HYPOTHALAMIC DYSFUNCTION AND NEUROTRANSMITTER ABNORMALITIES IN THE POSTVIRAL FATIGUE SYNDROME

by

Abdel Magid Osman Bakheit, PhD (Glasg.), MSc (Lond.)

A thesis submitted for the degree of Doctor of Medicine (MD) of the University of Glasgow

The research work for this thesis was carried out in the University Department of Neurology, Institute of Neurological Sciences, Glasgow

1992

List of contents

DEDICATION

Dedication

With affection and gratitude I dedicate this work to my teachers, past and present.

Acknowledgements

The research on which this thesis is based was carried out in the Academic Department of Neurology, University of Glasgow & the Institute of Neurological Sciences, Southern General Hospital, Glasgow, during my tenure as lecturer in Clinical Neurology. All the studies which are reported here were supervised by Professor P.O. Behan, Professor of Clinical Neurology, University of Glasgow.

I am very grateful to Professor Behan for his advice, guidance and unfailing support in the last few years. During this period Professor Behan introduced me to a great many concepts in the theory and practice of Clinical Neurology and helped me on the way to becoming a better clinician and scientist.

Professor PGE Kennendy, Burton Chair of Neurology, University of Glasgow, has kindly offered me support and advice.

I am indebted to many other collaegues. Dr. WMH Behan, Senior Lecturer in Pathology, University of Glasgow, taught me the technique of needle muscle biopsy and was always available to advise on muscle pathology. Dr. WS Watson, Principal Physicist, Southern General Hospital, Glasgow, helped with the measurements of total body

water and potassium. Dr JJ Morton of the MRC Blood Pressure Unit, Glasgow, and Dr CE Gray, Consultant Biochemist at Glasgow Royal Infirmary, helped with the assays of arginine vasopressin and serum prolactin, respectively. Drs. TG Dinan and V. O'Keane of St. James' Hospital, Dublin, assisted with the buspirone test.

My thanks also go to the sisters and nurses on ward 68, Southern General Hospital, to Professor Behan's laboratory staff and to all patients and control subjects who agreed to take part in these studies.

Publications resulting from this work

1. Behan PO, Bakheit AMO. Clinical spectrum of postviral fatigue syndrome. British Medical Bulletin 1991, 47: 793-808

2. Bakheit AMO, Behan WMH, Gow J, Behan PO. Persistent enteroviruses and abnormal hypothalamic and neurotransmitter function in postviral fatigue syndrome (abstract). Annals of Neurology 1991, 30: 315

3. Bakheit AMO, Behan PO, Dinan TG, Gray CE, O'Keane V. Possible upregulation of hypothalamic 5-HT receptors in patients with the postviral fatigue syndrome. British Medical Journal 1991, 304: 1010-1012

4. Smith AP, Behan PO, Bell W, Millar K, Bakheit M. Behavioural problems associated with the chronic fatigue syndrome. British Journal of Psychology (in press)

5. Bakheit AMO, Behan PO, Watson WS, Morton JJ. Abnormal arginine vasopressin secretion and water metabolism in patients with postviral fatigue syndrome. Acta Neurologica Scandinavica (in press)

Chapter one

INTRODUCTION

Postviral fatigue syndrome is a disorder that follows a viral infection and is characterised by severe, persistent or relapsing fatigue, usually with myalgia, and numerous neuro-psychiatric symptoms (see section on clinical features of PVFS).

Many viruses have been implicated in the disorder, e.g. Epstein-Barr virus (Hamblin et al, 1983), enteroviruses (Bell et al, 1988, Yousef et al, 1988, Miller et al, 1991), varicella and rubella (Behan et al, 1985). The main reason for the implication of a putative role of viruses in PVFS was that in the original descriptions the illness was considered only to occur in epidemics, however it is now known to occur as sporadic cases.

a) Historical review of postviral fatigue syndrome:

The first descriptions of the postviral fatigue syndrome were made in the eighteenth century and interest in this disorder was revived in the 1930s when Evans (1947) suggested that the syndrome is due to chronic brucellosis. Renewed interest in PVFS in the 1950 and 60s coincided with reports of many epidemics worldwide. The last two decades also represent an important milestone in the history of PVFS. First, more attention has been paid to the sporadic form of the disease and, secondly, extensive research programmes to study all aspects of this disorder have been established (see section on the organic nature of PVFS).

The first detailed account in the English literature of an illness resembling the PVFS was given by Manningham more than 240 years ago (Manningham, 1750). The disorder, which was known as febricula, was characterised by low-grade fever, excessive physical and mental exhaustion and fleeting muscle pains. It occured mostly in wealthy people, especially females. Subsequently, a condition identical to PVFS was described in the past century under the name of neurasthenia (Beard, 1869). Beard, who believed the condition to be organic in nature, stressed that the core symptom is physical and mental fatigue and he drew attention to the cardiovascular and gastro-intestinal features of the disease. He also confirmed the observation of Mannigham (1750) that the illness occurs more in females than in males. Two years later DaCosta (1871) redescribed the disease under the name "the effort syndrome". He emphasised the role of infection in intiating the symptoms and documented that the disease affects children as well as adults. He also made reference to other common features of the syndrome, such as palpitations, chest pain, dizziness, gastro-intestinal symptoms and sleep disturbances.

In the 1930s Evans (Evans, 1947) suggested that the disease is a form of chronic brucellosis. However, this hypothesis was subsequently challenged as serological evidence of continued brucella infection could not be demonstrated in most patients (Spink, 1951).

Many epidemics of PVFS were reported worldwide in the 1950s and 60s under different names including atypical poliomyelitis, abortive poliomyelitis, Icelandic disease, Akureyri disease, benign myalgic encephalomyelitis, Royal Free disease, epidemic malaise, epidemic pseudomyasthenia, chronic infectious mononucleosis syndrome, chronic fatigue and immune dysfunction syndrome. In recent years postviral fatigue syndrome and chronic fatigue syndrome have become the most widely used names for this condition (Behan & Behan, 1988, Holmes et al, 1988, Sharpe et al, 1991) and, in our opinion, the term postviral fatigue syndrome is the most helpful and correct one since it emphasises the role of viral infections in initiating the disease.

Most of the older literature on PVFS refers to the epidemic form of the disease. The main epidemics are summarised in this section (also see table 1/1).

Icelandic epidemic 1948-1949:

This occured in the town of Akureyri (population 6,900). 6.9% of the population was affected, mostly females between the ages of 15 and 30 years. There were no fatalities, but some paralytic cases were reported.

In addition to fatigue, myalgia, especially on exertion, was a prominent feature. Nervousness, palpitations and

excessive sweating were present in 42% and insomnia occured in 16% of patients (Sigurdsson et al, 1950).

Although a causative agent for this epidemic was not isolated , poliomyelitis and Coxsackie B viruses were suspected. A pandemic of poliomyelitis in 1955 swept the whole of Iceland except Akureyri district, which suggests that the population in this district had developed immunity against poliomyelitis following exposure to "epidemic neuromyasthenia" (Sigurdsson et al, 1958).

Six years after the Akureyri outbreak only 13% of the 39 patients studied had recovered completely. In the remaining patients the course of the disease was characterised by relapses and remissions. Fatigue remained the most disabling symptom and most patients continued to complain of myalgia, insomnia, nervousness and poor memory (Sigurdsson & Gudmundsson, 1956).

The outbreak in Alaska (1954):

Deisher (1957) reported an epidemic of myalgic encephalomyelitis in Seward, Alaska, which occured several weeks after an outbreak of poliomyelitis. The disease predominantly affected young females and was characterised by lethargy, muscle pain and stiffness, headaches, poor memory, anxiety and depression. Paraesthesiae, hyperacusis and photophobia were

prominent features in this epidemic. Interestingly, these symptoms increased in severity during menstruation and in some patients there was a clear pattern of relapses and remissions of symptoms in relation to the menstrual cycle.

In contrast to the patients who had developed typical poliomyelitis in the previous epidemic, those who suffered from myalgic encephalomyelitis had a normal cell and protein content in the cerebrospinal fluid and viral studies showed low antibody titres against the three strains of polio virus.

The Royal Free Hospital outbreak:

This occured in 1955. More than 300 members (or 9%) of the staff at the Royal free Hospital in London were affected by a flu-like illness. The commonest symptoms were fatigue and prostration, sore throats, headaches, neck stiffness, dizziness, blurred vision, myalgia and depression. Most patients were females (female/male ratio was approximately 4:1). Cerebrospinal fluid examination was performed in 8 patients and it was normal.

Of great interest is a 32 year old female patient who was affected in this epidemic (see Crowley et al, 1957). This patient died 7 months after the Royal Free outbreak. Postmortem exmination revealed multiple

microscopic areas of demyelination in the peri-ventricular white matter, brain stem and spinal cord. In addition, there was intense lymphocytic perivascular infiltration of the hypothalamus. Although it is likely that this patient had multiple sclerosis, it is unusual for the hypothalamus to be affected in this disease and it is possible that these changes were related to the "Royal Free Disease". Similar changes in the hypothalamus of a patient with PVFS have recently been documented at postmortem examination (PO Behan, personal communication). A study by Daugherty et al (1991) lends indirect support to the hypothalamic involvement in patients with PVFS. These authors found disseminated white matter lesions on magnetic resonance imaging (MRI) brain scans of 15 patients with PVFS. However, their findings have to be interpreted with caution as they have not yet been replicated.

Shortly after this epidemic 8 sporadic cases of the Royal Free disease were reported in North London (Ramsay & O'Sullivan, 1956). Although the occurrence of these cases coincided with an increased incidence of poliomyelitis in the same area, the disease was clinically distinct from polio. There were no paralytic cases and the cerebrospinal fluid was normal.

Seven of the eight patients were females. The initial symptoms were fever, sore throats, myalgia, anorexia and vomiting. All patients had severe fatigue which

persisted for 4-6 months after the onset of the disease. Emotional lability, "mental fatigue" and tinnitus were prominent features. Laboratory investigations were all normal except electromyography (see under " electrophysiological studies) and some non-specific changes on the electroencephalogram.

Other epidemics:

Several smaller epidemics of PVFS have been reported from various countries. These include an outbreak in California, USA, in 1934 (Wilson & Walker, 1936), Pennsylvania, USA (McConnell, 1945), Adelaide, Australia (Pellow, 1951), Middlesex hospital, London (Acheson, 1954), Coventry, England (Macrae & Galpine, 1954) and many others. The clinical presentation, course and outcome of these epidemics were similar to those of the Akureyri, Alaska and Royal Free epidemics. In addition, a common feature in all of these epidemics is that an enterovirus (polio or Coxsakie) had been suspected as the cause of the epidemic.

Table 1/1

The main epidemics of postviral fatigue syndrome:

Epidemic	year	reference
Los Angeles, USA	1934	Wilson & Walker, 1936
Harefield, Middlesex, UK	1939	Houghton & Jones, 1942
Akureyri, Iceland	1948	Sigurdsson et al, 1950
Adelaide, Australia	1949	Pellew, 1951
New York, USA	1950	White & Burtch, 1954
London, UK	1952	Acheson, 1954
Coventry, UK	1953	Macrae & Galpine, 1954
Maryland, USA	1953	Shelokov et al, 1957
Berlin, Germany	1954	Sumner, 1956
Royal Free Hosp. London, UK	1955	Compston, 1956
Perth, Australia	1955	Steen, 1956
Durban, S. Africa	1955	Hill, 1955

Table 1/1 (continued)

Epidemic	year	reference
Johannesburg, S. Africa	1954	Jackson et al, 1957
London, UK	1955	Ramsay & O'Sullivan, 1956
Florida, USA	1956	Poskazner et al, 1957
Newcastle upon Tyne, UK	1959	Pool et al, 1961
London, UK	1966	Ramsay, 1978
S.W. Ireland	1976	Corridan, 1978
Southampton, UK	1979	May et al, 1979
West Kilbride, Sctoland, UK	1980	Fagan et al, 1983

b) Clinical features of PVFS:

i: the diagnostic criteria of PVFS:

The most important factor which impeded progress of research in PVFS until recently was the lack of a universally accepted case definition, making comparison of reports by different investigators virtually impossible. The recent attempts to lay down guidelines for the diagnosis of PVFS (Holmes et al, 1988, Lloyd et al, 1988, Sharpe et al, 1991) are a welcome step in the right direction, although some problems still remain. Holmes et al (1988) classified the symptoms of PVFS into major, minor and physical criteria. The major criteria are:
1. fatigue - which must be persistent or relapsing, not improved by bed rest, reducing the patient's activity by 50% or more and present for six months or more
and 2. all other chronic illnesses which might produce the same symptoms must be excluded.

There are 11 minor criteria, e.g. myalgia, depression and sore throat. The physical criteria are: low grade fever, nonexudative pharyngitis and palpable cervical or axillary lymph nodes. Holmes stresses that both major criteria plus either 8 minor or 6 minor and 2 or more physical criteria must be present to make the diagnosis of PVFS.

In our opinion the working case definition of Holmes et al (1988) which is summarised in the previous paragraphs has the following weaknesses:

1. in contrast to psychiatric and most other conditions which cause chronic fatigue, PVFS is always precipitated by a viral infection. The definition of Holmes et al (1988) does not take this into consideration.

2. a past or family history of psychiatric illness, a poorly-adjusted premorbid personality and a poor work record are common in patients with psychiatric disease. Although patients with psychiatric illnesses may develop PVFS (like anybody else), we feel that patients with this history should not be included in clinical trials.

3. The working case definition (Holmes et al, 1988) does not include some frequently encountered psychiatric symptoms in patients with PVFS such as anxiety and hypochondriasis. Furthermore, "prolonged generalized fatigue following previuosly tolerable levels of exercise" is classified as a minor criterion. We think the fact that fatigue is precipitated or made worse by exercise is an important characteristic of the fatigue in PVFS. In our experience it is present in all patients with PVFS. This characteristic should, therefore, be incorporated in the definition of fatigue (which is a major criterion), rather than relegating it to a minor criterion.

It is essential that the criteria used for the case definition for research purposes are strict, especially if the disease in question is of unknown aetiology or can not be confirmed with objective laboratory tests, as is the case of PVFS. This is important to narrow the differential diagnosis, although such restriction will inevitably exclude some patients with the disease under investigation. In the following section we propose alternative criteria for the diagnosis of PVFS.

Our diagnostic criteria incorporate those of Holmes et al (1988) and also the diagnostic guidelines of the Medical Research Council (Sharpe et al, 1991). In addition, these criteria emphasise the important role of a viral infection in initiating PVFS and they also attempt to exclude patients with possible or known psychiatric illness. We have previously published these criteria (Behan, 1991, Behan & Bakheit, 1991) which we summarise below.

1) a history of a definite or probable viral infection preceding the onset of symptoms. This history must be corraborated by the appropriate laboratory tests;

2) the presence of severe fatigue for six months or more which reduced the patient's premorbid level of activity by 50% or more. The fatigue is precipitated or made worse by physical or mental effort and is not improved with bed rest;

3) at least three of the following symptoms must be present: myalgia, dizziness, sleep disturbances, poor memory and concentration, anxiety, hypochondriasis, the typical depression as described earlier (see section on psychiatric symptoms, pages 27-31), early satiety, diarrhoea alternating with constipation, abdominal bloating, poor appetite, fluctuations in body weight and menstrual irregularities;

4) chronic infections, malignancy, autoimmune disorders and other medical conditions which may cause fatigue must be excluded with the appropriate investigations;

5) patients with a past medical or family history of depression or any other major psychiatric illness are excluded;

6) all patients must have a well-adjusted premorbid personality (defined as ability to cope with every day life stress) and all must have a good work record.

We make the diagnosis of PVFS if the patient fulfils all of these criteria.

ii: General symptoms of PVFS:

PVFS occurs in both sexes, but the incidence is higher in females. In most series, including ours, 60-70% of all patients were females (Tobi et al, 1982, Jones et

al, 1985, Komaroff & Buchwald, 1991). The disease affects all age groups. Most patients are between 25-40 years old. Although children with PVFS may have some atypical features (see below), the clinical picture of the disease is essentially the same in all age groups.

The course of the disease is often protracted with relapses and remissions occuring at irregular intervals. We have seen patients who have had the disease for more than 20 years. A chronic relapsing and remitting course was also reported in the epidemic form of the disease (Sigurdsson & Gudmundsson, 1956, Compston 1956, Ramsay & O'Sullivan, 1956).

In addition to incapacitating fatigue (see below), patients with PVFS have a number of other symptoms which occur in various combinations. These include myalgia, disturbances of sleep, mood, memory and concentration. gastrointestinal symptoms (see below), fluctuations in body weight, excessive sweating, especially at night, intermittent low grade fever and headaches. Interesingly, the headaches are often of migraine type. Recurrent sore throats and cervical lymphadenopathy are common. Most patients also complain of tinnitus, dizziness, lightheadedness, cold extremities and sensitivity to bright light and noise. Occasionally, acroparaesthesia may be a prominent feature. Some patients present with chest pain identical to that of Bornholm disease (Stevenson & Hamblin, 1971) and

ischaemic type chest pain may also occur. Indeed several of our patients had elaborate cardiological investigations, including exercise tests and coronary angiography. Some of these patients also complain of palpitations.

iii: Fatigue:

Fatigue, as defined by physiologists, refers to a progressive decrement in force generation that occurs during muscular activity (Lancet, 1988). To the clinician fatigue is a state in which a required or expected muscle activity can not be maintained (Edwards, 1978). It is a subjective perception of lassitude or lack of energy. In other words, fatigue is "an overwhelming sustained sense of exhaustion and decreased capacity for physical and mental work" (Piper, 1989).

Two types of fatigue are distinguished: central and peripheral. Central fatigue occurs in the absence of muscle weakness or dysfunction of the neuromuscular junction and presumably results from poor motivation and lack of drive. On the other hand, peripheral fatigue is due to structural damage of skeletal muscle or the neuromuscular apparatus or interference with muscle energy metabolism.

By definition all patients with the PVFS must have fatigue as their main complaint. Characteristically, the

fatigue in these patients is of the "central" type. It is persistent or relapsing and does not improve with bed rest. It is generally agreed that the fatigue must reduce the patient's premorbid level of activity by 50% or more (Holmes et al, 1988, Behan & Bakheit, 1991, Sharpe et al, 1991).

Fatigue is a common symptom in many general medical and neurological conditions. It is commonly reported by patients with bacterial and viral infections, endocrine disturbances, e.g. Addison's and Cushing's disease, acromegaly and hypothyroidism. It is also a frequent complaint during pregnancy and the premenstrual tension syndrome (Pelosi et al, 1986). Fatigue may be troublesome in patients with the irritable bowel syndrome.

Fatigue is often a prominent symptom in patients with multiple sclerosis and Parkinson's disease and following head injuries and stroke.

In multiple sclerosis fatigue was listed as the most troublesome symptom by 28% of patients (Krupp et al, 1988, Krupp et al, 1989). In a third of these patients fatigue preceded other symptoms of the disease and patients described their fatigue as qualitatively different from that experienced before their illness. Interestingly, fatigue in multiple sclerosis was found to be independent of the presence or absence of

depression and it affected both males and females.

Fatigue is also common in patients with Parkinson's disease. Like fatigue in multiple sclerosis, it is also of the "central" type and may be, at least in part, due to the diurnal fluctuations of nigro-striatal dopamine concentrations (see Critchley et al, 1991). Fatigue and sleep disturbances are also present in patients with encephalitis lethargica and other parkinsonian syndromes which follow viral infections such as rubella, mumps and poliomyelitis.

iv: Sleep disturbances in PVFS:

Sleep disturbances have been reported in all epidemics of PVFS. For example, in the Akureyri epidemic 16% of patients had severe insomnia (Sigurdsson et al, 1950). Disorders of sleep are also common in the sporadic form of the disease and occur in 15-90% of patients (Komaroff & Buchwald, 1991).

Three patterns of sleep disturbances are observed in patients with the PVFS. In adults the most frequently reported sleep abnormality is that of excessive sleep. Patients usually go to sleep early and stay in bed for 10 hours or more. The sleep is not fully refreshing and patients often have difficulties with concentration during the day. A similar pattern of sleep disturbances has been reported in patients with the premenstrual

tension syndrome and seasonal affective disorder (SAD) which are probably associated with central 5-HT abnormalities (Wurtman & Wurtman, 1989). A smaller group of adult patients with PVFS suffer from insomnia, whilst the commonest form of sleep disturbaces in children is reversal of sleep pattern with insomnia at night and hypersomnolence during the day.

v: Psychiatric Symptoms:

Psychiatric symptoms are frequently encountered in patients with PVFS. These include poor concentration and memory, anxiety, irritability, hypochondriasis and depression. The reported incidence of these symptoms varies from 50 to 90% (Behan et al 1985, Straus, 1988, Komaroff & Buchwald, 1991).

The multiple symptomatology and the paucity of abnormal physical signs in patients with PVFS has led to the view that the syndrome is a somatisation disorder in patients with predisposition to psychiatric illness, especially depression (Cluff, 1991). The issue is complicated further by two other factors. These are:

a) the significant overlap between the symptoms of PVFS and those of depressive illness (Crowley et al, 1957, Behan & Bakheit, 1991, Thase, 1991);

b) the heterogeniety of the depressive syndromes.

Although patients with PVFS share a number of symptoms with those suffering from major (primary) depressive illness, there are also important differences. According to the current diagnostic criteria of the American Psychiatric Association (1987) a diagnosis of major depressive illness is made when the patient has a depressed mood and at least four of the following symptoms:

1. markedly diminished interest or pleasure in all or most activities;
2. 5% or more change in body weight in a period of 4 weeks. Increase or decrease in appetite;
3. sleep disturbances, characteristically insomnia and early morning waking;
4. psychomotor agitation or retardation;
5. feelings of guilt or worthlessness;
6. suicidal ideation or a recent suicidal attempt;
7. poor concentration/ indecisiveness;
8. fatigue.

In contrast to patients with major depressive illness, those with PVFS do not experience guilt or feelings of worthlessness, nor do they have suicidal ideation, hallucinations, delusions, psychomotor agitation or retardation. In our experience, these patients are capable of experiencing pleasure and continue to have interest in all their hobbies and leisure activities, i.e. they remain hedonic (Behan & Bakheit, 1991). Our

observations are in agreement with results of a recent study which showed that depressive symptoms in patients with PVFS are indistinguishable from those seen in patients with chronic rheumatoid arthritis (Procter, 1991). Anhedonia was not reported by patients in either group. Hypochondriasis is usually present in most patients with PVFS. Interestingly, this symptom is not regarded as a diagnostic criterion for major depressive illness (American Psychiatric Association, 1987).

The heterogeniety of the depressive syndromes is illustrated by the complex classification of these disorders. In addition to major depressive disorder, the third (revised) edition of the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 1987) distinguishes another two less severe conditions in which a depressed mood is a prominent feature. These are: adjustment disorder and dysthymic disorder. The first condition is characterised by a depressed mood plus one or two of the symptoms listed above. If these symptoms persist for 2 or more years, the condition is named dysthymic disorder. The Association also recognises a group of unclassifiable depressive disorders.

To define the psychiatric symptoms in patients with PVFS more clealy we have studied 57 patients with PVFS and 19 healthy control subjects. The two groups were matched for age, sex, socio-economic status and level of

education.

Mood (affective state) was assessed using standard questionnaires. Depression, anxiety and physical symptoms were measured using the Middlesex Hospital Questionnaire (Crown & Crisp, 1966), the Spielberg State Anxiety Inventory (Spielberg et al, 1970) and the Cohen-Hobermann Index of Physical Symptoms (Cohen & Hobermann, 1983), respectively. The Cognitive Failures Questionnaire (Broadbent et al, 1982) was used to document the self-reported errors of memory, attention and action. In addition, a battery of performance tests which have been previously used to assess the effects of acute viral infections on memory, attention and psychomotor function were performed (Smith et al, 1987, Smith, 1990). These were variable-fore-period simple reaction time, five choice serial response, detection of repeated numbers, free recall, delayed recognition memory, digit span, logical reasoning and semantic processing tasks. To measure distractability the stroop colour-word test was used (Smith & Broadbent, 1985).

Analysis of the self-rating questionnaires showed that patients with PVFS reported significantly higher levels of depression and anxiety compared to control subjects. As expected, they also had more physical symptoms . The psychomotor tests revealed that patients with PVFS were slower than control subjects in retrieval of information from general knowledge (semantic memory task) and

working memory (logical reasoning task). They also had problems maintaining attention and showed increased sensitivity to visually disturbing stimuli.

In an attempt to establish the effects of depression on the cognitive deficits mentioned above patients with PVFS were divided into two groups: those with low or high levels of depression. Comparison between the two groups showed similar impairments in patients with high and low levels of depression. This indicates that the mental impairments in patients with PVFS are independent of depression.

As stated earlier, the characteristics of depressive symptoms in patients with PVFS are distinct from those of primary depressive illness. Anhedonia, hallucinations and delusions, feelings of guilt and self-depreciation, which are the hallmark of major depressive illness, are not reported by patients with the PVFS. Nevertheless, it has been argued that PVFS represents a somatisation disorder in subjects with a premorbid vulnerabilty to depression (Taerk et al, 1987). This hypothesis was based on the finding of an increased prevalence of life-time psychiatric illness in patients with PVFS in comparison to control subjects. However, methodological bias accounts for this finding as the reported diffrence between the two groups disappears when only the premorbid psychiatric events are considered. Two recent studies of carefully selected groups of patients with

PVFS and control subjects did not demonstrate a significant difference in the prevalence of premorbid psychiatric illness between the two groups (Hickie et al, 1990, Procter, 1991).

vi: Gastrointestinal symptoms in patients with PVFS:

Abdominal bloating, nausea and early satiety are common symptoms of PVFS. In our series of 287 patients 55% complained of gastrointestinal symptoms, an incidence which is similar to that reported by Komaroff and Buchwald (1991). We have seen some patients in whom these symptoms are accompanied by diarrhoea which alternated with constipation. The clinical picture in these patients is identical to that of the irritable bowel syndrome.

The clinical similarities between PVFS and the irritable bowel syndrome are intriguing. Indeed the two disorders may share a common pathogenetic mechanism. In a recent study Dinan and collaborators (Dinan et al, 1990) have shown that the symptoms of the irritable bowel syndrome (also known as the syndrome of non-ulcer dyspepsia) may be due to dysfunction of central 5-HT neurones. Using the buspirone challenge test (see chapter two), these authors have demonstrated a significantly greater prolactin response to buspirone in patients with the irritable bowel syndrome than in healthy control subjects or patients with peptic ulcer disease. This

observation suggests hypersensitivity of hypothalamic 5-HT receptors and is similar to our findings in patients with PVFS which are dicussed in the following chapters.

Several of our patients reported craving for chocolate and sweets which they did not have prior to their illness. Interestingly, craving for carbohydrates is known to occur in patients with seasonal affective disorder and also in women with the premenstrual tension syndrome (Wurtman & Wurtman, 1989).

vii: The effects of menstruation on the symptoms of PVFS:

Menstruation exacerbates all symptoms of the PVFS. Deisher (1957) noted that myalgia, fatigue, fluid retention and irritability markedly increased in female patients immediately before menstruation. Interestingly, these patients had never experienced premenstrual tension before the onset of PVFS. In some patients the exacerbation of symptoms at mid cycle was so severe and occured with such regularity that a pattern of relapses and remissions in relation to menstruation was clearly recognisable by the patient. Similar observations have been reported by other investigators (Leon-Sotomayor, 1969, Behan & Bakheit, 1991). The exacerbation of PVFS in mid cycle appears to be a common complication. 50-60% of patients with PVFS in one series had this

complication (Komaroff & Buchwald, 1991).

The mechanism by which menstruation causes exacerbation of the symptoms of PVFS is not clear. Studies in the rat have shown that hypothalamic 5-HT receptors undergo regular functional changes during the oestrous cycle being upregulated following ovulation (Biegon et al, 1980) and it is possible that a similar mechanism accounts for the exacerbation of PVFS in humans (see following sections).

vii: Symptoms in children:

PVFS is less common in children than in adults. Bell et al (1991) have found that the risk of developing the disease is higher in children with an allergic disorder or a family history of autoimmune disease and also in those who have an adult family member with the disease.

Although the symptoms of PVFS in children and adults are essentially the same, there are some differences between the two groups. For example, the psychiatric symptoms of PVFS are more prominent in children than in adults. Patients are usually tearful and very dependent on their mothers. Eating disorders are also more frequently encountered in childern than in adults with PVFS. The problem is usually that of anorexia rather than overeating. Finally, sleep disturbances in children are characterised by reversal of sleep pattern with insomnia

during the night and excessive sleep during the day (Behan & Bakheit, 1991).

c) Conditions with some clinical resemblance to PVFS:

The clinical features of PVFS closely resemble those of the fluid retention syndrome (FRS) and the premenstrual tension syndrome (PMT), suggesting a common pathogenetic mechanism for these disorders.

The fluid retention syndrome (also known as cyclic oedema and idiopathic oedema) is a relatively common disorder of unknown aetiology. The diagnosis is based on the presence of the following three features: fluid retention, autonomic and psychiatric symptoms (Dunnigan, 1991). In addition, fatigue may be a prominent complaint (Pelosi et al, 1986) and symptoms of the irritable bowel syndrome are frequently reported (Dunnigan 1985). The psychiatric symptoms include depression, irritability, poor memory and concentration and sometimes anxiety (Pelosi et al, 1986).

The pathogenesis of FRS is not fully understood. Although previous studies did not demonstrate abnormalities in the levels of circulating hormones (Smith et al, 1972), more recent evidence points to a possible dysfunction of the hypothalamic-pituitary axis in patients with this disorder. For example, the syndrome often occurs in obese women and abnormalities

of glucose metabolism, such as insulin resistence or frank diabetes, are common in patients and their relatives (Dunnigan, 1990). Mild dysfunction of the thyroid gland may be present in some of these patients (Al-Khader & Aber, 1979). Furthermore, abnormal levels of circulating prolactin and sex hormones have been documented in these patients (Young et al, 1983).

PMT is a disorder characterised by variable increase in body weight, bloating, abdominal colic, mastalgia, excessive fatigue, mood swings, and sleep disturbances. In contrast to FRS, PMT reccurs regularly in the same phase of the menstrual cycle and the patient is invariably completely symptom-free postmenstruum.

PMT frequently occurs in patients with migraine (Tindall, 1987), a condition which is associated with central 5-HT abnormalities. It has been shown that fluctuations of 5-HT levels occur in the different phases of the menstrual cycle and that the observed changes correlate with behavioural abnormalities which occur in some women in mid cycle (Rausch & Janowsky, 1982). More recently Rickels et al (1989) have shown that buspirone, a 5-HT receptor agonist (and also a partial dopamine receptor antagonist), enhances prolactin release in patients with PMT but not in control subjects. Furthermore, buspirone alleviated the symptoms of PMT in these patients. These findings suggest a relationship between PMT and abnormalities of

5HT secretion or dysfunction of central 5HT receptors and are similar to our findings in patients with PVFS which are described in chapter 5.

Many patients with PVFS complain of increase in body weight and exacerbation of their fatigue and other symptoms premenstrually. In at least four of our patients the severity of premenstrual bloating and peripheral oedema was similar to that of the classical FRS (Behan & Bakheit, 1991). The following case history illustrates the clinical overlap between PVFS, FRS and PMT.

Case history:

The patient is a 44 year old married branch manageress with British Telecom who had not worked since the onset of her symptoms seven years ago. Her main symptoms are excessive fatigue and lethargy, depression, poor memory, excessive sleep (12-14 hrs/day), joint and chest pains, abdominal bloating, diarrhoea alternating with constipation, intermittent swelling of the hands and feet and weight gain. Her body weight fluctuated by two stones within a few days and this coincided with periods of severe depression and weepiness. These symptoms are worse premenstrually.

The symptoms followed an attack of hepatitis A which she developed while in Gambia. The course of her illness was

initially characterised by relapses and remissions lasting several weeks. However, two years ago she developed herpes zoster, following which she did not enjoy any further remissions.

There was no past medical or family history of note. She is a non-smoker and does not drink alcohol.

General physical and neurological examination was entirely normal. A full blood count, serum urea and electrolytes, liver and thyroid fuction tests were normal. The erythrocyte sedimentation rate was 26 mm in the first hour. Serum electrophoresis showed the IgM to be slightly depressed but was otherwise normal. CSF cell and protein content were normal. Function of the pituitary-hypothalamic-adrenal axis was normal. Barium studies of the gastrointestinal tract and a chest X ray were normal (interestingly the patient was diagnosed by a general physcian as having the irritable bowel syndrome). The buspirone challenge test and measurements of total body water in the follicular and luteal phases of the menstrual cycle were grossly abnormal (see chapters three & four). A needle muscle biopsy from vastus lateralis of the quadriceps femoris muscle revealed type two fibre atrophy. No viral particles were detected in muscle biopsy with the polymerase chain reaction using an enterovirus-specific probe.

e) The organic nature of PVFS:

As discussed earlier, chronic fatigue is a common complaint. In one study fatigue was reported as the main complaint by 25% of patients attending a primary care clinic (Kroenke et al, 1988). Fatigue is also common in psychiatric patients. As many as 15% of those with somatisation disorders complain of incapacitating fatigue (White, 1989). Controversy about the organic nature of PVFS (for examples see Imboden et al, 1961, Swartz, 1988, David et al, 1988, Cluff, 1991) has arisen because of three reasons. Patients with PVFS seldom have physical signs on clinical examination and at present there are no simple laboratory (i. e. objective) methods which can confirm the diagnosis in these patients. An additional problem is the overlap of symptoms of PVFS and major depressive illness, e.g. depressed mood, anxiety and hypochondriasis. In this section we summarise the currently available evidence for the organic nature of PVFS.

i: Viral studies:

As discussed earlier, reviews of epidemics of PVFS, as well as studies of sporadic cases show that the disease is precipitated by viral infection. Several serological studies have demonstrated higher Coxsackie B or Epstein-Barr viral titres in patients with PVFS than in the control populations but this may be only a

reflection of high background infection in the community (Miller et al, 1991). Furthermore, rises of antibody titres in response to viral infections have been shown to vary depending on age and gender (Biggar et al, 1981). The yield from viral culture of faeces and body fluids is relatively small. Enteroviruses were isolated (after removal of neutralising antibodies) from only a quarter of patients with PVFS (Yousef et al, 1988). Only recently the use of more reliable markers of viral infection has been introduced as a research tool in patients with PVFS. These include molecular hybridization techniques and the polymerase chain reaction.

Using an enterovirus group-specific probe, Archard et al. (1988) were able to detect enterovirus RNA particles in 26 out of 111 muscle biopsies of patients with PVFS. None of 30 healthy control subjects was positive. The authors suggested that persistence of infection may be an important pathogenetic mechanism of muscle fatiguability in these patients.

As shown by a study in this department (Gow et al, 1991) the polymerase chain reaction is a more sensitive method of detection of viral RNA than molecular hybridization techniques. In this study muscle biopsy material obtained from the vastus lateralis of the quadriceps femoris muscle of 60 patients with PVFS was studied with the polymerase chain reaction. An enterovirus-specific

RNA probe was used. Enteroviral genomic RNA was detected in 32 out of 60 biosies (53%). This contrasts with a 23% detection rate with mollecular hybridization methods (Archard et al, 1988).

We extended the observations of Gow et al (1991). Since publication of the original report, we have studied another group of patients with PVFS with the polymerase chain reaction using the same enterovirus-specific probe. Needle muscle biopsy material was processed in the same way as in the previous study. Of 61 patients, 28 were positive for genomic RNA and in 33 patients the virus particles were not detected with the polymerase chain reaction. These findings show that despite the sensitivity of the polymerase chain reaction, virus particles are not detected in almost half the patients with PVFS. However, it must be realised that other viruses, such Epstein-Barr, varicella zoster and rubella virus may also be associated with PVFS. It is also possible that the distribution of viral particles in muscle is patchy and that the needle biopsy may not be representative.

ii: Immunological studies:

Various immunological abnormalities have been reported in patients with PVFS but there is some lack of consistency between reports. For example, Kibler et al (1985) have demonstrated depressed in vitro production

of lymphokines in patients with PVFS but other workers did not agree (Straus et al, 1989, Behan & Bakheit, 1991). This may be due to bias in the selection of patients. In recent years two abnormal immunological findings have been well-documented: increased circulating immunoglobulins and decreased function of natural killer cells (Caligiuri et al, 1987, Murdoch, 1988, Morrison et al, 1991, Buchwald et al, 1991).

In this regard, Morrison, Behan and Behan (1991) studied the function of natural killer cells in 23 patients with PVFS and 19 age and sex-matched healthy control subjects. Using fluorochrome-conjugated specific monoclonal antibodies, these authors found a reduction in the numbers of CD56- and an increase in CD56+ natural killer cells in patients with PVFS in comparison to control subjects. There was also a decrease in CD16+ cells (CD56+ Fc gamma receptor bearing cells) which suggests a reduced antibody-dependent cellular immunity in these patients. A previous study of natural killer cells function in patients with chronic fatigue following Epstein-Barr virus infection has also demonstrated low natural killer cell cytotoxicity in these patients (Caligiuri et al, 1987).

As many as 50% of patients with PVFS give a history of atopy and allergic conditions and a similar history can often be elicited in these patients' relatives (Olson et al, 1986). Several of our patients also have

relatives with PVFS.

i: Genetic studies in PVFS:

The familial clustering of cases of PVFS and a high incidence of atopic disorders in these patients and their relatives are well documented. Deisher (1957) found a high frequency of multiple cases in the same household during the epidemic of myalgic encephalomyelitis in Alaska and similar findings were reported in the Royal Free Hospital (Crowley et al, 1957) and Akureyri epidemics (Sigurdsson et al, 1950). More recently, Bell et al (1991) reported seven cases of PVFS occuring in two families. We have also seen the disease affecting more than one member of the same family in at least three occasions.

A history of atopy in patients with PVFS and their first degree relatives is common. 21 out of 187 consecutive cases of PVFS admitted to our unit had a past or a family history of an allergy or an atopic disorder. The incidence of these disorder was reported to be between 40 and 70% by other investigators (Komaroff & Buchwald, 1991, Bell et al, 1991).

In order to examine the possible role of genetic factors in the pathogenesis of PVFS we studied the HLA expression in a well-defined group of 41 patients with this disorder (17 males and 24 females). 500 healthy

subjects from the same area were used as controls. We did not find any differences in HLA-A, HLA-B and HLA-DR antigen frequencies between patients and controls. However, in about a third of our patients HLA-DR antigen typing was not possible because of poor viability of B lymphocytes in patients with PVFS. We believe that the poor viability of B lymphocytes in our patients is due to some inherent property of these cells rather than due to methodological artefacts as there was no delay in processing the blood samples. Interestingly, a recent study from South Africa has also demonstrated abnormal HLA-DR antigen expression in 3 out of 10 patients with PVFS (van Greune & Bouic, 1990). The significance of these findings is at present not clear and more research in this field is required.

iii: Abnormalities of skeletal muscle mitochondria in PVFS:

In a study of 60 muscle biopsies of patients with PVFS morphological abnormalities of skeletal muscle were found in the majority of patients (Gow et al, 1991, Gow & Behan, 1991). Light microscopy revealed non-specific type two fibre atrophy in 50 out of 60 patients. Ragged red fibres, i.e. aggregates of mitochondria seen on light microscopy when the tissues are stained with Gomori trichrome stain, were also present in most cases. In 75% of patients electron microscopy showed that the mitochondria were pleomorphic, swollen and vacuolated.

In addition, there was obvious "compartmentalisation" and proliferation of cristae (Figure 1/1).

Figure 1/1

Mitochondrial abnormalities in skeletal muscle of patients with PVFS.

a) Patient with postviral fatigue syndrome. Skeletal muscle biopsy showing increased number of enlarged and pleomorphic mitochondria in the subsarcolemmal and intermyofibrillar regions. x 7,800

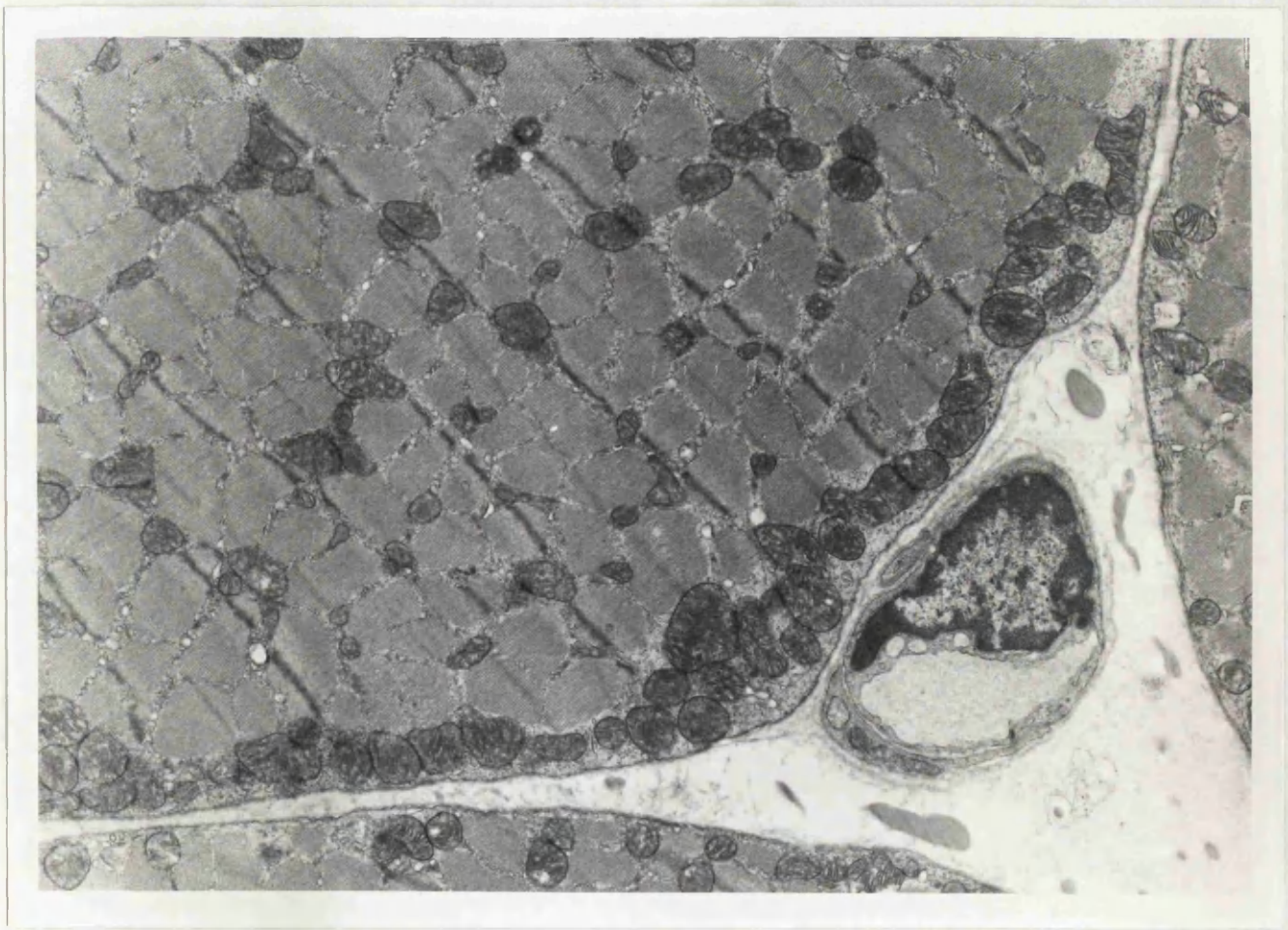
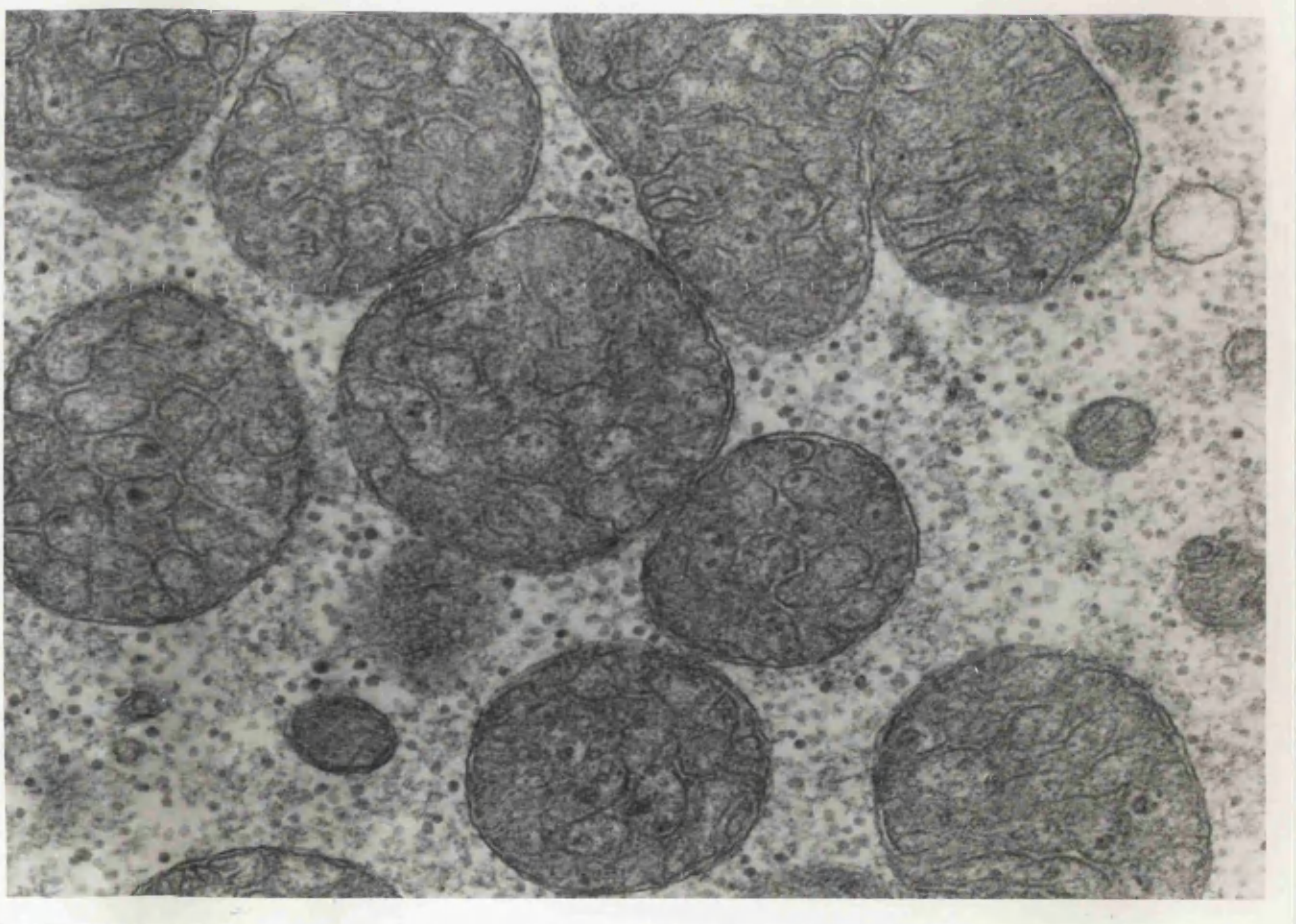


Figure 1/1

b) Same patient as in figure 1/1 a. Greatly enlarged and pleomorphic mitochondria showing proliferation of the cristae ("compartmentalization"). Five normal sized mitochondria are also present. x 78,000



iv: Electrophysiological studies:

Ramsay and O'Sullivan (1956) carried out electromyographic (EMG) studies in 8 patients suffering from the Royal Free disease. Although the EMG findings were essentially normal, these authors found a reduced number of motor unit potentials, i.e. reduced interference pattern. In addition, the motor units were of long duration, polyphasic and often of large amplitude.

In a later study of single fibre electromyography (Jamal & Hansen, 1986) abnormal jitter values were demonstrated in 75% of patients with PVFS. A subsequent study replicated the findings of Hansen & Jamal in four out of 30 patients studied (Roberts, 1990). This low incidence of jitter abnormalities in this group may be due to bias in patients' selection. In contrast to patients with myasthenia gravis and other neuromuscular disorders, impulse blocking has not been reported in patients with PVFS.

v: Metabolic studies in patients with PVFS:

The excessive muscle fatigue and myalgia of PVFS (which are characteristically precipitated or made worse by exercise) suggest an abnormality of intermediate muscle metabolism. This possibility was investigated by a number of authors.

A study of muscle metabolism with nuclear magnetic resonance spectroscopy in a patient with PVFS (Arnold et al, 1984a) revealed an abnormally early intracellular acidosis during exercise which was out of proportion to high energy phosphate metabolism.

Lactic acid may accumulate in the cell for one or more of the following three reasons:

a) reduced cytoplasmic buffering capacity;
b) impaired elemination of acid from the cell;
c) defects of the regulation of the relative contribution of glycolytic and oxidative processes in muscle energy provision.

Arnold and colleagues argued that neither reduced cytoplasmic buffering capacity, nor the impaired elemination of acid from the cells were responsible for their findings because the pH did not continue to fall as the intensity of exercise increased and also because the rate of pH recovery after exercise was normal. These authors suggested that the observed abnormality of muscle energy metabolism in PVFS is due to a disorder of metabolic regulation. The same investigators documented similar observations in a further group of patients with PVFS and postulated that persistence of virus in skeletal muscle may be responsible for the observed abnormalities (Arnold et al, 1984b, Taylor, 1989).

Riley et al. (1990) used a symptom limited exercise treadmill test paradigm to evaluate the aerobic work capacity in 13 patients with PVFS, 13 healthy control subjects and another group of controls consisting of 7 patients with the irritable bowel syndrome. The aerobic work capacity was assessed using clinical and laboratory criteria, such as heart rate, length of time on treadmill (an indication of fittness and exercise tolerance), serum lactate, blood glucose, creatine kinase, oxygen consumption and gas exchange before and at regular intervals during the test.

Patients with PVFS had a significantly higher heart rates at submaximal levels of exercise and also higher serum lactate concentrations than the control groups. These findings show that patients with PVFS have a reduced aerobic work capacity in comparison to controls. Furthermore, these patients perceived the work load at peak exercise to be greater than did the control subjects. In this study the end tidal CO2 concentration was normal at the begining and also at peak exercise which rules out the presence of hyperventilation in these patients.

Chapter two

THE HYPOTHALAMUS AND 5-HYDROXYTRYPTAMINE

a) Hypothesis and aims of the study:

The symptoms of PVFS were described in the previous chapter. They suggest dysfunction of brain structures which control circadian rhythms, food intake, water balance, gut motility, temperature regulation, cardiac function and mood and emotions. The control of all of these functions is regulated by the hypothalamus, and thus we undertook a study of hypothalamic function in patients with PVFS.

The hallmark of PVFS is overwhelming persistent or recurrent fatigue. As described in chapter one, the fatigue in this disorder is due to lack of central drive and is similar to that reported in patients with multiple sclerosis (Krupp et al, 1988) or Parkinson's disease (Critchley et al, 1991).

In addition to integration of visceral and endocrine function, the hypothalamus also influences drive, mood and behaviour. The control exerted by the hypothalamus on visceral and brain structures is mediated mainly via 5-HT and dopamine (see below). In theory, therefore, all the symptoms of PVFS could be explained by hypothalamic dysfunction. In an attempt to test this hypothesis we studied different aspects of hypothalamic function in a well-defined group of patients with PVFS. In this study we assessed the functional status of hypothalamic 5-HT receptors and evaluated the water metabolism and

melatonin secretion in these patients.

b) Structure and function of the hypothalamus:

The hypothalamus is a small structure situated below the hypothalamic sulcus. The anterior border of the hypothalamus is formed by the optic chiasm. Posteriorly it is bounded by the mamillary bodies. The median eminence of the hypothalamus forms the floor of the third ventricle.

Three groups of hypothalamic nuclei can be distinguished. These are:

1) the anterior group, i.e. the supraoptic and paraventricular nuclei;

2) the middle group which consists of the tuberal, arcuate, ventromedial and ventrolateral nuclei;

3) the posterior group.

The hypothalamus has extensive reciprocal fibre connections with all brain structures, including the cerebral cortex, the limbic system and brain stem. It also has a strong anatomical relationship with the pituitary gland by means of neural pathways and a system of portal blood vessels (Brodal, 1969).

Hypothalamic 5-HT receptors:

5-hydroxytryptamine (5-HT) or serotonin is a ubiquitous

neurotransmitter. 90% of the total body 5-HT is contained in the chromaffin cells of the small intestine and most of the remaining neurotransmitter is present in blood platelets. Less than 5% of the total body 5-HT is found in brain.

5-HT neurones, as mapped by immunocytochemical methods, are clustered along the midline in the pons and upper brain stem (raphe nuclei), the area postrema, locus ceruleus and around the interpeduncular nucleus.

Neurones of the raphe complex are the main source of brain 5-HT. Fibres from the raphe medianus largely project to limbic structures, whereas cells of the dorsal raphe complex send their fibres to the neostriatum, cerebral and cerebellar cortex. The hypothalamus receives serotoninergic fibres from the raphe complex. In addition, about 30% of hypothalamic 5-HT is produced locally by neurones and a group of specialised ependymal cells of the third ventricle (Brownstein et al, 1976, Brownstein, 1981).

Research in 5-HT receptor pharmacology is a rapidly advancing field and, although an attempt has been made to give a comprehensive review of the literature, this account is unlikely to be up to date in a few months' time.

In man, three different types of 5-HT receptors have

been identified so far and preliminary evidence suggests a possible fourth receptor type (Montgomery & Finberg, 1989). These are designated 5-HT1, 5-HT2, 5-HT3 and 5-HT4 receptors. 5-HT receptors are further subdivided into 5-HT1A, 5-HT1B, 5-HT1C and 5-HT1D subtypes (Bradley et al, 1986, Conn & Sanders-Bush, 1987). This classification is based on the anatomical distribution of these receptors, their possible physiological function and the selective binding of ligands to the diffrent receptor subtypes (see table 2/1). These receptor subtypes are present in brain and peripheral tissues, principally in platelets and smooth muscle of the gut and blood vessels.

5-HT1A receptors in brain are mainly presynaptic and are present on nerve terminals that release neurotransmitters other than 5-HT, e.g. dopamine, acetylcholine etc. Their function appears to be the control of release of these neurotransmitters. 5-HT1A receptors are also found in the internal carotid artery and its branches (Lancet, Editorial, 1989). 5-HT has an inhibitory action on all 5-HT1A receptors. Thus, it causes vasodilatation and increases intestinal peristalsis.

5-HT1B receptors have been described in rodents (Conn & Sanders-Bush, 1987). However, these receptors have not yet been found in human brain.

Brain 5-HT2 receptors are either inhibitory or excitatory and are probably responsible for most of the central effects of 5-HT. In the periphery these receptors are excitatory and their activation leads to contraction of smooth muscle in blood vessels and gut and also causes platelet aggregation. In brain these receptors are abundant in the hippocampus and frontal cortex (Hoyer et al, 1985). To date there are no selective drugs which act on these receptors. Ritanserin (which has agonist action on 5-HT2 receptors) also acts on dopamine receptors.

5-HT3 receptors are localised in the limbic system and gut. These receptors are indirectly involved in behaviour, memory and emotional responses. Barnes and collaegues (Barnes et al, 1989) have shown that 5-HT3 receptor antagonists enhance cholinergic transmission in the limbic system which is essential for memory formation and retrieval (Deutsch, 1971).

Despite the major advances in 5-HT receptor pharmacology in recent years, the pharmacological manipulation of these receptors has clinical application in only a few disorders at present. For example, 5-HT antagonists such as pizotifen and methysergide and more recently the 5-HT reuptake inhibitor, sumatriptan are used in the treatment of migraine, while cyproheptidine and ondansetron (Platt et al, 1992) are effective in reliefing the symptoms of the carcinoid syndrome. In

both cases these drugs' beneficial effect is achieved by blocking the peripheral actions of 5-HT. Interference with central 5-HT receptors with buspirine has been shown to be useful in the treatment of anxiety states (Cohn et al, 1986) and the premenstrual tension syndrome (Rickels et al, 1989).

In addition to the therapeutic use of drugs acting on 5-HT receptors, some of these agents, e.g. buspirone and metergoline, are now widely used to study the functional activity of 5-HT receptors in different psychiatric disorders. In this study we used buspirone to assess the hypothalamic 5-HT receptors in patients with PVFS. The rationale for using this agent is discussed in a later section.

Table 2/1

Properties of 5-HT receptor subtypes:

Receptor	agonist	antagonist
5-HT1A	8-OHDAP*	not available
	buspirone	
	sumatriptan	
5-HT1B**		
5-HT1C	mesulergine	
	metergoline	
	mianserin	
5-HT1D	ergot alkaloids	
	sumatriptan	
5-HT2	ritanserin	pizotifen
	ketanserin	methysergide
5-HT3	ICS 205-930***	ondansetron
		granisetron

* 8-hydroxy-N, N-dipropyl-2-aminotetralin

** 5-HT1B receptors have not yet been found in human brain.

*** 3-alpha-tropanyl-1H-indole-3-carboxylic ester

Functions of the hypothalamus:

Until relatively recently the function of the hypothalamus was thought to be limited to the integration and control of autonomic functions and the regulation of the secretory activity of the pituitary gland (Brooks, 1988). However, in recent years a wider role for the hypothalamus in neuroendocrine regulation has been appreciated and three more functions have been recognised. It is now well established that the hypothalamus is involved in emotional expression and adaptation to stress, consolidation of short term memory and also immune regulation.

i: The autonomic functions of the hypothalamus:

Experimental studies and clinical observations show an important role for the hypothalamus in the regulation of food intake and fluid balance. Ishibashi and collaegues studied the effects of neuropeptides on the feeding behaviuor in animals. These authors have shown that direct application of thyrotropin releasing hormone (Ishibashi et al, 1979a) or cholecystokinin (Ishibashi et al, 1979b) on ventromedial hypothalamic neurones of the rat has an anorexogenic effect. Similar observations were reported in patients with hypothalamic disease. For example, destruction of the lateral hypothalamic nuclei by tumours has been reported to cause a syndrome identical to anorexia nervosa (White & Hain, 1959,

Stricker & Anderson, 1980, Weller & Weller, 1982). By contrast, bilateral lesions of the venteromedial nucleus lead to hyperphagia and gross obesity (Reeves & Plum, 1969, Beal et al, 1981). Intrestingly, Kleine-Levin syndrome (Levin, 1936), which is characterised by hypothalamic hypogonadism, obesity, hyperphagia and increased somnolence, is thought to be due to hypothalamic dysfunction during puberty (Critchley, 1962). More recently, Carpenter et al (1982) have found inflammatory changes in the hypothalamus of a patient with this disorder. This is in accord with a previous postmortem report of a patient with a ventromedial hypothalamic neoplasm who presented with hyperphagia, behavioural changes and memory impairment (Reeves & Plum, 1969).

Hypothalamic-pituitary regulation of water balance:

50-60% of the total body weight of an adult is water. The total body water content is 5-10% more in females than in males. This depends largely on the differences in body fat content between the two sexes. Extracellular fluid (plasma, CSF, gastro-intestinal juices etc.) constitutes a third of the total body water content. The remaining two thirds is contained in the intracellular space. This ratio is kept constant thanks to the continuous fluid shifts between the extra and intracellular fluid copmartments. Similarly, the total body water content is maintained within a narrow range

by achieving a balance between fluids intake (via the thirst mechanism) and water loss, e.g. in urine and sweat. Water homeostasis is regulated by the hypothalamic-pituitary axis via the release of the antidiuretic hormone arginine vasopressin and the thirst mechanism.

Arginine vasopressin:

Arginine vasopressin (AVP) is a nonapeptide which is synthesised and released by the posterior lobe of the pituitary gland, i.e. the neurohypophysis. This hormone selectively increases the permeability of the collecting ducts of the nephron to water, thus enhancing the back diffusion of solute-free water. The end result of this effect is the production of concentrated urine.

The synthesis and release of AVP is regulated by a group of cells in the anterior hypothalamus known as the osmoreceptor. Immunocytochemical studies have shown that the perikarya of these neurones are the large cells in the supraoptic and paraventricular nuclei and the small cells in the dorsal pole of the suprachiasmatic nuclei of the hypothalamus (Gainer & Brownstein, 1981, Thasher, 1985). Fibres from these neurones project to the posterior lobe of the pituitary gland via the supraoptic hypophysial tract.

Hypothalamic osmoreceptors regulate AVP release in

response to changes in plasma osmolality. In addition, they are also sensitive to a number of other stimuli, including nausea (Rowe et al, 1979), cigarette smoking (Rowe et al, 1980), hypoglycaemia (Baylis et al, 1981) and some drugs, e.g. histamine, acetylcholine and catecholamines. Changes in the circulating blood volume also influence the sensitivity of the osmoreceptor via pressure sensitive receptors in the heart and the large blood vessels (Robertson, 1987).

Changes in the osmotic gradient across the cell membrane is the most potent stimulus of the osmoreceptor. Increase in plasma osmolality per se does not have any effects on the hypothalamic-pituitary axis. For example, a rise in osmolality due to a permeable solute such as urea does not stimulate AVP release, whereas impermeable solutes (sodium, mannitol) are potent stimuli for AVP secretion (Vokes et al,1987).

Although a one per cent change in plasma osmolality is sufficient to cause changes in circulating AVP concentrations, only a fall in plasma osmolality to 280 mosmol/kg or an increase to 295 mosmol/kg is considered a critical threshold for the functional "set" of the osmoreceptor (Kovacs & Robertson, 1992). At a plasma osmolality of 280 mosmol/kg the plasma AVP is often undetectable and the urine flow rate increases to its maximum (>10 ml/min) to prevent further dilution of body fluids. The opposite changes occur when plasma

osmolality approaches 295 mosmol/kg. These are summarised below (table 2/1).

Table 2/1

Plasma osmolality thresholds which alter the functional "set" of the osmoreceptor:

Plasma osmol mosmol/kg	urine osmol mosmol/kg	AVP pmol/l	thirst	urine flow rate ml/min
280	<100	v. low/ undetectable	no	>10
295	>800	0.5-7	yes	<0.5

The thirst mechanism:

The group of hypothalamic neurones which trigger thirst are adjacent to but completely distinct from those responsible for the regultion of AVP synthesis and release (Hammond et al, 1986). It is not surprising that the thirst "centre" responds to the same stimuli as the osmoreceptor. However, the osmotic threshold which triggers thirst exceeds that for AVP release by 10-15 mosmol/kg (Robertson, 1983). In other words, a healthy subject feels thirst only when the plasma osmolality exceeds 310 mosmol/kg.

The hypothalamus also plays an important role in the regulation of circadian rhythms, including

sleep-wakefulness cycles and reproduction in all animal species (Shilling et al, 1986) and also in man (see below).

ii: Memory and emotions:

Thanks to the close anatomical relationship of the hypothalamus with all parts of the limbic system, this organ plays an important role in the regulation of function of limbic structures. The hypothalamus receives extensive fibre connections from limbic structures, in particular the amygdaloid and septal complex and sends fibres to the brain stem and cerebral cortex, thus linking the limbic system to the reticular formation and cerebral cortex (Swanson & Sawchenko, 1983, Steriade & Llinas, 1988, Guyton, 1991).

Studies of the physiological responses to emotional stress have shown that expression of these responses in man consists of three inter-related reactions (Folkow, 1988). These are a situation-specific behavioural response, a somato-motor and an autonomic response. These responses are associated with metabolic and endocrine changes which provide the optimal milieu for the behavioural expression and are regulated by the hypothalamus.

In addition, the hypothalamus appears to have a more direct influence on human and animal emotional

reactions. For example, stimulation of the anterior ventral group of hypothalamic neurones in man evokes fear and anxiety (Monroe & Heath, 1954), whilst stimulation of postero-medial neurones causes obsessional behaviour (Sano, 1975). Stimulation of the lateral hypothalamus increases the animal's general activity. The opposite occurs with stimulation of the medial nuclei (Givens, 1984).

iii: Immune regulation and the hypothalamus:

The hypothalamus regulates the function of the immune system by two mechanisms. First, by exerting modulating influences on the limbic system and, secondly, by directly controlling the function of the sympathetic and parasympathetic systems.

The negative effects of severe emotional trauma on the human defence mechanisms and resistence to infections are well known. For example, it has been shown that bereavement is associated with transient impairment of function of T lymphocytes and increased susceptibility to infection (Bartrop et al, 1977). Sustained emotional stress may also increase the risk of disease by decreasing the numbers of circulating lymphocytes (Spry, 1972). In addition to these effects (which are probably mediated via the limbic system), experimental evidence suggests that the hypothalamus has a direct influence on immune regulation. Lesions of the anterior hypothalamic

nuclei have been shown to inhibit antibody production (Tyrey & Nalbandov, 1972) and decrease anaphylactic reactions (Schiavi et al, 1975).

iv: The regulation of neuroendocrine function:

Monoamine neurotransmitters and neuropeptides such as corticotropin releasing hormone and cholecystokinin (Ishibashi et al, 1979b) have been shown to mediate the function of hypothalamic neurones. In animal studies the local application of noradrenaline (Calogero et al, 1988a) or acetylcholine (Calogero et al, 1988b) onto the hypothalamus caused the secretion of corticotropin releasing hormone, whereas the application of gamma aminobuteric acid resulted in the opposite effect (Calogero et al, 1988c).

A detailed review of the effects of various neurotransmitters on neuroendocrine regulation is beyond the scope of this thesis. In this section we briefly summarise the effects of those neurotransmitters which influence prolactin release.

The control exerted by the hyopthalamus on pituitary lactotrope cells is mediated mainly by two different neurotransmitters. These are 5-HT and dopamine. Hypothalamic 5-HT fibres have been implicated in the control of various hypothalamic functions (Kato et al, 1974) as well as the stimulation prolactin (Lamberts &

MacLoed, 1978) and growth hormone release (Rolandi et al, 1982). By contrast, dopaminergic neurones inhibit prolactin release. Administration of specific dopamine agonists, e.g. bromocriptine and apomorphine (Mac Loed & Lehmeyer, 1974) suppresses prolactin release. On the other hand, drugs which deplete dopamine (reserpine, methyldopa, etc) or increase 5-HT concentrations (e.g. tryptophan) stimulate prolactin release. Similarly, 5-HT antagonists such as methysergide and metergoline (Gregory et al, 1990) block prolactin release. Thus, the use of pharmacological agents with specific effects on hypothalamic 5-HT or dopamine receptors and the serial measurements of serum prolactin concentrations may provide an accurate in vivo method of functional assessment of hypothalamic neurones.

c) The use of neuroendocrine challenge tests in the study of hypothalamic function:

The family of drugs known as the azapirones are well-tolerated agents which cause the release of prolactin, ACTH, cortisol and growth hormone via the selective stimulation of pre- and postsynaptic central 5-HT1A receptors (Tuomisto & Mannisto, 1985). These drugs include buspirone, gepirone and ipsapirone. The selectivity of the azapirones has been disputed. For example, McMillen et al (1983) have suggested that buspirone stimulated prolactin release by blocking dopamine receptors, while Meltzer and Fleming (1982)

have demonstrated mixed dopamine agonist-antagonist properties for buspirone. Another complicating factor is that in brain the azapirones metabolise to a piperazine derivative with alpha2 adrenergic receptor antagonist properties (Caccia et al, 1986). However, it is unlikely that the effects of the azapirones are mediated via adrenergic receptors. This is because 5-HT1A receptor antagonists such as Pindolol (Ceccaro et al, 1990, Cowen et al, 1990) and metergoline (Gregory et al, 1990) abolish the plasma prolactin increase produced by buspirone. Further evidence suggests that prolactin release is mediated by 5-HT pathways and is independent of dopaminergic function. For example, chemical or surgical destruction of central 5-HT pathways inhibits prolactin release (van de Kar & Bethea, 1982). The prolactin releasing effect of fenfluramine can be abolished by pretreatment with the 5-HT antagonist, cyproheptidine (Lewis & Sherman, 1985). Similarly, ritanserin (5-HT2 receptor antagonist) inhibits prolactin release in females whose dopamine 2 receptors were blocked by sulpiride (Falaschi et al, 1989). Although buspirone has effects on both dopaminergic and 5-HT receptors, the current evidence suggests that the prolactin response to buspirone is largely mediated via 5-HT1A receptors.

The azapirone most widely used in psycopharmacology research is buspirone. Following oral administration of this drug the serum prolactin levels rise in a

dose-dependent fashion and reach their peak one hour after the dose (Seppala et al, 1987). This effect was not observed with chronic administration of therapeutic doses of buspirone to healthy subjects or patients with anxiety (Tollefson et al, 1989).

The administration of a single oral dose of buspirone and serial measurements of serum prolactin is now accepted as a simple and reliable method of studying central 5-HT receptors. As discussed earlier, the symptoms of PVFS suggest hypothalamic dysfunction. Many patients with PVFS suffer from conditions such as migraine, fluid retention syndrome and the irritable bowel syndrome in which 5-HT metabolism is known or suspected to be abnormal (see chapter one). This is suggestive of a common underlying pathogenetic mechanism for all of these disorders. We decided, therefore, to study the function of hypothalamic 5-HT neurones in a well-defined group of patients with PVFS and compared them with healthy control subjects and patients with primary depressive illness.

d) The pineal gland and melatonin:

Study of the pineal gland and its hormone melatonin may give important clues as to the pathogenesis of PVFS. Experimental evidence suggests that melatonin has effects on sleep regulation, alertness, mood and gonadal function. The effects of the diurnal variations of

plasma melatonin concentrations on fatigue are also well documented.

The pineal gland is the main site of melatonin synthesis and release (Lesnick et al, 1985). It also has the highest concentration of 5-HT in brain (Brownstein, 1981, Vaughn, 1984). The synthesis and release of melatonin is regulated by the hypothalamus via two neural circuits: fibres from the superior cervical ganglion and the retino-hypothalamic tract (Moore, 1978, Moore & Klien, 1984).

The main sites of action of melatonin are thought to be the hypothalamus and brain stem. These areas have the highest density of melatonin receptors as shown by radioactive melatonin uptake studies (Anton-Tay & Wurtman, 1969, Bittman & Weaver, 1990).

Current evidence suggests that the effect of melatonin on hypothalamic neurones is mediated via 5-HT release. For example, an intraperitoneal injection of melatonin results in a rapid rise in brain 5-HT concentrations (Fraschini et al, 1971), whereas direct infusion of melatonin into the pituitary gland has no effect (Kamberi et al, 1971). In the rat subcutaneously injected melatonin binds mainly to hypothalamic neurones (Lang et al, 1981). Furthermore, 5-hydroxyindoleacetic acid: 5-HT ratio (an index of 5-HT turnover) in hypothalamic neurones of blinded hamsters is increased

(Vriend, 1989), suggesting an important role of hypothalamic 5-HT neurones in the control of behaviour mediated via the photoneuroendocrine system.

Melatonin and sleep:

Under physiological conditions darkness increases endogenous melatonin secretion and induces sleep (Sander et al, 1972, Wetterberg, 1978). The reverse is also true (Hansen et al, 1979). Similarly, administration of melatonin to healthy subjects (Anton-Tay et al, 1971, Armstrong et al, 1982, Arendt et al, 1985a) and to patients with chronic insomnia (Beck-Friis et al, 1984, Lieberman et al, 1984) induces sleep.

Melatonin and mood:

In 1979 Wetterberg and co-workers drew attention to the possible effects of melatonin on mood when they demonstrated abnormal melatonin levels in a patient with major depressive illness (Wetterberg et al, 1979). Subsequently, a strong correlation was found between the incidence of depression and the relatively short hours of day light in winter, while hypomania occured mostly in the spring as the day lengthened (Weller & Jauhar, 1981, Parker & Walter, 1982, Rosenthal et al 1984). Furthermore, high plasma melatonin levels in hypomanic patients and low concentrations in patients with depression have been reported by many investigators (for

review see Checkley & Arendt, 1984).

v: Melatonin and endocrine function:

The role of melatonin in animal reproductive physiology has been known for many years (see Lincoln, 1983). In humans melatonin delays the onset of puberty and inhibits the maturation of sex organs and pineal tumours may cause sexual precocity (Ringertz et al, 1954, Martin & Reichlin, 1987). Variations in plasma melatonin concentrations throughout the menstrual cycle have also been reported (Wetterberg et al, 1976).

Melatonin and fatigue:

A close correlation between the incidence and severity of fatigue in healthy subjects and changes in the concentration of urinary melatonin has been established (Wetterberg, 1978). A similar relationship between fatigue and disruption of melatonin circadian rhythms, e.g. following air travel over several time zones (jet lag syndrome) or when light-darkness cycles were increased or decreased under experimental conditions, has also been reported (Arendt & Marks, 1982, Arendt et al, 1985a). Interestingly, the fatigue of the jet lag syndrome can be alleviated by manipulating the circadian rhythms with exogenous melatonin (Arendt et al, 1986).

CHAPTER THREE

MATERIALS AND METHODS

Materials & methods

As can be seen from the preceding discussion, all the symptoms of PVFS could be explained as due to hypothalamic dysfunction. We therefore devised a set of three experimental studies to test various aspects of this part of the central nervous system. The experiments were:

Experiment 1: study of the functional status of hypothalamic 5-HT neurones

Experiment 2: study of water metabolism, including the total body water in patients and arginine-vasopressin secretion. Two patients with PVFS who had severe fluid retention were also studied during the various phases of the menstrual cycle

Experiment 3: study of melatonin excretion

Materials:

Selection of patients:

The following criteria were used in making the diagnosis of PVFS:

1) the symptoms of PVFS were preceded by an acute viral illness in all patients.

2) all patients had fatigue for at least six months and the level of fatigue was such that it reduced the patient's premorbid level of activity by 50% or more. The fatigue in these patients is made worse by exercise and is not affected by bed rest

3) in addition to the typical chronic fatigue, patients had three or more of the following symptoms: myalgia, sleep disturbances (insomnia, excessive sleep or reversed sleep pattern), depression, excessive sweating, constipation alternating with diarrhoea, palpitations and unsteadiness, menstrual irregularities and minor fluctuations in body temperature.

4) lack of co-morbidity: none of the patients had clinical or laboratory evidence of chronic illnesses, e.g. infection, malignancy, auto-immune disease, metabolic or endocrine disturbances.

5) none of the patients had a past or family history of a major psychiatric illness and all patients had a good work record and a well-adjused premorbid personality as shown by the patients' previous ability to cope with everyday life stress.

In addition to these strict diagnostic criteria, an objective confirmation of the organicity of the patients' illness was sought. All patients but one had a needle muscle biopsy from the vastus lateralis of the

quadriceps femoris muscle. The biopsy was processed for routine and electron microscopy and also for detection of genomic RNA sequences using the polymerase chain reaction.

Experiment 1: study of hypothalamic 5-HT receptors in patients with PVFS:

Patients and control subjects:

15 consecutive patients admitted to the Institute of Neurological Sciences between January and June 1991 for the diagnostic work-up of postviral fatigue syndrome gave an informed consent to participate in the study. There were 9 males and 6 females. The mean age of males was 39.8 yrs (range 29-59) and that of females was 33.1 yrs (range 25-40). The demographic characteristics of these patients are given in table 3/1.

Table 3/1

The demographic characteristics of patients with PVFS:

Age (yrs)	sex	duration of PVFS (yrs)	p.c.r*	mitochondrial damage**
34	M	3	-	++
39	M	10	+	-
59	M	2	-	++
49	M	5	-	++
42	M	2	-	++
32	M	1	+	+
30	M	4	+	++
29	M	1.5	+	-
45	M	2	+	++
28	F	2.5	-	+
39	F	3	+	++
40	F	6	NA	NA
28	F	1.5	+	-
25	F	2	+	-
39	F	2	-	+

* p.c.r. = polymerase chain reaction

** + = mild, ++ = moderately severe mitochondrial damage

NA = data not available

The control subjects consisted of two groups - a group of healthy volunteers and another one of patients with primary depressive illness. The healthy volunteers were recruited from the laboratory staff. There were 7 males and 6 females. The mean age of males was 36 yrs and that of females was 30.6 yrs.

The group of patients with primary depressive illness consisted of 7 males and 6 females with a mean age of 35 and 31.1 yrs, respectively. Depressive illness was diagnosed according to the DSM-III-R criteria (American Psychiatric Association, 1987) and all patients had a Hamilton depression score of more than 20 (Hamilton, 1960).

None of the patients or control subjects was taking any medication for at least six weeks prior to the study. Female patients who were menstruating normally were studied during the luteal phase of the menstrual cycle (see discussion).

Methods:

Patients and control subjects were fasted overnight. At 8:30 am the following morning a canula was inserted into the anticubital vein of the non-dominant arm and a blood sample for baseline prolactin levels was taken 15 minutes later. Subjects were then given a single oral dose of 60 mgs of buspirone at 9 am. Further blood

samples were collected hourly for three hours. Subjects remained in the recumbent position during the test.

Prolactin levels were measured blind to diagnosis by immunometric assay. Assays were standardised against NIBSC Third International Standard 84/500. The within and between batch co-efficient of variation were 3% and 6%, respectively over the concentration range 200 to 3000 mU/l.

The response to buspirone was determined by subtracting baseline from peak prolactin levels and the latter value was expressed as a percentage of baseline prolactin. For reasons discussed later the data for males and females were analysed seperately.

The statistical analysis of data was performed using Minitab Statistical Software (release 7.2).

Experiment 2: study of water metabolism in patients with PVFS:

i: The pilot study

We carried out a pilot study in which patients with PVFS were challenged with the water loading test under standard conditions as described by Robinson et al. (1941) and Soffer & Gabrilove (1952).

We selected 9 patients with PVFS and 8 healthy control subjects for this study. The patients and control subjects were matched for body weight. The patients' selection was based on the diagnostic criteria described above. All patients had evidence of mitochondrial damage consistent with PVFS (see chapter one) and enteroviral genomic RNA was detected with the polymerase chain reaction in muscle of these patients. There were 3 male and 6 female patients. The mean age for males was 38.6 and that of females was 34.3 years. The control group consisted of 4 males and 4 females with a mean age of 27 and 26 years, respectively. All patients and control subjects had normal blood urea and electrolytes and a random blood glucose result. None of the patients or control subjects was taking any medication for four weeks prior to the study. Smoking was not allowed for the duration of the study.

All subjects fasted from mid night. At 8 am the following morning they were asked to empty their bladders and the urine passed was discarded. The body weight of each subject was then recorded. Subjects were then given 20 ml/kg body weight of tepid water to drink over 45 minutes. To eleminate the effects of posture and exercise on urine excretion the test was carried out with subjects in the recumbant position. However, they were allowed to sit or stand up when voiding. Urine was collected hourly for four hours.

Patients with PVFS excreted 64.6 ± 8.2% (mean ± SD) of the water load, compared to 96.9 ± 26.2% in healthy control subjects (raw data in tables 3/2 & 3/3). The difference between the two groups is statistically significant (Student's t test, $p < 0.01$). We therefore decided to study water metabolism in greater detail in a well-defined group of patients with PVFS.

The study consists of three parts. In the first part we measured the total body water content in nine randomly selected patients with PVFS. For the second part of the study we identified two female patients with PVFS who exhibited features of the fluid retention syndrome. Water metabolism in these patients was studied at various stages of the menstrual cycle. In the third part of the study we evaluated the hypothalamic-pituitary regulation of vasopressin release in patients with PVFS using the water loading and water deprivation tests. Details of these studies are now described.

Table 3/2

Water loading test in nine patients with PVFS

Patient	age (yrs)	sex	duration of PVFS (yrs)	% urine output of water load
1.	41	F	5	63
2.	39	F	15	56
3.	49	M	10	69
4.	36	F	9	70
5.	29	M	9	60
6.	45	F	4	50
7.	38	M	2	73
8.	24	F	1	75
9.	21	F	2	66

Table 3/3

Water loading test in 8 healthy control subjects

Subj.	age (yrs)	sex	water load (ml)	% urine output of water load
1	30	M	1,350	96.2
2	30	F	2,000	98.7
3	26	F	1,350	111.4
4	28	M	1,400	81.4
5	24	F	1,000	107
6	24	F	1,500	115
7	26	M	1,400	40.7
8	24	M	1,625	125

ii: measurement of total body water and total body potassium in patients with PVFS:

Patients: 9 patients with PVFS gave an informed consent to take part in this study. There were 4 males and 5 females. The demographic characteristics of these patients are given in table 3/4.

Methods: Total body water was measured by tracer dilution in plasma samples taken 4 hours after an oral dose of 4 MBq tritiated water as described by Poon et al (1989). The measured values were compared with published predicted values based on height, weight, age and sex (Skrabal et al, 1973).

Total body potassium was measured using a whole body monitor to assess the body content of the naturally occuring radio-isotope of potassium, i.e. potassuim 40. The scanner type whole body monitor has sodium iodide detectors and is calibrated to take account of the differences in radiation absorption by subjects of varying size and body fat content (Runcie & Hilditch, 1974). The measured values were also compared with predicted values available from standard charts (Boddy et al, 1972).

Table 3/4

The demographic characteristics of patients selected for measurements of total body water and potassium content

Patient	age(yrs)	sex	height(cm)	weight(kg)
1.	66	M	169.5	67.0
2.	19	M	184.0	65.5
3.	57	M	176.5	76.5
4.	27	M	168.5	72.4
5.	37	F	150.5	51.9
6.	23	F	169.5	61.9
7.	45	F	159.0	49.1
8.	44	F	159.0	86.4
9.	27	F	162.5	52.3

iii: study of total body water content in female patients with PVFS and severe symptoms of fluid retention:

For this part of the study we selected two female patients with PVFS (not included in the first part of the study) who exhibited gross features of fluid retention. The case history of one of these patients is given in page 39. The second patient is a 46 year old woman who had PVFS for 10 years.

Baseline total body water, total body potassium and glomerular filtration rate were measured in these patients in the follicular and repeated in the luteal phase of the menstrual cycle using the methods described above. In addition, the extracellular and intracellular water content were also measured in the first patient in both phases of the menstrual cycle. In the second patient it was possible to measure these values only in the follicular phase of the cycle because of technical difficulties. The results are expressed in the standard way as percentage of the predicted values.

iv: study of arginine-vasopressin release in patients with PVFS:

Patients: nine patients with PVFS and eight healthy control subjects gave an informed consent to participate in the study. The demographic characteristics of

patients and controls are given in table 3/5. The diagnosis of PVFS was based on the criteria described above.

Table 3/5

Water loading and water deprivation tests: the demographic characteristics of patients with PVFS and control subjects.

	Patients	controls
Males	4	4
Females	5	4
mean age (yrs)	38.3	26.5
mean duration of illness	6.2 yrs	

Methods:

Arginine-vasopressin (AVP) secretion in response to changes in plasma osmolality was assessed using the water loading and water deprivation tests.

Water loading test:

Patients were fasted from mid night and at 8 a.m. the following morning their body weight was recorded and a canula was inserted into the antecubital vein of the non-dominant arm. A blood sample for baseline measurements of blood urea and electrolytes, blood glucose and plasma AVP was then taken. Baseline plasma and urine osmolality were also measured. Subjects were then given 20 ml/kg body weight of tepid water to drink

over 30-45 minutes; urine and blood samples were then collected hourly for four hours.

Water deprivation test:

This was carried out on a seperate day. Patients were fasted from mid night till 3 pm the following day. Samples for urea and electrolytes, plasma osmolality and AVP and urine osmolality were taken at 9 am and every two hours thereafter.

Urine and plasma osmolality were determined by depression of freezing point. Blood samples for AVP assay were centrifuged at 4 ^{o}C within 30 minutes of venosection and were stored at - 70 ^{o}C. Because AVP binds to platelets (Preibisz et al, 1983), the plasma immediately above the sediment was not removed. The plasma AVP was measured by the immunoassay method described by Morton et al. (1985).

Experiment 3: study of melatonin excretion in patients with PVFS:

Patients and control subjects:

Eight patients with PVFS (2 males and 6 females) and six age and sex-matched control subjects (2 males and 4 females) were recruited for the study. Patients with PVFS were selected according to the diagnostic criteria described earlier. The control subjects were patients

with various neurological conditions. All control subjects complained of excessive fatigue but none of them had a viral illness in the six months preceding the study. None of the patients or control subjects was on any medication during or at least four weeks prior to the study. The demographic characteristics of patients and control subjects are given in table 3/5.

Table 3/5

Melatonin in PVFS: the demographic characteristics of 8 patients with PVFS and 6 control subjects.

Patients with PVFS:

No	age	sex	duration of PVFS (yrs)
1	40	F	3
2	40	F	4
3	28	F	1.5
4	46	M	6
5	35	F	2
6	34	F	1
7	44	M	3
8	29	F	

Control subjects:

No	age	sex	diagnosis
1	55	F	mixed connective tissue disease
2	47	M	proximal myopathy
3	34	F	possible multiple sclerosis
4	47	F	non-metastatic breast carcinoma
5	39	M	spastic paraparesis ? cause
6	32	F	multiple sclerosis

Methods:

Patients and control subjects were admitted to hospital for the duration of the study. They followed their normal daily routine and were allowed to go to bed whenever they wished to do so. Urine for melatonin measurements was collected continuously every four hours for 48 hours. The amount of urine passed every four hours was recorded and then a 20 ml sample was taken and the rest of urine was discarded. The urine samples were stored immediately after collection in -70 °C until analysed.

Urine was analysed for 6-sulphatoxy melatonin using the immunoassay method described by Arendt et al (1985b). 6-sulphatoxy melatonin is the main metabolite of melatonin and its concentration in urine correlates closely with plasma melatonin levels (Matthews et al, 1991). Thus, measuring urinary 6-sulphatoxy melatonin concentrations over a period of time is as reliable as serial plasma melatonin estimations in evaluating pineal function. In addition, it has the advantage of being more convenient for patients than plasma sampling.

The urinary melatonin excretion over each 4 hour period in day one was added to that of the corresponding period in day two and the sum was divided by two to obtain the mean urinary melatonin excretion for that period. The urinary melatonin excretion in patients with PVFS was

then compared with that of control subjects using analysis of variance for repeated measures (ANOVA).

CHAPTER FOUR

RESULTS

Results

a) Buspirone challenge test:

All patients and control subjects completed the study satisfactorily. The administration of buspirone caused excessive fatigue and lightheadedness in patients but not in the control groups.

Electron microscopy of muscle biopsy showed mitochondrial changes similar to those previously described in patients with PVFS (Gow et al, 1990) in all patients studied and RNA enteroviral sequences were detected with the polymerase chain reaction in 6 patients.

The mean prolactin values before and after the oral administration of 60 mgs of buspirone are given in tables 4/1 & 4/2 and are graphically shown in figures 4/1 and 4/2. Summary statistics are shown in tables 4/3 and 4/4.

Peak prolactin values occured at one hour in patients with PVFS and in female patients in the control groups and at two hours in healthy and depressive males. As shown in table 4/1 and 4/2 there was no statistically significant difference in baseline prolactin values between patients with PVFS, healthy individuals and patients with primary depressive illness. Similarly, comparison between patients with PVFS and each of the

control groups did not reveal a statistically significant difference (PVFS vs healthy subjects: $p = 0.6$ for males and 0.09 for females, PVFS vs depressives: $p = 0.9$ and 0.2 for males and females, respectively). However, the percentage difference between peak and baseline prolactin levels was significantly higher in patients with PVFS than in healthy control subjects and patients with primary depression (see table 2/6). There was also a statistically significant difference when patients with PVFS were compared with each control group seperately (PVFS vs healthy subjects: $p = 0.02$ for males and 0.01 for females, PVFS vs depressives: $p = 0.01$ for males and 0.004 for females).

Table 4/1

Prolactin response (mU/L) to 60 mgs of buspirone in 7 healthy males, 7 male patients with primary depressive illness and 9 male patients with PVFS:

No	baseline	1	2	3 hrs	difference*
Healthy control subjects:					
1.	90	199	186	144	121
2.	151	364	492	286	225
3.	212	512	501	476	141
4.	112	496	473	475	342
5.	160	320	310	180	100
6.	220	460	456	381	109
7.	196	396	290	282	102
Patients with depressive illness:					
1.	180	660	570	531	266
2.	164	424	411	168	159
3.	244	264	320	300	31
4.	86	186	186	120	116
5.	188	210	440	286	134
6.	210	318	306	215	51
7.	184	164	384	216	108

* the percentage difference between peak and baseline prolactin concentrations.

Table 4/1 (continued)

Patients with PVFS:

No	baseline	1	2	3	difference
1.	220	550	340	350	150
2.	280	820	400	220	192
3.	88	890	370	210	911
4.	140	680	350	210	385
5.	150	780	390	220	420
6.	89	230	210	180	158
7.	180	950	600	320	427
8.	160	1300	850	670	712
9.	300	1740	900	490	480

Table 4/2

Prolactin response (mU/L) to 60 mgs of buspirone in 6 healthy females, 6 female patients with primary depression and 6 female patients with PVFS:

Nc	baseline	1	2	3hrs	difference*
Healthy females:					
1.	180	400	420	360	133
2.	160	680	684	540	327
3.	80	556	550	424	595
4.	290	1590	1386	1111	448
5.	200	660	556	486	230
6.	220	461	492	451	123
Patients with primary depression:					
1.	155	557	387	385	259
2.	112	386	386	299	244
3.	280	684	681	493	144
4.	264	1268	1118	760	380
5.	298	584	580	410	95
6.	256	1376	1300	720	437

* the percentage difference between peak and baseline prolactin concentrations

Table 4/2 (continued)

Female patients with PVFS:

No	baseline	1	2	3 hrs	difference
1.	350	3060	1140	940	774
2.	360	5300	3310	1134	1100
3.	160	1210	820	390	656
4.	290	1980	650	320	582
5.	180	2000	1070	540	1011
6.	620	2520	1210	630	306

Table 4/3

summary statistics - mean (standard deviation) of prolactin response to 60 mgs of buspirone in healthy subjects, patients with depressive illness and patients with PVFS:

Males:

Time	healthy subj.	depressives	PVFS
baseline	163 (49)	179 (48)	178 (65)
1 hr	392 (110)	318 (175)	882 (434)
2 hrs	401 (116)	373 (120)	490 (240)
3 hrs	317 (132)	262 (134)	318 (164)
% differ.	163 (49)	123 (77)	426 (256)

Females:

	healthy subj.	depressives	PVFS
baseline	188 (69)	227 (75)	326 (166)
1 hr	725 (438)	809 (410)	2678 (1425)
2 hrs	681 (356)	742 (384)	1367 (975)
3 hrs	562 (276)	511 (188)	659 (318)
% differ.	309 (186)	260 (131)	738 (292)

Table 4/4

One-way analysis of variance of baseline prolactin values and the percentage diffrence between peak and baseline prolactin concentration in healthy subjects, patients with depressive illness and patients with PVFS.

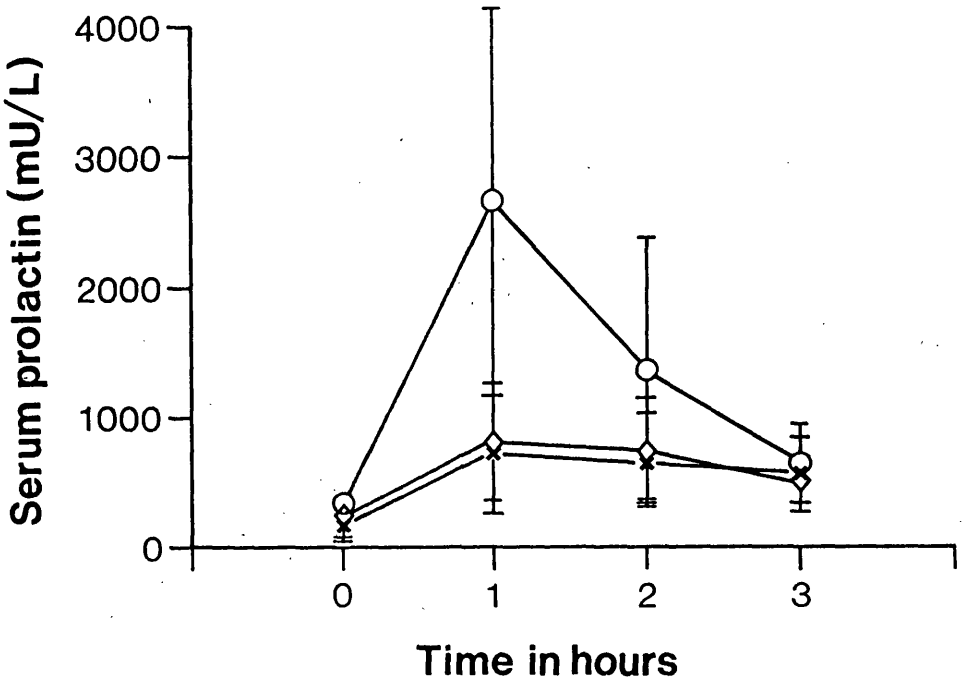
Males:

	DF	SS	MS	F	P
Baseline	2	1240	620	0.17	0.848
% differ	2	444079	222040	7.29	0.004

Females:

baseline	2	61008	30504	2.40	0.125
% differ	2	828671	414336	9.07	0.003

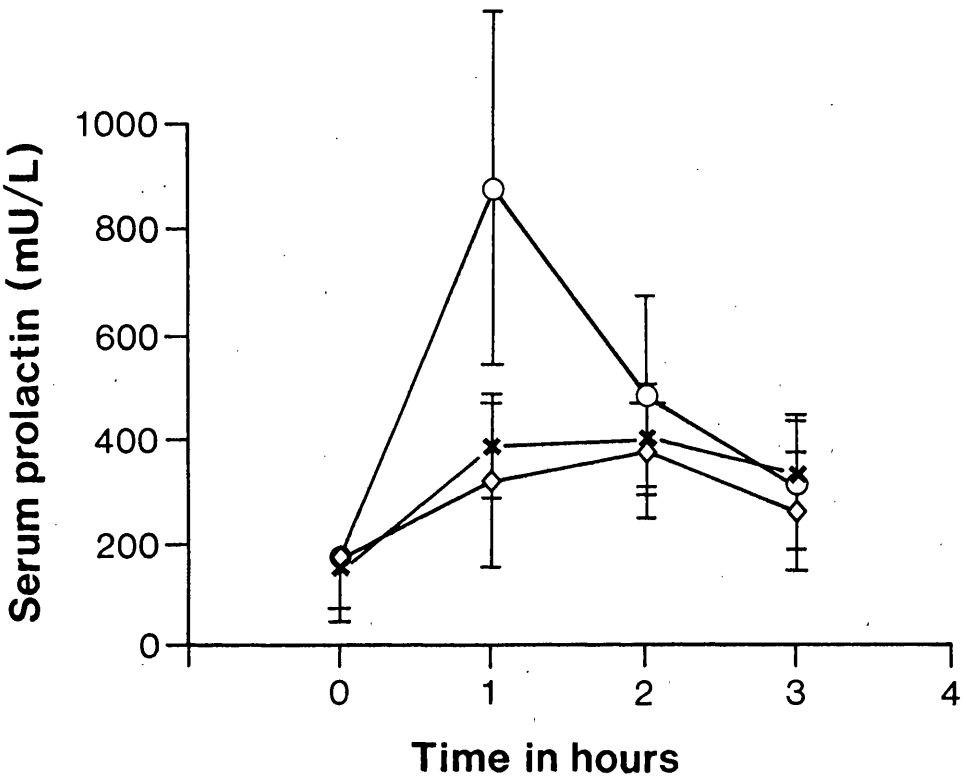
Figure 4/1



Prolactin response to 60mg of buspirone in 6 females with PVFS (—○—) and an equal number of healthy subjects (—×—) and patients with primary depression (—◇—).

Points and bars represent mean and 95% confidence intervals.

Figure 4/2



Prolactin response to 60mg of buspirone in 9 male patients with PVFS (—○—) 7 healthy subjects (—×—) and 7 patients with primary depression (—◇—).

Points and bars represent mean and 95% confidence intervals.

b) Water metabolism in patients with PVFS:

i: total body water and potassium:

The raw data of the total body water and potassium content in patients with PVFS and the predicted values are given in table 4/5 and 4/6.

The total body water for the group of patients with PVFS was 5.3% higher than predicted, however, no individual result fell outwith the normal range of 84-116% of the predicted value. Because the predicted total body water values (TBW) depend on body weight which will increase with water retention, expressing the measured TBW as a percentage of the predicted TBW underestimates water retention by about 30%.

The mean total body potassuim was 9% lower than predicted, with one result falling outwith the normal range, i.e. 84-116% the predicted value.

Table 4/5

Measured and predicted total body water (TBW) content in nine patients with PVFS:

Patient (age/sex)	height (cm)	weight (kg)	total TBW (litres)	% predicted TBW
66M	169.5	67.0	37.5	107
19M	184.0	65.5	43.5	101
57M	176.5	76.6	42.1	104
27M	168.5	72.4	45.0	108
37F	150.5	51.9	27.0	107
23F	169.5	61.9	35.8	114
45F	159.0	49.1	27.1	103
44F	159.0	86.4	36.5	104
27F	162.5	52.3	30.9	107

Table 4/6

Measured and predicted total body potassium content in nine patients with PVFS

Patient (age/sex)	height (cm)	weight (kg)	TBK (mmol)	% predicted TBK
66M	169.5	67.0	2663	88
19M	184.0	65.5	3507	87
57M	176.5	76.6	2914	81
27M	168.5	72.4	3769	105
37F	150.5	51.9	1850	89
23F	169.5	61.9	2761	99
45F	159.0	49.1	1844	85
44F	159.0	86.4	2445	90
27F	162.5	52.3	2625	107

ii: total body water content in female patients with PVFS with severe symptoms of fluid retention

As shown in the table below (table 4/7), the total body water content was greater in the luteal phase of the menstrual cycle than in the follicular phase in both patients. In one patient it was possible to measure the intracellular and extracellular water distribution in both phases of the menstrual cycle. In this patient the intracellular water content was 91% of the predicted value in follicular phase of menstruation and 111% of the predicted value in the luteal phase.

Table 4/7

Total body water (TBW) and extracellular (ECW) and intracellular water (ICW) content in two patients with PVFS who exhibited severe symptoms of fluid retention during the follicular and the luteal phases of the menstrual cycle:

Patient 1

	Follicular phase		luteal phase	
	measured	% predicted	measured	% predicted
TBW	35.0 L	94%	38.6 L	102%
ECW	18.1 L	97%	17.6 L	93%
ICW	16.9 L	91%	20.9 L	111%

Patient 2

TBW	32.7 L	93%	33.6 L	96%
ECW	16.2 L	90%	*	*
ICW	16.5 L	95%	*	*

iii: arginine vasopressin secretion in patients with PVFS

All patients and control subjects completed this part of the study satisfactorily. Nausea or vomiting was not reported and blood pressure remained unchanged throughout the test.

Baseline blood urea and electrolytes and blood glucose were normal in all patients and controls. The urine and plasma osmolality and the corresponding plasma AVP concentrations during the water loading and the water deprivation tests in patients and healthy controls are given in tables 4/8 - 4/11 and are graphically shown in figures 4/3 and 4/4.

These results show that AVP values were significantly lower in patients with PVFS when compared with healthy control subjects. The mean ± standard error of the mean (SEM) in control subjects was 0.9 ± 0.2 pmol/l, whereas the corresponding values for patients with the PVFS were 0.1 ± 0.03 pmol/l (Student's t test, $p = 0.001$). Furthermore, there was also a clear difference between the two groups during the water loading and water deprivation tests.

As shown in figures 4/3 and 4/4 the plasma AVP levels correlated with the serum and urine osmolality in healthy controls. However, the plasma AVP values in

patients with PVFS were consistently low at high urine and plasma osmolality concentrations. This suggests either a global deficit of AVP secretion or an abnormal hypothalamic response to osmotic stimuli in patients with PVFS (see discussion).

Table 4/8

Urine and plasma osmolality (mmol/kg) and the corresponding plasma arginine-vasopressin (AVP) concentration (pmol/l) in nine patients with PVFS during the water loading test

Patient/time		baseline	1	2	3	4 hrs
1.	urine	440	220	95	83	107
	plasma	*	282	283	283	287
	AVP	0.03	0.03	0.25	0.03	0.06
2.	urine	758	183	62	103	401
	plasma	282	286	286	285	*
	AVP	0.27	0.04	0.04	0.20	0.17
3.	urine	905	178	66	78	271
	plasma	280	284	283	283	*
	AVP	0.33	0.29	0.28	*	0.13
4.	urine	840	759	116	87	178
	plasma	281	282	286	282	*
	AVP	0.28	0.29	0.2	0.16	0.03
5.	urine	509	280	64	125	438
	plasma	*	278	283	287	288
	AVP	0.06	0.06	0.04	0.1	0.1

Table 4/8 (continued)

Patient/time		baseline	1	2	3	4 hrs
6.	urine	727	201	46	46	86
	plasma	276	278	282	283	*
	AVP	*	0.17	0.31	0.17	0.22
7.	urine	496	575	87	73	178
	plasma	*	280	288	282	288
	AVP	0.12	0.25	0.25	0.14	*
8.	urine	431	400	73	64	202
	plasma	*	281	284	287	287
	AVP	0.03	0.38	0.10	0.18	0.01
9.	urine	690	724	310	241	299
	plasma	*	278	276	275	273
	AVP	0.20	0.24	0.14	0.10	0.03

Table 4/9

Urine and plasma osmolality (mmol/kg) and the corresponding plasma arginine-vasopressin (AVP) concentration (pmol/l) in eight healthy control subjects during the water loading test

Subject/time		baseline	1	2	3	4 hrs
1.	urine	846	70	63	230	*
	plasma	285	279	275	280	278
	AVP	1.61	0.16	0.03	0.25	0.17
2.	urine	1039	128	221	703	*
	plasma	287	281	279	280	279
	AVP	1.44	0.11	*	0.29	0.13
3.	urine	517	73	120	238	*
	plasma	287	283	281	282	281
	AVP	1.04	0.23	0.08	0.04	0.17
4.	urine	683	103	71	81	*
	plasma	277	274	270	272	275
	AVP	1.49	*	0.11	0.04	0.19
5.	urine	505	63	74	312	*
	plasma	284	278	277	280	282
	AVP	0.19	*	0.05	0.08	0.23

Table 4/9 (continued)

Subject/time		baseline	1	2	3	4 hrs
6.	urine	705	116	63	94	*
	plasma	282	275	271	273	283
	AVP	1.07	0.34	0.5	0.29	0.27
7.	urine	720	176	78	79	202
	plasma	283	275	275	277	277
	AVP	0.12	*	0.02	0.01	*
8.	urine	1173	268	69	68	277
	plasma	289	283	283	289	277
	AVP	0.96	0.11	0.04	0.17	0.27

Table 4/10

Urine and plasma osmolality (mmol/kg) and the corresponding plasma arginine-vasopressin (pmol/l) in nine patients with PVFS during the water deprivation test

Patient/time		09	11	13	15 hrs
1.	urine	207	240	424	637
	plasma	288	289	289	290
	AVP	0.03	0.06	0.06	0.10
2.	urine	813	794	400	282
	plasma	291	286	285	285
	AVP	0.27	0.01	0.12	0.06
3.	urine	917	637	787	703
	plasma	284	287	283	285
	AVP	0.33	0.41	0.28	0.19
4.	urine	207	219	640	*
	plasma	292	285	*	*
	AVP	0.28	0.38	0.19	*
5.	urine	221	308	514	796
	plasma	288	289	290	279
	AVP	0.06	0.05	0.05	0.11

Table 4/10 (continued)

Patient/time		09	11	13	15 hrs
6.	urine	940	796	861	757
	plasma	286	282	288	284
	AVP	*	0.26	0.26	0.26
7.	urine	657	411	688	694
	plasma	293	290	288	287
	AVP	0.12	0.18	0.18	0.22
8.	urine	878	306	395	291
	plasma	294	296	292	290
	AVP	0.03	0.32	0.26	0.44
9.	urine	698	375	451	427
	plasma	285	290	290	291
	AVP	0.20	0.09	0.04	0.01

Table 4/11

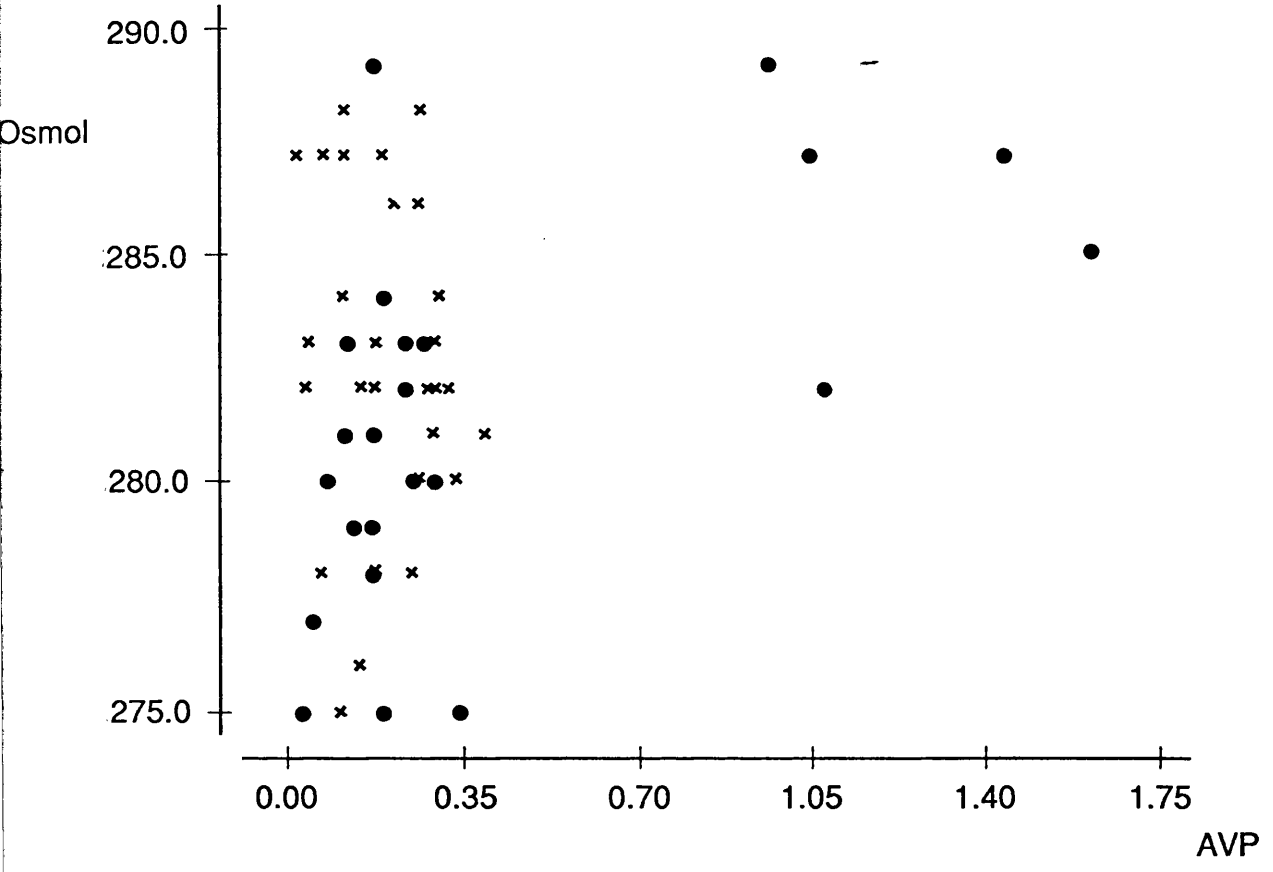
Urine and plasma osmolality (mmol/kg) and the corresponding AVP concentration (pmol/l) in eight healthy control subjects during the water deprivation test.

Subject/time		9	11	13	15 hrs.
1.	urine	846	876	920	*
	plasma	278	282	285	280
	AVP	1.60	2.4	0.6	0.25
2.	urine	1050	912	904	*
	plasma	287	287	285	285
	AVP	1.40	1.19	0.5	0.42
3.	urine	*	700	647	723
	plasma	*	278	277	278
	AVP	*	0.36	0.19	0.19
4.	urine	900	920	789	617
	plasma	277	276	278	276
	AVP	1.20	0.86	0.09	0.12
5.	urine	684	918	761	856
	plasma	280	281	281	276
	AVP	0.18	0.22	0.11	0.14

Table 4/11 (continued)

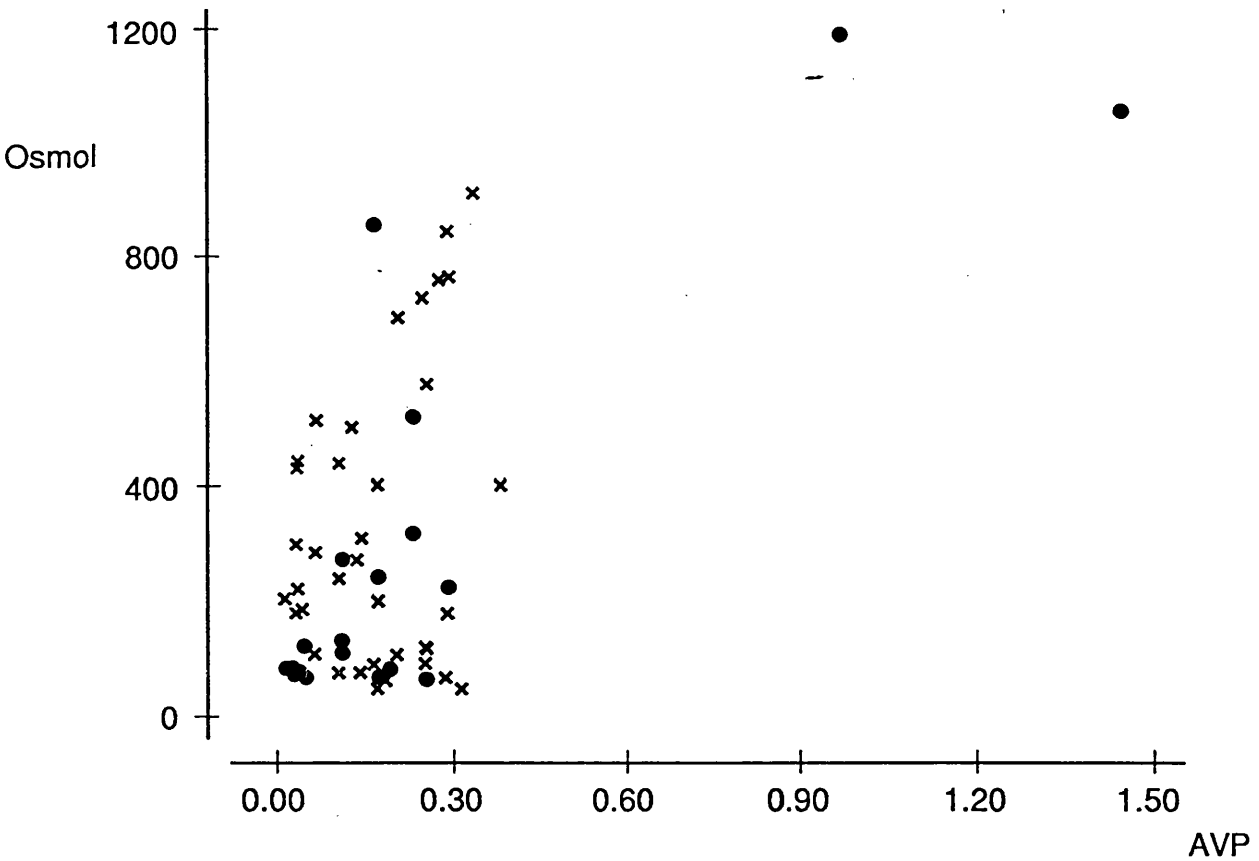
Subject./time		09	11	13	15 hrs
6.	urine	968	705	487	318
	plasma	282	280	279	280
	AVP	1.01	0.69	0.18	0.31
7.	urine	*	431	679	734
	plasma	*	275	277	280
	AVP	*	0.03	0.12	0.28
8.	urine	286	601	799	1063
	plasma	284	285	287	289
	AVP	0.10	0.13	0.39	0.48

Figure 4/3



Plasma AVP and the corresponding serum osmolality in 8 healthy control subjects (●) and 9 patients with PVFS (×)

Figure 4/4



Plasma AVP and the corresponding urine osmaolality in 8 healthy subjects (●) and 9 patients with PVFS (×)

Experiment 3: melatonin excretion in patients with PVFS and control subjects:

The two groups were comparable in respect of age and total body weight. The mean age (± standard deviation) of male and female patients with PVFS was 45.0 ± 1.0 and 35.4 ± 4.9 years, respectively compared to 43.0 ± 4.0 years for males and 39.2 ± 12.6 years for females in the control group. Also there was no statistically significant difference in body weight between the two groups. In females the body weight (± standard deviation) was 57.4 ± 4.4 kg in patients with PVFS and 56.7 ± 6.1 kg in control subjects. The mean body weight for male pateints and control subjects was 71.2 ± 6.7 and 70.4 ± 10 kg, respectively.

All patients and control subjects completed the study. The results of urinary melatonin excretion over a period of 48 hours are given in tables 4/12 & 4/13.

As summarised in table 4/14 and graphically shown in figure 4/5, the 4 hourly urinary excretion of 6-sulphatoxy melatonin was higher in patients with PVFS than in control subjects. However, the difference between the two groups did not reach statistical significane. The increased excretion of melatonin in patients with PVFS was observed throughout the day, but more so between mid-night and 8 am.

The pattern of melatonin excretion in both groups was the same and is similar to that observed in healthy subjects with maximum concentrations late at night and in the early hours of the morning. However, in one patient with PVFS and in two control subjects urinary 6-sulphatoxy melatonin levels reached their peak in the afternoon.

Table 4/12

Urinary melatonin excretion (ng/ml) over 48 hours period in 8 patients with PVFS:

Patient	day	0-4	4-8	8-12	12-16	16-20	20-24 hours
1	1	-	24.9	15.8	0.9	2.5	-
	2	-	-	13.0	-	27	-
2	1	-	23.3	17.9	4.0	2.8	7.7
	2	-	22.7	13.8	3.2	0.1	3.8
3.	1	-	1.6	11.1	2.1	2.3	3.5
	2	2.2	2.3	1.6	7.7	21.1	4.3
4	1	-	-	-	1.9	1.8	14.3
	2	26.8	35.5	17.0	1.9	0.9	16.2
5	1	-	25.2	3.4	-	-	1.6
	2	-	40.2	-	1.8	0.9	2.4
6	1	12.8	-	0.3	13.1	-	0.5
	2	-	-	-	7.3	1.7	4.1
7	1	5.4	7.4	2.8	1.6	-	-
	2	-	-	-	0.1	0.1	2.4
8	1	-	11.2	-	0.8	0.3	1.0
	2	9.6	7.6	2.6	-	-	5.4

Table 4/13

Urinary melatonin excretion over 48 hours period in 6 control subjects:

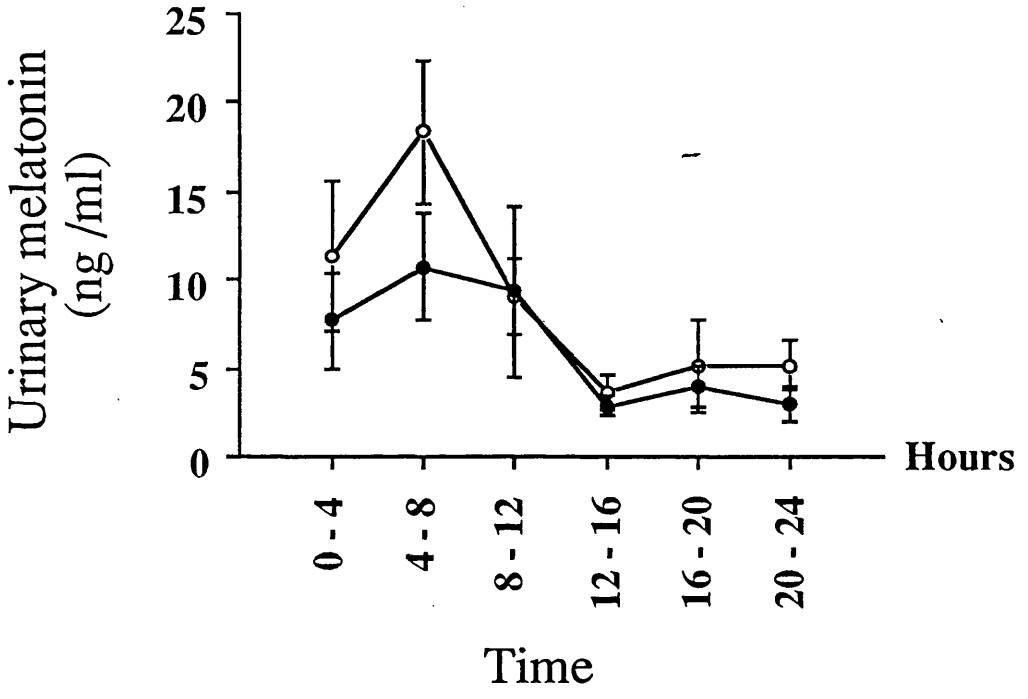
Subj.	day	0-4	4-8	8-12	12-16	16-20	20-24
1	1	4.9	9.1	1.0	1.2	-	-
	2	-	6.4	0.8	1.2	0.9	0.2
2.	1	-	-	-	1.5	1.9	6.2
	2	10.4	24.8	10.1	5.8	4.0	2.4
3	1	-	-	-	-	2.2	0.9
	2	-	-	37.0	3.7	1.2	0.9
4	1	-	-	-	5.7	13.1	0.7
	2	-	0.9	0.8	-	5.2	1.6
5	1	-	5.3	-	2.0	2.6	1.4
	2	-	13.3	9.8	-	7.5	5.0
6	1	-	15.5	-	3.1	-	-
	2	-	-	5.7	1.5	1.1	10.1

Table 4/14

Summary statistics: 24 hour urinary melatonin excretion (ng/ml) in 8 patients with PVFS and 6 control subjects. The values represent the mean ± standard error of the mean.

	0-4	4-8	8-12	12-16	16-20	20-24
PVFS	11.3±4.2	18.3±3.9	9.0±2.0	3.5±1.0	5.1±2.5	5.1±1.3
Cont.	7.6±2.7	10.7±2.9	9.3±4.8	2.8±0.6	3.9±1.2	2.9±1.0
p =	0.6	0.1	0.9	0.6	0.7	0.2

Figure 4/5



24 hour urinary melatonin excretion (ng / ml) in 8 patients with PVFS (—o—) and 6 control subjects (—•—). Values represent mean ± SEM

CHAPTER FIVE

DISCUSSION AND SUMMARY

Discussion:

There are a number of reasons for studying hypothalamic function in patients with PVFS. First, the hypothalamus controls circadian rhythms, food intake, fluid balance, gut motility temperature regulation and cardiac function. All of these are affected in patients with PVFS. Secondly, the hypothalamus influences mood, drive and behaviour (see pages 61-68). Central fatigue, i.e. fatigue which is not the result of structural muscle disease or abnormalities of neuromuscular transmission, is the core feature of this syndrome and is due to lack of drive. Thirdly, the hypothalamus plays an important role in consolidation of short term memory, emotional expression, adaptation to stress and immune regulation. As described in chapter one, depressed mood, anxiety, hypochondriasis and poor short term memory are frequently encountered in patients with PVFS. Immunological abnormalities have been documented in these patients (Caligiuri et al 1987, Morrison et al, 1991). Finally, whilst postmortem studies have not been conclusive, they suggest hypothalamic involvement in these patients (see chapter two). We, therefore, undertook the present study, which is the first formal evaluation of hypothalamic function in patients with PVFS.

In the first part of the study, we used a neuroendocrine challenge test to see if there was increased

sensitivity of hypothalamic 5-HT receptors (which suggests depletion of 5-HT in these patients). We then studied water metabolism, including measurements of total body water content and arginine-vasopressin release in response to water deprivation and water loading. Finally, we measured melatonin excretion in these patients.

In the first study we demonstrated a significantly increased prolactin response to buspirone challenge in patients with PVFS in comparison with healthy individuals and also with patients suffering from primary depressive illness. As discussed earlier (see pages 69-71), prolactin response to buspirone administration appears to be mediated via hypothalamic 5-HT receptors. The findings of the present study, therefore, suggest an increased sensitivity of central 5-HT receptors in patients with PVFS.

Our results also show that there was no significant increase in serum prolactin levels in patients with primary depression following the administration of 60 mg of buspirone. The buspirone challenge test may, therefore, be a useful clinical test to differentiate between PVFS and major depressive illness.

The use of neuroendocrine challenge tests to assess the hypothalamic function has now become a standard research method in biological psychiatry and is widely used in

the assessment of hypothalamic function in patients with a variety of psychiatric disorders, including panic disorder (Kahn et al, 1988) and major depressive illness (Cowen & Charig, 1987, Golden et al, 1990). The validity of the data obtained using neuroendocrine challenge tests has been corraborated by other methods. For example, decreased 5-HT mediated prolactin release has been demonstrated in patients with major depressive illness with the 5-HT reuptake inhibitor clomipramine (Anderson et al, 1992) but not in response to thyrotropin-releasing hormone (which acts directly on the pituitary to release prolactin). This suggests decreased function of central 5-HT neurones in depressed patients, an observation which was confirmed in postmortem studies using autoradiography (Yates et al, 1990). Neuroendocrine challenge tests have also proved to be more valuable in assessing the function of the pituitary-hypothalamic axis than the standard pituitary stimulation tests (see below).

Hypothalamic dysfunction has been suspected as a major pathogenetic mechanism in primary depression because symptoms of hypothamic disease, e.g. change in appetite, body weight, sleep pattern and libido, and also endocrine changes, such as hypercortisolism, increased corticotropin releasing hormone (which acts as a neurotransmitter in the limbic system and cerebral cortex) and the blunted ACTH response to corticotropin releasing hormone (Sachar et al, 1970, Amsterdam et al,

1987, Cowan & Wood, 1991) occur in these patients. Decreased brain concentrations of 5-HT (which is involved in the regulation of hypothalamic function as described in chapter two) has been extensively reported in patients with primary depressive illness (Shaw et al, 1967, Goodwin & Post, 1983, Cowen et al, 1987, Cowen & Charig, 1987). This data heralded a period of extensive research in psychiatry using routine endocrine tests, in particular the pituitary function tests, in an attempt to elucidate the pathogenetic mechanisms of mental illness.

However, the use of pituitary function tests (which are relatively cheap and easy to do) as an indirect method of assessing hypothalamic function in patients with depressive illness has resulted in contradictory and misleading data. This is largely because of the multiplicity of the feedback and feedforward mechanisms which regulate pituitary function. In recent years these tests have, therefore, been replaced by the more robust neuroendocrine challenge tests, such as the buspirone test.

In all studies we selected patients who fulfilled strict diagnostic criteria of PVFS as described earlier (Behan 1991, Behan and Bakheit 1991). All patients have had fatigue and other symptoms for more than 6 months. The onset of their symptoms followed a viral illness and conditions such as chronic infection, malignancy etc,

which can cause chronic fatigue and lassitude have been excluded. The patients had a well-adjusted premorbid personality as suggested by their previous ability to cope with every day stress and had good work records. None of the patients had a past or family history of psychiatric illness. Primary depression can of course occur de novo in individuals with a previously well-adjusted premorbid personality and a good family history, but the depressive symptoms in our patients were different from those which occur in primary depression (Behan & Bakheit, 1991, Procter, 1991). For example, none of our patients expressed feelings of guilt, self-depreciation or had anhedonia.

In addition to the typical clinical features of PVFS in the patients selected for this study, the organic nature of their symptoms was supported by the presence of the characteristic mitochondrial damage previously reported in patients with the PVFS (Gow & Behan 1991, Behan et al, in press) and the presence of enteroviral genomic RNA in muscle biopsy (Gow et al, 1991).

In this study we also carefully controlled for factors which affect prolactin secretion. One of these factors is the influence of female sex hormones on prolactin release. The variability of buspirone-induced prolactin release during the various phases of the menstrual cycle are well documented. Yatham et al (1989) studied the prolactin responses to a single oral dose (60 mgs) of

buspirone in 6 healthy women during the follicular and luteal phases (defined as day 2 and day 24 of the menstrual cycle, respectively) and also at mid cycle (day 14). These authors found a significantly higher prolactin response in the luteal phase of the menstrual cycle than at mid cycle or in the follicular phase. Similar observations were confirmed by Dinan et al (1990).

We took two measures to overcome the possible bias due to the effects of female gonadal hormones on prolactin secretion. First, data for males and females were analysed separately. Secondly, all female patients (who were menstruating normally) were studied in the luteal phase of the menstrual cycle.

From our results it can be seen that, in contrast to patients with PVFS, those suffering from primary depressive illness had a blunted prolactin response to buspirone. This finding is in agreement with the results of a recent study (Upadhyaya et al, 1991) and indicates a fundamental difference in central 5-HT receptor function between patients with PVFS and those suffering from primary depressive illness. In the study of Upadhyaya and colleagues the prolactin response to drugs which increase 5-HT concentrations in brain, e.g. L-tryptophan, clomipramine and fenfluramine, was found to be reduced. Interestingly, the normal response was restored with the successful treatment of depression.

For this reason buspirone challenge test may be useful in the differential diagnosis of these two conditions.

In contrast to our findings, at least two studies have reported significantly higher baseline serum prolactin values in depressed patients than in healthy controls (Golden et al, 1989, Whalley et al 1989). Interestingly, despite the raised baseline prolactin values observed in depressive patients, the response to the 5-HT inhibitor clomipramine in these studies (Golden et al, 1989, Anderson et al, 1992) was identical to that seen in our patients.

Whalley et al. (1989) measured the plasma concentration of prolactin in 98 psychotic patients and 35 healthy control subjects over a period of 17 hours and found significantly increased serum prolactin concentrations in patients with schizoaffective mania and psychotic depression, especially in morning blood samples. However, 32 of these patients received neuroleptic or sedative drugs prior to admission and 30 of the remaining patients were given amylobarbitone on the day of blood sampling because of behavioural problems. The discrepancy between our results and those of Whalley et al (1989) can be accounted for by methodological artefacts in their study. First, these authors studied patients on their first admission to a psychiatric unit. It is usually difficult to make a definitive diagnosis of depressive illness on the

patients' first admission to hospital. The diagnosis of depressive illness, for example, may be changed to schizophrenia at a later date. Secondly, in this study patients were receiving antipsychotic medication during the study. Almost all of these drugs are known to interfere with brain neurotransmitters and neuropeptides which regulate hypothalamic-pituitary function.

In conclusion, in this study we have shown that patients with PVFS have a significantly higher prolactin response to buspirone challenge when compared with healthy subjects and patients with primary depression. This suggests upregulation of hypothalamic 5-HT receptors.

Because of the clear difference in prolactin response between patients with PVFS and depressives the buspirone challenge test can be exploited as a diagnostic test to differentiate between these two conditions.

In our next experiment we measured the total body water content in a well-defined group of patients with PVFS. We also measured the total body water content and the volume of the intracellular and extracellular fluid compartments in the luteal and follicular phases of the menstrual cycles in two female patients with this disorder who also exhibited features identical to those of the fluid retention syndrome. For further evaluation of the hypothalamic-pituitary regulation of water

metabolism we studied the secretion of AVP in response to plasma and urine osmolality changes during the water loading and water deprivation tests.

Our results showed that patients with PVFS have an increased total body water content when compared with the predicted values in age and sex-matched healthy control subjects. The mean total body water in patients was 5.3% higher than that in controls. Because total body water values depend on body weight which will increase with water retention, expressing the total body water as a percentage of the predicted values underestimates water retention by about 30%. Patients with PVFS, therefore, have a significantly higher total body water than healthy subjects.

We studied two women with PVFS and signs of fluid retention which always became worse in the preovulatory phase of the menstrual cycle. In these patients the total body water and the intracellular water content were significantly greater in the luteal than in the follicular phase of the menstrual cycle.

Finally, the baseline AVP values were significantly lower in patients with PVFS than in healthy subjects and they tended to remain low at relatively high plasma and urine osmolality during the water deprivation test. This suggests either a global deficit of AVP secretion or reduced sensitivity of the osmoreceptor to osmotic

stimuli. In some patients there was no correlation between plasma or urine osmolality and plasma AVP concentrations, suggesting failure of hypothalamic regulation of AVP secretion.

The findings reported in this study could not have been due to methodologiacl artefacts. We measured the plasma rather than serum osmolality. This is because the methods used for serum preparation may lead to apparent increase in plasma AVP concentrations (Redetzki et al, 1972). Similarly, venous stasis was avoided when blood samples for osmolality were collectd as artefactual increase in osmolality by up to 20 mosmol/kg may result from the use of a tourniquet (Robertson et al, 1976).

High blood urea and/or hyperglycaemia distort the relationship between plasma osmolality and plasma AVP concentrations (Robertson, 1976). All our patients had normal blood urea and electrolytes and a normal blood glucose.

Nausea and vomiting, which are potent stimuli of AVP release (Zerbe & Robertson, 1987), were not reported by either the patients or the control subjects.

We were also careful to control for all the other factors which influence AVP release. These are cigarette smoking (Rowe et al, 1980) , the effects of posture, hypovolaemia and the phase of the menstrual cycle.

Smoking was not allowed during the study. The tests were carried out with the patients and control subjects lying down except when voiding.

Dehydration during the water deprivation test may cause hypovolaemia. A reduction in circulating blood volume by 7-15% is a strong stimulus of AVP release (Robertson & Athar 1976). However, such a large decrease in circulating blood volume inevitably triggers compansotary mechanisms, e.g. tachycardia, and is accompanied by a fall in arterial blood pressure. All of our patients and control subjects had a stable blood pressure and pulse rate throughout the study. We can, therefore, safely assume that there was no significant decrease in blood volume in these subjects.

The hypothalamic-pituitary response to the various stimuli which inhibit or trigger AVP release varies according to the phase of menstrual cycle and is thought to be due to the effects of female sex hormones. The basal plasma osmolality and the osmotic threshold for thirst and AVP release were found to be significantly lower in the luteal than in the follicular phase of the menstrual cycle (Spruce et al, 1985). However, the normal correlation between plasma osmolality and plasma AVP concentration is preserved in both phases of the menstrual cycle. In one study (Spruce et al. 1985) the correlation coefficient between plasma osmolality and AVP values was 0.95 in the luteal phase and 0.93 in the

follicular phase. To eliminate the possible effects of female gonadal hormone variations in the different phases of the menstrual cycle on AVP release all female patients were studied in the luteal phase of the cycle.

In this study we chose to use water loading and water deprivation rather than saline infusion to induce plasma osmolality changes because, as stated by Hammer et al (1980), fluid deprivation is the natural physiological stimulus for AVP release and also because it does not interfer with other systems which regulate renal function, e.g. the renin-angiotensin-aldosterone system.

Although hospitalisation and fasting for the test may cause some degree of emotional stress in some patients, stress per se has no effect on AVP release. Experimental evidence in healthy subjects (Edelson et al, 1986) and also in patients with anxiety neurosis (Raskind et al, 1978) shows that stress increases AVP release only when it is accompanied by nausea or hypovolaemia.

One of the findings in this study, namely the delayed excretion of a water load, has been previously reported in 10 out of 33 patients with "chronic nervous exhuastion" (Levy et al, 1946). These patients were described as having " a definite reduction of vigour if not loss of strength" as their main presenting symptom. Although the authors do not mention a history of a viral illness preceding the symptoms of fatigue in these

patients, their description of the disorder which they named chronic nervous exhaustion fits the diagnosis of PVFS.

In conclusion, this study has shown that patients with PVFS have an increased total body water content, deficiency of AVP secretion and an erratic AVP response to osmotic stimuli. Taken together with our observation of an increased prolactin response to buspirone in these patients, these abnormalities of water metabolism suggest hypothalamic dysfunction in patients with the PVFS.

Finally, we measured the melatonin excretion over a 48 hour period in patients with PVFS and a group of control subjects. Analysis of our data shows that patients with PVFS excreted more 6-sulphatoxy melatonin than the control subjects, especially between mid night and 8 am. However, the difference did not reach the level of statistical significane. An important finding in this study is that the pattern of melatonin excretion in patients with PVFS and control subjects was the same with peaks early in the morning and low values in the afternoon. This profile is similar to that reported in healthy subjects (Wetterberg, 1978) and was seen in all patients with PVFS except one.

Age, sex and body weight have important effects on pineal function (Ferrier, 1982, Iguchi et al, 1982). To

eliminate the effects of these factors patients with PVFS in this study were carefully matched for these parameters with control subjects.

One can not draw hard conclusions from this part of the study, of small sample. However, the observed differences between patients with PVFS and control subjects suggest a possibility of melatonin disturbances in these patients.

d) Summary and concluding remarks:

The clinical features of PVFS suggest hypothalamic dysfunction in these patients. The role of the hypothalamus in regulating food intake, fluid balance, temperature regulation, gut motility, cardiovascular, sexual and endocrine functions is well-documented.

5-HT is the main neurotransmitter in the hypothalamus and abnormalities of 5-HT secretion may underlie hypothalamic dysfunction. Disoders, such as migraine and the fluid retention syndrome, in which disturbances of central 5-HT are known or suspected to play an important role are common in patients with PVFS. Patients with PVFS often have symptoms identical to those of the irritable bowel syndrome. Recent evidence suggests that the latter disorder may be due to hypersensitivity of hypothalamic 5-HT receptors (Dinan et al, 1990). We have seen several patients with PVFS who developed craving

for chocolates and sweets following the onset of their illness. The basis for this curious symptom of craving for carbohydrates is thought to be an abnormality of central 5-HT regulation (Wurtman & Wurtman, 1989).

In the present study we have shown upregulation of hypothalamic 5-HT receptors in these patients and subtle abnormalities in hypothalamic regulation of water metabolism in patients with PVFS. The persistence of enteroviral genomic RNA particles in skeletal muscle of more than 50% of patients with PVFS studied and structural abnormalities of muscle mitochondria have been reported (Gow et al, 1991, Gow & Behan, 1991)). It is possible that similar changes are also present in the hypothalamus of these patients.

The association of PVFS with poliomyelitis has also been extensively reported (see review of epidemics of PVFS) and may be an important clue in the aetiopathogenesis of PVFS. Hypothalamic involvement may occur in patients with poliomyelitis where there are subtle disturbances of temperature regulation, gut motility and sexual function (Baker et al, 1950). In addition, these patients often have sleep disturbances and emotional lability. Similar observations were reported by Brown et al. (1947) in their description of patients dying of poliomyelitis.

Hypothalamic dysfunction is also known to occur in

patients with mitochondrial disease (Fitzsimons et al, 1981, Herzberg, 1990). Of great interest is recent work showing that viruses and other microorganisms can cause subtle hormonal and neurotransmitter abnormalities in the hypothalamus without producing local structural damage. For example, although trypanosomes, which cause African sleeping sickness with fatigue and endocrine disturbances, do not cross the blood-brain barrier, they can cause selective induction of MHC class I in hpothalamic neurones in the early stages of the disease (Kristensson, 1990).

Oldstone and colleagues (Oldstone et al, 1984, Oldstone, 1989, de la Torre et al, 1991) have demonstrated conclusively that viruses may interefere with the production of hormones, neurotransmitters and cytokines without causing cell lysis. This is of great importance in considering the fact that no morphological evidence of hypothalamic damage has been seen in some cases of PVFS examined at postmortem (personal communication: Dr J. McLaughland)).

The raphe nuclei (and 5-HT systems) are particularly susceptible to viral infections. Infection of rats with viruses via the nasal mucosa causes alteration in central 5-HT concentrations and behavioural changes in these animals (Kristensson, personal communication). It is worthy of note that these biochemical and behavioural changes in these animals may persist for many months,

while the cellular infiltrate in the hypothalamus disappears two weeks after inoculation.

In conclusion, the studies discussed in this thesis demonstrate subtle abnormalities of hypothalamic function and upregulation of hypothalamic 5-HT receptors in patients with PVFS. Taken together with the evidence that enterovirus persistence has been established in the muscle of patients with this disorder, and that persistent viruses can interfere with cell function without causing cell death, we postulate that the neuroendocrine and 5-HT abnormalities reported here are due to viral persistence in hypothalamic neurones. We are at present attempting to establish an animal model of persistent enteroviral infection in the nervous system, to test this hypothesis.

REFERENCES

Acheson ED. Encephalomyelitis associated with poliomyelitis virus. Lancet 1954, 2: 1044-1048

Adams RD. Lassitude and asthenia. In: Issebacher KJ (ed.). Harrison's Princ iples of Internal Medicine. 9th ed. McGraw-Hill, New York 1980, pp 76-77

Al-Khader AA, Aber GM. The relationship between the "idiopathic oedema syndrome" and subclinical hypothyroidism. Clinical Endocrinology 1979, 10: 271-279

American Psychiatric Association. Diagnostic and statistical manual of mental disorders (DSM-III-R). 3d ed. Washington DC, 1987

Amsterdam JD, Maislin G, Winokur A, Kling M, Gold P. Pituitary and adrenocortical responses to the ovine corticotropin releasing hormone in depressed patients and healthy volunteers. Archives of General Psychiatry 1987, 44: 775-781

Anderson IM, Ware CJ, da Roza Davis JM, Cowan PJ. Decreased 5-HT mediated prolactin release in major depression. British Journal of Psychiatry 1992, 160: 372-378

Anton-Tay F, Wurtman RJ. Regional uptake of H-melatonin from blood or cerebrospinal fluid by rat brain. Nature 1969, 221: 474-476

Anton-Tay F, Diaz JL, Fernandez-Guardiola A. On the effects of melatonin upon the human brain. Its possible therapeutic implications. Life Science 1971, 10: 841-850

Archard LC, Bowles NE, Behan PO, Bell EJ, Doyle D. Postviral fatigue syndrome: persistence of enterovirus RNA in muscle and elevated creatine kinase. Journal of the Royal Society of Medicine 1988, 81: 326-329

Arendt J. Melatonin assays in body fluids. Journal of Neural Transmission 1978, Suppl. 13: 265-278

Arendt J, Marks V. Physiological changes underlying jet lag. British Medical Journal 1982, 284: 144-146.

Arendt J, Bojkowski C, Folkard S et al. Some effects of melatonin and the control of its secretion in humans. In: Photoperiodism, melatonin and the pineal. Pitman, London, 1985a, (Ciba Foundation Symposium, vol. 117) pp 266-283

Arendt J. Bojkowski C, Franey C, Wright J, Marks V. Immunoassay of 6-sulphatoxy melatonin sulphate in human plasma and urine. Abolition of the urinary 24 hour rhythm with atenolol. Journal of Clinical Endocrinology & Metabolism 1985b, 60: 1160-1173

Arendt J, Aldhous M, Marks V. Alleviation of jet lag by melatonin: preliminary results of a double-blind trial. British Medical Journal 1986, 292: 1170

Armstrong S, Ng KT, Coleman GJ. Influence of the pineal gland on brain-behaviour relationships. In: Reiter RJ (ed.). The pineal gland, vol.3 (Extra-reproductive effects). CRC Press Inc, Boca Raton, 1982, pp 81-106

Arnold DL, Bore P, Radda GK, Styles P, Taylor D. Excessive intracellular acidosis of skeletal muscle on exercise in a patient with post viral exhaustion/fatigue syndrome: a 31 P NMR study. Lancet 1984a, i: 1367-1369

Arnold DL, Bore P, Radda GK, Styles P, Taylor D. Excessive acidosis of skeletal muscle on exercise in the post viral exhaustion syndrome: A 31 P NMR study. Proceedings of the 3rd annual meeting of the Society of Magnetic Resonance in Medicine. New York. society of Magnetic Resonance in Medicine, Berkely, Ca, USA, p 12.

Axelrod J. The pineal gland: a neurochemical transducer. Science 1974, 184: 1341-1348

Baker AB, Matzke HA, Brown JR. Poliomyelitis. III Bulbar poliomyelitis, a study of medullary function. Archives of Neurology & Psychiatry, 1950, 63: 257-281

Barnes JM, Barnes NM, Costall B, Naylor RJ, Tyers MB. 5-HT3 receptors mediate inhibition of acetylcholine release in cortical tissue. Nature 1989, 338: 762-763

Bartrop RW, Lazarus L, Luckhurst E, Kiloh LG. Depressed lymphocyte function after bereavement. Lancet 1977, i: 834-836

Baylis PH, Zerbe RL, Robertson GL. Arginine-vasopressin response to insulin-induced hypoglycaemia in man. Journal of Endocrinology & Metabolism 1981, 53: 935-940

Beal MF, Kleiman GM, Ojemann RG, Hochberg FH. Gangliocytoma of the third ventricle: hyperphagia, somnolence and dementia. Neurology 1981, 31: 1224-1228

Beck-Friis J, von Rosen D, Kjellman BF, Ljunggren JG, Wetterberg L. Melatonin in relation to body measures, sex, age, season and the use of drugs in patients with major affective disorders and healthy subjects. Psychoneuroendocrinology 1984, 9: 261-277

Behan PO, Behan WMH, Bell EJ. The postviral fatigue syndrome - an analysis of the findings in 50 cases. Journal of Infection 1985, 10: 211-222

Behan PO, Behan WMH. Postviral fatigue syndrome. CRC Critical reviews in Neurobiology 1988, 4: 157-178

Behan PO, Bakheit AMO. Clinical spectrum of the postviral fatigue syndrome. British Medical Bulletin, 1991, 47: 793-808

Behan PO. Myalgic encephalomyelitis: postviral fatigue syndrome. Diagnostic and clinical guidlines for doctors. EME Association, 1991

Behan WMH, More IAR, Behan PO. Mitochondrial abnormalities in the postviral fatigue syndrome. Acta Neuropathologica Scandinavica (in press)

Bell EJ, McCartney RA, Riding MH. Coxsackie B viruses and myalgic encephalomyelitis. Journal of the Royal Society of Medicine 1988, 81: 329-331

Bell KM, Cookfair D, Bell DS, Reese P, Cooper L. risk factors associated with chronic fatigue syndrome in a cluster of pediatric cases. Reviews of Infectious Diseases 1991, 13 (Suppl. 1): S32-S38

Berga SL, Yen SS. Circadian pattern of plasma melatonin concentrations during four phases of the human menstrual cycle. Neuroendocrinology 1990, 51: 606-612

Biegon A, Bercovitz H, Samuel D. Serotonin receptor concentration during the oestrous cycle of the rat. brain Research 1980, 187: 221-225

Biggar RJ, Gardiner C, Lennette ET, Collins WE, Nkrumah FK, Henle W. Malaria, sex and place of residence as factors in antibody response to Epstein-Barr virus in Ghana, West Africa. Lancet 1981, 2: 115-118

Bittman EL, Weaver DR. The distribution of melatonin binding sites in neuroendocrine tissue of the ewe. Biology of Reproduction 1990, 43: 986-993

Boddy K, King PC, Hume R, Weyers E. The relation of total body potassium to height, weight and age in normal subjects. Journal of Clinical Pathology 1972, 25: 512-517

Bojkowski CJ, Arendt J. Factors influencing urinary 6-sulphatoxy melatonin, a major melatonin metabolite, in normal human subjects. Clinical Endocrinology 1990, 33: 435-444

Bradley PB, Engel G, Feniuk W et al. Proposal for classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology 1986, 25: 563-576

Broadbent DE, Cooper PJ, Fitzgerald PF, Parkes KR. The cognitive failures questionnaire (CFQ) and its correlates. British Journal of Clinical Psychology 1982, 21: 1-6

Brodal A. Neurological anatomy in relation to clinical Medicine. Second edition. Oxford University Press, London, 1969, pp 587 & 597

Brooks CM. The history of thought concerning the hypothalamus and its functions. Brain Research Bulletin 1988, 20: 657-667

Brown JR, Baker AB, Adams J, McQuarrie I. The bulbar form of poliomyelitis. 1. Diagnosis and the correlation of clinical with physiologic and pathologic manifestations. Journal of the American Medical association 1947, 134: 757-762

Brownstein MJ, Palkovits M, Kizer JS. Effect of surgical isolation of the hypothalamus on neurotransmitter content. Brain Research 1976, 117: 287-295

Brownstein MJ. Serotonin, histamine and the purines. In: Siegel GJ, Albers RW, Agranoff BW & Katzman (eds). Basic neurochemistry. 3d ed. Little, Brown & Company, Boston, 1981, pp 220

Buchwald D, Komaroff A. Review of laboratory findings in patients with chronic fatigue syndrome. Reviews of Infectious Diseases 1991, 13 (Suppl. 1): S12-S18

Caccia S, Conti I, Vigano et al. 1-(2-pyrimidinyl)-piperazine as active metabolite of buspirone in rat and man. Pharmacology 1986, 33: 46-50

Caligiuri M, Murray C, Buchwald D, Levine H, Cheney P, Peterson D, Komaroff AL, Ritz J. Phenotypic and functional deficiency of natural killer cells in patients with chronic fatigue syndrome. Journal of Immunology 1987, 139: 3306-3313

Calogero AE, Bernardini R, Gold PW, Chrousos GP, Regulation of rat hypothalamic corticotropin releasing hormone secretion in vitro: potential clinical implications. Advances in Experimental Medical Biology 1988a, 245: 167-281

Calogero AE, Gallucci WT, Bernardini R, Saoutis C, Gold PW. Effect of cholinergic agonists and antagonists on rat corticotropin releasing hormone secretion in vitro. Neuroendocrinology 1988b, 47: 303-308

Calogero AE, Gallucci WT, Chrousos GP, Gold PW. interactions between GABAergic neurotransmission and rat corticotropin releasing hormone secretion in vitro: theoretical and clinical implications. Brain Research 1988c, 463: 28-36

Cardinali DP. Melatonin. A mammalian pineal hormone. Endocrinogy Research 1981, 2: 327-346

Carpenter S, Yassa R, Ochs R. A pathologic basis for Kleine-Levin syndrome. Archives of Neurology 1982, 39: 25-28

Checkley S, Arendt J. Pharmacoendocrine studies of GH, PRL and melatonin in patients with affective illness. In: Brown GL et al (Eds.). Neuroendocrinology and psychiatric disorders. Raven Press, 1984, pp 165-190

Cluff E. Medical aspects of delayed convalescence. Reviews of Infectious Diseases 1991, 13 (Suppl.1): S138-S140

Coccaro EF, Gabriel S, Mahon T, Macaluso J, Siever LJ. Preliminary evidence of serotonin (5-HT-1-like) component to the prolactin response to buspirone challenge in humans. Archives of General Psychiatry 1990, 47: 594-595

Cohen S, Hobermann H. Positive events and social supports as buffers of life change stress. Journal of Applied Social Psychology 1983, 13: 99-124

Cohn JB, Wilcox CS, Meltzer HY. Neuroendocrine effects of buspirone in patients with generalized anxiety disorder. The American Journal of Medicine 1986, 80 (Suppl. 3B): 36-40

Compston ND. Epidemic at the Royal Free Hospital. British Medical Journal 1956, 2: 157

Conn PJ, Sanders-Bush E. Central serotonin receptors: effector systems, physiological roles and regulation. Psychopharmacology 1987, 92: 267-277

Corridan JP. Epidemic neuromyasthenia in Southwest Ireland. Postgraduate Medical Journal 1978, 54: 731-736

Cowen PJ, Charig EM. Neuroendocrine responses to intravenous tryptophan in major depression. Archives of General Psychiatry 1987, 44: 958-966

Cowen PJ, Charig EM, Fraser S, Elliot JM. 5-HT receptor binding during depressive illness and tricyclic antidepressant treatment. Journal of Affective Disorders 1987, 13: 45-50

Cowen PJ, Anderson IA, Grahame-Smith DG. Neuroendocrine effects of azapirones. Journal of Clinical Psychopharmacology 1990, 10(Suppl.): 21S-25S

Cowen PJ, Wood AJ. Biological markers of depression. Psychological Medicine 1991, 21: 831-836

Critchley M. Periodic hypersomnia and megaphagia in adolescent males. Brain 1962, 85: 627-656

Critchley PHS, Malcom GP, Malcom PN, Gibb WR, Arendt J, Parkes JD. Fatigue and melatonin in Parkinson's disease. Journal of Neurology, Neurosurgery & Psychiatry 1991, 54: 91-92

Crowley N, Nelson M, Stovin S. Epidemiological aspects of an outbreak of encephalomyelitis at the Royal free Hospital, London, in the summer of 1955. Journal of Hygeine (London), 1957, 55: 102-122

Crown S, Crisp AH. A short clinical diagnostic self-rating scale for psychoneurotic patients. British Journal of Psychiatry 1966, 112: 917-923

DaCosta JM. On irritable heart: a clinical study of a form of functional cardiac disorder and its consequence. American Journal of Medical Science 1871, 121: 17-52

David A, Wessely S, Pelosi A. Postviral fatigue syndrome: time for a new approach. British Medical Journal 1988, 296: 696-699

Daugherty SA, Henry BE, Peterson DL, Swarts RL, Bastien S, Thomas RS. Chronic fatigue in Northern Nevada. Reviews of Infectious Diseases 1991, 13 (Suppl. 1): S39-S44

Deicher JB. Benign myalgic encephalomyelitis (Iceland disease) in Alaska. Nortwest Medicine 1957, 56: 1451-1456

Deutsch JA. The cholinergic synapse and the site of memory. Science 1971, 174: 788-794

Den Boer JA, Westenberg HG. Serotonin function in panic disorder: a double-blind placebo-controlled study with fluvoxamine and ritanserin. Psychopharmacology 1990, 102: 85-94

Dinan TG, Barry S, Yatham LN, Mobayed M, O'Hanlon M. The reproducibility of the prolactin response to buspirone: relationship to the menstrual cycle. International Clinical Psychopharmacology 1990, 5: 119-123

Dinan TG, Yatham LN, Barry S, Chua A, Keeling PWN. Serotonin supersensitivity: the pathophysiologic basis of non-ulcer dyspepsia ? Scandinavian Journal of Gastroenterology 1990, 25: 541-544

Dunnigan MG. Recognition and management of the fluid retention (idiopathic or cyclical oedema) syndromes. In: McNaughton MC (ed.). Medical Gynaecology. Blackwell Scientific, Edinburgh, 1985, pp 27-55

Dunnigan MG. Management of the fluid retention syndrome in women. Hospital Update 1990, 16: 653-662

Karl CR, D'Occhio MJ, Kennaway DJ, Seamark RF. Temporal changes in the pattern of melatonin secretion in sheep held in constant darkness. Journal of Pineal Research 1990, 8: 115-121

Edelson JT, Robertson GL. The effect of the cold pressor test on vasopressin secretion in man. Psychoneuroendocrinology 1986, 11: 307-316

Edwards RHT. Physiological analysis of skeletal muscle weakness and fatigue. Clinical Science & Molecular Medicine 1978, 54: 463-470

Ehrenkrantz JR, Tamarkin L, Comite et al. Daily rhythms of plasma melatonin in normal and precocious puberty. Journal of Clinical Endocrinology & Metabolism 1982, 55: 307-310

Evans AC. Brucellosis in the United States. American Journal of Public Health 1947, 37: 139-151

Fagan KG, Behan PO, Bell EJ. Myalgic encephalomyelitis - report of an epidemic. Journal of the Royal College of General Practitioners 1983, 33: 335-337

Falaschi P, Rosa M, Rocca A et al. Role of serotonin in the control of prolactin secretion: effect of antiserotonergic drugs in normal subjects and in hyperprolactinaemic states. Proceedngs of the International Symposium on Serotonin. March 29 - April 1, 1989.

Ferrier IN, Arendt J, Johnstone EC, Crow TJ. Reduced nocturnal melatonin secretion in chronic schizophrenia: relationship to body weight. Clinical Endocrinology 1982, 17: 181-187

Fitzsimons RB, Clifton-Bligh P, Wolfenden WH. Mitochodrial myopathy and lactic acidaemia with myoclonic epilepsy, ataxia and hypothalamic infertility: a variant of Ramsay-Hunt syndrome ? Journal of Neurology, Neurosurgery & Psychiatry 1981, 44: 77-82

Folkow B. Stress, hypothalamic function and neuroendocrine sequences. Acta Medica Scandinavica, 1988 (Suppl. 14K), 723: 61-69

Fraschini F, Cullo R, Martini L. Mechanisms of inhibitory action of pineal principles on gonadotrophin secretion. In: Wolsteinholme GEW & Knight J (eds). The pineal gland. Churchill, London, 1971, p 259

Gainer H, Brownstein MJ. Neuropeptides. In: Siegel GJ, Albers RW, Agranoff BW, Katzman R (eds). Basic Neurochemistry. 3d ed. Little Brown & Company, Boston, 1981, pp 279

Givens JR. The hypothalamus in health and disease. Year Book Medical Publishers, Chicago, 1984.

Gold PW, Kaye W, Robertson GL, Ebert M. Abnormalities in plasma and cerebrospinal-fluid arginine vasopressin in patients with anorexia nervosa. New England Journal of Medicine 1983, 308: 1117-1123

Gold PW, Gwirtsman H, Avgerinos PC, Nieman LK, Galluci WT, Kaye W, Jimerson D, Ebert M, Rittmaster R, Loriaux DL, Chrousos GP. Abnormal hypothalamic-pituitary-adrenal function in anorexia nervosa. New England Journal of Medicine 1986, 314: 1335-1342

Golden RN, Hsiao JK, Lane E, Ekstrom D, Rogers S, Hicks R, Potter WZ. Abnormal neuroendocrine responsivity to acute I.V. clomipramine challenge in depressed patients. Psychiatry Research 1990, 31: 39-47

Goodwin FM, Post RM. 5-hydroxytryptamine and depression: a model for the interaction of normal variance with pathology. British Journal of Clinical Pharmacology 1983, 15: 3935-404S

Gow JW, Behan WMH. Amplification and identification of enteroviral sequences in the postviral fatigue syndrome. British Medical Bulletin 1991, 47: 827-885

Gow JW, Behan WMH, Clements GB, Woodall C, Behan PO. Enteroviral sequences detected by polymerase chain reaction in muscle biopsies of patients with postviral fatigue syndrome. British Medical Journal 1991, 302: 692-696

Gregory CA, Anderson IM, Cowen PJ. Metergoline abolishes the prolactin response to buspirone. Psychopharmacology 1990, 100: 283-284

van Gruene CHJ, Bouic PJD. Abberant in vitro HLA-DR expression in patients with chronic fatigue. South African Medical Journal 1990, 78: 219-220

Guerin MV, Watson R, McLoughney J, Earl CR, Seamark RF, Matthews CD. The normal patterns of serum melatonin in Romney March sheep held in natural photoperiodic conditions. In: Reiter R (ed.). Advances in pineal research, vol. 3, John Libbey, London & Paris, 1988, pp 137-141

Guyton A. Behavioral and motivational mechanisms of the brain - the limbic system and the hypothalamus. In: Textbook of Medical Physiology, WB saunders Company, Philadelphia, 1991, pp 648-658

Hamblin TJ, Hussain MH, Akbar AN, Tang YC, Smith JL, Jones DB. immunological reason for chronic ill health after infectious mononucleosis. British Medical Journal 1983, 287: 85-88

Hamilton M. A rating scale for depression. Journal of Neurology, Neurosurgery & Psychiatry 1960, 23: 56-62

Hammer H, Ladefoged J, Olgaard K. Relationship between plasma osmolality and plasma vasopressin in human subjects. American Journal of Physiology1980, 238: E313-E317

Hammond DN, Moll GW, Robertson GL. Hypodipsic hypernatraemia with normal osmoregulation of vasopressin. New England Journal of Medicine 1986, 315: 433-436

Hansen T, Birkeland AJ, Lingjaerde O. Melatonin and sleep in man: a preliminary report. In: Priest RG, Pletscher A, Ward J (ed.). Sleep Research. MTP Press Ltd, Lancaster, England, 1979, pp 43-44

Herzberg L. Endocrine aspects of mitochondrial cytopathy: marked phenotypic variation in two affected siblings. Quarterly Journal of Medicine, New Series, 1980, 74: 41-48

Hickie I, Lloyd A, Wakefield D, Parker G. The psychiatric status of patients with the chronic fatigue syndrome. British Journal of Psychiatry, 1990, 156: 534-540

Hill RCJ. Memorandum on the outbreak amongst the nurses at Addington hospital, Durban. South African Medical Journal 1955, 29: 344-345

Hoffmann K. Photoperiodism in vertebrates. In: Handbook of behavioural neurobiology. Vol. 4, Plenum Press, New York, London, 1981, pp 449-473

Holmes GP, Kaplan JE, Gantz NM, Komaroff AL, Schonberger LB, Straus SE, Jones JF, DuBois RE, Cunningham-Rundles C, Pahwa S, Tosato G, Zegans LS, Purtilo DT, Brown N, Schooley RT, Brus I. Chronic fatigue syndrome: a working case definition. Annals of Internal Medicine 1988, 108: 387-389

Houghton LE, Jones EI. Persistent myalgia following sore throat. Lancet 1942, i: 196-198

Hoyer D, Engel G, Kalkman HO. Characterization of the 5-HT1b recognition site in rat brain: bindi ng studies with (-) [125I]-iodocyanopindolol. European Journal of Pharmacology 1985, 118: 1-12

Iguchi H, Kato KI, Ibayashi H. Age-dependent reduction in serum melatonin concentrations in healthy subjects. Journal of Clinical Endocrinology & Metabolism 1982, 55: 27-29

Illnerova H, Zvolsky P, Vanecek J. The circadian rhythm in plasma melatonin concentration of the urbanized man: the effect of summer and winter time. Brain Research 1985, 328: 186-189

Imboden JB, Canter A, Cluff LE. Convalescence from influenza: a study of the psychological and clinical determinants. Archives of Internal Medicine 1961, 108: 393-399

Ishibashi S, Oomura Y, Okajima T. Facilitatory and inhibitory effects of THR on lateral hypothalamic and ventromedial neurones. Physiological Behaviour 1979a, 22: 785-787

Ishibashi S, Oomura Y, Okajima T, Shibata S. Cholecystokinin, motilin secretion effects on the central nervous system. Physiological Behaviour 1979b, 23: 401-403

Jackson AL, Jacobson S, cooper B. A disease resembling poliomyelitis. Report of an outbreak in Johannesburg. South African Medical Journal 1957, 31: 514-517

Jamal G, Hansen S. Electrophysiological studies in the postviral fatigue syndrome. Journal of Neurology, Neurosurgery & Psychiatry 1986, 48: 691-694

Jones JF, Ray CG, Minnich LL, Hicks MJ, Kibler R, Lucas DO. Evidence for active Epstein-Barr virus infection in patients with persistent, unexplained illness: elevated anti-early antigen antibodies. Annals of Internal Medicine 1985, 102: 1-7

Kahn RE, Asnis GM, Wetzler S, van Praag HM. Neuroendocrine evidence for serotonin receptor hypersensitivity in panic disorder. Psychopharmacology 1988, 96: 360-364

Kambari IA, Mical RS, Porter JC. Effects of melatonin and serotonin on the release of FSH and prolactin. Endocrinology 1971, 88: 1288-1299

Kappers JA. The pineal organ - an introduction. In: Wolsteinholme GEW & Knight J (eds). The pineal gland. Churchill, London, 1971, p 3

Kato Y, Nakai Y, Imura H, Chihara K, ogo S. effect of 5-hydroxytryptophan (5-HTP) on plasma prolactin levels in man. Journal of Clinical Endocrinology & Metabolism 1974, 38: 695-697

van de Kar LD, Bethea CL. Pharmacological evidence that serotonergic stimulation of prolactin release is mediated via the dorsal raphe nucleus. Neuroendocrinology 1982, 35: 225-230

Kibler R, Lucas PO, Hicks MJ, Poulos BT, Jones JF. Immune function in chronic active Epstein-Barr virus infection. Journal of Clinical Immunology 1985, 5: 46-54

Koenig JI, Meltzer HY, Gudelsky GA. 5-hydroxytryptamine 1A receptor-mediated effects of buspirone, gepirone and ipsapirone. Pharmacolgy, Biochemistry & Behaviour 1988, 29: 711-715

Komaroff AL, Buchwald D. Symptoms and signs of chronic fatigue syndrome. Reviews of Infectious Diseases 1991, 13 (Suppl 1): S8-S11

Kovacs L, Robertson GL. Disorders of water balance - hyponatraemia and hypernatraemia. Bailliere's Clinical Endocrinology and metabolism 1992, 6: 107-127

Kristensson K. Induction of MHC-antigens in hypothalamic nuclei during experimental trypanosome infection. Paper presented at the Annual Meeting of the Irish Neurological Association, Cork, 18 May 1990

Kroenke K, Wood DR, Mangelsdorff D, Meier NJ, Powell JB. Chronic fatigue in primary care: prevalence, patient characteristics and outcome. Journal of the American Medical Association 1988, 260: 929-934

Krupp LB, Alvarez LA, La Rocca NG, Scheinberg LC. Fatigue in multiple sclerosis. Archives of Neurology 1988, 45: 435-437

Krupp LB, La Rocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Annals of Neurology 1989, 46: 1121-1123

Kuchel O, Cuche JL, Buu NT, Guthrie JP, Unger T, Nowaczynski W, Boucher R, Genest J. Catecholamine excretion in "idiopathic" oedema: is decreased dopamine excretion a pathogenetic factor?. Journal of Clinical Endocrinology & Metabolism 1977, 44: 639-646

Lamberts SWJ, Mac Loed RM. The interaction of serotonergic and dopaminergic systems on prolactin secretion in the rat. Endocrinology 1978, 103: 287-295

Lancet (anonymous editorial). Fatigue. Lancet 1988, 2: 546-548

Lancet (anonymous editorial). Drugs acting on 5-hydroxytryptamine receptors. Lancet 1989, ii: 717-719

Lang U, Aubert, Sizonenko PC. Location of melatonin in receptors (abstract). Pediatric Research 1981, 15: 80

Larsson SE, Bengtsson A, Bodegard L, Henriksson KG, Larsson J. Muscle changes in work-related chronic myalgia. Acta Orthopedica Scandinavica 1988, 58: 552-556

Leon-Sotomayor L. Epidemic diencephalomyelitis: a possible cause of neuropsychiatric, cardiovascular and endocrine disorders. Pageant Press International Corp., New York, 1969

Lesnick JE, Chayt KJ, Bruce DA, Roorke LB, Trojanowski J, Savino PJ, Schantz NJ. Familial pinealoblastoma. Journal of Neurosurgery 1985, 62: 930-932

Levin M. Periodic somnolence and morbid hunger. Brain 1936, 59: 494-504

Levy MS, Power MH, Kepler EJ. The specificity of the "water test" as a diagnostic procedure in Addison's disease. J. of Clinical Endocrinology & Metabolism 1946, 6: 607-632

Lewis DA, Sherman BM. Serotonergic regulation of prolactin and growth hormone secretion in man. Endorinology 1985, 110: 152-157

Lewy AJ, Wehr TA, Goodwin FK, Newsome DA, Markey SP. Light suppresses melatonin secretion in humans. Science 1980, 210: 1267-1269

Lewy AJ, Newsome DA. Different types of melatonin circadian secretion rhythms in some blind subjects. Journal of Clinical Endocrinology & Metabolism 1983, 56: 1103-1107

Lieberman HR, Waldhauser F, Garfield G, Lynch HJ, Wurtman RJ. Effects of melatonin on human mood and performance. Brain Research 1984, 323: 201-207

Lincoln G. Melatonin as a seasonal time cue: a commercial story. Nature 1983, 302: 755

Lloyd AR, Wakefield D, Boughton C, Dwyer J. What is myalgic encephalomyelitis ? Lancet 1988, i: 1286-1287

MacLoed RM, Lehmeyer JE. Studies on the mechanism of the dopamine-mediated inhibition of prolactin secretion. Endocrinology 1974, 94: 1077-1085

Macrae AD, Galpine JF. An illness resembling poliomeyelitis observed in nurses. Lancet 1954, 2: 350-352

Manningham R. The symptoms, nature, causes and cure of the febricula or little fever: commonly called the nervous or hysteric fever; the fever on the spirits; vapours; hypo;, or spleen. 1750, 2d ed., London, J.Robinson, pp 52-53

Martin JB, Reichlin S. Clinical neuroendocrinology. 2d ed. FA Davies Company, Philadelphia, 1987, p 355

Matthews CD, Guerin MV, Wang X. Human plasma melatonin and urinary 6-sulphatoxy-melatonin: studies in natural annual photoperiod and extended darkness. Clinical Endocrinology 1991, 35: 21-27

May PGR, Donnan SPB, Ashton JR, Ogilvie MM, Rolles CJ. Personality and medical perception in benign myalgic encephalomyelitis. Lancet 1980, 2: 1122-1124

McConnell J. An epidemic of pleurodynia with prominent neurologic symptoms and no demonstrable cause. American Journal of Medical Science 1945, 209: 41-48

McMillen BA, Matthews RT, Sanghera MK, Shepard PD, German DC. Dopamine receptor antagonism by the novel antianxiety drug, buspirone. Journal of Neuroscience 1983, 3: 733-738

Meltzer HY, Fleming R. Effect of buspirone on prolactin and growth hormone secretion in laboratory rodents and man. Journal of Clinical Psychiatry 1982, 43: 76-79

Miller NA, Carmichael HA, Calder ED, Behan PO, Bell EJ, McCartney RA, Hall FC. Coxsackie B serology does not help diagnose postviral fatigue syndrome. British Medical Journal 1991, 302: 140-143

Monroe RR, Heath RC. Psychiatric observations on the patient group. In: Heath RC (ed.). Studies in schizophrenia: a multidisciplinary approach to mind - brain relationships. Harvard University Press, Cambridge, 1954, pp 345-385

Montgomery SA, Finberg N. Is there a relationship between serotonin receptor subtypes and selectivity of response in specific psychiatric illnesses ? British Journal of Psychiatry 1989, 155 (Suppl. 8) 63-70

Moore RY. Central neural control of circadian rhythms. In: Ganong WF & Martini L (ed.). Frontiers in neuroendocrinology, vol. 5. Raven Press, New York, 1978, pp 185-206

Moore RY, Klein DC. Visual pathways and the central neural control of a circadian rhythm in pineal serotonin N-acetyltransferase activity. Brain Research 1984, 71: 17-33

Morrison LJA, Behan WHM, Behan PO. Changes in natural killer cell phenotype in patients with post-viral fatigue syndrome. Clinical experimental Immunology 1991, 83: 441-446

Morton JJ, Connell JMC, Hughes MJ, Inglis GC, Wallace ECH. The role of plasma osmolality, angiotensin II and dopamine in vasopressin release in man. Clinical Endocrinology 1985, 23: 129-138

Moses AM, Gabrilove JS, Soffer LJ. Simplified water loading test in hypoadrenocorticism and hypothyroidism. Journal of Clinical Endocrinology & Metabolism 1958, 18: 1413-1417

Murdoch JC. Cell-mediated immunity in patients with myalgic encephalomyelitis syndrome. New Zeland Medical Journal 1988, 101: 511-512

Nir I, Hirschmann N. A thin-layer chromatographic assay for measuring pineal hydroxyindole-o-methyltransferase activity. Experientia 1979, 35: 1426-1427

Oldstone MBA, Rodriguez M, Daughaday WH, Lamperet PW. Viral perturbation of endocrine function: disorder of cell function leading to disturbed homeostasis and disease. Nature 1984, 307: 278-280

Oldstone MBA. Viral alteration of cell function. Scientific American, 1989, 260: 42-48

Olson GB, Kanaan MN, Gersuk GM, Kelley LM, Jones JF. Correlation between allergy and persistent Epstein-Barr virus infections in chronic-active Epstein-Barr virus infected patients. Journal of Allergy & Clinical Immunology 1986, 78: 308

Parker G, Walter S. Seasonal variation in depressive disorders and suicidal deaths in New South Wales. British Journal of Psychiatry 1982,140: 626-632

Pellew RAA. A clinical description of a disease resembling poliomyelitis seen in Adelaide, 1949-1951. Medical Journal of Australia 1951, 1: 944-946

Pelosi AJ, Sykes RA, Lough JR, Muir WJ, Dunnigan MG. A psychiatric study of idiopathic oedema. Lancet 1986, 2: 999-1001

Piper BF. Fatigue: current basis for practice. In: Funk SG, Tornquist EM, Champagne MT, Copp LA, Wiese RA (eds.). Key aspects of comfort: management of pain, fatigue and nausea. Springer Publishing Company, New York, 1989, pp 187-198

Platt AJ, Heddle RM, Rake MO, Smedley H. Ondansetron in carcinoid syndrome. Lancet 1992, 339: 1416-1417

Pool JH, Walton JN, Brewis EG, Uldall PR, Wright AE, Gardner PS. Benign myalgic encephalomyelitis in Newcastle upon Tyne. Lancet 1961, 1: 733-737

Poon WS, Mendelow AD, Davies DL, Watson W, Easton J, Morton J. Secretion of antidiuretic hormone in neurological patients: appropriate or inappropriate ? Australia & New Zeland Journal of Surgery 1989, 59: 173-180

Procter CA. Depression and disability in the chronic fatigue syndrome. MSc thesis. University of Glasgow, 1991

Poskanzer DC, Henderson DA, Knukle FC, Kaltzer SS, Clement WB, Bond JO. Epidemic neuromyasthenia. An outbreak in Punta Gorda, Florida. New England Journal of Medicine 1957, 257: 356-364

Preibisz JJ, Sealey JE, Laragh JH, Cody RJ, Weksler BB. Plasma and platelet vasopressin in essential hypertension and congestive heart failure. Hypertension 1983, 5: 129-138

Ramsay AM, O'Sullivan E. Encephalomyelitis simulating poliomyelitis. Lancet 1956, i: 761-764

Ramsay AM. Epidemic neuromyasthenia 1955-1978. Postgraduate Medical Journal 1978, 54: 718-721

Raskind MA, Weitzman RE, Orenstein H, Fisher DA, Courtney N. Is antidiuretic hormone elevated in psychosis ? A pilot study. Biological Psychiatry 1978, 13: 385-390

Rausch JL, Janowsky DS. Premenstrual tension: etiology. In: Friedman RC (ed). Behaviour and the menstrual cycle. Marcel Dekker, New York, 1982, p397

Redetzki HM, Hughes JR, Redetzki JE. Differences between serum and plasma osmolalities and their relationship to lactic acid values. Proceedings of The Society of Experimental Biological Medicine 1972, 139: 315-318

Redman J, Armstrong S, Ng KT. Free-running activity rhythms in the rat: entrainment by melatonin. Science 1983, 219: 1080-1081

Reeves AG, Plum F. Hyperphagia, rage and dementia accompanying a ventromedial hypothalamic neoplasm. Archives of Neurology 1969, 20: 616-624

Reppert SM, Duncan MJ, Goldman BD. Photic influences on the developing mammal. In: Photoperiodism, melatonin and the pineal. (Ciba Foundation Symposium, vol. 117) Pitman, London, 1985, pp 116-128

Rickels K, Freeman E, Sondheimer S. Buspirone in the treatment of premenstrual syndrome. Lancet 1989, i: 777

Riley MS, O'Brien CJ, McCluskey DR, Bell NP, Nicholls DP. Aerobic work capacity in patients with chronic fatigue syndrome. British Medical Journal 1990, 301: 953-956

Ringertz N, Nordenstam H, Flyger G. Tumours of the pineal region. Journal of Neuropathology & Experimental Neurology 1954, 13: 540-561

Robinson FJ, Power MH, Kepler EJ. Two new procedures to assist in the recognition and exclusion of Addison's disease. A preliminary report. Proceedings of the Staff Meetings, Mayo Clinic 1941, 16: 577

Roberts L. Single fibre EMG studies in the chronic fatigue syndrome. Journal of Neurological Sciences 1990, 98 (Suppl): 97

Robertson GL, Athar S. The interaction of blood osmolality and volume in regulating plasma vasopressin in man. Journal of Clinical Endocrinology & Metabolism 1976, 42: 613-620

Robertson GL. Thirst and vasopressin function in normal and disordered states of water balance. Journal of Laboratory & Clinical Medicine 1983, 101: 351-371

Robertson GL. Physiology of vasopressin secretion. Kidney International 1987, 32 (Suppl. 21): S20-S26

Rojdmark S, Berg A, Rossner S, Wetterberg L. Nocturnal melatonin secretion in thyroid disease and obesity. Clinical Endocrinology 1991, 35: 61-65

Rolandi E, Franceschini R, Cataldi A, Barreca T. Endocrine effects of sumatriptan. Lancet 1992, 339: 1365

Rosenthal NE, Sack DA, Gillin JC, Lewy AJ, Goodwin FK, Davenport Y, Mueller PS, Newsome DA, Wehr TA. Seasonal affective disorder. A description of the syndrome and preliminary findings with light therapy. Archives of General Psychiatry 1984, 41: 72-80

Rowe JW, Shelton RL, Helderman JH, Vestal RE, Robertson GL. Influence of emetic reflex on vasopressin release in man. Kidney International 1979, 16: 729-735

Rowe JW, Kilgore A, Robertson GL. Evidence in man that cigarette smoking induces vasopressin release via an airway specific mechanism. Journal of Endocrinology & Metabolism 1980, 51: 170-172

Runcie J, Hilditch TE. Energy provision, tissue utilization, and weight loss in prolonged starvation. British Medical Journal 1974, i: 352-356

Sachar EJ, Hellman L, Fukushima DK, Gallagher TF. Cortisol production in depressive illness: a clinical and biochemical clarification. Archives of General Psychiatry 1970, 23: 289-298

Sander LW, Julia HL, Stechler G, Burns P. Continuous 24-hour interactional monitoring in infants reared in two caretaking environments. Psychosomatic Medicine 1972, 34: 270-282

Sano K. Posterior hypothalamic lesions in the treatment of violent behaviuor. In: Fields WS, Sweet WH (eds). Neural basis of violence and aggression. Warren H. Green, St. Louis, 1975, pp 401-427

Schiavi RC, Macris NT, Camerino MS, Stein M. Effect of hypothalamic lesions on immediate hypersensitivity. American Journal of Physiology 1975, 228: 596-601

Seppala T, Ranta T, Shrotriya RC. Serum prolactin levels after buspirone in man. Medical Biology 1987, 65: 61-63

Sharpe MC, Archard LC, Banatvala JC et al (24 authors). A report - chronic fatigue syndrome: guidelines for research. Journal of Royal Society of Medicine 1991, 84: 118-121

Shaw DM, Camps FE, Eccleston EG. 5-hydroxytryptamine in the hind brain of depressive suicides. British Journal of Psychiatry 1967, 113: 1407-1411

Shelokov A, Habel K, Verder E, Welsh W. Epidemic neuromyasthenia. An outbreak of poliomyelitis-like illness in student nurses. New England Journal of Medicine 1957, 257: 345-355

Shilling NV, Belich AI, Karmanova IG. Role of the hypothalamus in the organization of the cycle of wakefulness and primary sleep in the frog Rana temporaria. Journal of Evovolution & Biochemical Physiology 1984, 20: 130-136

Sigurdsson B, Sigurjonsson J, Sigurdsson JHJ, Thorkelsson J, Gudmandsson KR. A disease epidemic in Iceland simulating poliomyelitis. American Journal of Hygeine 1950, 52: 222-238

Sigurdsson B, Gudmundsson KR. Clinical findings six years after outbreak of Akureyri disease. Lancet 1956, i: 766-767

Sigurdsson B, Gudnadottir M, Petursson G. response to poliomyelitis vaccination. Lancet 1958, 1: 370-371

Skrabal F, Arnot RN, Joplin GF. Equations for the prediction of normal values for exchangeable sodium, exchangeable potassium, extracellular fluid volume and total body water. British Medical Journal 1974, i: 37-38

Smith AP, Broadbent DE. The effect of noise on the naming of colours and reading of colour names. Acta Psychologica 1985, 58: 275-285

Smith AP, Tyrrell DAJ, Coyle K, William JS. Selective effects of minor illnesses on human performances. British Journal of Psychology 1987, 78: 183-188

Smith AP. Respiratory virus infections and performance. Philosophical Transactions of the Royal Society 1990, London, B 327: 519-528

Smith CK, Barish J, Carrea A, Williams RH. Psychiatric disturbances in endocrinologic disease. Psychosomatic Medicine 1972, 34: 69-86

Soffer LJ, Gabrilove JC. A simplified water loading test for the diagnosis of Addison's disease. Metabolism 1952, 1: 504-510

Spielberg CD, Gorsuch RL, Lushene RE. STAI Manual for the State-Trait Anxiety Inventory. Consulting Psychologists Press, Palo Alto, 1970.

Spink WW. What is chronic brucellosis? Annals of Internal Medicine 1951, 35: 358-374

Spruce BA, Baylis PH, Burd J, Watson MJ. Variation in osmoregulation of arginine-vasopressin during the human menstrual cycle. Clinical Endocrinology 1985, 22: 37-42

Spry CJF. Inhibition of lymphocyte recirculation by stress and corticotropin. Cell Immunology 1972, 4: 86-92

Steen AE. Virus epidemic in recurrent waves. British Medical Journal 1956, 1: 235

Steriade M, Llinas RR. The functional states of the hypothalamus and the associated neuronal interplay. Physiological Reviews 1988, 68: 649-742

Stevenson J, Hembling MH. A case of Bornholm disease associated with Coxsackie virus group [A Type 9 infection]. Lancet 1971, 2: 873

Straus SE. The chronic mononucleosis syndrome. Journal of Infectious Diseases 1988, 157: 405-412

Straus SE, Dale KJ, Peter JB, Dinarello CA. Circulating lymphokine levels in the chronic fatigue syndrome (letter). Journal of Infectious Diseases 1989, 160: 1085-1086

Stricker EM, Andersen AE. The lateral hypothalamic syndromes: comparison with the syndrome of anorexia nervosa. Life Science 1980, 26: 1927-1934

Sumner DW. Further outbreak of a disease resembling poliomyelitis. Lancet 1956, 1: 764-766

Swanson LW, Sawchenko PE. Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. Annual Review of Neuroscience 1983, 6: 269-324

Swartz MN. The chronic fatigue syndrome - one entity or many ? New England Journal of Medicine 1988, 319: 1728-1728

Taerk GS, Toner BB, Salit IE et al. Depression in patients with neuromyasthenia (benign myalgic encephalomyelitis). International Journal of Psychiatry and Medicine 1987, 17: 49-56

Taylor DJ. Cellular energetics in skeletal muscle of patients with with postviral fatigue syndrome. Paper presented at the symposium: Postviral fatigue syndrome and myalgic encephalomyelitis (ME): areas of controversy. Kensington and Chelsea New Town Hall, London, 23 June 1989

Tindall VR. Jeffcoate's Principles of Gynaecology. 5th ed. Butterworths, London 1987, pp 542-544

Thase ME. Assessment of depression in patients with chronic fatigue syndrome. Reviews of Infectious Diseases 1991, 13 (Suppl 1): S114-S118

Thasher TN. Circumventricular organs, thirst and vasopressin secretion. In: Schrier RW (ed.). Vasopressin. Raven Press, New York 1985, pp 311-318

Tobi M, Morag A, Ravid Z, Chowers I, Fedman-Weiss V, Michaeli Y, Ben-Chetrit E, Shalit M, Knobler H. Prolonged atypical illness associated with serological evidence of persistent Epstein-Barr virus infection. Lancet 1982, 1: 61-64

Tollefson GD, Godes M, Montague-Clouse J, Lancaster SP, Garvey MJ. Buspirone: effects on prolactin and growth hormone as a function of drug level in generalised anxiety. Journal of Clinical Psychopharmacology 1989, 9: 132-136

de la Torre JC, Borrow P, Oldstone MBA. Viral persistence and disease: cytopathology in the absence of cytolysis. British Medical Bulletin 1991, 47: 838-851

Tuomisto J, Mannisto P. Neurotransmitter regulation of anterior pituitary hormones. Pharmacology Reviews 1985, 37: 249-332

Tyrey L, Nalbandov AV. influence of anterior hypothalamic lesions on circulating antibody titres in the rat. American Journal of physiology 1972, 222: 179-185

Upadhyaya AK, Pennel I, Cowan PJ, Deakin JFW. Blunted growth hormone and prolactin responses to L-tryptophan in depression: a state dependent abnormality. Journal of Affective Disorders 1991, 21: 213-218

Vaughn MK. Pineal peptides - an overview. In: Reiter R (ed.). The pineal gland. Raven Press, New York, 1984, pp 39-82

Vigersky RA, Loriaux D, Anderson AE, Lipsett MR. Anorexia nervosa: behavioural and hypothalamic aspects. Clinical Endocrinology & Metabolism 1976, 5: 517-535

Vriend J. Endocrine effects of blinding in male Syrian hamsters are associated with increased hypothalamic 5-hydroxyindolacetic acid/ serotonin ratio. Journal of Pineal Research 1989, 7: 401-409

Waldhauser F, Weiszenbacher G, Frisch H, Zeitlhuber U, Waldhauser M, Wurtman R. Fall in nocturnal serum melatonin during prepuberty and pubescence. Lancet 1984, 1: 362-365

Waldhauser F, Vierhapper H, Pirich K. Abnormal circadian melatonin secretion in night-shift workers. New England Journal of Medicine (letter), 1986, 315: 1614

Weller MPJ, Jauhar P. Travel induces distrubances in circadian rhythms as precipitants of affective illness. In: Perris C et al (eds). Biological Psychiatry. Elsevier/North Holland, Biomedical Press, Amsterdam, 1981, pp 1253-1256

Weller RA, Weller EB. Anorexia nervosa in a patient with an infiltrating tumour of the hypothalamus. American Journal of Psychiatry 1982, 139: 824-825

Wetterberg L, Arendt J, Paunier L, Sizonenko PC, van Donselaar W, Hyden T. Human serum melatonin changes during the menstrual cycle. Journal of Clinical Endocrinology & Metabolism, 1976, 42: 752-764

Wetterberg L. Melatonin in humans. Physiological and clinical studies. Journal of Neural Transmission 1978, Suppl. 13: 289-310

Wetterberg L, Beck-Friis J, Aperia B, Patterson U. Melatonin/cortisol ratio in depression. Lancet 1979, 2: 1361

Wetterberg L, Beck-Friis J, Kjellman BF, Ljunggren JG. Circadian rhythms in melatonin and cortisol secretion in depression. Advances in Biochemical Psychopharmacology 1984, 39: 197-205

Wilson JC, Walker PJ. Acute anterior poliomyelitis: orthopedic aspects of California epidemic of 1934. Archives of Internal Medicine 1936, 54: 477-491

Whalley LJ, Christie JE, Blackwood DHR, Bonnie J, Dick H, Blackburn IM, Fink G. Disturbed endocrine function in psychoses. British Journal of Psychiatry 1989, 155: 455-461

White DN, Burtch RB. Iceland disease - a new infection simulating acute anterior poliomyelitis. Neurology 1954, 4: 506-516

White P. Management of fatigue syndromes: psychiatric considerations. Paper presented at the symposium: Postviral fatigue syndrome and myalgic encephalomyelitis (ME): ares of controversy. Kensington & Chelsea New Town Hall, London, 23 June, 1989

Wurtman RJ, Wurtman JJ. Carbohydrates and depression. Scientific American 1989, 260: 50-57

Yates M, Leake A, Candy JM, Fairbrain AF, McKeith IG, Ferrier IN. 5HT2 receptor changes in major depression. Biological Psychiatry 1990, 27: 489-496

Yatham LN, Barry S, Dinan TG. Serotonin receptors, buspirone, and premenstrual syndrome. Lancet 1989, i: 1447-1448

Yousef GE, Bell EJ, Mann GF, Murugesan V, Smith DG, McCartney RA, Mowbary JF. Chronic enterovirus infection in patients with postviral fatigue syndrome. Lancet 1988, i: 146-150

Young JB, Brownjohn AM, Chapman C, Lee MR. Evidence for hypothalamic disturbance in cyclical oedema. British Medical Journal 1983, 286: 1691-1693

Zerbe RL, Robertson GL. Osmotic and non-osmotic regulation of thirst and vasopressin secretion. In: Maxwell MH, Kleeman CR, Narins RG (eds.). Clinical disorders of fluid and electrolyte metabolism. 4th ed. McGraw-Hill, New York, 1987, pp 61-68