have found a significant correlation between the decrease of brain 5-HT resulting from PCPA administration and the decrease of SWS.

PCPA+5-HTP

Since PCPA inhibits only the first step in 5-HT synthesis, it is possible to bypass this blocking action by injecting 5-HTP. Thus, in PCPA-treated cats whose brain 5-HT levels were decreased by 85 per cent, the injection of 5 mg/kg of 5-HTP resulted in an increase of brain 5-HT levels to 60 per cent of the base-line level after one hour (Hoyland et. al., 1970).

If such a dose of 5-HTP is given when PCPA-produced insomnia is at its maximum, then normal sleep is restored for several hours (Mouret et. al., 1967; Koella et. al., 1968; Jouvet, 1968; Pujol et. al., 1971). In addition to reversing the insomnia, the injection of 5-HTP restricted the PGO spiking to REM periods (Dement, 1969).

This depressing effect of PCPA upon sleep and its restoration by low doses of 5-HTP is one of the main arguments implicating 5-HT with the sleep mechanisms. However, there are several issues which do not strengthen the above argument:

i) During PCPA-induced insomnia there is continuous PGO spiking (Jouvet, 1969; Dement et. al., 1969). In other words, this does not constitute normal wakefulness and suggests that other factors than 5-HT are involved in the sleep-wakefulness cycle.

ii) After eight consecutive days of PCPA administration, sleep returned to approximately 70 per cent of baseline values in the cat even though brain 5-HT levels were maximally depressed. (Dement, 1969).

iii) The recovery of brain 5-HT after PCPA administration lags considerably behind the recovery of sleep. For example, 11 days after PCPA administration, SWS had returned to about 85 per cent of baseline whereas brain 5-HT had returned to only about 55 per cent of base-line values (Mouret et. al., 1965).

For this last point it can be argued that a 1 to 1 correlation between brain 5-HT and sleep would not be expected. Low measures of brain 5-HT levels do not necessarily imply lowered levels of functional activity; they could equally imply unimpared functional activity, even though storage levels were low, if turnover was sufficiently rapid. However, the second point cannot be dismissed so easily. Dement (1969) suggested that the change in PGO spiking might explain their findings. He proposed that PGO spiking is regulated by 5-HT and, under normal circumstances, the presence of 5-HT confines the spikes to REM sleep. A corollary of this hypothesis is that when 5-HT levels are decreased, PGO spikes become dispersed throughout the 24 hour period where they serve as a disruptive influence resulting in decreased sleep. However, the author further suggested that the animals habituate to the arousing stimuli after several days, thereby allowing for the reversal of the PCPA-induced insomnia.

Human Studies. The effect of PCPA administration in man is not so sleep-disruptive as it is in animals and only REM sleep appears to be reduced. Wyatt (1972) administered doses of 2-4 g/24 hour PCPA, over periods from two weeks to three years, to 17 patients suffering from a variety of medical disorders. PCPA was found to decrease REM sleep by 20-70 per cent. The maximum decrease took place after 2-3 weeks of treatment and appeared to be both dosage and time dependent. Some patients exhibited small increases in NREM sleep during PCPA administration, so that total sleep time was not affected. When the PCPA treatment was discontinued, REM sleep remained at a low level for about three weeks before returning to normal levels. Furthermore, no compensatory REM rebound was elicited by PCPA administration as is often the case with drugs that suppress REM sleep. This finding strongly suggests that PCPA interferes with a major pathway for the appearance of REM sleep rather than just suppressing its occurrence.

PCPA was also found to have effects on the phasic aspects of REM sleep. The number of rapid eye movements over the whole night and per minute of REM sleep were reduced whereas during stage 2 sleep they were increased. In addition, the amount of phasic integrated potentials (PIPs), monitored from the extra-ocular muscles in two subjects, were increased during NREM sleep. These PIPs may well be analogous to the PGO spikes in cats, and in this respect, the effect of PCPA is similar in both animals and humans. This provides further evidence for Dement's hypothesis that one of the functions of serotonin is to confine such phasic events to REM sleep.

PCPA+5-HTP

Wyatt (1972) also reported on the effects of the administration of 400-800 mg d, l 5-HTP for 5-6 consecutive nights to four patients with carcinoid tumours and whose REM sleep was maximally depressed by PCPA treatment. Three of the patients showed a moderate-to-total return of REM sleep whilst the fourth patient showed no change. Total rapid eye movement activity also returned toward normal. NREM sleep was not found to be affected by the drugs. When the 5-HTP administration was

discontinued, the amount of REM sleep again decreased. This finding is consistent with those from the animal studies in that the administration of 5-HTP can reverse the effects of PCPA upon sleep. This clearly implicates serotonin with the regulation of sleep although these are certain major differences in its influence between humans and animals.

6.5 Administration of storage inhibitors - reserpine.

Reserpine is thought to inhibit the intra-neuronal storage of the monoamines thereby allowing for their increased catabolism by MAO. Thus, reserpine administration decreases the concentrations of serotonin, dopamine and noradrenaline, although it may also increase their rate of turnover (Brodie et. al., 1966). The individual actions of 5-HT, DA or NA upon sleep are very difficult to interpret from the studies of reserpine administration.

Animal studies. Delormé et. al. (1965) found that a single dose (0.5 mg/kg) of reserpine suppressed EEG synchronization for 6-8 hours and REM sleep for 24 hours in cats. As with PCPA, the administration of reserpine was associated with continuous PGO activity. Interestingly, cortical synchronization was restored in the respirized cat by the administration of 5-HTP (30-50 mg/kg) and REM sleep by the administration of DOPA (30-50 mg/kg). This finding led to the hypothesis that SWS and REM sleep were associated with 5-HT and CA metabolism, respectively (Matsumoto and Jouvet, 1964). Hoffman and Domino (1969), in a carefully designed dose-response study, confirmed the effect of reserpine in the cat and showed that the decrease of both stages of sleep was a function of dose. However, in rabbits (Khazan and Sawyer, 1964) and monkeys (Reite et. al., 1969), moderate to large doses of reserpine were reported to increase REM sleep.

Human studies. In humans, reserpine is reported to increase REM sleep and decrease SWS with doses in the order 0.01 to 0.14 mg/kg (Tissot, 1965; Hartmann, 1966; Hoffman and Domino, 1969; Coulter et. al., 1971).

It seems unlikely from the PCPA studies that the reserpine-decreased levels of 5-HT are responsible for the increase in REM sleep. It can be argued that, as the turnover of serotonin may be increased, there may be an increase of functional serotonin available at the synapse. The reserpine effect may be due, in part, to the decreased levels or increased turnover of the catecholamines.

6.6 Administration of receptor blockers - methysergide

Methysergide is a blocker of serotonin receptor sites (Graham, 1967; Roberts and Straughan, 1967) and its administration appears to have similar effects to PCPA administration. Thus, in rabbits, methysergide decreases REM sleep and, to a lesser extent, NREM sleep (Tabushi & Himwich, 1971). This decrease in REM sleep is still evident if methysergide is administered to cats who have been REM deprived for 48 hours (Jackman and Radulovacki, 1976).

The administration of methysergide does not appear to alter the rate of 5-HT turnover as 5-HIAA levels have been reported to be unaffected in the cisternal CSF of cats (Jackman and Radulovacki, 1976) and in the whole brain of rats (Jacoby and Bryce, 1976).

In humans, methysergide is generally associated with increased REM latency and/or reduced REM sleep (Oswald et. al., 1966; Pack and Schmidt, 1972; Mendelson et. al., 1975) whereas SWS has been found to be decreased (Pack and Schmidt, 1972) or unchanged (Mendleson et. al., 1975).

Lysergic acid diethylamide (LSD) is also thought to block serotonin receptors (Gaddum, 1953) although recent work suggest that it may mimic serotonin at synaptic terminals (Byck, 1975). This latter theory is consistent with the findings of increased REM period length or decreased REM periodicity after LSD administration just before sleep or during sleep (Muzio et. al., 1966; Torda, 1968).

6.7 Administration of re-uptake blockers - cocaine, tricyclic anti-depressants

Cocaine and the tricyclic anti-depressants are able to block

the re-uptake of serotonin and the catecholamines by the pre-synaptic neuron. Therefore, as is true of the reserpine studies, the findings from the use of such drugs can only be generalised to the combined effects of the monoamine neurotransmitters.

Cocaine, administered to depressed patients, has been shown to decrease REM sleep (Post et. al., 1974). Upon withdrawal a REM rebound occurred,although it should be noted that these changes in REM sleep were associated with parallel changes in sleep length. As the sleep length was not held constant in the analysis, it is difficult to conclude whether cocaine primarily affects REM sleep or sleep length.

The tricyclic anti-depressant drugs have been reported to decrease REM sleep in normal volunteers (Toyoda, 1964; Ritvo et. al., 1967; Zung, 1969; Hartmann, 1969; Carlsson et. al., 1969; Dunleavy et. al. 1971). It appears that REM sleep partially returns to normal levels if the drugs are administered for several weeks and, upon discontinuation, a large REM rebound is elicited (Dunleavy et. al., 1971).

6.8 Administration of catabolism inhibitors - monoamine oxidase inhibitors (MAOI)

Monoamine oxidase inhibitors (MAOI) inhibit the breakdown of 5-HT, DA and NA. It has been suggested (Grahaeme-Smith, 1974) that the normal synthesis of the monoamine neurotransmitters is greater than that required to fulfill the functional needs of the brain, and the excess is metabolised by intra-neural MAO and is thus inactivated. Therefore, if a MAOI is administered, the levels of the monoamine-neurotransmitters may exceed their intraneuronal storage capacity whereupon the excess will "spill out into functional activity" (Grahaeme-Smith 1971). This increase in functional activity is seen to be a consequence of the increased availability of the monoamines to the synaptic cleft.

The findings suggest that MAOI have a dose-dependent effect upon REM sleep in humans. Low doses of phenelzine (5-15 mg) increased REM sleep by 3 to 44 per cent (Wyatt, 1972) whereas high does of phenelzine (60-105 mg), isocarboxazid (60 mg); mebamazine (15 mg), and pargyline (60-100 mg) greatly decreased

REM sleep by 50-100 per cent. However, these large decreases in REM sleep were often preceded by small increases in REM sleep during the first few days of administration.

As these findings show a 2-way effect upon REM sleep by altering the metabolism of at least 3 neurotransmitters, it is difficult to incorporate these findings into the body of knowledge gained from the precursor-loading studies. It has been suggested (Pscheidt, 1964; Sourkes, 1972) that the MAOI have a greater influence on 5-HT, as compared to DA and NA, turnover because very little 5-HT is degraded by methylation compared to DA and NA (Snyder et. al. (1972).

Kupfer and Bowers (1972) have reported the appearance of a REM rebound following the discontinuation of a relatively high dose of phenelzine (45-60 mg/day).

### 6.9 Other factors affecting 5-HT metabolism

Nicotinamide has been found to increase brain 5-HT synthesis (Scherer and Kramer, 1972). Two papers report on the chronic effects of nicotinamide administration upon sleep in the mouse (Beaton et. al., 1972) and in the human (Robinson et. al., 1977).

The authors suggest that nicotinamide administration results in a build-up of nicotinamide adenine dinucleotide (NAD) which then, by feedback inhibition, decreases the activity of tryptophan pyrrolase. This enzyme catalyses the conversion of tryptophan to kynurenine and other compounds and its reduced activity would therefore allow more tryptophan to be available for metabolism to 5-HT in the brain.

Both these studies reported an increase in REM sleep only but, before this can be accepted as a consequence of increased 5-HT synthesis, the effects of nicotinamide administration will have to be assessed on the other neurotransmitter systems.

## 7. A CORRELATIONAL APPROACH TO THE STUDY OF SLEEP

This approach is diametrical to those discussed before. Instead of manipulating neurotransmitter metabolism and observing the resultant effect upon sleeping behaviour (a causal approach), the correlational

approach observes changes in neurotransmitter metabolism whilst manipulating sleep (i.e. REM deprivation) or during sleep. The advantage of this method is that the researcher can examine changes in neurotransmitter metabolism under normal physiological conditions during natural sleep. This approach relies heavily upon the availability of precise and sensitive techniques which can be used for monitoring biochemical and physiological activity.

## 7. CHANGES IN SEROTONIN METABOLISM DURING VARIOUS BEHAVIOURAL STATES

### 7.1 Sleep

Rodden et. al. (1973) compared the relative rate of decarboxylation of [$^{14}$C] carboxyl labelled tryptophan and tyrosine in human subjects during wakefulness and sleep.

The $^{14}CO_2$ specific activity of the subject's breath was monitored every 10 seconds over a 5 hour period after the intravenous administration of either labelled l-tryptophan or l-tyrosine. The results showed that there was no significant or consistent difference in overall $^{14}CO_2$ elimination after the administration of labelled tyrosine between sleep and wakefulness. However, $^{14}CO_2$ elimination was found to be reduced by an average of 50% during sleeping in the three subjects who received the labelled tryptophan.

It is very difficult to interpret this finding because the rate of $^{14}CO_2$ elimination following the administration of a labelled precursor is affected by several variables, apart from its rate of catabolism. These factors include the size of the metabolic pool in the blood and tissues, the blood flow, the rate of entry into cells and the rate of excretion of $CO_2$ from the blood bicarbonate pool. The authors established that there was no difference in the kinetics of $^{14}CO_2$ elimination between sleep and wakefulness after $^{14}C$ bicarbonate administration and, therefore, the reduction of $^{14}CO_2$ elimination after $^{14}C$ l-tryptophan administration cannot be attributed to changes in turnover of the bicarbonate pool. The variables blood flow and rate of entry into cells would not appear to be a cause of the reduced $^{14}CO_2$ elimination because this finding was not observed after the administration of $^{14}C$ l-tyrosine.

The authors suggest that the finding may be a result of an increase of the tryptophan pool during sleep although Wurtman et. al. (1968) has shown that, in normal humans, the plasma tryptophan (and also plasma tyrosine) levels decrease to a minimum during the middle of the night. However, it is not yet known whether the tissue pool changes during sleep.

Wyatt et. al. (1974) reported on the rate of 5-HT turnover during various behavioural states in four patients with presenile dementia by taking ventricular fluid samples during wakefulness, NREM and REM sleep periods. The authors found that the concentration of 5-HIAA varied slightly but, significantly, during the three states of consciousness. The levels of 5-HIAA were highest during NREM sleep and lowest during REM sleep. This suggests that 5-HT turnover, as compared to waking levels, is accelerated during SWS and decreased during REM sleep.

Chen et. al. (1974) have also studied the relationship between sleep and tryptophan. Their study measured free (FPT), bound (BPT) and total plasma tryptophan (TPT) levels throughout sleep, which was polygraphically monitored in six young female subjects. Plasma tryptophan can be divided into two categories depending on whether or not it is bound to albumin. As tryptophan has a great affinity for albumin, the BPT levels normally amount to about 90% of the TPT levels. No significant correlations were found between plasma tryptophan levels (either free, bound or total) and specific sleep stages. However, the mean FPT levels were positively correlated with REM sleep and negatively correlated with NREM sleep. The converse was found for the mean BPT levels, suggesting that an inverse relationship existed between the FPT and BPT levels. This relationship was found to be significant. The data also revealed a decrease in FPT levels, but not TPT levels, during the night.

Interpreting these findings, the authors concentrated upon the significance of the changes in FPT levels as opposed to the covarying BPT levels. This is because the free fraction is regarded as being more metabolically active than the bound fraction, due to the fact that only free amino-acids can pass through cell membranes. As

increased FPT levels have been associated with increased brain tryptophan levels leading to an increase in 5-HT levels or 5-HT turnover (Tagliamonte et. al., 1973; Knott and Curzon, 1972), the authors suggested that their findings of a positive correlation between FPT levels and REM sleep was evidence that 5-HT metabolism was closely linked with the REM sleep mechanism.

The authors noted that the FPT levels may be dependent upon, in part, the diurnal rhythm of plasma corticosteroids. Adrenocortical hormones have been shown to induce tryptophan pyrrolase activity, which is the first enzyme in the kynurenine pathway (Knox and Auerbach, 1955). Therefore, an increase in adrenocortical hormones would be expected to be associated with a decrease in FPT levels. Plasma corticoids have been found to increase from a minimum at approximately midnight to a maximum about six to nine hours later (Loraine and Bell, 1971) and this rhythm could explain the decrease of FPT levels found during the night by Chen et. al.

The findings by Chen et. al. (1974) do not appear to be consistent with those of Wyatt et. al. (1974). Whilst Chen et. al. provide evidence for a relationship between increased 5-HT synthesis and increased REM sleep, the other study suggests that 5-HT turnover is reduced during REM sleep. It could be argued that these apparent inconsistencies occur because the techniques used to monitor levels of metabolites (i.e. 5-HIAA) provide only gross information from time periods of minutes or even hours. During this time the serotonergic system may have effected a variety of neural functions. For example, 5-HT has been linked with the control of such diverse activities as sleep, body temperature and affective disorders. Therefore, the knowledge of the exact occasions when 5-HT is released into the synapse would be of great value, so that its release can be associated with specific behaviourial and electrophysiological events. Recordings of the firing rate of serotoninergic neurons enables occasions of 5-HT release to be recorded and correlated to ongoing behavioural events.

Thus, McGinty and Harper (1973) recorded the firing rate of serotoninergic neurons of the dorsal raphe nucleus in cats during

both sleep and wakefulness. Whereas the firing rate was stable during a variety of waking behaviours, it was markedly depressed during REM sleep and moderately depressed during SWS as compared to waking levels. The average firing rate during REM sleep was reduced by 92% from awake levels and 85% from NREM levels. Also, it was found that the seroton-inergic neurons exhibited a pause in firing immediately preceding the ocurrence of PGO spikes. This effect was also present in PCPA-treated cats providing further evidence for Dement's hypothesis linking 5-HT with the regulation of PGO spikes.

This finding of a large decrease in the firing rate of the raphe neurons during REM sleep is consistent with the reported decrease in 5-HIAA levels during this stage of sleep (Wyatt et. al., 1974). However, the data during NREM sleep is not consistent. This may well be due to the fact that one study used animals whereas the other used human patients. Another variable is the type of waking behaviour during which the "control" data were recorded, although McGinty and Harper found little change in the raphe firing rate with various waking behaviours.

Again, we are faced with the problem of cause or effect. Does the decreased firing rate of the raphe neurons induce REM sleep or vice-versa, The answer to this problem is unknown at present.

### 7.2 Sleep deprivation

Kuhn et. al. (1976) monitored FPT, BPT and TPT levels in six young subjects undergoing 120 hours of total sleep deprivation. They found that prolonged sleep deprivation was associated with a significant increase in FPT levels only. As free fatty acids (FFA) have a greater affinity for albumin than tryptophan (Curzon et. al., 1973), an increase in plasma FFA as a result of stress, including sleep deprivation (Kuhn et. al., 1965), could have mediated this reported increase in FPT levels during sleep deprivation. This could explain why Knott and Curzon (1972) have observed increases in FPT levels in the rat following immobilisation stress. The weak point of all sleep deprivation experiments is that the deprivation has to be carried out by stressful or pharmalogical means and, consequently, the experimenter cannot ascertain the effect of sleep deprivation per se.

Post et. al. (1976) collected lumbar CSF from 17 depressed patients who had been sleep deprived for one night. The results indicated that 26 to 34.5 hours of sleep deprivation had little effect upon brain serotonin metabolism. The 5-HIAA levels were increased in 12 patients but this did not constitute a significant increase for the group as a whole.

Hery et. al. (1970) observed an increase in the formation of [$^3$H] 5-HT from [$^3$H]tryptophan, both in vivo and in vitro, in the rat brain after they had been deprived of REM sleep for 96 hours by the flowerpot method. This method involves placing the animal upon a small platform surrounded by water. The area of the platform is just sufficient to allow the animal to remain dry during wakefulness and NREM sleep but not during REM sleep, when muscular tonus is lost and the animal falls into the water thereby effecting REM sleep deprivation.

This method is obviously very stressful and numerous experiments have not incorporated realistic control procedures, such as placing other animals in the same situation but on larger platforms so that they can enjoy REM sleep. The better experiments also control for the stress of body wetting by immersing the animals for similar periods of time as the REM sleep deprived animals. This study by Hery et. al. only incorporated the platform control and no control was made for water immersion which is possibly a major source of stress.

Stern et. al. (1971), using rats, found that both REM deprivation (by the flowerpot method) and stress (by immersion in cold water for 1 hour each day for 5 days) increased the rate of metabolism of 5-HT and NA to a similar extent when their catabolism was blocked by the MAOI, pargyline. These results suggest that REM deprivation by the flowerpot method is a chronic stressor and that the increased metabolism of 5-HT and NA is most likely a result of this stress rather than changes in REM sleep per se.

Radulovacki (1973) came to a similar conclusion. He found that the levels of 5-HIAA in the CSF of cats rose significantly in those who deprived of REM sleep and also in those cats who were placed upon a platform sufficiently large to allow for normal sleeping. This again

suggests that the increased turnover of 5-HT associated with REM deprivation is due to non-specific stress effects.

The effects of stressful stimuli upon brain serotonin synthesis have been studied by several investigators, using mainly rats and mice as their subjects. The results show general agreement that the synthesis is accelerated by these stimuli (Corrodi et. al., 1968; Bliss et. al., 1968; Reid et. al., 1968: Thiery et. al., 1968; Tagliamonte et. al., 1971; Weiss and Aghajanian, 1971; Curzon et. al., 1972; Bliss et. al., 1972; Bliss, 1973; Bourgoin et. al., 1973; Azmitia and McEwen, 1974; Palkovits et. al., 1976), although the actual serotonin levels have been found to be increased, decreased or unchanged. Examples of the stressor used include electric foot shock, immobilisation, formalin administration, heat and cold.

However, Cramer et. al. (1973) deprived intact and hypophysectomized rats of REM sleep and found that 5-HT turnover was increased by both groups. The effects of REM sleep deprivation were found to be more rapid and pronounced in the hypophysectomized rats and the authors concluded that the increase in 5-HT turnover was not due to pituitary-adrenal stimulation or "non-specific stress" as suggested by Stern et. al., (1971). As adrenalectomy has been shown not to modify 5-HT synthesis in the rat telencephalon and to reduce it in the brain stem (Azmitia and McEwen, 1974) it would appear that the increase in 5-HT turnover in the hypophysectonized rats during REM sleep deprivation was not a consequence of the functional inactivation of the adrenal cortex.

## APPENDIX V - DATA FOR STUDY IV

### 1. Experimental Details

| | |
|---|---|
| Number of subjects: | 8 |
| Diets studied: | Control |
| | High carbohydrate and fat, low protein (HCF) |
| | Low carbohydrate and fat, high protein (LCF) |
| Number of nights each diet studied: | 3 consecutive nights, plus one post-diet night |
| Sleep period: | 450 minutes for subjects 1, 2, 3, 5, 7, 8. |
| | 425 minutes for subject 4. |
| | 400 minutes for subject 6. |

### 2. Individual data

#### 2.1 Data for dietary periods

The data is presented for the whole night and for the first and second halves of the night. The duration of each stage of sleep or sleep variable, in minutes, is presented in the following format (as used in the computer analysis):

$S_1D_1N_1$ $S_1D_1N_2$ $S_1D_1N_3$ $S_1D_2N_1$ $S_1D_2N_2$ $S_1D_2N_3$ $S_1D_3N_1$ $S_1D_3N_2$ $S_1D_3N_3$

$S_2D_1N_1$

$S_3D_1N_1$

$S_4D_1N_1$

$S_5D_1N_1$

$S_6D_1N_1$

$S_7D_1N_1$

$S_8D_1N_1$

where

$S_{1-8}$ denotes the subjects

$D_1$, $D_2$, $D_3$ denote the control, HCF and LCF diets, repsectively

$N_{1-3}$ denotes the night of administration

2.2 Post-diet data (N=5)

The post-diet data is presented in the following format:

$S_1H$ $S_1L$ $S_1H_1$ $S_1L_1$ $S_1H_2$ $S_1L_2$

$S_2$

$S_3$

$S_5$

$S_8$

where

$S_1$, $S_2$, $S_3$, $S_5$ and $S_8$ are the five subjects

H,L denote the whole night data from the post-HCF and post-LCF diet nights, respectively.

$H_1$,$L_1$ denote the first half of the night data

$H_2$,$L_2$ denote the second half of the night data

STAGES 3+4 WHOLE NIGHT DATA (MINUTES)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 120 | 127 | 136 | 98 | 144 | 151 | 119 | 136 | 126 |
| 102 | 11 | 99 | 98 | 75 | 98 | 89 | 91 | 87 |
| 181 | 132 | 151 | 130 | 135 | 156 | 118 | 107 | 114 |
| 117 | 91 | 79 | 73 | 89 | 61 | 96 | 90 | 93 |
| 149 | 131 | 107 | 120 | 130 | 144 | 146 | 145 | 139 |
| 86 | 96 | 111 | 86 | 95 | 80 | 95 | 72 | 104 |
| 128 | 111 | 108 | 94 | 132 | 114 | 111 | 103 | 124 |
| 72 | 89 | 107 | 99 | 93 | 95 | 92 | 97 | 99 |
| STAGE 4 | | | | | | | | |
| 86 | 67 | 58 | 55 | 82 | 73 | 77 | 66 | 65 |
| 48 | 51 | 21 | 19 | 42 | 40 | 30 | 46 | 40 |
| 124 | 69 | 86 | 104 | 91 | 105 | 79 | 75 | 99 |
| 75 | 36 | 56 | 48 | 58 | 33 | 63 | 69 | 66 |
| 89 | 89 | 59 | 59 | 77 | 76 | 77 | 89 | 85 |
| 67 | 56 | 67 | 74 | 79 | 38 | 65 | 57 | 68 |
| 92 | 74 | 83 | 69 | 78 | 74 | 76 | 71 | 87 |
| 45 | 44 | 62 | 58 | 44 | 64 | 61 | 60 | 44 |
| STAGE 3 | | | | | | | | |
| 34 | 60 | 78 | 43 | 62 | 78 | 42 | 70 | 61 |
| 54 | 62 | 78 | 79 | 33 | 58 | 59 | 45 | 47 |
| 57 | 63 | 65 | 26 | 44 | 51 | 39 | 32 | 15 |
| 42 | 55 | 23 | 25 | 31 | 28 | 33 | 21 | 27 |
| 60 | 42 | 48 | 61 | 53 | 68 | 69 | 56 | 54 |
| 19 | 40 | 44 | 12 | 16 | 42 | 30 | 15 | 36 |
| 36 | 37 | 25 | 25 | 54 | 40 | 35 | 32 | 37 |
| 27 | 45 | 45 | 41 | 49 | 31 | 31 | 37 | 55 |
| STAGE REM | | | | | | | | |
| 81 | 67 | 83 | 120 | 115 | 92 | 96 | 84 | 99 |
| 88 | 89 | 84 | 105 | 108 | 98 | 90 | 98 | 101 |
| 80 | 69 | 92 | 104 | 98 | 93 | 91 | 90 | 74 |
| 103 | 92 | 84 | 120 | 109 | 127 | 96 | 102 | 99 |
| 103 | 97 | 121 | 93 | 120 | 108 | 85 | 103 | 105 |
| 92 | 80 | 85 | 112 | 89 | 107 | 63 | 98 | 93 |
| 81 | 63 | 80 | 100 | 89 | 115 | 81 | 92 | 96 |
| 71 | 69 | 67 | 100 | 78 | 83 | 83 | 66 | 74 |
| STAGE 2 | | | | | | | | |
| 213 | 197 | 205 | 188 | 176 | 194 | 196 | 180 | 190 |
| 194 | 220 | 218 | 217 | 249 | 233 | 227 | 209 | 214 |
| 171 | 223 | 197 | 191 | 205 | 180 | 229 | 211 | 235 |
| 178 | 212 | 208 | 200 | 208 | 204 | 195 | 194 | 194 |
| 166 | 206 | 206 | 225 | 184 | 180 | 194 | 182 | 190 |
| 188 | 196 | 180 | 173 | 194 | 200 | 194 | 210 | 178 |
| 154 | 192 | 165 | 193 | 179 | 194 | 210 | 209 | 207 |
| 272 | 250 | 237 | 222 | 254 | 244 | 251 | 257 | 258 |
| STAGE 1 | | | | | | | | |
| 35 | 59 | 26 | 44 | 16 | 13 | 39 | 50 | 34 |
| 46 | 29 | 49 | 30 | 17 | 21 | 44 | 52 | 46 |
| 17 | 26 | 10 | 24 | 12 | 17 | 12 | 41 | 27 |
| 27 | 30 | 54 | 32 | 19 | 32 | 38 | 39 | 38 |
| 24 | 16 | 16 | 12 | 16 | 15 | 25 | 20 | 16 |
| 30 | 30 | 23 | 29 | 22 | 11 | 48 | 20 | 25 |
| 60 | 76 | 95 | 52 | 47 | 27 | 41 | 44 | 23 |
| 34 | 42 | 36 | 29 | 25 | 26 | 16 | 30 | 19 |
| STAGE 0 | | | | | | | | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| 2 | 0 | 0 | 1 | 0 | 3 | 0 | 3 | 0 |
| 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| 4 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 |
| 27 | 8 | 2 | 11 | 3 | 0 | 7 | 2 | 0 |
| 1 | 0 | 3 | 0 | 0 | 2 | 8 | 0 | 0 |

STAGE 4 LATENCY

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 11 | 10 | 11 | 13 | 10 | 12 | 9 | 14 | 13 |
| 13 | 22 | 19 | 20 | 9 | 14 | 18 | 15 | 13 |
| 5 | 11 | 12 | 11 | 8 | 7 | 12 | 9 | 5 |
| 13 | 18 | 26 | 15 | 16 | 25 | 24 | 15 | 17 |
| 15 | 18 | 20 | 15 | 20 | 14 | 15 | 17 | 27 |
| 14 | 15 | 11 | 13 | 12 | 12 | 11 | 14 | 10 |
| 7 | 27 | 11 | 10 | 9 | 10 | 17 | 11 | 9 |
| 18 | 22 | 17 | 21 | 19 | 13 | 18 | 13 | 21 |

REM LATENCY

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 76 | 82 | 105 | 58 | 59 | 65 | 66 | 107 | 73 |
| 73 | 73 | 58 | 55 | 60 | 69 | 55 | 66 | 57 |
| 62 | 40 | 130 | 58 | 59 | 62 | 106 | 60 | 117 |
| 60 | 54 | 120 | 53 | 64 | 65 | 63 | 51 | 58 |
| 81 | 173 | 61 | 67 | 84 | 126 | 86 | 59 | 95 |
| 75 | 158 | 84 | 62 | 77 | 88 | 73 | 67 | 114 |
| 80 | 84 | 75 | 62 | 71 | 68 | 135 | 68 | 135 |
| 69 | 126 | 155 | 163 | 72 | 66 | 80 | 79 | 155 |

REM PERIODICITY

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 246 | 256 | 238 | 236 | 237 | 233 | 245 | 244 | 192 |
| 243 | 204 | 187 | 206 | 190 | 219 | 217 | 181 | 207 |
| 231 | 147 | 163 | 133 | 193 | 162 | 194 | 146 | 211 |
| 200 | 216 | 188 | 167 | 155 | 141 | 239 | 170 | 203 |
| 159 | 224 | 162 | 176 | 219 | 189 | 223 | 184 | 154 |
| 134 | 208 | 168 | 163 | 179 | 145 | 188 | 172 | 172 |
| 180 | 140 | 149 | 177 | 166 | 112 | 192 | 207 | 174 |
| 213 | 190 | 168 | 161 | 174 | 209 | 181 | 163 | 217 |

NUMBER OF REM PERIODS

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 3 | 3 | 3 | 4 | 4 | 4 | 5 | 3 | 4 |
| 4 | 4 | 5 | 4 | 4 | 4 | 4 | 5 | 4 |
| 4 | 5 | 5 | 6 | 5 | 5 | 4 | 6 | 4 |
| 4 | 4 | 4 | 5 | 5 | 5 | 4 | 4 | 4 |
| 5 | 3 | 5 | 5 | 4 | 4 | 4 | 5 | 5 |
| 5 | 3 | 4 | 4 | 4 | 5 | 4 | 4 | 4 |
| 4 | 6 | 5 | 5 | 5 | 5 | 4 | 5 | 4 |
| 4 | 4 | 4 | 4 | 5 | 5 | 5 | 5 | 3 |

STAGES 3+4 FIRST HALF DATA (MINUTES)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 84 | 78 | 101 | 69 | 104 | 88 | 99 | 96 | 99 |
| 86 | 105 | 76 | 78 | 74 | 70 | 81 | 88 | 78 |
| 139 | 79 | 133 | 91 | 94 | 111 | 106 | 102 | 85 |
| 73 | 56 | 55 | 65 | 79 | 49 | 73 | 62 | 66 |
| 113 | 91 | 92 | 104 | 105 | 113 | 102 | 113 | 103 |
| 86 | 82 | 83 | 85 | 95 | 63 | 89 | 70 | 83 |
| 101 | 70 | 90 | 77 | 90 | 73 | 90 | 79 | 101 |
| 50 | 72 | 94 | 92 | 75 | 59 | 68 | 77 | 75 |
| STAGE 4 | | | | | | | | |
| 60 | 45 | 51 | 35 | 52 | 44 | 64 | 47 | 56 |
| 42 | 51 | 21 | 19 | 42 | 34 | 30 | 46 | 40 |
| 103 | 58 | 73 | 74 | 79 | 69 | 79 | 75 | 75 |
| 48 | 20 | 43 | 48 | 58 | 31 | 48 | 47 | 39 |
| 73 | 64 | 52 | 54 | 66 | 61 | 69 | 74 | 63 |
| 67 | 56 | 61 | 74 | 79 | 37 | 65 | 57 | 68 |
| 77 | 51 | 72 | 65 | 63 | 47 | 66 | 58 | 81 |
| 40 | 42 | 62 | 58 | 43 | 41 | 48 | 51 | 44 |
| STAGE 3 | | | | | | | | |
| 24 | 33 | 50 | 34 | 52 | 44 | 35 | 49 | 43 |
| 44 | 54 | 55 | 59 | 32 | 36 | 51 | 42 | 38 |
| 36 | 21 | 60 | 17 | 15 | 42 | 27 | 27 | 10 |
| 25 | 36 | 12 | 17 | 21 | 18 | 25 | 15 | 27 |
| 40 | 27 | 40 | 50 | 39 | 52 | 33 | 39 | 40 |
| 19 | 26 | 22 | 11 | 16 | 26 | 24 | 13 | 15 |
| 24 | 19 | 18 | 12 | 27 | 26 | 24 | 21 | 20 |
| 10 | 30 | 32 | 34 | 32 | 18 | 20 | 26 | 31 |
| STAGE REM | | | | | | | | |
| 43 | 25 | 31 | 51 | 37 | 43 | 42 | 22 | 33 |
| 34 | 27 | 13 | 43 | 32 | 36 | 29 | 16 | 34 |
| 33 | 39 | 20 | 44 | 28 | 28 | 32 | 35 | 31 |
| 49 | 55 | 20 | 37 | 23 | 32 | 56 | 21 | 38 |
| 19 | 46 | 28 | 29 | 44 | 11 | 57 | 33 | 95 |
| 20 | 33 | 26 | 27 | 41 | 46 | 24 | 18 | 19 |
| 20 | 29 | 24 | 21 | 19 | 48 | 31 | 16 | 17 |
| 28 | 21 | 6 | 24 | 22 | 41 | 28 | 12 | 13 |
| STAGE 2 | | | | | | | | |
| 86 | 102 | 89 | 84 | 82 | 89 | 75 | 90 | 86 |
| 90 | 84 | 122 | 93 | 117 | 111 | 95 | 101 | 93 |
| 52 | 98 | 72 | 84 | 102 | 84 | 85 | 82 | 96 |
| 74 | 98 | 120 | 98 | 103 | 115 | 73 | 115 | 79 |
| 84 | 87 | 103 | 89 | 69 | 92 | 56 | 72 | 23 |
| 92 | 78 | 83 | 80 | 54 | 83 | 72 | 106 | 93 |
| 61 | 88 | 89 | 109 | 96 | 94 | 100 | 115 | 100 |
| 130 | 118 | 103 | 100 | 116 | 116 | 127 | 134 | 129 |
| STAGE 1 | | | | | | | | |
| 12 | 20 | 4 | 21 | 2 | 5 | 9 | 17 | 7 |
| 11 | 9 | 14 | 11 | 2 | 8 | 20 | 20 | 19 |
| 2 | 9 | 0 | 6 | 1 | 2 | 2 | 6 | 13 |
| 17 | 4 | 18 | 13 | 8 | 16 | 11 | 15 | 30 |
| 9 | 1 | 2 | 3 | 7 | 9 | 10 | 7 | 4 |
| 2 | 7 | 8 | 8 | 10 | 6 | 15 | 6 | 5 |
| 23 | 35 | 20 | 15 | 19 | 10 | 4 | 14 | 7 |
| 16 | 14 | 22 | 9 | 12 | 9 | 2 | 2 | 8 |
| STAGE 0 | | | | | | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| 20 | 3 | 2 | 3 | 1 | 0 | 0 | 1 | 0 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

STAGES 3+4 SECOND HALF DATA (MINUTES)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 36 | 49 | 35 | 29 | 40 | 63 | 20 | 40 | 27 |
| 16 | 8 | 23 | 20 | 1 | 28 | 8 | 3 | 9 |
| 42 | 63 | 18 | 39 | 41 | 45 | 12 | 5 | 29 |
| 44 | 35 | 24 | 8 | 10 | 12 | 23 | 28 | 27 |
| 36 | 40 | 15 | 16 | 25 | 31 | 44 | 32 | 36 |
| 0 | 14 | 28 | 1 | 0 | 17 | 6 | 2 | 21 |
| 27 | 41 | 18 | 17 | 42 | 41 | 21 | 24 | 23 |
| 22 | 17 | 13 | 7 | 18 | 36 | 24 | 20 | 24 |
| STAGE 4 | | | | | | | | |
| 26 | 22 | 7 | 20 | 30 | 29 | 13 | 19 | 9 |
| 6 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 |
| 21 | 11 | 13 | 30 | 12 | 36 | 0 | 0 | 24 |
| 27 | 16 | 13 | 0 | 0 | 2 | 15 | 22 | 27 |
| 16 | 25 | 7 | 5 | 11 | 15 | 8 | 15 | 22 |
| 0 | 0 | 6 | 0 | 0 | 1 | 0 | 0 | 0 |
| 15 | 23 | 11 | 4 | 15 | 27 | 10 | 13 | 6 |
| 5 | 2 | 0 | 0 | 1 | 23 | 13 | 9 | 0 |
| STAGE 3 | | | | | | | | |
| 10 | 27 | 28 | 9 | 10 | 34 | 7 | 21 | 18 |
| 10 | 8 | 23 | 20 | 1 | 22 | 8 | 3 | 9 |
| 21 | 42 | 5 | 9 | 29 | 9 | 12 | 5 | 5 |
| 17 | 19 | 11 | 8 | 10 | 10 | 8 | 6 | 0 |
| 20 | 15 | 8 | 11 | 14 | 16 | 36 | 64 | 65 |
| 0 | 14 | 22 | 1 | 0 | 16 | 6 | 2 | 21 |
| 12 | 18 | 7 | 13 | 27 | 14 | 11 | 11 | 17 |
| 17 | 15 | 13 | 7 | 17 | 13 | 11 | 11 | 24 |
| STAGE REM | | | | | | | | |
| 38 | 42 | 52 | 69 | 78 | 49 | 54 | 62 | 66 |
| 54 | 62 | 71 | 62 | 76 | 62 | 61 | 82 | 67 |
| 47 | 30 | 72 | 60 | 70 | 65 | 59 | 55 | 43 |
| 54 | 37 | 64 | 83 | 86 | 95 | 40 | 81 | 61 |
| 84 | 51 | 93 | 64 | 76 | 97 | 28 | 70 | 10 |
| 72 | 47 | 59 | 95 | 48 | 61 | 39 | 80 | 74 |
| 61 | 34 | 36 | 79 | 70 | 67 | 50 | 76 | 79 |
| 43 | 48 | 61 | 76 | 56 | 42 | 55 | 54 | 61 |
| STAGE 2 | | | | | | | | |
| 127 | 95 | 116 | 104 | 94 | 105 | 121 | 90 | 104 |
| 104 | 136 | 96 | 124 | 132 | 122 | 132 | 108 | 121 |
| 119 | 125 | 127 | 107 | 103 | 96 | 144 | 129 | 139 |
| 104 | 114 | 88 | 102 | 105 | 89 | 122 | 79 | 116 |
| 82 | 119 | 103 | 136 | 115 | 88 | 138 | 110 | 167 |
| 96 | 118 | 97 | 93 | 140 | 117 | 122 | 104 | 85 |
| 93 | 104 | 76 | 84 | 83 | 100 | 110 | 94 | 107 |
| 142 | 132 | 134 | 122 | 138 | 128 | 124 | 123 | 129 |
| STAGE 1 | | | | | | | | |
| 23 | 39 | 22 | 23 | 14 | 8 | 30 | 33 | 27 |
| 35 | 20 | 35 | 19 | 15 | 13 | 24 | 32 | 27 |
| 15 | 17 | 10 | 18 | 11 | 15 | 10 | 35 | 14 |
| 10 | 26 | 36 | 19 | 11 | 16 | 27 | 24 | 9 |
| 15 | 15 | 14 | 9 | 9 | 6 | 15 | 13 | 12 |
| 28 | 23 | 15 | 21 | 12 | 5 | 33 | 14 | 20 |
| 37 | 41 | 75 | 37 | 28 | 17 | 37 | 30 | 16 |
| 18 | 28 | 14 | 20 | 13 | 17 | 14 | 28 | 11 |
| STAGE 0 | | | | | | | | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| 2 | 0 | 0 | 1 | 0 | 3 | 0 | 3 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| 4 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 5 | 0 | 8 | 2 | 0 | 7 | 1 | 0 |
| 0 | 0 | 3 | 0 | 0 | 2 | 8 | 0 | 0 |

STAGES 3+4 POST-DIET DATA (MINUTES)

| | | | | | |
|---|---|---|---|---|---|
| 135 | 136 | 107 | 88 | 28 | 48 |
| 91 | 73 | 90 | 63 | 1 | 10 |
| 102 | 84 | 92 | 76 | 10 | 8 |
| 139 | 124 | 113 | 97 | 26 | 27 |
| 116 | 111 | 76 | 88 | 40 | 23 |

STAGE 4

| | | | | | |
|---|---|---|---|---|---|
| 83 | 93 | 73 | 59 | 10 | 34 |
| 47 | 26 | 47 | 24 | 0 | 2 |
| 74 | 65 | 71 | 57 | 3 | 8 |
| 93 | 83 | 90 | 58 | 3 | 25 |
| 76 | 60 | 56 | 48 | 20 | 12 |

STAGE 3

| | | | | | |
|---|---|---|---|---|---|
| 52 | 43 | 34 | 29 | 18 | 14 |
| 44 | 47 | 43 | 39 | 1 | 8 |
| 28 | 19 | 21 | 19 | 7 | 0 |
| 46 | 41 | 23 | 39 | 23 | 2 |
| 40 | 51 | 20 | 40 | 20 | 11 |

STAGE REM

| | | | | | |
|---|---|---|---|---|---|
| 93 | 107 | 29 | 34 | 64 | 73 |
| 106 | 89 | 36 | 23 | 70 | 66 |
| 77 | 49 | 25 | 10 | 52 | 39 |
| 81 | 122 | 25 | 29 | 56 | 93 |
| 63 | 102 | 25 | 30 | 38 | 72 |

STAGE 2

| | | | | | |
|---|---|---|---|---|---|
| 183 | 179 | 74 | 98 | 109 | 81 |
| 214 | 261 | 89 | 128 | 125 | 133 |
| 246 | 286 | 106 | 156 | 140 | 130 |
| 211 | 163 | 82 | 82 | 129 | 81 |
| 190 | 224 | 117 | 106 | 73 | 118 |

STAGE 1

| | | | | | |
|---|---|---|---|---|---|
| 39 | 27 | 15 | 5 | 24 | 22 |
| 39 | 27 | 10 | 11 | 29 | 16 |
| 21 | 13 | 2 | 5 | 19 | 8 |
| 19 | 39 | 5 | 15 | 14 | 24 |
| 13 | 13 | 7 | 1 | 6 | 12 |

STAGE 0

| | | | | | |
|---|---|---|---|---|---|
| 0 | 1 | 0 | 0 | 0 | 1 |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 18 | 0 | 3 | 4 | 15 |
| 0 | 2 | 0 | 2 | 0 | 0 |
| 68 | 0 | 0 | 0 | 68 | 0 |

STAGE 4 LATENCY

| | |
|---|---|
| 11 | 12 |
| 15 | 23 |
| 9 | 9 |
| 13 | 32 |
| 13 | 15 |

REM LATENCY

| | |
|---|---|
| 58 | 61 |
| 126 | 63 |
| 105 | 43 |
| 81 | 74 |
| 69 | 62 |

REM PERIODICITY

| | |
|---|---|
| 134.5 | 103.0 |
| 118.5 | 104.5 |
| 134.5 | 112.0 |
| 97.5 | 90.0 |
| 94.5 | 78.5 |

NUMBER OF REM PERIODS

| | |
|---|---|
| 4 | 5 |
| 3 | 4 |
| 3 | 5 |
| 5 | 4 |
| 4 | 5 |

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# Exercise and human sleep

IT has been proposed that slow wave sleep (SWS) in humans is associated with somatic restitution[1], and findings of increased plasma somatotrophin during SWS[2], and of the greater amounts of SWS following exercise[3,4] support this proposal. Others have, however, failed to show any exercise effects of sleep[5,6]. Exercise has been found to increase sleep somatotrophin levels, but not SWS[7]. These contradictory findings may in part be attributed to the large interstudy variations of administered exercise and fitness of subjects (in no study has exercise been standardised against individual work capacity); the time of day of exercise; the need for further qualification of SWS, defined[8] here as sleep stages 3 and 4, respectively containing 20–50% by time of delta activity, and more than 50% delta activity. Up to 20% delta activity is included in the non-SWS stage 2. We have investigated the effects of the time of day of individually standardised amounts of exercise on subsequent sleep in which stage 2 was subdivided further between 10% and 20% delta activity (2ii).

Eight healthy male subjects (18–22 yr), of average build and fitness, were assessed for individual maximum aerobic power during steady-state exercise on a bicycle ergometer by the Nomogram method[9]. From the maximum work capacity calculated, each subject was prescribed a workload 45% of this capacity. It was further established that this load could be maintained without undue stress for 85 min, with a 15 min break at the half way point. In the main study the exercise was performed by each subject on two separate days, once in the morning, and once in the afternoon. Electroencephalograms were obtained throughout the sleeping period for each exercise night, and for a following 'carryover' night. There were initial adaptation nights and two baseline nights. Subjects were measured in pairs with one performing morning exercise and the other evening exercise on any one day. Diet, naps, extraneous exercise and alcohol intake were controlled. Sleep records were independently scored into the recognised sleep stages[8] and stage 2ii. Group data were compiled for the sleep variables shown in Tables 1 and 2. Related $t$ tests were used to compare the data for each of the experimental nights with the average baseline values.

Tables 1 and 2 show that there are few significant findings. Whole-night percentages of each sleep stage and parameter, including 2ii, during all experimental nights remained within baseline limits. During the first half of the afternoon exercise night, and particularly before the first rapid eye movement (REM) period, stage 3 showed a significant increase. Although stages 4 and 2ii before the first REM period tended to show increases, they did not individually reach significance. On this night three subjects missed their normally expected first REM period, producing an unusually long delay to a first REM period and enabling more SWS to occur before the first REM

**Table 1** Group means (%) for sleep stages

| | Baseline | Morning exercise | | Afternoon exercise | |
|---|---|---|---|---|---|
| | | Recovery night | Carryover night | Recovery night | Carryover night |
| Whole night | | | | | |
| Stages 0 + 1 | 5.2 | 6.2 | 5.7 | 5.4 | 4.8 |
| 2ii | 9.9 | 8.8 | 8.9 | 11.7 | 9.6 |
| 3 | 9.5 | 9.7 | 10.8 | 10.8 | 8.5 |
| 4 | 18.2 | 19.1 | 17.2 | 17.2 | 16.8 |
| 3 + 4 | 27.7 | 28.8 | 27.9 | 27.9 | 25.3 |
| 2ii + 3 + 4 | 37.6 | 37.6 | 36.8 | 39.6 | 34.9 |
| REM | 23.5 | 25.2 | 24.1 | 23.7 | 24.4 |
| First half | | | | | |
| Stages 0 + 1 | 2.8 | 3.2 | 3.8 | 2.7 | 2.6 |
| 2ii | 11.6 | 8.0† | 10.0 | 14.6 | 12.2 |
| 3 | 13.2 | 11.5 | 13.6 | 16.2† | 11.0 |
| 4 | 31.6 | 35.3 | 26.1 | 29.3 | 30.8 |
| 3 + 4 | 45.1 | 46.7 | 39.7 | 45.3 | 41.9 |
| 2ii + 3 + 4 | 56.7 | 54.7 | 49.7* | 59.9 | 54.1 |
| REM | 13.7 | 15.9 | 16.3 | 13.2 | 14.5 |
| Second half | | | | | |
| Stages 0 + 1 | 7.8 | 9.2 | 7.6 | 8.2 | 7.1 |
| 2ii | 8.2 | 9.5 | 8.1 | 8.8 | 7.1 |
| 3 | 5.7 | 8.0 | 7.9 | 5.3 | 6.1 |
| 4 | 4.9 | 2.8 | 8.2 | 5.1 | 2.8 |
| 3 + 4 | 10.4 | 10.9 | 16.1 | 10.8 | 8.7 |
| 2ii + 3 + 4 | 18.6 | 20.4 | 24.3 | 19.6 | 15.8 |
| REM | 33.3 | 34.4 | 31.8 | 34.7 | 34.4 |

*Significant at 0.05 level (two-tailed).
†Significant at 0.01 level (two-tailed).

**Table 2** Group means for REM parameters

| Time (min) before first REM period | Baseline | Morning exercise | | Afternoon exercise | |
|---|---|---|---|---|---|
| | | Recovery night | Carryover night | Recovery night | Carryover night |
| Stages 0 + 1 | 1.0 | 0.7 | 1.4 | 2.1 | 1.7 |
| 2ii | 10.6 | 5.4* | 7.8 | 16.6 | 9.3 |
| 3 | 14.4 | 10.7 | 12.5 | 19.6* | 11.6 |
| 4 | 47.9 | 50.2 | 42.0 | 56.2 | 40.0 |
| 3 + 4 | 62.1 | 60.9 | 54.5 | 75.7 | 51.6 |
| 2ii + 3 + 4 | 72.7 | 66.3 | 62.3 | 92.3* | 60.9 |
| REM latency | 91.9 | 79.4 | 79.4 | 112.9 | 89.5 |
| First REM period length | 14.4 | 11.9 | 9.0 | 15.8 | 10.9 |
| REM periodicity | | | | | |
| First period | 88.4 | 87.9 | 77.2 | 77.1 | 81.7 |
| Mean of first and second periods | 92.3 | 92.0 | 90.4 | 89.9 | 89.6 |

*Significant at 0.05 level (two-tailed).

period. These three REM period delays seem to be mainly responsible for the longer, but non-significant, REM latency. There are no significant effects for any parameter during the afternoon exercise carryover night. Stage 2ii shows two related significant decreases on the morning exercise night, one before the first REM period and the other for the first half of the night. Any possible loss of delta activity here, however, may have been made up in the slight increase of stage 4, the more substantial delta activity sleep, during the first half of the night. On the morning exercise carryover night, summated delta activity stages 2ii–3–4 showed a significant decrease during the first half of the night, which seems to be reciprocated by a significant increase during the second half. This seems to be a real temporal displacement, rather than an artefact of the division line of sleep into halves. The reason for this is not known.

Our findings seem to show a time of day effect of exercise on recovery sleep. The main SWS delta activity increase was for stage 3 during the first half of the night following afternoon exercise, and this seems to be partially counterbalanced by a non-significant decrease during the second half of the night, resulting in no significant overall changes. If SWS reflects enhanced protein synthesis and body restitution[1], then in view of the high workload imposed, more substantial changes in SWS might have been expected. It must be concluded that if a heavy, but tolerable, workload is imposed early in the day then ensuing wakefulness is sufficient for recovery. If this exercise is given later in the day, however, then ensuing wakefulness may not be adequate for recovery, and some of the recovery process may intrude into the earlier part of sleep and be reflected in the sleep EEG.

J. A. Horne
J. M. Porter

*Department of Human Sciences,*
*Loughborough University,*
*Loughborough,*
*Leicestershire, UK*

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# TIME OF DAY EFFECTS WITH STANDARDIZED EXERCISE UPON SUBSEQUENT SLEEP

J.A. HORNE and J.M. PORTER

*Department of Human Sciences, University of Technology, Loughborough, Leicestershire (Great Britain)*

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The functions of slow wave sleep (SWS) stages 3 and 4 in humans have attracted less attention and debate than the functions of REM. It is considered by many sleep researchers, for example, Oswald (1969) and Hartman (1973), that SWS is concerned with the processes of tissue regeneration and general body restitution. Findings by Sassin et al. (1969) of increased levels of plasma somatotrophin during SWS, and the greater amounts of SWS following exercise reported by Baekeland and Lasky (1966), and Zloty et al. (1973), can be given in support of this proposal. On the other hand, Hauri (1968), Baekeland (1970) and Zir et al. (1971) have failed to show these SWS increases. In a recent qualification, Adamson et al. (1974) found that the levels of plasma somatotrophin during sleep following strenuous daytime exercise increased significantly above normal sleep levels. But SWS remained unchanged. Ryback and Lewis (1971), in a more unusual study, reduced exercise by confining subjects to 5 weeks of bed rest. Whilst the experimental group was allowed no exercise in bed, a similarly confined control group did regular exercise. Interestingly, the no-excercise group showed significant increases in SWS, whereas the exercise-in-bed group did not. This finding may in part be due to the low SWS levels obtained from the one baseline night which was recorded prior to bed rest.

Although from all these findings it appears that there is equivocality on the effects of exercise upon SWS, the evidence is amassing for a negative effect. However, these studies are difficult to equate. The exercise given to subjects varies considerably, with exhaustive and stressful loads given by Hauri (1968) on the one hand, and the more moderate levels given by Zir et al. (1971) on the other. In no study is the exercise standardized against each subject's work capacity. Furthermore, the studies of Baekeland and Lasky (1966) and Zloty et al. (1973) use very fit individuals, whereas Hauri (1968) and Ryback and Lewis (1971) use subjects not selected for high fitness levels. Thus, the substantial variation in maximum aerobic power of the subjects used in these studies, and the differences in work load imposed, must contribute to the equivocality of findings.

A second methodological variable is the time of day of exercise. If, according to Webb and Agnew (1971), SWS is essentially a circadian phenomenon, and if there is a relationship between exercise and SWS, then time of day of exercise might be of importance. Baekeland and Lasky (1966) compared afternoon and evening exercise, and found that SWS was only increased after the former. The authors suggested that the evening exercise was a stressor and produced a more disturbed sleep and CNS activation, which opposed any possible increase in SWS.

Further quantification of SWS itself may provide qualification of exercise and SWS. The present standardized method of sleep stage scoring described by Rechtschaffen and Kales (1968) divides SWS into stage 3, containing between 20% and 50% by time of delta activity, and stage 4, containing greater than 50% by time of delta. Epochs containing less than 20% delta are classified as stage 2. Thus, an

unknown amount of the delta activity outside the umbrella of SWS may be missed. Division of stage 2 into a substage containing between, for example, 10% and 20% delta activity would be particularly desirable for studies concerned with SWS.

The present study investigated time-of-day effects of standardized exercise upon subsequent sleep. Stage 2 was additionally quantified in respect of delta activity.

## Method

Eight healthy male, average build undergraduates, within the age group 18—22 years, performed the study. Subjects all smoked less than five cigarettes per day, were medication free, and slept normally.

An initial plot study was conducted in order to: (i) calculate the work capacity of each subject, and to determine a fixed percentage of this capacity, which could be prescribed as an individual workload; (ii) determine a length of time, constant for all subjects, for how long this load could be maintained without great discomfort. Work capacity was calculated by the sub-maximal exercise nomogram method of Astrand (1960). The bicycle ergometer method was selected as it is independent of body weight. Subjects pedalled on a bicycle ergometer set at a fixed work load for 6 min and had their heart rate (HR) measured, by means of EKG, during the last minute. For each subject, this task was performed at three different work loads, with adequate rest between. The three HRs, which were within the range 130—175 beats/min, were individually used to calculate a maximum aerobic power ($VO_2$ max). From these, an average $VO_2$ max was assessed for each subject and, finally, the maximum work load. During measurements, environmental temperature and humidity were kept constant. The work load chosen for the main study was 45% of each subject's maximal work capacity. The duration of exercise was determined by requiring all subjects to work at the prescribed load until a break was urgently required. A 15 min break was given and exercise was continued until subjects felt exhausted. The break allowed subjects to do more work over the total exercise period. From these results it was decided that for the main study, using the bicycle ergometer, the optimal exercise duration would consist of two 42 min periods, with a 15 min break in between. The prescribed work loads set on the ergometer for the group of subjects ranged from 90 to 150 watts, with a mean of 126 watts, at a pedal rate of 60 revolutions/min. An experimenter ensured that this rate was strictly adhered to. During exercising, subjects wore sports kit, and had the saddle height adjusted to their specific requirements.

For the main study, subjects were taken in pairs and underwent 9 nights in a sleep laboratory. All-night sleep records were taken, using the standardized techniques of EEG, EOG and EMG recording for sleep, described by Rechtschaffen and Kales (1968). Electrode placements were as follows: A1, A2, C3, C4, lateral to each eye, and left and right EMG located at the submental area medial to the mandible. A ground electrode was used. Electrodes were silver/silver chloride discs attached by "colloidon". EKG paste was used for electrode—skin contact. For each subject the following montage was used: Left eye, Al; right eye, A1; C3, A2; C4, A1; and left and right EMG. Recordings were made at a paper speed of 15 mm/min on a Grass model 78 12-channel EEG machine.

Each experimental run of two subjects was spread over 2 weeks, with the interposed weekend free. Weekdays 1 and 2 of the first week, and weekday 1 of the second week acted as adaptation nights, with full electrode attachment, but no recording. Weekdays 3 of week 1 and 2 of week 2 were used as baseline recording nights. Exercise, followed by a sleep recovery night, was given on weekdays 4 and 3 of weeks 1 and 2 respectively. Exercise carryover nights were recorded on the respective weekdays 5 and 4. Subjects performed their prescribed exercise load either between 10.00 and 12.00 h (a.m.) or 16.00—18.00 h (p.m.).

On any one exercise day one subject of each pair performed a.m. exercise, and the other p.m. exercise. For the second exercise day each subject underwent the alternative exercise condition.

For all days, subjects were required to go about their normal daytime activities. They were instructed to refrain from: (i) any extra-experiment heavy exercise; (ii) changes in diet; (iii) evening tea or coffee; (iv) all alcohol; (v) naps.

The first 7.5 h of each sleep record were used for analysis. This length was the lowest common denominator in natural sleep length for the group. Under home circumstances all subjects reported sleeping between 7.5 h and 8 h 15 min per night. For each night in the laboratory, subjects were allowed up to 8 h 45 min of sleep before being awoken. Sleep records were divided up into 1 min epochs and were independently scored by two experimenters, according to the criteria of the APSS standards for sleep stage scoring (Rechtschaffen and Kales 1968). In addition, stage 2 was scored into two subdivisions: stage 2i, containing less than 10% by time delta activity, and stage 2ii, containing between 10% and 20% by time delta activity.

For each night, group data were compiled for the sleep variables described in Tables I and II. For each of these variables the recovery and carryover nights following both exercise

TABLE I

Group means for sleep stages.

| | Baseline | a.m. exercise | | p.m. exercise | |
|---|---|---|---|---|---|
| | | Recovery night | Carryover night | Recovery night | Carryover night |
| *Whole night* (%) | | | | | |
| Stages 0+1 | 5.2 | 6.2 | 5.7 | 5.4 | 4.8 |
| 2(ii) | 9.9 | 8.8 | 8.9 | 11.7 | 9.6 |
| 3 | 9.5 | 9.7 | 10.8 | 10.8 | 8.5 |
| 4 | 18.2 | 19.1 | 17.2 | 17.2 | 16.8 |
| 3+4 | 27.7 | 28.8 | 27.9 | 27.9 | 25.3 |
| 2(ii)+3+4 | 37.6 | 37.6 | 36.8 | 39.6 | 34.9 |
| REM | 23.5 | 25.2 | 24.1 | 23.7 | 24.4 |
| *First half* (%) | | | | | |
| Stages 0+1 | 2.8 | 3.2 | 3.8 | 2.7 | 2.6 |
| 2(ii) | 11.6 | 8.0** | 10.0 | 14.6 | 12.2 |
| 3 | 13.2 | 11.5 | 13.6 | 16.2** | 11.0 |
| 4 | 31.6 | 35.3 | 26.1 | 29.3 | 30.8 |
| 3+4 | 45.1 | 46.7 | 39.7 | 45.3 | 41.9 |
| 2(ii)+3+4 | 56.7 | 54.7 | 49.7* | 59.9 | 54.1 |
| REM | 13.7 | 15.9 | 16.3 | 13.2 | 14.5 |
| *Second half* (%) | | | | | |
| Stages 0+1 | 7.8 | 9.2 | 7.6 | 8.2 | 7.1 |
| 2(ii) | 8.2 | 9.5 | 8.1 | 8.8 | 7.1 |
| 3 | 5.7 | 8.0 | 7.9 | 5.3 | 6.1 |
| 4 | 4.9 | 2.8 | 8.2 | 5.1 | 2.8 |
| 3+4 | 10.4 | 10.9 | 16.1 | 10.8 | 8.7 |
| 2(ii)+3+4 | 18.6 | 20.4 | 24.3** | 19.6 | 15.8 |
| REM | 33.3 | 34.4 | 31.8 | 34.7 | 34.4 |

* Significant at 0.05 level (2-tailed).
** Significant at 0.01 level (2-tailed).

TABLE II

Group means for REM parameters.

| | Baseline | a.m. exercise | | p.m. exercise | |
|---|---|---|---|---|---|
| | | Recovery night | Carryover night | Recovery night | Carryover night |
| *Number of minutes prior to first REMP* | | | | | |
| Stages 0+1 | 1.0 | 0.7 | 1.4 | 2.1 | 1.7 |
| 2 ii | 10.6 | 5.4* | 7.8 | 16.6 | 9.3 |
| 3 | 14.4 | 10.7 | 12.5 | 19.6* | 11.6 |
| 4 | 47.9 | 50.2 | 42.0 | 56.2 | 40.0 |
| 3+4 | 62.1 | 60.9 | 54.5 | 75.7 | 51.6 |
| 2 ii+3+4 | 72.7 | 66.3 | 62.3 | 92.3* | 60.9 |
| *REM latency (min)* | 91.9 | 79.4 | 79.4 | 112.9 | 89.5 |
| 1st REMP length | 14.4 | 11.9 | 9.0 | 15.8 | 10.9 |
| REM periodicity | | | | | |
| 1st period | 88.4 | 87.9 | 77.2 | 77.1 | 81.7 |
| Mean of 1st & 2nd period | 92.3 | 92.0 | 90.4 | 89.9 | 89.6 |

* Significant at 0.05 level (2-tailed).

conditions were individually compared with the baseline values, by means of related "t tests".

## Results

From Tables I and II it can be seen that there are few significant findings. Whole night percentages of each sleep stage and parameter, including stage 2ii, during all experimental nights remain within baseline values. However, for the first half of the night following p.m. exercise, stage 3 shows a significant increase to the $P = 0.01$ level and, related to this, stage 3 prior to the first REM period (REMP) is significantly increased to $P = 0.05$. Although stages 4 and 2ii prior to this first REMP tend to show increases above baseline, these changes do not individually reach significant levels, but collectively, with stage 3, reach an increase significant to $P = 0.05$. Although 2ii does not show significant changes per se, some subjects display increases during the p.m. exercise night. The p.m. exercise carryover night produces no significant results for any parameter.

Stage 2ii on the a.m. exercise recovery night shows two related significant decreases. One, at the $P = 0.01$ level for the first half of the night, and the other at the $P = 0.05$ level prior to the first REMP. On the a.m. exercise carryover night, summated delta activity stages 2ii + 3 + 4 display a significant decrease at the $P = 0.05$ level during the first half of the night. This reduction appears to be made up with a significant increase at the $P = 0.01$ level during the second half of the night.

## Discussion

The findings from this study appear to show time-of-day effects with daytime exercise and subsequent sleep. Following a.m. exercise, the decrease in stage 2ii during the first half of the recovery night is the only significant finding. Any reduction in the comparatively small amount of delta activity to be found in

this sub-stage may have been made up in the non-significant increase in stage 4 found during the same half of the night. The finding of a significant decrease followed by a significant increase of summated stages 2ii + 3 + 4 during the respective first and second halves of the a.m. exercise carryover night, may be in part due to the artificiality of dividing sleep up into first and second halves of the night. However, the slight decreases of most sleep stages prior to the first REMP, suggests that there is a real time displacement of these summated sleep stages. The reason for this finding is not known. Interestingly, the p.m. exercise carryover night shows no significant changes whatsoever.

The p.m. exercise recovery night does produce a significant increase in stage 3 during the first half of the night. This stage also shows a slight decrease during the second half of the night, which may account for the overall lack of significant change for stage 3 and other SWS stages for the whole night. It must be noted that on this recovery night three out of the eight subjects missed their normally expected first REMP, producing an unusually long delay to a first REMP and enabling more SWS to occur prior to a first REMP. This may be the reason for the significant increase of summated 2ii + 3 + 4 prior to the first REMP. These long REMP delays for the three subjects are mostly responsible for the long REM latency of 112.9 min for this night, and the large standard deviation makes this delay not significantly different from the baseline value of 91.9 min.

Although subjects were allowed up to 8 h 45 min sleep, no subject ever appeared to require this amount of sleep for any night during the study. Total sleep time was not affected by exercise.

No doubt the level of work load incurred during exercise is an important independent variable. A work load considerably greater than voluntarily contemplated by a subject would be physiologically very stressful. Pushing the body to extreme tolerance limits would probably produce different post-exercise effects, whether they be in wakefulness or sleep, than for recovery after a work load within tolerable limits of a subject's ability. The load imposed by this investigation fell within the latter category, and because of the standardization of this load, all subjects felt equally exhausted after exercise. Although this exercise was in excess of the level they would normally entertain, subjects were not completely physically exhausted. After a rest and refreshment they were able to go about their studies. Thus, if SWS reflects body restitution and increased levels of protein re-synthesis in the adult, then the high work load incurred by the subjects might have been expected to produce a more definite SWS change than actually found. It must be concluded from these findings that if a tolerable exercise load is taken early in the day, then from the EEG manifestations of sleep, ensuing wakefulness is sufficient for recovery, and subsequent sleep is not affected. If exercise is given later in the day, then ensuing wakefulness may not be adequate, and some of the recovery process may intrude into the earlier part of sleep. However, from the findings of Baekeland and Lasky (1966), discussed earlier, it may appear that if exercise is taken in the evening, near to the sleep period, sleep is disrupted. This might be due to an exercise disturbance of various physiological processes, for example, basal metabolism, which normally declines prior to sleep.

The validity of the nomogram method for assessing $VO_2$ max has been assessed on several occasions. In a more recent evaluation, Davies (1968) found that compared with the direct method, the sub-maximal technique using the bicycle ergometer is accurate to within ±15% of the true figure. Therefore, for the present type of study, inter-individual work loads can be considered quite comparable.

This investigation may have been enhanced by assays of somatotrophin levels during sleep, but this was not possible. Although Zir et al. (1971) found no significantly higher levels of sleep somatotrophin following exercise, compared with control nights, Adamson et al.

(1974) take their findings of an increased amount of somatotrophin following a.m. exercise to support the anabolic and restitutional processes of sleep. But whether this particular somatotrophin increase does increase protein synthesis during sleep is not actually known. Increased cellular intake of amino acids does not necessarily infer ongoing protein synthesis. From recent work of, for example, Young et al. (1975), it appears that protein synthesis is performed at a very high energy cost, and is probably the main contributor to metabolic rate. However, from such measures of metabolic rate as $O_2$ consumption, Brebbia and Altshuler (1965), and Webb and Hiestand (1975) have shown that $O_2$ consumption during sleep is low compared with daytime values. Therefore, it may be difficult to conceive of particularly large amounts of protein synthesis taking place during sleep, especially SWS.

It is felt that stage 2 as presently described is a very heterogenous sleep stage and that the sub-division used here helped further to qualify delta activity during sleep, giving a more complete picture of SWS.

## Summary

The effect standardized exercise had upon sleep was studied with eight subjects. A pilot study assessed individual work capacity by the sub-maximal estimation of $VO_2$ max. In the main study each subject performed the exercise, once a.m. and once p.m., on different days. Sleep was scored into stages and an additional sub-division of stage 2 containing 10—20% by time of delta. Comparison with baseline showed no significant whole night changes with any criteria following either a.m. or p.m. exercise. After p.m. exercise there was a significant increase in stage 3 for the first half of the night. It was concluded that ensuing wakefulness following early daytime exercise is sufficient for recovery, but late daytime exercise may result in an intrusion of recovery into initial sleep. Sleep is not seen to be necessary for recovery from muscular fatigue.

## Résumé

*Influence de la période du jour où s'effectue un exercice standard sur le sommeil subséquent*

On a recherché à propos de huit sujets, l'effet d'un exercice standard sur leur sommeil. Une étude préliminaire a permis de déterminer à propos de chaque sujet sa capacité individuelle au travail par l'estimation de $VO_2$ max. Dans l'étude principale, chaque sujet effectuait l'exercice, une fois le matin, une fois l'après-midi, à des jours différents. Le sommeil était évalué stade par stade, avec une subdivision du stade 2 selon le contenu en activité delta (moins de 10%; entre 10 et 20%). Par rapport au sommeil témoin, aucune modification significative du sommeil total n'a pu être constatée selon que l'exercice avait été effectué le matin ou l'après-midi, quel que soit le critère utilisé. A la suite de l'exercice d'après-midi, une augmentation significative du stade 3 a pu être constatée, dans la première moitié de la nuit. On conclut que l'état de veille qui fait suite à un exercice pratiqué tôt dans la journée suffit à la récupération, qu'un exercice effectué tard entraîne une récupération en cours de début de sommeil. Finalement donc, le sommeil ne semble pas indispensable à la restauration après fatigue musculaire.

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