SOME BIOCHEMICAL AND BEHAVIOURAL EFFECTS OF FEMALE GONADAL HORMONES.

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SUMMARY

SOME BIOCHEMICAL AND BEHAVIOURAL EFFECTS OF FEMALE GONADAL HORMONES.

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This thesis investigates some of the possible factors involved in the aetiology of the psychological syndromes seen at times of hormonal flux in human females. The thesis is split into two sections, firstly human clinical studies and secondly animal behavioural and biochemical studies.

Putative aetiological factors of post partum 'blues' were investigated, however no relationship between this syndrome and either sociological factors such as marital status or age or biochemical factors such as plasma progesterone or oestradiol was seen. Significantly decreased plasma total tryptophan on the first day post partum was found amongst 'blues' cases. The incidence of 'blues' was significantly greater amongst primiparae than multiparae, although there were no consistent significant biochemical differences between these two parity groups.

The same factors were investigated in relation to those subjects who exhibited depressed affect within 9 months of parturition. The incidence of such depressive symptoms was significantly greater amongst mothers of male infants than amongst mothers of female infants, although there were no consistent biochemical differences between these two groups. Puerperal plasma non esterified fatty acids were also significantly increased in those subjects who later exhibited depressed affect, there were no differences in any of the other biochemical variables measured. There was no relationship between the incidence of 'blues' and the incidence of depression post partum.

Plasma total tryptophan and non esterified fatty acids were also studied in respect to menstrual mood changes. There was no relationship between these parameters and the psychological symptoms. Plasma tryptophan was also studied in relation to perimenopausal depression. There were no differences in plasma free or total tryptophan between depressed and non depressed subjects, although plasma free tryptophan was significantly decreased amongst post menopausal subjects.

Studies of the sedative effects of progestational hormones in mice showed that both natural progesterone and synthetic progestogens were capable of prolonging thiopentone anaesthesia in female mice, however there was no such effect in male mice. Chronic administration of high doses of progestational hormones to female mice produced significantly increased plasma tryptophan, plasma non esterified fatty acids and brain indoleamines. There was no effect of this treatment in male mice. Acute withdrawal of the chronic progesterone treatment in female mice (as a simulation of parturition) had no effects on plasma tryptophan or non esterified fatty acids, brain indoleamines were all significantly decreased. There was no effect of this treatment in male mice. Behavioural studies performed during this hormone treatment suggested that the changes in brain indoleamines involved 'non functional' rather than 'functional' 5HT stores.

The conclusions drawn were that variations in plasma progesterone concentrations were unlikely to be involved in the changes of plasma tryptophan seen during human pregnancy or puerperium, or to be involved in the mood changes frequently seen at these times.

Key words: Tryptophan, Indoleamines, Progestational hormones, Depressed affect

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INTRODUCTION

This introduction is a description of the state of knowledge in this field at the outset of this thesis. Factors involved in the background, and influencing the design of the studies are presented within this section. New data published since the outset of any projects, and hence playing no part in their initial design will be presented and discussed at the end of this thesis.

1 GENERAL INTRODUCTION AND AIMS OF THE THESIS

Depression is a state of mind which is frequently characterised as a mental illness, it is however, also a normal mood state which everybody experiences from time to time, and it would be wrong to label all individuals who sometimes experience non-incapacitating, but nevertheless unpleasant, states of mental distress as psychiatrically ill or in need of treatment. On the other hand, some depressive mood swings may generate so much misery as to totally disrupt a person's life style, or even in the extreme, to precipitate suicide. Major psychiatric disturbances may thus completely destroy an individual's ability to live a satisfactory life.

Some forms of severe mental distress are, however, quite normal states of mood, for example the depression following bereavement, but where these depressive feelings exist for very long periods of time, or where their onset was not preceded by some "triggering" factor, then it is reasonable to suggest that some form of mental illness exists. Such forms of depressive illness that lack a triggering factor are frequently termed "endogenous", in that the depression appears to arise from within the person concerned. It is these endogenous illnesses that are widely believed to be brought on by some biochemical change within the central nervous system of the patient, and many theories as to the nature of this biochemical change have been proposed.

In women however, there are certain times of life when reports of depression or disrupted mood are prevalent. Such times are premenstrually, post-partum, perimenopausally and following treatment with certain hormonal preparations. These disturbances are rarely of sufficient severity as to warrant medical treatment, but they are frequently severe enough to disrupt the individual's life style. The fact that these mood changes are endogenous in nature, and that they occur at times of great physical and biochemical change, lends support to the hypothesis that there is a biochemical basis to the mental changes.

This thesis therefore aims to investigate some of the biochemical changes that occur at these times of mood change in women, and to attempt to explain these mood changes in terms of chemical changes within the central nervous system (CNS).

2 THE PUTATIVE ROLE OF MONOAMINE NEUROTRANSMITTERS IN THE AETIOLOGY OF ENDOGENOUS DEPRESSIVE DISORDERS.

In the 1950s it was noted that patients receiving rauwolfia alkaloids eg Reserpine, for the treatment of hypertension, frequently reported the symptoms of depression as a side effect (Muller et al.,1955; Harris, 1957). However the mechanism of this effect was not understood at that time. Subsequently, in 1959 it was noted that the antituberculous drugs, iproniazid and isoniazid, produced mood elevations in tuberculous patients. Iproniazid was the more effective and this was later introduced for the treatment of depression (Pare and Sandler, 1959). Again the mechanism of this effect was not fully understood. Amphetamine had also been used for the alleviation of depression (Leake, 1958).

The modes of action of these drugs then became apparent and the monoamine hypothesis of depression was proposed. Reserpine was seen to prevent the storage of monoamine transmitters in the presynaptic terminals. Hence there was a depletion of such stores, leading to a decreased release of neurotransmitters (Pletsher et al.,1955). In addition, iproniazid was shown to inhibit the activity of monoamine oxidase (Smith, 1964). This enzyme is the major enzymic route by which the neurotransmitters are "deactivated" following their release and action on the post-synaptic receptor. This enzyme inhibition therefore produced increased concentrations of the neurotransmitter within the synaptic cleft.

Amphetamine also causes the release of neurotransmitter from the presynaptic terminal (Leake, 1959). A picture of the biochemical basis of depression was therefore being developed. What was seen was that drugs that decreased the amount of transmitter at the post synaptic terminal produced depression, whilst drugs which increased the amount of neurotransmitter present could relieve depressive symptoms.

The development of a new class of antidepressant drugs in the late 1950s, the tricyclic antidepressants, added much weight to this idea. These drugs were seen to produce their effects by preventing the re-uptake of neurotransmitters following their release, back into the presynaptic terminal (Glowinski and Axelrod, 1964). This re-uptake of neurotransmitter is the major route by which their action is normally terminated. It was therefore seen that these drugs were producing their effect by increasing the amount of transmitter present at the post-synaptic receptor (Iversen et al, 1975).

The hypothesis therefore developed was that depression was caused by a deficit of a neurotransmitter within the CNS, and that antidepressant drugs acted by increasing the amount of transmitter present.

There was however some debate as to which transmitter was deficient. The main contenders for the rôle were 5-Hydroxytryptamine (5HT) and Noradrenaline (NA). Both of these were implicated since reserpine depletes stores of both whilst the antidepressant drugs (with a few exceptions, see later) produce effects on both.

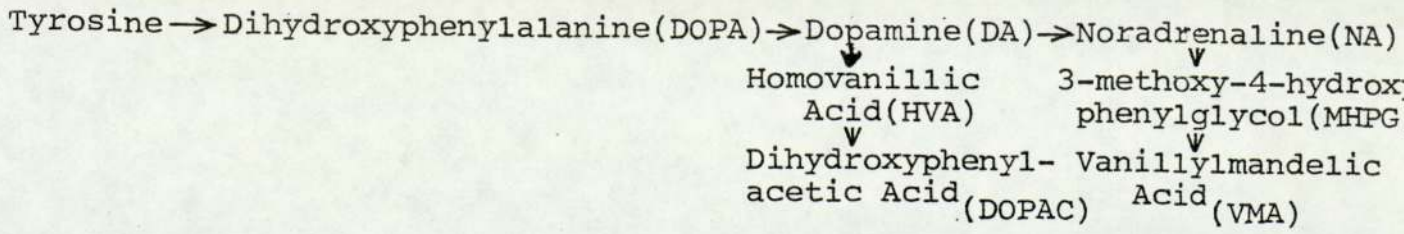
The next problem was therefore to demonstrate a functional deficit of either neurotransmitter in depressed patients. The evidence for an NA deficit will be discussed first.

Studies measuring NA in post mortem brains of depressives have been unsuccessful in showing any deficit (Van Praag, 1980 b). Studies of NA in cerebrospinal fluid (CSF) have also been inconclusive (ibid). However determinations of the NA metabolites 3-methoxy-4-hydroxy phenylglycol (MHPG) and vanillylmandelic acid(VMA)(See FigI)have produced some positive results. In the CNS (including the spinal cord) MHPG is the major NA metabolite, whilst VMA is the major metabolite in the peripheral nervous system. Therefore measurements of these metabolites in the CSF indicate levels or turnover of NA in the CNS.

Post and Goodwin, 1978, demonstrated a subgroup of endogenous depressed patients that had decreased CSF MHPG and VMA concentrations. However, other studies have not repeated these findings (Van Praag, 1980 b).

Urinary excretion of MHPG has also been compared between depressives and controls. This approach is valid since it is estimated that over 50 per cent of MHPG found in the urine is formed in the CNS (Van Praag, 1980b), hence urinary MHPG may be used as an indicator of NA metabolism in the CNS.

Figure I : Simplified Diagram of Catecholamine Metabolism.



Several studies have reported decreased urine MHPG in depression (Maas et al., 1968; Schildkraut et al., 1978). VMA excretion was seen to be normal, suggesting a reduced central NA turnover with an undisturbed peripheral NA metabolism. However, only certain subgroups of the depressed patients exhibited the decreased urine MHPG. These subgroups were indistinguishable from other depressed patients in terms of the psychiatric symptomatology (Schildkraut et al., 1978).

A further method for investigating monoamine function *in vivo* is the neuroendocrine approach. Hormone release by the anterior lobe of the pituitary is regulated by the releasing and inhibiting factors of the hypothalamus. The production of these factors is at least partially controlled by monoaminergic neurones, therefore functional disorders in the activity of these neurones would manifest themselves in an altered release of one or more of the anterior pituitary hormones. In this context, certain endocrine disorders have been demonstrated for some sub-groups of depressed patients.

The first of these disorders is in the secretion of cortisol. Some depressed patients have been shown to have a hypersecretion of cortisol into the urine (Board et al., 1957; Bunney et al., 1965), with the loss of normal diurnal urine cortisol secretory patterns (Sachar, 1973). This loss of the diurnal rhythm was seen to return to normal on recovery from depression (ibid).

In normal subjects, cortisol secretion parallels adrenocortico-trophic hormone (ACTH) secretion. Hence in these depressive subgroups, an ACTH hypersecretion is likely. Normally ACTH secretion is under inhibitory control by noradrenergic neurones of the hypothalamus via corticotropin releasing factor (CRF). Hence the hypersecretion of cortisol may be a reflection of an underactive noradrenergic system of the hypothalmus.

Some depressed patients have also been shown to lack the normal response to the dexamethasone suppression test (Carroll et al., 1976). Dexamethasone is a synthetic steroid which normally inhibits the release of ACTH. However this ACTH secretion inhibition is not seen in some depressive patients, adding further support to the hypothesis of a disorder of the CRF/ACTH system in depression. Checkley and Crammer (1977) further demonstrated a decreased release of plasma cortisol following methamphetamine administration. Increased plasma cortisol in depression has also been seen following insulin-induced hypoglycaemia (Perez-Reyes, 1969).

Hypothalamic noradrenergic systems also control the release of Growth Hormone (GH) from the anterior pituitary via GH-releasing factor. GH release may be stimulated by insulin induced hypoglycaemia. Using this method, depressed patients have been shown to have decreased GH release (Mueller et al., 1969; Sachar et al., 1971). Similar results have been seen following GH release stimulation by amphetamine (Langer et al., 1976) and clonidine,

and α_1 and α_2 adrenoceptor agonist (Matussek, 1980). This again suggests a deficit in the hypothalamic noradrenergic system.

Luteinizing hormone (LH) is another hormone partially noradrenergically controlled, via LH-releasing factor. Plasma levels of this hormone have also been reported as being decreased in some endogenous depressives (Altman et al., 1976), again suggesting a disturbed noradrenergic system.

There is a similar body of evidence implicating 5HT in the aetiology of endogenous depression. One of the first studies performed was that of Shaw and co-workers (1967), in which 5HT concentrations in the hind brains of depressive suicides were measured and compared with control subjects that had died from accidents. The results showed that depressed suicides had decreased levels of hind brain 5HT. The same was found to be true for the brainstem (Pare et al., 1969) and the raphe nuclei (Lloyd et al., 1974). Similar results have been seen for depressed patients who died from causes other than suicide (Birkmeyer and Riederer, 1975). However, not all workers have been able to produce such differences between normals and suicides (eg Beskow et al., 1976; Cochran et al., 1976). As with the noradrenergic research, extrapolations concerning the central metabolism of 5HT have been made from measurements of its metabolite. The major metabolite of 5HT is 5-Hydroxyindoleacetic Acid (5HIAA). (See Fig II).

Figure II - Simplified Diagram of 5HT Metabolism.

Tryptophan(TP) ⟶ 5Hydroxytryptophan(5HTP) ⟶ 5Hydroxytryptamine (5HT)
↓
5Hydroxyindoleacetic Acid(5HIAA)

Asburg and colleagues (1976) reported that in some depressed patients there was a decrease in lumbar CSF 5HIAA concentrations. However, endogenous depressed patients with decreased CSF 5HIAA were indistinguishable in terms of psychiatric symptoms from depressed patients with normal CSF 5HIAA. Previous workers had also seen decreased CSF 5HIAA in depression which did not return to normal on recovery from the depression (Coppen et al., 1972; Ashcroft et al., 1973).

Bridges and colleagues (1976) measured 5HIAA and tryptophan (TP), the precursor of 5HT, in cerebroventricular CSF. This group found that the mean levels of TP and 5HIAA were decreased in depressed patients as compared with other psychiatric and neurosurgical controls.

A better method for the measurement of CSF 5HT turnover uses probenecid. Probenecid blocks the efflux of carboxylic acids, including 5HIAA, from the CSF. Using this method therefore, a better indication of 5HT turnover over a longer time is obtained. Using this method, 40 per cent of endogenous depressed patients showed decreased CSF 5HIAA (Van Praag et al., 1973). This is seen as indicating a decreased turnover of 5HT in the CNS. This deficit did not return to normal on clinical recovery (ibid, 1980). Again those depressed patients with decreased CSF 5HIAA were indistinguishable from other endogenous depressives in terms of psychiatric symptoms (Van Praag, 1980a).

Another approach to studying the 5-Hydroxytryptaminergic system has been to measure plasma TP.

The conversion of TP to 5HTP is the rate limiting step in the production of 5HT (See later). Therefore decreased levels of plasma free TP results in decreased 5HT synthesis (Knott and Curzon, 1972). Measurements of plasma TP therefore give some information concerning the 5-Hydroxytryptaminergic system.

Coppen and co-workers (1972, 1973, 1978) reported that depressed patients exhibited decreased levels of plasma free TP when compared with controls. Decreased free TP was also seen by Kishimoto and Hama (1976). The deficit was seen to return to normal on recovery from the depression (Kishimoto & Hama, 1976; Shaw et al., 1979). However, Coppen and co-workers (1973) reported that the free TP concentrations rose on recovery from depression, but not to normal levels. Peet and co-workers (1976) found no differences in plasma free or total TP between depressed and recovered patients.

Decreased plasma free TP was also seen in the mild depressive mood changes frequently seen immediately post partum (Stein et al., 1976; Handley et al., 1977).

No differences were found in plasma free TP between depressed and non-depressed patients by Wirz-Justice and co-workers (1975), Riley and Shaw (1976) or Garfinkel and co-workers (1976). Handley and colleagues (1980) found no difference in plasma free TP in cases of depressive mood change post partum, however plasma total TP was significantly decreased. Coppen and co-workers (1973) found no difference in plasma total TP between depressed and non-depressed patients.

Niskanen and colleagues (1976) found no difference in total TP between depressed and non-depressed patients, however they found that depressed patients had significantly increased plasma free TP.

Using radio-labelled TP no differences were found in metabolism of TP between depressed and non-depressed patients (Coppen et al., 1974).

Following the reports that plasma free TP was decreased in depression, attempts were made at using TP supplements as an antidepressant. However results were generally negative (see review, Van Praag, 1980 a.), however in a double blind trial against amitriptylline, the two were found to be therapeutically equivalent (Herrington et al., 1976). TP has also been seen to potentiate the therapeutic efficacy of monoamine oxidase inhibitors (MAOI) and tricyclic antidepressants (Cooper, 1979; Walinder, 1976). Van Praag and co-workers (1971) also reported that 5HTP was of use as an antidepressant. MAOI are also potentiated by 5HTP (ibid).

The fact that post-probenecid CSF 5HIAA remained abnormal in patients following recovery from depression (Van Praag (1980a) and that some workers reported that plasma free TP remained decreased following recovery from depression (Peet et al., 1976) supported the earlier hypothesis of Kety, 1971. This hypothesis was that the actual depressive episode was due to an NA deficit, whilst a 5HT deficit was a chronic predisposing factor for depression. This led Van Praag (1979) to use 5HTP therapy as a prophylaxis for depression, in order to overcome the chronic 5HT deficit. However, due to the small number

of patients studied, the results were inconclusive, although the patients with abnormal CSF 5HIAA appeared to respond well to this therapy. Moller and colleagues (1979), reported that some patients respond well to TP replacement therapy. However, those patients that responded well could not be predicted by prior investigation of plasma free or total TP values. Therefore these results suggest that there is a subgroup of depressed patients with a replaceable 5HT deficit.

However, not all workers believe that depression is caused by a 5HT deficit. It is proposed that individuals prone to depression have decreased synthesis and turnover of central 5HT. This decrease in 5HT turnover may result in supersensitivity of the 5HT receptors. Therefore at times of stress there would be a release of 5HT which would act on the supersensitive receptors. It is postulated that it is this action of 5HT on supersensitive receptors which produces the symptoms of depression. This would explain why TP supplements do not alleviate the symptoms of depression (Aprison et al., 1976; Segawa et al., 1979). This theory however cannot explain why antidepressant drugs which produce increased levels of 5HT can actually alleviate depression. Hence this view point has attracted very few supporters to date.

The monoamine hypothesis of depression is based mainly on the fact that the MAOI and tricycle antidepressant drugs were demonstrated to produce an increase in the amount of neurotransmitter at the receptor. However, these drugs can cause the downfall of this hypothesis as well as being its foundation. Firstly, the hypothesis of Kety (1971) proposed that a 5HT deficit was a predisposing factor for depression, whilst the actual episodes of depression were produced by an NA deficit. However, certain of the tricyclic antidepressants are more selective for 5HT reuptake inhibition (eg Clomipramine), others selectively inhibit NA reuptake (eg Maprotiline), whilst the remainder inhibit reuptake of both NA and 5HT. However, they are all therapeutically useful in the treatment of depression.

Another antidepressant, Mianserin, is a weak inhibitor of 5HT reuptake and has some 5HT and α_2-adrenoceptor blocking activity. Its antidepressant effect is possibly due to blockade of pre-synaptic receptors, producing increased release of transmitter from the presynaptic terminal (Matussek, 1980).

The other factor that is probably the major argument against the monoamine hypothesis is that the MAOI and tricyclic antidepressants produce the increased concentrations of transmitter at the synapse within hours of administration (Schildkraut et al., 1967). However the therapeutic effect of these drugs does not occur until about 2 weeks after the initiation of treatment. The only way in which the monoamine hypothesis of depression

can overcome this problem is by assuming that the anti-depressants require this length of time to reach the target synapses. This pharmacokinetic explanation however seems unlikely.

It is possible however that depression is caused not by the alteration of transmitter release from the presynaptic terminal, but rather by a decreased sensitivity of the post-synaptic receptor. Hypothetically both cases could produce the same effect. An increase in presynaptic receptor sensitivity could also produce similar effects. In connection with this, a number of studies have shown alteration of central receptor sensitivity or density following chronic antidepressant treatment in animals. This will be discussed in more detail in the general discussion.

3 OTHER BIOCHEMICAL CORRELATES OF ENDOGENOUS DEPRESSION.

As stated previously, there have been many findings to support the monoamine hypothesis of depression. However, there are other factors which suggest a different mechanism in the aetiology of endogenous depression.

One factor that has been repeatedly seen in depressed patients is some kind of biological membrane transport dysfunction. Wood and co-workers (1979) reported that TP accumulation by blood platelets was increased in acute depression. It was hypothesised that this factor may be important in the regulation of plasma TP, and may therefore explain the decreased plasma free TP seen in depression (Coppen et al., 1972). A delayed clearance of intravenous radiolabelled tryptophan has also been seen in depressives (Spano et al., 1975) again suggesting some transport mechanism disruption.

Sandler and co-workers (Sandler et al., 1975; Bonham-Carter et al., 1978) have also shown that depressed patients show a deficit in conjugated tyramine excretion following an oral tyrosine load. This is hypothesised as being due to either a monoamine oxidase over-activity, or due to some membrane transport mechanism deficit. Support for the membrane transport deficit can also be found in the finding that depressed patients show decreased red blood cell lithium concentrations (Elizur et al., 1972).

Plasma large neutral amino acid concentrations, expressed as a ratio against TP, have also been seen to be increased in depression (Moller et al., 1976). This would produce a profound effect on transport of TP into the CNS (see later).

Another theme running through the research into the biochemical causes of depression is that of some abnormal stress response in depressed patients. One of the commonly measured markers of stress are plasma concentrations of non-esterified fatty acids (NEFA). These are normally rapidly mobilised from fat stores at times of stress following the action of noradrenaline on the β-adrenoceptors of adipose tissue. Hence measurement of changes in NEFA concentrations can give some information concerning sympathetic nervous system activity. However, NEFA themselves can affect the rate of entry of TP into the CNS for conversion to 5HT (See later). Therefore, NEFA may also give us some information concerning 5HT synthesis and metabolism. Van Praag and Leijnse (1966) reported that endogenous depressed patients had increased NEFA metabolism. This they presumed to be due to decreased glucose utilisation, and not due to any increased emotion or perception of stress. Mueller and co-workers (1970) however, using strict dietary controls, failed to find any significant differences between depressed and non-depressed patients with respect to early morning fatty acids following overnight fasting. However, an increased rise in plasma NEFA following mild stress has been seen in endogenous depressives, this is seen as possibly reflecting an abnormality in the sympathetic nervous system (Curzon et al., 1979).

Still on the theme of possible abnormal stress responses in depressed patients, cortisol is released from the adrenal cortex at times of stress. As mentioned previously, raised urinary cortisol has been seen in depressives (Board et al., 1957; Bunney et al., 1965). The latter group reported that those patients with the highest cortisol excretion showed a struggle with the illness, with "ego alienation", whilst a group of patients with slightly lower cortisol excretion showed extensive denial of the illness.

Another hormone rapidly released following mild stress is prolactin (Bentley, 1980). Twenty-four hour secretions of this hormone have been seen to be increased in depressed patients (Halbreich et al., 1979). Hence again some abnormal stress response may be present in depressed patients.

One of the striking features about the present and the previous section is the fact that researchers report that certain sub groups of patients show the various abnormalities. For example, Asburg and colleagues (1976) discuss the idea that there are two groups of depressed patients, those with low CSF 5HIAA and those with high CSF 5HIAA. Van Praag went on toreport that those patients with low CSF5HIAA responded well to treatment with 5HTP (Van Praag, 1980a). The two groups of patients showed identical psychiatric symptoms.

Schildkraut and colleagues (1978) also reported a subgroup of depressed patients with decreased urinary MHPG. Again those patients with a low MHPG could not be distinguished from other depressives in terms of psychiatric symptomatology.

Therefore, perhaps depression is a mixture of illnesses, with multiple underlying causes, or perhaps there is a single underlying cause which may produce a variety of satellite biochemical changes. Perhaps it is these satellite changes that have been measured to date.

Whilst accepting and acknowledging other hypotheses of depression, this thesis concentrates on the rôle of TP and the 5-Hydroxytryptaminergic system in the aetiology of depressive mood changes. The mood changes that are studied are those changes seen at the times of the female reproductive cycle that are associated with large fluctuations in hormone levels. Hence this thesis investigates some possible biochemical factors involved in the mood changes seen premenstrually, post-partum and perimenopausally.

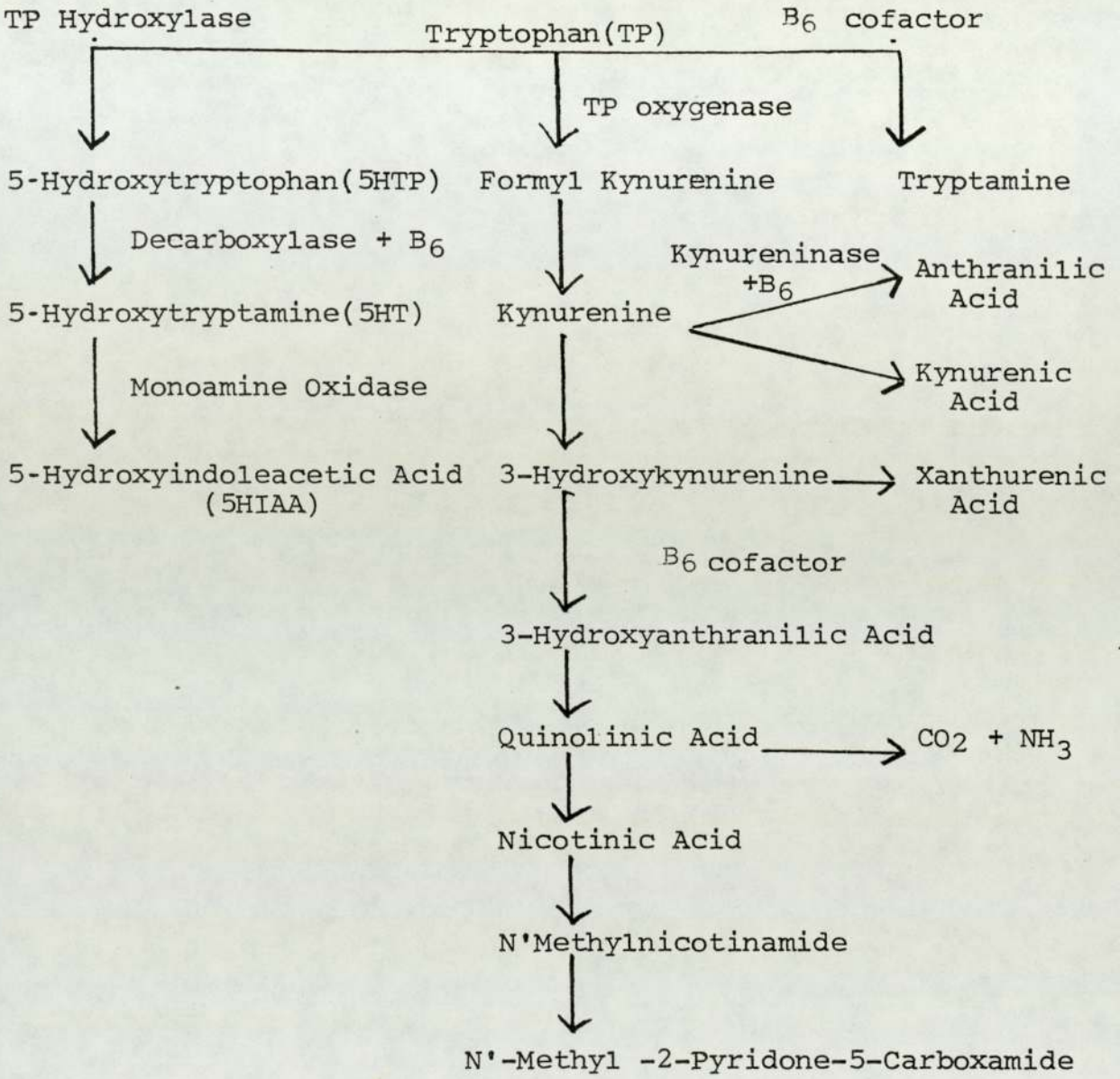
4 5-HYDROXYTRYPTAMINE SYNTHESIS AND METABOLISM, AND ITS CONTROLLING FACTORS.

The synthesis and metabolic pathway for 5HT is illustrated in Figure III. 5HT is formed and stored in specific brain neurones, the initial step of the synthesis being the uptake of TP from the plasma. TP exists in the plasma in two forms; the free (unbound) form, and the bound form. Approximately 90 per cent of plasma TP is normally bound to the plasma protein albumin (McMenamy & Oncly, 1958). Many workers believe that it is only this free TP that can enter the brain (Christensen & Handlogten, 1979), hence plasma free TP concentrations are a better determinant of brain TP concentrations than the total plasma TP concentrations (Gessa & Tagliamonte, 1974; Bloxham and Curzon, 1978). This simple picture however, is complicated by the fact that the active transport mechanisms into the brain may be able to 'strip' TP from the albumin, hence entry of TP into the brain is not solely dependant on plasma free TP levels (Yuwiler et al., 1977; Pardridge, 1979).

Once TP has entered the neurone it is acted upon by the enzyme Tryptophan hydroxylase to produce 5-Hydroxytryptophan (5HTP). This enzyme hydroxylation is the rate limiting step of 5HT production (Green & Sawyer, 1966). 5HTP is then decarboxylated to 5HT.

Under normal circumstances the tryptophan hydroxylase is not saturated with its substrate TP (Jecquier et al., 1969), therefore increased 'supply' of TP to the enzyme would cause increased production of 5HT.

FIGURE III : Synthesis and Metabolism of TP and 5HT.



Hence the rate limiting step of 5HT production is actually the entry of TP into the brain (Knott & Curzon, 1974). Increases in plasma free TP produce increased brain TP (Knott & Curzon, 1972), and this in turn produces increased brain 5HT (Fernstrom & Wurtman, 1971). However, small doses of TP have been shown to produce increases in brain 5HT without affecting either plasma or brain TP (ibid).

Thus the production of brain 5HT may be influenced by two basic factors. Firstly, the rate at which the uptake process transports TP into the brain (Hamon et al., 1974), and secondly the availability of TP, probably in the free form in the plasma (Fernstrom &Wurtman, 1971). Changes in either of these factors will therefore affect 5HT synthesis.

The first of these to be discussed will be the transport of TP into the brain. There are two carrier systems for the entry of TP into the brain (Christensen & Handlogten, 1979), one active system and one passive system (Sourkes, 1979). The passive system is a saturable, low affinity carrier, whilst the active system is high affinity, high capacity (Mans et al., 1979a; Hamon et al., 1974). Normally the high affinity system accounts for 40 per cent of the total TP transport (Mans et al., 1979). It is believed that alterations in the activity of the high affinity transport system may be responsible for the diurnal variations of 5HT synthesis seen in the rat (Hery et al., 1974). Both systems are however subject to competition by large neutral amino acids (LNAA) such as tyrosine, phenylalanine,

leucine, isoleucine and valine (Fernstrom & Wurtman, 1972a). Hence decreased plasma concentrations of these amino acids have been shown to produce increased levels of brain TP, probably due to decreased competition for entry at the carrier sites (James et al., 1976). Hence dietary intake of amino acids may influence TP uptake and 5HT synthesis (Fernstrom, 1979), also post prandial free TP may be of no use in predicting brain TP due to the high dietary LNAA (Fernstrom et al., 1975).

Progesterone has also been shown to increase brain uptake of TP in vivo (Glowinski et al., 1973).

Entry of TP into the brain is also influenced by the concentration of TP in the plasma, probably in the free form (Knott & Curzon , 1972) (Vide Supra). TP is normally 90% bound to plasma albumin (McMenamy & Oncly, 1958) however, non esterified fatty acids (NEFA) bind to plasma albumin with a greater affinity than TP does. Therefore NEFA compete with TP for albumin binding, and will displace TP from albumin (Curzon et al., 1974; Knott & Curzon, 1972). Hence increased plasma NEFA produce increased free TP, which in turn produces increased brain TP and 5HT (Knott and Curzon, 1972).

NEFA and LNAA however do not act independently. Studies involving the administration of a carbohydrate diet show that carbohydrate intake results in the lowering of plasma NEFA levels. This therefore reduced plasma free TP . However, the carbohydrate diet also produced insulin release, which induces amino acid uptake by tissues.

There is therefore less competition for the transport of TP into the brain. The nett result of such treatment is actually an increase of brain TP and 5HT (Fernstrom & Wurtman, 1972b; Madras et al., 1974). The complex nature of factors influencing 5HT production can therefore be seen.

The production of 5HT, however is not the major metabolic pathway for TP. The major metabolic route is via the production of Kynurenine in the liver (Fig III). TP may also be used for protein synthesis. Therefore the relative activity of the other two metabolic pathways have profound effects on the utilisation of TP for 5HT synthesis.

The utilisation of TP for the production of Kynurenine may be increased by the acceleration of the enzyme Tryptophan oxygenase, this is the initial and rate limiting enzyme in the production of Kynurenine (Mangoni, 1974). Hence acceleration of this enzyme may lead to a diversion of TP along this pathway, possibly leaving a deficit of TP for 5HT synthesis. Acceleration of this pathway would also produce a deficit of pyridoxine (Vitamin B_6), this cofactor being required for the synthesis of 5HT, and the decarboxylation of TP to tryptamine (See Fig III), hence again producing a deficit of TP for 5HT synthesis.

Evidence for increased metabolism along the Kynurenine pathway would be the excretion of increased amounts of the pathway metabolites, eg Kynurenic acid and xanthurenic acid. Some evidence of increased Kynurenine metabolite excretion has been seen in depressed patients (Rubin, 1967; Curzon & Bridges, 1970).

These results however, were not replicated by Frazer and colleagues (1973), who found no such increase in metabolite excretion.

Cortisol, oestrogens and oestrogen-progesterone combinations may increase Kynurenine production (Altman & Greengard, 1966; Rose, 1969; Rose & Braidman, 1969), and cortisol has been seen to temporarily decrease brain 5HT in rats (Curzon & Green, 1968). However, Kovacs and Telegdy (1979) reported that low doses of corticosterone produced increased concentrations and turnover of 5HT in the hypothalamus and mesencephalon, whilst high doses decreased 5HT in the hypothalamus and mesencephalon. Bond (1979) reported increased mouse brain 5HT following chronic administration of an oestrogen/progestogen combination. However, this effect was not seen by Algeri and co-workers (1977) or by Gould (1979). Decreased brain 5HT after 3 months chronic administration of oestrogen or oestrogen/progestogen combination was reported by Shetty and Gaitonde (1980). This effect of oestrogen/progestogen decreasing brain 5HT (Shetty & Gaitonde, 1980) may be due to acceleration of the Kynurenine pathway. Gould (1979) reported that chronic oestrogen/progestogen produced increased plasma Kynurenine. This effect was not seen however for cortisol (ibid), hence the decreased brain 5HT following cortisol administration (Curzon & Green, 1968) cannot be explained by this mechanism.

The effects of endogenous hormonal changes on brain 5HT have also been studied.

In the silver fox,brain 5HT was found to be increased during the proestrous phase of the oestrous cycle, and decreased at oestrous (Voitenko et al., 1979). In mice, brain 5HT was seen to be at a maximum during dioestrous, and minimum at oestrous (Greengrass & Tonge, 1971; Bond, 1979).

During pregnancy and immediately post partum in rats, no changes were found in brain 5HT, however, increased 5HIAA was seen immediately post partum suggesting increased turnover of 5HT (Rowland et al., 1978). In mice however, brain 5HT and 5HIAA was seen to decrease immediately post partum, although there was a slight elevation of brain TP. This suggests decreased synthesis and turnover of 5HT (Greengrass & Tonge, 1972).

In human perimenopausal patients it has been seen that decreased plasma oestrogens produce decreased plasma free TP, possible because oestrogens normally compete with TP for the albumin binding (Aylward, 1976). Gould (1979) however failed to show any effect of oestradiol on tryptophan binding to albumin in vitro. In female mice,gonadectomy produced decreased brain 5HT and 5HIAA, although brain TP was unaltered (Greengrass & Tonge, 1975).

Hence it can be seen that factors that affect plasma TP binding to albumin, TP entry into the brain and TP metabolism via the Kynurenine pathway may all affect central 5HT synthesis and metabolism. The complex nature of these effects can also be appreciated when it is remembered that cortisol and NEFA secretion are increased in depression, these two factors are also controlled by NA (See previous section) and these

factors may affect 5HT synthesis (vide supra). Hence a relationship between the NA and 5HT systems can be seen.

In connection with this, the 5HT transmitter systems is not the only transmitter system that has been shown to be affected by alterations of hormone levels. Shetty and Gaitonde (1980) reported decreased levels of brain NA following chronic adminstration of contraceptive steriod hormones to rats. However, no change in NA levels, but decreased turnover of NA was seen in the preoptic region following oestradiol treatment (Hiemke et al., 1980). Decreased hypothalamic dopamine levels and turnover were also reported (ibid). Algeri and co-workers (1977) found no effect of steroid contraceptive drugs on whole brain NA in rats or guinea pigs, however dopamine turnover was increased. Supersensitive dopamine receptors in the striatum have also been reported following six days of oestrogen treatment in the rat (Hruska & Silbergend, 1980).

Decreased levels of NA and dopamine in the fore and mid brains of mice, due to increased transmitter release, have been reported five days post parturition (Greengrass & Tonge, 1972) and following gonadectomy (Greengrass & Tonge, 1975).

A further factor that would affect both 5HT and NA, monoamine oxidase (See previous section), has also been shown to be decreased premenstrually, during pregnancy and post partum in humans (Wirz-Justice et al., 1975) and following oestrogen treatment in humans (Klaiber et al., 1979).

5 MOOD CHANGES CONCURRENT WITH CHANGES IN THE REPRODUCTIVE SYSTEM OF HUMAN FEMALES

This thesis concentrates on the investigation of biochemical correlates of the mood changes that occur throughout the reproductive life cycle of human females. Such mood changes occur premenstrually, post-partum, perimenopausally and following the use of steroid hormone contraceptives. Such mood changes all occur at times of 'upheaval' of the female reproductive endocrine system, and they tend to exhibit similar symptoms. It is therefore feasible to suggest that the disturbances may be caused by similar processes and that these processes may be hormonal in nature.

In addition to being of value in its own right, the study of these mood changes may also produce further information concerning the biochemical bases of clinical endogenous depressions. This does not assume that these 'reproductive' mood changes are models of more severe depression, but it accepts that some knowledge may be gained.

The 'reproductive' mood changes are also of special value in that they have a predictable frequency with a predictable time of onset, hence prospective studies are possible, and biochemical changes preceding the mood changes may be studied. This is generally not possible with more severe psychiatric illness.

The following section describes the syndromes being studied.

5.1 Description of Symptoms.

5.1 a) Menstrual Mood Changes.

The term premenstrual tension was first used by Frank (1931). This term has however now been superceded, and 'Premenstrual Syndrome' is the phrase more commonly used. The syndrome described by Frank consisted of symptoms of nervous tension, irritability, anxiety, depression, bloated feelings of the abdomen, swelling of fingers and legs, tightness and itching of skin, headaches, dizziness and palpitations. Frank also reported that less commonly there may be some hypersomnia, excessive thirst and appetite, increased libido, asthma, migraine, vasomotor rhinitis, urticaria and epilepsy. These symptoms were seen to develop during the second half of the menstrual cycle, ie following ovulation, and to disappear shortly after the onset of the menses.

Wessman and co-workers (1960) gave subjects a 10 - point self rating scale to complete daily for 6 weeks. They reported that although there was no clear periodicity in the mood fluctuations, and that the moods were markedly irregular, there were depressed periods of short duration, generally with a poor premenstruum. Depressed mood was also frequently reported for the first day of the menstrual phase.

Banks and Beresford (1979) however reported that if subjects are required to complete a health diary throughout the menstrual cycle, headache is the most frequently reported symptom, followed by changes in energy, backache, colds and sore throats. The reporting of symptoms was seen to be more common during the menstrual phase. These symptoms are however more physical in nature and this may explain the disparity between this and other studies. Further evidence for the existance of a premenstrual syndrome comes from Cooke (1945). This paper reported that 84% of crimes of violence in women were committed during the premenstrual phase. Mackinnon and Mackinnon in 1956 found that there was an increased frequency of death by accident or suicide during the premenstrual phase. One of the most prolific authors on this subject, Dalton (1959, 1960, 1961) also reported an excess of accidents, crimes and visits to the GP for minor complaints during the premenstrual and menstrual phases. Therefore, there is a large body of literature to support the existance of such a 'premenstrual syndrome'.

Rees (1953) reported the incidence of premenstrual syndrome, from a sample of 145 subjects, he reported that 56.5 per cent had no significant premenstrual symptoms,

whilst 24.8 per cent had moderate symptoms, and 15.6 per cent had severe premenstrual symptoms. Dalton(1964) however reports a somewhat lower incidence. She states that 50 per cent of women, some time between puberty and menopause, suffer from premenstrual syndrome, of which 30 per cent suffer to a significant degree and 10 per cent suffer to a severe degree. Therefore it is clear from these figures that a high proportion of women do experience some degree of premenstrual syndrome.

5.1 b) Post Partum Mood Changes.

These changes are commonly grouped together under the single heading of "Post Natal Depression". However the symptoms would suggest that rather than there being one single illness, there are at least three separate entities : Puerperal 'blues', post natal depression and a post natal psychotic depression.

Puerperal ('maternity') Blues :- A period of dysphoria immediately post-partum has been long recognised. Savage (1875) termed the disturbance 'milk fever' due to its coincidence with the onset of lactation. The 'blues' is normally seen as occurring on the third or fourth day post-partum, and lasting for about one day or less. Yalom and co-workers (1968) studied mothers over the first

ten days post-partum, their description of the 'blues' is still one of the most widely quoted. The syndrome which they describe had the most characteristic sign of short-lived, sporadic, crying which was inexplicable to the patient. The crying was frequently described as being inappropriate to the patient's mood. Lability of mood was also reported, with exaggerated sensitivity and empathy, leading to weeping over news stories or in response to minor problems or comments. Fatigue, irritability, confusion and anxiety were also reported.

Estimates of the incidence of 'blues' range from 5% to 80% (Hamilton, 1962), but the real incidence is probably of the order of 50% (Yalom et al., 1968).

Post Natal Depression :- This syndrome possesses the same symptoms as classical depression, with sleep disturbances, loss of appetite and libido, increased irritability, depressed affect and anxiety. Suicidal or infanticidal impulses are, however, rare (Pitt, 1968). The reported time of onset varies however, the peak time of onset seems to be at about three months post partum (Kumar & Robson, 1978). The incidence of this type of illness is reported as being approximately 10 per cent of pregnancies (Pitt, 1968 - 10.8 per cent; Dalton, 1970 - 7 per cent; Kumar & Robson, 1978 - 11 per cent).

Post Natal Psychotic Depression :- This is a rare condition of extreme severity. The symptoms are frequently those of severe depression or mania. Delusions such as guilt may also be present. Infanticidal and suicidal tendancies are also common. However whether this illness is a separate entity or whether it is the same as other similar non-puerperal psychoses is still being debated (See discussion), the prognosis for the puerperal psychosis is better than that for similar non-puerperal psychoses (Brockington 1978). The characterisic feature of this type of depression is its sudden onset within a few days post partum (Paffenbarger et al., 1961). The incidence is reported as being between 0.05 - 0.3 per cent of pregnancies with a hundred fold increase if psychosis has followed a previous pregnancy (ibid).

5.1 c) Perimenopausal Mood Changes.

Aylward (1976) studied 596 post menopausal women, with the period of climacteric amenorrhoea ranging from 6 - 28 months. From this population the symptoms most commonly reported were : Inability to make decisions, 79%; Apathy or inner unrest, 65%; Irritability and aggression, 51%; Depressive thought content, 47%; Sleep disturbances, 46%; Psychomotor retardation, 38%; Loss of Libido, 37% and

loss of emotional reaction, 18%. This therefore presents a good picture of the range of perimenopausal symptoms. As can be seen from the figures, on average 80% of women are affected by at least one symptom. Aylward however does not comment on the severity or debilitating extent of the symptoms.

5.1 d) Mood Changes Concurrent With The Use Of Steroid Hormone Contraception.

There are a multitude of reports of psychological side effects of oral contraceptives (eg Kane et al., 1967; Huffer et al., 1970; Herzberg & Coppen, 1970). All report cases of depression, Kane and co-workers (1967) reported an incidence of 34% following use of a combined oral contraceptive, this figure was supported by Huffer and colleagues (1970) who reported an incidence of 28 per cent. Herzberg and Coppen (1970)however reported that the depression only occurred for one or two days premenstrually as opposed to the cyclical mood changes seen in control subjects. Another commonly reported symptom is decreased libido, this was seen in about 15% of users (Kane et al., 1967 - 15%; Huffer et al., 1970 - 13%). Both Kane and colleagues (1967) and Marcotte and co-workers (1970) report decreased orgasm as another effect. Other symptoms reported are irritability, headaches, fatigue (Herzberg & Coppen, 1970),

sadness, melancholia and lethargy (Marcotte et al., 1970).

However, beneficial effects have also been reported, including increased well being, increased libido and increased orgasm. The incidence of beneficial effects being reported as 22 per cent (Kane et al., 1967).

However, biochemical explanations of these mood changes are not the only explanations possible. Therefore before biochemical hypotheses and factors are described, other factors will be considered.

6 HISTORICAL VIEWS OF FEMININITY.

A belief that women are especially vulnerable to emotion or psychic disturbance is one that has been held for many centuries. However, if the earliest records for admissions to lunatic asylums are consulted, it can be seen that since 1844, the ratio of male to female admissions is only 4 : 5, however, women were more frequently admitted to pauper asylums or workhouses (Skultans, 1979). Skultans however states that this disparity between medical opinion and hard statistics is due to the fact that many female complaints such as "hysteria", were dealt with privately at home. Therefore the asylum admission figures present an incorrect picture.

The medical literature is rife with descriptions of feminine illnesses. Haslam (1817) wrote "In females who become insane

the disease is often related to the peculiarities of their sex", by which he meant, emotional and reproductive problems. Laycock (1840) wrote that the emotional vulnerability of women was equal to that of a child. The 'weakness'of women being universally linked with menstruation, thus, the "fulginous exhalation of corrupt seed" was seen to give rise to many symptoms :

> "a brutish kind of dotage, troublesome sleep, terrible dreams in the night, a foolish kind of bashfulness to some, perverse conceits and opinions, dejection of mind, much discontent, preposterous judgement. They are apt to loathe, dislike, disdain, to be weary of every object, etc. each thing almost is tedious to them, they pine away, void of counsel, apt to weep and tremble, timorous, fearful, sad and out of all hope of better fortunes".

> "now their breasts, now their hypochondries, belly and sides, then their heart and head aches, now heat, then wind, now this, now that offends, they are weary of all, and yet will not, cannot again tell how, where or what offends them though they be in great pain and agony, and frequently complain, grieving, sighing, weeping and discontented still, sine causa manifesta".
>
> (Burton, 1621)

Burton does however note that such afflictions only occur in wealthy ladies of leisure and "seldom do hired servants or handmaids suffer thus".

Another cause of the weakness of women was believed to be the movement of the uterus about the body, thus producing hysteria. However, Willis (1664) noted that the uterus of non-pregnant women was small and 'so strictly tied to its neighbouring parts, that it cannot of itself be moved or ascend from its place'. He believed that hysteria was rather a disease of the brain or the nervous system.

Carter (1853) defines hysteria as :

> "a disease which commences with a convulsive paroxysm commonly called 'hysteria'. This paroxysm is witnessed under various aspects, and in various degrees of severity, being limited, in some cases, to a short attack of laughter or sobbing; and in others, producing very energetic involuntary movements, maintained during a considerable time, and occasionally terminating in a period of catalepsy or coma".

This illness, Carter believed, was brought on by the repression of 'sexual passion' due to the restraints of society.

It is however possible that the 'illnesses' are brought on not so much by the physical symptoms of menstruation, or by the repression of 'sexual passions', but instead by the attitude of society to women. Perhaps it was the historic cultural and social beliefs concerning women that gave rise to the beliefs concerning the feminine 'characteristics' described previously. One of the strongest factors determining a society's attitude to women would be its attitude to menstruation.

6.1 Cultural Attitudes to Menstruation, Childbirth, the Menopause and Contraception, and their Influences on Mood.

Fluhmann (1956) writes that amongst primitive man, the appearance of blood was inevitably associated with the mortal wounds that he inflicted upon animals of the hunt, or with injuries, often painful, that he received himself. Therefore, the cyclical haemorrhages which befell the women of the tribe were the source of much consternation, and hence,

through the centuries, the subject of menstruation has remained cloaked in mystery and superstition.

Menstruating women were frequently "taboo", and even in the twentieth century, the term, 'curse' is used colloquially. Pliny (AD 23 - 79) remarked that 'on the approach of women in this state, new wine will become sour, seeds which are touched by her become sterile, grass withers away, garden plants are parched up, and fruit will fall from the tree beneath which she sits'. The menstruating women were objects of great dread, and special huts were provided for them away from the tribal village. Also these women were sometimes required to smear brightly coloured dyes on their faces, or to wear special garments (Fluhmann, 1956).

Hebrew law also legislated against menstruating women, stating that 'she shall be put apart for seven days'. Also, 'anything on which she sits or lies, or anyone who touches her, shall be declared unclean until evening' (Leviticus 15,vv 19 - 30).

Some societies also had special regulations concerning young girls at the time of their first menstrual period. Two of the more stringent being that 'their feet shall not touch the ground, nor shall the sun shine upon them', the same regulations appear in the customs of the Romans, the Mexicans and the Japanese. Sometimes special precautions were taken with girls believed to be approaching menarche in that they were housed in cages protected

from the sun, and raised on bamboo shelves for as long as four or five years, in anticipation of the first menses (Fluhmann, 1956).

A multitude of other restrictions were also seen amongst different cultures, the Council of Nicaea in AD325 prohibited women from entering a church during menstruation, in France, they were prohibited entrance to sugar refineries, since the proximity of a menstruating woman would cause sugar to turn black. In Mexico they were not allowed into silver mines in case the ore should disappear, and in Indo China, no woman could work in the opium industry for fear that the opium would turn bitter. In Portugal it was believed that menstruating women were more likely to be bitten by lizards, and up until the eighteenth century, in Germany it was believed that the hair of a menstruating woman, if buried in the ground would turn into a snake (Fluhmann, 1956). Therefore it can be seen that throughout history, the time of the menses would be a difficult time for any woman. It is possible therefore that the premenstrual syndrome is a mood change produced by some 'expectancy' of the problems and taboos associated with menstruation. If this were so, it would be probable that different cultures, with their different taboos, would produce different symptoms and severities of premenstrual syndrome. This problem was addressed by Janiger and colleagues (1972), in their study they issued menstrual symptom questionnaires to women of a variety of cultures

and compared the responses. They found that the principal symptoms of the premenstrual syndrome were present in all of the cultural groups studied. However, the frequency and severity of the symptoms differed, the rôle of cultural variations in pain response was mentioned with respect to these differences. However, it would seem that although the reported severity of premenstrual symptoms differs across cultural boundaries, the syndrome was present in all cultures, it is therefore unlikely that cultural attitudes concerning menstruation are involved in the aetiology of premenstrual syndrome. However, the major symptoms of its presentation may be culturally influenced.

Following the same lines of reasoning, it may be that differing cultural attitudes concerning childbearing and motherhood may affect the incidence and symptoms of post-partum mental illness. For example, in Ugandan society an unwanted pregnancy would not be acknowledged, since childbearing was seen to define a woman's existance (Cox, 1978). Also, children may be required to 'create' the parents (ibid) and in the West Indies, the pregnant state is seen as being the most attractive state, with fears of loss of attractiveness following the weight loss concurrent with parturition , also pregnancy, as a proof of fertility, increases a woman's 'value' (Davidson, 1972). However, the incidence of puerperal 'blues' in the West Indies was found to be 60.4% (Davidson, 1972), with the same symptoms as described for 'blues'

in Western societies, the incidence being very close to that reported by Yalom and co-workers (1968).

The incidence of mild depression post-partum in Ugandan women was found to be 9.7% (Cox, 1978), a figure very similar to that of 10.8% reported for London women (Pitt, 1968). It therefore appears that the differing cultural attitudes to pregnancy and motherhood do not affect the incidence of post-partum mental changes.

The importance of fertility and childbearing in the definition of womanhood (Vide Supra) may also play an important rôle in the aetiology of perimenopausal dépression. The menopause signifying the end of the function of maternity. However, no figures for the cross-cultural variations in the incidence of menopausal depression are available.

The comparitively recent introduction of hormonal oral contraceptives means that there is no 'cultural' basis for problems arising from the use of these preparations, there are however, religious objections to their use. It is this fact that led Bottella-Llusia (1973) to state that psychological changes concurrent with oral contraceptive use are due to personal conflicts, arising from religious and moral attitudes and that biochemical factors play no part in these disturbances.

Paige (1971) reported that the beneficial psychological effects of oral contraceptives, such as a reduction

of anxiety, were due to decreased menstrual flow, hence lessening the anxiety due to blood flow taboos. Interestingly, the removal of the fear of pregnancy was not mentioned as having any rôle in the decrease of anxiety.

Reports of differing estimates of psychological changes concurrent with the use of oral contraceptives for different cultures are unavailable.

From the preceding sections, it can be seen that the incidences of these 'reproductive' mood changes are constant across cultures, and historically within the same cultures. The basic symptoms are also similar between cultures. However, some ethnic variation of symptom presentation does occur eg assessment of pain, such an ethnic variation in psychiatric symptom presentation is well known (Waldron, personal communication). Therefore slight differences in the reported menstrual symptoms may be expected. It therefore seems feasible to propose that the aetiology of these 'reproductive' mood changes is due to some other reason than cultural attitudes and expectations. However, the individual symptom presentation may be culturally influenced.

However, some social factors have been shown to influence mood.

6.2 Social Origins of Depression.

Sociological variables have been shown to be involved in the aetiology of depressive illness (Brown & Harris, 1978).

The hypothesis of the social origins of depression involves the interaction of factors called vulnerability factors and life events. Vulnerability factors involve such variables as poor socio-economic class, lack of an intimate confidant or early loss of mother. Meanwhile, life events involve more recent events such as death of a spouse, or some important news, etc. The hypothesis is that the summation of vulnerability factors, together with life events may cause depression. Thus, Brown and Harris noted that, in their sample population of women, all of the women with vulnerabilty factors of: lack of a confidant (husband or boyfriend), early loss of mother, with three or more children aged below 14 living at home, and unemployed, suffering one or more life events, became depressed. However, only 1% of women with a confidant, regardless of the number of children or employment status, not suffering from any life events, developed depression. Therefore these sociological factors were seen to be important in the aetiology of depressive illness.

It is therefore possible that the 'life events' approach may be used to explain premenstrual syndrome, puerperal mood changes, etc.

When considering premenstrual syndrome, it is important to remember that the mood changes occur premenstrually, therefore physical symptoms such as dysmenorrhoea cannot be involved in its aetiology.

However, other physical symptoms such as feelings of bloatedness and mastalgia are so frequently seen in premenstrual syndrome that they are considered as symptoms required for its diagnosis. However, it is possible that the somatic complaints are in fact involved in the aetiology of the psychological changes, acting as 'life events', and are not just symptoms.

When considering parturition and its associated mood changes, there are again many factors that may be considered as being either life events or vulnerability factors. Such factors would be socio-economic class, marital status, parity, previous obstetric history, sex of infant as compared with desired sex of infant, length and difficulty of labour, obstetric complications and health of baby. However, these factors were not seen to play any rôle in the incidence of 'blues' (Pitt, 1973). Although the incidence of the 'blues' has been reported as being greater amongst primiparae (Nott et al., 1976). When post-natal depression is considered, again these factors did not appear to affect its incidence (Pitt, 1968). However, Kumar and Robson (1978) reported that depressed patients tended to report greater marital tension during pregnancy, and to have a history of infertility. Kendall and co-workers (1976) reported no effect of any of the variables listed above on the incidence of puerperal psychosis.

It therefore appears on balance, that life events play no rôle in the aetiology of post-natal mental illness. However childbirth per se may be a factor which causes depression in women that are predisposed by some vulnerability factors.

When considering menopausal depression there are many 'life events' that may be related to the mental changes, ranging from physical symptoms such as hot flushes, etc.,to family events such as children leaving home. However, no data concerning life events and menopausal depression is available.

Thus it would seem that cultural and sociological variables do not play a major rôle in the aetiology of the mental illnesses seen at times of hormonal fluctuations, however they may affect the way in which the syndromes present.

Another factor that may be involved in the aetiology of these mood changes is personality. However, little research has been performed to investigate this. Probably one of the most influential papers is that of Coppen and Kessel (1963). These workers investigated reports of dysmenorrhoea and premenstrual irritability with respect to personality as measured by the Maudsley Personality Inventory. Their results showed that those patients reporting moderate or severe premenstrual irritability scored more highly on the Neuroticism scale than patients not making such reports. This differentiation was not so for

Extraversion scores. Interestingly however, there was no differentiation on either personality scale, between subjects reporting dysmenorrhoea, and patients not making such reports.

These findings supported the earlier work of Rees (1953) who showed that neurotic women, as defined by the Maudsley medical questionnaire, reported premenstrual syndrome more frequently than normal controls, however, some very neurotic women did not suffer from premenstrual syndrome.

Even less research has been performed to investigate the relationship between personality types and post-partum mental illness. There have been several reports of a psychoanalytic nature (eg Lomas, 1960; Hayman, 1962; Douglas, 1963) and factors such as immaturity, inadequacy, vulnerability and low self-esteem, shyness, aggression, denial, obsessionalism and excessive compliance have been linked with puerperal illness. Pitt (1968) using the Maudsley personality inventory, reported that patients that developed puerperal depression were more neurotic and less extrovert.

No data concerning relationships between personality and mood changes at the menopause, or following oral contraceptive use appears to have been published.

7 ENDOCRINOLOGICAL AND BIOCHEMICAL CHANGES OF THE FEMALE REPRODUCTIVE SYSTEM.

At the times of mood disturbance mentioned, ie premenstrually, puerperally and perimenopausally, there are great fluctuations in the state of many physiological and biochemical systems within the body. It is these changes that have frequently been postulated as being causative of the psychological symptoms.

7.1 Menstrual Cycle

Mood is not the only parameter that is seen to vary cyclically throughout the menstrual cycle, a whole plethora of biochemical variables show a similar pattern of changes and it is this similarity in the fluctuations that has led to manyhypotheses of biochemical causative factors of mood changes.

The earliest theories concerned the cycling of the female gonadal hormones and the factors that control this cyclical pattern.

The periodicity and cyclicity of the menstrual cycle is controlled by the hypothalamus. This cycling is inherent to the hypothalamus and its associated nuclei. Via the hypothalamic peptide hormone, Luteinizing Hormone-Releasing Hormone (LH-RH), the hypothalamus controls the release of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) from the pituitary (Butt, 1979).

The first of these pituitary gonadotrophins, Luteinizing Hormone, controls the development of the corpus luteum. It is this corpus luteum which secretes the progesterone. Thus, LH controls plasma progesterone levels (Bell et al., 1976). When the plasma progesterone profile throughout the menstrual cycle is studied (Fig IV), it can be seen that progesterone levels are low during the follicular stage of the cycle, rising rapidly following ovulation, then declining suddenly pre-menstrually. The peak plasma levels of progesterone, seen during the luteal phase, are in the order of 0.5 nmol/dm^3 (Bell et al., 1976).

The second gonadotrophic hormone, Follicle Stimulating Hormone, controls the development and maturation of the ovarian follicle, it also produces an increase in oestrogen production (Bell et al., 1976). The plasma oestradiol profile therefore shows a rise during the follicular stage, falling suddenly at ovulation, followed by a smaller, secondary rise during the luteal phase, falling again premenstrually. (Fig V). The peak plasma level of oestradiol is about 3.5 nmol/dm^3, seen during the follicular stage.

There are also cyclical variations in the urine excretion patterns of these hormones. Oestrogens are excreted throughout the cycle, rising from an initial value of 70 nmol/24 hours to a peak of 250 nmol/24 hours prior to ovulation and a secondary peak of 150 nmol/24 hours during the luteal phase (Bell et al., 1976).

Fig IV Plasma progesterone during the menstrual cycle (after Bell et al 1976)

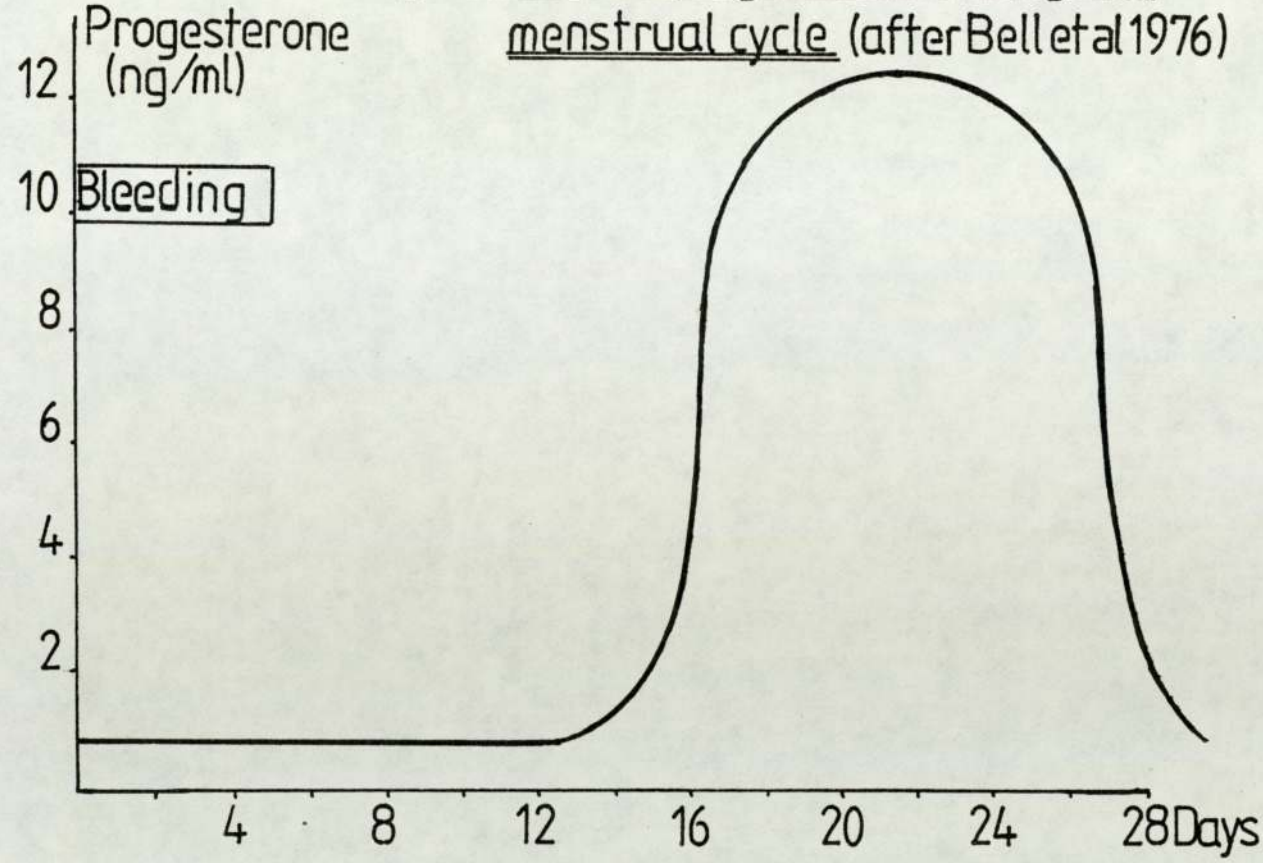
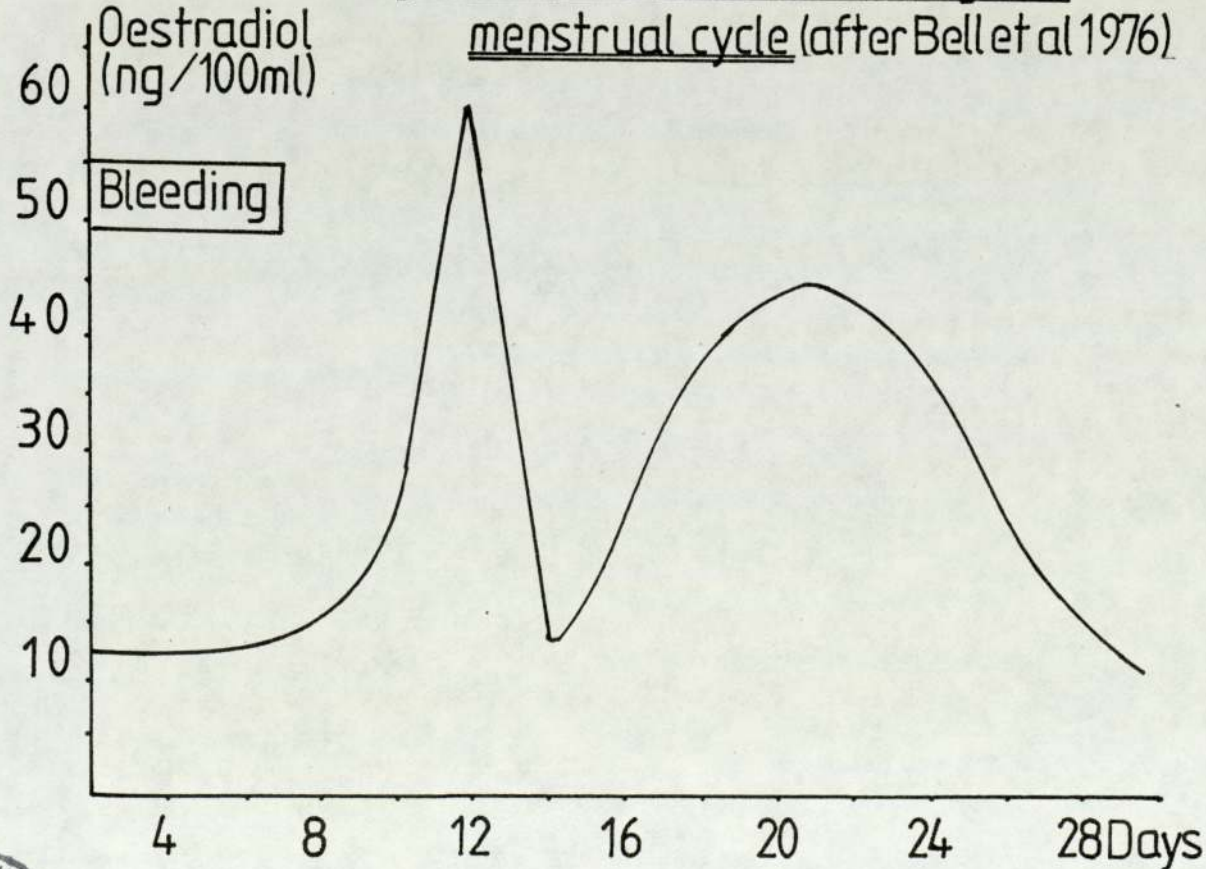


Fig V Plasma oestradiol during the menstrual cycle (after Bell et al 1976)



The progesterone metabolite, pregnanediol, appears in the urine at levels of about 5 nmol/24 hrs in the early stages of the cycle, rising to 13 nmol/24 hrs during the luteal phase (Bell et al., 1976).

Prolactin secretion also varies during the menstrual cycle , being influenced by oestrogen levels such that fluctuations of prolactin levels reflect the changes of oestrogens. The general pattern of prolactin secretion is such that prolactin levels are greater at ovulation and during the luteal phase than they are during the luteal phase. (Table 1, Butt, 1979)

Aldosterone secretion also rises during the luteal phase. This is probably as a result of plasma renin and angiotensin increases at this time (Bell et al., 1976). Sodium excretion, sodium : potassium ratio and urinary volume also vary throughout the cycle (O'Brien et al., 1980) possibly due to the aldosterone fluctuations.

Table I Serum Concentrations of Prolactin throughout the Menstrual Cycle.

Cycle Phase	Mean(mU/dm^3)	Actual Range
Follicular (Days 1-6)	303	140 to 618
Ovulation (Day of LH peak $\pm$ 2)	354	180 to 795
Luteal (Days 21-24)	352	184 to 799

After Butt, 1979

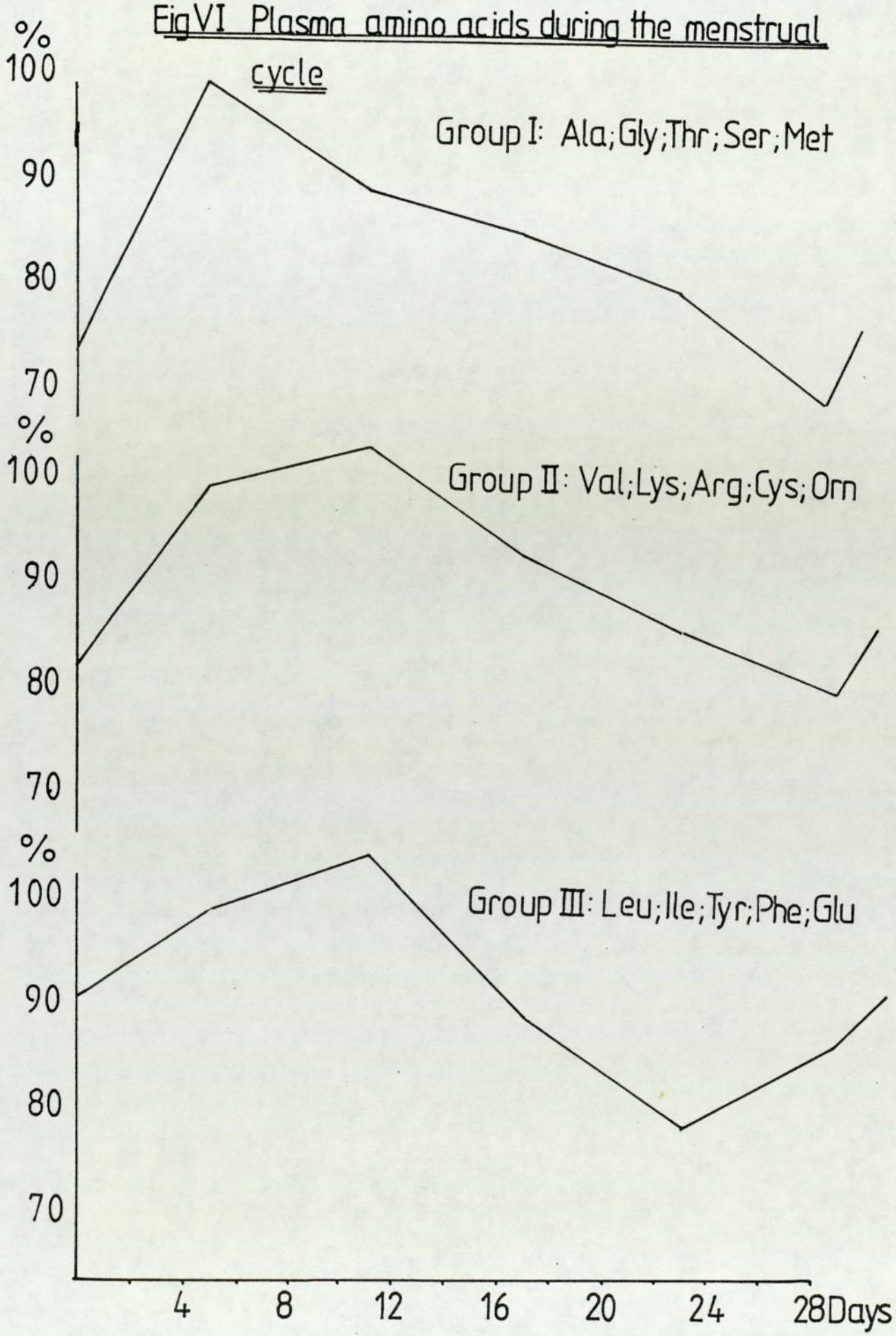
In addition to these endocrinological factors, a host of other factors also vary throughout the menstrual cycle. Bond (1979) reported that plasma TP varies throughout the menstrual cycle. Total TP was seen to show a peak of 14 μg/ml at ovulation, with a nadir of 5 μg/ml during the menstrual phase. Plasma free TP showed a similar pattern, with a peak of 2.0 μg/ml at ovulation, and a nadir of 1.0 μg/ml during the follicular stage. Wirz-Justice and co-workers (1975) however, reported that free TP was greater during the premenstrual phase than during the ovulatory stage.

Cox and Calame (1978) studied the plasma profiles of 18 amino acids throughout the menstrual cycle. On the basis of the plasma profiles, the amino acids were divided into three groups, (Fig VI). However, in general, the amino acids were seen to rise during the follicular phase, and fall to a nadir during the luteal phase (ibid).

Monoamine oxidase activity has also been shown to be decreased by 20 per cent premenstrually (Wirz-Justice et al., 1975).

As mentioned in previous sections, many of these changes (eg Oestrogens, progestogens, TP, monoamine oxidase, etc.) are capable in affecting 5HT synthesis and metabolism. In connection with this, many of these factors have been postulated as being causative of the mood changes of the premenstruum. Probably the oldest hormonal hypothesis involved progesterone,

Fig VI Plasma amino acids during the menstrual cycle



after Cox & Calame, 1978

a deficiency of progesterone during the luteal phase being believed to produce mood changes (Dalton, 1964 & 1977). However, it is now evident that although a 'substantial percentage' of premenstrual syndrome sufferers do have a deficiency of progesterone during the luteal phase, there are some non-sufferers with equally low progesterone concentrations (Brush, 1979).

Plasma oestradiol and plasma progesterone/oestradiol ratios have also been studied, however, no significant differences between premenstrual syndrome sufferers and non-sufferers were seen (Brush, 1979).

Plasma aldosterone was postulated as being involved in the fluid retention often seen during premenstrual syndrome.

However, no differences in aldosterone levels were seen between premenstrual syndrome sufferers and non-sufferers (Munday et al., 1977).

Plasma prolactin has also been studied in premenstrual syndrome, however, no consistent significant differences were seen between premenstrual syndrome sufferers and non-sufferers (Brush, 1979).

A final hypothesis originates from the empirical use of pyridoxine (Vitamin B_6) in the treatment of premenstrual syndrome. Good results for its clinical use have been reported (Kerr, 1977). As described previously, pyridoxine supplements may allow TP to move away from the kynurenine pathway, and into

the 5HT synthesis pathway. However, as yet there has been no demonstration of a pyridoxine deficit in premenstrual syndrome sufferers (Brush, 1979).

However, if oestrogen or progesterone is producing an acceleration of the kynurenine pathway, then pyridoxine supplements may revert the balance without there being a previous pyridoxine deficit (See previous).

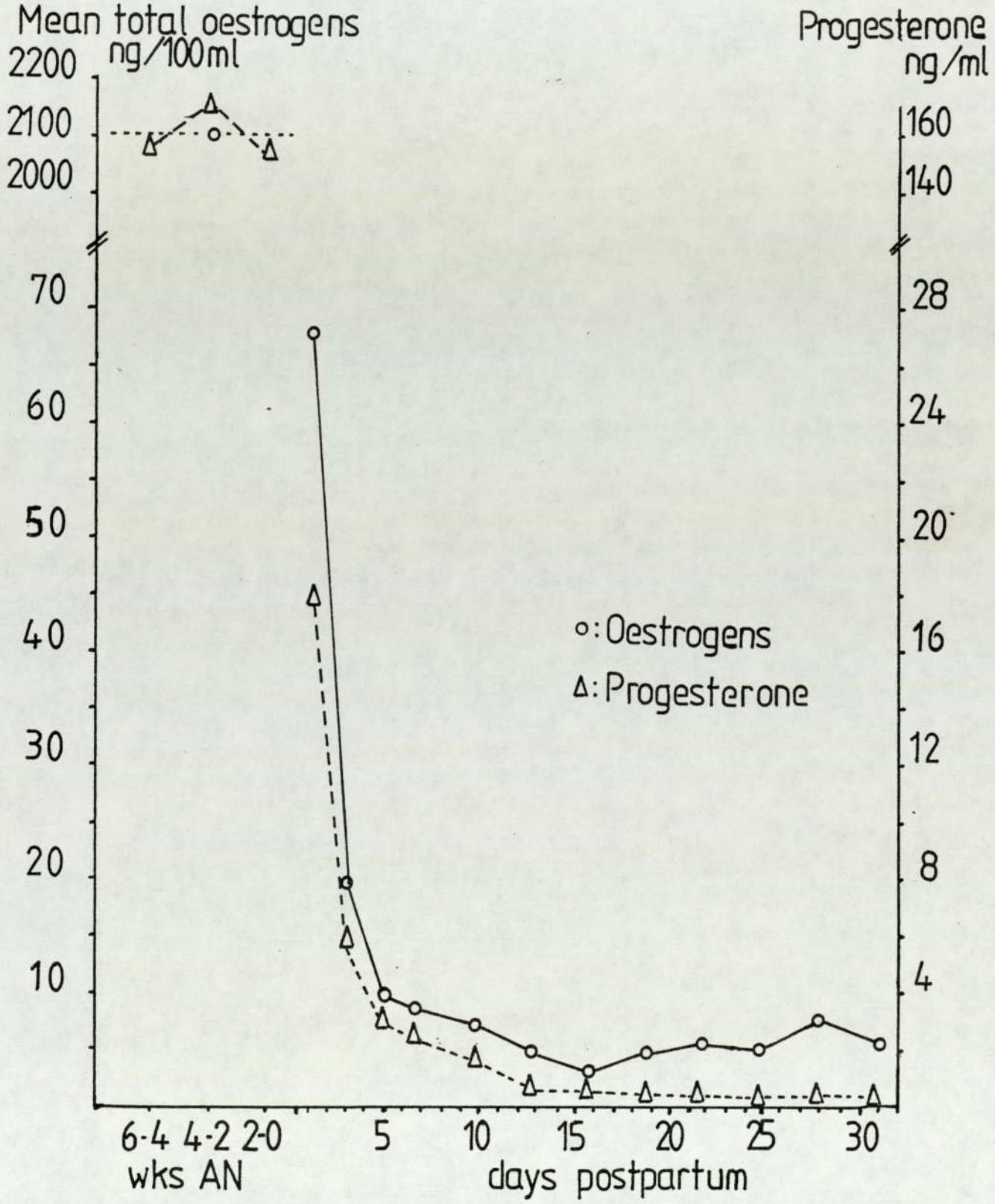
Therefore, from the data above, there is as yet no conclusive evidence for a particular hypothesis of a biochemical aetiological factor in premenstrual syndrome.

7.2 Pregnancy and the Puerperium.

As with the menstrual cycle, there are a great many endocrine and biochemical changes that occur during pregnancy and the puerperium.

During pregnancy, plasma levels of total oestrogens may rise to a level 1000 fold greater than that of the non-pregnant state (Nott et al., 1976). These levels drop rapidly to pre-pregnant levels following parturition. The source of the additional oestrogens is the foeto-placental unit. The placenta cannot synthesise oestrogens from simple precursors, however, the foetus converts pregnenolone to epiandrosterone, and it is this epiandrosterone which the placenta converts to oestrone (E_1) and oestradiol (E_2). The third oestrogen, Oestriol (E_3) is produced by the foetus (Bell et al., 1976).

Fig VII Plasma total oestrogens & progesterone at parturition after Nott et al., 1976



Plasma levels of progesterone also rise dramatically during pregnancy, reaching a peak of about 500 nmol/dm^3 at 38 weeks of pregnancy. The levels then fall slightly over the last two weeks of pregnancy (Bell et al., 1976). This finding is disputed by Batra and Grundsell (1978) who report that there is no fall in progesterone in late pregnancy. Progesterone levels remain constant during labour and then fall to about 50% of the pregnancy levels within one hour of delivery, after 24 hours, the levels are 25% of pregnancy level, falling to menstrual levels by 72 hours post-partum (Yonone et al., 1968; Dawood and Teoh, 1975) (Fig VII).

Prolactin also rises greatly during pregnancy, the concentrations reached by term of pregnancy being about 300 ng/ml (Normal levels are about 9.0 ng/ml). This rise of prolactin is probably due to the high oestrogen levels. Post-partum the prolactin levels remain high, with peak levels being reached immediately following suckling. Prolactin levels remain high until weaning and the cessation of breast feeding (Williams, 1974). Cox and Calame (1978) studied 18 amino acids during pregnancy and showed that with the exception of alanine and glutamic acid, the plasma amino acid levels fell throughout pregnancy.

Similarly, plasma tryptophan, both free and total, has been shown to be decreased during pregnancy. The levels for total and free tryptophan at the end of pregnancy being about 8 μg/ml

and 1.0 µg/ml respectively, these levels rose to levels of 10 µg/ml and 1.3 µg/ml respectively by the third day post-partum (Handley et al., 1980).

Plasma cortisol also rises about three fold during pregnancy and it again rises during labour and falls rapidly post-partum (Batra & Grundsell, 1978; Handley et al., 1980). The cortisol rise during pregnancy is due to an oestrogen induced increase in cortico-steroid-binding-globulin concentrations (Doe et al., 1967).

Plasma Non-Esterified Fatty Acids (NEFA) are also elevated during pregnancy, reaching levels of about 1.3 m Equiv/dm^3, the level then falls to about 0.6 m Equiv/dm^3 by day 5 post-partum (Burt, 1960).

Several of these factors have been postulated as being involved in the aetiology of puerperal mental illness. Dalton (1971) postulated that the sudden fall of progesterone at parturition was causative of post-partum mood swings. A failure to adapt to the sudden hormonal changes was seen as the underlying problem. Dalton (1971) also reported that 'blues' sufferers were more frequently reporting elation during pregnancy than non-sufferers. This elation during pregnancy, she postulated, was due to increased levels of circulating progesterone during pregnancy. However, this hypothesis was not upheld by the findings of Nott and co-workers (1976). These workers found that there was no difference in

plasma progesterone levels, either in the last month of pregnancy or the first month post-partum, between 'blues' sufferers and non-sufferers. The same negative results were also shown for antepartum and post partum levels of total oestrogens, oestrogen/progesterone ratio, LH, FSH and prolactin (ibid). This study did however show a relationship between progesterone levels and self-reported mood. It was found that those patients that had the greatest relative fall in progesterone at parturition, tended to report greater mood changes.

In contradiction to this study however, George and Wilson (1980) reported a significant relationship between plasma prolactin levels over the first few days post partum and the incidence of 'blues'. It was found that prolactin levels were increased in 'blues' cases.

Ballinger (1980) studied urine levels of adenosine - 3′, 5′ - cyclic monophosphate (cAMP) in relation to mood changes in the puerperium. It was found that cAMP levels were high during pregnancy, fell immediately post partum, then rose within a few days of parturition. It was reported that women reporting the greatest mood changes post partum showed the greater rise in cAMP excretion in the few days immediately post partum. However, the interpretation of these results was difficult (See discussion).

There has also been much interest shown in the relationship between plasma TP and mood changes in the puerperium. Stein and co-workers (1976) studied TP levels on day 6 post partum in relation to mood. They showed that there was no difference between 'blues' cases and non-cases with respect to plasma total TP, however plasma free TP was found to be decreased in 'blues' cases. This finding was replicated by Handley and colleagues (1977), who measured daily TP levels for 5 days post partum. Again free TP was found to be significantly lower post-partum in 'blues' cases. There was no difference in plasma total TP. This same group also reported that plasma cortisol levels were increased in those patients that became elated in the puerperium (ibid).

The study of TP and cortisol in the puerperium was then expanded (Handley et al., 1980). This study showed that there was a seasonal variation in the incidence of 'blues', and also that there was a seasonal variation of plasma cortisol and free TP. This meant that relationships between mood and post-partum free TP and antenatal cortisol were only seen at certain times of the year. However, it was found that 'blues' cases and non-cases did differ with respect to plasma total TP. Two distinct groups of subjects were found, those that exhibited the characteristic rapid rise of plasma total TP immediately post-partum and those that failed to show the early rapid rise. The results showed that there was an increased incidence of 'blues' cases amongst those subjects that failed to show the early

TP rise as compared with those subjects who showed the normal rise.

Further variables studied with respect to puerperal mood were those of weight loss and electrolyte excretion (Stein, 1980). It was found that although 'blues' cases and non-cases did not differ with respect to these variables, those patients that showed a sudden onset of 'blues' symptoms tended to show a rapid weight loss concurrent with the onset. It was postulated that the rapid weight loss may be involved with the onset of the 'blues' phenomenon.

Relatively fewer studies have been performed concerning biochemical correlates of post-natal depressive illness within the first few months post-partum. Gelder (1978) reported that there were no differences in the levels of progesterone, oestrogens, prolactin LH or FSH either during the last month of pregnancy or the first month post-partum between depressed and non-depressed patients. However, Handley and colleagues (1980) reported that there was a signficantly greater tendancy for total TP non-risers (see above) to visit their family doctor complaining of depression in the first six months post-partum than for the total TP risers.

The low incidence of puerperal psychosis has meant that few biochemical studies have been performed. Cernik (1980) measured plasma levels of oestrogen, progesterone, prolactin, LH, FSH, cortisol and thyroid

hormone in post-partum psychotic patients as soon as the psychosis presented. When compared with normal puerperal women, it was found that the psychotics showed increased levels of oestrogen and prolactin and decreased levels of progesterone, LH, FSH and cortisol. However, most of the differences were seen to be non-significant, and the gonadotrophic hormone levels were linked with the changes in progesterone. Hence the only definite changes seen were that puerperal psychotics presented increased oestrogens and decreased cortisol and progesterone. Progesterone supplements have also been reported to be of use in the treatment of post-partum psychosis (Schmidt, 1943).

Riley (1979) noted that puerperal psychotics with no previous personal or family history of psychiatric illness, showed significantly raised serum calcium during the illness, as compared with other psychiatric patients, and that this raised serum calcium returned to normal on clinical recovery. The significance of this finding is unclear.

7.3 The Menopause.

At the time of the menopause, there is a decline of ovarian activity, this is not an abrupt change, but takes place over a number of years. Concurrent with this decline of ovarian activity, levels of the gonadotrophins, LH and FSH rise considerably by about ten fold (Bell et al., 1976), however even 20

years before the actual cessation of menstruation, the LH levels may be elevated (ibid).

Excretion of oestrogens also rises temporarily at the time of the menopause. The origin of this oestrogenic material is unknown since excretion also continues following oophorectomy and adrenalectomy, and traces are excreted many years after the menopause (ibid).

TP has also been seen to be altered at the time of the menopause, Aylward (1976) reported decreased plasma free TP in post menopausal subjects. Coppen and Wood (1978) however reported that whilst free TP levels were decreased perimenopausally, there was no difference between pre-and post-menopausal values.

7.4 Hormonal (Oral Contraceptive) Therapy.

There are only small changes in oestrogenic and progestogenic activity concurrent with the use of oral contraceptives, however generally, natural hormones are replaced by synthetic hormones. The follicular and luteal peaks of FSH and the luteal peak of LH are therefore abolished following the use of combined oestrogen/progestogen contraceptives (Swerdloff & Odell, 1969). Progesterone-only pills do not block the LH surge, the contraceptive action probably being due to an effect on cervical mucus. (Baird, 1976).

TP has also been studied during oral contraceptive therapy (Bond, 1979). The results show that oral contraceptive users show nadirs of 5 µg/ml total TP and 1.2 µg/ml free TP at the mid-point of the cycle,

with peaks of 10 μg/ml total TP and 1.6 μg/ml free TP during the premenstrual phase.

Adams and co-workers (1973) reported that vitamin B_6 (pyridoxine hydrochloride) may be deficient in patients suffering depression associated with oral contraception. In these patients with the vitamin B_6 deficiency, supplements were given and the depression was seen to remit. It therefore appears that some of the mood changes seen following use of oral contraceptives are due to a pyridoxine deficiency.

8 THE EFFECTS OF CONTRACEPTIVE STEROIDS ON ANIMAL BEHAVIOUR.

Progesterone has a sedative action in animals (Selye, 1941) and in humans (Merryman, 1954). This effect is much more marked in females than in males (ibid). The synthetic progestogen, norethisterone acetate (20 µg/kg) has also been seen to decrease locomotor activity in female mice following chronic treatment (Bond, 1979). The same result was seen for a norethisterone acetate/ethinyl oestradiol combination (ibid). Locomotor activity has also been seen to vary throughout the oestrous cycle, being increased at the oestrous phase in untreated mice (ibid).

High doses of oestrogens also increase the head twitch response to 5HTP in mice, signifying a possible reduction in 5HT availability. The same effect is seen with an oestrogen/progestogen combination. High doses of progesterone alone (250 mg/kg) however lead to a potentiation of the head twitch response (Brotherton & Doggett, 1978). There is however no alterations in the head twitch response throughout endogenous hormone changes of the oestrous cycle (Boulton & Handley, 1973).

Hence it can be seen that the reproductive hormones are able to affect behaviour in animals. These behavioural changes are possibly due to altered central 5HT metabolism.

METHODS

METHODS

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METHODS

General Introduction

This method section describes the methods used in experiments and studies performed during the preparation of this thesis. Additional information such as drug dosages, sample sizes and subject population characteristics are given at the beginning of each results chapter. Protocols for studies are also described within the results section.

1 BIOCHEMICAL METHODS

1.1 Plasma Total Tryptophan.

The method used was that of Denkla and Dewey (1967), as modified by Bloxam and Warren (1974). This assay involved the conversion of tryptophan to norharman, a fluorophore.

50 µl of plasma was added to 2ml ice cold 10% trichloroacetic acid (TCA). This caused denaturation and precipitation of the plasma proteins. The precipitate was then pelletted by centrifugation at 4°C, 11,000 rpm (12000g) for 10 minutes. The supernatent was decanted into glass boiling tubes and the level of liquid in the tubes was marked by means of a felt-tipped marker. 200µl of 2% (w/v) formaldehyde solution and 100µl of 6×10^{-3}M Ferric Chloride Solution (in 10% TCA) were added and the solutions were immediately mixed for 1 second by vortex. The tubes were then stopped by means of a glass marble (to prevent excessive build-up of pressure within the tube) and placed into a water bath at 99 - 101 °C. The tubes were incubated for 1 hour.

Following the reaction (Fig 1) the tubes were removed from the water bath and allowed to cool to room temperature. Fluid lost during incubation was then replenished by the addition of 10% TCA until the level of liquid in the tube returned to the marked level and the solution was mixed by vortex. The product

was read in a glass cuvette using an Aminco-Bowman spectrophotofluorimeter, excitation and emission wavelengths being 368 nm and 448 nm respectively. Excitation and emission slits were 3mm.

Norharman has characteristic excitation and emission spectra, (Fig 2) and the wavelengths chosen for use when measuring TP were those of peak fluorescence. Comparison of the excitation and emission spectra for norharman and for the product of the TP assay showed that norharman was being produced by the assay reaction (Fig 3).

The relationship of norharman concentration and fluorescence was linear over the range of norharman concentrations achieved from physiological concentrations of TP, Pearson's r = .9910 (Fig 4)

Standard Solutions

The standard solution used was 10 µg/ml TP in distilled water. This was stored at -30°C as a stock solution of 100 µg/ml, and freshly diluted 1 : 10 for each assay.

Recovery of Tryptophan

From the assay reaction (Fig 1) 1 mole Tryptophan (TP) produces 1 mole norharman (NH). In one study, 50µl of tryptophan (10µg/ml) gave a fluorescence reading of 3.5 units following the reaction.

Initial Concentration TP = 50 μl x (10 μg/ml) in 2ml TCA

= .25 μg/ml

[Divide by MWTP: 204] = $\frac{.25 \times 10^{-3}}{204}$ mols/ml TP

$\therefore$ If yield = 100%, reaction should produce

$\frac{.25 \times 10^{-3}}{204}$ mols/ml NH

As stated above, NH produced fluorescence of 3.5 units.

From Fig 4, 3.5 units fluorescence was produced by

8.5×10^{-7} mols/ml NH

$\therefore$ The Theoretical Yield = $\frac{.25 \times 10^{-3}}{204} = 1.225 \times 10^{-6}$ mols/ml

Actual Yield = 0.85×10^{-6} mols/ml

% Yield = $\frac{\text{Actual Yield}}{\text{Theoretical Yield}} \times 100$

= $\frac{0.85 \times 10^{-6}}{1.225 \times 10^{-6}} \times 100$

= 69.4%

Reproducibility

A single sample of plasma was assayed six times, the mean total tryptophan concentration was found to be 13.27 $\pm$ 0.57 μg/ml. The coefficient of variance was 0.15

Stability of Tryptophan/Norharman

Tryptophan was found to be stable when stored at -30°c. Results for the fluorescence produced by the same stock solution of tryptophan (100 μg/ml) being thawed daily, an aliquot removed, diluted 1 : 10 and assayed in duplicate were as follows :

Time	Day 1	Day 2	Day 3	Day 4	Day 5
Mean Fluor-escence (arbitrary units)	5.16	5.04	5.23	4.70	5.48

Hence, allowing for experimental variation, it was seen that TP was stable during storage and repeated freezing and thawing. Standards were also stable for up to two months as can be seen from these values for a two month old standard compared with a freshly made standard, each of 100 µg/ml, assayed in triplicate.

Old Standard : 46.7 ± 1.1 fluorescence units;

Fresh Standard: 49.0 ± 3.1 fluorescence units

t = 0.8699 p = 0.5634

Norharman was found also to be stable following production by the assay reaction. A plasma sample was assayed and the tryptophan concentration was read (against a standard) at varying times following removal of the tubes from the water bath. Each mean is the result of 6 determinations.

Time	15mins	30mins	45mins	60mins	After beingstored overnight at 4°C
µg/ml TP	13.27±0.57	12.76±0.56	13.33±0.56	12.67±0.55	12.93±0.55

It was therefore seen that norharman did not decay following the incubation stage.

Sample Storage

Plasma samples were stored for up to three weeks at -30°C.

1.2 Plasma Free (non-albumin bound) Tryptophan.

The non-bound portion of TP was separated by the method of Bloxam and co-workers (1977). This method involved the filtration of plasma through an ultra-filtration membrane by centrifugation. Free (unbound) TP freely passed through the membrane, but that bound to albumin was retained.

200 µl of plasma was placed into the filtration apparatus as described by Bloxam and colleagues (1977). The plasma was then gassed for 30 seconds with a humidified 5% CO_2/95% O_2 mixture, and shaken at room temperature for 10 minutes. This gassing stage was performed in order to ensure that the pH of the sample was constant (ibid). In the present study, using indicator paper, pH was found to be 7.5, it was not possible to measure the pH of a 200 µl plasma sample more accurately with the apparatus available.

Filtration through the membrane (pm10 membrane, Amicon Ltd) was produced by centrifugation at 4000 rpm (2000g) for 30 minutes at 20°C. The ultrafiltrate passed through the membrane into the collecting tube. Approximately 100 µl of ultrafiltrate was obtained, this was then assayed for tryptophan by the method described in section 1.1.

Standard solutions of TP were carried for each assay, and filtered by the same procedure, as the membrane was found to retain 16.46 ± 2.56 % (n = 20) of the TP.

The method described above varies slightly from that of Bloxam and co-workers (1977). Firstly, it was found that prior soaking of the membranes in distilled water, to remove the glycerol plasticiser was necessary otherwise insufficient filtrate was obtained. Bloxam and colleagues (1977) reported this to be unnecessary. Secondly, centrifugation was performed for 30 minutes. Bloxam and co-workers (1977) used 20 minutes, but it was found that this did not produce sufficient ultrafiltrate.

Reproducibility

A single plasma sample was assayed seven times, the mean value for the free TP obtained was 4.23 ± 0.146 µg/m
The coefficient of variance was therefore 0.04.

1.3 Plasma Non Esterified Fatty Acids.

The method used was that of Curzon and Kantamaneni (1977) which involved the reaction of fatty acids with a fluorophore, β - umbelliferone (7-Hydroxy-4-methyl-coumarin) (Fig 5).

Two forms of this assay were used for experiments reported within this thesis. The first was essentially the same as that reported by Curzon and Kantamaneni (1977) and was used for the assay of the human plasma.

The second version was developed to use a much smaller volume of plasma, and this was used for the assay of mouse plasma.

a) Human Plasma

400 µl of plasma (or 400µl of Palmitic Acid standard in heptane (standard), or 400 µl of distilled water (blank)) were added to 2.0 ml Dole's solution (see section 12). This was mixed by vortex for 5 minutes using a multivortex shaker (Denley V100). The solutions were then allowed to stand for 10 minutes. 1.2 ml Heptane and 0.8 ml distilled water were added to the samples and the blank (0.8ml Heptane + 1.2ml H_2O was added to the standards). The solutions were shaken for 5 minutes, allowed to stand for 10 minutes and centrifuged for 5 minutes at 2500 rpm (1000g) in a bench centrifuge (MSE Ltd). 1.0ml of the upper, organic layer was added to 1.0ml 0.025% Sulphuric Acid. The mixture was shaken for 5 minutes and centrifuged for 5 minutes as before. 0.3ml of the upper organic layer was then added to .45ml of the indicator solution (see section 12), gassed with dry nitrogen for 90 seconds and read immediately in a spectrophotofluorimeter at excitation 364 nm, emission 450nm (fig6), using a quartz cuvette.

It was found that palmitic acid decreased the fluorescence of β-umbelliferone in a linear fashion r = - .9965 (Fig 7).

Curzon and Kantamaneni (1977) reported that gassing for 10 seconds was sufficient to drive oxygen out of the indicator solution and give a consistent reading which decayed slowly by 5% over 2 minutes.

It was found however, that gassing the samples for 90 seconds, followed by capping of the cuvette with an airtight cap, increased the reproducibility of the results, and the stability of the fluorescence. Gassing samples with oxygen in the form of 95% O_2/ 5% CO_2 reduced the fluorescence of the indicator solution from 13.3 $\pm$ 0.3 fluorescence units before gassing to 1.3 $\pm$ 0.13 fluorescence units after gassing for 15 seconds.

Standard Solution

The standard solutions used were 1.0 and 0.5 mM palmitic acid solution in n-heptane. These were stored at 4°C.

Reproducibility

Four standard solutions were each assayed in triplicate. The mean coefficient of variance was found to be 0.13.

Stability of NEFA

Three plasma samples were obtained. These were assayed fresh and then after 1, 3, 6 and 8 days of storage at -30°C.

The mean and standard error for each sample was :

0.598 ± 0.022 mEq/dm^3 (n=5)

1.128 ± 0.060 mEq/dm^3 (n=5)

0.570 ± 0.034 mEq/dm^3 (n=5)

The mean coefficient of variance was 0.01. NEFA was therefore seen to be stable when stored at -30°C for 8 days, and to be stable to freezing and thawing.

b) Mouse Plasma

Because of the large volume of plasma required in the above method, and because of the small amount of plasma obtained from a mouse, it was necessary to develop a microassay for NEFA.

The following method was therefore used : 100 µl of plasma was added to 1.0 ml Dole's solution. The solutions were mixed and allowed to stand. 1.0ml n-Heptane and 0.9ml water (or vice-versa for standards) was added. Solutions were again mixed and centrifuged. 0.5 ml of the upper layer was added to 0.5ml sulphuric acid, the solutions were mixed and centrifuged. 0.3ml of the upper layer was added to 0.45ml indicator solution. The fluorescence was then read as before.

All solutions, and durations of mixing and centrifugation were the same as in section 1.3 (a).

Storage

Murine plasma was stored at -30°C and assayed within 3 days. Human plasma was similarly stored for up to 2 weeks.

Reproducibility

The calibration curve, and the range of duplicate readings were within acceptable limits (Fig 8). However, this microassay produced a calibration curve with a correlation coefficient of r=-.9897, p= <.001, as compared with a value of r=-.9965, p= <.001, for the standard (Human) plasma assay. Therefore, if the availability of plasma is such to allow the standard assay to be performed, this assay will give more accurate results.

1.4 Plasma Cortisol.

The plasma cortisol assays were kindly performed by Dr S L Handley and Mrs P Marklew. The method used was that of Mattingly (1962). This is a fluorimetric assay which involved the extraction of the corticoids into dichloromethane. Fluorescence was produced by the addition of sulphuric acid/Ethanol fluorescence indicator.

The method measured 11-hydroxycorticoids, and therefore assayed both cortisol and corticosterone.

1.5 Plasma Amino Acids.

Plasma amino acid analyses were kindly performed by the Department of Biological Sciences, University of Aston.

The assays were performed on a LOCARTE, amino acid auto analyser. This method utilised the ninhydrin

reaction of the amino acids. Ninhydrin reacts with primary amines to form a coloured complex known as Ruhemann's purple. It is this coloured complex which is measured. For imino acids, such as proline, a yellow coloured complex is formed and measured.

The amino acids were separated using a column (23cm) of Locarte 'microbead' resin (8% cross linkage). The amino acids were eluted from the column using two sodium citrate buffers. The first was pH 3.17 (0.255 M Sodium), with a run time of 30 minutes. The second was pH 4.25 (0.21 M Sodium) with a run time of 40 minutes.

The buffer flow rate was 1ml/minute, at a temperature of 55°C.

A sample analyser output is presented in Appendix I. The sample amino acid concentrations were calculated by comparison of the area under the curve for the sample with the area of a peak produced by a standard of known concentration. The area of the peak was calculated by the product of the peak height by the peak width at its half height.

Stability of Amino Acids

A single plasma sample was divided into aliquots and stored at -30°C. Auto analysis of the amino acids was performed on the fresh plasma, after 1 month and after 3 months. The results are presented in Figure 9.

As can be seen, the majority of the amino acids are stable, with the exception of Ornithine, Serine, Arginine and Cysteine. The concentrations of ammonia

are also variable.

1.6 Plasma Dihydrobiopterin.

These assays were kindly performed by Professor J A Blair, Department of Chemistry, University of Aston. The method used was that of Leeming and colleagues (1976) which is a bioassay involving the use of crithidea fasciculata.

1.7 Plasma Oestriol.

These assays were kindly performed by The Clinical Chemistry Department, Birmingham Maternity Hospital. The assay method used utilising antiserum raised against Oestriol-6-(O-Carboxymethyl)oxime bovine serum albumin. (Evans et al.,1981)

1.8 Plasma Oestradiol.

These assays were kindly performed by The Department of Clinical Endocrinology, Birmingham and Midland Hospital for Women. The method used was a specific radioimmune assay measuring 17-β Oestradiol. The antiserum used was raised in rabbits to a 17-β-Oestradiol 6-O-carboxymethyloxime bovine serum albumin conjugate. This method is routinely used within the above mentioned department and is documented by Shaw and co-workers, 1974.

1.9 Plasma Progesterone.

These assays were kindly performed by The Department of Clinical Endocrinology, Birmingham and Midland Hospital for Women. The method used was a specific radioimmune assay measuring progesterone. The antiserum used was raised in rabbits to a progesterone 11-α succinyl bovine serum albumin conjugate. This method is routinely used within the above mentioned department and is documented by Shaw and co-workers, 1974.

1.10 Urine 3-Methoxy-4-Hydroxy Phenyl Glycol (MHPG).

These assays were kindly performed by Dr G Baker, University of Alberta, Edmonton, CANADA. The following method was used:

a) Incubation of urine samples

To 2ml of urine was added:

0.66ml 1M Sodium Acetate, pH6.0

0.33ml 2% w/v EDTA

0.066ml β-Glucuronidase/Aryl Sulfatase

an internal standard of 6μg parahydroxybenzyl alcohol was also added. The pH was returned to 6.0, and the solution was incubated at 37^{o}C for 20-24 hours.

b) Assay

1.0ml of the incubated urine was placed in a 1.5ml microfuge tube and placed on ice for 5 minutes. 0.1ml 4M Perchloric Acid was added, mixed by vortex, and centrifuged for 1 minute.

The mixture was transferred to larger tubes, and neutralised to pH 7.8 with $KHCO_3$. This was centrifuged at 2500 rpm for 5 minutes and transferred to a fresh tube.

Acetylation was performed by adding 0.15ml acetic anhydride and $NaHCO_3$. (This stage was performed slowly over 20 minutes, due to the vigorous reaction).

The resultant solution was transferred to clean tubes and 3ml of Ethyl acetate was added. This was shaken for 2 minutes and centrifuged at 2500 rpm for 5 minutes.

The ethyl acetate was transferred to small tubes and dried under nitrogen. 25μl of ethyl acetate was then reacted with 75μl of Trifluoro-acetic anhydride for 30 minutes at room temperature. 300 μl of cyclohexane was added and the solutions mixed, followed by the addition of 3ml of saturated sodium borate solution. This was mixed by vortex and briefly centrifuged at 2000 rpm. The cyclohexane was then removed and used for Gas Chromatographical analysis.

c) Gas Chromatography

The column used was a 3% ov-101, 4 coil column.

The following conditions were used :

Injection port temperature : 250°C

Detection port temperature : 250°C

Carrier : Argon/methane : 90:10 at 40ml/minute

The temperature programme for the oven was : 130°C for 3 minutes, increasing by 10°C per minute to 150°C. The retention time for the internal standard was about 3.9 minutes and for MHPG was 8.7 minutes.

Standards

Internal Standard : parahydroxybenzyl alcohol 3 µg in methanol.

MHPG standard : 0.5 - 3.0 µg

Results were expressed both as µg MHPG per 24 hour urine volume, and as µg MHPG per mg urine creatinine.

1.11 Brain Indoleamines.

The method used was a modification of that used by Gould (1979), which itself was a modification of Curzon and Green (1970). This method involved the formation of a fluorophore using O-phthalaldehyde (OPT) and the measurement of TP by the Denkla and Dewey method (Methods Section 1.1).

a) Extraction

Mouse brains were weighed and then homogenised in 3ml ice-cold acidified n-butanol. The homogenate

was then shaken for 10 minutes on an automatic shaker (Griffin Ltd), allowed to stand on ice again for five minutes (in order to maintain the low temperature) and centrifuged at 2500 rpm (1000g) for 5 minutes in a bench centrifuge (MSE Ltd).

2.5ml of the supernatent were removed, and added to 5ml n-heptane and 0.8ml of 0.1M Hydrochloric Acid (containing 0.1% w/v cysteine). This was then shaken and centrifuged as above. 5ml of the supernatent was removed and added to 0.8ml Sorensen's Buffer (See Section 12) at pH 7.0. The remainder of the supernatent organic layer together with any tissue disc which may have formed at the interface, was then aspirated off, and the aqueous layer was assayed for 5HT and TP (See later).

The organic layer, together with the Sorensen's buffer, was shaken and centrifuged as above. The supernatent, together with any tissue disc at the interface was aspirated off, and the aqueous layer was assayed for 5HIAA.

0.3ml of Standard solutions were extracted by the same procedure.

(b) i <u>Assay of 5HT and 5HIAA</u>

This method involved the formation of a fluorophore by use of o-phthalaldehyde(OPT) reagent

(Maickel, 1972). This method assays various indoles which must be separated as above.

0.1ml of the aqueous phase (containing either 5HT or 5HIAA) was added to 0.65ml of OPT reagent and 0.05ml cysteine hydrochloride solution (1% w/v). The mixture was then incubated in a shaking water bath at 80°C for 20 minutes. The solutions were removed, allowed to cool and the fluorescence was read on an Aminco-Bowman spectrophotofluorimeter at excitation wavelength 360nm and emission wavelength 470nm. The reaction products for 5HT and 5HIAA were identical and these wavelength parameters produced the maximum fluorescence. (Fig 10 & 11). The relationship between indole concentration and fluorescence was found to be linear (Fig 12) 5HT r = .992; 5HIAA r = .989.

(b) ii Assay of Tryptophan

The method of Denkla and Dewey (1967), as in Methods 1.1 was used. Norharman was formed as before, and it can be seen that the emission and excitation spectra produced by this assay (Fig 13) are identical to those produced by the plasma tryptophan assay (Fig 3). This therefore suggests that there is no interference from other brain indoleamines. The relationship between tryptophan concentration and fluorescence was found to be linear (Fig 14) r = .982.

Standard Solutions

A stock solution containing :

Tryptophan 500 µg/ml

5-Hydroxytryptamine 50 µg/ml

5-Hydroxy Indole Acetic Acid 50 µg/ml

all in 0.1% Cysteine hydrochloride solution was used. This was split into aliquots and stored at -30°C. An aliquot was thawed only once as 5HT is unstable to freezing and thawing (Gould, 1979).

The stock solution was diluted 1/25, 1/50 and 1/100 for use in the assay. Recovery standards of 0.1 µg/ml 5HT or 5HIAA, or 1 µg/ml TP were also used.

Extraction Recoveries

Recovery standards were prepared by adding the 5HT, 5HIAA or TP solution directly to the OPT/ cysteine mixture. The percentage recovery of 5HT, 5HIAA and TP was then calculated. The theoretical recovery of the indoles was calculated assuming partition coefficients between aqueous and organic layers to be 100%, and the actual recoveries were expressed as a percentage of this value. The recovery values found were:

5HT	:	77.65 ± 3.37 % n=4
5HIAA	:	79.28 ± 8.46 % n=4
TP	:	92.01 ± 12.26 % n=4

Comments

a) The addition of cysteine hydrochloride at the stages indicated was important in order

to prevent oxidation of the indoles.

b) The pH of Sorenson's buffer was critical, a pH of 7.0 being required for extraction of 5HIAA. Extraction of 5HIAA was seen to be decreased by 5.8% with a buffer solution at pH 7.2 and a buffer at pH 7.4 decreased the 5HIAA extraction by 30.7%.

2 BEHAVIOURAL METHODS.

2.1 Behavioural Assessement by Observation in Mice.

This is a series of tests designed to evaluate the motor co-ordination, arousal and reflexes of the animal. The tests were selected and the scoring key was taken from Irwin, 1968. The tests were always performed in the same order.

A An Animal in Home Cage

The following observations and scores were made before the animals were handled:

Body Position :

	Score
Completely flattened	0
Lying on side	1
Lying upright	2
Sitting up	4
Standing on hind legs (rearing)	6
Repeated vertical leaping	8

Palpebral Closure :

Eyes wide open	0
¼ closed	2
½ closed	4
¾ closed	6
fully closed	8

Locomotor Activity :

None, resting	0
Casual scratch/groom/slow movement	2
Vigorous scratch/groom/mod. movement	4
Vigorous movement/rapid dart	6
Extremely vigorous movement	8

Bizarre Behaviour : note -

Head Flick (HF); Head search (HS);
Hallucinatory (H); Compulsive biting (B);
Self destructive bite (SB);

Compulsive licking (L); Prancing (P);
Upright walk (UW); Aimless wander (AW);
Circling (C); Waltzing (W); Retropulsion (R);
Spatial disorientation (D).

Exophthalmus :

\+ if present - if absent

Tremors :

None	0
Slight, fine body (1.5mm)	2
Moderately coarse (3mm)	4
Markedly coarse, impaired locomotion (4.5mm)	6
Extremely coarse, no locomotion, (6mm)	8

Twitches :

Slight, moderate, extreme 0 - 8

Convulsions :

Describe.

B Animals in Observation Arena

Animals were then transferred into an observation arena (29cm x 31cm) and the following tests were performed.

Transfer Arousal (appearance) :

Coma	0
Slow, dulled movement	2
Alert, active movement	4
Slightly excited	6
Extremely excited	8

Spatial Locomotion :

Duration of periods of movement(scored 0-4) multiplied by a speed of movement factor :

Slow = 1
Active = 1.5
Rapid = 2

Palpebral Closure :

As Above

Piloerection :

Slight, moderate, extreme 0 - 8

Startle Response :

Note of response to sudden noise produced by clapping of hands.

No jerk	0
¼cm jerk	2
½cm jerk	4
¾cm jerk	6
1cm or more	8

Pelvic Elevation :

Flattened	0
Barely touches	2
3mm elevated	4
6mm elevated	6
12mm elevated	8

Tail Elevation :

Flattened	0
Horizontally extended	2
Elevated 45°	4
Vertical 90°	6
Elevated 135°	8

Finger Approach :

A fingertip was moved slowly towards animal. The response was noted.

None	0
Head movement only, at distance	2
Movement towards finger, no contact	4
Contact, touches finger	6
Completely on finger	8

Finger Withdrawal :

The finger was then removed, the response noted.

None	0
Eye squint only	2
Slight head & body movement	4
Moderate head & body movement	6
Vigorous head & body movement	8

Touch Escape :

The mouse was stroked across the back, the response was noted.

None	0
Slow escape (firm stroke)	2
Moderate escape (light stroke)	4
Vigorous escape (light stroke)	6
Very vigorous escape (barely touch)	8

Gait :

The abnormality of the gait was recorded.

Normal = 0
Unable to move without rolling over = 8

C Tail Suspension

The animal was then removed and suspended by the tail above a bench.

Visual Placing :

The distance of the nose above the bench before the forelegs were extended.

No limb extension	0
After nose contact	1
6mm	2
12mm	4
18mm	6
25mm	8

Grip Strength :

The animal was dragged backwards across the cage top. The grip strength was estimated.

None	0
Semi-effective	2
Moderate grip	4
Active grip	6
Unusually effective	8

D On Cage Lid (grid) :

The animal was then placed on the cage lid, without restraint.

Pinna Reflex :

The inside of the pinna was gently stimulated using a fine wire, the response was noted.

No response	0
Slight flick of ear	2
Mod. ear flick, Head movement	4
Active head movement	6
Repetitive, hyperactive head flicks	8

Cornea Response :

The cornea was gently touched using a fine wire, and the response was noted.

No response	0
Sluggish closure	2
Active single eye blink	4
Active double eye blink	6
Multiple blinking of eye	8

Toe Pinch :

The toes on a hind limb were pinched using forceps.

No response	0
Slow limb withdrawal	2
Moderately rapid withdrawal	4
Brisk withdrawal	6
Very brisk repeated limb flexing	8

Tail Pinch :

The tail was pinched using forceps.

No response	0
Slow movement	2
Slight biting, escape, vocalisation	4
Moderate bite, escape, vocalisation	6
Vigorous biting, escape	8

E Supine Restraint

The animal was then gripped and held supine.

Limb Tone :

A limb was extended and flexed and the amount of resistance noted 0 - 8

Abdominal Tone :

The abdomen was pressed and the return of the abdominal position was noted.

Completely flaccid, no return	0
Slightly flaccid, no return	2
Slight resistance	4
Moderate resistance	6
Extreme resistance, board-like	8

Lacrimation :

Presence or absence noted.

Salivation :

Presence or absence was noted.

Provoked Biting :

A small, plastic rod, (2mm diam) was placed touching the mouth, the response was noted.

No response	0
Slight, weak	2
Moderately active	4
Vigorous, not immediate or continuous	6
Vigorous, continuous biting	8

Catalepsy :

The mouse was then placed against a catalepsy bar, and the length of time the posture was held was measured.

Righting Reflex :

The mouse was gently thrown about 20cm into the air and the presence or absence of a normal, steady landing on the feet was noted.

F General

Scores were also given for grasp irritability (0 - 8), the number of vocalisations, and defaecation and urination.

2.2 Locomotor Activity.

Locomotor activity of mice was measured by means of an Animex Activity Meter (L K B, Farad). (Svensson and Thieme, 1969). This apparatus measures movement of animals as they disturb electromagnetic fields produced by six coils under the animals' cage. The tuning of the apparatus was set as 40μA, with the sensitivity set at 25μA. At this setting, only gross locomotor movement was recorded, finer motor activity was not counted.

Unless otherwise stated, locomotor activity of animals was measured in their home cage. Animals were housed in cages of 42cm x 28cm x 15cm.

2.3 Plate-Crossing.

This test involves the measurement of the animals' exploratory behaviour in a novel environment. It is considered to be a measure of fear and anxiety-like behaviour (Marriott & Smith, 1972).

The apparatus consisted of a box of 21.5cm square, with walls 15cm high. On the floor of the box were 4 metal tiles (10cm square) separated from each other and from the walls by 0.5cm. The tiles were also raised 0.5cm from the floor.

A mouse was placed in one corner of the box facing the corner, and the number of times the

mouse crossed from one tile to another with all four feet, in a period of 90 seconds was recorded. The latency of the crossings was also recorded by noting of the time taken to achieve 1, 5 and 10 crossings.

2.4 Potentiation of Barbiturate Sleeping Time.

This test is a measure of a drugs sedative, hypnotic or anaesthetic properties.

Animals were pre-treated with either test-drug or control. Then sodium thiopentone (INTRAVAL) was injected intravenously into the tail vein. The dose of anaesthetic used was 30mg/kg in a volume of 5ml/Kg. The mouse immediately became anaesthetised, and was placed on its back, on cotton wool, under a microscope lamp. The time taken for the animal to recover from the anaesthesia as defined as the duration of the loss of righting reflex, was recorded. This method is a modification of the hexobarbital sleeping time as used by Brodie and colleagues (1955).

3 PSYCHOLOGICAL ASSESSMENTS.

General Introduction

Mood is generally measured in one of three ways : a) Mood Questionnaires, b) Interview by experimenter, and finally, c) self report diaries. All three of these methods have been used during the preparation of this thesis. The other form of psychological assessment performed was the assessment of personality, this again was a questionnaire type measurement. Each type of methodology will be described separately.

3.1 Mood Questionnaires.

On each occasion that such a questionnaire was employed, a standard procedure was followed. The rubric at the head of the questionnaire was read to the subject, and any queries were answered. In this way, it was ensured that correct method of answering the questionnaire was understood. The subject then completed the questionnaire, the experimenter did not watch the answering of any questions, nor was any time limit enforced. When the subject indicated that the questionnaire was completed, the papers were collected and, where relevant, it was checked that all the questions had been answered. If omissions had been made, the subject was requested to complete the remaining questions. At no time were the responses discussed with the subject. Questionnaires were always collected immediately following completion so that it was not possible for responses to be altered.

Four types of such questionnaires were used and these will be described separately. The first three to be described were designed to measure mood 'state', at a particular moment in time, the fourth questionnaire was designed to quantify personality which is a 'trait', or chronic quality that theoretically does nor vary with time.

3.1.1 Multiple Affect Adjective Checklist (MAACL).

This checklist was developed by Zuckerman and Lubin (1965) and was designed to measure three factors of mood : Depression, Anxiety and Hostility. The questionnaire gives a final score for each of these factors, these scores refer only to mood at the point in time that the questionnaire was completed. The scores obtained were designed to act as a descriptive measurement of each of the mood factors.

The questionnaire consists of 132 mood adjectives and the subject is required to mark those adjectives which describe his/her present mood state. The questionniare requires approximately five minutes for completion.

Scoring of the questionniare is by the use of standard scoring keys for each factor, these give numerical scores for each factor. Since the mood questionnaire contains both positive and negative mood adjectives, the scoring keys take both words marked, and words left

unmarked into account. The maximum scores possible for each factor are:

Depression 'D' : 40

Hostility 'H' : 28

Anxiety 'A' : 21

3.1.2 Beck Depression Inventory (BDI)

This questionnaire was developed by Beck and colleagues (1961) as a diagnostic tool for the diagnosis of clinical depression. It is therefore a measure of more severe depression than the MAACL.

This questionnaire is used in several forms. The form used in Chapter 1 consisted of 19 sets of statements each consisting of 3 or 4 sentences describing mood in varying degrees of depression. The subjects were required to indicate which sentence of each set of statements best described their mood at that point in time. Each sentence has a set score associated with it. The maximum depression score possible on the questionnaire was 57.

The form of the BDI used in Chapter 4 was similar in design, except that it contained only 13 sets of statements. The statements omitted in this second questionnaire were those concerning feelings of being punished, feelings

of self-blame, frequency of crying, irritability, insomnia and concern of health and bodily functions. Scores on this second questionnaire were reported to be highly correlated with score on the 19 item BDI (Beck & Bearnesderfer (1974)). The maximum possible score for this second questionnaire was 39.

3.1.3 Sabbatsberg Depression Questionnaire.

This questionnaire is of the type that poses a question and requires a reply of Never, Seldom, Often or Always. The original questionnaire has a maximum score of 50. However, in the study of Chapter 3, all scores were doubled in order to eliminate fractions. The maximum score was therefore 100. The reported criterion of clinically significant depression for this questionnaire was 50 or above (Fedor-Freybergh, 1977)

3.1.4 Blues Inventory.

This questionnaire was designed by Dr G Waldron, to be a self report form of the clinical interview previously used for the diagnosis of 'blues' in the study of Handley and colleagues (1980). This questionnaire together with its scoring schedule is presented in Appendix II. The maximum score on this questionnaire was 8.

3.2 Experimenter Interviews.

The method used throughout this thesis was that of semi-structured interviews. This method sets out a list of questions to be asked, but does not preclude the asking of extra questions, if extra information may be gained by doing so. As with normal interview procedure, the experimenter asked 'open-ended' questions such as 'How are you feeling today?' as opposed to directional questions such as 'Are you better today?' which could influence the direction of the answer. Care was also taken to show no undue reaction to answers to questions since this again can influence the responses.

Reports of interviews were not written up during the interview, but within 5 minutes of the end of the interview. This was because the writing up of the interview notes during the conversation disrupted the fluency of the conversation.

All interviews were performed by the same experimenter therefore a high degree of rapport existed between the subject and the experimenter.

3.2.1 Puerperal Interviews.

Subjects were interviewed in late pregnancy and throughout the puerperium, therefore different forms of the interview were required. Three forms of interview were used, the first form was used as 36 weeks gestation, a second form was used for the first four days

post partum, and the third form was used on the fifth day post partum. All of these were semi-structured interviews.

a) Antenatal Interview

The following factors were investigated : Previous menstrual history, planning of pregnancy (including history of infertility), general health during pregnancy, mood and emotions during pregnancy, particular anxieties concerning pregnancy amd parturition, concern over appearance and loss of attractiveness, husband's reaction to pregnancy, sexual activity and marital relations during pregnancy, preparations for parenthood.

b) Post-Partum Days 1 - 4

This interview contained questions relating to the subject's mood over the previous 24 hours, episodes of crying and weepiness, particular anxieties, reactions to visitors, problems with sleeping, appetite and problems with the feeding of the baby.

An observer rated desciption of mood was also recorded. This description was based on the subject's reaction to the experimenter, content of conversation, pressure of conversation, general liveliness and movement, facial expressions, etc. Examples of such descriptions would be 'appears upset today' or 'is much more cheerful today'.

c) Post-Partum Day 5

This interview was essentially the same as that described in section b previously, however, in addition, the subject was asked if she had previously heard of 'baby blues' and if so, whether she thought that she had suffered from the 'blues'. If the answer to this question was affirmative, the subject was asked if she had ever experienced similar feelings at any other time in her life.

Before these questions were asked, an observer rating of blues was recorded. This rating was based on whether the subject had either reported or exhibited the common symptoms of 'blues', ie weepiness, transient anxiety or depressed affect, mood lability, etc, at any time during the previous 4 days.

3.2.2 Post-Natal Follow-Up Interview.

This interview was conducted at about 9 months post-partum. It was carried out at the subject's own home. This interview was designed to investigate the subject's health and emotions during the preceding 9 month s post-partum. The questions asked concerned :

Any medical treatment received by the mother or baby since parturition, times of any mood disturbances, reactions of the husband and

family to the new baby, reaction of the mother to other children, effects of the baby on social life, etc., effects on marital relations and finally, would the mother happily go through another pregnancy, labour and delivery again.

In some cases (see chapter 4) subjects had moved away from the area, in these cases, the same questions were presented in a postal questionnaire. The interview schedules and postal questionnaire are presented in Appendices III & VIII

3.3 Self-Report Menstrual Diary.

This consisted of a simple diary-like form, which was completed daily. The subject was required to record any symptoms from a given list, which had troubled her on that particular day. Days of menstruation were also recorded.(Appendix IV).

3.4 Eysenck Personality Inventory.

This questionnaire was developed by Eysenck and Eysenck (1963) to measure factors of personality. It asks a variety of questions such as 'do you tend to find yourself in the background on social occasions' and the subject is required to answer 'yes' or'no'. The replies are then scored using a standard score key. The results produced give values for two personality traits, 'Extroversion' and 'Neuroticism'.
The maximum 'E' score possible was 24, the maximum 'N' score was 24.

4 SUBJECT HISTORY COLLECTION.

General Introduction

For studies concerning mood changes during pregnancy and the puerperium (chapters 1 & 4), personal, sociological and medical histories were collected for each subject. Sociological and general past medical histories were collected by interviewing the subjects. The more detailed information concerning the parturition, eg blood loss at delivery, birth weight of infant, etc. was obtained by referral to the subject's obstetric notes.

For the study of perimenopausal subjects (chapter 3) all data concerning personal medical histories were collected by interview by Dr A D Parsons.

4.1 Pregnancy and Puerperal Mood Studies.

4.1.1 Antenatal Data.

a) Basic Background Information

The following data was collected by interviewing the subjects:

Age	Previous Menstrual Difficulty
Nationality	Past Medical History
Marital Status	Past Psychiatric History (depressive)
Husband's Occupation	Past Psychiatric History (non-affective)
Age at menarche	Family Medical History

b) Antenatal History

This information was collected at the same time as the above :

Planning of Pregnancy

Reason for cessation of oral contraceptive

Oral contraceptive history

Other contraceptive history

4 1 2 Obstetric Data

The following information was otained by consulting the medical/obstetric notes.

a) Maternal Data

Parity

Induction (method)

Current Obstetric History (eg Hyperemesis)

Forceps (type and reason for use)

Duration of Pregnancy

Blood transfusion (total volume)

Date of delivery

Intravenous infusions (total volume)

Hour of delivery

b) Infant Data

Number of infants

Birthweight

Sex

Light for Dates +

Apgar Score *

Maturity (ie Post., Prem.)

+ * See over

* Criteria for Apgar Scores (Passmore et al., 1974)

Score	0	1	2
Heart rate	Absent	< 100	> 100
Respiratory rate	Absent	Slow/Irregular	Regular/ Crying
Muscle tone	Limited	Some flexion	Active movement
Response to stimuli (catheter)	Nil	Grimace	Cough/Sneeze
Colour	Blue/White	Pink body/Blue extremities	Pink

+ Criteria For Light for Dates

This measurement is derived from a comparison of the weight of the infant with tables of population norms of birthweight depending on the maturity of the infant. (See Catzel, 1976)

The aforementioned data were recorded onto a response proforma and coded to allow computer analysis. (See Appendix V).

4.2 Menopausal Study.

The following information was collected by interview :

Subject's date of birth

Past psychiatric history

Date of last menstrual period

Current psychotropic medication

Surgical history (Hysterectomy/Ovariectomy)

5. SUBJECT DIETARY AND MEDICATION RESTRICTIONS.

As described in the Introduction, plasma TP may be affected by the presence of NEFA and by the brain penetration of large neutral amino acids. The amino acids are normally obtained from a dietary source, whilst NEFA would be mobilised from the adipose tissue at times of fasting. Therefore, strict dietary control was enforced in all subjects prior to blood sampling for the determination tryptophan, fatty acids or amino acids.

For chapters 1, 2 and 4 blood samples were withdrawn during the morning. Therefore a standardised breakfast was required. The breakfast used was as follows :-

One bowl of cereal, or one slice of toast or bread. Cooked breakfasts were not permitted. There was no restrictions on fresh fruit, fruit juice, butter, margarine, jam, marmalade, milk, coffee or tea (with or without sugar). It was however stressed that patients were to eat some form of breakfast.

For blood samples taken in the afternoon (Chapter 3), a light, low fat lunch was enforced.

Aspirin is also known to interfere with TP binding to plasma albumin (McArthur, 1969). Therefore patients were not permitted to take aspirin for 24 hours prior to blood sampling, however Paracetamol was permitted.

6. HORMONAL CYCLE STAGE DETERMINATIONS.

6.1 Menstrual Cycle.

The human menstrual cycle is normally divided into five unequal stages. The first stage is the menstrual stage which begins with the onset of the menses. The second stage is the follicular phase which begins at the cessation of menstruation and lasts until ovulation. The third stage is the ovulatory phase which defines the immediate period of ovulation. This is followed by the luteal stage which lasts for about 8 days. The final stage is the premenstrual stage which immediately precedes the onset of the next menses.

The mean length of the menstrual cycle is 28 days (Bell et al., 1976), however there is a great variation between individuals, therefore, within this thesis certain arbitrary criteria were used in the determinations of the menstrual cycle stage (See below).

In order to determine the stages of the menstrual cycle, subjects were required to record their sublingual body temperature daily before they arose from bed, and before they ate or drank anything. The timing and duration of the menstrual flow was also recorded using the menstrual records described in Methods 3.3.

Body temperature varies cyclically throughout the menstrual cycle, with a slight fall of body temperature, about $0.5^{o}c$, at the time of ovulation. There is then a rise in body temperature during the luteal phase (Bell et al., 1976).

The ovulatory stage was therefore defined as the day of the dip in body temperature, together with one day either side of this dip. The menstrual stage stage was defined as the five days following the on-set of menses, regardless of the actual duration of menstruation. The premenstrual stage was defined as the five days preceding the onset of menstruation. The follicular stage was therefore those days between the cessation of menstruation and ovulation, whilst the luteal phase was those days between ovulation and the premenstruum.

6.2 Murine Oestrous Cycle.

The stage of oestrous cycle was determined by vaginal lavage with a small volume of normal saline. Microscopic inspection of the saline sample allowed determination of the vaginal cytology. On the basis of this cytology, the oestrous cycle was divided into 4 stages following the description of Allen (1922) :

1 Dioestrous : Small number of epithelial cells, in various stages of nuclear degeneration.

2 Proestrous : Large number of nucleated epithelial cells.

3 Oestrous : A mass of cornified, non-nucleated cells.

4 Metoestrous : In the early stages there were numerous cornified, non-nucleated cells, bunched together. In the later stage there was a decreased number of cornified cells, with a heavy infiltration of smaller leucocytes.

6.3 Menopausal Status.

The perimenopausal subjects used in this thesis (Chapter 3), were all referrals to a menopause research clinic. Therefore all subjects had either ceased to menstruate or had very irregular menstruation (Parsons, Personal communication). The classification of subjects into pre- or post menopausal groups was therefore made on the basis of plasma follicular stimulating hormone (FSH) concentrations.

FSH levels during the menstrual cycle normally vary between 1 - 20 IU/dm^3, the peak being at the mid-point of the cycle. However, post menopausally plasma levels rise to above 40 IU/dm^3 (Bell et al., 1976). Therefore, a criterion of 35 IU/dm^3 or above was used for the determination of menopausal status. Subjects with plasma FSH values of greater then 35 IU/dm^3 were classified as post menopausal, whilst subjects with values below 35 IU/dm^3 were classified as premenopausal. Subjects were easily classified into these groups with premenopausal FSH values ranging from 4 - 27 IU/dm^3 and post menopausal FSH values ranging from 36 - 50 IU/dm^3

NB All FSH values of 50 IU/dm^3 or greater were recorded as 50 IU/dm^3.

7 ANIMAL HUSBANDRY.

The animals used were bred within our colony at the University of Aston. They were all albino TO strain mice. Subsequent to weaning, mice were kept in single sex groups of 25 in polypropylene cages (50cm x 30cm x 15cm). Female mice were divided into groups of 5 in cages (30cm x 13cm x 11cm) 14 days before experimentation in order to ensure synchronisation of the oestrous cycles.

The experimental room was maintained at a temperature of 21 ± 1°C with a fixed 12 hour light/12 hour dark cycle. Animals received diet cubes (Heygates Ltd) and tap water ad libitum.

8 BODY FLUID AND TISSUE SAMPLE COLLECTION AND STORAGE.

8.1 Human Plasma Samples.

Blood samples were taken by venepuncture of the ante cubital vein with minimum stasis. The blood was then placed into a heparinised tube and mixed thoroughly. Plasma was obtained by centrifugation for 10 minutes at 2500 rpm (1000g). The plasma was decanted and stored at -30°C until required. After thawing of the plasma, occasionally strands of fibrin were seen to have been precipitated, these were pelletted by brief centrifugation (2-3 minutes) at 2500 rpm.

Because of the possible danger of infective hepatitis (especially Australia antigen) to the experimenter, strict hygiene standards were maintained and no subject was allowed to take part in any study if there was any

possibility of a history of hepatitis.

8.2 Human Urine Samples.

24 hour urine collections were performed. The urine being stored in 2 litre polypropylene bottles containing a preservative (20ml 2.0% w/v EDTA/0.5g Sodium Metabisulphate per litre of urine). At the end of the 24 hour collection the volume was recorded and a 20ml sample was retained and stored deep frozen at -30°C, the remainder of the urine was discarded.

8.3 Animal Plasma Samples.

Animals were sacificed by cervical dislocation and an incision was made in the jugular vein. The blood was then allowed to drain via a heparinised funnel into a small heparinised collecting tube. The blood was immediately centrifuged, the plasma removed and stored at -30°C until required.

8.4 Animal Brain Samples.

Animals were sacrificed by cervical dislocation, and a deep incision was made at the level of the cervical vertebrae. Further incisions were made along the mid-dorsal line of the skull and at the level of the orbits. The upper portion of the skull was removed and the brain was lifted out, using a small spatula, leaving the olfactory lobes *in situ*. The complete process took appoximately 30 seconds. Brains were immediately weighed and homogenised into 3ml ice cold acidified n-butanol.

The homogenate was then stored for up to 3 days at -30°C.

9. DRUG ADMINISTRATION TECHNIQUES.

9.1 Subcutaneous Route

Injection was made via a 25G 5/8 hypodermic needle into the loose skin at the scruff of the neck. The injection volume was 10ml/kg.

9.2 Intravenous Route

The mouse was restrained in a glass cylinder, with the tail being left accessible. A microscope lamp was shone onto the tail in order to produce vasodilatation. The injection was then made into one of the four tail veins via a 26G 3/8 hypodermic needle. The injection volume was 5ml/kg.

9.3 Oral Route

The oesophagus was intubated beyond the glottis using an oral dosing needle. Hence the drug was effectively delivered directly to the stomach. The volume administered was 5ml/kg, unless otherwise stated.

10 ETHICAL COMMITTEE APPROVAL.

All investigations involving human volunteers were accepted by the relevant Human Science Ethical Committee. Protocols for Chapters 1, 2 and 4 were accepted by the Human Science Ethical Committee of the University of Aston. Chapters 3 and 4 were accepted by the Ethical Committee of the University of Birmingham Medical School and Chapter 1 was accepted by

the Ethical Committee of the London Hospital Medical College.

11. DRUGS AND VEHICLES USED.

Dydrogesterone (DUPHASTON) - a kind gift from DUPHAR LABORATORIES LTD.

This drug was administered in Gum Acacia, and stored at 4°C

Ethinyl Oestradiol (Sigma Ltd)

Administered in Gum Acacia suspension and stored at 4°C

Gum Acacia (Sigma Ltd)

This was used as a vehicle and as a control. The concentration used was 25 mg/ml of double distilled water. This vehicle was stored for up to 7 days at 4°C.

Norethisterone Acetate (Sigma Ltd)

This was administered either in suspension in Gum Acacia, or in 0.9% w/v saline solution. The drug was dissolved in a few drops of ethanol before being diluted with saline. This compound was stored at 4°C.

Progesterone (Sigma Ltd)

This was administered in Gum Acacia and stored at 4°C in darkness.

Saline (AnalaR grade NaCl, BDH Chemicals)

0.9% w/v NaCl in double distilled water. This was made in large volumes and stored at room temperature.

Sodium Thiopentone (INTRAVAL) (May & Baker)

This was administered in double distilled water and prepared fresh for each experiment.

L-Tryptophan (Sigma Ltd)

This was prepared in a solution of 0.9% w/v NaCl and 0.5% Tween 80 (BDH). This compound was prepared freshly for each experiment.

12 CHEMICALS AND SOLVENTS USED

Chemicals		Supplier
Acetone(CH_3COCH_3)	Laboratory Reagent Grade	BDH Chemicals, Poole.
n-Butan-1-ol($CH_3(CH_2)_2OH$)	AnalaR Grade	BDH Chemicals, Poole.
L-Cysteine Hydrochloride Monohydrate		Sigma Chemicals
Decon 90		Decon Laboratories Ltd, Hove.
Disodium ethylenediamine tetraacetate dihydrate(EDTA)	AnalaR Grade.	BDH Chemicals, Poole.
Disodium Hydrogen Orthophosphate($Na_2HPO_4 \cdot 12H_2O$)	AnalaR Grade	BDH Chemicals, Poole.
Ethanol(CH_3CH_2OH) 99.9%	BP	James Burrough Ltd, London.
Ferric Chloride($FeCl_3$)	AnalaR Grade	BDH Chemicals, Poole.
Formaldehyde(HCHO)	AnalaR Grade	BDH Chemicals, Poole.
n-Heptane($CH_3(CH_2)_5CH_3$)	AnalaR Grade	BDH Chemicals, Poole.
Norharmane		Sigma Ltd.
Hydrochloric Acid(HCl)	AnalaR Grade	BDH Chemicals, Poole.
5-HydroxyIndole Acetic Acid (5HIAA)		Sigma Ltd
5-Hydroxytryptamine(5HT) as the creatinine Sulphate Complex		Sigma Ltd
Nitric Acid (HNO_3)	AnalaR Grade	BDH Chemicals, Poole.
Palmitic Acid ($CH_3(CH_2)_{14}COOH$)		Sigma Ltd
o-Phthalaldehyde (OPT)		Sigma Ltd
Potassium dihydrogen orthophosphate (KH_2PO_4)		Griffin&George Ltd, Birmingham
Propan-2-ol ($CH_3CHOH-CH_3$)	AnalaR Grade	BDH Chemicals, Poole.
Sodium Barbital		Sigma Ltd
Sodium Chloride(NACl)	AnalaR Grade	BDH Chemicals, Poole.
Sodium Metabisulphate($Na_2S_2O_5$)		BDH Chemicals, Poole.
Sulphuric Acid (H_2SO_4)	Laboratory Reagent Grade.	BDH Chemicals, Poole.

Teepol — BDH Chemicals, Poole

Trichloro Acetic Acid (TCA) AnalaR Grade — BDH Chemicals, Poole

β- Umbelliferone (7-hydroxy-4-methylcoumarin) — Sigma Ltd.

The following reagents were made and stored in bulk :

Acidified n-Butan-1-ol:- 0.85ml conc HCI per litre Butan-1-ol

OPT indicator solution:- OPT 40 µg/ml in 10M HCI

Sorensen's Buffer pH 7.0 :- 2.3g/100ml Disodium hydrogen orthophosphate & 0.908g/100ml potassium dihydrogen orthophosphate.

β-Umbelliferone Indicator Solution:-

Stock Solution:- 20mg 7-hydroxy-4-methyl coumarin in 10ml Ethanol.

Working Soltion:-0.3ml stock solution together with 8ml 2.5% w/v sodium barbital in water.

0.1ml working solution added to 10ml Ethanol +

20ml n-Heptane immediately before use.

Doles Solution:- Propan-2-ol/n-Heptane/0.5M H_2SO_4 (40:10:1)

13 STATISTICAL ANALYSIS OF DATA

All mean values quoted within this thesis are expressed as the arithmetic mean plus or minus one standard error of that mean. Unless otherwise stated, all probabilities quoted are values for two-tailed probability. In cases of a directional hypothesis, one-tailed probabilities were used.

Parametric data was tested using the student's-t test, for most experimental designs the independent means t-test was appropriate, in the case of repeated measures design of experiment the related means t-test was used. Analysis of variance was used for the analysis of parametric data from more than two sample populations.

For non-parametric data, the Mann-Whitney U Test was used for independent samples, and the Wilcoxon test was used for related sample analyses.

Comparison of non-parametric frequency data was performed using the Chi^2 test except in cases of small sample sizes when the Fisher's exact test was used.

Correlational analysis of parametric data utilised the pearson product-moment correlation coefficent.

The data from Chapter 4 was analysed, using the tests mentioned above, utilising the Statistical Package for Social Sciences (SPSS) (Nie et al., 1975).

14. CLEANSING OF GLASSWARE.

All glassware used for assays involving fluorimetric analysis was soaked overnight in Decon solution, then rinsed twice in tap water and once in double distilled water. It was then dried in a hot air oven.

General glassware was soaked overnight in Teepol solution and then rinsed and dried as above.

Fluorimetric cuvettes, both glass and quartz, were rinsed in tap water, then stored in conc. Nitric Acid. Immediately before use, the cuvettes were rinsed in double distilled water and finally dried by rinsing in acetone.

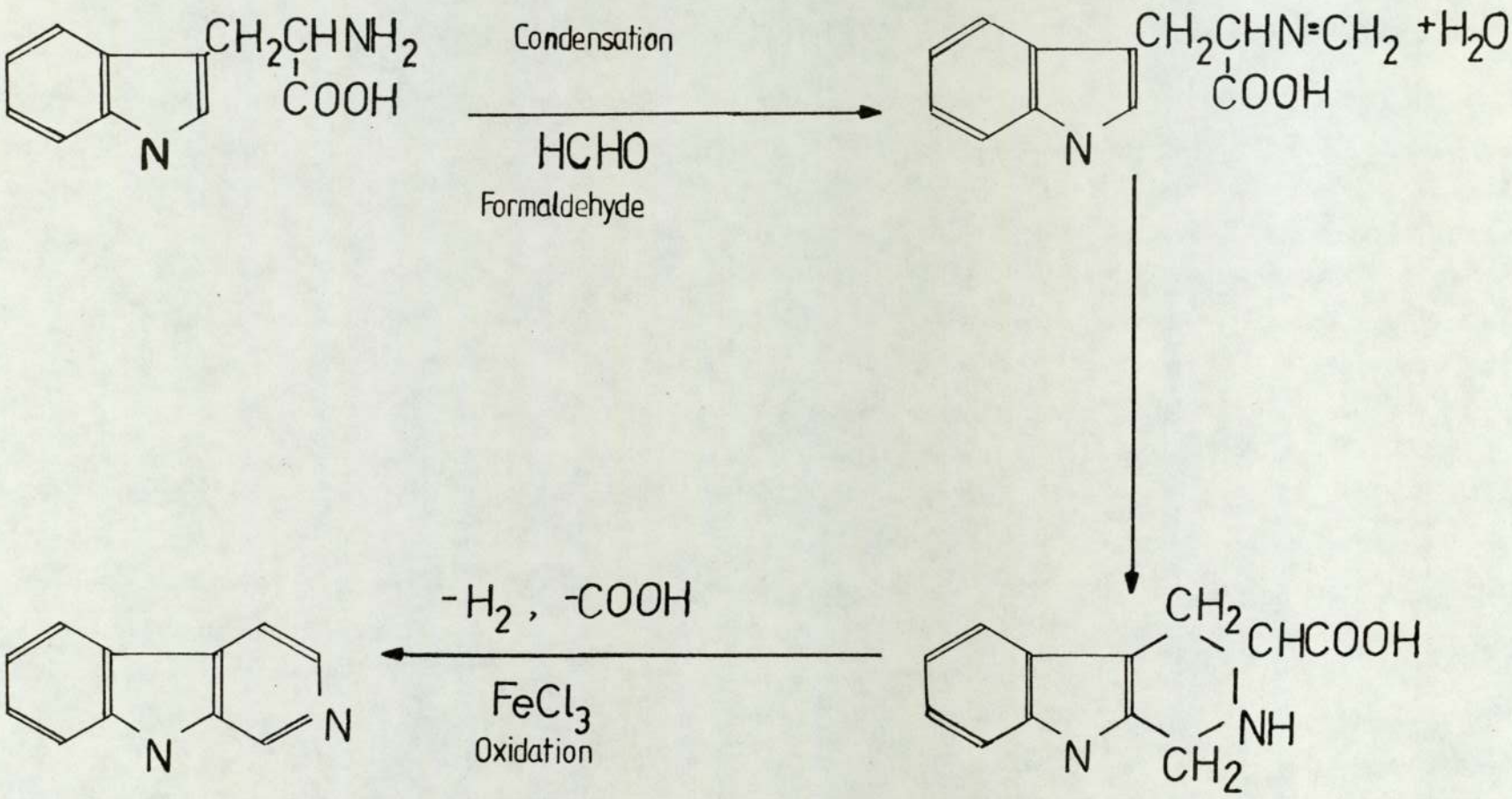
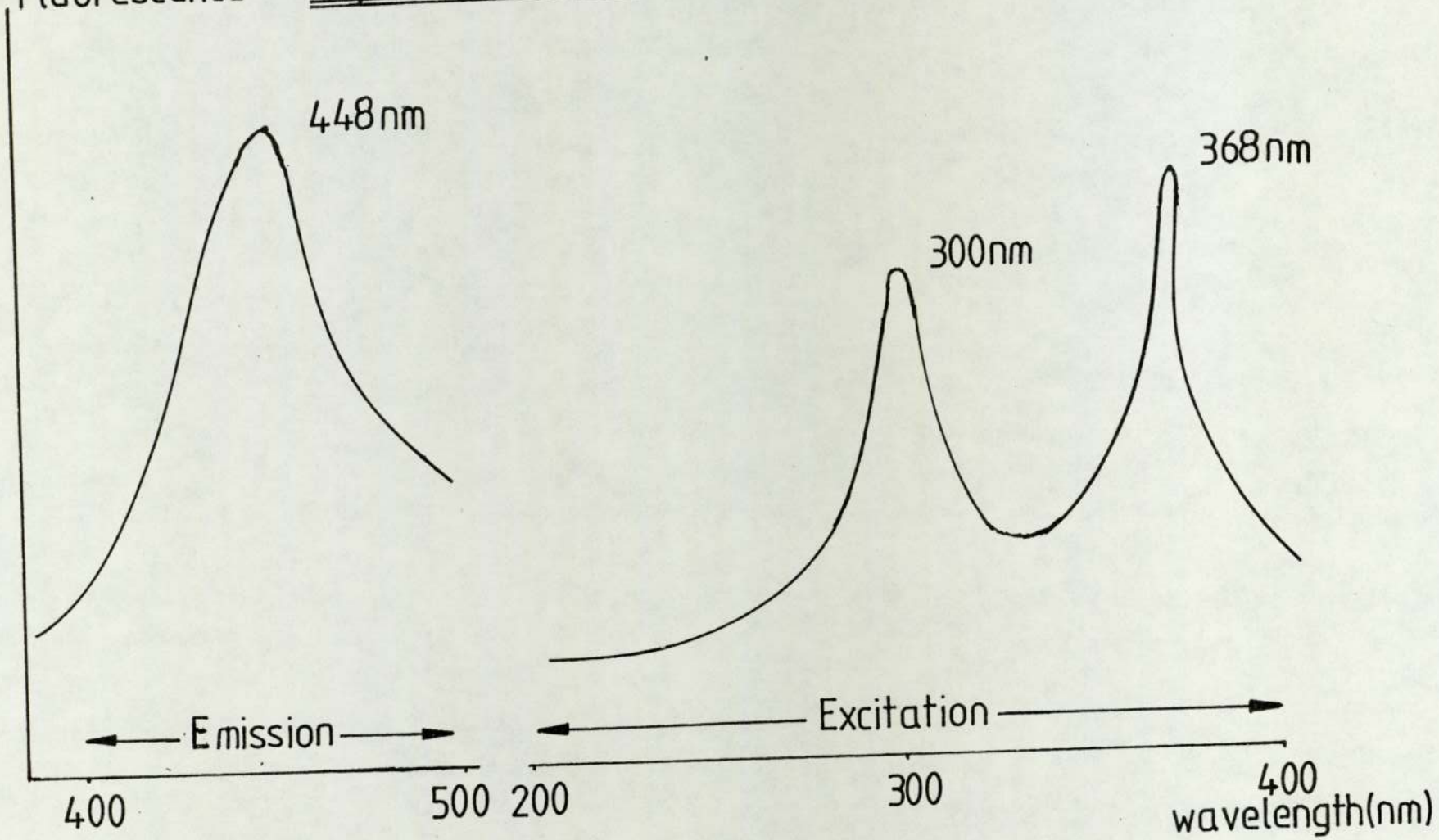


Fig1 Reaction involved in the assay of trytophan

Fig2 Excitation& emission spectra of norharman



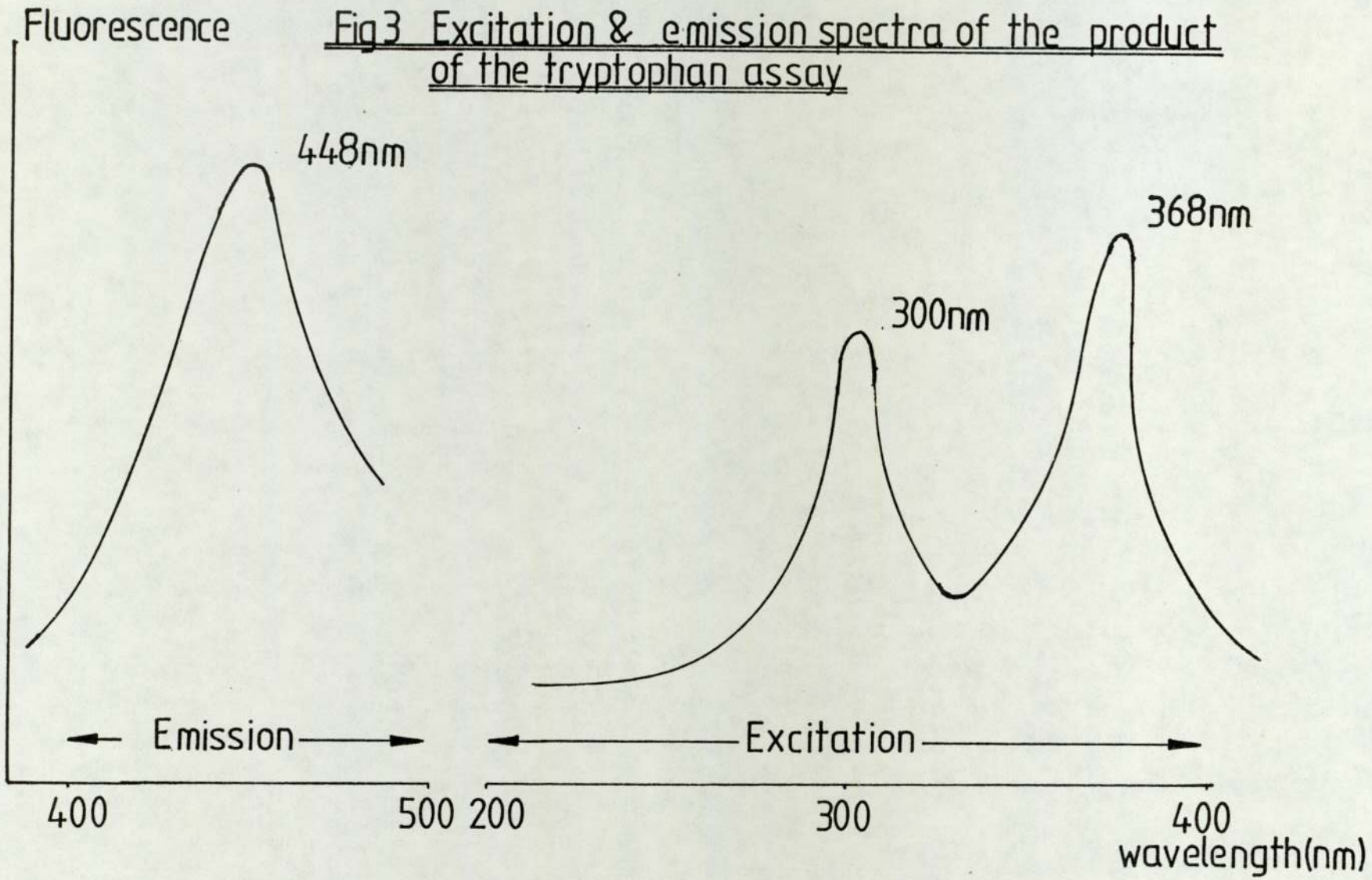


Fig 3 Excitation & emission spectra of the product of the tryptophan assay

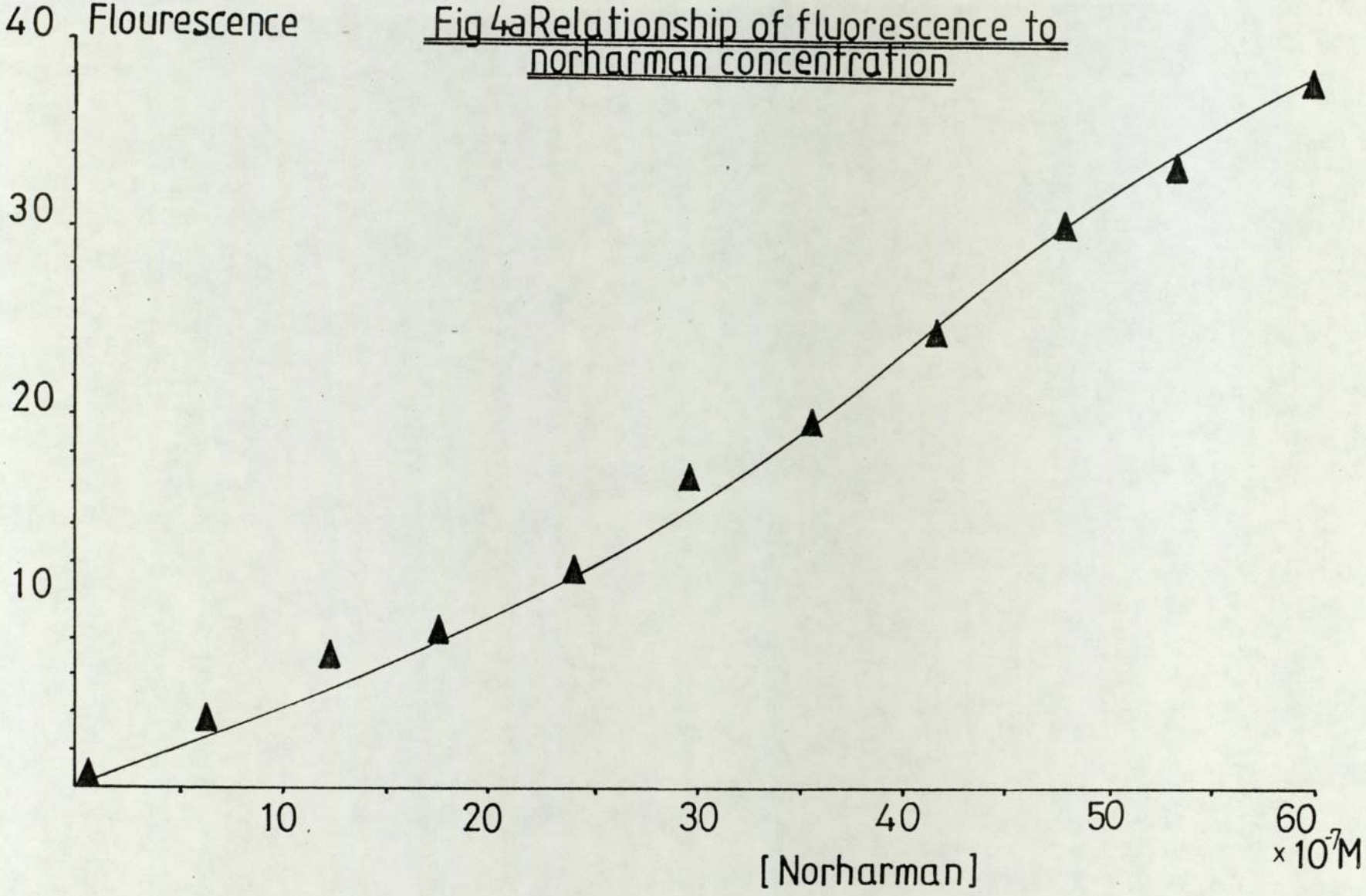
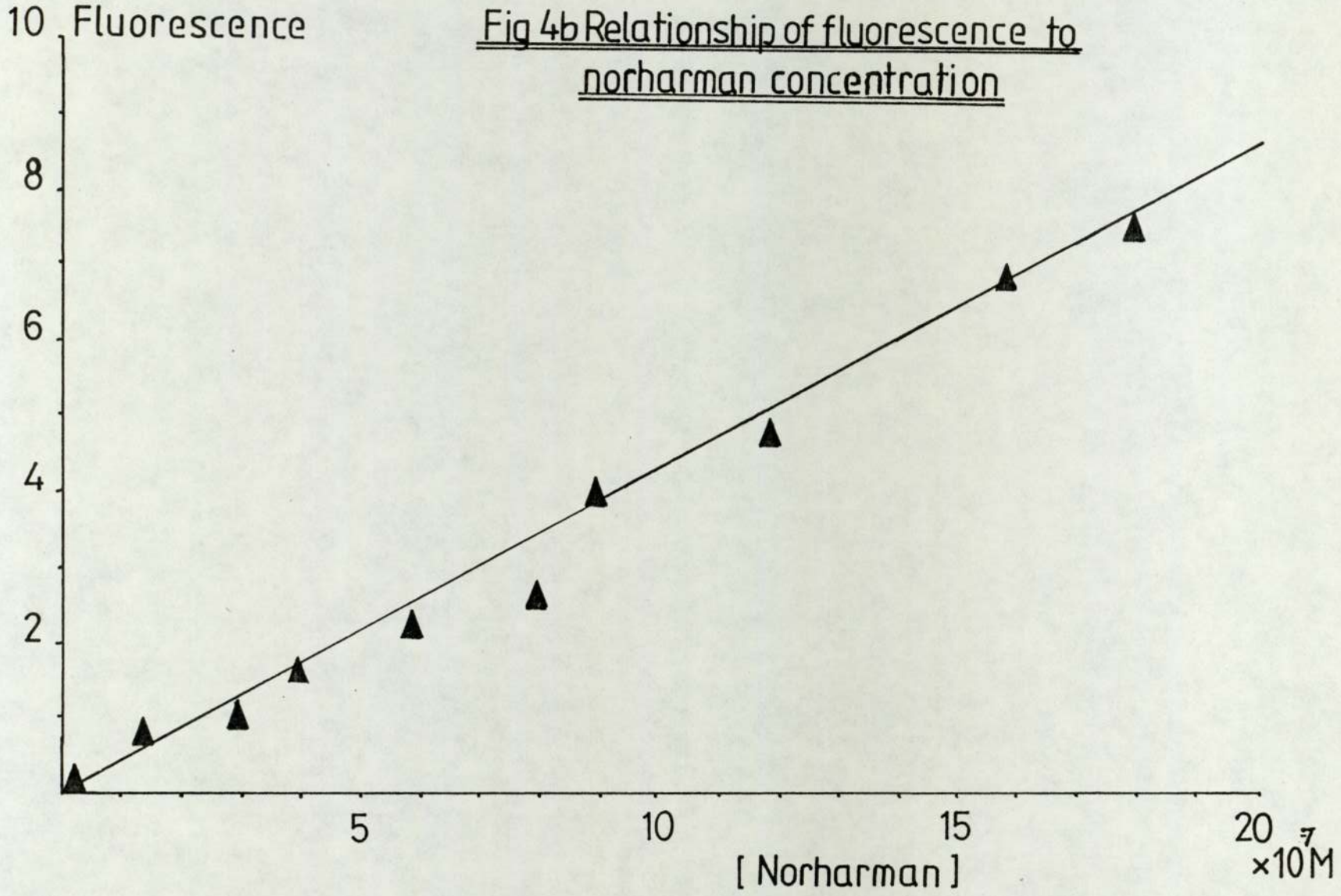


Fig 4a Relationship of fluorescence to norharman concentration

Fig 4b Relationship of fluorescence to norharman concentration



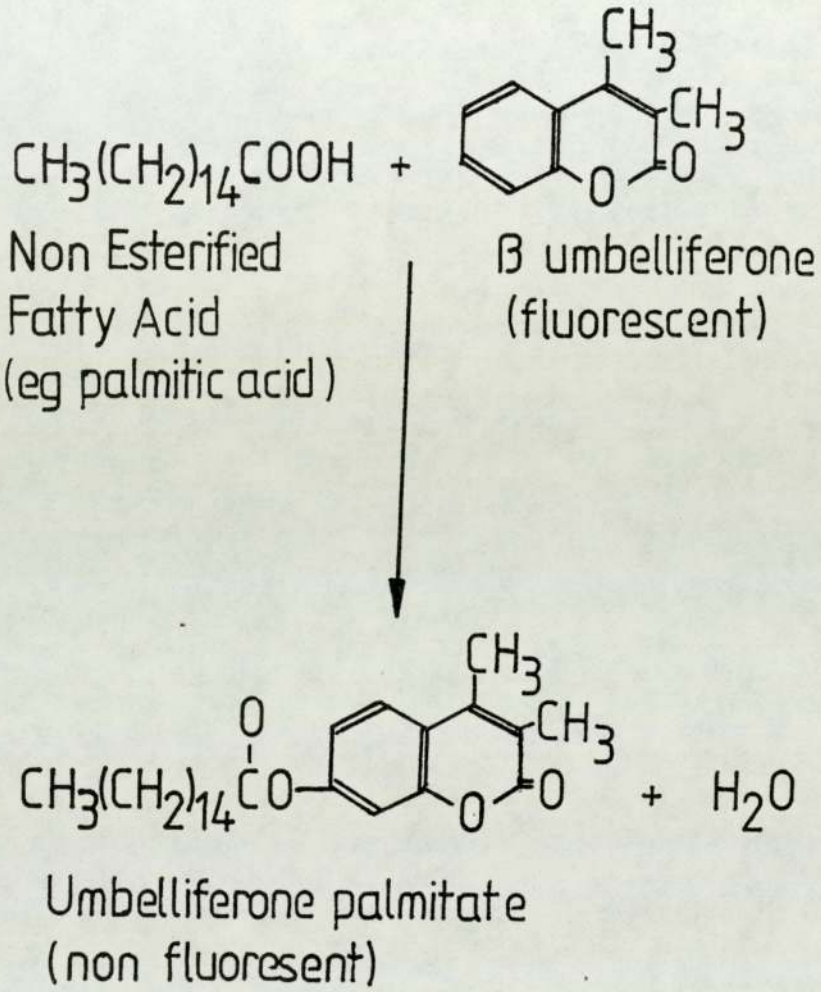
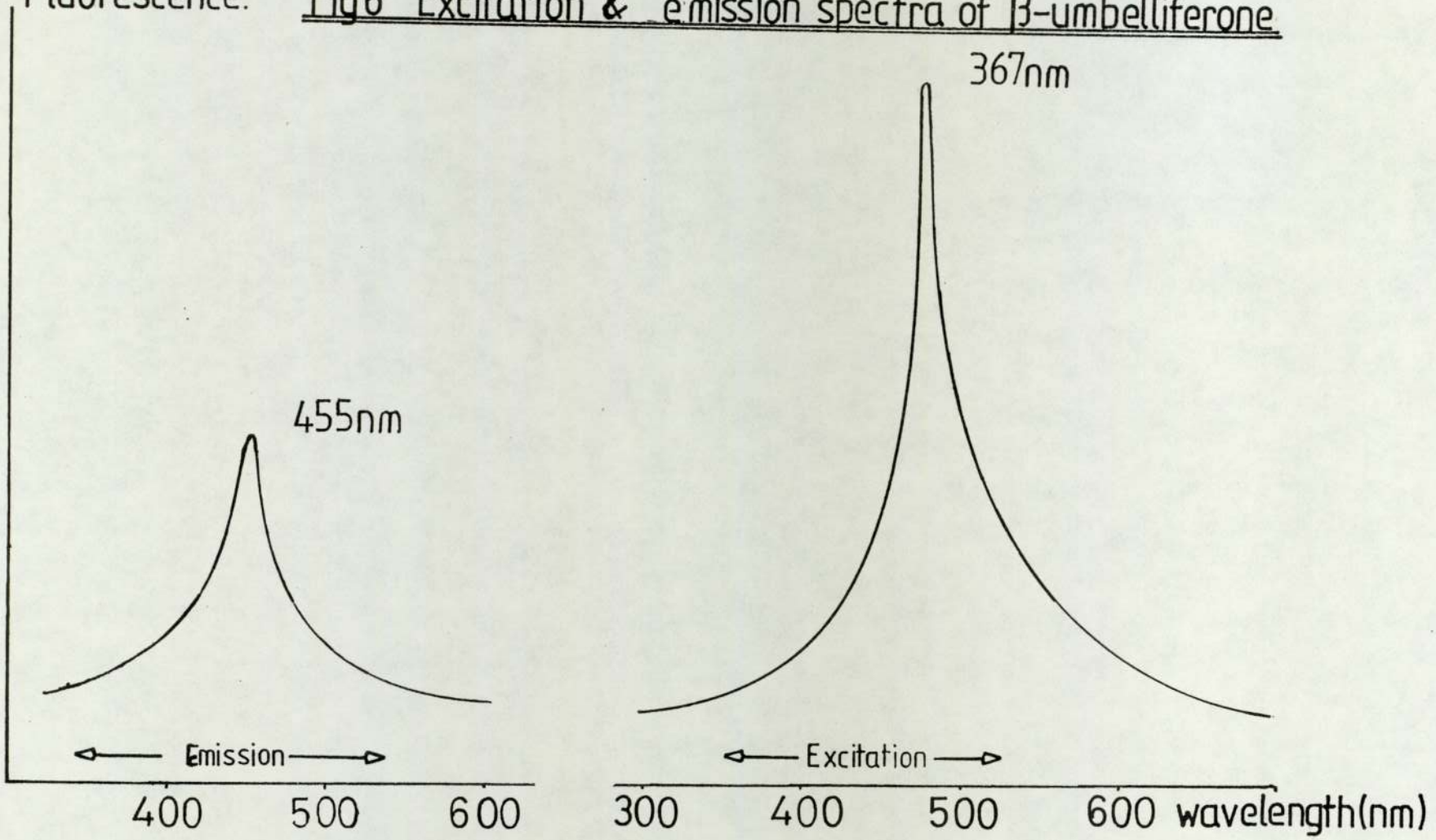


Fig 5 Reaction involved in NEFA assay


Fluorescence.
Fig6 Excitation & emission spectra of β-umbelliferone
455nm
367nm
Emission
Excitation
400
500
600
300
400
500
600
wavelength(nm)

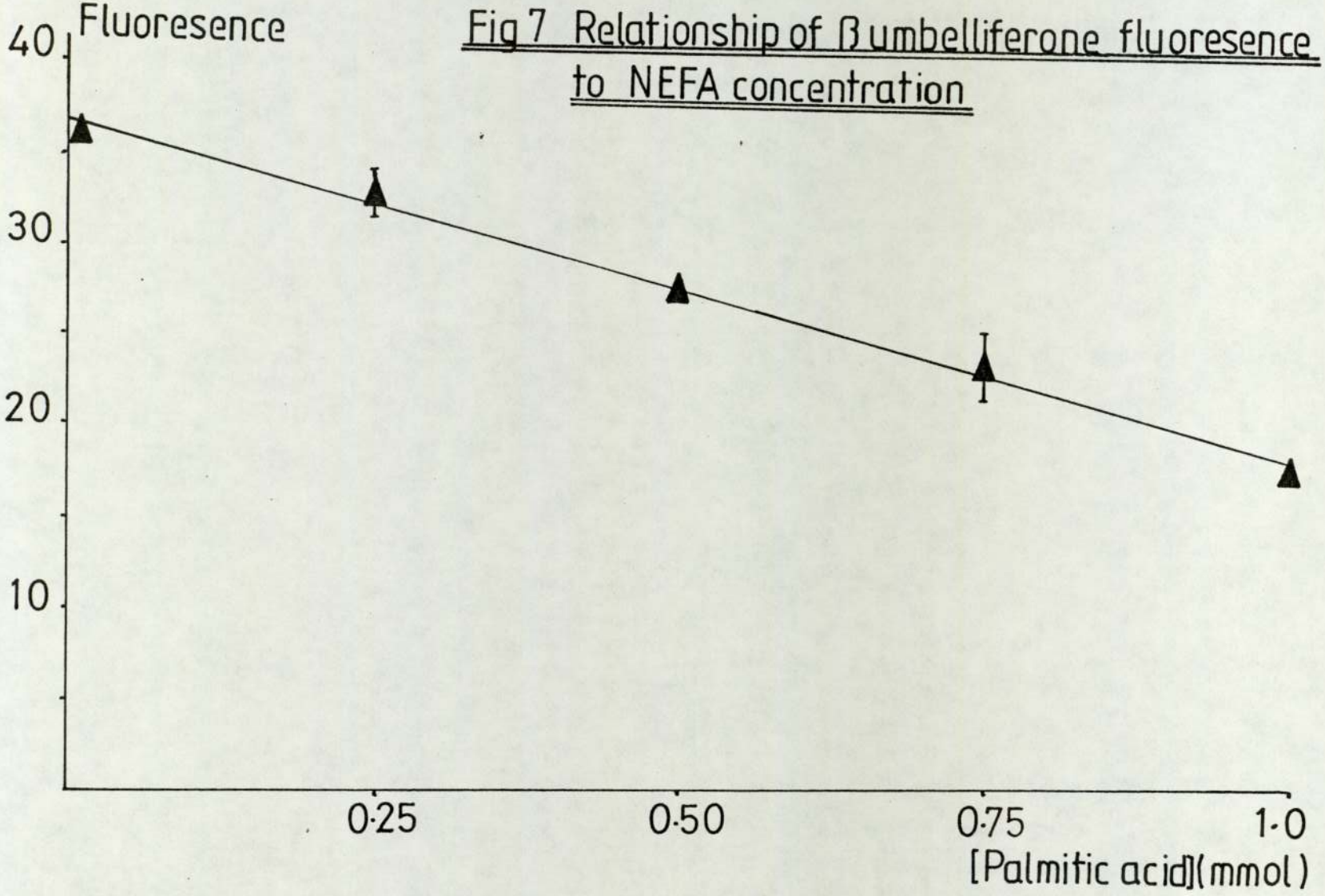


Fig 7 Relationship of Bumbelliferone fluoresence to NEFA concentration

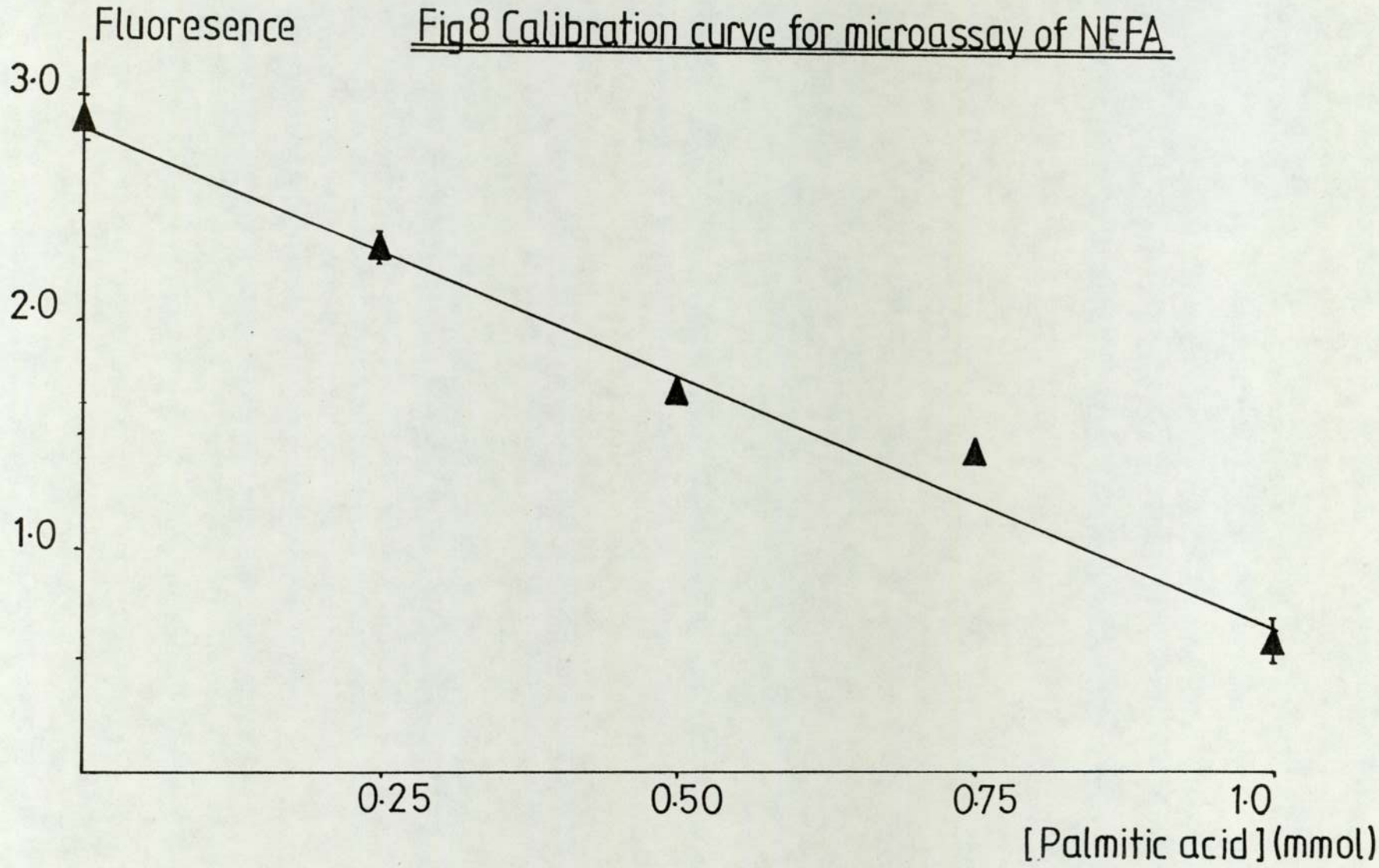
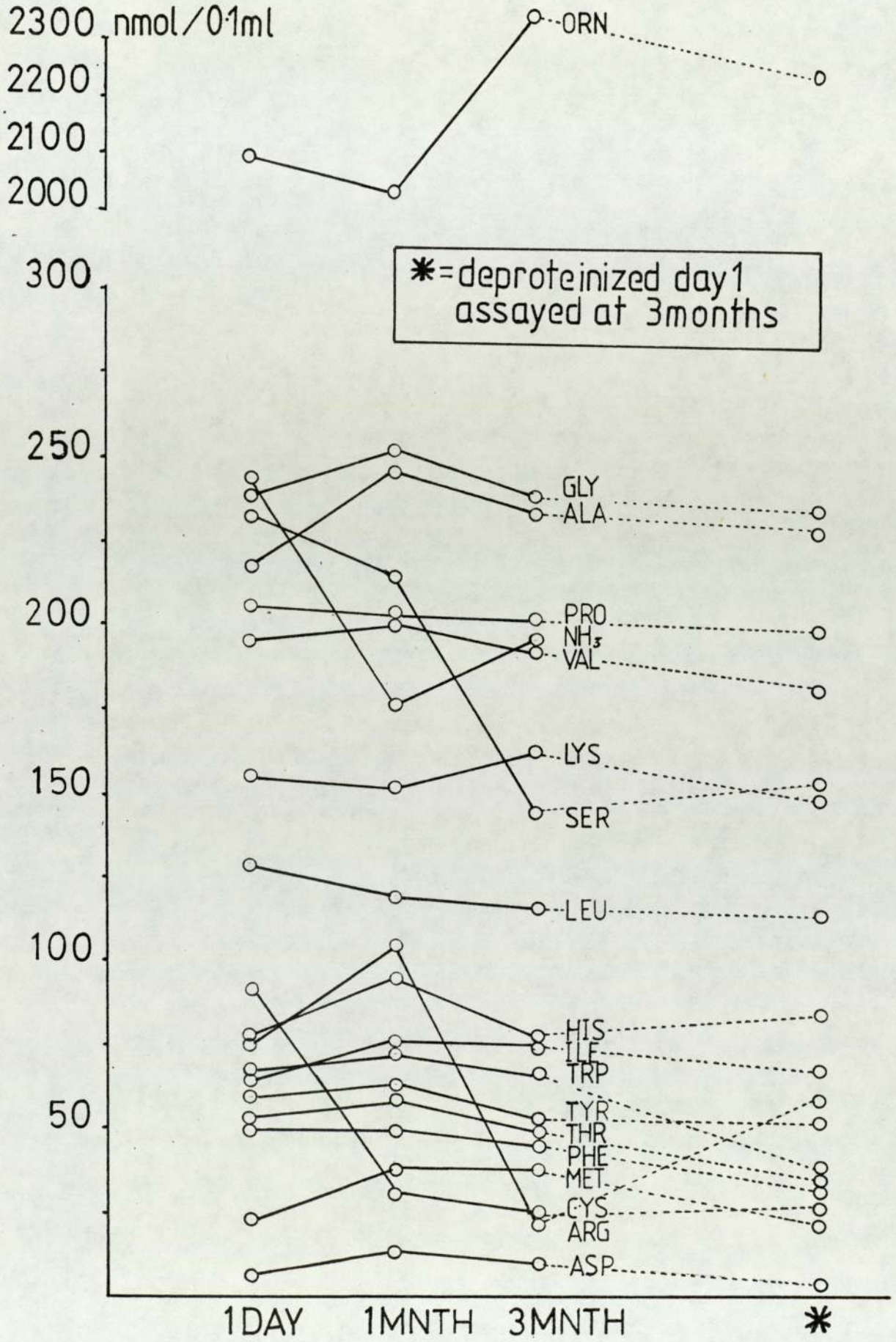


Fig 8 Calibration curve for microassay of NEFA

Fig 9 Stability of amino acids during storage



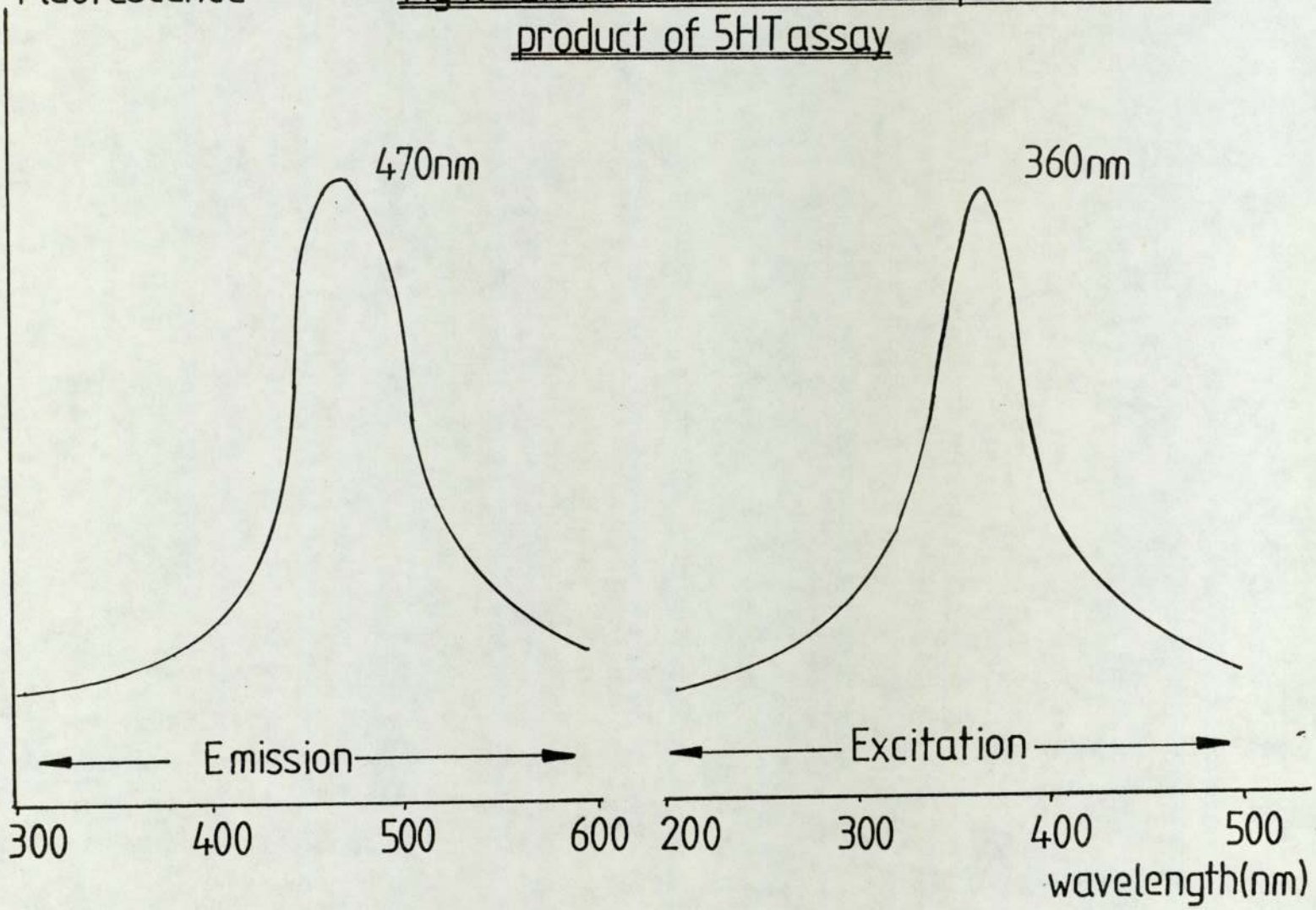


Fig 10 Excitation & emission spectra of the product of 5HT assay

Fig11 Excitation & emission spectra of the product of 5HIAA assay

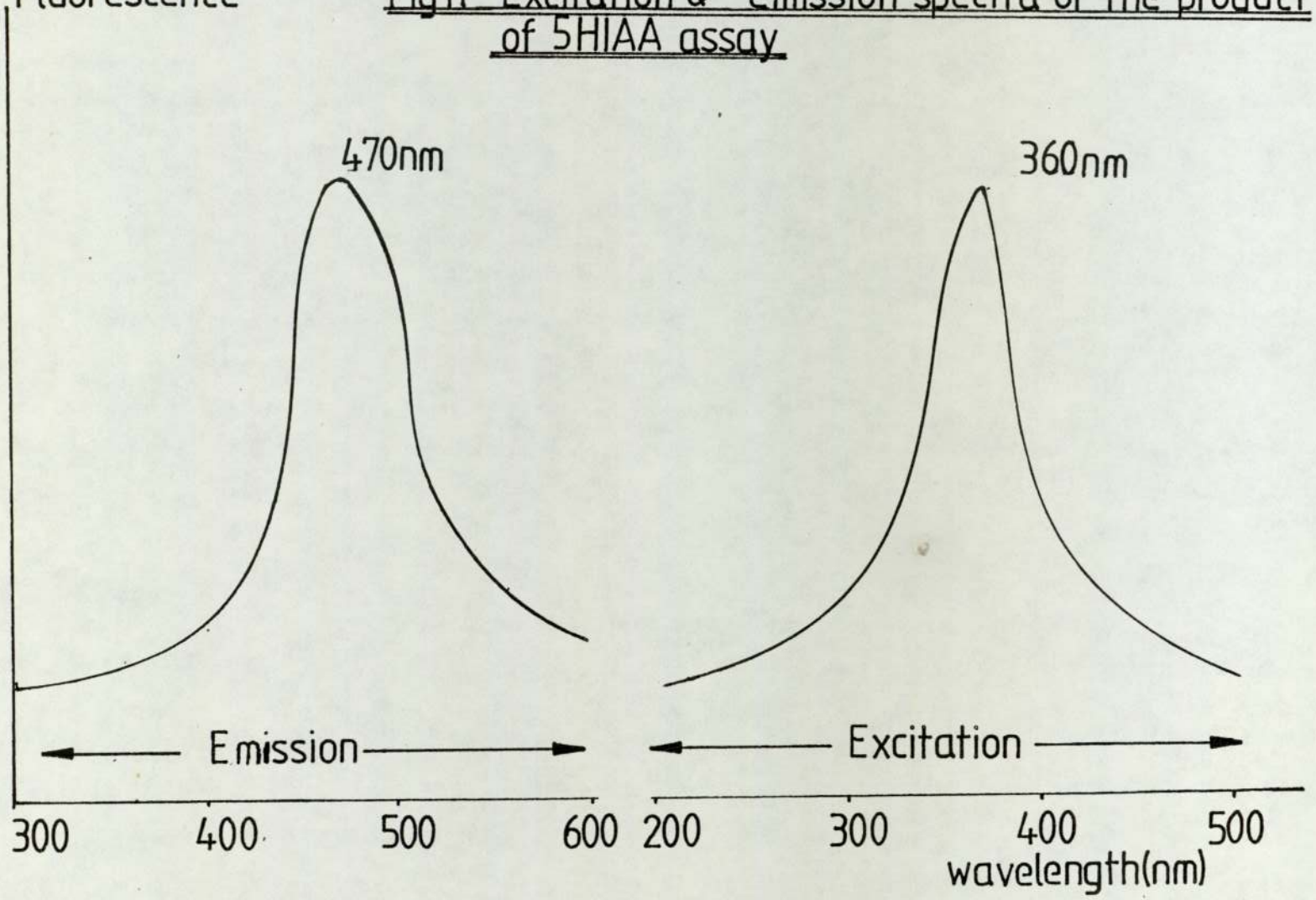
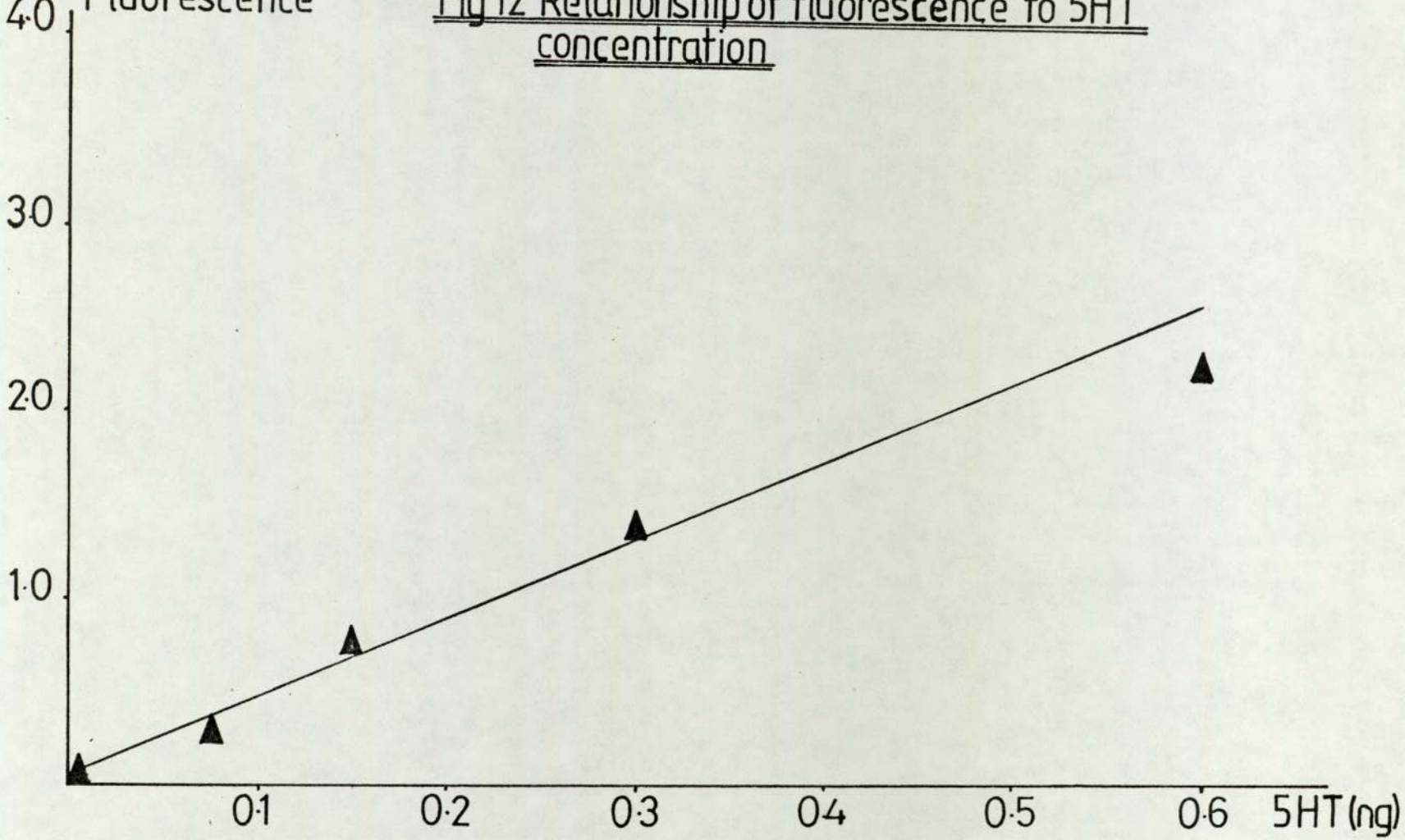


Fig 12 Relationship of fluorescence to 5HT concentration



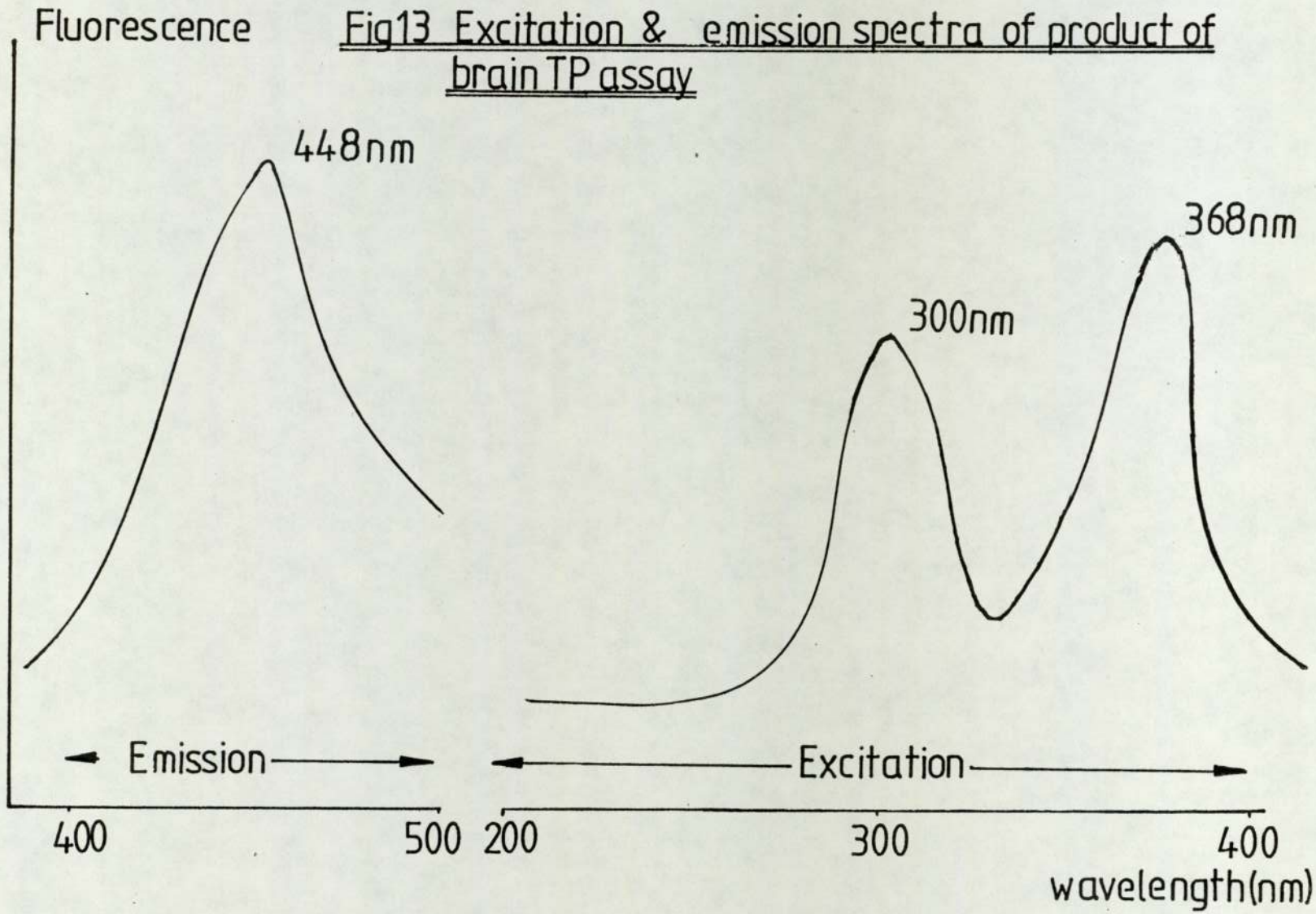
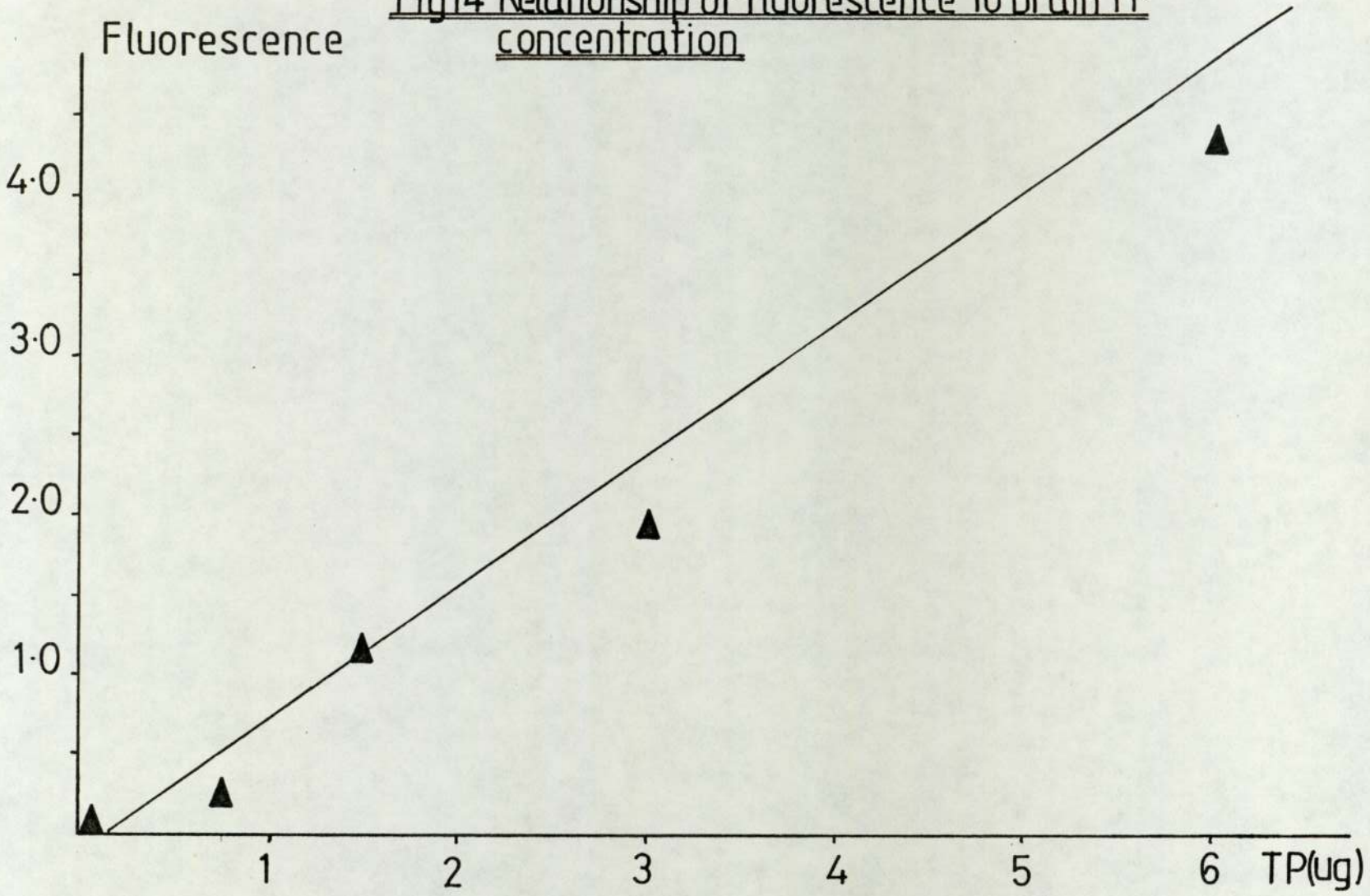


Fig13 Excitation & emission spectra of product of brain TP assay

Fig14 Relationship of fluorescence to brain TP concentration



RESULTS

CHAPTER 1

CHAPTER 1

RELATIONSHIP BETWEEN PLASMA TRYPTOPHAN, NON ESTERIFIED FATTY ACIDS AND MOOD IN HUMAN FEMALES IN LATE PREGNANCY AND THE EARLY PUERPERIUM.

1.12 Plasma Amino Acid Profiles at Parturition

1.13 Summary and Comments

Chapter 1

Relationship Between Plasma Tryptophan, Non-esterified Fatty Acids and Mood in Human Females in Late Pregnancy and the Early Puerperium.

1.1 INTRODUCTION

This study was designed to replicate the work of Handley and co-workers (1977 & 1980). Handley and colleagues (1977) had shown that subjects suffering from the 'blues' syndrome immediately post partum exhibited decreased plasma free TP during the puerperium, relative to puerperal women not showing such transient mood changes. However, this result was not replicated by the same group in the 1980 study. The 1980 study did however demonstrate a relationship between plasma total TP and post-partum mood. As described in the Introduction, a subgroup of subjects were identified, these subjects failed to exhibit the normal rise in plasma total TP immediately post-partum, it was shown that members of this subgroup were more likely to suffer from post-partum depressive mood changes than subjects showing the normal plasma total TP profiles immediately post-partum (See Introduction).

This failure of the plasma total TP rise post-partum was not postulated as being causative of the psychological changes but rather it was seen as a possible marker of some underlying abnormality.

In addition to this correlate of puerperal mood change, Curzon and colleagues (1979) had shown that subjects suffering from non-puerperal endogenous depression responded to mild stress with an increased elevation of plasma NEFA relative to non-depressed controls.

This study therefore investigated the possibility that subjects who develop either puerperal 'blues' or the more serious post-partum depression may show increased NEFA following the stress of parturition, relative to subjects not showing such mood changes.

In addition, plasma NEFA compete with TP for binding to plasma albumin (See Introduction). Hence, plasma NEFA may play some rôle in the plasma TP changes immediately post-partum. The involvement of NEFA in the aetiology of the TP 'riser' or 'non riser' subgroups (See Introduction) was therefore investigated.

1.2 Subject Population Characteristics.

Subjects for this study were drawn from women attending a routine antenatal clinic at Mile End Hospital, London, between February and June, 1980. The selection criteria for these subjects were that they should be at between 36 - 38 weeks gestation between the above mentioned dates, they should be UK born, having received an English Language education, and that they be currently free from any medical or psychiatric illness. Dietary and medication restrictions were also enforced (Methods 5).

A total of 26 women gave their consent to take part in the study, however due to organisational difficulties, sufficiently complete biochemical and psychiatric data was obtained from only 11 of these.

1.3 Protocol.

Subjects were approached by the psychiatrist (Dr G Waldron) at a routine antenatal appointment. The nature of the study was described and the relevant consent form (Appendix VI) was signed. The study was described as an investigation of mood, at no time was any reference made to 'post-natal depression'

or 'baby blues'.

Following recruitment into the study, subjects gave a 10ml. venous blood sample and completed a MAACL mood questionnaire and a Beck depression inventory. Following parturition, subjects were visited daily for the first five days post-partum between 0830 - 0930 hours. On each occasion a 10ml. venous blood sample was obtained and an MAACL questionnaire and a 'blues' inventory were completed. On the third day post-partum, a Beck depression inventory was also completed (See Methods 3.1).

On the fifth day post-partum a short, retrospective interview was performed by the psychiatrist. Subjects were classified as either 'blues' cases or non cases by the psychiatrist on the basis of the retrospective interview and the mood questionnaires.

At six months post-partum, the subjects were visited at their homes and a retrospective psychiatric interview was performed. On the basis of this interview, subjects were classified as either depression cases or non cases*.

Plasma samples were assayed for free and total TP and NEFA (Methods 1.1, 1.2 and 1.3). Plasma samples from 4 subjects, selected on the criterion of there being sufficiently complete biochemical data for these subjects, were also subjected to amino acid analysis (Methods 1.5).

* NB All psychiatric diagnoses presented within this chapter were made by the psychiatrist, Dr G Waldron. The interviews used in this chapter are not those described in Methods and used in chapter 4. However, the interviews described within Methods were developed under the guidance of Dr Waldron, and are similar in nature and content to those used by Dr Waldron in the present chapter.

All assays were performed blind with respect to the psychiatric data.

1.4 Psychiatric Results.

Of the 11 subjects, 1 was considered to be depressed antenatally and 3 others were considered to be borderline antenatal depression cases. Of these, the three borderline cases subsequently suffered from 'blues'. All of these antenatally depressed subjects were considered to be depressed at 6 months post-partum, but only 1 was considered to be suffering from 'child birth related' symptoms. The others were considered to be either chronic depressives or to have other aetiological factors.

Five of the eleven subjects were classified as being 'blues' cases, and seven of the eleven subjects were considered to have shown some depressive symptoms at the time of the six month follow-up interview. Using the Mann-Whitney U Test, there were no significant differences in the MAACL 'A', 'D' or 'H' scores, either antenatally or puerperally, or in the BDI scores antenatally, puerperally or 6 months post partum between 'blues' cases and non cases. The same was true for the mood test scores of depression cases.

1.5 Personal and Sociological Factors.

1.5.1 Blues.

There were no differences between 'blues' cases and non cases with respect to age ($t=1.12$, $p=.292$) or age of menarche ($t=.0294$, $p=.976$). Only one subject was unmarried, she was classified as a 'blues' case. There were no significant differences in parity,

gravidity or incidence of a previous psychiatric illness between cases and non cases. Table 1.1

Table 1.1

	Prev.Psych.History	No History	Primiparae	Multiparae
Blues' Case	2	3	1	4
Blues' Non Case	3	3	2	2

NS (Fisher's Exact) NS (Fisher's Exact

1 5 2 Depression

There were no differences between those subjects that were considered to have been depressed within the 6 months post-partum relative to subjects not exhibiting such symptoms with respect to age ($t=.523$ $p=.618$), age of menarche ($t=1.966$, $p=.079$), parity, gravidity or history of previous psychiatric illness (Table 1.2). Only one subject was unmarried, she was not considered to be depressed at the 6 month follow-up interview.

Table 1.2

	Prev.Psych.History	No History	Primiparae	Multiparae
ession ase	2	4	1	5
ession Case	3	2	2	1

NS (Fisher's Exact) NS (Fisher's Exact)

1.6 Biochemical Results.

1.6.1 Total Tryptophan.

Plasma total TP was found to be low in late pregnancy and then to increase immediately post-partum. Fig 1.1.

1.6.2 Free Tryptophan.

Plasma free TP showed a similar profile to total TP in that it was low antenatally, and rose immediately post partum. Fig 1.2.

1.6.3 Non Esterified Fatty Acids.

Plasma NEFA were seen to be high antenatally, the levels then fell considerably immediately post-partum and continued to fall gradually. Fig 1.3.

1.7 Biochemical Correlates of 'Blues'

1.7.1 Total Tryptophan.

There were no significant differences in antenatal total TP between 'blues' cases and non cases. However 'blues' cases exhibited significantly decreased total TP on day 2 post partum ($p=.0487$) relative to 'blues' non cases. Fig 1.4.

1.7.2 Free Tryptophan.

There were no significant differences in free TP either antenatally or post-natally between 'blues' cases and non cases. Fig 1.5.

1.7.3 Non Esterified Fatty Acids.

There were no significant differences at any point between 'blues' cases and non cases with respect to plasma NEFA either antenatally or post partum. Fig 1.6.

1.8 Biochemical Correlates of Post Partum Depression

1.8.1 Total Tryptophan

There were no significant differences at any point between those subjects that went on to show depressive

symptoms post partum and those patients which did not show such symptoms, in respect to plasma total TP. Fig 1.8.

1.8.2 Free Tryptophan.

As with plasma total TP, there were no differences between depressed subjects and non-depressed subjects, with respect to plasma free TP. Fig 1.9.

1.8.3 Non Esterified Fatty Acids.

Those subjects that went on to show depressive symptoms in the 6 months post partum were seen to have increased concentrations of NEFA in the final month of pregnancy when compared with mothers who did not become depressed ($p=0.0192$). These depressed patients also showed increased NEFA levels post partum, however these differences failed to attain statistical significance. Fig 1.10.

1.9 Relationship Between 'Blues' and Depression.

There was no significant relationship between the incidence of 'blues' and the incidence of depression. Fisher's exact test $p=0.3000$. Table 1.3

Table 1.3 Relationship Between 'blues' and depression.

	'Blues' cases	'Blues' Non cases
Depression Cases	4	3
Depression Non Cases	1	3

1.10 Classification of Tryptophan 'Risers' and 'Non Risers'.

The total TP profile for each individual subject was investigated. It was possible to divide the subjects into 3 categories : Risers, Non-Risers and Non-Defined. Risers were those subjects who exhibited the usual rise of total TP to levels above the antenatal value, immediately post-partum. Non Risers were those subjects who did not show the TP rise until after the second obstetric day. Due to missing data some subjects could not be classified.

1.10.1 Relationship of Tryptophan Rise to 'Blues'.

Only seven subjects could be definitely classified as either risers and non-risers. It was found that there was no difference in the incidence of 'blues' between the two groups. Table 1.4.

Table 1.4 Relationship of Tryptophan Rise to 'Blues'.

	TP Riser	TP Non Riser
'Blues' Case	3	1
'Blues' Non Case	2	1

p=.5700 (Fisher's Exact)

1.10.2 Relationship of Tryptophan Rise to Depression.

Of the seven classifiable subjects, there were no significant difference in the incidence of depression between risers and non-risers (Table 1.5)

Table 1.5 Relationship of Tryptophan Rise to Depression

	Riser	Non Riser
Depression Case	4	0
Depression Non Case	1	2

p=.1100 (Fisher's Exact)

1.11 Relationship of Plasma Tryptophan to NEFA.

In order to investigate whether plasma NEFA might be involved in the aetiology of the TP rise phenomenon, the relationship between plasma TP and NEFA was investigated.

1.11.1 Total Tryptophan.

Using all the available data from the 26 subjects, the relationship between NEFA and total TP was investigated using linear regression, It was found that antenatally there was a negative correlation between these two parameters. However, post partum this relationship was disrupted so that it became a weak positive relationship. The negative relationship was seen to be returning by the fifth day post-partum.

Table 1.6 Relationship of NEFA to Total TP.

	All data	Antenatal	Day 1	Day 2	Day 3	Day 4	Day 5
Pearson r	-.1585	-.2978	.2979	.3827	-.1588	-.5940	-.5090
Intercept	11.229	10.706	9.101	8.786	11.576	12.135	12.074
Slope	-2.216	-2.693	6.574	6.850	-1.930	-5.096	-10.14
N	78	26	12	14	17	4	5
Students t	1.65	1.53	0.99	1.44	0.62	1.04	1.02
Probability	.0992	.1357	.6528	.1730	.5507	.4087	.3844

1.11.2 Free Tryptophan.

There did not appear to be any consistent relationship between free TP and NEFA. Table 1.7

Table 1.7 Relationship of NEFA to Free TP.

	All data	Antenatal	Day 1	Day 2	Day 3	Day 4	Day 5
Pearson r	.1203	-.0054	.3221	.2061	.5239	-.7332	-.5184
Intercept	3.070	3.198	2.652	2.949	2.477	6.827	3.554
Slope	.562	-.268	2.121	1.227	2.353	-9.632	-2.998
N	73	24	10	14	15	3	5
Student's t	1.03	0.03	0.96	0.73	2.23	1.08	1.05
Probability	.3072	.9744	.6324	.5142	.042	.4763	.3722

1.12 Plasma Amino Acid Profiles at Parturition.

The plasma amino acid profiles, for the days immediately post partum, for four selected subjects are presented in Fig 1.11. Of these subjects, A and B were TP risers, C was a non riser and D was a 'non classifiable', possible riser.

Another factor to be noted is that Serine and Cysteine have previously been shown to be unstable during storage (Methods 1.5).

TP risers and non risers were seen to exhibit similar amino acid profiles, with the exception of proline and cysteine. However cysteine has previously been shown to be unstable during storage, therefore, the importance of this finding is unclear. Proline was found to be increased in the TP non-riser relative to the TP risers.

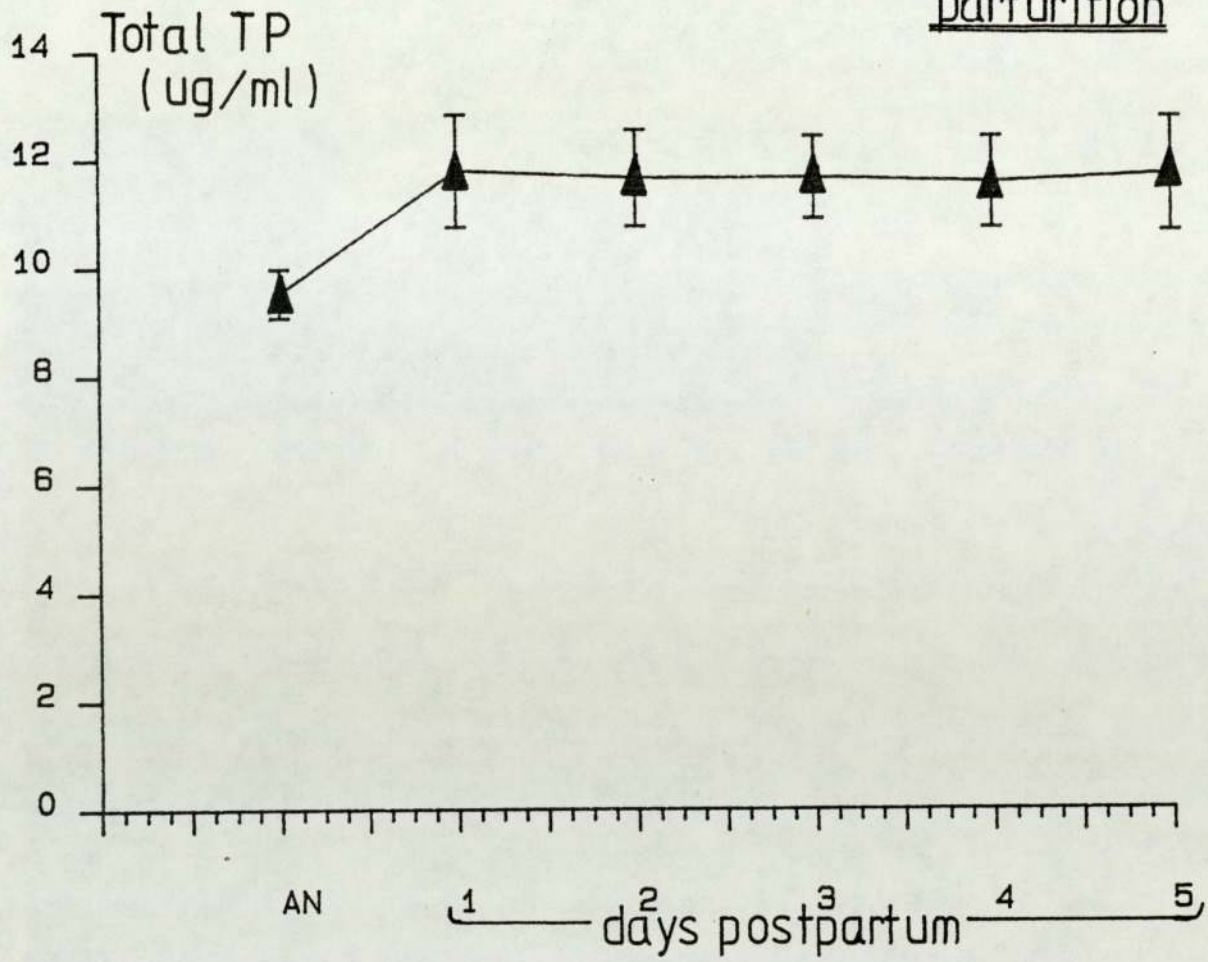
There were no differences in the amino acid profiles between the 'blues' cases (B and C) and non cases (A and D), or the depression cases (A and B) and non cases (C and D).

1.13 Summary and Comments.

Due to organisational difficulties, this study was conducted on a very small sample size. An extended study of a similar

nature is presented in chapter 4. However, several points are worth noting from this study. Firstly, there was a high incidence of depression antenatally, and non child birth related depression post-natally. Secondly, total TP and NEFA were seen to be weakly negatively related antenatally. This relationship was disrupted immediately post-partum, but was seen to be returning by the fifth day post-partum. It is therefore possible that under normal, non pregnant physiological conditions, there is a close relationship between plasma total TP and plasma NEFA.

Fig1·1 Total trytophan profile at parturition



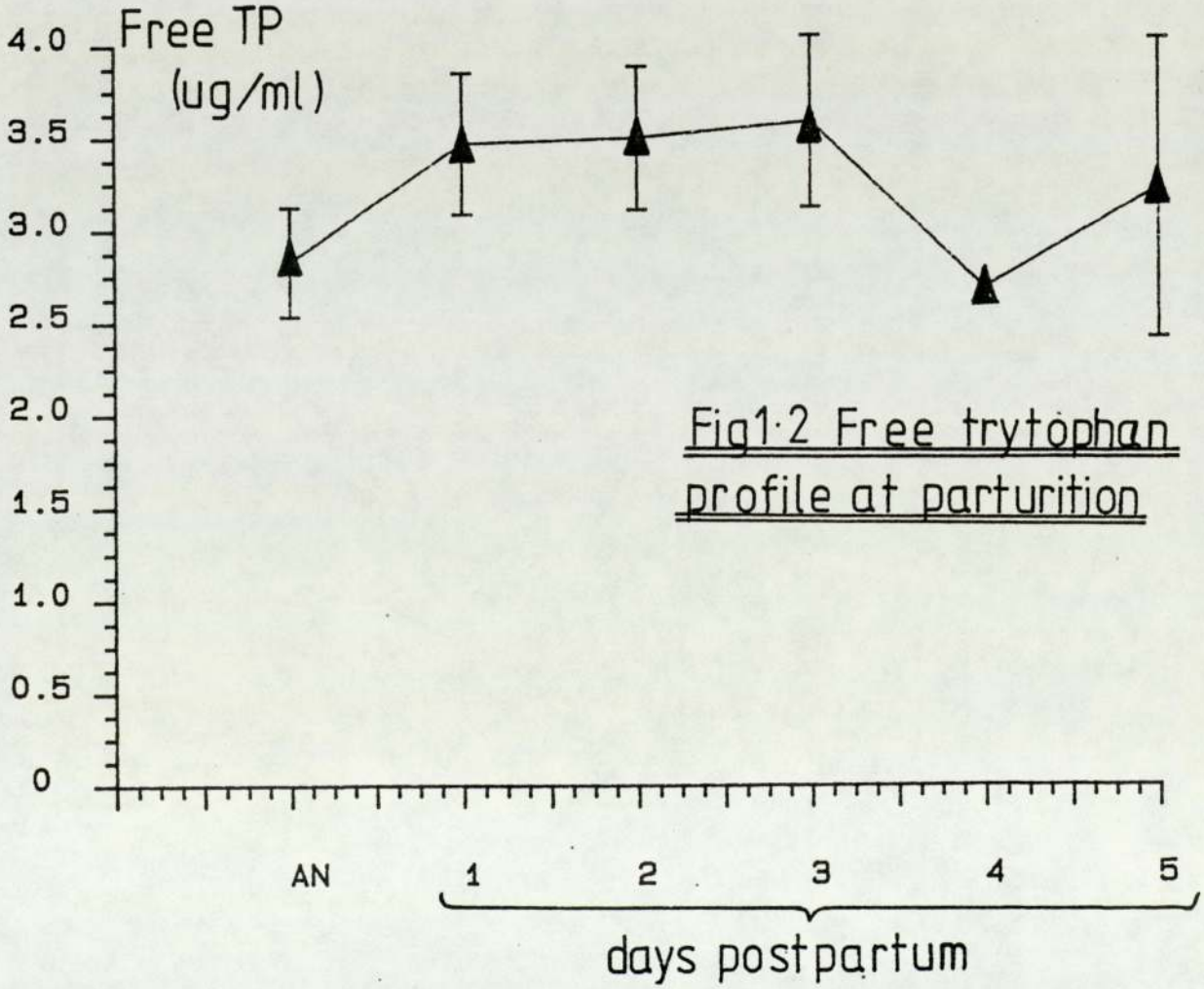


Fig1·2 Free trytophan profile at parturition

Fig1·3 NEFA profile at parturition

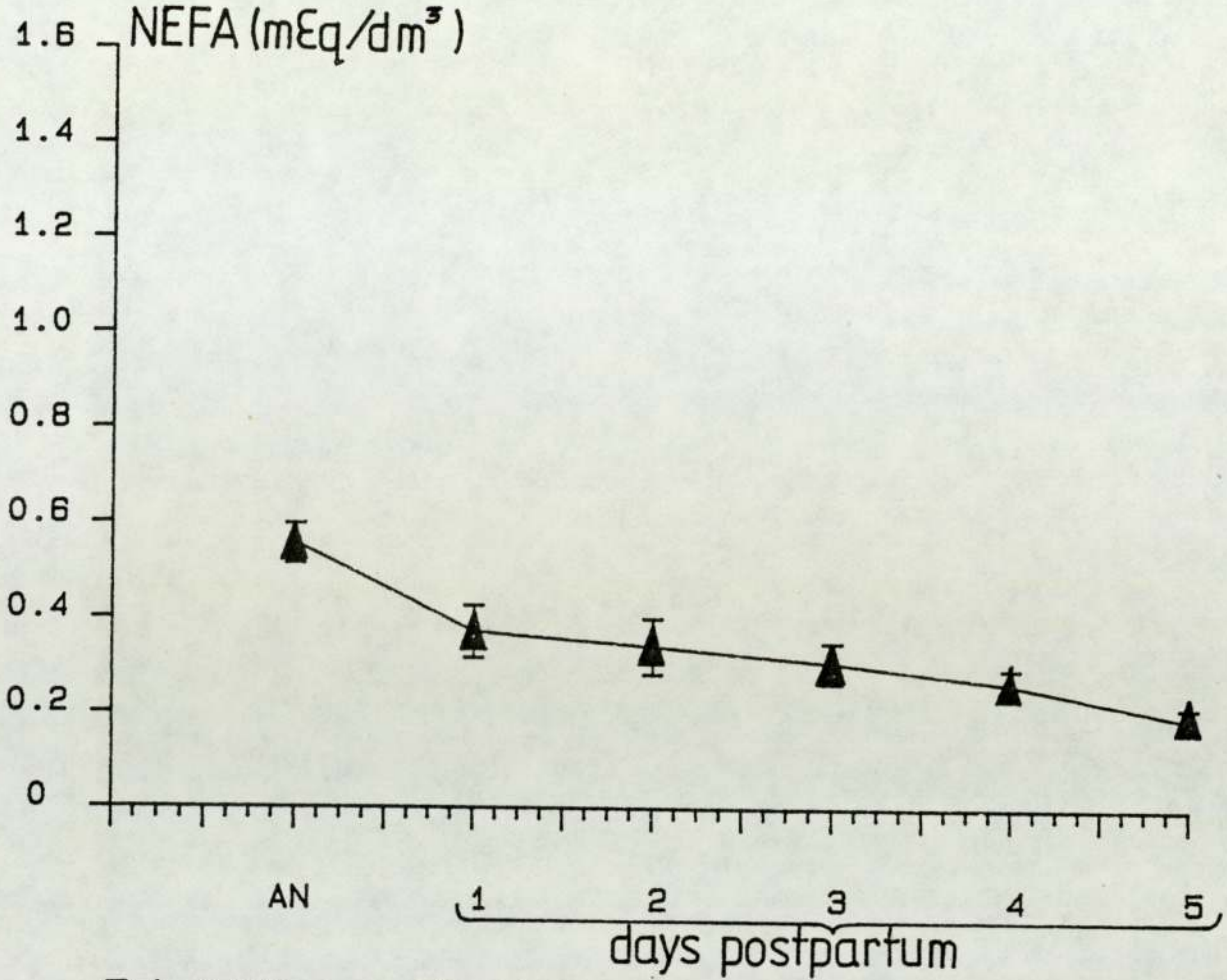


Fig1·4 Relationship of total TP to 'blues'

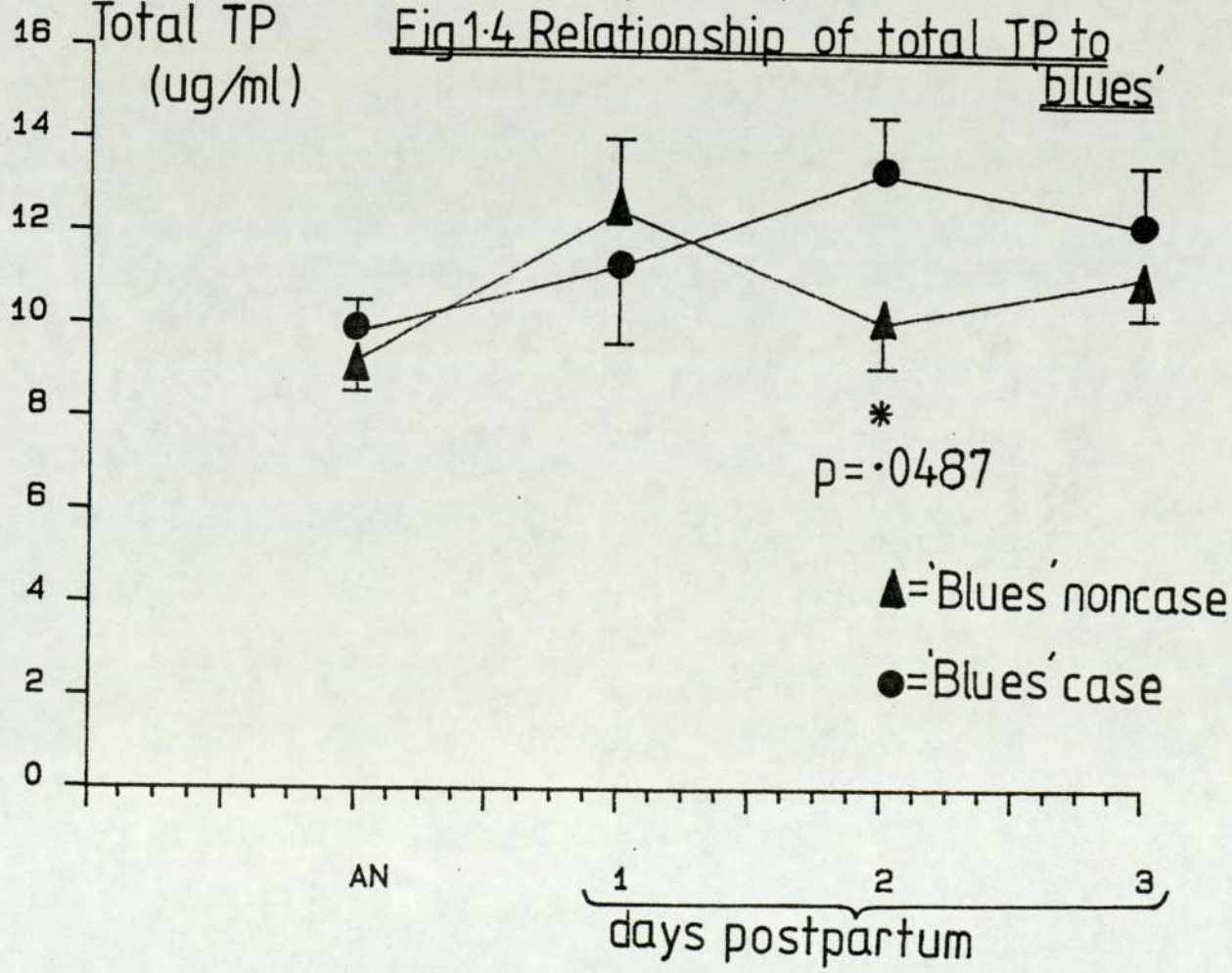
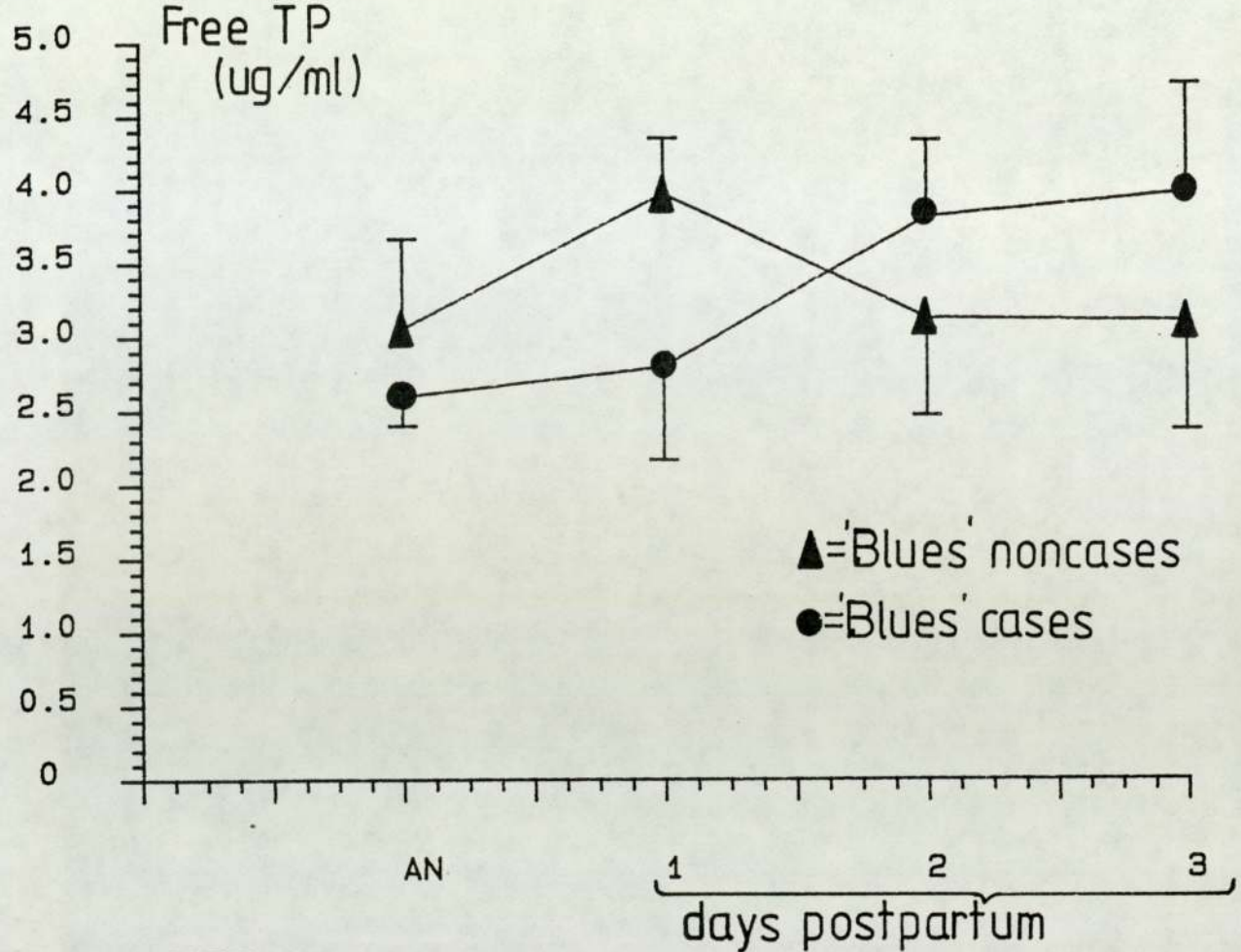


Fig 1·5 Relationship of free TP to 'blues'



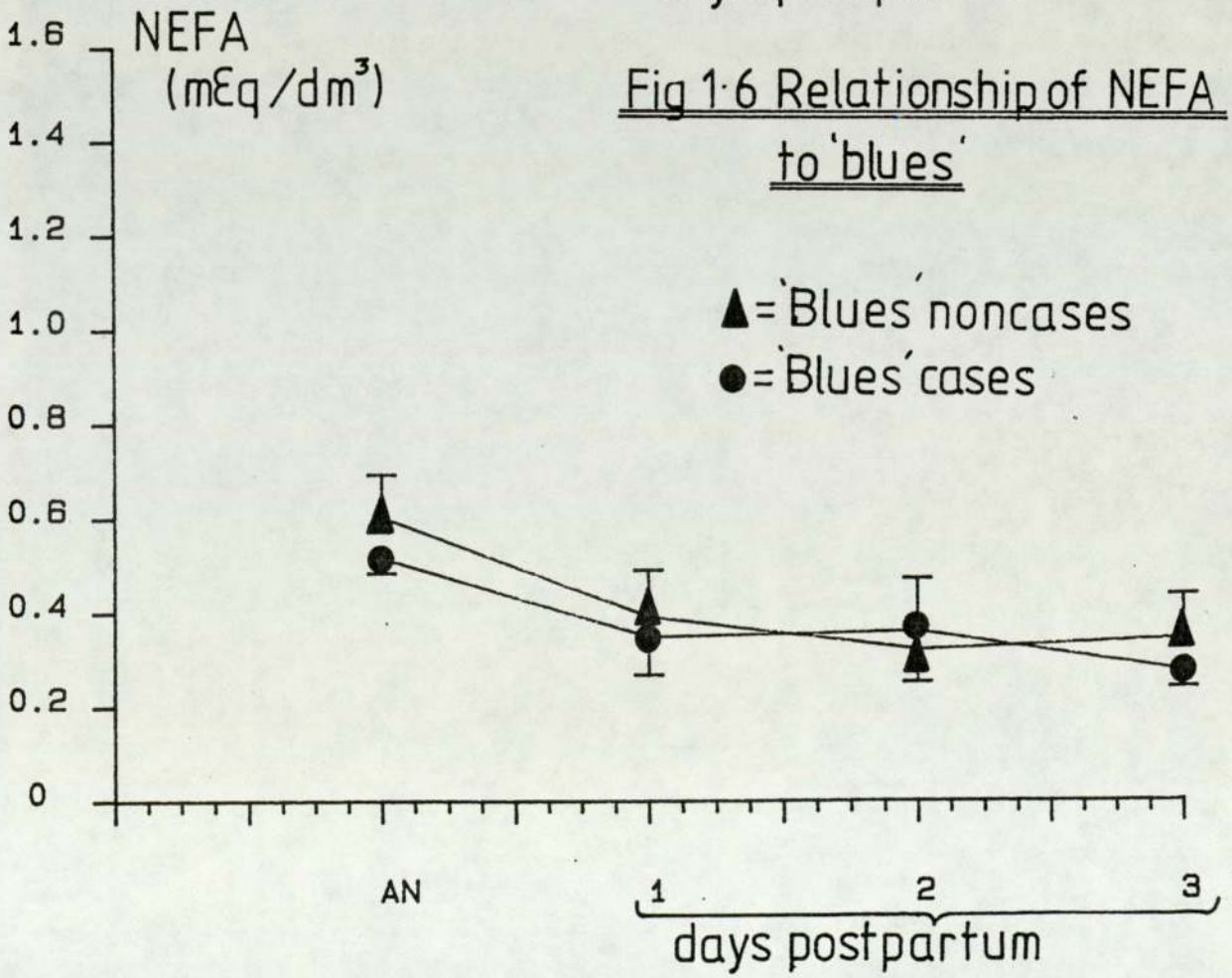


Fig 1·6 Relationship of NEFA to 'blues'

Fig 1·8 Relationship of total TP to depression

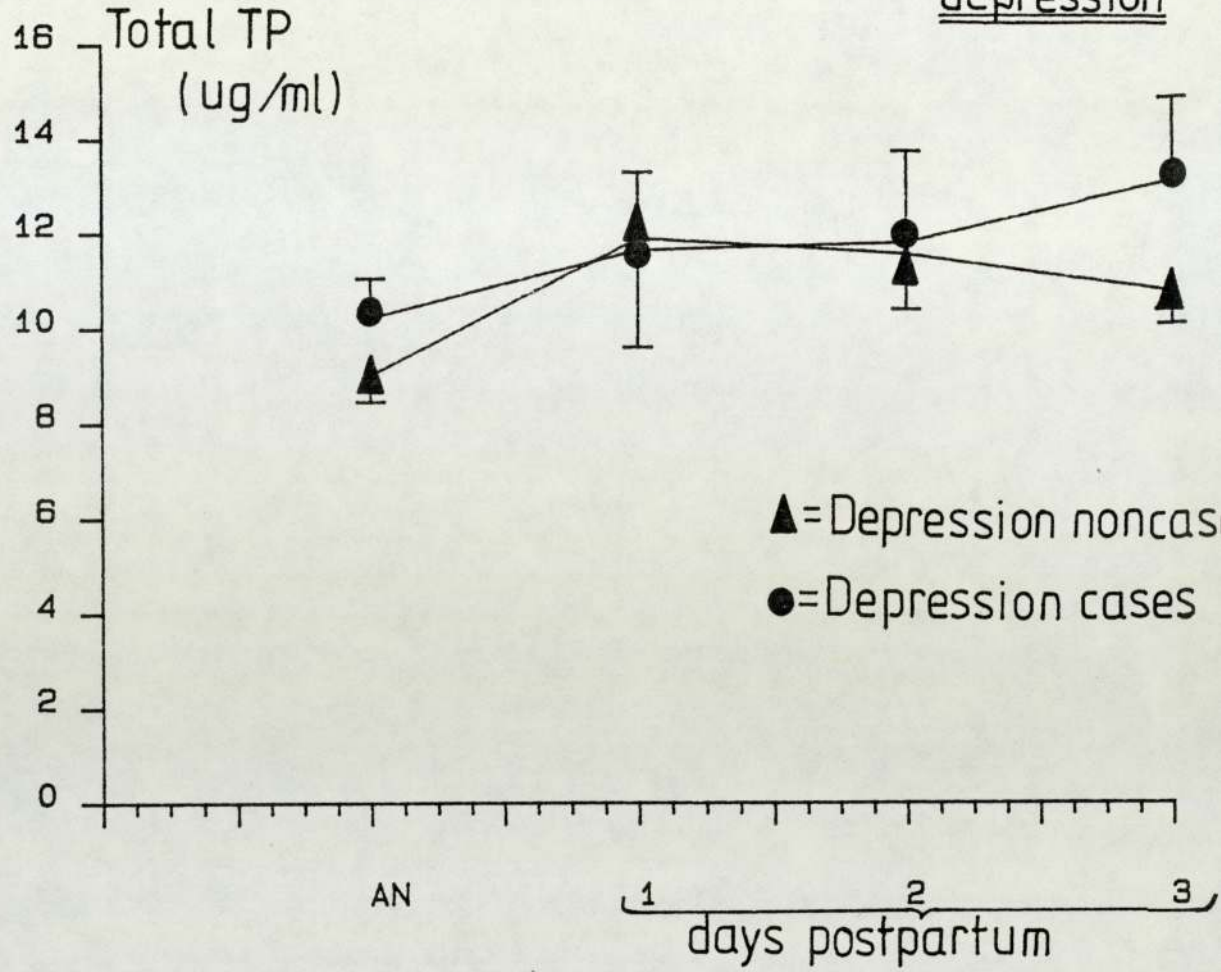


Fig 1·9 Relationship of free TP to depression

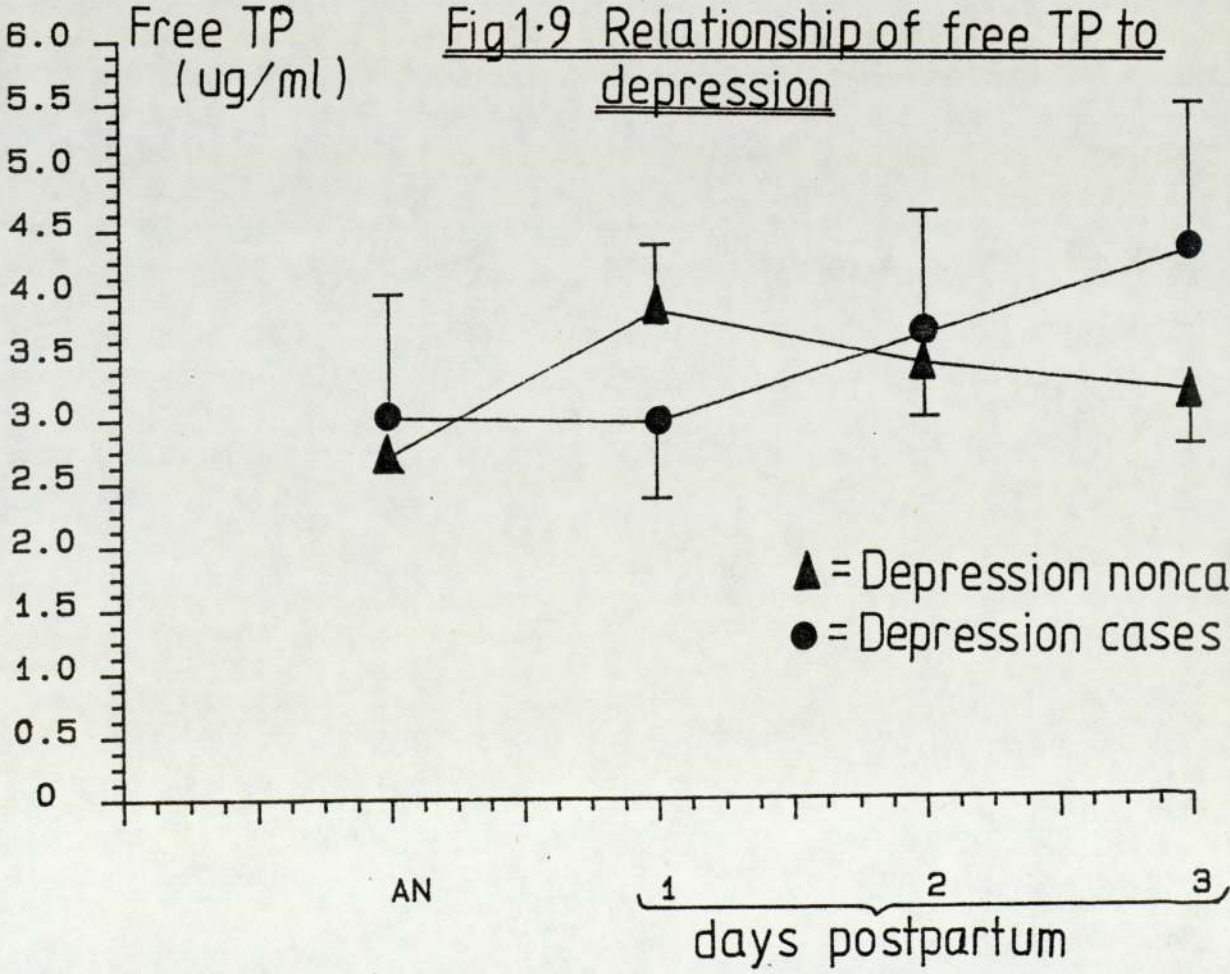


Fig 1.10 Relationship of NEFA to depression

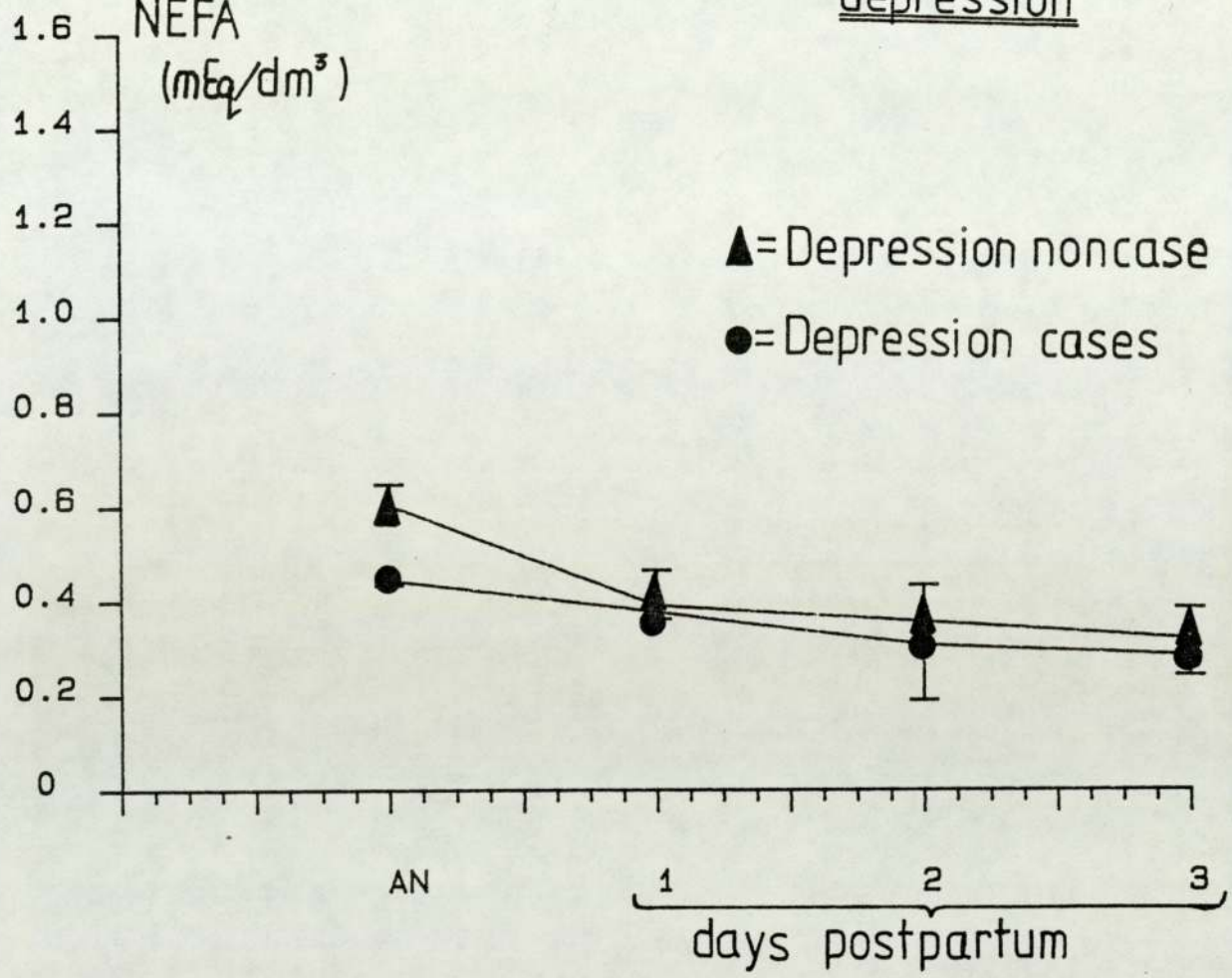
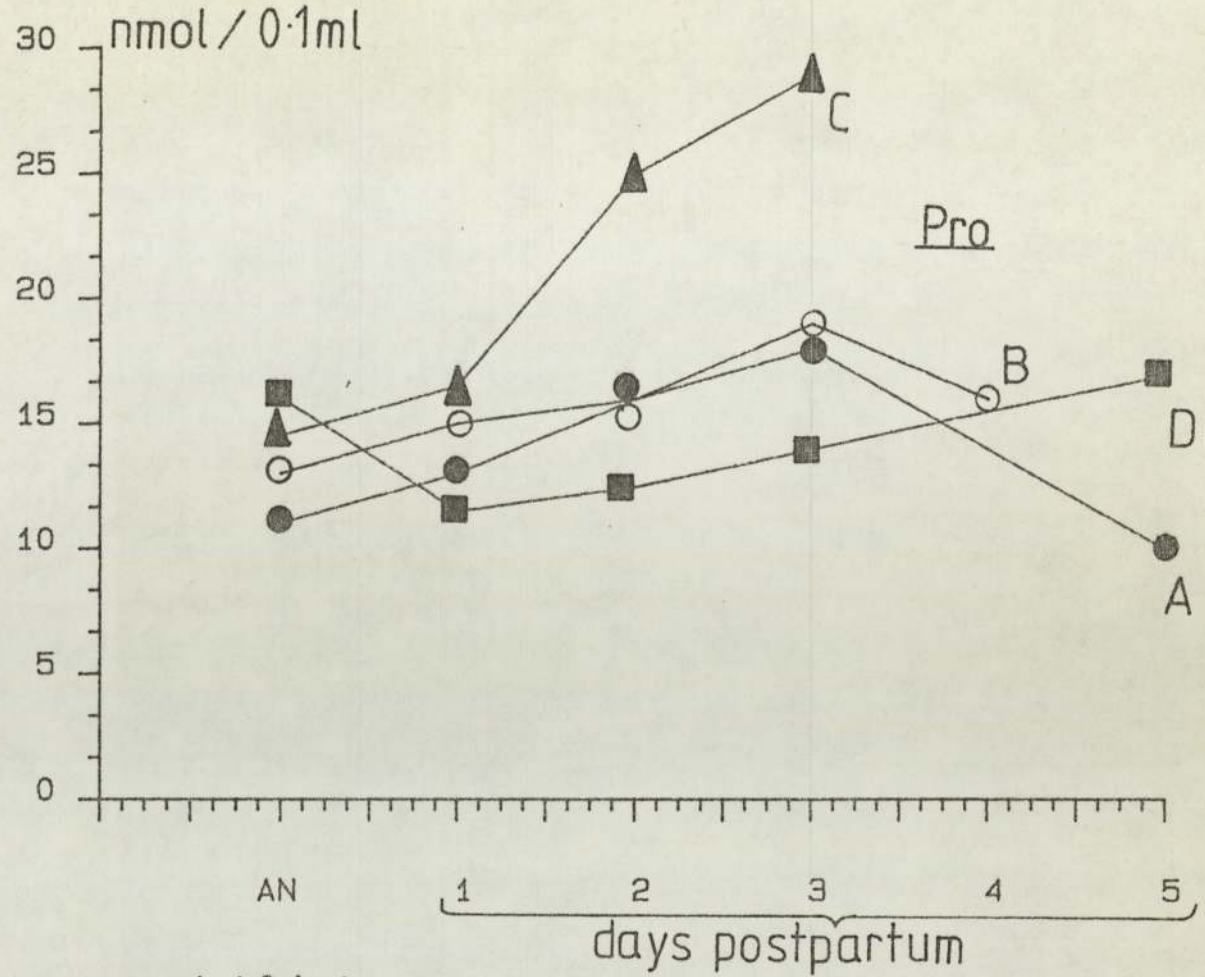


Fig 1·11 Amino acid profiles at parturition



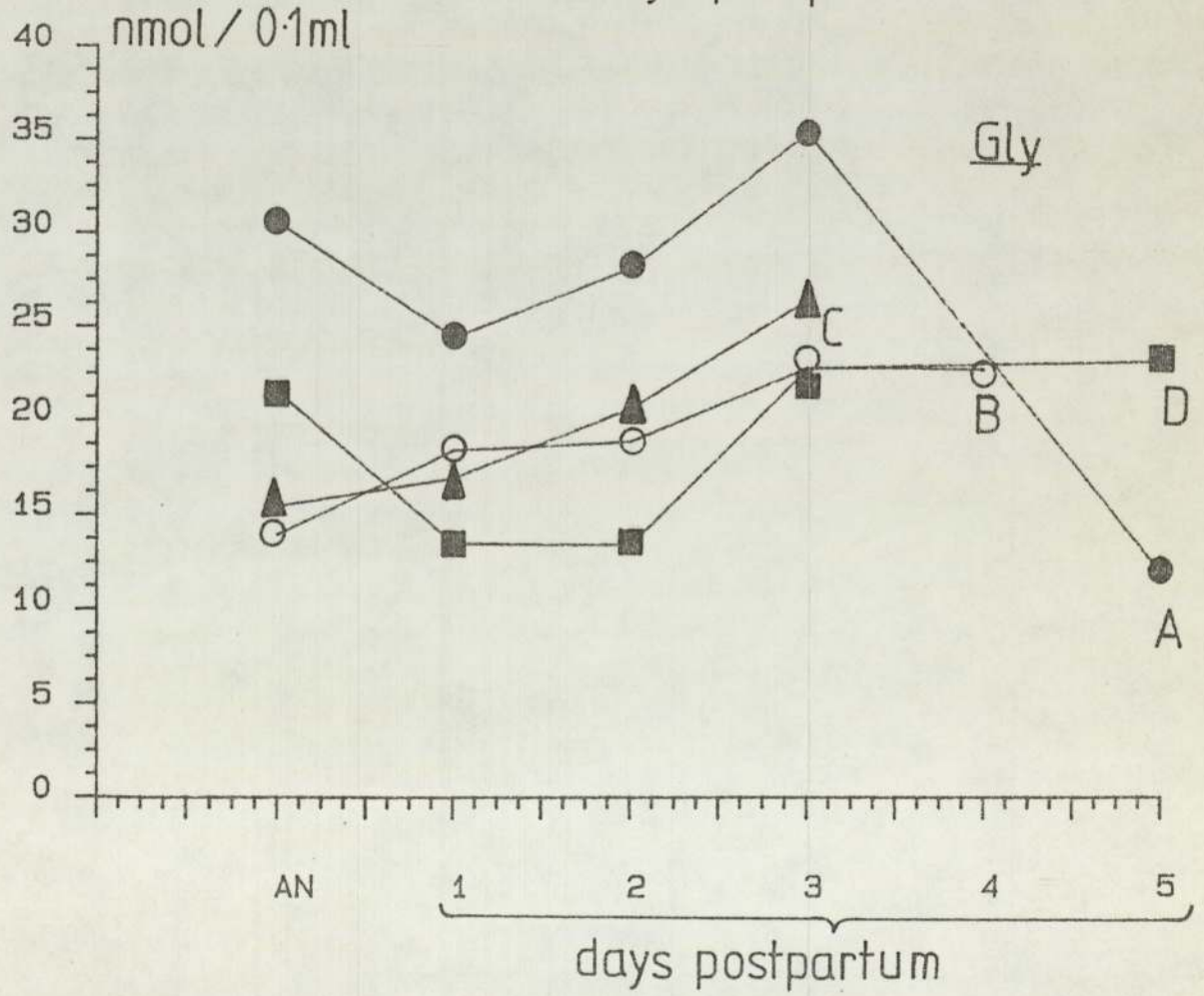


Fig 1·11 Amino acid profiles at parturition

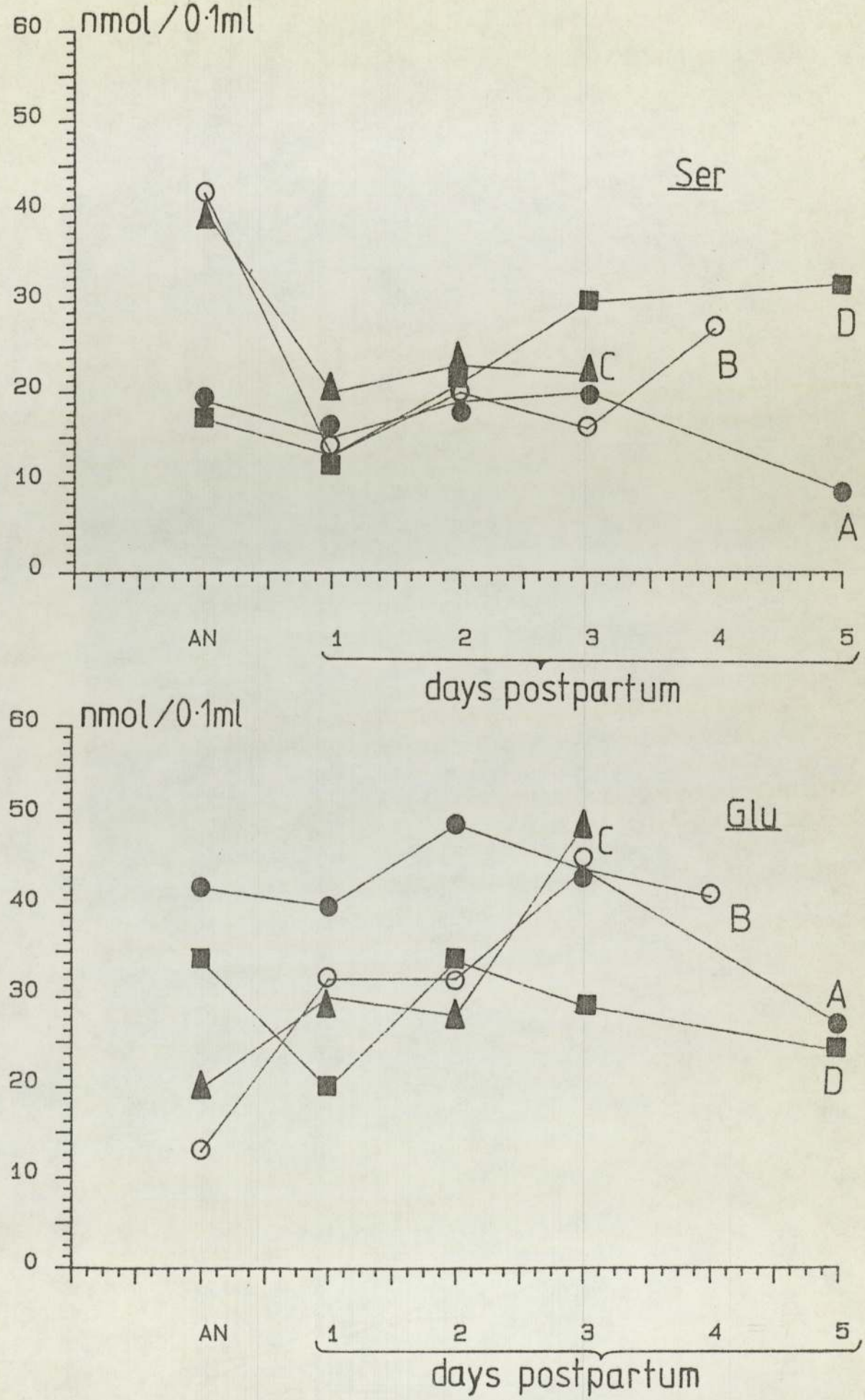
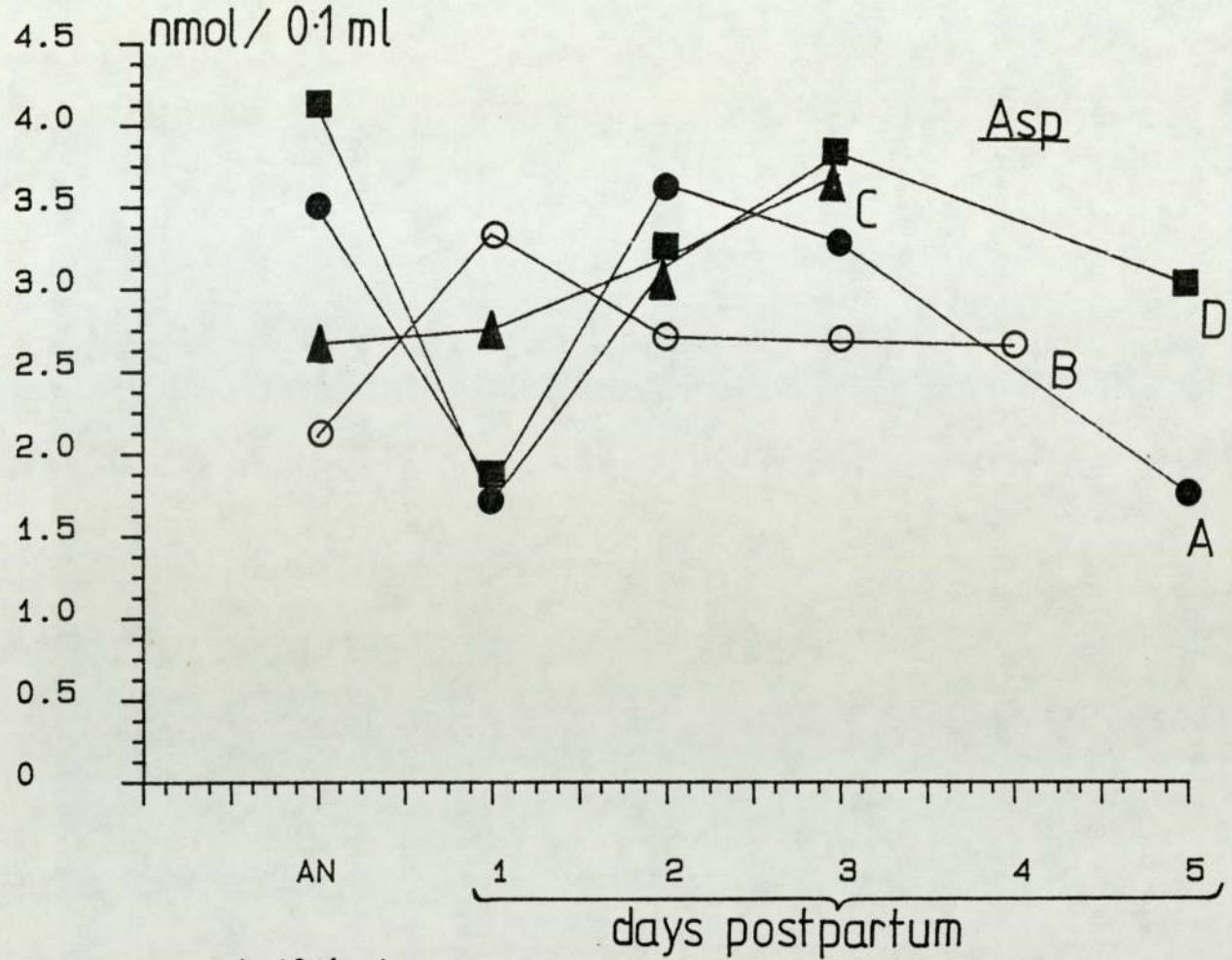


Fig 1·11 Amino acid profiles at parturition



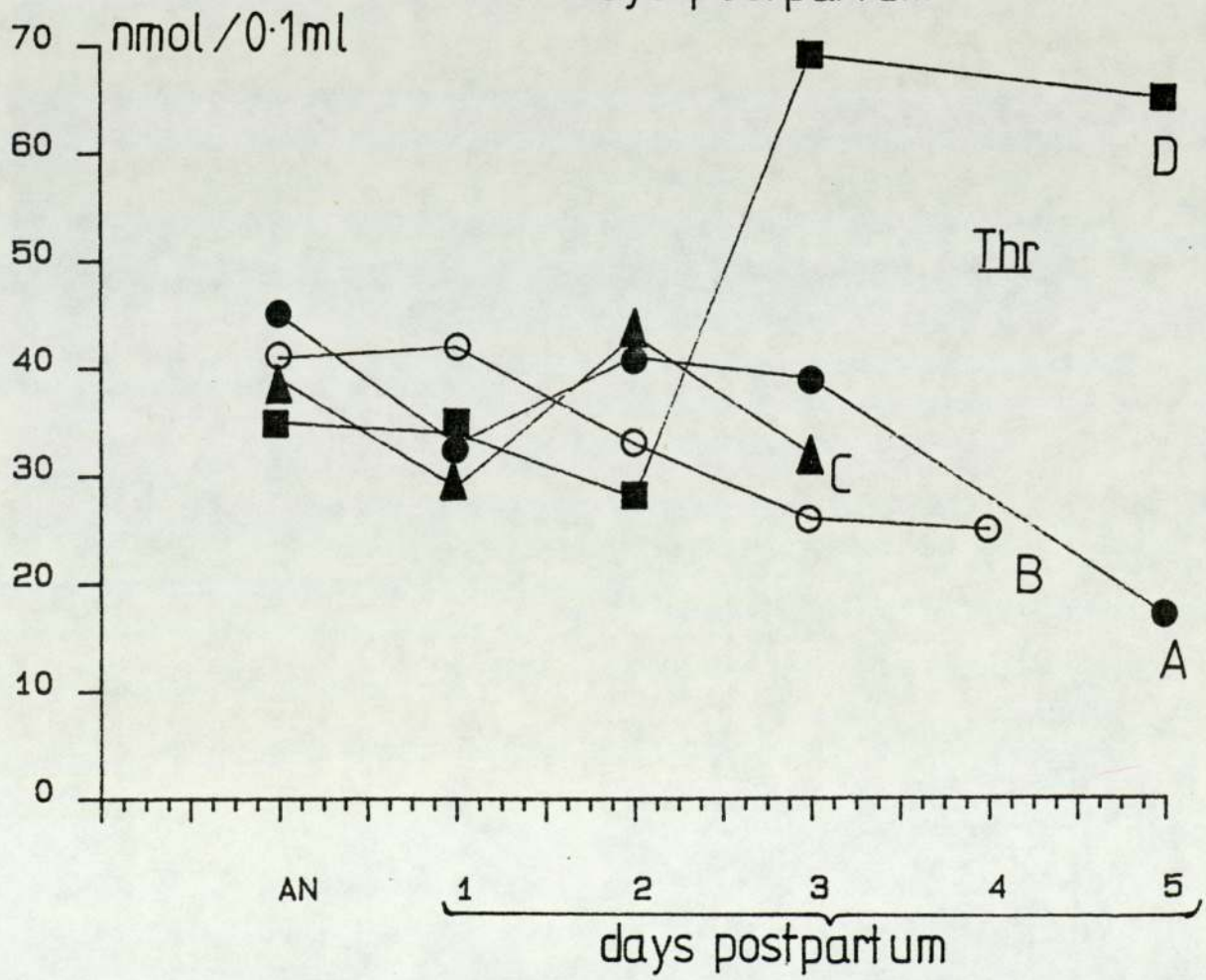
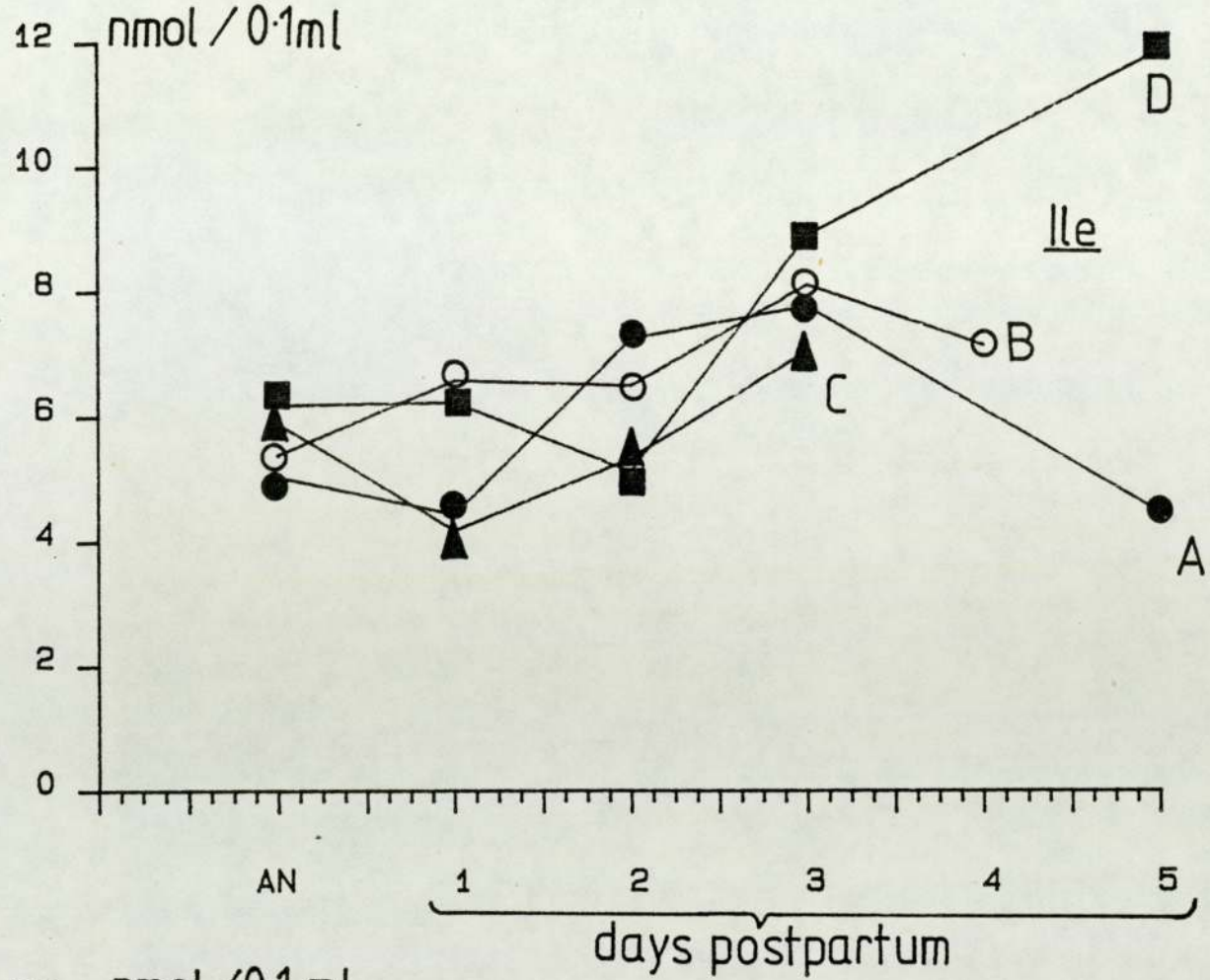


Fig 1·11 Amino acid profiles at parturition



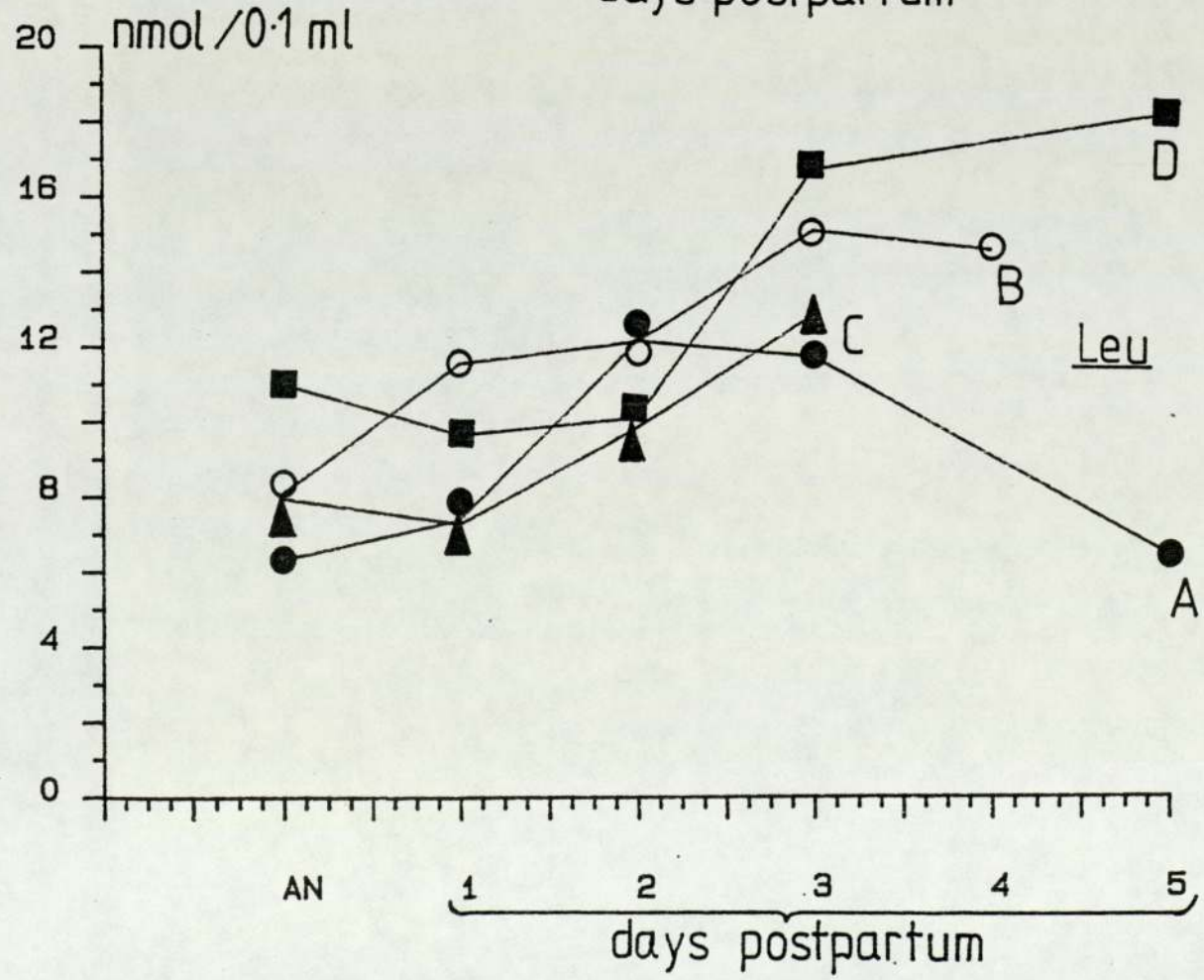
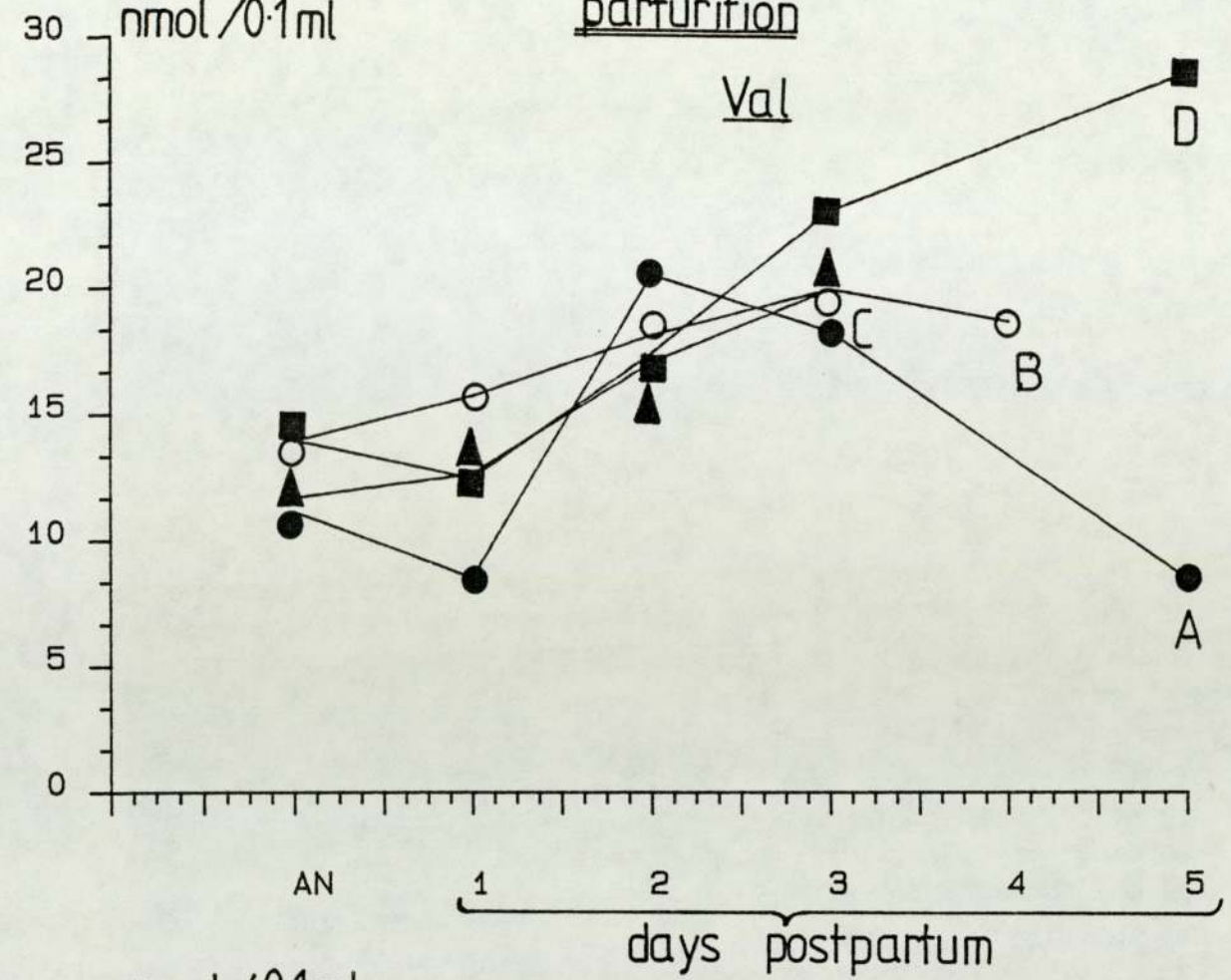


Fig 1·11 Amino acid profiles at parturition



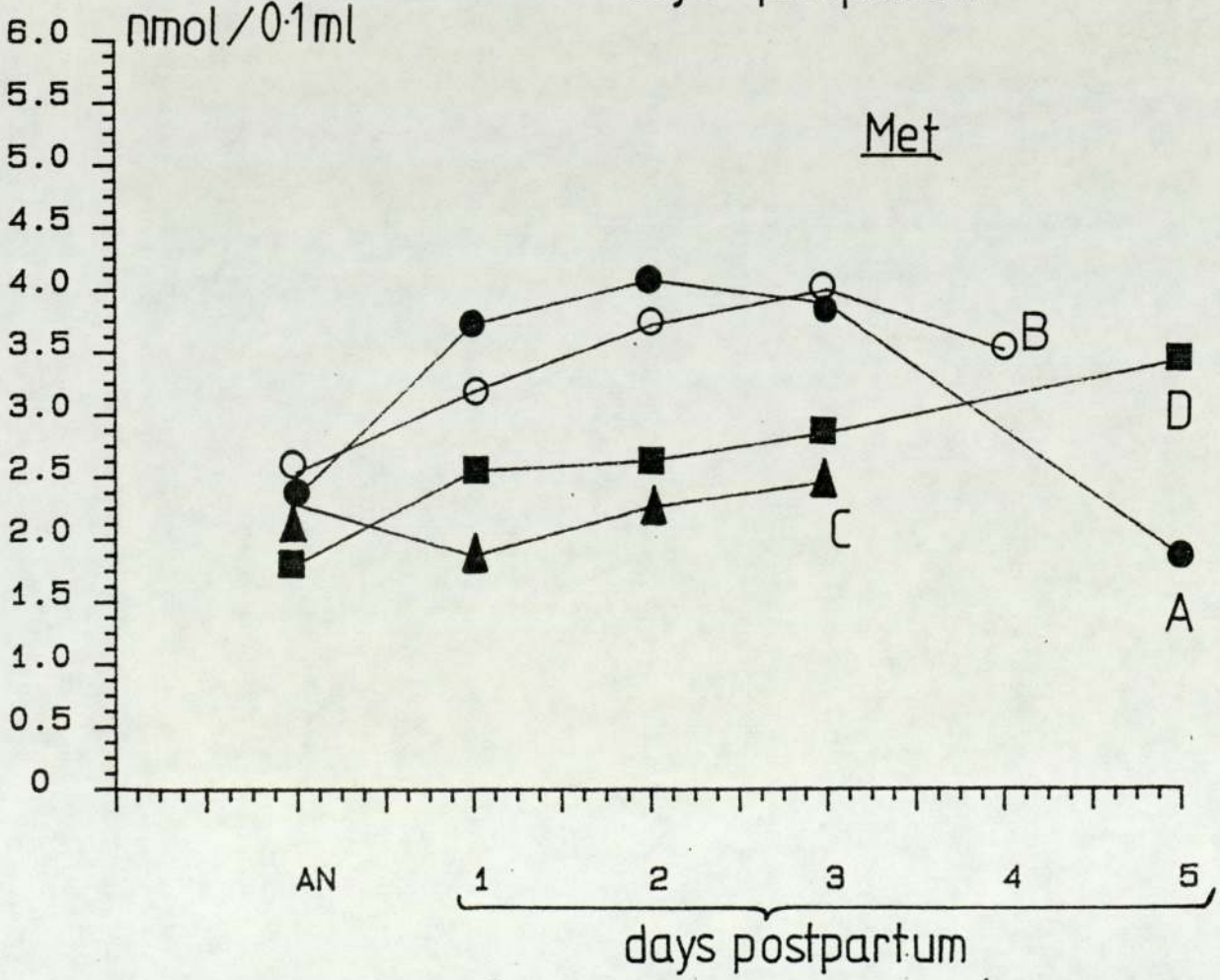
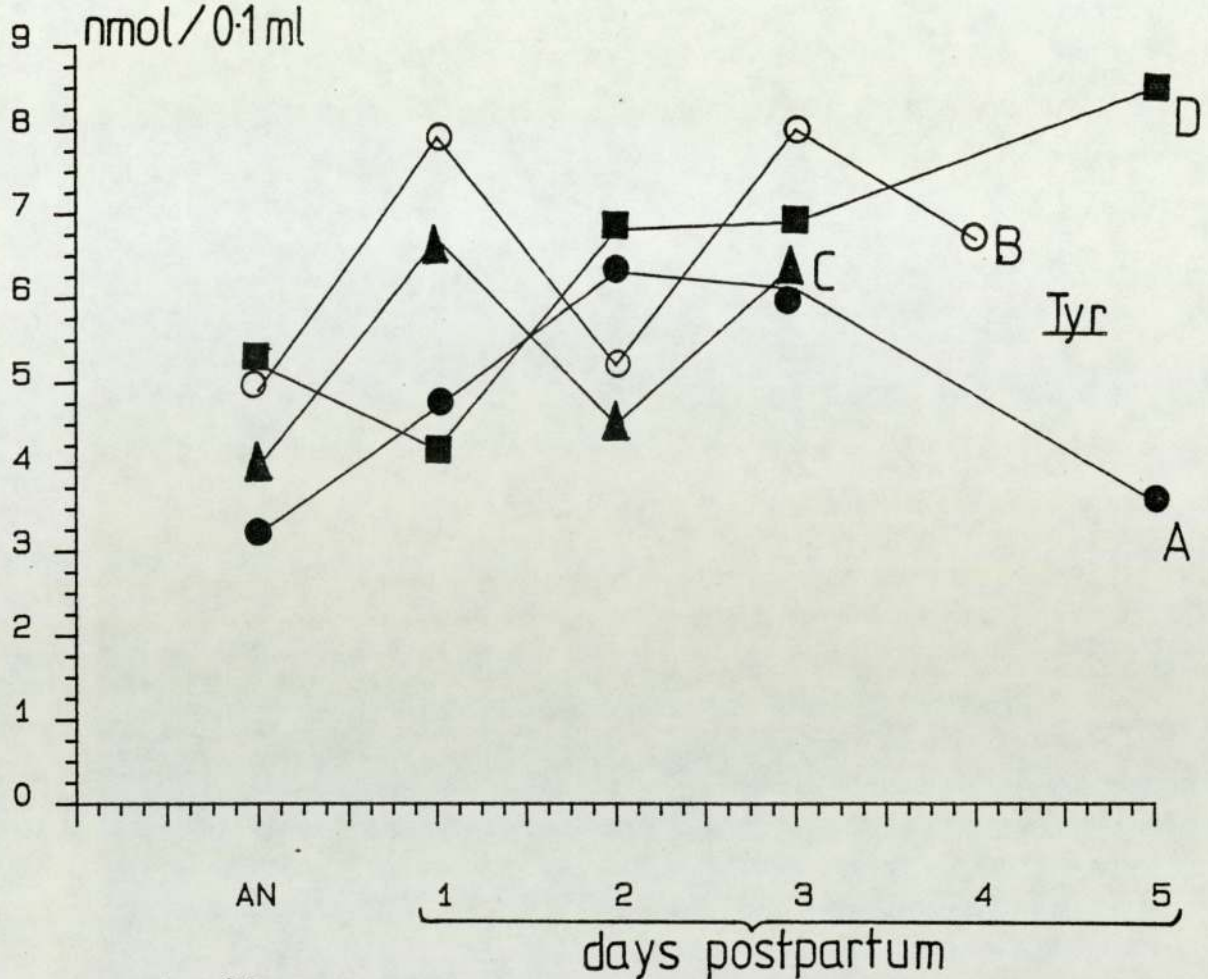


Fig 1·11 Amino acid profiles at parturition



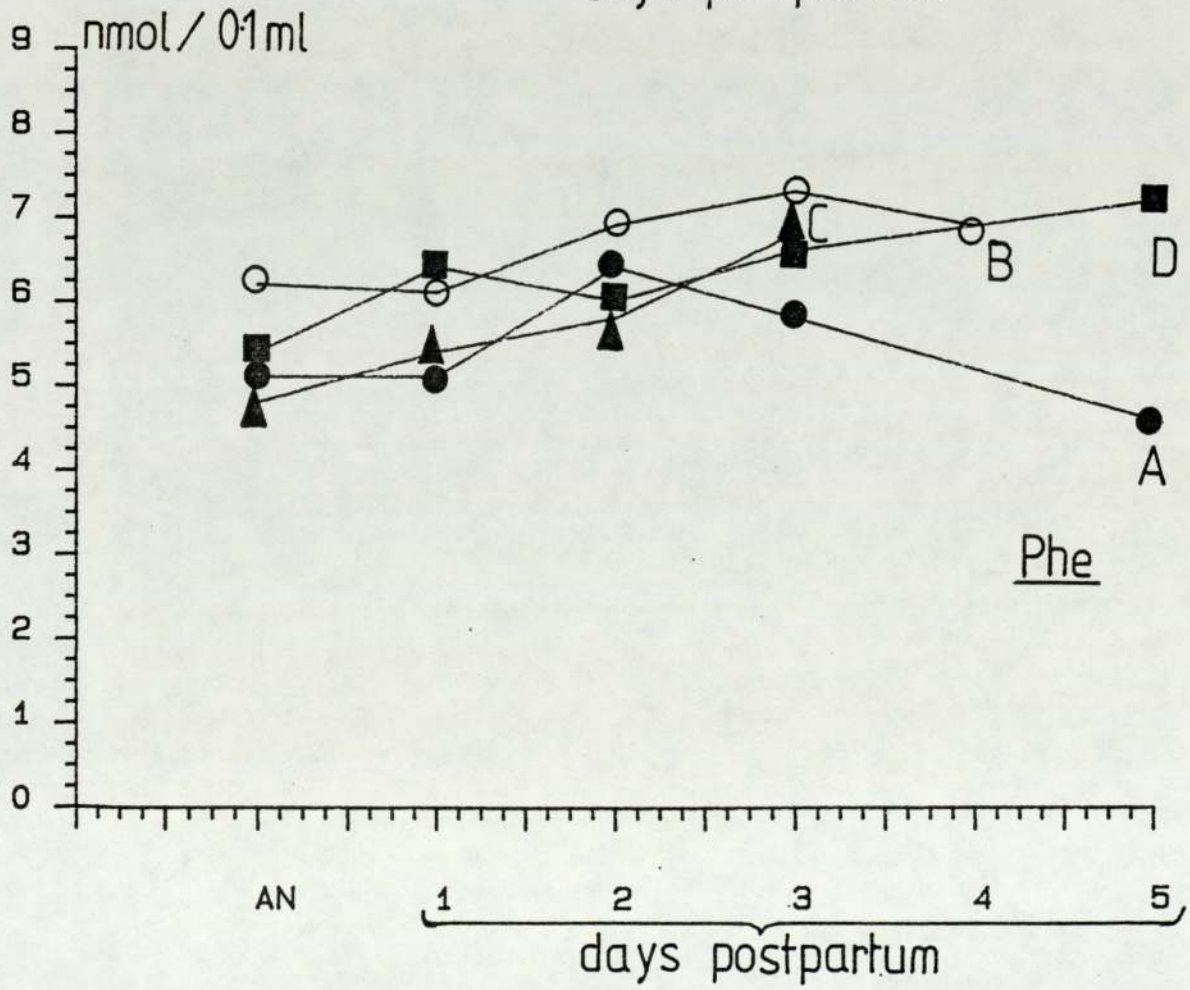
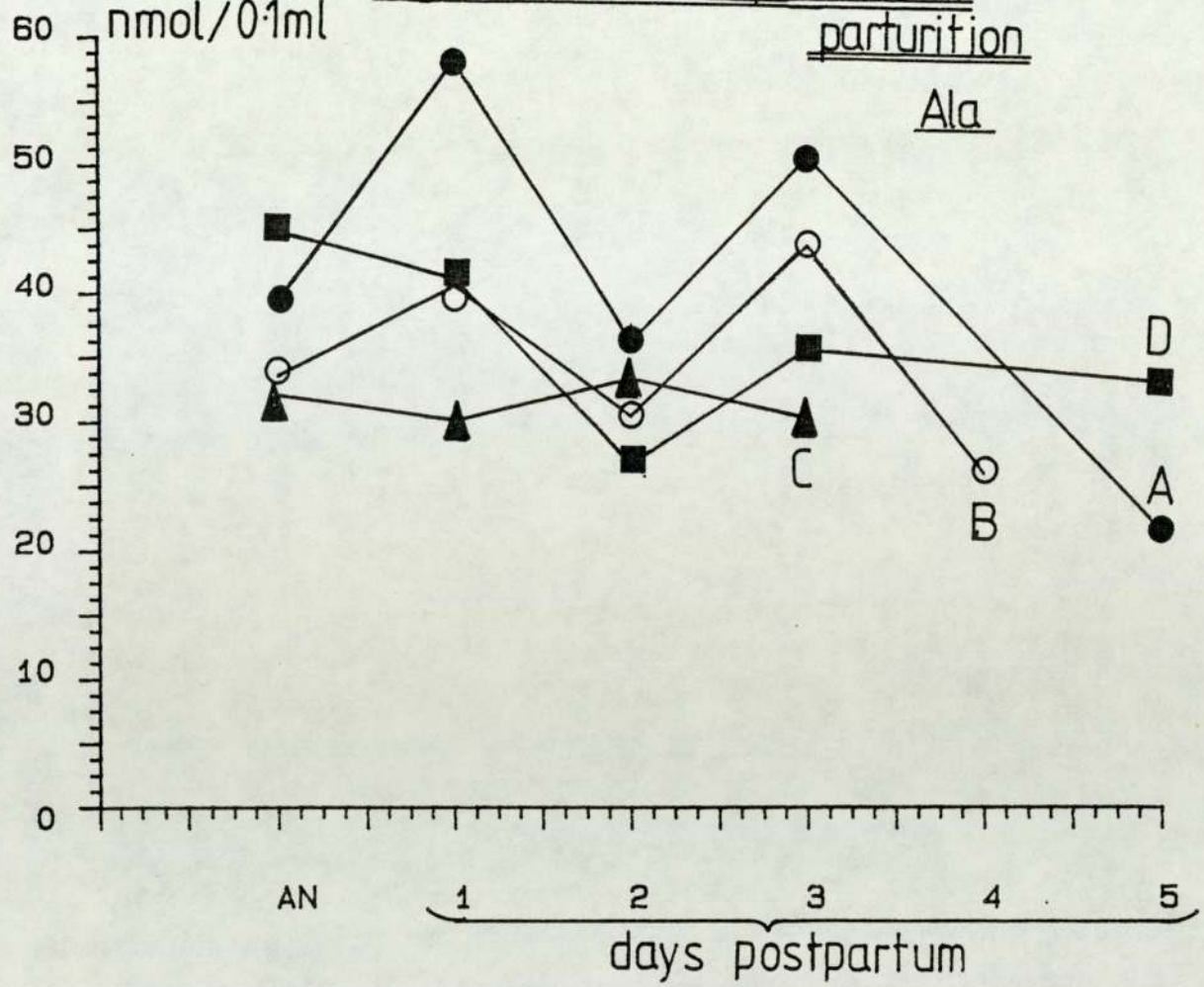
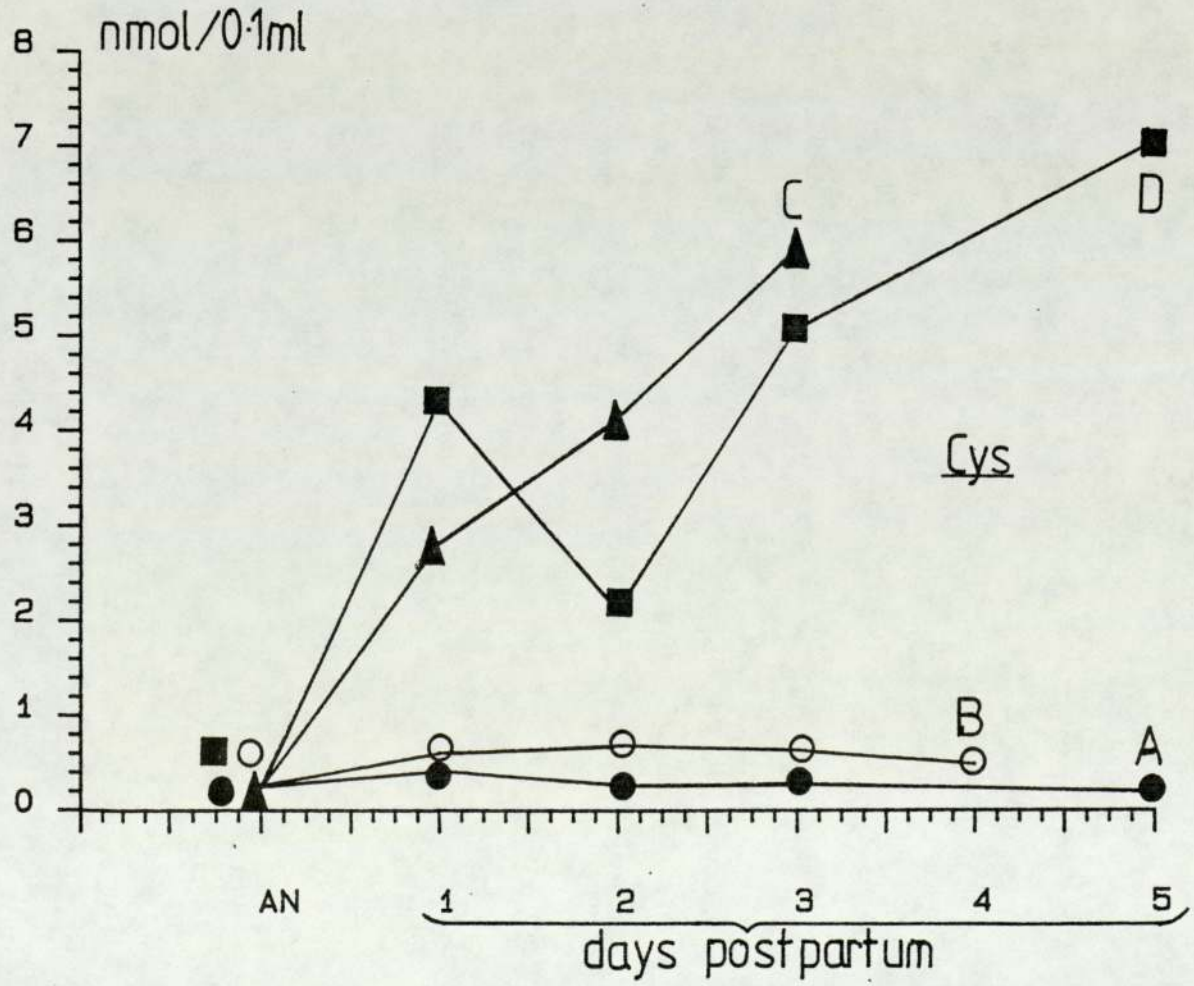


Fig 1·11 Amino acid profiles at parturition





CHAPTER 2

CHAPTER 2

THE RELATIONSHIP BETWEEN PLASMA TOTAL TRYPTOPHAN AND NON-ESTERIFIED FATTY ACIDS IN HUMANS AND IN MICE, AND THEIR RELATIONSHIPS WITH MOOD THROUGHOUT THE HUMAN MENSTRUAL CYCLE.

Chapter 2

The Relationship between Plasma Total Tryptophan and Non-Esterified Fatty Acids in Humans and in Mice, and their Relationships with Mood throughout the Human Menstrual Cycle.

2.1 INTRODUCTION.

The results of the previous chapter showed that there was some possible correlation between plasma total TP and NEFA during late pregnancy. This relationship was seen to be disrupted immediately post partum, but to return by about day 5 post-partum. This study was therefore designed to investigate whether such a relationship existed in Human Males and non-pregnant females.

Handley and colleagues (1980) reported that subjects suffering from puerperal mood swings, ie 'blues' exhibited abnormal plasma total TP profiles immediately post-partum (See Introduction It was therefore postulated that the abnormal plasma TP profiles, and possibly the mood changes, may be the result of the disruption of a relationship between plasma total TP and NEFA. This relationship was therefore studied throughout the menstrual cycle to investigate whether there was any alteration of this relationship premenstrually, a time of increased reports of mood change.

In addition, following the work of Riley and Shaw (1976) and Handley and colleagues (1980), the relationship between mood and plasma total TP was studied. Curzon and colleagues (1979) have also reported that depressed patients show an increased

rise of plasma NEFA following mild stress as compared with control subjects. It was therefore investigated as to whether subjects reporting depressive mood changes throughout the menstrual cycle exhibited abnormal plasma NEFA throughout the menstrual cycle.

2.2 SUBJECT POPULATION CHARACTERISTICS.

The subjects for this study were 18 male students and 16 female students (none of whom were currently using oral contraceptives). The age range was 18 to 24 years. Dietary and medication restrictions were enforced as described in Methods Section 5.

2.3 METHODS.

Blood samples were taken between 0900 and 1030, once for male subjects, and approximately 10 times throughout one complete menstrual cycle for females. (For determination of stage of menstrual cycle see Methods 6.1)

Assessment of depressed affect was by use of the Multiple Affect Adjective Checklist 'D' Score and a menstrual symptom chart. (See Methods Section 3.1 and 3.3). These were completed at the same time each day by the female subjects throughout one complete menstrual cycle. Female subjects also completed an Eysenck Personality Inventory. (See Methods Section 3.4). These were completed at the outset of the experiment, and therefore were completed at different stages of the menstrual cycle.

Following the work of Handley and colleagues (1980), depressed mood was defined by a cut-off point of a score of 22 on the MAACL 'D' scale. This score had previously been shown to correlate with a clinically significant puerperal mood change

(ibid). Therefore the magnitude of the mood changes being studied throughout the menstrual cycle was comparable with the puerperal mood changes studied by Handley and colleagues (1980).

2.4 RESULTS.

2.4.1 Males.

The mean plasma total TP was 11.70 ± 0.50 μg/ml (≙ 0.06 mMol/dm^3). The mean plasma NEFA was 0.59 ± 0.03 mEqiv/dm^3. The correlation coefficient of plasma total TP against NEFA was r = + 0.547, this was significant at the 0.018 level.

2.4.2 Females.

2.4.2.a Plasma Total Tryptophan.

Plasma Total TP showed a slight variation throughout the menstrual cycle, the peak being during the ovulatory phase (12.71 ± 0.43 μg/ml) and the nadir being during the menstrual phase (11.20 ± 0.26 μg/ml) (Table 2.1). Analysis of variance was non significant

Table 2.1 Variation of Plasma Total Tryptophan During the Menstrual Cycle.

Stage of Cycle	Mean Plasma Total Tryptophan ± SEM(μg/ml)
Menstrual	11.20 ± 0.26 (n=22)
Follicular	11.87 ± 0.25 (n=32)
Ovulatory	12.71 ± 0.43 (n=11)
Luteal	11.84 ± 0.25 (n=40)
Premenstrual	11.62 ± 0.32 (n=23)

2.4.2.b Plasma Non Esterified Fatty Acids.

Plasma NEFA showed very little variation throughout the menstrual cycle, except during the follicular stage, directly following menstruation, when values were increased. (Table 2.2). Follicular NEFA was significantly greater than that of the premenstrual stage ($p = .0119$), but not significantly greater than the NEFA levels for the menstrual phase ($p = .0866$). The overall analysis of variance was non-significant.

Table 2.2 Variation of Plasma NEFA During the Menstrual Cycle.

Stage of Cycle	Mean Plasma NEFA $\pm$ SEM(mEquiv/dm^3)
Menstrual	0.44 $\pm$ 0.03 (n=22)
Follicular	0.51 $\pm$ 0.03 (n=31)
Ovulatory	0.40 $\pm$ 0.04 (n=11)
Luteal	0.44 $\pm$ 0.02 (n=40)
Premenstrual	0.40 $\pm$ 0.03 (n=23)

2.4.2.c Relationship of NEFA to Total TP.

Correlation coefficients for total TP against NEFA were calculated for each stage of the menstrual cycle (Table 2.3). However none of the stages showed any significant correlation.

Table 2.3 Relationship Between Plasma Total TP and Plasma NEFA During The Menstrual Cycle.

Stage of Cycle	Correlation coefficient, r	Slope	Intercept	2-Tailed Significance
Menstrual	-.0238	-0.003	0.470	0.088(n=22)
Follicular	+.3121	+0.033	0.116	0.084(n=31)
Ovulatory	-.4744	-0.048	1.010	0.138(n=11)
Luteal	+.1234	+0.012	0.298	0.440(n=40)
Premenstrual	-.0870	-0.009	0.498	0.305(n=23)

2.4.3 Variations in Mood Throughout the Menstrual Cycle.

2.4.3.a MAACL 'D' Score.

There was very little variation in the MAACL 'D' Scores throughout the menstrual cycle however, there was a peak premenstrually with the nadir during the follicular stage (Table 2.4). From the study of individual mood records however, it was seen that there was no cyclicity of mood variation.

Table 2.4 Variation of MAACL 'D' Score During the Menstrual Cycle.

Stage of Cycle	MAACL 'D' Score ± SEM
Menstrual	18.8 ± 0.6 (n=56)
Follicular	17.5 ± 0.7 (n=68)
Ovulatory	18.5 ± 0.8 (n=35)
Luteal	18.5 ± 0.5 (n=83)
Premenstrual	19.6 ± 0.6 (n=51)

2.4.3.b Self Reports of Depression.

Throughout the whole study, there were only 25 self reports of depression. 12 of these were during the premenstrual phase, 9 were during the menstrual phase and 4 during the luteal phase. When the total number of days for each stage of the menstrual cycle for all subjects were considered, it was found that the increased incidence of self reports of depression could not be accounted for by considering the length of the cycle stages. In fact during the follicular stage for which a total of 103 days were studied, there were no reports of depression, whilst during the 72 premenstrual days studied, there were 11 reports of depression. (Table 2.5)

Table 2.5 Frequencies of Self Reports of Depression During The Menstrual Cycle.

Stage Of Cycle	Total Number of days studied	Total Number of Reports of Depression	Percentage of Total Days on which depress -ion reported
Menstrual	77	9	11.7%
Follicular	103	0	0%
Ovulatory	41	0	0%
Luteal	129	4	3.1%
Premenstrual	72	11	15.3%

2.4.3.c Relationship of MAACL 'D' Score to Self Reported Depression.

Within each cycle stage, the MAACL 'D' scores for individuals for days on which depression was self reported was compared with the MAACL 'D' scores for days on which there were no such reports. It was found that there was no significant difference between the MAACL 'D' scores for days of self-reported depression, and days with no such report. (Table 2.6). A 1-tailed test of significance was used.

Table 2.6 Relationship of MAACL 'D' Score to Self Report of Depression.

Stage of Cycle	Mean MAACL 'D' Score ± SEM	1-tailed significance (Mann-Whitney U Test)
Premenstrual Self reported depression	20.73±0.98(range17-26) (n=11)	
No self reported depression	19.5 ± 0.72(range7-21) (n=38)	p=.35
Menstrual Self reported depression	19.75±1.92(range12-25) (n=8)	
No self reported depression	18.57±0.71(range7-37) (n=47)	p=0.15

There were no reports of depression during the follicular and ovulatory stages. There was insufficient data for a similar analysis of the 4 reports of the luteal phase.

2.4.3.d Relationship of Personality with Mood.

The extroversion 'E' and neuroticism 'N' scores (Methods 3.4) were studied with respect to mood. It was found that there were no significant differences in terms of either EPI 'N' score or 'E' scores between those subjects that self reported depression at any point during the menstrual cycle, and those subjects not making such reports (Mann-Whitney U Test). Fig 2 1..

Considering the individual subjects' peak MAACL 'D' scores, there were no differences either in the EPI 'E' score or the 'N' score between subjects scoring 21-22 on the 'D' scale and subjects scoring either 23-26 or 27-37 on the 'D' scale (Mann-Whitney U Test). Fig 2 2

2.4.4 Relationship of Total Tryptophan with Mood.

2.4.4.a MAACL 'D' Score.

The criterion for depression used was an MAACL 'D' score of 22 or greater (See Section 2.3). Therefore if a subject scored 2 2 or greater during any stage of the cycle she was considered as being depressed during that stage. Plasma total tryptophan values for 'depressed' cycle stages were compared with values for 'non-depressed' stages. (Table 2.7) There was no significant difference in values

of plasma total TP during the menstrual, follicular and premenstrual stages. However during the luteal phase, plasma total TP was decreased in 'depressed subjects', the differenc however was not significant. (t=1.6256; p=.1086). During the ovulatory phase, plasma total TP was significantly increased in 'depressed subjects', p=.01.

Table 2.7 Relationship of Plasma Total TP to MAACL 'D' Score During the Menstrual Cycle.

Stage of Cycle	Mean Plasma Total TP±SEM(µg/ml)		2-tailed Significance
	'Depressed'	'Non Depressed'	
Menstrual	11.38±0.35(n=10)	11.30±0.49(n=10)	t=0.6095; p=.5562
Follicular	11.62±0.37(n=14)	12.23±0.38(n=16)	t=1.1800; p=.2466
Ovulatory	13.57±0.49(n=6)	11.66±0.41(n=5)	t=3.2353; p=.0100
Luteal	11.57±0.29(n=28)	12.45±0.51(n=12)	t=1.6256; p=.1086
Premenstrual	11.50±0.50(n=12)	11.75±0.47(n=11)	t=0.3735; p=.1867

2.4.4.b Self Reports of Depression.

If a subject reported depression during any stage of the menstrual cycle she was regarded as being depressed for that stage. The mean plasma total TP values were compared for 'depressed' and 'non depressed' stages, (Table 2.8). It was found that there were no significant difference in plasma total TP betwe

'depressed' and non-depressed' cases on the basis of self-reports of depression.

Table 2.8 Relationship of Plasma Total TP to Self-Reports of Depression During the Menstrual Cycle.

Stage of Cycle	Mean Plasma Total TP±SEM(μg/ml)		2-tailed Significance
	'Depressed'	'Non Depressed'	
Menstrual	11.20±0.41(n=10)	11.27±0.35(n=13)	t=0.1296; p=.8934
Premenstrual	11.91±0.55(n=13)	11.21±0.32(n=9)	t=1.0101; p=.3258

There were no reports of depression during the follicular and ovulatory stages and insufficient data for the 4 reports of depression during the luteal phase.

2.4.5 Relationship of NEFA with Mood.

2.4.5.a MAACL 'D' Score.

The criterion for depression was the same as in section 2.4.4.a. Values of plasma NEFA were compared for 'depressed' and 'non depressed' cases for each stage of the menstrual cycle (Table 9).

It was found that there was no significant difference in plasma NEFA values between 'depressed' and 'non depressed' subjects for the menstrual, follicular, ovulatory and premenstrual phases. However, plasma NEFA values were significantly decreased in 'depressed' subjects during the luteal phase, t = 2.6191, p = .0121

Table 2.9 Relationship of Plasma NEFA to MAACL 'D' Score During the Menstrual Cycle

Stage of Cycle	Mean Plasma NEFA±SEM(mEquiv/dm^3)		2 tailed significance
	Depressed	Non Depressed	
Menstrual	0.462±0.060(n=10)	0.417±0.047(n=10)	t=0.6241; p=.5468
Follicular	0.477±0.038(n=12)	0.522±0.039(n=17)	t=0.8434; p=.5888
Ovulatory	0.360±0.054(n=6)	0.444±0.079(n=5)	t=1.0055; p=.3426
Luteal	0.399±0.023(n=28)	0.522±0.048(n=12)	t=2.6191; p=.0120
Premenstrual	0.429±0.049(n=12)	0.363±0.045(n=11)	t=1.0358; p=.3130

2 4 5 b Self Reports of Depression

Plasma NEFA values were compared for 'depressed' and 'non depressed' cases for each stage of the menstrual cycle (Table 2 10)

The same criterion for depression was used as in section 2.4.4.b.

It was found that those subjects who reported 'depression' premenstrually had increased plasma NEFA values, t=3.2112, p=.0044. This difference was not seen during the menstrual stage.

Table 2.10 Relationship of NEFA to Self Reports of Depression During the Menstrual Cycle.

Stage of Cycle	Mean Plasma NEFA±SEM(mEquiv/dm^3)		2-tailed Significance
	'Depressed'	'Non Depressed'	
Menstrual	0.420±0.081(n=8)	0.445±0.031(n=14)	t=0.3637; p=.7200
Premenstrual	0.467±0.045(n=14)	0.290±0.014(n=9)	t=3.2112; p=.0044

There were no reports of depression during the follicular or ovulatory stages, and there was insufficient data for the 4 reports of depression during the luteal phase.

2.5 THE EFFECTS OF INCREASED PLASMA TRYPTOPHAN ON PLASMA NEFA IN MALE MICE.

From the present and previous chapter, it seems possible that there is some ill-defined relationship between plasma total TP and plasma NEFA. Fernstrom and Wurtman (1971) showed that increased plasma NEFA produced a decrease in plasma total TP (See Introduction). There is however no data on the possibility of a reciprocal relationship between plasma total TP and NEFA.

2.5.1 Methods.

The initial stage of the experiment was a study of plasma TP Kinetics following subcutaneous administration. Male mice were injected with TP 400mg/kg s.c., plasma concentrations of total TP were then assayed at varying times after the injection. The results showed that the peak plasma TP concentrations were achieved 30 minutes after the injection (See later).

The second stage of the experiment consisted of injecting male mice with varying doses of TP s.c. and measuring plasma NEFA concentrations 30 minutes after the TP

administration. The remaining plasma was then pooled for plasma total TP assays.

2.5.2 Results.

The plasma profile of total TP, following injection of 400mg/kg L-tryptophan s.c., is presented in Table 2.11 and Figure 2.3. The results show that the peak plasma concentrations occurred 30 minutes after injection.

Increased plasma total TP produced significantly increased plasma NEFA (Table 2.12). However, there was no direct correlation between plasma total TP and plasma NEFA concentrations (r=.6415, p=.085, 2-tailed).

2.6 SUMMARY AND COMMENTS.

The results of this study show that there is a significant positive correlation between plasma total TP and NEFA in Human Males. No such relationship exists in Human Females at any stage of the menstrual cycle. Plasma total TP and NEFA however, do vary throughout the menstrual cycle, although these changes were non-significant following analysis of variance

Mood was also seen to vary throughout the menstrual cycle, with self reports of depression being more frequent during the premenstruum and menstruum. There was no such cyclical variation in the MAACL 'D' scores.

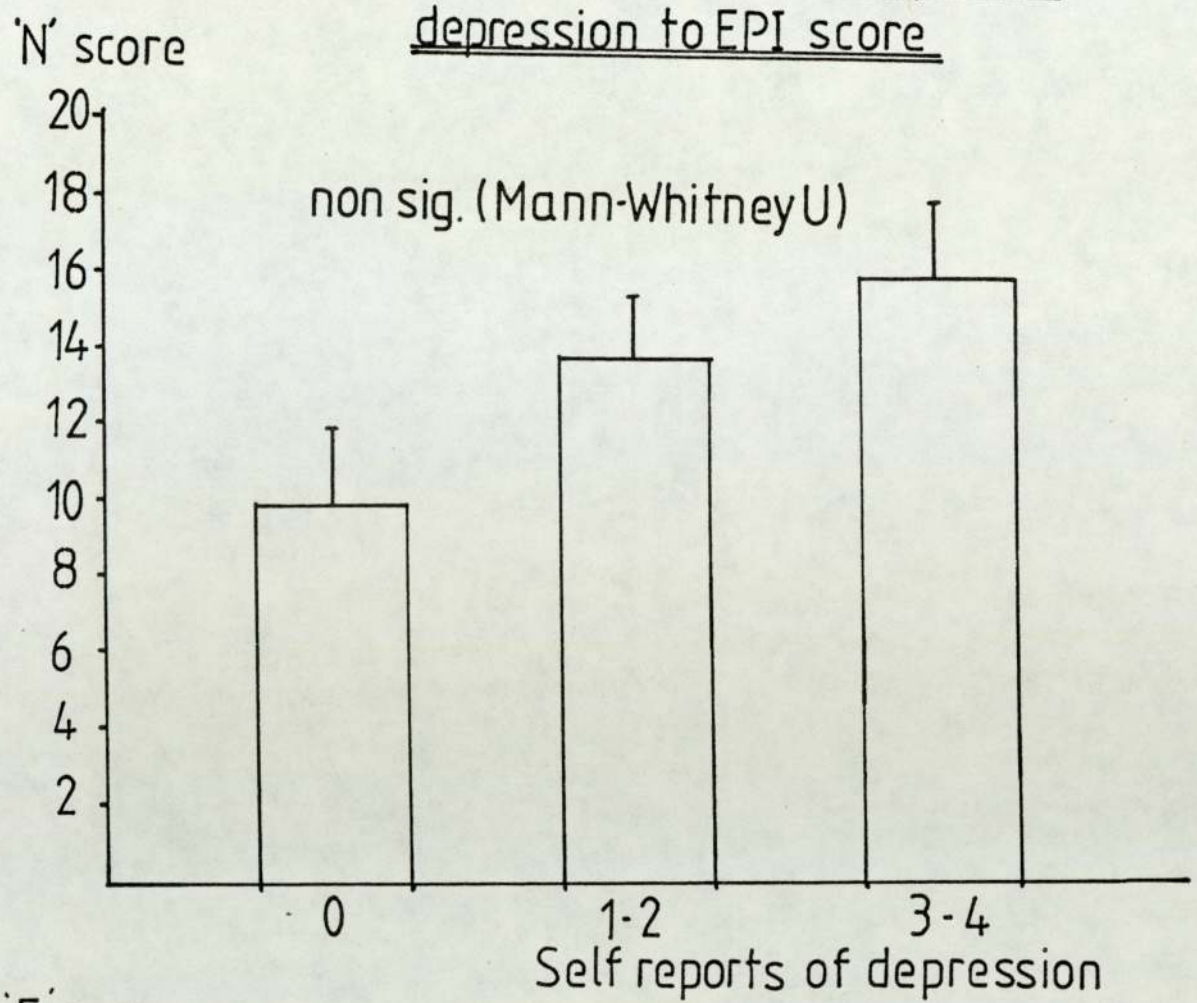
Plasma total TP was seen to be significantly increased in depression during the ovulatory stage of the menstrual cycle. Plasma NEFA was seen to be significantly decreased in depression during the luteal phase of the menstrual cycle. Subjects

self reporting depression during the premenstruum were seen to have significantly increased plasma NEFA during that stage.

In mice, increased plasma total TP was seen to significantly increase plasma NEFA concentrations.

No measurements of plasma free TP were made in this study as the experiments were designed to investigate the relationship between plasma total TP and plasma NEFA.

Fig 2.1 Relationship of self reported depression to EPI score



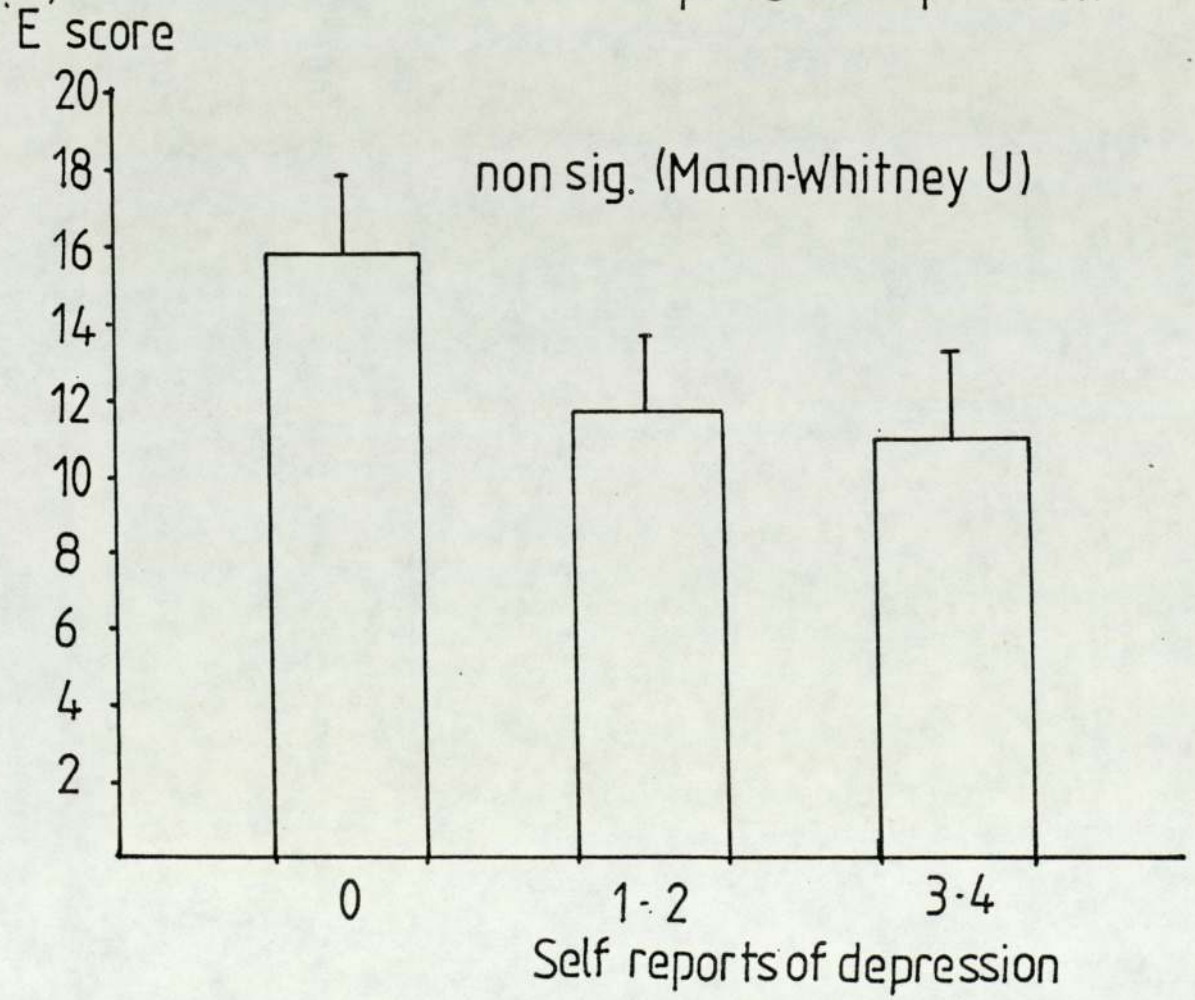
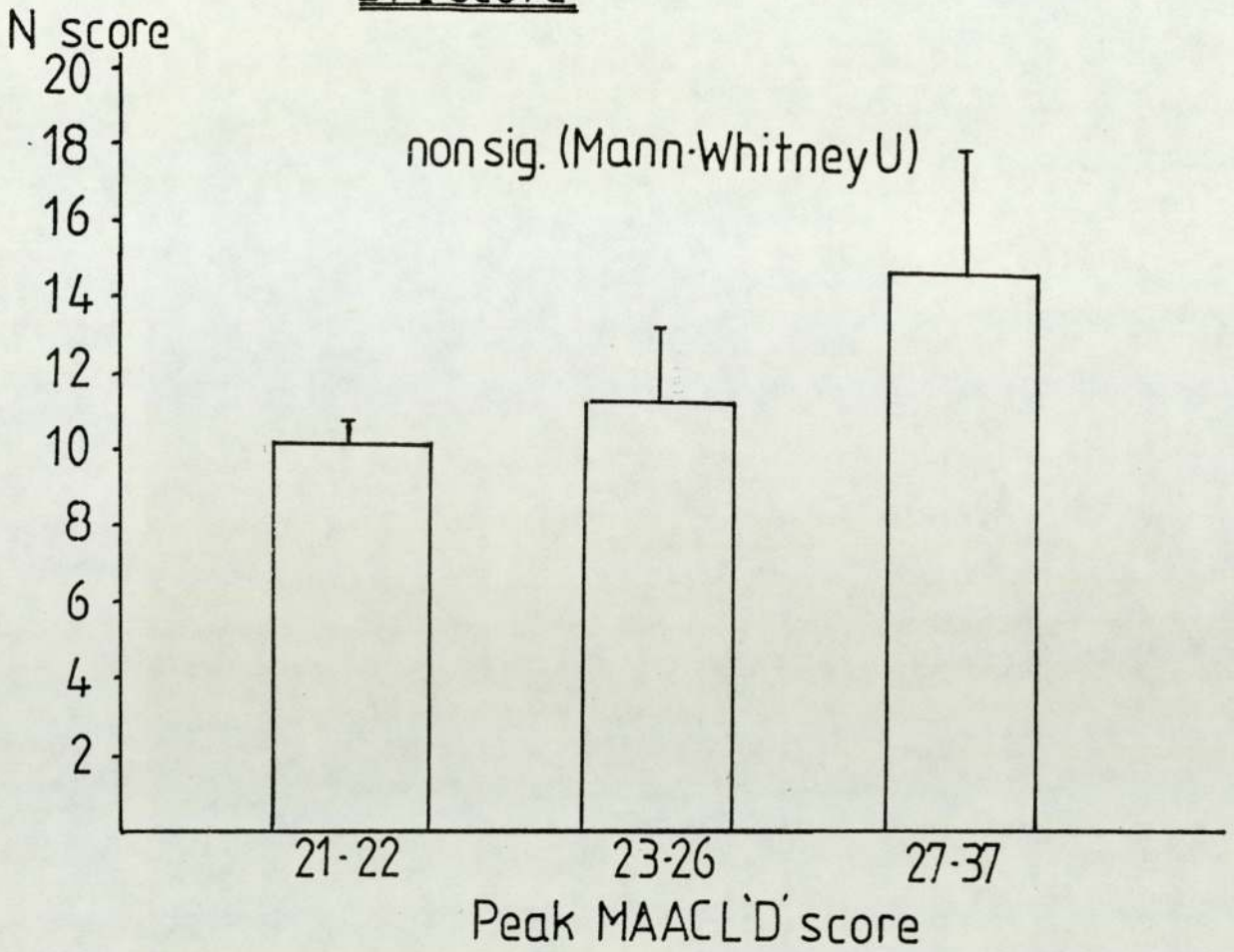
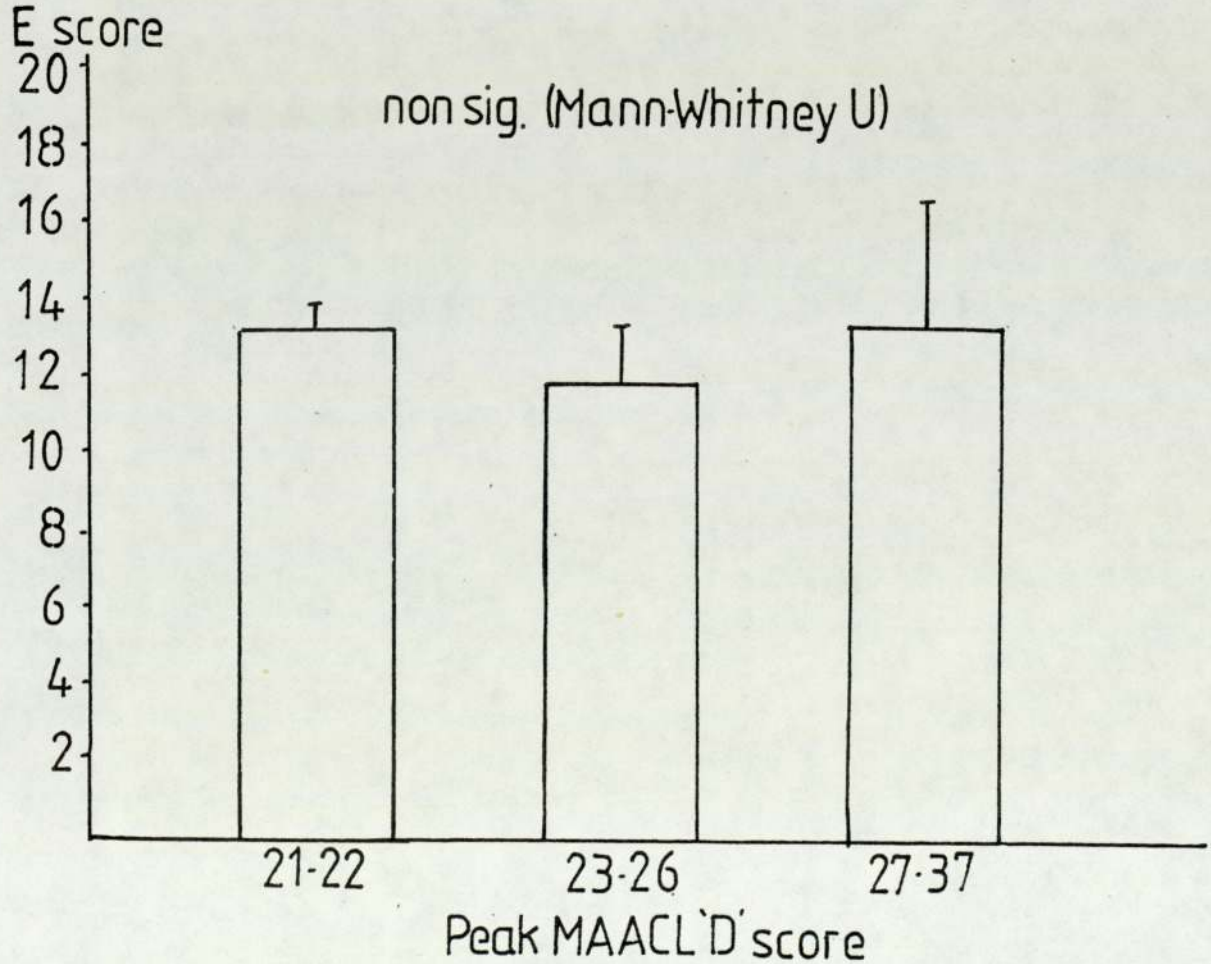


Fig 2·2 Relationship of MAACL 'D' score to EPI score





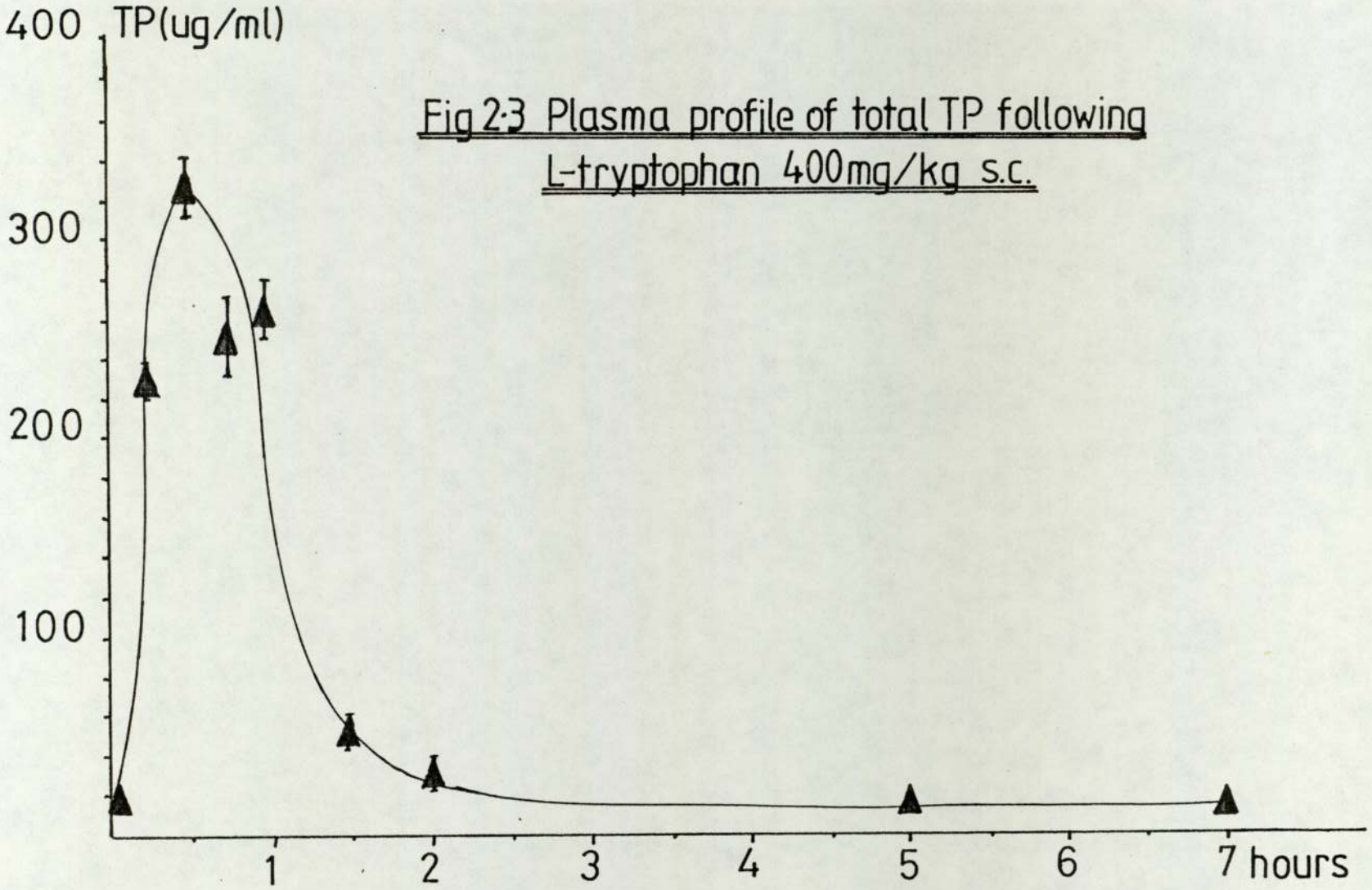


Fig 2·3 Plasma profile of total TP following L-tryptophan 400mg/kg s.c.

Table 2 11 : Plasma Total Tryptophan Following Administration of L-Tryptophan 400 mg/kg s c

Time after administration	Plasma Total Tryptophan (µg/ml) ± S E M	Number of samples
0	18.31 ± 1.65	6
15 mins	229.30 ± 6.08	5
30 mins	325.85 ± 11.45	6
45 mins	150.90 ± 21.52	6
60 mins	162.60 ± 12.00	4
90 mins	50.58 ± 7.62	5
120 mins	32.20 ± 7.28	5
5 hours	13.52 ± 1.67	5
7 hours	13.00 ± 2.25	5

Table 2 12 Effect of Increased Plasma Total Tryptophan Concentrations on Plasma NEFA Concentrations

Dose of Tryptophan (mg/ml)	Pooled Plasma total tryptophan (μg/ml)	Mean Plasma NEFA (mEq/dm^3) ± S E M	2 tailed probability
0	36.00	0.767±0.053(n=6)	
10	30.11	0.590±0.121(n=4)	.1203
50	55.17	0.960±0.423(n=3)	.5543
100	93.10	0.548±0.131(n=4)	.0745
150	98.85	1.177±0.041(n=6)	.0002
200	119.54	1.165±0.050(n=6)	.0003
300	212.64	1.040±0.082(n=6)	.0117
400	258.62	1.238±0.120(n=5)	.0024

CHAPTER 3

CHAPTER 3

AN INVESTIGATION OF THE RELATIONSHIP BETWEEN PLASMA TRYPTOPHAN AND DEPRESSED AFFECT IN PERIMENOPAUSAL WOMEN.

CHAPTER 3

An Investigation of the Relationship Between Plasma Tryptophan and Depressed Affect in Perimenopausal Women

3.1 INTRODUCTION.

Coppen and colleagues (1972 & 1973) showed that plasma free TP was decreased in depressed patients. Coppen and Wood (1978) also showed that plasma free TP was decreased in perimenopausal women, when compared with either pre- and post- menopausal women. This, they state, is probably due to the changes in the levels of circulating oestrogens.

In addition to this, Aylward (1976) demonstrated that in a group of post-menopausal patients that had previously undergone hysterectomy, plasma free TP was decreased in those patients who were depressed.

This study attempted to replicate these results by studying the plasma free and total TP characteristics of perimenopausal women and by studying the relationship of the plasma TP characteristics with plasma Follicle Stimulating Hormone (FSH). FSH is increased at the menopause in response to the decreased circulating oestrogen levels and therefore following from the work of Coppen and Wood (1978) and Aylward (1976) it is possible that FSH and plasma tryptophan are related.

3.2 PATIENT POPULATION CHARACTERISTICS.

The subjects used for this study were 100 consecutive GP referrals to a menopause research clinic at Birmingham Maternity Hospital.

All of the subjects were of white, caucasian origin, and the distribution of the socio-economic class was skewed towards classed I and II (Parsons, personal communication). Of the 100 subjects, 35 had a previous history of depressive illness and 32 were currently undergoing psychotropic drug therapy. The mean age of the subjects was 47.35 $\pm$ 0.69 years.
Subjects were divided into pre- and post-menopausal groups using the criteria described in Methods section 6.3. Dietary restrictions were also enforced, Methods section 5.

Each subject completed the Sabbatsberg Depression Inventory (Methods 3.1) in order to determine their depressive status, and blood samples were obtained for the determination of FSH, TotalTP and FreeTP.

3.3 RESULTS.

3.3.1 Incidence of Depression.

There was no significant difference in the incidence of depression between premenopausal and post-menopausal subjects (Table 3.1).

χ^2 = 0.0401, df = 1, Non significant

Table 3.1 : Incidence of Depression in pre- and post-menopausal women.

	Premenopausal	Postmenopausal
Depressed	15	17
Non Depressed	27	28

Again, there was no significant difference in the incidence of depression either pre- or post-menopausally when the subjects were divided on the basis of having a previous history of depressive illness. (Tables 3.2 and 3.3). Premenopausally X^2 = 0.9583, df =1, Non Significant; Postmenopausally X^2 = 0.1358, df=1, Non Significant.

Table 3.2 : Effects of a Previous Depressive History on The Incidence of Depression in Premenopausal Women.

	Depressed	Non Depressed
Previous Depressive History	9	12
No Previous Depressive History	6	15

Table 3.3 : Effects of a Previous Depressive History on The Incidence of Depression in Postmenopausal Women.

	Depressed	Non Depressed
Previous Depressive History	7	10
No Previous Depressive History	10	18

3.3.2 RELATIONSHIP OF PLASMA TOTAL TRYPTOPHAN AND DEPRESSION.

When all patients were considered, whether pre- or post-menopausal, there was no difference in plasma total TP

levels between depressed and non-depressed patients (Table 3.4) t = 0.8693. 2 tailed significance level = 0.6090.

Table 3.4 : Relationship of Plasma Total Tryptophan to Depression in Perimenopausal Women.

	Mean Plasma Total Tryptophan ± SEM (μg/ml)
Non-Depressed	6.767 ± 0.168 (n=60)
Depressed	6.507 ± 0.245 (n=35)

This lack of difference between depressed and non-depressed subjects was also present when the subjects were divided into pre- and post- menopausal groups (Table 3.5). Premenopausal : t = 0.9819, 2 tailed significance = 0.6668; Postmenopausal : t= 0.0865, 2 tailed significance = 0.929.

Table 3.5 : Relationship of Plasma Total Tryptophan to Depression in Pre- and Post- Menopausal Women.

	Mean Plasma Total Tryptophan ± SEM (μg/ml)
Premenopausal Non Depressed	6.838 ± 0.283 (n=28)
Premenopausal Depressed	6.384 ± 0.372 (n=15)
Postmenopausal Non Depressed	6.643 ± 0.218 (n=28)
Postmenopausal Depressed	6.609 ± 0.360 (n=17)

32 of the subjects were currently receiving pharmacotherapy. These subjects were therefore divided into a separate group. However, there was still no significant difference in plasma total TP between drug free depressed and non-depressed subjects, either pre- or post-menopausally. Nor was there any difference between treated depressed and non depressed groups either pre- or post-menopausally (Table 3.6). However, patients receiving medication had significantly decreased total TP ($p=.027$, 2 tailed). Results of the analysis of variance were :

Source	Sum of Squares	df	Mean Square	F ratio	Signific.
Menopausal Status	0.004	1	0.004	0.002	.961
Drug Treatment	9.209	1	9.209	5.084	.027
Depression Status	0.283	1	0.283	0.156	.694
2-way interactions:					
Menopause/Treatment	0.029	1	0.029	0.016	.900
Menopause/Depression	0.390	1	0.390	0.216	.644
Treatment/Depression	0.967	1	0.967	0.534	.467
3-way interaction:					
Menopause/Depression/ Treatment	0.056	1	0.056	0.031	.860
Within groups	143.110	79	1.812		

Table 3.6 : Effects of the Menopause, Depressive Illness and Psychotopic Drug Treatment on Plasma Total Tryptophan.

		Plasma Total Tryptophan±SEM(μg/ml)	
Premenopausal	Drug Free Non Depressed	6.935 ± 0.323	(n=23)
	Drug Free Depressed	6.891 ± 0.638	(n=6)
	Treated Non Depressed	6.395 ± 0.479	(n=5)
	Treated Depressed	5.936 ± 0.420	(n=9)
Postmenopausal	Drug Free Non Depressed	6.757 ± 0.244	(n=20)
	Drug Free Depressed	7.075 ± 0.517	(n=9)
	Treated Non Depressed	6.359 ± 0.448	(n=8)
	Treated Depressed	6.085 ± 0.416	(n=8)

Subjects were also divided as to whether they had undergone hysterectomy or whether they had undergone a natural menopause, and again, there was no difference in plasma total TP between depressed or non-depressed subjects within these groups (Table 3.7).

ource	Sum of Squares	df	Variance Estimate	ratio	df	Signif
atural Menopause.	2.2512	1	2.2512	0.988	1,41	NS
epressed/Non Dep.	0.012	1	0.012	0.005	1,41	NS
enopause/Depress-on Interaction.	0.602	1	0.602	0.264	1,41	NS
ithin Groups	93.4135	41	2.2784			

Table 3.7 : Effects of Hysterectomy and Depression on Plasma Total Tryptophan.

	Plasma Total Tryptophan ± SEM (µg/ml)
'Natural'Post Menopausal Depressed.	6.691 ± 0.361 (n=16)
'Natural'Post Menopausal Non Depressed.	6.722 ± 0.237 (n=24)
Hysterectomy Post Menopausal Depressed.	5.302 (n=1)
Hysterectomy Post Menopausal Non Depressed.	6.172 ± 0.464 (n=4)

3.3.3 Relationship of Plasma Free Tryptophan and Depression.

Plasma free TP was determined for 14 subjects with depression scores of 65 or greater (ie severely depressed) and for 11 subjects with scores of less than 30. However there was no difference between these 2 groups. (Table 3.8)

Table 3.8 : Relationship of Plasma Free Tryptophan to Depression In Perimenopausal Women

t= 0.3088, p= .758

	Free Tryptophan(µg/ml) ± SEM
Depressed Patients	3.40 ± 0.32 (n=13)
Non Depressed Patients	3.56 ± 0.50 (n=8)

However, post-menopausal women were seen to have lower concentrations of plasma free TP than pre-menopausal women ($t = 3.48$, $p = .0026$), but there were no significant differences between depressed and non-depressed patients within either the premenopausal group or the post-menopausal group. (Tables 3.9 and 3.10).

Table 3.9 : Comparison of Plasma Free Tryptophan in Pre- and Post- Menopausal Women.

	Free Tryptophan (μg/ml) ± SEM
Premenopausal	4.05 ± 0.32
Postmenopausal	2.68 ± 0.24

$t = 3.48$, $p = .0026$

Table 3.10 : Relationship of Plasma Free Tryptophan to Depression in Pre- and Post-Menopausal Women.

	Plasma Free TP (μg/ml) ± SEM	2-tailed Probability
Premenopausal Non Depressed	4.68 ± 0.33	
Premenopausal Depressed	3.74 ± 0.43	t=1.51, p=.1595
Postmenopausal Non Depressed	2.53 ± 0.28	
Postmenopausal Depressed	2.84 ± 0.44	t=0.67, p=.5297

3.3.4 Relationship of Plasma FSH to Depression.

Plasma FSH rises sharply following the menopause (Bell et al., 1976) and this fact was used as a criterion for the determination of menopausal status. However, there was no difference in FSH levels between depressed and non depressed subjects (Table 3.11).

Table 3.11 : The Relationship Between FSH and Depression.

	Mean FSH ± SEM(IU/dm^3)	2-tailed Significance
Premenopausal Non Depressed	9.67 ± 1.88 (n=18)	t=0.07
Premenopausal Depressed	9.52 ± 1.23 (n=25)	p=0.9429
Postmenopausal Non Depressed	48.82 ± 0.60 (n=28)	t=1.89
Postmenopausal Depressed	46.65 ± 1.16 (n=17)	p=0.0623

NB Plasma FSH values of 50 IU/dm^3 signify FSH $\geq$ 50 IU/dm^3

3.3.5 Relationship of FSH to Plasma Total Tryptophan.

There was no correlation between plasma total tryptophan and plasma FSH levels. (r = -.0621, 2 tailed significance = 0.441, n=94)

3.3.6 Relationship of FSH to Plasma Free Tryptophan.

Free tryptophan was found to be significantly correlated with FSH. The correlation coefficient was found to be - 0.3855, this was significant at the 0.05 level.

3.4 SUMMARY AND COMMENTS.

There was no difference in plasma total or free TP or FSH between depressed and non-depressed subjects. Nor was there any difference in the incidence of depression either pre- or post- menopausally.

Plasma free tryptophan was found to be significantly decreased in post menopausal subjects and this was also manifested in a significant negative correlation between free TP and FSH.

CHAPTER 4

Chapter 4

A MULTIFACTORIAL ANALYSIS OF FACTORS INVOLVED IN POST-PARTUM MOOD CHANGES.

Chapter 4

A Multifactorial Analysis of Factors Involved in Post-Partum Mood Changes.

4.1 Introduction.

As described in the Introduction, there have been many studies of sociological and biochemical factors, and their involvement in the aetiology of post-partum depressive mood changes. The only sociological variable that has been seen to show any significant relationship with either 'blues' or depression is parity (Nott et al., 1976), other factors appear to be irrelevant (See Introduction). However, there have been several studies of the putative biochemical factors involved in the aetiology of the depressive symptoms, and several factors have been isolated. For example, plasma free TP has been found to be decreased in cases of the 'blues' (Stein et al., 1976; Handley et al., 1977), a lack of the early rise of plasma total TP has been seen to be correlated with 'blues' (Handley et al., 1980), plasma prolactin has been shown to be increased in 'blues' sufferers (George et al., 1980) and a relationship between weight loss, electrolyte excretion and puerperal mood change has also been demonstrated (Stein, 1980). However endocrinological parameters were seen to have no significant relationship to puerperal mood, except that the relative fall of plasma progesterone at parturition was correlated with self reports of depressive mood change in the puerperium (Nott et al., 1976).

When the more severe post natal depression was investigated, it was found that there was no relationship between endocrinological factors and depression (Nott et al., 1976). Handley and colleagues (1980) however, reported that subjects showing a failure of the early plasma total TP rise post-partum exhibited a greater incidence of depression within the six months post-partum. A fuller description of previous work in this field is presented in the Introduction.

In addition to these factors that have been studied in puerperal and post-partum depression, there are some biochemical correlates of non-puerperal depression that have not been studied for the particular case of post-partum depression. Such factors are plasma NEFA, plasma LNAA and urinary MHPG (for fuller details see Introduction).

This study was therefore designed to replicate the previous studies of Handley and colleagues (1977 & 1980) and Nott and co-workers (1976), and to extend these studies by investigating additional biochemical parameters. The other important factor of this study was that all of the biochemical variables were measured in the same individuals, hence the interrelationships between the biochemical variables could be investigated. The ultimate aim of the study was therefore to investigate whether previous work from this laboratory could be replicated and whether work from other laboratories could be repeated in this situation. Also, if multiple biochemical markers of either depression or 'blues' were identified, it would be possible to investigate whether all of the biochemical markers of the mood change occurred in the same individuals. If this was so it would indicate the possibility of one single factor underlying both the mood change and all of the biochemical changes.

However, if the biochemical markers did not occur together then a multiplicity of underlying causative factors would be implicated.

4.2 Protocol.

Sixty-five women attending a routine antenatal clinic at Birmingham Maternity Hospital between February and May 1981 were asked to take part in the study. Criteria for inclusion into the study were that they should be in the final month of pregnancy, between the above mentioned dates and that they should be English Language educated and free from any concurrent medical or psychiatric illness. Those volunteering for the study had the aims and procedures of the study described to them, they then signed the relevant consent form (Appendix VII). The study was described as an investigation of post-natal mood, at no time were 'baby blues' or post-natal depression mentioned.

A brief personal history was then obtained from the volunteers (Methods 4.1). At 36 weeks gestation patients were interviewed in order to ascertain information concerning health and emotions during the pregnancy, they also completed an MAACL and a 30 ml. blood sample was withdrawn from the antecubital vein (Methods 3.1 & 8.1). Then at 38 weeks gestation, a further blood sample was withdrawn and the patients completed an MAACL and a Beck Depression Inventory (BDI) (Methods 3.1). Following parturition, the subjects were visited daily on the post natal ward and on these occasions, a blood sample was withdrawn, a MAACL completed and a brief clinical interview conducted (Methods 3.2). On the third day post-partum, the above procedure was performed, however a BDI was also administered and a 24 hour urine collection was initiated

(Methods 8.2). On the fifth day post-partum, a blood sample was withdrawn, an MAACL administered and a concluding clinical interview was conducted (Methods 3.2).

Information concerning operative procedures, etc. at parturition were obtained from the medical records (Methods 4.1).

Antenatal blood samples were collected between 09.30 - 12.00 hours, following a set breakfast (Methods 5). Post-Natal blood samples were obtained between 08.30 and 09.30, following the same set breakfast. Blood samples were centrifuged and the plasma withdrawn within one hour of collection, they were then stored at -30°C. Plasma samples were later divided into aliquots and distributed to the various laboratories for biochemical analysis. Urine samples were also stored at -30°C. Plasma samples were assayed for free TP, total TP, NEFA, amino acids, progesterone, oestradiol, oestriol and dihydrobiopterin. Antenatal plasma samples were also assayed for cortisol. The 24 hour urine sample was assayed for creatinine and MHPG (See Methods). All biochemical assays were performed blind to the psychiatric data.

At nine months post-partum subjects were visited at their home and a follow-up interview was conducted (Methods 3.2). The follow-up interview was conducted blind with respect to the biochemical data.

4.3 Subject Population Characteristics.

Of the sixty-five women approached, sixty two volunteered to take part in the study. No information at all was collected from the three who declined to take part. Of the remaining sixty-two, one subject withdrew from the study at 39 weeks

gestation, one subject gave birth to a spina bifida infant and was therefore discounted from the study, two more had perinatal complications and were lost to the study, the remaining five subjects were lost due to the hospital failing to notify the author of their delivery, and due to them being moved to a ward different to the usual one. Therefore post-natal data was collected from fifty-three subjects. Only these fifty-three subjects are included in the statistical analyses.

The mean age of the subjects was 28.04 ± 0.63 years (range 17 - 36). All subjects were of British nationality except for one Iranian, one Canadian and one fron Eire. Of the classifiable subjects, 14 were of social class I, 10 were of class II, 15 were of class III, 2 of class IV and 1 of class V. 41 of the subjects were married, 5 were single, 4 were divorced, 1 separated and 2 were cohabiting.

26 subjects were primiparous, 12 were para. 2, 3 were para. 3 and 8 were para. 4 or greater (there was missing data from 4 cases). 19 subjects were primagravida, 16 had one previous pregnancy and 14 had 2 or more pregnancies (there was missing data from 4 cases). The mean length of pregnancy was 39·78 weeks , with a range of 35 - 42 weeks. (Delivery was normally induced if pregnancy extended beyond 42 weeks).

Two subjects delivered by caesarian section, the rest were by vaginal delivery. Forceps were used in 8 cases. Two subjects gave birth to twins, however, in one of the cases, 1 infant died <u>in utero</u>, the other died aged two days. No psychiatric data was collected from this subject. 32 subjects gave birth to male infants and 19 to females (2 were unrecorded). 34 mothers breast fed their infants.

4.4 Subject Population Psychiatric Characteristics.

4.4.1 MAACL Hostility Scores.

Hostility scores were slightly higher at the initial antenatal meeting than on subsequent meetings, however, there was very little variation in the mean post-partum scores (Fig 4.1).

4.4.2 MAACL Anxiety Scores.

Anxiety scores were markedly higher antenatally than post-natally. However, scores were increased again on the fifth day post-partum (Fig 4.1).

4.4.3 MAACL Depression Scores.

There was very little difference in the depression scores antenatally and postnatally (Fig 4.1).

4.4.4 BDI Depression Scores.

Depression scores were markedly higher antenatally than postnatally. Antenatally the mean BDI score was 4.167 ± 0.464 (Range 0 - 11), postnatally the mean score was 2.298 ± 0.336 (range 0 - 11). Significance < 0.001 (Wilcoxon matched pairs test).

4.4.5 Self Reported Symptoms.

22 subjects reported feelings of depression during pregnancy, with a further 2 reporting severe depression. 6 subjects reported elation during pregnancy, with 1 further reporting extreme elation. 3 subjects reported anxiety during pregnancy, however when questioned further, 12 reported apprehension concerning labour and pain with a further 9 reporting other specific anxieties such as fear of hospitals, worries concerning the normality of the baby, etc.

15 subjects reported labile mood during pregnancy with 28 reporting tearfulness.

Post-partum, 1 subject reported crying on day 1, with 2 patients reporting crying on day 2 and 4 and 6 patients reporting crying on days 3 and 4 respectively. 1 subject reported feelings of misery on day 1, with 4, 3 and 4 similar reports on days 2, 3 and 4 respectively.

Only 1 subject reported anger post-natally, that was on day 4. However irritability was more frequently reported with 2 reports on day 2, 1 report on day 3 and 4 reports on day 4.

The most frequently reported emotion was that of elation. There were 8 reports on day 1, with 8, 6 and 3 on respective subsequent days.

When asked if they thought that they had suffered from the 'blues', 14 (33.3%) reported in the affirmative.

At the time of the 9 month follow-up interview, 19 subjects (38.8%) reported feelings of depression post-partum, with an additional 4 subjects reporting severe depression. Of these only 2 had visited a doctor for treatment of the symptoms, and one additional subject was married to a GP who did not treat the depression due to the fact that "post-natal depression is known to be self-limiting".

4.5 Subject Population Biochemical Characteristics.

4.5.1 Plasma Total Tryptophan.

Total TP was lower in pregnancy that at any time post-partum. The levels rose immediately post-partum,

then fell slightly on post-partum days 2 and 3 before rising again on days 4 and 5 (Fig 4.2).

4.5.2 Plasma Free Tryptophan.

This showed the same pattern as total TP (Fig 4.2).

4.5.3 Plasma Non Esterified Fatty Acids.

Plasma NEFA were high during pregnancy, the levels then fell markedly at parturition and continued to fall until day 5 post-partum (Fig 4.3).

4.5.4 Plasma Dihydrobiopterin.

Dyhydrobiopterin did not vary between pregnancy and post-partum (Fig 4.4).

4.5.5 Plasma Oestriol.

Plasma oestriol was high during pregnancy, being in the region of 50 nMol/dm^3. However, post-partum levels were down to approximately 3 nMol/dm^3 by the time of the first post-partum sample, 13.33$\pm$2.27 hours post-partum. These low levels were below the accurate range of the assay.

4.5.6 Plasma Oestradiol.

During pregnancy oestradiol was in the region of 70 nMols/dm^3. This fell to 2nMols/dm^3 by day 1 post-partum and 0.2 nMol/dm^3 by the fifth day post-partum (Fig 4.5).

4.5.7 Plasma Progesterone.

Plasma progesterone reached a level of about 1100 nMol/dm^3 at the end of pregnancy, this fell to about 49 nMol/dm^3 by the first day post-partum and the fall continued until a level of 4.5nMol/dm^3 was

achieved on the fifth day post-partum (Fig 4.5).

4.5.8 Plasma Amino Acids.

All of the amino acids measured exhibited low levels during pregnancy, followed by a rise during the puerperium (Fig 4.6).

4.6 Diagnosis and Definition of 'Blues'.

A global 'blues' score was computed for each subject using their own self reports, the observer rating, the peak post-natal MAACL 'D' score and the post-natal Beck depression inventory score.

The scores assigned for the self-reports of 'blues' were 0 for no 'blues', 1 for mild, 2 for moderate and 3 for severe. The same scoring system was used for the observer rating of 'blues'. Cut off points were used for the mood questionnaires. Following the previous work of Handley and co-workers (1980) a cut off point of 22 or greater was used for the MAACL 'D' score. This corresponded to the 88th percentile. The 88th percentile for the BDI was a score of 4. (Handley and co-workers (1980) previously found that an MAACL 'D' score of 22 corresponded to the 80th percentile, in this present study the 80th percentile MAACL 'D' score was 21 and the 80th percentile BDI score was 4)

Scores of 1 were assigned to subjects scoring above 4 on the BDI or above 22 on the MAACL 'D' scale. Therefore the maximum global 'blues' score possible was 3 for the subject rating, 3 for the observer rating and 1 each from the the MAACL 'D' score and BDI score, hence the total was 8.

From the observer rating of 'blues', a clinically significant degree of 'blues' was seen to correspond to a score of 2.

This observer score of 2 corresponded with a global 'blues' score of 4 or greater. The criterion for a 'blues' case was therefore a global 'blues' score of 4 or greater, subjects scoring 1, 2 or 3 were classified as borderline, and subjects scoring zero on all scales were classified as non-cases.*

This criteria gave 14 (27%) 'blues' cases, 18 (35%) borderline cases and 20 (38%) non-cases. In three instances subjects reported initial weepiness at home immediately after leaving hospital, these were classified as border-line cases. One subject suffering from twin perinatal mortality was excluded from the psychiatric analysis.

4.6.1 Psychiatric Characteristics of the 'Blues'.

'Blues' cases scored more highly on the MAACL Hostility scale on post-partum days 3, 4 and 5, ($p = 0.0214$, 0.0467 and 0.0118 respectively, Mann-Whitney U Test). There were no significant differences antenatally or immediately post-partum. 'Blues' cases also scored significantly more highly on the MAACL anxiety scale at 36 weeks gestation ($p = 0,0334$ Mann-Whitney U) and on days 3 and 5 post-partum ($p=0.0037$ & 0.0202 respectively, Mann-Whitney U).

There were no differences between 'blues' cases and non-cases on the antenatal depression scale scores, however cases scored significantly more highly on

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*NB if an 80th percentile MAACL 'D' score of 21 or above had been used, this would not have affected the final classification of 'blues' cases.

days 2 and 3 post-partum (p=0.031 & 0.0165 respectively Mann-Whitney U). 'Blues' cases also scored more highly on the post-natal Beck Depression Inventory (p= 0.007, Mann-Whitney U), however there was no significant difference between 'blues' cases and non cases on the antenatal BDI scores (Table 4.1).

'Blues' cases also reported tearful episodes during pregnancy more frequently (χ^2, $p = 0.0428$).

4.6.2 Sociological and Personal Correlates of 'Blues'

There were no significant differences between 'blues' cases and non-cases on any of the following variables: Age, Age at menarche, Marital Status, Nationality, Socioeconomic Class, Duration of Pregnancy, Blood loss at delivery, Birth weight of infant, Apgar score of infant, Sex of infant, Reports of previous premenstrual syndrome or menstrual difficulties, Reports of illness during pregnancy, Planning of Pregnancy, Sexual activity during pregnancy, Specific anxieties concerning pregnancy and labour, Considerate nature of husband, Previous history of the 'blues', Previous personal or family psychiatric history or Problems of delivery, ie use of forceps or induced labour.

However, 'blues' cases more frequently reported tears during pregnancy (χ^2 significant, $p = 0.0428$), there were more frequent reports of a previous medical history (usually of a gynaecological nature) (χ^2 significant, $p = 0.0436$). 'Blues' cases were more frequently gravida 1 or 2 (χ^2 significant, $p = 0.0043$) and were more frequently primiparae (χ^2

significant, p=0.0109). 'Blues' cases also more frequently breast-fed their infants (χ^2 significant, p = 0.0267). Of the subjects that had previously used oral contraceptives, the 'blues' non-cases more frequently reported that they stopped using this form of contraception due to side-effects or other reasons (eg religious grounds or 'just not liking taking tablets') than 'blues' cases who generally stopped contraception due to the planning of a pregnancy (p=.03.). Table 4.2.

4.6.3 Biochemical Correlates of 'Blues'. (Table 4.3)

Plasma Total Tryptophan.

There were no differences between 'blues' cases and non cases in antenatal plasma total TP, however, 'blues' cases were found to have significantly decreased plasma total TP on day 1 post-partum (t = 2.68, p = 0.014). (Fig 4.7)

Plasma Free Tryptophan.

There were no significant differences in plasma free TP either antenatally or post-natally, or in free TP expressed as a percentage of total TP, between 'blues' cases and non cases.

Plasma Non Esterified Fatty Acids.

There were no significant differences in plasma NEFA either antenatally or postnatally between 'blues' cases and non cases.

Plasma Dihydrobiopterin.

There was no differences in plasma dihydrobiopterin either antenatally or post natally between 'blues' cases and non cases.

Plasma Oestriol.

There were no differences in antenatal plasma oestriol between 'blues' cases and non cases. Post partum concentrations of oestriol were found to be below the accurate range of the assay.

Plasma Oestradiol.

There were no differences in either antenatal or post-natal oestradiol between 'blues' cases and non cases.

Plasma Progesterone.

There were no significant differences in either antenatal or post natal plasma progesterone between 'blues' cases and non cases. Nor was there any difference in the relative drop of progesterone from the final antenatal sample to the first post-partum sample either between 'blues' cases and non cases or between subjects self-reporting 'blues' and subjects not making such reports.

Antenatal Plasma Cortisol.

There were no differences in antenatal plasma cortisol between 'blues' cases and non cases.

Urinary 3-Methoxy-4-hydroxy phenyl glycol.

There were no differences in the post-partum day 3 24hour urine MHPG values expressed as either mg/g creatinine, or mg/unit volume urine, between the 'blues' cases and non cases.

Plasma Amino Acids (Other than Tryptophan).

'Blues' cases were found to exhibit increased plasma glycine at 38 weeks of pregnancy ($p = .027$) and on

day 4 post-partum ($p = .004$). Plasma alanine was also found to be increased in 'blues' cases at 38 weeks of pregnancy ($p = .045$). There were no other significant differences in plasma amino acids between 'blues' cases and non cases, and there was no difference in the ratio of TP to the other LNAA at any time point, between 'blues' cases and non cases.

4.7 Diagnosis and Definition of Post-Partum Depression.

Subjects were interviewed nine months post-partum and retrospective information concerning moods and emotions, etc. since the delivery was collected (See Methods 3.2). Notes were made at the time of the interview and subjects were classified as being either depressed, non depressed or border-line, depending on their presentation of the clinical symptoms of depression such as appetite loss, sleep disturbance, loss of libido, depressed affect, etc.
The interview reports were then classified into case, non case or border-line by the author and independently by the psychiatrist, Dr Gillian Waldron. On only three occasions did the diagnoses differ, these were differences between case and border-line or non case and border-line. These anomalies were resolved by discussion, with two being classified as border-line and 1 classified as a case.
40 subjects (75.5%) were visited at their homes for the interviews. A further 9 subjects (17%) could not be visited, therefore they completed a postal questionnaire (Appendix VIII). 4 subjects were not followed up. Of these, one was the case of the twins dying perinatally, one subject returned to

Canada and could not be traced, the remaining two subjects had left the area and could not be traced from hospital records or electoral register. Therefore a total of 92.5% of subjects were followed up at 9 months post-partum.

Of these 49 subjects, 26 (53.1%) were classified as non cases, 12(24.5%) were classified as border-line and 11 (22.4%) were classified as having been depressed.

The most commonly reported time of depression was 12 weeks post-partum (mean 13.6 $\pm$ 3.15 weeks). The mean duration of the depression was 7.125 $\pm$ 2.84 weeks.

4.7.1 Psychiatric Characteristics of Depression. (Table 4.4) There were no significant differences between depression cases and non cases on any of the MAACL scales or the BDI questionnaire either antenatally or puerperally.

4.7.2 Sociological and Personal Correlates of Depression. None of the following factors showed any differences between depression cases and non cases : Age, Marital status, Nationality, Socioeconomic Class, Parity, Gravidity, Duration of Pregnancy, Planning of Pregnancy, Previous use of Oral Contraception, Previous Menstrual Problems, Route of Delivery, Use of Forceps, Induction of Labour, Blood loss at Delivery, Birthweight of infant, Apgar score of infant, Mode of Feeding, Emotional and Physical Symptoms during Pregnancy, Previous Psychiatric History, Family medical and psychiatric history,

Helpfulness of husband, Sexual activity and Marital relations post-partum, Incidence of transient post-partum mood change (ie 'blues').

However, differences were seen in the following variables : Depression cases reported decreased sexual activity during pregnancy more frequently (Fisher's Exact p = 0.0245). Depression cases reported a greater incidence of a previous medical history (usually of a gynaecological nature, ie treatment for infertility or D & C) (χ^2 , p = .0005). Depression cases had also given birth to male infants more frequently (χ^2, p = 0.0407). Depression cases also reported a higher age of menarche than non cases (Cases : 13.73±0.30 years; Non cases : 12.31±0.28 years; p = .005). (Table 4.5)

4.7.3 Biochemical Correlates of Depression. (Table 4.6)

Plasma Total Tryptophan.

There were no differences in plasma total TP between depression cases and non cases at any point either antenatally or post-partum.

Plasma Free Tryptophan.

There were no differences in plasma free TP between depression cases and non cases antenatally, however, depressed cases were found to have significantly increased plasma free TP on the fifth day post-partum, t = 3.41, p = 0.008. (Fig 4.8). There were no differences in free TP, expressed as a percentage of total TP, between depression cases

and non cases.

Plasma Non Esterified Fatty Acids.

There were no significant differences in plasma NEFA between depression cases and non cases antenatally. However post-partum depressed cases were found to have significantly increased plasma NEFA on day 3 post-partum, $t = 2.51$, $p = 0.017$. (Fig 4.9)

Plasma Dihydrobiopterin.

There were no significant differences in plasma dihydrobiopterin at any point either antenatally or post natally between depression cases and non cases.

Plasma Oestriol.

There were no differences in plasma oestriol between depression cases and non cases antenatally. Due to the low concentrations of oestriol present in the plasma post-partum, these measurements were not performed as they were below the accurate range of the assay.

Plasma Oestradiol.

There were no differences in plasma oestradiol, either antenatally or post-natally, between depression cases and non cases.

Plasma Progesterone.

There were no differences in plasma progesterone, either antenatally, or post natally, or in the relative fall of plasma progesterone between the second antenatal sample and the day 1 post-partum sample, between depressed and non-depressed subjects.

Antenatal Plasma Cortisol.

There were no differences in the antenatal plasma cortisol concentrations between depression cases and non cases.

Urinary 3-Methoxy-4-hydroxy phenyl glycol.

The urine MHPG on day 3 post-partum was found to be significantly increased in depressed subjects, when expressed as μg per unit volume ($t = 2.35$, $p = 0.037$). This was not so if the MHPG was expressed as μg/g creatinine. However urine creatinine was found to be significantly increased in subjects who went on to become depressed ($t = 2.66$, $p = 0.021$).

Plasma Amino Acids (other than Tryptophan).

Subjects that became depressed post-partum were found to exhibit increased plasma threonine at 36 weeks of pregnancy ($p = .030$) and on the first day post-partum ($p = .049$). Isoleucine was also found to be increased on day 4 post-partum ($p = .008$) in depression cases. There were no other differences in any of the plasma amino acids between depression cases and non cases, nor was there any difference in the ratio of TP to the other LNAA between depression cases and non cases.

4.8 Characteristics of Tryptophan 'Risers' and 'Non Risers'.

Plasma total TP is low during pregnancy, but rises immediately post-partum (Handley et al., 1980). A small subgroup of subjects however fail to show the early rise in total TP, the rise being delayed until after the second day post-partum.

(See Introduction).

From the plasma total TP profiles for each case, subjects were classified as being either TP risers, TP non risers, or unclassifiable, by Dr S L Handley. These classifications were made blind with respect to any other biochemical or psychiatric data.

24 subjects were classified as risers, 9 subjects were classified as non-risers, the remainder could not be classified due to missing data.

Other variables were compared for risers and non risers.

4.8.1 Biochemical Correlates of the Tryptophan Rise. (Table 4.7)

Plasma Total Tryptophan.

TP non risers were found to have significantly increased plasma total TP at 38 weeks of gestation ($t = 3.57$, $p = 0.002$). Post-partum, on day 1 non risers had decreased total TP, this difference was not significant ($t = 1.73$, $p = 0.093$). On days 2 and 3 however, non risers had significantly decreased plasma total TP ($t = 3.91, 2.85$, $p = >0.001$ and 0.008 respectively). There were no significant differences between the groups on days 4 or 5 post-partum.

Plasma Free Tryptophan.

TP non risers were found to have significantly increased plasma free TP at 36 weeks gestation ($t = 3.04$, $p = 0.005$). There were no differences between the groups post-partum, except that free TP, expressed as a percentage of total TP was found to be significantly increased in non-risers on post-partum day 2 ($t = 2.08$, $p = .046$).

Plasma Non-Esterified Fatty Acids.

There were no differences in plasma NEFA between risers and non-risers antenatally, however, post-partum, non risers were found to have decreased fatty acids, these differences were significant on day 1 ($t = 2.35$, $p = 0.026$) and on day 3 ($t = 2.19$, $p = 0.037$).

Plasma Dihydrobiopterin.

There were no differences in plasma dihydrobiopterin between risers and non risers either antenatally or post-natally.

Plasma Oestriol.

There were no differences in antenatal oestriol between risers and non risers, post-partum oestriol concentrations were outside the accurate range of the assay used.

Plasma Oestradiol.

There were no differences in plasma oestradiol between risers and non risers either antenatally or post-partum.

Plasma Progesterone.

There were no differences in plasma progesterone between risers and non risers either antenatally or post-partum.

Antenatal Plasma Cortisol.

There were no differences in antenatal plasma cortisol between risers and non risers.

Urinary 3-Methoxy-4-hydroxy phenyl glycol.

There were no differences in the third day post-partum 24 hour urine MHPG between risers and non risers.

Plasma Amino-Acids (other than tryptophan).

TP non risers were found to exhibit significantly decreased valine and phenylalanine on day one post-partum ($p = .016$ & $.038$ respectively) and to show decreased tyrosine and phenylalanine on day 2 post-partum ($p = .016$ & $.022$ respectively).

All other differences were non-significant.

TP non risers also showed an increased ratio of TP to the other LNAA at 38 weeks of pregnancy ($p = .041$).

4.8.2 Psychiatric Characteristics of Tryptophan 'non risers'

There were no significant differences between TP 'risers' and non risers on any of the psychiatric questionnaire scales either antenatally or post-partum (Mann-Whitney U). Nor was there any significant increase in the incidence of 'blues' or depression between TP 'risers' and 'non risers'. (Table 4.8).

4.8.3 Sociological and Personal Characteristics of Tryptophan 'non risers'

There were no differences between TP 'risers' and 'non risers' on any of the following variables :

Age, Age of menarche, Parity, Gravidity, Nationality, Socioeconomic class, Previous medical or psychiatric history, family medical or psychiatric history,

Previous use of oral contraceptives, Duration of Pregnancy, Use of forceps or Induction of Labour, Blood loss at delivery, Hour of delivery (with respect to hour of blood sampling) or sex of infant. TP non risers reported a higher proportion of irregular menstruation ($p = .03$), also non risers more frequently bottle-fed their infants ($p = .02$) relative to TP risers. (Table 4.9).

4.9 Comparison of Primiparae with Multiparae.

There were no significant differences in any of the psychiatric questionnaire scores for any individual time point either antenatally or post natally, between primiparae and multiparae. However, primiparae showed a significantly greater incidence of 'blues' ($p = 0.0109$).

Primiparae were seen to exhibit significantly increased plasma total TP at 36 weeks of pregnancy ($p = .001$), and on day 2 post-partum ($p = .048$) and increased phenylalanine at 36 weeks pregnancy ($p = .036$). Primiparae also exhibited decreased alanine on day 2 post-partum ($p = .039$) and on day 3 post-partum ($p = .001$), all other biochemical differences between primiparae amd multiparae were non significant. (Table 4.10).

4 10 Comparison of Mothers of Male Infants with Mothers of Female Infants.

Male infants were found to be of a significantly greater birth weight ($p = .047$). Mothers giving birth to male infants were found to exhibit significantly increased glutamic acid at 36 weeks of pregnancy ($p = .031$), decreased glycine at 36 weeks of pregnancy ($p = .008$), increased proline and tyrosine

on day 1 post-partum (p = .019 & .035 respectively), and decreased NEFA on day 4 post-partum (p = .026). All other biochemical differences were non-significant. Mothers giving birth to male infants were seen to exhibit a greater incidence of depression post-partum (p = .0407). (Table 4.11)

4.11 Characteristics of Subjects Seeking Medical Assistance for Depression Post-Partum.

Two subjects were treated for depression post-partum, and one further subject was married to a GP who decided not to treat the illness due to its self-limiting nature. Two of these subjects were TP risers, with the third being unclassified. These 3 subjects also exhibited significantly increased plasma cortisol at 36 weeks gestation (t = 2.66, p = .011); increased alanine on post-partum day 2 and valine on post-partum day 3 (t = 2.37, p = .023 & t = 2.85, p = .008 respectively) and relatively increased oestradiol on post-partum days 2 and 3 (t = 2.66, p = .011 & t = 2.12, p = .04 respectively). 1 of these subjects was a 'blues' case, the other two were non cases.

4.12 Interrelationship Between Biochemical Variables at Parturition.

Total TP was found to be significantly correlated with the other LNAA at 38 weeks of gestation (r = 0.4511, p = .018, 1 tailed (n = 22)). There was no significant correlation between these two parameters at any other time point. Total TP was also significantly correlated with oestradiol on post-partum day 3 (r = 0.3353, p = .024, 2 tailed (n = 22)). There was no relationship between these variables at any other time point. There was no significant relationship between total TP

and either progesterone or NEFA at any time point. Similarly there was no relationship between free TP and either oestradiol, progesterone or NEFA . Plasma progesterone was significantly correlated with plasma oestradiol on post-partum day 1 ($r = 0.6384$, $p = .001$ ($n = 40$)) and on day 2 ($r = 0.2987$, $p = .046$ ($n = 45$)), there was no significant correlation between these variables at any other time point (Table 4.12).

Fig 4·1 Puerperal MAACL scores

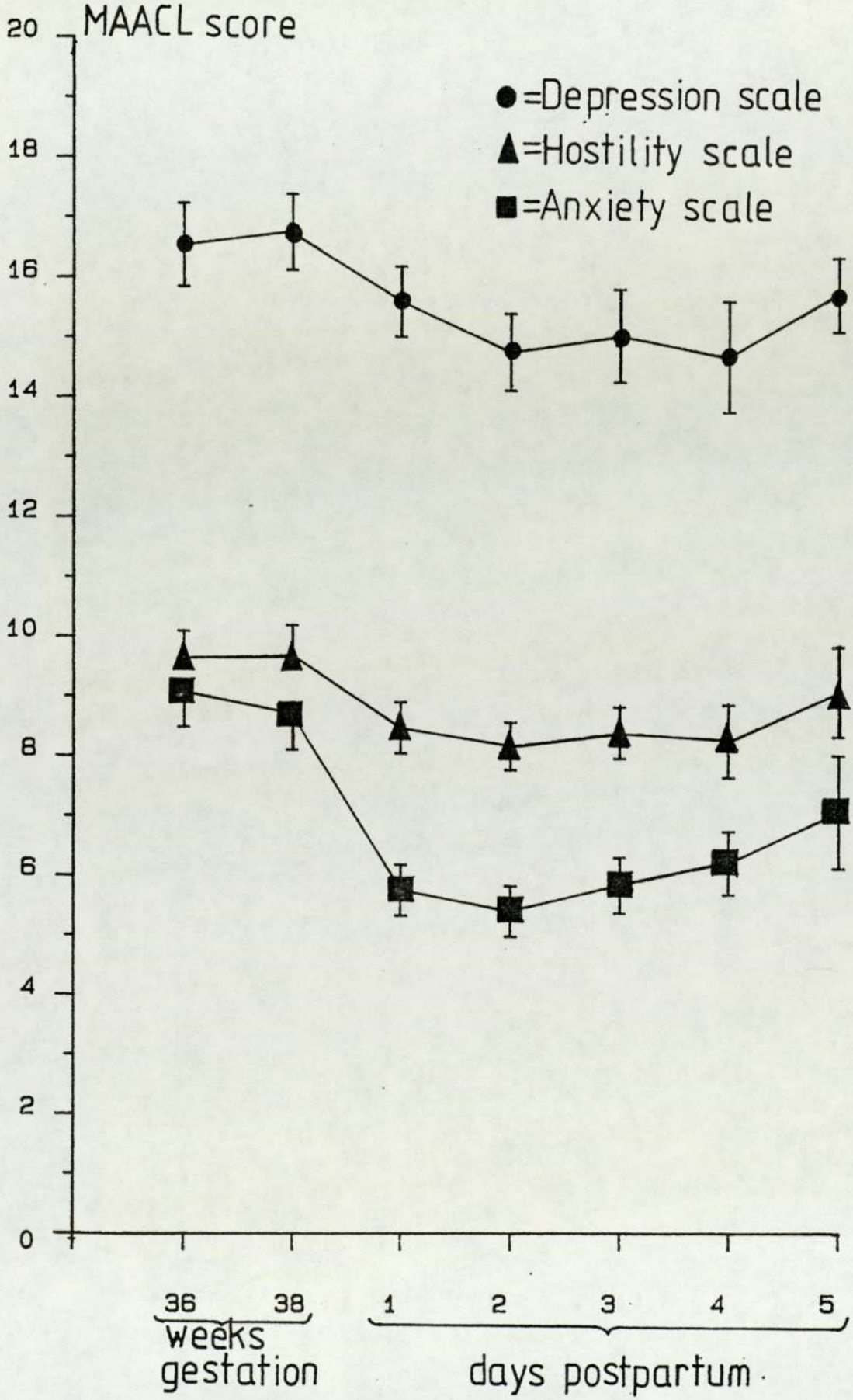


Fig 4·2 Variation of plasma TP at parturition

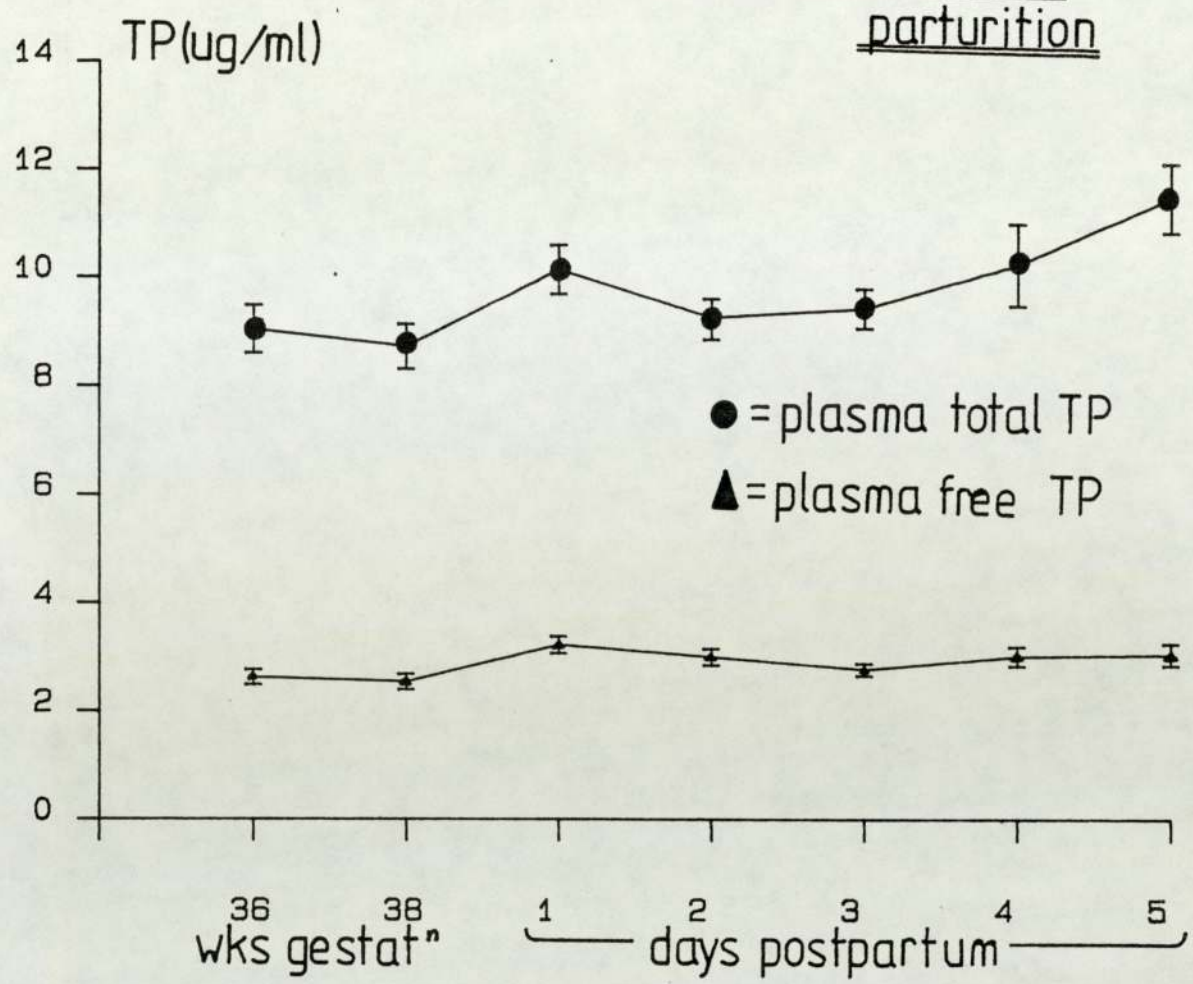


Fig 4·3 Variation of NEFA at parturition

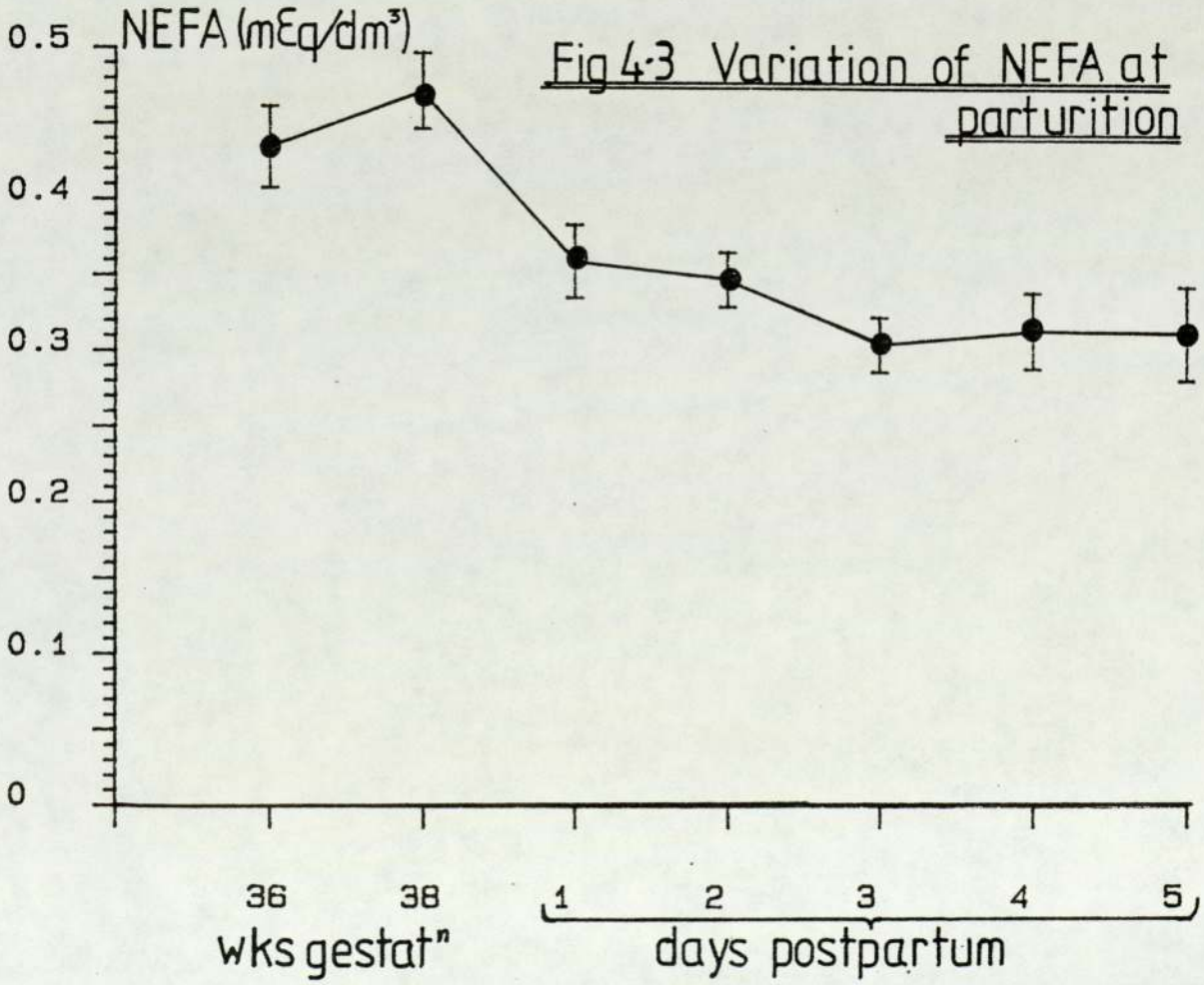


Fig 4·4 Plasma dihydrobiopterin profile at parturition

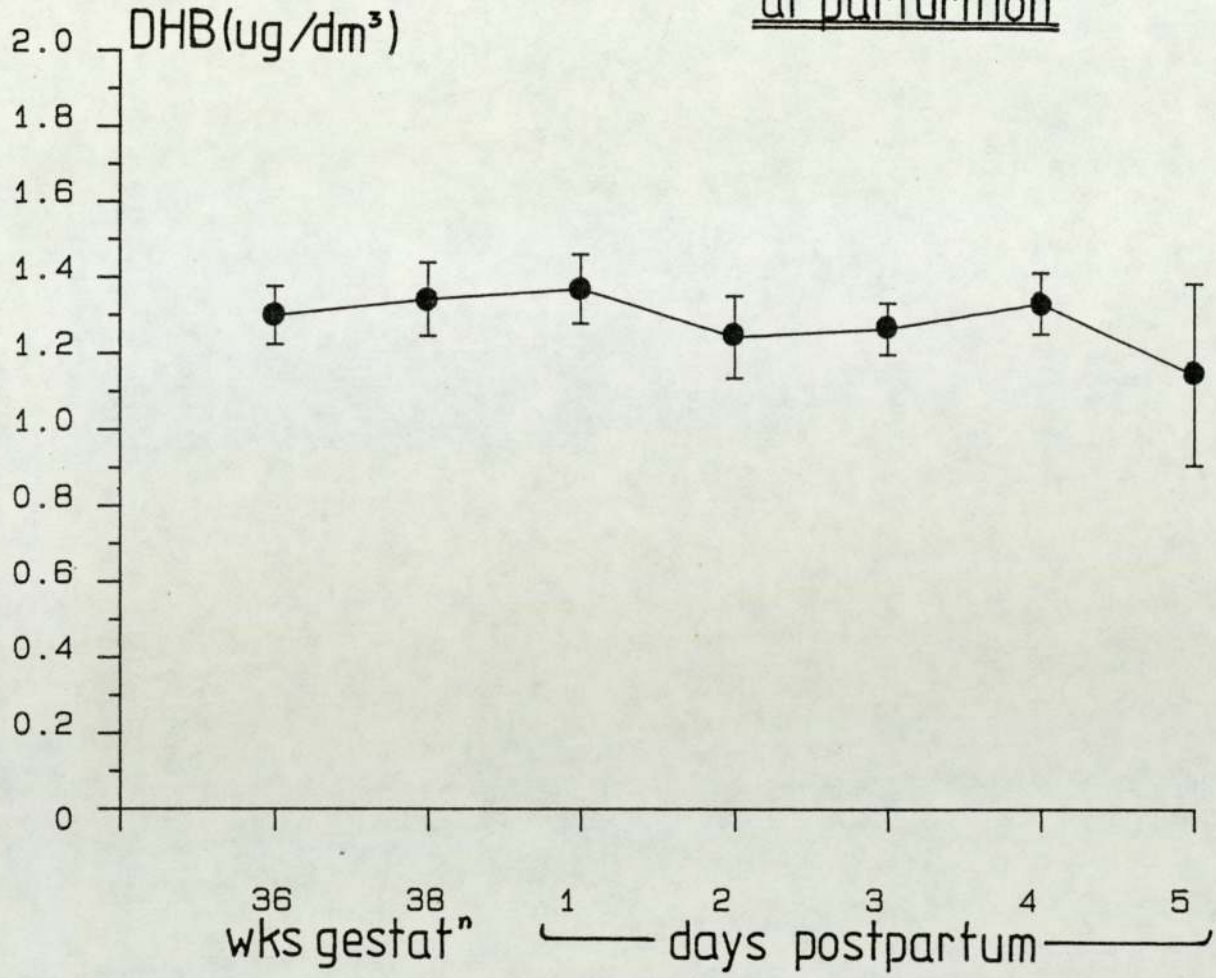


Fig 4·5 Variations of plasma progesterone & oestradiol at parturition

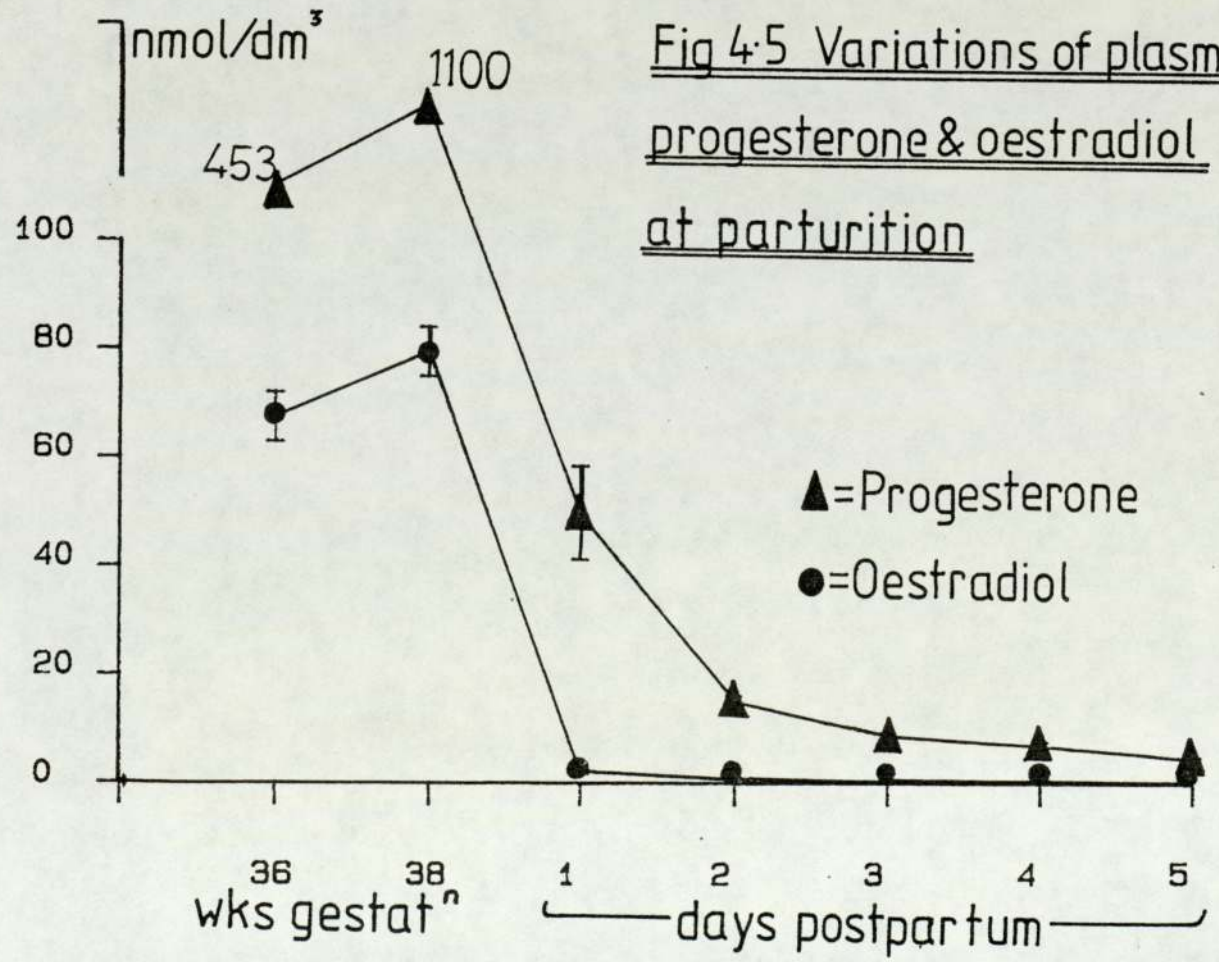


Fig 4·6 Plasma amino acid profiles at parturition

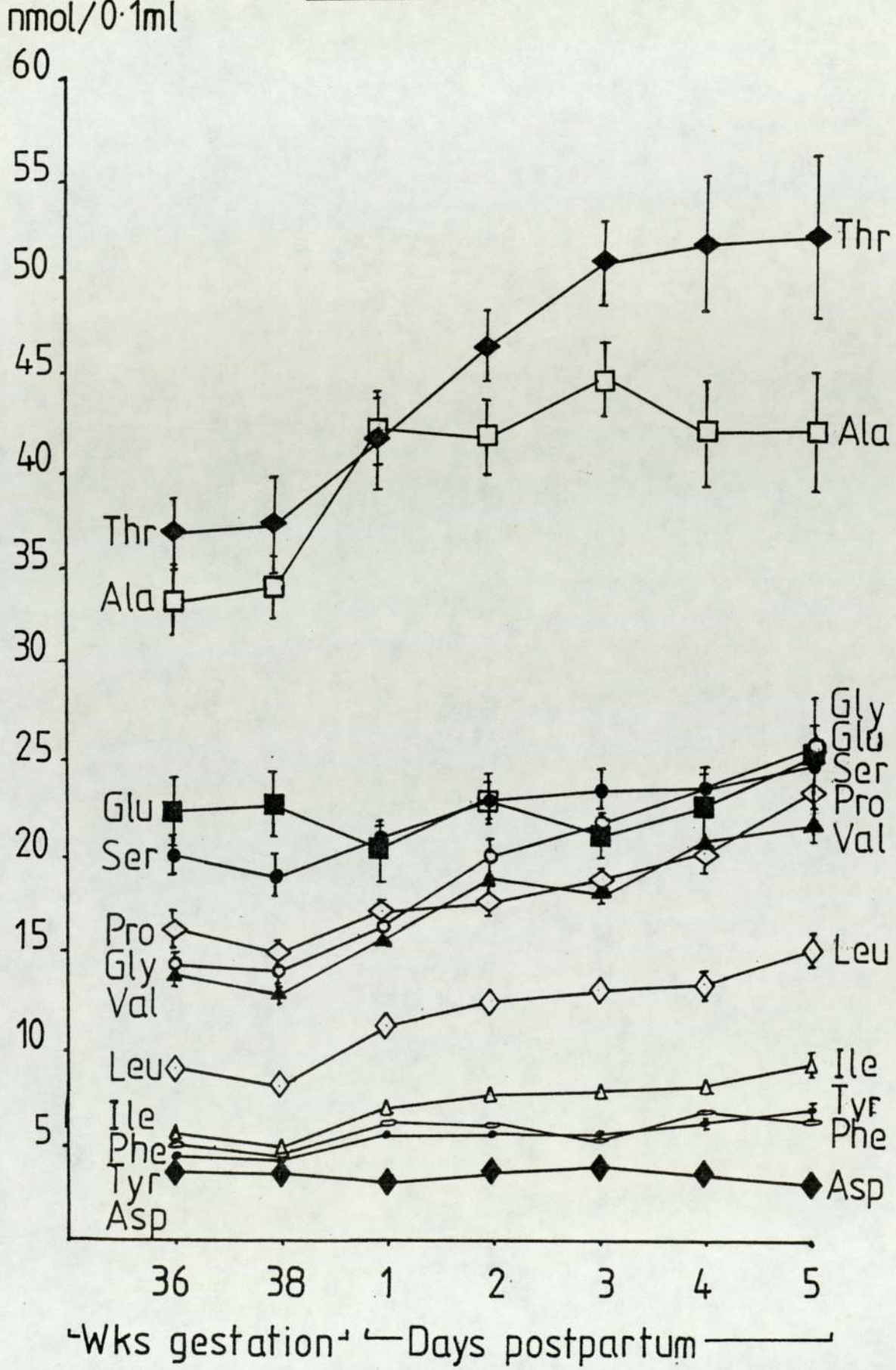


Fig 4·7 Relationship of total TP to 'blues'

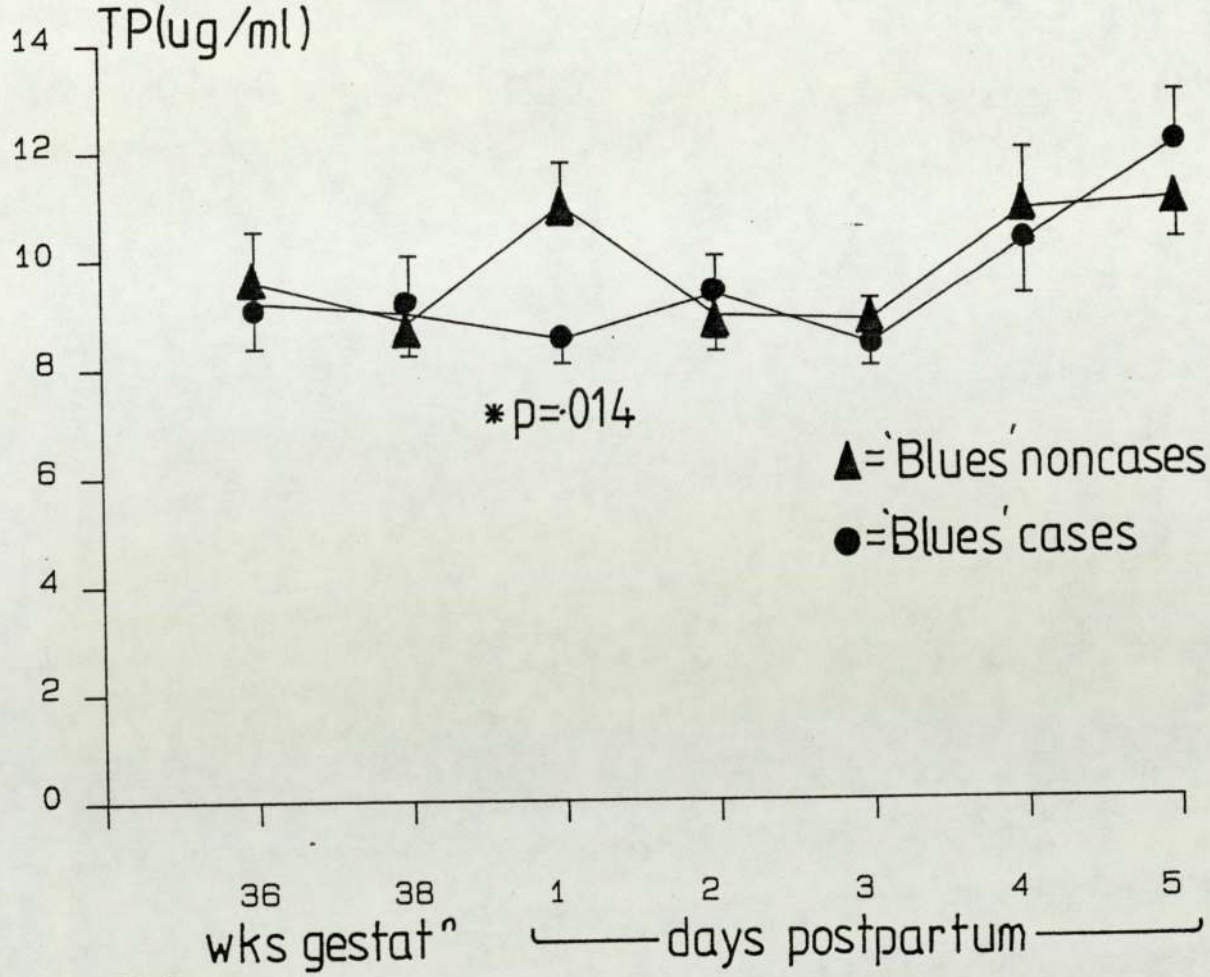


Fig 4·8 Relationship of free TP to depression

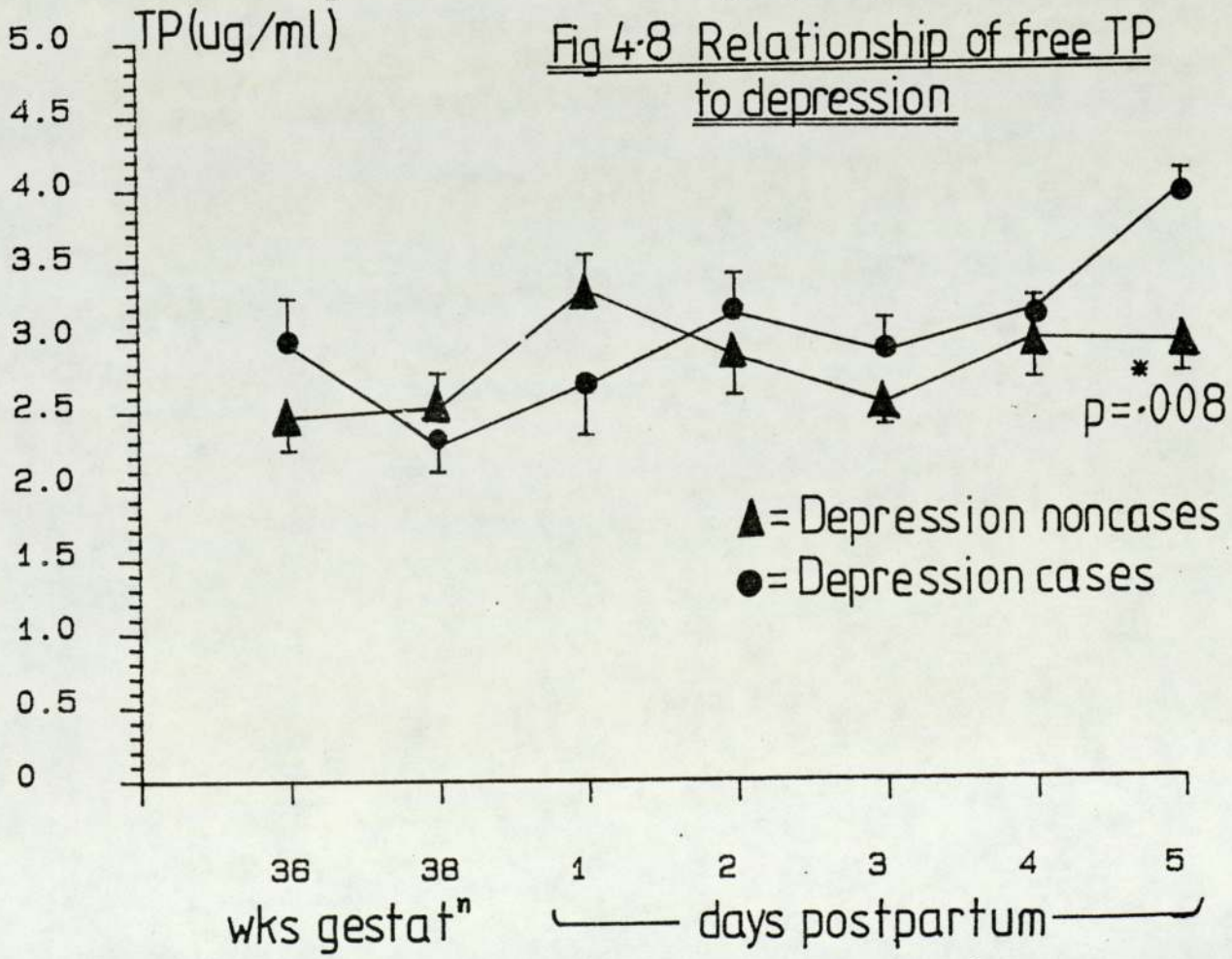
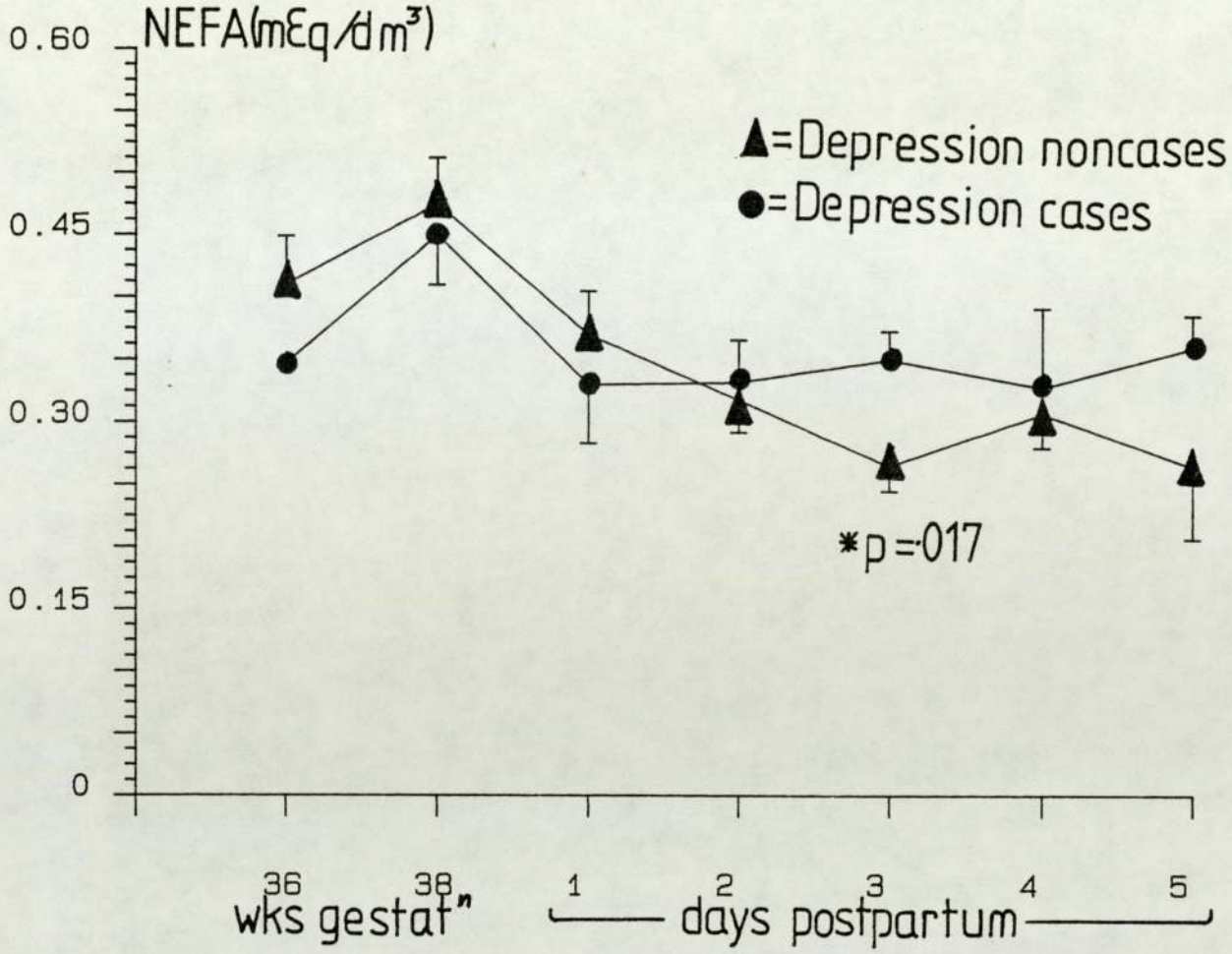


Fig 4·9 Relation of NEFA to depression



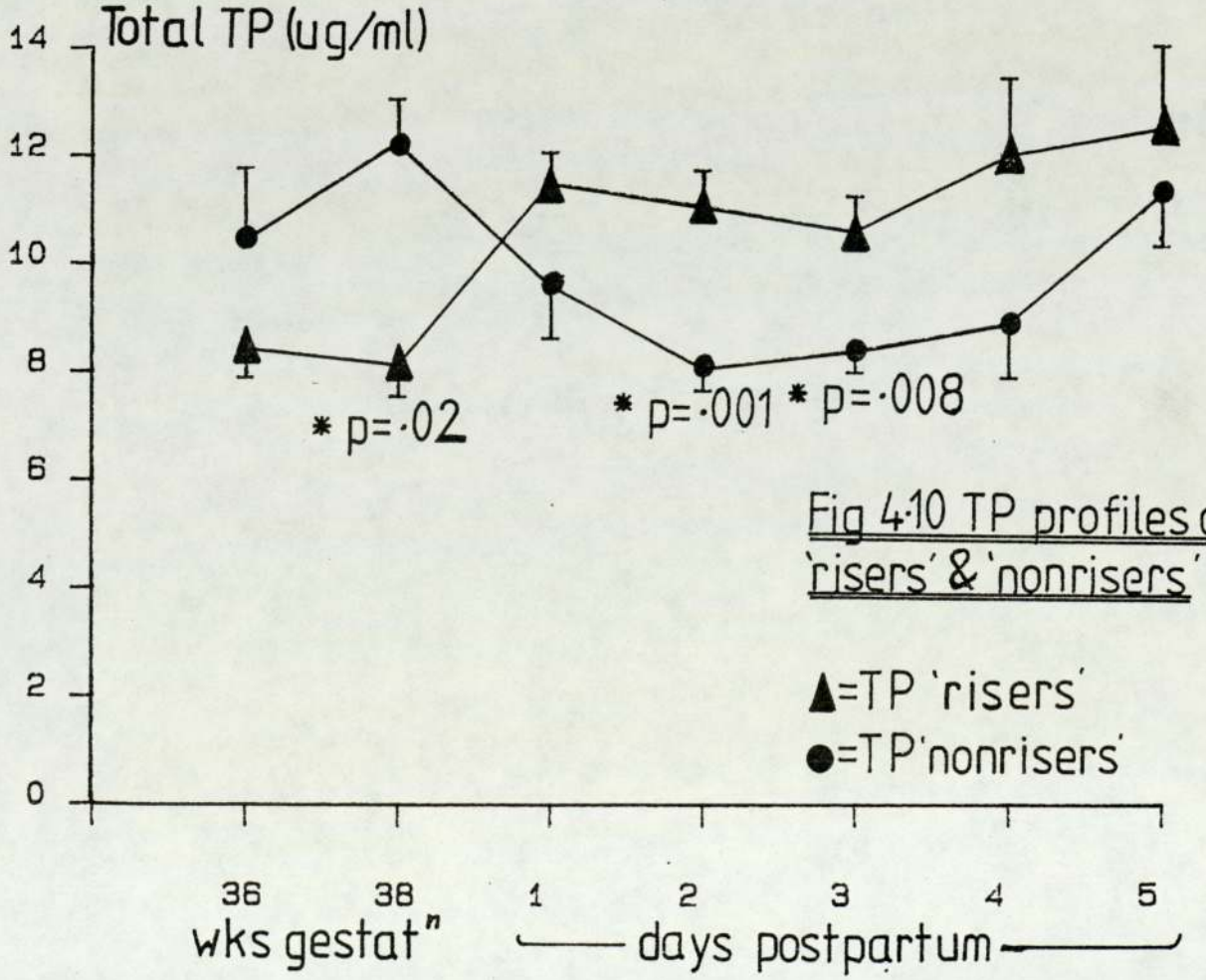


Fig 4·10 TP profiles of 'risers' & 'nonrisers'

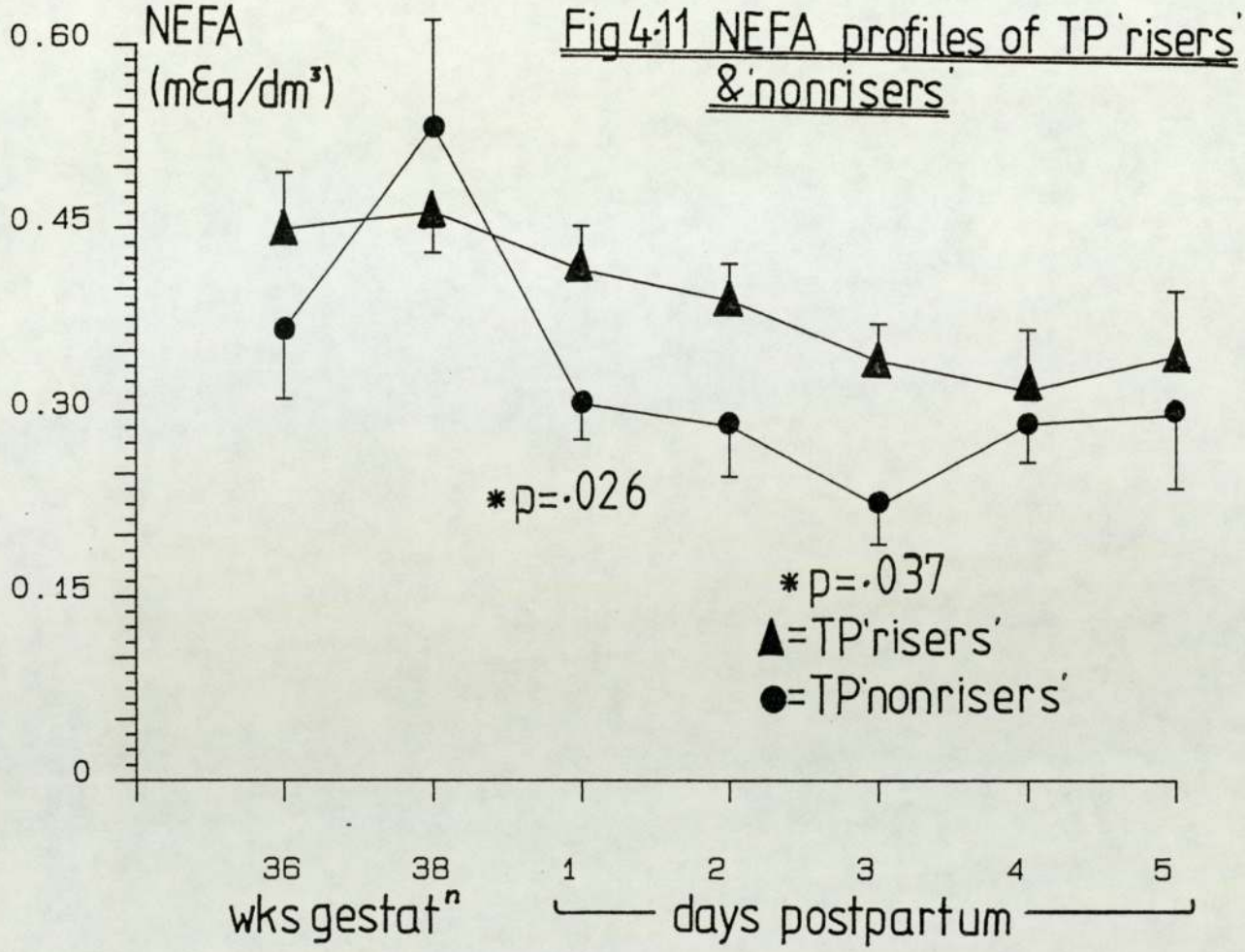


Fig 4.11 NEFA profiles of TP 'risers' & 'nonrisers'

TABLE 4.1 Comparison of mood scale scores between 'blues' cases and noncases.

Variable	Time	Mean rank(N)		Mann-whitney	2-tailed
		cases	noncases	U score	probability
MAACL'A'	AN1	15.92(13)	13.27(15)	79.0	.3880
	AN2	12.28(9)	11.82(14)	60.5	.8730
	PN1	14.00(12)	13.07(14)	78.0	.7557
	PN2	20.00(14)	14.79(19)	91.0	.1220
	PN3	19.43(14)	12.06(16)	57.0	.0214
	PN4	11.04(13)	5.50(5)	12.5	.0467
	PN5	8.00(9)	2.00(3)	00.0	.0118
MAACL'H'	AN1	12.30(15)	17.04(13)	64.5	.0334
	AN2	15.72(9)	9.61(14)	29.5	.0334
	PN1	15.63(12)	11.68(14)	58.5	.1835
	PN2	20.29(14)	14.58(19)	87.0	.0912
	PN3	20.43(14)	11.19(16)	43.0	.0037
	PN4	10.69(13)	6.40(5)	17.0	.1234
	PN5	7.89(9)	2.33(3)	1.0	.0202
MAACL'D'	AN1	14.77(13)	14.27(15)	94.0	.8714
	AN2	12.61(9)	11.61(14)	57.5	.7277
	PN1	14.88(12)	12.32(14)	67.5	.3927
	PN2	21.21(14)	13.89(19)	74.0	.0310
	PN3	19.61(14)	11.91(16)	54.5	.0165
	PN4	10.58(13)	6.70(5)	18.5	.1660
	PN5	7.67(9)	3.00(3)	3.0	.0514
BDI	AN	11.31(8)	9.96(9)	41.5	.6095
	PN	19.32(14)	10.97(15)	44.5	.0070

Table 4.2 Comparison of sociological variables between 'blues' cases and noncases.

	'Blues' cases (Number of samples in parentheses)	'Blues' noncases	
Age	26.57+/-1.49 (19)	28.53+/-0.97 (14)	t=1.14 p=.262
Age of menarche	12.57+/-0.39 (14)	12.74+/-0.34 (19)	t=0.32 p=.752
Duration of pregnancy (wks)	39.43+/-0.44 (14)	39.94+/-0.41 (17)	t=0.85 p=.401
Blood loss at delivery (ml)	345+/-55 (11)	329+/-67 (12)	t=0.19 p=.852
Birth weight of infant (g)	3175+/-213 (14)	3422+/-150 (17)	t=0.97 p=.401
Apgar score	9.29+/-.13 (14)	9.18+/-.13 (17)	t=0.60 p=.552

C:'Blues' cases NC:'Blues' noncases

Marital Status

	Single	Married	Divorced	Cohabiting
C	2	13	2	2
NC	2	17	1	0

$X^2 = 1.78, p=.62$

Socioeconomic Class

	I	II	III	IV	V
C	3	2	7	0	1
NC	5	3	2	2	0

$X^2 = 6.45, p=.17$

Gravidity

	1	2	>3
C	4	5	10
NC	8	6	0

$X^2 = 10.92, p=.004$

Parity

	0	1	2	>3
C	12	2	0	0
NC	6	4	3	6

$X^2 = 11.17, p=.01$

Nationality

	GB	Other
C	13	1
NC	18	1

$X^2 = .05, p=.82$

Sex of infant

	M	F
C	7	7
NC	11	7

$X^2 = .40, p=.53$

Table 4.2 Comparison of sociological variables between 'blues' cases and noncases.

Previous PMS

	No	Yes
C	9	5
NC	13	6

X^2=.06, p=.80

Previous dysmenorrhoea

	No	Yes
C	18	1
NC	11	3

X^2=2.0, p=.16

Previous irregular menstruation

	No	Yes
C	10	4
NC	14	5

X^2=.02, .89

Previous use of oral contraception

	Never	>6mnth ago	<6mnth ago
C	2	7	5
NC	2	11	6

X^2=.23, .89

Reason for stopping use of oral contraceptives

	N/A	Side effects	Planned Preg	Other
C	3	2	9	0
NC	2	6	5	6

X^2= 8.78, .03

Physical fitness during preg.

	Good	Poor
C	3	11
NC	9	10

X^2=2.34, p=.13

Depression during preg.

	No	Yes
C	7	7
NC	10	8

X^2=.08, .75

Tearfulness during preg.

	No	Yes
C	3	11
NC	12	7

X^2=4.1, p=.043

Sexual activity during preg.

	NC	Decreased
C	3	3
NC	3	5

Fishers exact= .53

Planning of pregnancy

	Planned	No precautions	Inadequate prec.
C	11	2	1
NC	9	7	3

X^2=3.3, p= .19

Table 4.2 Comparison of sociological variables between 'blues' cases and noncases.

Mode of feeding

	Breast	Bottle	Mixed
C	12	0	0
NC	10	5	0

$X^2 = 4.91$, p=.027

Previous Medical History

	Non relevant	Mod.	Sev^r.
C	6	6	2
NC	16	2	1

$X^2 = 6.27$, p=.04

Previous Psychiatric History

	No	Yes
C	13	1
NC	16	3

$X^2 = .57$, p=.45

Family psychiatric history(affective)

	No	Yes
C	14	0
NC	18	1

$X^2 = .76$, p=.38

Induced Labour

	No	Yes
C	12	2
NC	15	3

$X^2 = .03$, p=.85

Use of Forceps

	No	Yes
C	11	3
NC	16	2

$X^2 = .64$, p=.43

Table 4.3 Comparison of biochemical variables between 'blues' cases and non-cases.

Variable	Time	Mean +/- SEM (N)				Student's t	2-tailed prob.
		cases		noncases			
		mean	SEM	mean	SEM		
Total TP (ug/ml)	AN1	9.20	0.83(13)	9.58	0.94(14)	0.300	.761
	AN2	8.99	1.07(9)	8.85	0.63(15)	0.120	.903
	PN1	8.52	0.45(12)	10.97	0.80(15)	2.68	.014
	PN2	9.33	0.07(14)	8.92	0.65(19)	0.420	.679
	PN3	8.39	0.41(14)	8.83	0.40(15)	0.76	.454
	PN4	10.28	0.99(12)	10.86	1.90(5)	0.30	.770
	PN5	12.08	0.96(8)	11.05	0.75(2)	0.51	.625
Free TP (ug/ml)	AN1	2.862	.262(13)	2.639	.213(13)	0.66	.515
	AN2	2.400	.212(9)	2.671	.295(14)	0.67	.512
	PN1	3.000	.292(12)	3.367	.232(15)	1.00	.328
	PN2	3.071	.248(14)	2.916	.212(19)	0.48	.174
	PN3	2.550	.224(14)	3.013	.243(15)	1.40	.174
	PN4	3.208	.286(12)	3.520	.188(5)	0.67	.514
	PN5	3.325	.251(8)	3.500	.800(2)	0.29	.782
NEFA (mEq/ml)	AN1	.4041	.038(13)	.3982	.045(13)	0.10	.921
	AN2	.5051	.063(11)	.4372	.035(15)	1.01	.323
	PN1	.3548	.036(12)	.4063	.049(15)	0.82	.422
	PN2	.3525	.036(14)	.3526	.031(19)	0.00	.998
	PN3	.3391	.033(16)	.2663	.024(16)	1.83	.078
	PN4	.3323	.035(12)	.3124	.030(5)	0.34	.738
	PN5	.3160	.038(7)	.3315	.038(2)	0.21	.841
DHB (ug/dm^3)	AN1	1.354	.153(11)	1.111	.162(9)	0.64	.530
	AN2	1.533	.182(6)	1.344	.151(9)	0.80	.440
	PN1	1.473	.208(11)	1.480	.136(10)	0.03	.977
	PN2	0.978	.076(9)	1.173	.096(11)	1.53	.142
	PN3	1.289	.114(9)	1.223	.145(11)	0.32	.750
	PN4	1.222	.109(9)	1.267	.158(6)	0.24	.814
Oestriol ($nmol/dm^3$)	AN1	40.70	7.66(10)	45.82	5.18(11)	0.56	.580
	AN2	49.60	4.24(5)	49.31	3.79(13)	0.04	.966
Oestradiol ($nmol/dm^3$)	AN1	74.00	9.45(13)	69.40	7.56(10)	0.36	.720
	AN2	81.00	2.89(3)	80.64	6.16(14)	0.03	.980
	PN1	2.64	1.01(12)	2.64	0.93(13)	0.01	.996
	PN2	0.75	0.15(12)	0.58	0.07(17)	1.05	.309
	PN3	0.45	0.07(13)	0.39	0.04(14)	0.86	.395
	PN4	0.36	0.05(12)	0.27	0.02(5)	1.63	.124
	PN5	0.33	0.06(8)	0.17	0.00(1)	0.94	.380

Table 4.3 Comparison of biochemical variables between 'blues' cases and noncases.

Variable	Time	Mean +/- SEM(N)				Student t	2-tailed prob.
		cases		noncases			
		mean	SEM.	mean	SEM		
Progesterone ($nmol/dm^3$)	AN1	484.2	48.5(12)	409.0	38.1(10)	1.18	.251
	AN2	560.0	87.2(3)	906.7	313.(12)	1.07	.307
	PN1	49.8	12.2(12)	39.62	10.7(13)	0.63	.533
	PN2	17.3	2.01(13)	14.71	2.11(17)	0.87	.393
	PN3	9.1	0.76(13)	7.86	0.65(14)	1.30	.205
	PN4	6.3	0.56(12)	5.80	1.39(5)	0.43	.673
	PN5	4.8	0.48(8)	3.00	0.00(1)	1.30	.234
Relative fall of progesterone at parturition:		77.29	69.0(14)	545.5	221.0(19)	2.02	.057
ASP (nmol/0.1ml)	AN1	2.71	.56(7)	3.21	.18(10)	0.83	.434
	AN2	4.25	1.73(4)	2.71	.36(9)	0.88	.445
	PN1	2.73	.47(13)	2.89	.32(10)	0.26	.799
	PN2	2.91	.45(12)	3.18	.32(15)	0.50	.627
	PN3	3.01	.59(9)	3.38	.39(14)	0.55	.590
	PN4	2.96	.38(10)	2.02	.03(4)	2.47	.036
THR (nmol/.1ml)	AN1	42.20	4.23(7)	33.80	2.43(10)	1.84	.085
	AN2	44.83	7.29(4)	30.94	3.09(10)	2.10	.057
	PN1	43.10	3.41(12)	38.40	2.27	1.10	.285
	PN2	43.59	3.66(12)	46.33	3.47(14)	.54	.592
	PN3	50.48	4.00(10)	50.06	3.96(12)	.07	.941
	PN4	53.71	5.79(11)	51.94	5.77(3)	.15	.883
SER (nmol/.1ml)	AN1	21.00	2.27(7)	18.46	.61(9)	1.10	.306
	AN2	24.03	3.77(4)	16.31	.86(9)	2.00	.139
	PN1	20.86	2.02(12)	21.63	1.33(10)	.30	.746
	PN2	22.83	1.67(12)	22.46	1.10(14)	.22	.828
	PN3	22.46	1.54(10)	22.54	1.26(12)	.05	.961
	PN4	24.53	1.33(11)	24.46	3.64(3)	.02	.983
GLU (nmol/.1ml)	AN1	17.77	3.4(7)	24.75	3.0(9)	1.54	.147
	AN2	24.33	5.92(4)	23.35	2.12(9)	.20	.845
	PN1	19.93	2.56(12)	21.23	2.40(10)	.36	.719
	PN2	19.45	2.3(12)	23.07	2.22(14)	1.13	.270
	PN3	19.84	2.83(10)	22.05	2.37(12)	.60	.552
	PN4	24.05	2.93(11)	21.52	4.21(3)	.42	.685
PRO (nmol/.1ml)	AN1	16.03	1.06(7)	15.32	1.30(9)	.40	.695
	AN2	15.09	.31(4)	14.98	1.35(9)	.08	.940
	PN1	17.07	1.02(12)	16.13	1.19(10)	.61	.550
	PN2	17.80	1.55(12)	18.02	.91(14)	.13	.898
	PN3	18.92	1.47(10)	18.77	1.04(12)	.08	.934
	PN4	20.87	1.34(11)	19.70	2.53(3)	.41	.692

Table 4.3 Comparison of biochemical variables between 'blues' cases and noncases.

Variable	Time	Mean +/- SEM (N)				Student t	2-tailed prob.
		cases		noncases			
		mean	SEM	mean	SEM		
GLY (nmol/.1ml)	AN1	13.11	.77(7)	13.12	.54(9)	.01	.990
	AN2	17.05	2.02(4)	12.42	.85(9)	2.55	.027
	PN1	15.35	1.06(12)	15.90	1.25(11)	.34	.740
	PN2	19.89	1.81(12)	19.93	1.48(14)	.04	.971
	PN3	20.90	1.22(10)	20.57	1.59(11)	.16	.874
	PN4	26.10	1.77(11)	19.56	.09(2)	3.70	.004
ALA (nmol/.1ml)	AN1	29.01	3.5(7)	31.65	1.7(9)	.71	.488
	AN2	40.96	4.61(4)	31.80	1.85(9)	2.26	.045
	PN1	41.03	2.67(12)	40.61	3.21(11)	.10	.921
	PN2	38.74	3.56(12)	43.81	3.4(14)	1.03	.315
	PN3	39.16	4.48(10)	47.96	3.31(11)	1.60	.126
	PN4	41.63	5.28(11)	34.33	3.76(3)	.69	.502
VAL (nmol/.1ml)	AN1	13.21	.79(7)	13.32	.76(9)	.10	.923
	AN2	13.44	.51(4)	12.73	.67(9)	.66	.521
	PN1	17.40	1.04(12)	15.17	.90(10)	1.59	.128
	PN2	17.81	1.20(12)	18.50	.78(14)	.50	.621
	PN3	18.22	1.31(10)	19.52	.71(12)	.91	.372
	PN4	21.99	1.51(11)	21.02	2.66(3)	.30	.768
ILE (nmol/.1ml)	AN1	4.61	.36(7)	4.80	.32(9)	.38	.710
	AN2	5.14	.52(4)	4.32	.33(9)	1.35	.204
	PN1	6.19	.41(12)	6.75	.53(10)	.84	.412
	PN2	7.33	.43(12)	7.18	.47(14)	.24	.812
	PN3	8.04	.50(10)	7.66	.38(12)	.62	.541
	PN4	8.60	.39(11)	8.76	.78(3)	.20	.848
LEU (nmol/.1ml)	AN1	7.40	.13(7)	8.67	.56(9)	2.20	.056
	AN2	8.90	.79(4)	7.66	.40(9)	1.57	.144
	PN1	11.26	.77(12)	10.99	.74(10)	.25	.807
	PN2	11.46	.70(12)	12.71	.54(14)	.42	.680
	PN3	12.53	.98(10)	12.99	.59(12)	.42	.680
	PN4	14.22	.76(11)	14.46	2.73(3)	.12	.903
TYR (nmol/.1ml	AN1	3.84	.28(7)	3.95	.28(9)	.27	.794
	AN2	4.61	.44(4)	4.11	.25(9)	1.07	.308
	PN1	5.20	.33(12)	5.67	.42(10)	.90	.379
	PN2	5.07	.58(12)	5.50	.35(14)	.66	.513
	PN3	5.40	.37(10)	5.61	.20(12)	.51	.617
	PN4	5.96	.46(11)	6.07	.73(3)	.11	.913
PHE (nmol/.1ml)	AN1	4.85	.44(7)	4.86	.38(9)	.02	.987
	AN2	5.08	.65(4)	4.75	.17(9)	.68	.509
	PN1	6.23	.37(12)	5.56	.38(10)	1.24	.231
	PN2	5.42	.36(12)	5.43	.26(14)	.03	.974
	PN3	5.52	.59(10)	5.52	.13(12)	.01	.996
	PN4	6.46	.47(11)	5.90	.70(3)	.58	.573

Table 4.3 Comparison of biochemical variables between 'blues' cases and noncases.

Variable	Time	Mean +/- SEM				student t	2-tailed prob.
		cases		noncases			
		mean	SEM	mean	SEM		
TP/LNAA Ratio	AN1	.324	.037(7)	.241	.016(7)	2.06	.062
	AN2	.232	.020(4)	.248	.021(9)	.47	.646
	PN1	.192	.017(12)	.251	.033(10)	1.65	.115
	PN2	.205	.020(12)	.174	.013(13)	1.31	.204
	PN3	.176	.023(10)	.164	.009(10)	.47	.647
	PN4	.181	.016(11)	.252	.022(2)	1.77	.104
CORTISOL ($nmol/dm^3$)	AN1	30.15	2.39(13)	31.98	1.30(14)	.69	.498
	AN2	31.25	2.00(9)	30.17	1.24(14)	.48	.634
MHPG (mg/ml)		1242	199(9)	1071	266(4)	.49	.634
MHPG (mg/g creat)		3027	1286(9)	1605	462(4)	1.04	.323

Table 4.4 : Comparison of mood scale scores between depression cases and noncases.

Variable	Time	Mean rank(N)		Mann-whitney U score	2 tailed probability
		cases	noncases		
MAACL'A'	AN1	19.32(11)	15.24(21)	84.5	.2128
	AN2	19.25(6)	13.20(22)	37.5	.1059
	PN1	21.50(7)	14.40(24)	45.5	.0665
	PN2	20.77(11)	17.50(25)	112.5	.3865
	PN3	19.95(10)	15.72(23)	85.5	.2436
	PN4	12.30(5)	10.59(16)	33.5	.5892
	PN5	6.75(4)	6.38(8)	15.0	.8642
MAACL'H'	AN1	19.64(11)	14.86(21)	81.0	.1678
	AN2	15.58(6)	14.20(22)	59.5	.7139
	PN1	17.29(17)	15.63(24)	75.0	.6678
	PN2	18.55(11)	18.48(25)	137.0	.9862
	PN3	16.70(10)	17.13(23)	112.0	.9053
	PN4	12.30(5)	10.59(16)	33.5	.5885
	PN5	4.75(4)	7.38(8)	9.0	.2295
MAACL'D'	AN1	19.77(11)	14.79(21)	79.5	.1514
	AN2	17.58(6)	13.66(22)	47.5	.2968
	PN1	15.64(7)	16.10(24)	81.5	.9055
	PN2	19.45(11)	18.08(25)	214.0	.7173
	PN3	16.25(10)	17.33(23)	107.5	.7675
	PN4	11.70(5)	10.78(5)	36.5	.7719
	PN5	6.50(4)	6.50(8)	16.0	1.0000
BDI	AN	14.17(6)	12.63(19)	50.0	.6528
	PN	19.40(10)	15.18(22)	81.0	.2219

4.5 Comparison of sociological variables between depression cases and noncases.

	Depression cases (Number of samples in parentheses)	Depression noncases	
Age	27.91+/-1.52 (11)	28.12+/-0.83 (26)	t=0.13 p=.899
Age of menarche	13.73+/-0.30 (11)	12.31+/-0.28 (26)	t=3.02 p=.005
Duration of pregnancy (wks)	39.70+/-0.42 (10)	39.76+/-0.32 (25)	t=0.11 p=.911
Blood loss at delivery (ml)	281+/-50 (8)	326+/-33 (19)	t=0.74 p=.464
Birth weight of infant (g)	3576+/-247 (10)	3251+/-108 (24)	t=1.20 p=.250
Apgar score	9.20+/-.13 (10)	9.28+/-.12 (25)	t=0.00 p=1.00

C:Depressn cases NC:Depressn noncases

Marital Status

	Single	Married	Divorced	Cohabiting	Separated
C	1	8	1	1	0
NC	3	19	2	1	1

$X^2 = 0.87, p=.93$

Socioeconomic Class

	I	II	III	IV	V
C	3	2	3	1	0
NC	5	6	8	0	0

$X^2 = 2.52, p=.47$

Gravidity

	1	2	>3
C	3	5	3
NC	11	8	7

$X^2 = 0.94, p=.630$

Parity

	0	1	2	>3
C	5	3	1	2
NC	14	6	2	4

$X^2 = 0.22, p=.97$

Nationality

	GB	Other
C	10	1
NC	24	1

$X^2 = .02, p=.89$

Sex of infant

	M	F
C	10	1
NC	14	11

$X^2 = 4.2, p=.04$

Table 4.5 Comparison of sociological variables between Depression cases and noncases.

Previous PMS

	No	Yes
C	5	6
NC	19	7

$X^2 = 2.6, p = .11$

Previous dysmenorrhoea

	No	Yes
C	10	1
NC	23	3

$X^2 = .05, p = .83$

Previous irregular menstruat^n

	No	Yes
C	8	3
NC	10	8

$X^2 = .05, .83$

Previous use of oral contracept^n

	Never	>6mnth ago	<6mnth ago
C	2	6	3
NC	3	12	11

$X^2 = .74, \rho = .86$

Reason for stopping use of oral contraceptives

	N/A	Side effects	Planned Preg	Other
C	3	2	4	2
NC	5	6	12	3

$X^2 = 0.74, \rho = .86$

Physical fitness during preg.

	Good	Poor
C	3	8
NC	10	16

$X^2 = 0.42, p = .51$

Depression during preg.

	No	Yes	Severe
C	6	5	0
NC	17	8	1

$X^2 = 1.04, \rho = .59$

Tearfulness during preg.

	No	Mild	Severe
C	5	4	1
NC	14	12	0

$X^2 = 5.0, p = .170$

Sexual activity during preg.

	N.C.	Decreased
C	8	2
NC	1	5

Fishers exact = .024

Planning of pregnancy

	Planned	No precautions	Inadequate prec.
C	7	4	0
NC	17	4	5

$X^2 = 3.7, p = .16$

Table 4.5 Comparison of sociological variables between Depression cases and noncases.

Mode of feeding

	Breast	Bottle	Mixed
C	9	0	0
NC	15	2	1

$X^2 = 3.50, p = .320$

Previous Medical History

	Non relevant	Mod.	Sev^r,
C	3	6	2
NC	23	1	2

$X^2 = 15.4, p = .0005$

Previous Psychiatric History

	No	Yes
C	10	1
NC	24	2

$X^2 = .02, p = .89$

Family psychiatric history(affective)

	No	Yes
C	0	11
NC	1	25

$X^2 = .43, p = .51$

Induced Labour

	Yes	No
C	1	10
NC	4	21

$X^2 = .30, p = .58$

Use of Forceps

	Yes	No
C	1	10
NC	5	20

$X^2 = .65, p = .42$

Incidence of 'blues'.

	'blues' case	'blues' noncase
C	5	6
NC	3	10

$X^2 = 1.34, p = .25$

Table 4.6 Comparison of biochemical variables between depression cases and noncases.

Variable	Time	Mean +/- SEM(N) cases mean	cases SEM	noncases mean	noncases SEM	Students t	2-tailed prob.
Total TP (ug/ml)	AN1	8.78	1.08(8)	9.10	0.55(22)	0.28	.785
	AN2	7.16	.78(8)	8.85	.59(22)	1.55	.132
	PN1	10.29	.78(8)	9.75	.66(24)	.43	.671
	PN2	8.95	.79(11)	9.06	.59(25)	.11	.909
	PN3	8.80	.48(11)	9.32	.59(21)	1.08	.294
	PN4	11.49	1.97(5)	9.26	1.00(15)	.98	.348
	PN5	12.43	2.06(4)	10.68	.77(8)	1.17	.253
Free TP (ug/ml)	AN1	2.95	.32(8)	2.47	.23(20)	1.17	.234
	AN2	2.28	.18(8)	2.52	.24(20)	.81	.427
	PN1	2.69	.34(8)	3.32	.24(24)	1.38	.178
	PN2	3.16	.27(11)	2.87	.26(24)	.68	.502
	PN3	2.89	.23(11)	2.55	.15(22)	1.29	.206
	PN4	3.18	.09(5)	2.98	.27(15)	.42	.678
	PN5	4.00	.12(4)	2.96	.22(7)	3.41	**.008**
NEFA (mEq/dm^3)	AN1	.349	.058(8)	.410	.039(21)	1.41	.169
	AN2	.452	.040(8)	.475	.037(23)	.34	.739
	PN1	.330	.047(8)	.369	.035(24)	.59	.562
	PN2	.332	.034(11)	.318	.026(25)	.30	.768
	PN3	.350	.023(11)	.265	.021(23)	2.51	.017
	PN4	.325	.064(5)	.307	.027(15)	.31	.761
	PN5	.360	.026(4)	.265	.058(6)	1.26	.242
DHB (ug/dm^3)	AN1	1.23	.16(8)	1.39	.13(8)	.77	.452
	AN2	1.64	.23(5)	1.35	.13(5)	1.11	.281
	PN1	1.36	.15(7)	1.42	.14(7)	.39	.702
	PN2	1.39	.44(7)	1.16	.10(7)	.51	.628
	PN3	1.11	.07(9)	1.39	.14(9)	1.75	.100
	PN4	1.10	.10(4)	1.45	.13(4)	1.60	.136
	PN5	1.05	.15(2)	1.25	.55(2)	.35	.759
Oestriol ($nmol/dm^3$)	AN1	53.17	7.87(6)	38.64	4.76(14)	1.63	.120
	AN2	48.83	4.35(6)	56.46	3.95(13)	1.16	.261
Oestradiol ($nmol/dm^3$)	AN1	78.13	11.30(8)	69.59	6.37(17)	.71	.486
	AN2	71.00	6.40(5)	85.59	7.06(17)	1.07	.298
	PN1	4.31	1.81(6)	1.94	.52(24)	1.73	.094
	PN2	.79	.16(10)	.67	.08(21)	.78	.441
	PN3	.44	.07(11)	.45	.04(18)	.12	.908
	PN4	.34	.07(5)	.33	.04(15)	.10	.919
	PN5	.31	.11(4)	.32	.04(7)	.13	.901

Table 4.6 Comparison of biochemical variables between depression cases and noncases.

Variable	Time	Mean +/- SEM(N) cases mean	cases SEM	noncases mean	noncases SEM	Students t	2-tailed prob.
Progest. (nmol/dm^3)	AN1	491.7	65.3(10)	463.8	23.9 (16)	.40	.696
	AN2	470.0	65.1(3)	1406.9	603.0 (16)	1.54	.143
	PN1	75.0	27.4(4)	47.8	11.7 (24)	.88	.385
	PN2	19.1	2.2(10)	15.6	1.8 (22)	1.16	.254
	PN3	9.6	.7(11)	9.1	1.0 (18)	.49	.627
	PN4	6.6	.4(5)	6.9	.6 (15)	.31	.763
	PN5	4.3	.6(4)	5.3	.4 (7)	1.42	.189
Relative fall of prog. at parturition.		100.9	72.1(11)	821.6	39.2 (26)	1.81	.082
ASP (nmol/.1ml)	AN1	3.399	.422(7)	3.096	.367 (14)	.50	.620
	AN2	2.943	.967(3)	2.610	.306 (10)	.45	.663
	PN1	2.645	.356(8)	2.855	.254 (22)	.44	.662
	PN2	3.738	.484(11)	2.886	.287 (21)	1.61	.117
	PN3	4.137	.599(9)	3.123	.458 (17)	1.35	.198
	PN4	3.125	.581(6)	2.555	.214 (14)	1.15	.264
	PN5	3.600	1.124(3)	2.634	.768 (4)	.74	.493
THR (nmol/.1ml)	AN1	31.55	4.14(7)	40.97	1.98 (14)	2.34	.030
	AN2	36.73	3.00(4)	38.64	4.16 (10)	.27	.788
	PN1	33.78	4.56(7)	43.21	2.15 (22)	2.06	.049
	PN2	44.06	3.45(10)	45.82	2.53 (21)	.40	.691
	PN3	45.93	4.88(10)	50.81	2.53 (17)	.18	.861
	PN4	47.53	5.19(4)	51.64	4.58 (15)	.44	.667
	PN5	49.13	7.13(2)	52.07	7.78 (5)	.23	.829
SER (nmol/.1ml)	AN1	18.11	1.31(6)	21.09	1.42 (14)	1.27	.221
	AN2	19.59	1.28(4)	18.31	1.98 (10)	.39	.706
	PN1	22.73	3.83(6)	20.60	.74 (22)	.54	.610
	PN2	21.68	1.06(10)	22.85	.98 (21)	.74	.466
	PN3	21.53	1.45(10)	23.48	1.07 (17)	1.10	.283
	PN4	23.37	1.71(4)	22.77	1.22 (15)	.24	.816
GLU (nmol/.1ml)	AN1	24.34	3.33(6)	21.48	2.82 (13)	.60	.555
	AN2	24.91	4.86(4)	20.60	2.26 (10)	.93	.373
	PN1	20.37	2.92(6)	19.67	1.69 (21)	.20	.845
	PN2	22.05	2.26(10)	20.79	1.73 (21)	.43	.673
	PN3	23.32	2.35(10)	20.91	2.03 (17)	.75	.457
	PN4	25.60	2.80(4)	22.32	2.45 (15)	.65	.523
	PN5	29.54	2.46(2)	24.18	5.17 (6)	.52	.594

Table 4.6 Comparison of biochemical variables between depression cases and noncases.

Variable	Time	Mean +/- SEM(N)				Students t	2-tailed prob.
		cases		noncases			
		mean	SEM	mean	SEM		
PRO (nmol/.1ml)	AN1	16.67	1.18(6)	15.15	1.10(14)	.81	.424
	AN2	14.68	.73(4)	14.68	.73(4)	.30	.771
	PN1	17.33	1.34(6)	17.25	.79(22)	.05	.964
	PN2	17.31	1.41(10)	17.25	.66(21)	.05	.962
	PN3	18.35	.85(10)	17.41	.76(17)	.80	.434
	PN4	19.86	1.58(4)	20.58	1.24(15)	.28	.782
	PN5	20.06	.64(2)	25.12	4.91(5)	.62	.565
GLY (nmol/.1ml)	AN1	13.75	1.09(6)	13.89	.92(14)	.09	.932
	AN2	13.33	1.23(5)	13.06	.68(10)	.21	.839
	PN1	16.60	1.89(7)	15.65	.89(22)	.50	.619
	PN2	18.84	1.37(10)	19.76	1.26(21)	.45	.659
	PN3	19.69	1.27(8)	21.69	1.06(17)	1.13	.271
	PN4	23.15	.99(4)	22.72	1.68(15)	.13	.898
	PN5	22.66	.48(2)	24.71	2.95(15)	.41	.696
ALA (nmol/.1ml)	AN1	31.28	4.72(6)	33.41	2.24(14)	.47	.647
	AN2	36.68	2.81(4)	32.58	2.83(10)	.84	.418
	PN1	46.25	5.13(7)	41.72	2.28(22)	.92	.368
	PN2	41.23	4.28(10)	40.66	2.12(21)	.13	.895
	PN3	41.39	4.31(9)	43.86	1.37(17)	.50	.620
	PN4	45.59	11.9(4)	39.79	2.62(15)	.47	.667
	PN5	49.26	6.16(2)	7.8	3.49(5)	.19	.115
VAL (nmol/.1ml)	AN1	13.44	.94(6)	14.01	.78(14)	.43	.675
	AN2	13.42	1.37(4)	12.12	.41(10)	1.24	.238
	PN1	16.17	1.17(6)	16.46	.62(22)	.22	.830
	PN2	18.45	1.40(10)	18.00	.57(21)	.30	.770
	PN3	18.64	1.05(10)	17.93	1.23(17)	.39	.697
	PN4	21.48	2.39(4)	20.15	1.32(15)	.47	.645
	PN5	22.64	3.36(2)	23.19	2.03(5)	.14	.892
ILE (nmol/.1ml)	AN1	4.352	.412(6)	5.544	.451(14)	1.60	.127
	AN2	4.533	.702(4)	4.433	.297(10)	.09	.927
	PN1	5.994	.672(6)	6.601	.294(22)	.92	.367
	PN2	6.973	.560(10)	7.592	.278(21)	1.11	.275
	PN3	8.012	.531(10)	7.078	.404(17)	1.40	.173
	PN4	9.722	.211(4)	7.408	.387(15)	3.00	.008
	PN5	9.999	.000(2)	8.677	.683(5)	1.16	.300
LEU (nmol/.1ml)	AN1	7.984	.608(6)	9.062	.722(14)	.91	.375
	AN2	7.603	1.028(4)	7.705	.369(10)	.12	.906
	PN1	10.383	1.088(6)	11.269	.463(22)	.84	.410
	PN2	11.929	.960(10)	12.701	.472(21)	.81	.422
	PN3	13.377	.853(10)	11.958	.662(17)	1.31	.202
	PN4	14.035	1.050(4)	12.741	.839(15)	.75	.465
	PN5	14.765	2.165(2)	14.865	2.027(5)	.05	.960

Table 4.6 Comparison of biochemical variables between depression cases and noncases.

Variable	Time	Mean +/- SEM(N)				Students t	2-tailed prob.
		cases		noncases			
		mean	SEM	mean	SEM		
TYR (nmol/ •1ml)	AN1	4.087	.502(6)	4.469	.333(14)	.63	.536
	AN2	4.356	.577(4)	3.841	.188(10)	1.12	.283
	PN1	5.288	.509(6)	5.447	.227(22)	.31	.756
	PN2	5.923	.727(10)	4.899	.209(21)	1.35	.203
	PN3	5.632	.395(10)	5.388	.227(17)	.58	.569
	PN4	5.614	.660(4)	5.962	.430(15)	.38	.705
	PN5	6.368	1.496(2)	6.924	1.027(5)	.31	.770
PHE (nmol/ •1ml)	AN1	5.079	.485(6)	5.248	.462(14)	.22	.830
	AN2	4.909	.471(4)	4.486	.140(10)	1.18	.262
	PN1	6.015	.399(6)	5.964	.218(22)	.11	.914
	PN2	5.471	.408(10)	5.408	.218(21)	.15	.883
	PN3	5.516	.588(9)	5.509	.240(17)	.00	.997
	PN4	6.489	.993(4)	6.090	.327(15)	.55	.584
	PN5	7.168	2.203(3)	6.984	.690(5)	.11	.916
Ratio TP/LNAA	AN1	.274	.037(5)	.268	.029(14)	.11	.915
	AN2	.236	.026(4)	.229	.014(10)	.25	.807
	PN1	.232	.026(6)	.219	.020(22)	.33	.746
	PN2	.192	.023(10)	.197	.016(20)	.19	.847
	PN3	.175	.016(9)	.217	.035(15)	1.09	.290
	PN4	.207	.040(4)	.188	.028(14)	.33	.744
	PN5	.215	.046(2)	.194	.029(5)	.38	.718
CORTISOL ($nmol/dm^3$)	AN1	33.92	2.83(9)	29.95	1.28(21)	1.49	.149
	AN2	28.21	1.92(7)	31.27	1.20(21)	1.30	.206
MHPG(mg/ml)		1570	368 (4)	880	121 (10)	2.35	.037
MHPG(mg/creat)		1738	383 (4)	2954	1151(10)	1.00	.338

Table 4.7 Comparison of biochemical variables between TP 'risers' and 'nonrisers'.

Variable	Time	Mean +/- SEM(N)				Students t	2-tailed prob.
		risers mean	SEM	nonrisers mean	SEM		
Total TP (ug/ml)	AN1	8.43	.54(19)	10.43	1.33(9)	1.67	.106
	AN2	8.12	.59(20)	12.25	.79(6)	3.57	.002
	PN1	11.50	.56(24)	9.60	.98(9)	1.73	.093
	PN2	11.08	.65(24)	8.07	.41(9)	2.73	.010
	PN3	10.61	.65(22)	8.43	.41(8)	2.85	.008
	PN4	12.04	1.42(12)	8.92	1.02(6)	1.45	.167
	PN5	12.52	1.55(6)	11.30	.94(4)	.63	.550
Free TP (ug/ml)	AN1	2.32	.15(18)	3.23	.31(9)	3.04	.005
	AN2	2.63	.23(20)	3.00	.37(5)	.76	.454
	PN1	3.36	.35(24)	3.28	.27(9)	.19	.848
	PN2	2.98	.26(24)	3.02	.18(9)	.15	.883
	PN3	2.77	.18(23)	2.65	.16(8)	.38	.704
	PN4	2.74	.27(12)	3.37	.41(6)	1.30	.211
	PN5	2.83	.35(4)	2.80	.47(4)	.04	.967
NEFA (mEq/dm^3)	AN1	.449	.046(18)	.437	.056(9)	.17	.870
	AN2	.463	.030(21)	.534	.087(7)	.98	.336
	PN1	.416	.036(24)	.307	.029(9)	2.35	.026
	PN2	.391	.030(24)	.289	.041(9)	1.85	.074
	PN3	.342	.030(23)	.224	.031(8)	2.19	.037
	PN4	.318	.050(12)	.291	.031(6)	.36	.723
	PN5	.347	.053(4)	.299	.060(4)	.60	.569
DHB (ug/dm^3)	AN1	1.34	.13(13)	1.44	.20(7)	.60	.569
	AN2	1.30	.13(12)	1.67	.09(3)	1.35	.199
	PN1	1.43	.15(15)	1.27	.16(9)	.71	.485
	PN2	1.11	.11(12)	1.26	.13(8)	.87	.397
	PN3	1.39	.12(13)	1.20	.16(6)	.93	.364
	PN4	1.44	.14(10)	1.53	.09(3)	.34	.739
Oestriol ($nmol/dm^3$)	AN1	47.7	6.3(12)	29.4	6.3(8)	1.96	.066
	AN2	53.5	4.9(11)	53.0	4.1(5)	0.06	.954
Oestradiol ($nmol/dm^3$)	AN1	65.8	8.0(12)	65.9	11.0(8)	0.00	.998
	AN2	72.5	6.2(14)	78.8	6.0(4)	.51	.615
	PN1	2.6	.8(20)	2.3	1.0(4)	.16	.878
	PN2	.6	.1(21)	.8	.2(8)	1.01	.321
	PN3	.4	.0(20)	.5	.1(7)	1.69	.104
	PN4	.3	.0(12)	.4	.1(6)	2.44	.051
	PN5	.2	.0(3)	.4	.1(4)	1.64	.161

Table 4.7 Comparison of biochemical variables between TP 'risers' and 'nonrisers'.

Variable	Time	Mean +/- SEM(N) risers mean	SEM	nonrisers mean	SEM	Students t	2-tailed prob.
Progest. ($nmol/dm^3$)	AN1	444.8	48.9(12)	416.3	73.2(8)	.34	.740
	AN2	1727	699 (14)	825.0	425 (4)	.67	.514
	PN1	56.5	15.4(20)	44.4	17.8(9)	.49	.631
	PN2	16.1	1.9(22)	13.6	2.8(8)	.68	.502
	PN3	8.4	.7(20)	7.7	1.3(7)	.47	.646
	PN4	5.9	.8(12)	8.8	3.2(6)	.88	.415
	PN5	3.3	.3(3)	4.0	.4(4)	1.20	.286
ASP (nmol/ .1ml)	AN1	3.03	.26(10)	2.58	.49(5)	.89	.389
	AN2	2.95	.64(12)	3.20	.51(3)	.19	.854
	PN1	2.70	.28(20)	2.20	.38(9)	1.02	.317
	PN2	2.93	.28(19)	2.35	.37(8)	1.19	.244
	PN3	2.96	.34(16)	2.53	.33(7)	.76	.457
	PN4	2.48	.30(13)	3.08	.53(6)	1.05	.309
	PN5	3.75	1.06(3)	2.14	.14(4)	1.50	.272
THR (nmol/ .1ml)	AN1	36.8	3 (10)	40.4	3.1(5)	.76	.461
	AN2	38.9	2.3(12)	41.4	3.0(3)	.52	.611
	PN1	41.0	2.5(20)	43.2	1.3(9)	.75	.457
	PN2	45.7	2.5(18)	47.4	2.5(8)	.41	.684
	PN3	53.5	3.1(17)	52.7	3.7(7)	.14	.889
	PN4	49.9	4.3(10)	52.0	2.7(6)	.35	.733
	PN5	51.1	9.1(2)	56.1	3.9(4)	.62	.567
SER (nmol/ .1ml)	AN1	19.1	1.0(9)	19.9	1.3(5)	.50	.628
	AN2	18.4	.7(12)	19.2	2.5(3)	.44	.667
	PN1	20.7	1.3(19)	20.1	1.9(9)	.24	.814
	PN2	22.3	.8(18)	21.2	1.5(8)	.71	.483
	PN3	25.6	1.2(17)	21.5	2.0(7)	1.79	.087
	PN4	23.7	1.5(10)	24.0	1.0(6)	.17	.864
	PN5	28.1	.4(2)	22.4	2.6(4)	1.44	.222
GLU (nmol .1ml)	AN1	22.5	3.4(9)	16.6	3.0(4)	1.06	.312
	AN2	21.2	2.3(12)	23.7	4.4(3)	.49	.630
	PN1	22.1	1.9(18)	16.0	2.0(9)	1.32	.198
	PN2	20.4	1.6(18)	16.7	1.9(8)	1.37	.184
	PN3	19.8	1.7(17)	18.1	2.5(7)	.54	.594
	PN4	21.5	1.6(10)	25.5	2.9(6)	.34	.743
	PN5	24.4	4.5(3)	20.0	3.3(4)	.82	.451
PRO (nmol/ .1ml)	AN1	15.3	.8(9)	16.9	2.0(5)	.87	.404
	AN2	14.8	.7(12)	16.1	3.6(3)	.37	.747
	PN1	17.5	.8(19)	15.1	1.3(9)	1.66	.109
	PN2	18.3	.6(19)	16.7	1.6(8)	1.15	.262
	PN3	18.7	.9(10)	19.7	1.7(7)	.52	.608
	PN4	21.0	1.5(16)	18.3	1.5(6)	1.19	.254
	PN5	22.4	3.0(2)	21.2	3.8(4)	.21	.847

Table 4.7 Comparison of biochemical variables between TP 'risers' and 'nonrisers'.

Variable	Time	Mean +/- SEM(N) risers mean	SEM	nonrisers mean	SEM	Students t	2-tailed prob.
GLY (nmol/ .1ml)	AN1	13.7	.8(9)	13.5	.7(5)	.17	.867
	AN2	14.3	1.0(12)	13.7	.4(3)	.30	.770
	PN1	16.4	1.0(19)	14.8	1.5(9)	.96	.347
	PN2	20.0	1.3(18)	16.6	.9(8)	2.09	.048
	PN3	22.6	1.2(17)	21.8	1.1(7)	.43	.669
	PN4	25.1	2.5(9)	22.8	1.7(6)	.70	.497
	PN5	28.8	4.8(2)	26.4	3.6(4)	.25	.813
ALA (nmol/ .1ml)	AN1	33.3	3.1(9)	30.7	3.4(5)	.54	.601
	AN2	32.4	1.5(12)	36.3	5.7(3)	.97	.349
	PN1	42.6	2.3(19)	38.4	3.2(9)	1.07	.296
	PN2	41.4	2.2(18)	41.3	4.1(8)	.02	.984
	PN3	44.6	3.0(16)	44.7	2.6(7)	.02	.982
	PN4	42.9	3.3(10)	42.4	7.7(6)	.07	.943
	PN5	47.0	3.9(2)	41.7	6.5(4)	.53	.625
VAL (nmol/ .1ml)	AN1	13.6	.8(9)	13.0	1.4(5)	.41	.692
	AN2	12.5	.4(12)	13.7	.8(3)	1.29	.221
	PN1	17.4	.6(19)	14.3	1.1(9)	2.59	.016
	PN2	18.2	.4(18)	16.8	1.0(8)	1.30	.227
	PN3	18.7	1.1(17)	18.9	.7(7)	.17	.869
	PN4	19.7	1.2(10)	21.2	1.2(6)	.23	.823
	PN5	23.3	2.7(2)	2.7	1.4(4)	.96	.390
LEU (nmol/ .1ml)	AN1	8.8	.5(9)	8.6	.9(5)	.16	.878
	AN2	7.9	.4(12)	9.4	.7(3)	1.68	.116
	PN1	11.7	.5(19)	10.2	.8(9)	1.68	.106
	PN2	12.6	.4(18)	11.4	.8(8)	1.6	.131
	PN3	13.6	.7(17)	13.7	.7(7)	.33	.745
	PN4	13.1	1.0(10)	13.8	.9(6)	.48	.636
	PN5	15.7	1.3(2)	13.9	1.1(4)	1.00	.373
ILE (nmol .1ml)	AN1	5.24	.44(9)	5.40	.47(5)	.24	.817
	AN2	4.73	.28(10)	4.82	.47(3)	.12	.910
	PN1	6.35	.28(20)	6.86	.70(9)	.81	.423
	PN2	7.53	.30(18)	6.49	.39(8)	1.97	.061
	PN3	7.60	.43(16)	7.77	.41(7)	.24	.812
	PN4	7.92	.52(10)	8.44	.45(6)	.69	.504
	PN5	9.37	.62(2)	8.97	.75(4)	.34	.752
TYR (nmol .1ml)	AN1	4.27	.22(9)	4.26	.62(5)	.03	.976
	AN2	3.91	.19(12)	4.85	.64(3)	1.92	.077
	PN1	5.75	.26(19)	4.94	.48(9)	1.61	.120
	PN2	5.75	3.30(18)	4.36	.33(8)	2.60	.016
	PN3	6.11	.28(17)	5.54	.22(7)	1.24	.229
	PN4	6.03	.19(10)	5.84	.64(6)	.27	.795
	PN5	6.52	.90(2)	5.92	.82(4)	.45	.676

Table 4.7 Comparison of biochemical variables between TP 'risers' and 'nonrisers'.

Variable	Time	Mean +/- SEM(N) risers mean SEM	Mean +/- SEM(N) nonrisers mean SEM	Students t	2-tailed prob.
PHE (nmol .1ml)	AN1	5.16 .43(9)	4.72 .53(5)	.63	.541
	AN2	4.38 .22(12)	5.21 .38(3)	1.75	.104
	PN1	6.17 .25(19)	5.19 .39(9)	2.19	**.038**
	PN2	5.69 .23(18)	4.64 .34(7)	2.45	**.022**
	PN3	5.97 .27(17)	5.29 .24(6)	1.39	.179
	PN4	6.03 .44(10)	6.14 .39(5)	.15	.884
	PN5	7.22 2.15(2)	5.53 .62(4)	1.05	.353
TP/LNAA ratio	AN1	.252 .025(7)	.301 .055(5)	.90	.389
	AN2	.232 .013(12)	.292 .008(3)	2.27	**.041**
	PN1	.245 .019(19)	.245 .035(9)	.01	.994
	PN2	.217 .015(18)	.193 .025(7)	.82	.421
	PN3	.229 .034(15)	.158 .011(6)	1.99	.063
	PN4	.244 .039(10)	.172 .028(5)	1.23	.242
	PN5	.234 .027(2)	.207 .017(4)	.89	.422
Cortisol ($nmol/dm^3$)	AN1	26.6 2.1 (19)	28.4 1.7 (9)	.40	.694
	AN2	30.1 1.3 (19)	30.4 1.8 (6)	.14	.886
MHPG mg/24 hr urine		1142 209 (10)	952 136 (5)	.60	.557
MHPG mg/ g creat.		1823 166 (10)	1818 355 (5)	.02	.988

Table 4.8 : Comparison of mood scale scores between Tryptophan risers and nonrisers.

Variable	Time	Mean rank(N)		Mann-whitney U score	2 tailed probability
		cases	noncases		
MAACL'H'	AN1	15.43(20)	14.06(9)	81.5	.6863
	AN2	13.53(20)	13.42(6)	59.5	.9754
	PN1	15.30(22)	16.06(8)	83.5	.8315
	PN2	17.65(24)	15.28(9)	92.5	.5279
	PN3	15.54(23)	15.36(7)	79.5	.9605
	PN4	10.64(14)	8.20(5)	26.0	.4016
	PN5	5.33(6)	4.33(3)	7.0	.5978
MAACL'D'	AN1	14.78(20)	15.50(9)	85.5	.8312
	AN2	12.83(20)	15.75(6)	46.5	.4075
	PN1	16.25(22)	13.44(8)	71.5	.4368
	PN2	17.40(24)	15.94(9)	98.5	.7002
	PN3	16.50(23)	12.21(9)	57.5	.2578
	PN4	10.32(14)	9.60(5)	30.5	.6765
	PN5	5.67(6)	3.67(3)	5.0	.2997
MAACL'A'	AN1	15.28(20)	14.39(9)	84.5	.7944
	AN2	13.43(20)	13.75(6)	58.5	.9266
	PN1	15.66(22)	15.06(8)	84.5	.8688
	PN2	16.75(24)	17.65(9)	102.0	.8073
	PN3	15.65(23)	15.00(7)	77.0	.8619
	PN4	9.79(14)	10.60(5)	32.0	.7774
	PN5	4.75(6)	5.50(3)	7.5	.6973
BDI	AN	12.25(18)	13.25(6)	49.5	.7625
	PN	15.80(23)	11.92(6)	50.5	.3070

Table 4.9 Comparison of sociological variables between Tryptophan risers and nonrisers.

	Nonrisers (Number of samples in parentheses)	Risers	
Age	28.56+/-1.30 (9)	27.83+/-0.96 (24)	t= .41 p=.685
Age of menarche	12.44+/-0.48 (9)	12.75+/-0.35 (24)	t=0.48 p=.636
Duration of pregnancy (wks)	39.78+/-0.64 (9)	39.88+/-0.27 (24)	t=0.17 p=.868
Blood loss at delivery (ml)	358+/-52 (6)	318+/-44 (19)	t=0.48 p=.638
Birth weight of infant (g)	3216+/-387 (9)	3372+/-104 (24)	t=0.39 p=.707
Apgar score	8.22+/-1.04 (9)	9.08+/-.10 (24)	t=0.83 p=.433

NR:Nonrisers R:Risers

Marital Status

	Single	Married	Divorced	Cohabiting	Separated
R	0	21	1	0	1
NR	0	9	0	0	0

X^2= 1.71, p=.64

Socioeconomic Class

	I	II	III	IV	V
R	8	6	5	1	0
NR	1	3	4	0	1

X^2=5.10, p=.28

Gravidity

	1	2	>3
R	11	6	7
NR	3	4	2

X^2= 1.17, p=.56

Parity

	0	1	2	>3
R	12	5	3	4
NR	6	2	1	0

X^2= 1.85, p=.60

Nationality

	GB	Other
R	21	3
NR	9	0

X^2=1.2, p=.27

Sex of infant

	M	F
R	14	10
NR	5	4

X^2=.02, p=.89

Table 4.9 Comparison of sociological variables between Tryptophan risers and nonrisers.

Previous PMS

	No	Yes
R	14	10
NR	8	1

$X^2 = 2.75, p = .10$

Previous dysmenorrhoea

	No	Yes
R	20	4
NR	9	0

$X^2 = 1.71, p = .19$

Previous irregular menstruat^n

	No	Yes
R	20	4
NR	4	5

$X^2 = 4.99, p = .03$

Previous use of oral contracept^n

	Never	>6mnth ago	<6mnth ago
R	3	9	12
NR	1	7	1

$X^2 = 4.71, p = .09$

Reason for stopping use of oral contraceptives

	N/A	Side effects	Planned Preg	Other
R	4	5	12	3
NR	1	2	5	1

$X^2 = 0.18, p = .98$

Physical fitness during preg.

	Good	Poor
R	7	17
NR	4	5

$X^2 = 0.69, p = .41$

Depression during preg.

	No	Mild	Severe
R	11	10	2
NR	4	5	0

$X^2 = 1.0, p = .61$

Tearfulness during preg.

	No	Mild	Severe
R	9	13	1
NR	16	3	0

$X^2 = 2.56, p = .46$

Sexual activity during preg.

	NC	Decreased
R	5	6
NR	2	0

Fishers exact= .29

Planning of pregnancy

	Planned	No precautions	Inadequate prec.
R	17	6	1
NR	7	2	0

$X^2 = .44, p = .80$

Table 4.9 Comparison of sociological variables between Tryptophan risers and nonrisers.

Mode of feeding

	Breast	Bottle	Mixed
R	18	0	0
NR	4	1	2

$X^2=9.37, p=.020$

Previous Medical History

	Non relevant	Mod.	Sevr.
R	19	5	0
NR	6	3	0

$X^2=0.56, p=.46$

Previous Psychiatric History

	No	Yes
R	20	4
NR	8	1

$X^2=.16, p=.69$

Family psychiatric history(affective)

	No	Yes
R	23	1
NR	9	0

$X^2=.39, p=.53$

Induced Labour

	No	Yes
R	22	2
NR	6	3

$X^2=3.2, p=.07$

Use of Forceps

	No	Yes
R	22	2
NR	7	2

$X^2=1.18, p=.28$

Incidence of 'Blues'

	Case	Border	Noncase
R	6	11	7
NR	3	1	4

$X^2=2.9, p=.24$

Incidence of depression

	Case	Border	Noncase
R	5	7	11
NR	1	2	5

$X^2=.57, p=.75$

Time of first blood sample after delivery.(Hrs)

R	$13.04^{+}/-1.9$ (24)
NR	$14.11^{+}/-3.2$ (9)

$t=.29, p=.77$

Table 4.10 Comparison of mood scale scores between primiparous and multiparous subjects.

Variable	Time	Mean ranks(N)		Mann-whitney U score	2-tailed probability
		cases	noncases		
MAACL'A'	AN1	22.68(22)	23.30(23)	246.0	.8724
	AN2	19.84(19)	20.15(20)	187.0	.9321
	PN1	23.78(20)	21.44(24)	214.5	.5455
	PN2	26.50(25)	26.50(27)	337.5	1.0000
	PN3	23.21(21)	25.50(27)	256.5	.5718
	PN4	14.60(5)	15.68(25)	58.0	.8011
	PN5	4.25(2)	9.63(15)	5.5	.1539
MAACL'H'	AN1	24.43(22)	21.63(23)	221.5	.4721
	AN2	21.68(19)	18.40(20)	158.0	.3660
	PN1	22.00(20)	22.92(24)	230.0	.8117
	PN2	24.10(25)	28.72(27)	277.5	.2686
	PN3	21.98(21)	26.46(27)	230.5	.2663
	PN4	13.60(5)	15.88(25)	53.0	.5942
	PN5	2.50(2)	9.87(15)	2.0	.0505
MAACL'D'	AN1	24.14(22)	21.19(23)	228.0	.5691
	AN2	22.76(19)	17.38(20)	137.5	.1383
	PN1	23.13(20)	21.98(24)	227.5	.7672
	PN2	26.18(25)	26.80(27)	329.5	.8831
	PN3	22.00(21)	26.44(27)	231.0	.2736
	PN4	14.60(5)	15.68(25)	58.0	.8018
	PN5	3.25(2)	9.77(15)	3.5	.0857
BDI	AN	17.26(17)	19.61(19)	140.5	.5006
	PN	23.55(21)	24.37(26)	263.5	.8358

Table 4.10 Comparison of biochemical variables between primiparae and multiparae.

Variable	Time	Mean +/- SEM(N)				Students t	2-tailed prob.
		primips. mean	SEM	multips. mean	SEM		
Total TP (ug/ml)	AN1	10.24	.64(24)	7.61	.43(20)	3.42	.001
	AN2	9.59	.65(19)	7.95	.49(21)	2.05	.048
	PN1	10.39	.71(24)	9.89	.55(21)	.55	.588
	PN2	9.64	.63(27)	8.87	.48(25)	.96	.334
	PN3	9.43	.53(27)	9.44	.51(21)	.01	.991
	PN4	10.39	.86(24)	9.45	1.40(4)	.43	.672
	PN5	11.66	.74(13)	10.77	1.29(3)	.54	.601
Free TP (ug/ml)	AN1	2.75	.18(24)	2.45	.20(18)	1.10	.277
	AN2	2.34	.15(18)	2.73	.24(20)	1.39	.174
	PN1	3.40	.23(24)	3.04	.21(21)	1.13	.266
	PN2	3.02	.15(26)	2.99	.28(25)	.11	.912
	PN3	2.79	.17(27)	2.76	.15(21)	.13	.894
	PN4	3.01	.20(24)	3.08	.42(4)	.12	.906
	PN5	3.07	.19(13)	3.00	1.30(2)	.05	.966
NEFA (mEq/ dm^3)	AN1	.412	.037(24)	.463	.041(19)	.91	.366
	AN2	.491	.042(21)	.451	.027(21)	.81	.422
	PN1	.352	.037(24)	.367	.032(21)	.29	.772
	PN2	.365	.029(27)	.329	.021(25)	.98	.337
	PN3	.314	.026(28)	.291	.023(22)	.63	.531
	PN4	.323	.027(24)	.256	.051(4)	.96	.348
	PN5	.314	.036(12)	.297	.047(2)	.19	.855
DHB (ug/dm^3)	AN1	1.40	.12(19)	1.18	.09(16)	1.44	.158
	AN2	1.28	.13(12)	1.40	.15(13)	.59	.560
	PN1	1.23	.10(18)	1.53	.15(16)	1.65	.110
	PN2	1.26	.19(17)	1.23	.10(5)	.15	.881
	PN3	1.33	.10(19)	1.17	.09(13)	1.17	.250
	PN4	1.29	.08(17)	1.48	.23(5)	.96	.350
Oestriol ($nmol/dm^3$)	AN1	39.3	4.9(19)	40.5	4.8(12)	.16	.871
	AN2	50.8	3.2(12)	51.9	4.1(15)	.22	.829
Oestradiol ($nmol/dm^3$)	AN1	68.1	6.0(21)	66.4	7.3(14)	.19	.851
	AN2	83.7	8.9(13)	76.1	4.4(17)	.76	.455
	PN1	1.5	.4(23)	3.0	.9(19)	1.59	.125
	PN2	.6	.1(24)	.7	.1(21)	1.18	.243
	PN3	.4	.0(24)	.4	.0(21)	.45	.656
	PN4	.3	.0(24)	.3	.0(4)	.09	.927
	PN5	.3	.0(12)	.2	.0(2)	1.03	.324

Table 4.10 Comparison of biochemical variables between primiparae and multiparae.

Variable	Time	Mean +/- SEM(N) primips. mean	SEM	multips. mean	SEM	Students t	2-tailed prob.
Progest. ($nmol/dm^3$)	AN1	454.7	34.7(19)	451.1	47.4(16)	.06	.950
	AN2	1274.	468.(13)	1032.	562.(15)	.32	.748
	PN1	40.4	8.2(22)	61.4	16.1(18)	1.16	.255
	PN2	12.3	1.7(24)	15.1	1.3(22)	.06	.959
	PN3	8.3	.5(24)	9.3	.9(21)	1.02	.315
	PN4	6.1	.4(24)	11.5	4.7(4)	1.14	.337
	PN5	4.8	.4(12)	3.0	0.0(2)	1.86	.088
ASP (nmol/ .1ml)	AN1	3.29	.36(15)	3.66	.46(11)	.68	.505
	AN2	3.11	.67(11)	2.95	.33(11)	.22	.831
	PN1	2.67	.29(23)	2.95	.29(17)	.68	.499
	PN2	2.93	.33(21)	3.27	.26(21)	.84	.406
	PN3	3.06	.43(20)	3.41	.30(19)	.66	.515
	PN4	2.65	.24(20)	2.92	.53(6)	.52	.607
	PN5	3.13	.61(7)	2.18	.23(2)	.79	.456
THR (nmol/ .1ml)	AN1	38.9	2.2(15)	32.2	2.9(11)	1.61	.120
	AN2	40.6	3.9(11)	33.4	2.5(12)	1.59	.127
	PN1	41.6	2.4(22)	40.7	2.4(17)	.26	.798
	PN2	43.2	2.3(20)	49.0	2.9(20)	1.56	.127
	PN3	50.5	2.7(19)	50.8	3.3(20)	.06	.953
	PN4	52.5	2.2(21)	45.4	9.1(3)	.76	.453
SER (nmol/ .1ml)	AN1	20.8	1.4(14)	18.6	1.1(10)	1.17	.253
	AN2	20.3	1.9(10)	17.5	.8(12)	1.41	.183
	PN1	19.4	.8(22)	22.2	1.6(16)	1.66	.105
	PN2	21.9	1.1(20)	23.6	.8(20)	1.21	.235
	PN3	22.7	1.3(19)	23.8	1.1(20)	.69	.497
	PN4	23.5	1.0(21)	22.5	.5(3)	.35	.727
GLU (nmol/ .1ml)	AN1	22.6	2.8(13)	21.4	2.2(10)	.31	.763
	AN2	23.0	2.8(10)	21.8	2.0(12)	.38	.708
	PN1	19.8	1.7(21)	19.3	2.0(16)	.19	.852
	PN2	20.5	1.7(20)	21.6	1.7(20)	.43	.667
	PN3	20.1	1.7(19)	22.2	1.7(20)	.87	.392
	PN4	22.6	1.9(21)	24.7	3.1(3)	.41	.684
PRO (nmol/ .1ml)	AN1	16.7	1.1(14)	14.7	1.2(10)	1.21	.238
	AN2	14.1	.6(10)	15.1	1.0(12)	.84	.412
	PN1	16.8	.9(22)	17.3	.9(16)	.4	.690
	PN2	16.7	.9(21)	18.3	.7(20)	1.34	.188
	PN3	17.5	.9(18)	19.4	.8(20)	1.66	.106
	PN4	20.3	.9(21)	20.7	3.9(3)	.15	.884

Table 4.10 Comparison of biochemical variables between primiparae and multiparae.

Variable	Time	Mean +/- SEM(N) primips. mean	SEM	multips. mean	SEM	Students t	2-tailed prob.
GLY (nmol/.1ml)	AN1	14.7	.9(14)	13.7	1.0(10)	.68	.502
	AN2	14.4	1.1(11)	13.5	.7(12)	.68	.506
	PN1	16.0	.9(22)	16.4	1.1(17)	.32	.747
	PN2	18.9	1.1(20)	20.6	1.2(20)	1.02	.314
	PN3	20.6	1.0(18)	22.8	1.1(19)	1.46	.154
	PN4	22.7	1.4(20)	22.8	3.7(3)	1.34	.193
ALA (nmol/.1ml)	AN1	32.2	2.6(14)	33.9	6.8(10)	.47	.645
	AN2	34.2	2.6(10)	33.4	1.8(12)	.25	.805
	PN1	39.0	1.7(22)	45.4	3.1(17)	1.79	.085
	PN2	37.9	2.1(20)	45.3	2.8(20)	2.14	**.039**
	PN3	28.3	2.2(19)	51.4	2.2(19)	4.24	**.000**
	PN4	41.0	2.9(21)	45.5	7.0(3)	.55	.585
VAL (nmol/.1ml)	AN1	14.4	.7(14)	12.8	.7(10)	1.54	.138
	AN2	12.7	.4(10)	12.7	.5(12)	.04	.966
	PN1	16.9	.6(22)	16.3	.9(16)	.51	.613
	PN2	17.7	.7(20)	18.7	.6(20)	1.04	.304
	PN3	18.3	.8(19)	19.2	1.0(20)	.61	.548
	PN4	20.4	1.1(21)	19.0	1.4(3)	.45	.656
LEU (nmol/.1ml)	AN1	9.0	.7(14)	8.3	.6(10)	.70	.492
	AN2	8.1	.5(10)	7.8	.4(12)	.52	.619
	PN1	11.4	.5(12)	11.2	.6(16)	.19	.850
	PN2	12.1	.5(20)	12.7	.5(20)	.82	.419
	PN3	12.6	.7(19)	13.3	.6(20)	.81	.422
	PN4	13.4	.7(21)	12.1	.2(3)	1.79	.087
ILE (nmol/.1ml)	AN1	5.41	.44(14)	4.81	.37(10)	.95	.338
	AN2	4.74	.29(10)	4.53	.30(12)	.50	.622
	PN1	6.68	.31(22)	6.47	.36(17)	.45	.655
	PN2	7.23	.31(20)	7.50	.35(20)	.59	.559
	PN3	7.57	.37(19)	7.55	.36(19)	.05	.963
	PN4	8.15	.34(21)	7.30	.80(3)	.89	.381
TYR (nmol/.1ml)	AN1	4.61	.35(14)	3.96	.26(10)	1.40	.175
	AN2	4.28	.23(10)	4.01	.21(12)	.87	.397
	PN1	5.52	.26(22)	5.43	.29(16)	.23	.816
	PN2	4.84	.28(20)	5.68	.33(21)	1.95	.059
	PN3	5.44	.28(19)	5.71	.35(20)	.58	.563
	PN4	5.92	.33(19)	6.64	.91(3)	.77	.448
PHE (nmol/.1ml)	AN1	5.60	.45(14)	4.47	.22(10)	2.25	.036
	AN2	4.64	.28(10)	4.65	.16(12)	.02	.984
	PN1	6.10	.24(22)	5.74	.28(16)	.98	.335
	PN2	5.36	.26(19)	5.45	.21(21)	.25	.803
	PN3	5.46	.36(17)	5.64	.14(21)	.47	.640
	PN4	6.12	.32(20)	5.74	.38(4)	.51	.614

Table 4.10 Comparison of biochemical variables between primiparae and multiparae.

Variable	Time	Mean +/- SEM(N)				Students t	2-tailed prob.
		primips. mean	SEM	multips. mean	SEM		
Cortisol ($nmol/dm^3$)	AN1	20.7	1.7 (24)	30.2	1.4 (20)	.20	.840
	AN2	30.1	1.5 (18)	30.7	1.1 (20)	.33	.744
MHPG (mg / 24 hr urine)		1010	109 (16)	1325	600 (3)	.52	.657
MHPG (mg/g creat.)		2487	732 (16)	1522	239 (3)	1.25	.227

Table 4.11 Comparison of biochemical variables between mothers of male infants and mothers of female infants.

Variable	Time	Mean +/- SEM(N)				Students t	2-tailed prob.
		male mean	SEM	female mean	SEM		
Total TP (ug/ml)	AN1	8.81	.50(26)	9.59	.84(17)	.85	.399
	AN2	8.14	.47(24)	9.74	.77(15)	1.89	.067
	PN1	10.00	.53(26)	10.49	.82(18)	.43	.672
	PN2	9.24	.53(31)	9.51	.62(20)	.32	.751
	PN3	9.51	.47(28)	9.43	.62(19)	.10	.918
	PN4	10.09	1.07(18)	10.56	.95(10)	.29	.772
	PN5	10.83	1.06(9)	12.34	.42(7)	1.32	.215
Free TP (ug/ml)	AN1	2.58	.17(26)	2.75	.24(15)	.58	.564
	AN2	2.53	.21(23)	2.55	.20(15)	.04	.968
	PN1	3.23	.21(26)	3.23	.29(18)	.02	.981
	PN2	3.00	.22(30)	3.00	.23(20)	.01	.992
	PN3	2.88	.16(28)	2.59	.18(19)	1.19	.241
	PN4	2.88	.21(18)	3.28	.33(10)	1.07	.296
	PN5	3.30	.31(8)	2.79	.24(7)	1.28	.220
NEFA (mEq/dm^3)	AN1	.418	.030(26)	.461	.056(16)	.74	.463
	AN2	.445	.029(25)	.503	.046(16)	1.14	.260
	PN1	.322	.029(24)	.417	.041(18)	1.95	.058
	PN2	.328	.024(31)	.379	.029(20)	1.37	.178
	PN3	.285	.020(29)	.334	.032(20)	1.35	.184
	PN4	.274	.029(18)	.388	.038(10)	2.36	**.026**
	PN5	.322	.042(7)	.301	.048(7)	.33	.749
DHB (ug/dm^3)	AN1	1.25	.10(22)	1.39	.13(13)	.92	.363
	AN2	1.34	.14(13)	1.30	.14(11)	.19	.850
	PN1	1.31	.10(21)	1.41	.19(12)	.49	.625
	PN2	1.28	.16(20)	1.23	.11(11)	.24	.810
	PN3	1.29	.09(21)	1.25	.13(10)	.23	.816
	PN4	1.33	.10(15)	1.34	.14(7)	.05	.958
	PN5	1.05	.15(2)	1.25	.55(2)	.35	.759
Oestriol ($nmol/dm^3$)	AN1	40.3	4.3(20)	38.6	6.9(15)	.21	.833
	AN2	53.9	2.6(19)	46.4	7.1(10)	1.24	.228
Oestradiol ($nmol/dm^3$)	AN1	64.4	5.4(23)	72.6	9.3(11)	.81	.422
	AN2	77.0	5.3(20)	85.8	9.7(9)	.87	.395
	PN1	2.2	.6(24)	2.2	.7(17)	.06	.953
	PN2	.6	.1(27)	.7	.1(17)	.17	.866
	PN3	.4	.0(26)	.4	.1(18)	.13	.893
	PN4	.3	.0(18)	.3	.0(10)	.47	.639
	PN5	.3	.1(8)	.3	.1(6)	.04	.970

Table 4.11 Comparison of biochemical variables between mothers of male infants and mothers of female infants.

Variable	Time	Mean +/- SEM(N)				Students t	2-tailed prob.
		male mean	male SEM	female mean	female SEM		
Progest. ($nmol/dm^3$)	AN1	483.2	35.9(24)	384.0	43.2(10)	1.59	.122
	AN2	880.0	317.2(17)	1681.	875.(10)	.86	.408
	PN1	57.4	13.9(22)	41.2	9.2(17)	.97	.338
	PN2	16.4	1.6(27)	13.4	1.3(18)	1.31	.196
	PN3	8.9	.6(26)	8.5	.9(18)	.37	.712
	PN4	6.6	.5(18)	7.2	2.0(10)	.25	.804
	PN5	4.5	.5(8)	4.5	.5(6)	.00	1.00
ASP (nmol/ .1ml)	AN1	3.79	.36(17)	2.66	.40(8)	1.92	.068
	AN2	2.82	.27(15)	3.48	1.03(7)	.63	.552
	PN1	2.69	.19(25)	2.87	.49(14)	.34	.735
	PN2	3.24	.27(25)	2.79	.33(16)	1.04	.304
	PN3	3.41	.30(22)	2.87	.47(16)	1.01	.318
	PN4	2.64	.26(16)	2.82	.39(10)	.41	.684
THR (nmol/ .1ml)	AN1	34.1	3.4(17)	41.8	2.5(8)	1.99	.059
	AN2	38.6	2.8(16)	32.8	4.1(7)	1.16	.261
	PN1	40.3	2.3(24)	43.0	2.3(14)	.74	.462
	PN2	45.6	2.2(23)	49.3	2.5(16)	1.48	.147
	PN3	50.0	2.9(22)	51.6	3.4(16)	.37	.712
	PN4	52.1	3.9(15)	50.8	5.1(9)	.21	.835
	PN5	55.5	3.4(5)	48.3	8.7(4)	.83	.434
SER (nmol/ .1ml)	AN1	20.5	1.4(15)	19.0	1.2(8)	.66	.516
	AN2	19.1	1.6(15)	18.2	1.1(7)	.43	.624
	PN1	20.1	10.7(23)	21.4	2.0(14)	.64	.523
	PN2	23.1	.8(23)	22.2	1.3(16)	.51	.573
	PN3	23.5	1.2(22)	22.5	1.1(16)	.59	.560
	PN4	22.9	1.1(15)	24.3	1.7(9)	.75	.463
	PN5	26.7	3.1(5)	22.0	2.8(4)	1.10	.306
GLU (nmol/ .1ml)	AN1	25.3	2.3(14)	17.0	2.5(8)	2.32	.031
	AN2	21.7	1.7(15)	23.7	3.8(7)	.53	.601
	PN1	20.0	1.4(22)	18.9	2.5(14)	.41	.681
	PN2	21.5	1.3(23)	20.3	2.3(16)	.47	.644
	PN3	22.4	1.4(22)	19.4	2.2(16)	1.23	.223
	PN4	21.7	1.5(15)	24.7	3.7(9)	.88	.388
	PN5	25.2	2.6(6)	24.8	7.5(4)	.07	.949
PRO (nmol/ .1ml)	AN1	17.0	1.1(15)	14.4	.8(8)	1.59	.128
	AN2	15.3	.8(15)	13.2	.9(7)	1.69	.106
	PN1	18.2	.8(23)	15.2	.9(14)	2.45	**.019**
	PN2	17.8	.8(24)	17.0	.9(16)	.64	.528
	PN3	19.0	.9(21)	17.8	.8(16)	.99	.331
	PN4	21.3	1.3(15)	18.8	1.1(9)	1.26	.220
	PN5	25.9	3.7(5)	20.2	4.0(4)	1.03	.336

Table 4.11 Comparison of biochemical variables between mothers of male infants and mothers of female infants.

Variable	Time	Mean +/- SEM(N)				Students t	2-tailed prob.
		male mean	SEM	female mean	SEM		
GLY (nmol/ .1ml)	AN1	15.5	.9(15)	12.4	.6(8)	2.96	.008
	AN2	13.6	.6(16)	14.6	1.7(7)	.57	.588
	PN1	16.4	.9(24)	15.7	1.3(14)	.57	.571
	PN2	19.6	.9(23)	19.7	1.5(16)	.06	.592
	PN3	21.5	1.0(20)	22.0	1.2(16)	.32	.754
	PN4	22.4	1.5(15)	25.3	2.5(8)	1.09	.290
	PN5	26.3	1.9(5)	25.4	4.1(4)	.21	.840
ALA (nmol/ .1ml)	AN1	33.3	2.6(15)	32.1	2.3(8)	.31	.760
	AN2	34.9	2.0(15)	31.2	1.7(7)	1.17	.257
	PN1	42.7	2.3(24)	39.7	2.8(14)	.85	.402
	PN2	40.9	2.2(23)	41.9	3.4(16)	.27	.700
	PN3	44.2	2.6(21)	44.2	2.5(16)	.00	1.000
	PN4	44.3	3.7(15)	37.1	3.1(9)	1.36	.189
	PN5	46.4	2.9(5)	36.3	4.6(4)	1.93	.095
VAL (nmol/ .1ml	AN1	14.5	.7(15)	12.7	.8(8)	1.57	.132
	AN2	12.8	.4(15)	12.4	.6(7)	.50	.624
	PN1	16.6	.6(23)	16.7	1.0(14)	.10	.919
	PN2	17.8	.7(23)	18.7	.6(16)	.95	.348
	PN3	18.4	.7(22)	19.1	1.2(16)	.55	.587
	PN4	20.5	1.5(15)	19.7	1.0(9)	.41	.688
	PN5	24.2	1.8(5)	20.9	1.3(4)	1.35	.218
LEU (nmol/ .1ml)	AN1	9.3	.7(15)	8.0	.4(8)	1.42	.171
	AN2	7.7	.4(15)	8.3	.5(7)	.99	.334
	PN1	11.4	.5(23)	11.5	.7(14)	.08	.937
	PN2	12.2	.5(23)	12.5	.6(16)	.43	.667
	PN3	12.7	.6(22)	13.3	.6(16)	.57	.574
	PN4	13.0	.9(15)	13.8	.9(9)	.60	.558
	PN5	15.3	.8(5)	14.4	1.0(4)	.74	.485
ILE (nmol/ .1ml)	AN1	5.39	.44(15)	4.90	.31(8)	.77	.450
	AN2	4.55	.26(15)	4.79	.32(7)	.52	.610
	PN1	6.55	.31(23)	6.73	.37(15)	.39	.700
	PN2	7.04	.32(23)	7.72	.32(16)	1.47	.150
	PN3	7.46	.34(22)	7.55	.39(15)	.19	.850
	PN4	8.14	.44(15)	7.88	.45(9)	.39	.698
	PN5	9.69	.24(5)	8.30	.74(4)	1.97	.090
TYR (nmol/ .1ml)	AN1	4.60	.33(15)	4.05	.24(8)	1.15	.265
	AN2	4.09	.18(15)	4.22	.29(7)	.42	.682
	PN1	5.81	.25(23)	4.96	.29(14)	2.19	.035
	PN2	5.21	.27(24)	5.36	.42(16)	.32	.748
	PN3	5.41	.36(22)	5.81	.23(16)	.92	.362
	PN4	6.14	.42(15)	5.81	.44(9)	.51	.616
	PN5	7.37	.88(5)	6.06	.80(4)	1.07	.319

Table 4.11 Comparison of biochemical variables between mothers of male infants and mothers of female infants.

Variable	Time	Mean +/- SEM(N) male mean	SEM	female mean	SEM	Students t	2-tailed prob.
PHE (nmol/.1ml)	AN1	5.44	.43(15)	4.69	.29(8)	1.19	.248
	AN2	4.59	.18(15)	4.77	.27(7)	.57	.574
	PN1	5.88	.19(23)	6.10	.39(14)	.50	.623
	PN2	5.25	.22(23)	5.61	.25(16)	1.04	.304
	PN3	5.42	.30(21)	5.73	.16(16)	.87	.393
	PN4	5.95	.39(15)	6.22	.33(9)	.47	.641
	PN5	7.21	.91(5)	5.85	.61(4)	1.16	.283
Cortisol ($nmol/dm^3$)	AN1	30.7	1.6(27)	30.1	1.8(16)	.22	.824
	AN2	29.2	1.0(22)	31.7	1.6(15)	1.41	.168
MHPG(mg/24hr urine)		921	131(11)	1251	224(8)	1.34	.196
MHPG(mg/g creat.)		1675	214(11)	3241	1432(8)	1.08	.315
Birth weight of infant(g)		3470	125(29)	3063	159(20)	2.04	.047

Sex of infant		male	female
depression	cases	10	1
	noncase	14	11

$X^2 = 4.19$ p= .041

Table 4.12 Interrelationships between biochemical parameters at parturition.

Time / Correlat'n		AN1	AN2	PN1	PN2	PN3	PN4	PN5
		(Number of samples in parentheses)						
Total TP v LNAA	r=	-.148	.451	.127	-.052	.036	.114	.155
	p=	.26(22)	.02(22)	.31(18)	.38(39)	.42(35)	.30(23)	.35(9)
Total TP v prog.	r=	-.146	.037	-.076	.025	-.055	.114	-.008
	p=	.42(33)	.85(28)	.64(40)	.87(46)	.72(45)	.57(28)	.98 14
Total TP v oest.	r=	.292	-.050	-.216	-.274	-.353	-.007	-.340
	p=	.09(35)	.80(80)	.17(42)	.07(45)	.02(45)	.97(28)	.23(18)
Total TP v NEFA	r=	-.296	.032	.157	.212	.138	.352	.008
	p=	.05(33)	.85(40)	.30(45)	.13(52)	.35(48)	.07(28)	.98(14)
Free TP v prog.	r=	.016	-.220	.031	-.189	.121	.269	.295
	p=	.93(33)	.27(27)	.85(40)	.21(45)	.44(44)	.17(28)	.31(14)
Free TP v oest.	r=	-.047	.045	-.278	-.135	-.294	.214	.001
	p=	.79(35)	.82(29)	.08(42)	.39(44)	.05(44)	.28(28)	.99(14)
Free TP v NEFA	r=	.185	.182	.156	.046	.128	.027	.209
	p=	.24(42)	.28(37)	.31(45)	.75(51)	.39(44)	.89(28)	.47(14)
Progest. v oest.	r=	.091	.037	.638	.299	.150	.230	.154
	p=	.62(33)	.86(27)	.00(40)	.05(45)	.33(45)	.24(28)	.60(14)

DISCUSSION OF CHAPTERS 1 - 4

DISCUSSION OF CHAPTERS 1 - 4.

The following section is a discussion of the preceding clinical studies. The results will be discussed in the light of results reported by other workers. A further discussion of these chapters is presented in the general discussion at the end of this thesis.

Psychological Findings of the Syndromes Studied.

Mood Variations Throughout the Menstrual Cycle.

There was no significant variation in the MAACL 'D' scores between the different stages of the menstrual cycle. There was however a greater incidence of self reports of depression during the premenstrual and menstrual stages of the cycle. There are several possible explanations for the variation of self reported depression without a variation in depressed affect as measured by the MAACL 'D' scale. Firstly, the MAACL was designed for the measurement of mood at a particular moment in time, the self-reports however were made from a retrospective global view of the previous day. It is therefore possible that the MAACL questionnaires were completed at a time of day before the negative aspects of that day became apparent. This may explain the incongruity between the self report and the MAACL scores.

An alternative explanation may be that the MAACL 'D' scale is in fact measuring something other than depression. This explanation is unlikely in that this questionnaire has been validated against interview and against similar mood questionnaires (Zuckerman & Lubin, 1965). A final explanation for the incongruity between the mood scale and the self reports may rest on the subject's perception of depressed affect.

During the premenstruum and menstruum, there is a liklihood of a variety of somatic symptoms such as abdominal pain, feelings of bloatedness, etc. (See Introduction), it is possible that, due to the media coverage, subjects accept such symptoms as being synonymous with premenstrual syndrome. This may lead to the reporting of somatic symptoms using psychological terminology. This process may have been encouraged further by the subjects 'guessing' that the study concerned premenstrual mood changes, and therefore reporting depression premenstrually in order to 'help the experimenter'. In contrast, the MAACL scores are more difficult to influence by volition, and they take no account of somatic symptoms, they are therefore less influenced by the subjects' preconceptions of premenstrual syndrome.

It is probably a combination of these factors which produces the incongruity between the mood questionnaire scores and the subjective reports.

In addition, those subjects self reporting depression within this study also tended to score more highly on the Eysenck neuroticism scale. This finding is in agreement with the earlier work of Rees (1953) and Coppen and Kessel (1963), which showed a relationship between neuroticism and reports of physical amd mental symptoms of the menstrual cycle. These results may indicate that when mood, as measured by mood questionnaire, is equal, the more neurotic subjects are more likely to report the symptoms as being troublesome. Therefore there may be a subgroup of neurotic subjects that require a lower threshold of symptom severity before reporting that symptom as being troublesome. Such a suggestion has previously been made by Gelder (1978).

Post-Partum Mood Changes.

There was little difference in the MAACL mood scale scores between the antenatal and the post-natal time points, except that subjects scored more highly on the anxiety scale antenatally than postnatally. This may be a reflection of the concern over the outcome of the pregnancy. In contrast antenatal BDI scores were significantly greater than the post-natal BDI scores. This may reflect a greater severity of depression in late pregnancy that during the puerperium. The MAACL scores do not support this finding. The difference in in BDI scores may therefore be a consequence of the somatic questions of the BDI scale, as questions concerning appetite, sleep, libido and concern over personal appearance may not be appropriate for subjects in late pregnancy and the early puerperium.

Those subjects classified as 'blues' cases scored more highly on all of the MAACL scales and the BDI post-natally. These subjects also scored more highly on the anxiety scale antenatally, when compared with 'blues' non cases.

These results therefore show that while a subgroup of subjects ie 'blues' cases, show marked changes in the mood scores at parturition , there is not such a general trend of mood change for all subjects at parturition.

The fact that the mood changes of the 'blues' syndrome produced significant differences in the mood questionnaire scores, unlike the reported mood changes of the premenstruum suggests that puerperal mood changes and premenstrual mood changes are different in nature and severity.

Perimenopausal Mood Changes.

There was no significant difference in the incidence of depression between pre-menopausal and post-menopausal subjects. This finding may reflect the heterogeneity of the sample population in that no differentiation was made between reactive and endogenous depressions. Therefore, if there is a greater incidence of endogenous depression post-menopausally, the result may have been obscured by the presence of reactive depressions. As there was no 'breakdown' of the symptoms of the pre- and post- menopause depressed subjects, it is not possible to state whether there is any difference in the symptomatology of pre- and post- menopause depressive illness.

Non Biochemical Factors Involved in the Aetiology of Hormone-Related Mood Changes.

Menstrual Mood Variations.

As described in the previous section, using the MAACL, there was no evidence of a greater incidence of negative affect premenstrually or menstrually, as compared with the other menstrual cycle stages. There was however a greater incidence of self reports of depression during these stages. These self reports of depression possibly reflect a greater severity of somatic symptoms, or a possible relationship with a neurotic personality trait (see previous). No record was made of the self reported severity of the premenstrual or menstrual somatic symptoms therefore it was not possible to test any such relationship between somatic symptoms and affect.

Post-Partum Mood Changes.

'Blues'

'Blues' cases reported a greater incidence of tearfulness during pregnancy than non cases. Following from the previous section on premenstrual mood changes, it is possible that this increased reporting of tearfulness amongst 'blues' cases reflects either a greater willingness to report such symptoms as being troublesome, or a lower threshold for crying amongst these subjects. This is similar to the suggestion that that reports of depression premenstrually are related to a neurotic personality and an increased willingness to report symptoms. If these increased reports of tearfulness during pregnancy in 'blues' cases are a reflection of an increased willingness to report symptoms then it would be plausible to expect an increase in the reports of premenstrual mood changes or other menstrual problems amongst these subjects. However,this study did not find an increased incidence of such reports amongst 'blues' cases. Personality inventories were not used in the present study, but they have been used by previous workers, and no relationship has been found between personality and the incidence of 'blues' (See Introduction). It is therefore unlikely that the increased incidence of tearfulness during pregnancy in 'blues' cases is related to a neurotic personality trait.

In agreement with the previous work of Pitt (1973), Dalton (1971), Kumar & Robson (1978) and Nott and co-workers (1976), the present study found no difference between 'blues' cases and non cases on any variables such as age, age of menarche, marital status, socioeconomic class, previous menstrual history, personal or family psychiatric history or the length

or difficulty of the pregnancy, labour or delivery. This fact therefore supports the hypothesisthat the aetiological factors underlying the 'blues' are biochemical in nature rather than being sociological. However, as in previous studies, (eg Nott et al., 1976), the present study found a greater incidence of 'blues' amongst primiparae than multiparae. This difference was also manifested as a greater incidence of 'blues' amongst gravida 1 or 2 subjects than amongst those of gravida 3 or above. This may be a causal relationship in that the experience (whether pleasurable or traumatic) of the pregnancy, labour and delivery of the first child may produce the emotional and affective changes characteristic of the 'blues', whilst the birth of subsequent children may not involve so many novel experiences. Therefore a knowledge of 'what to expect', together with previous experience may offer some protection against the 'blues'.

Another explanation for this finding of an increased incidence of 'blues' amongst primiparae may be that women that suffer 'blues' following the birth of their first child are not willing to undergo the same experience again. They may therefore limit themselves to one child. The results of the present study however found no difference between 'blues' cases and non cases in their willingness to go on to have further children, when questioned at 9 months post-partum. As there were no consistent biochemical differences between primiparae and multiparae the initial explanation for the increased incidence of 'blues' amongst primiparae is probably the more likely.

'Blues' cases also reported a significantly greater incidence of a previous medical history, usually of a gynaecological nature such as treatment for infertility or dilatation and curettage. This finding may reflect an association between 'blues' and previous gynaecological problems, although no previous studies have reported such an association. There was however no significant relationship between the incidence of 'blues' and minor gynaecological problems such as menstrual irregularity or dysmenorrhoea. Hence any relationship with 'blues' is concerned only with more major gynaecological problems. There is no data to suggest the nature of this relationship. It is interesting to note in this context that 'blues' non cases more frequently stopped using oral contraceptives for reasons other than a planned pregnancy, than 'blues' cases.

The final correlate of 'blues' found in the present study was that of an increased incidence of 'blues' amongst breast-feeders. A possible biochemical explanation for this finding will be presented in a later section. However it is probable that the biochemical changes associated with breast-feeding are unrelated to the aetiology of 'blues' as 'blues' symptoms are short lived whilst breast-feeding continues for several months. It is therefore probably that the association between breast-feeding and 'blues' involves some factor other than a biochemical one, for example there may be a certain personality type which breast feed and that are also more susceptible to 'blues', there is however no further data to suggest the true nature of the association between 'blues' and breast-feeding.

Post-Partum Depression.

When considering the more severe post-partum depression, a similar pattern to that for 'blues' was seen in that there was no significant association between the incidence of depression and age, parity, marital status, socioeconomic class, etc. These findings were in full agreement with the previous work of Pitt (1968) and Kendell and colleagues (1967). Nor was a previous personal or family history of psychiatric illness in any way associated with the incidence of depression post-partum. This finding is contrary to that of Paykel and colleagues (1980). In the present study the only factors found to be significantly related to the incidence of post-partum depression were sexual activity during pregnancy, a history of previous medical/gynaecological illness, the sex of the infant and the age of menarche.

Subjects that went on to become depressed post-partum more frequently reported decreased sexual activity during pregnancy. This decreased sexual activity was not related to either parity or gravidity. Similarly, there was no relationship between depression and post-partum sexual activity. Such a decrease in antenatal sexual activity may therefore be indicative of latent fears of harming the foetus, or possibly due to altered body image during pregnancy. There wee however no significant relationships between post-partum depression and reports of anxiety during pregnancy, or concern over personal appearance during pregnancy. Recent work by Kumar and Robson (1982) reported that those subjects that became depressed post-partum reported a greater incidence of pregnancy-related marital conflict. The result may be associated with the present finding of decreased antenatal sexual activity amongst those subjects that subsequently became depressed post-partum.

Elliot (1982) reported that subjects that become depressed post-partum score more highly on the EPI 'N' scale, and report greater anxiety and somatic discomfort during pregnancy, than subjects who do not become depressed. Again these results may be associated with the decreased sexual activity during pregnancy in depression cases seen in the present study.

Subjects that went on to become depressed post-partum also more frequently gave birth to male infants. No previous studies have reported a similar finding. This effect does not appear to be due to male infants having any differential biochemical effect on the mothers as there were no significant biochemical differences between subjects giving birth to male and female infants. There may however be differences in some biochemical factor not measured in the present study. There is no data to suggest that nature of the relationship between the incidence of depression and the sex of the infant, it is therefore unclear whether this effect may be due to male infants being more 'troublesome' to care for in some way. This finding would need to be replicated to ensure that the result had not occurred by chance.

There was also a greater incidence of previous medical/gynaecological problems amongst subjects that became depressed post-partum. This result may be related to that of Kumar and Robson (1982) who found that subjects who became depressed post-partum were more frequently elderly or sub-fertile primiparae. The present study found no relationship between depression and age or parity, but the previous gynaecological problems were frequently concerned with sub-fertility. Therefore the findings of these two studies may be associated.

Depression cases also reported a later age of menarche. This may also be associated with the subfertility. Such a finding has not previously been reported, although Dalton (1971) reported a higher age of menarche amongst 'blues' cases.

From the results of the smaller 'pilot' study of puerperal and post-partum mood change (chapter 1), it appeared that sociological variables may play a rôle in the aetiology of post-partum depressive illness. Of the 11 subjects studied, 7 were diagnosed as being depressed at 6 months post-partum. This figure represents 64% of the total and is therefore much greater than the incidence of about 10% reported by earlier studies (Pitt 1968; Dalton 1971; Kumar & Robson 1978), or the 22.4% reported in the larger study of this thesis. Of the 11 subjects of the smaller study, 4 were diagnosed as being depressed antenatally, whilst 2 subjects were diagnosed as suffering from non childbirth related depressions at 6 months post-partum. This very high incidence of psychiatric illness is probably a characteristic of the particular population studied. These subjects were taken from the Tower Hamlets region of the east end of London, and in many cases, the living conditions of the subjects were very poor (Waldron-personal communication). No records were made of the vulnerability factors or recent life events of the subjects, but it is possible that the high proportion of depression found amongst subjects of this study was a summation of true post-partum depression together with a depression incidence inherent to the population, due to the social conditions of the area. This explanation is supported by the work of Brown

and Harris (1978) who studied a population of women in a similar area of East London and reported a high incidence of depression, caused, it was believed, by the interaction of sociological vulnerability factors and the life events of the subjects studied (See Introduction).

The explanation is also supported by the work of Paykel and colleagues (1980) who suggested that psychosocial stress was a causal factor in the development of post-partum depression. Similar results have also been reported by Martin (1982). This latter study reported that life events related to the pregnancy and birth may be associated with the aetiology of post-partum depression, whilst there was no such association for life events unrelated to the pregnancy or birth. These results therefore suggest that sociological factors may play a rôle in the aetiology and presentation of post-partum depressive illness.

Interrelationships Between the Hormone-Related Mood Changes.

There has been much research investigating the biochemical factors underlying the mood changes seen in women at times of hormonal flux (See Introduction). Because all of the mood changes occur at times of similar hormonal fluctuations it is attractive to suggest that there is a single, common, underlying change responsible for all of the mood variations. For example, Dalton (1971) suggested that the fall in progesterone may be the underlying factor in both premenstrual syndrome and post-natal depression. If such a hypothesis were correct, it might be expected that women who suffer from premenstrual syndrome due to the presence of some biochemical factor, may also suffer from post-partum mood changes, due

to the presence of that same biochemical abnormality. By the same reasoning, it might be expected that subjects suffering mental changes following the birth of one child due to the presence of the biochemical predisposition, would suffer the same symptoms following the birth of subsequent children. In support of such a hypothesis, Dalton (1971) reported that subjects suffering from post-partum mood changes more frequently reported premenstrual syndrome. Also, subjects suffering from post-partum depression following the birth of one child have been seen to more frequently suffer similar symptoms following subsequent births (Dalton, 1982, b). In the case of the more florid post-partum psychosis, the chance of the occurrence of post-partum psychosis is increased by a factor of one hundred if a previous delivery has been followed by a psychiatric episode (Paffenberger, 1961).

The findings of the present study do not support these previous reports. Subjects that suffered from 'blues' or post-partum depression did not report a greater incidence of premenstrual syndrome, nor was there an increased incidence of 'blues' amongst subjects that subsequently suffered from post-partum depression. There was no difference in reports of previous 'blues' between 'blues' cases and non cases. These results therefore suggest that there is no common underlying factor in the aetiology of the hormonally-related psychological changes.

Similarly, there was no increased incidence of previous personal or family psychiatric illness (either affective or non affective) in cases of 'blues', post-partum depression, or perimenopausal depression, as compared with non cases.

These results therefore suggest that as well as the individual hormonally-related psychological symptoms being produced by separate, underlying changes, all of the hormonally-related syndromes are separate from non-hormonal psychiatric illness in terms of aetiology.

Biochemical Factors Involved in the Aetiology of Hormone-Related Mood Changes.

As described in the introduction, many biochemical factors have previously been associated with hormone-related mood changes. Each of these factors will be discussed separately, and the implications of the results from the present studies together with results from other workers will be described.

Plasma Prolactin.

George and Wilson (1980) reported a strong correlation of post-partum anxiety and depression with plasma prolactin. However, the significance of this finding must be questioned on several bases. Firstly, plasma prolactin is highly variable with marked diurnal variations (Sassin et al., 1972) and a pulsatile release following suckling. This would mean that any plasma measurements of prolactin would need to be made at a fixed time following suckling. Secondly, prolactin is released at times of stress, the effect being so marked that the procedure of the venepuncture itself may produce an increase in plasma prolactin (Raud et al., 1971). George and Wilson (1980) reportedly controlled for the effects of whether the subjects were breastfeeding or bottle-feeding and for diurnal variations. However, the observed correlation between anxiety and prolactin was calculated using parametric statistics, whereas MAACL scores are ordinal scales and therefore non-parametric in nature. In addition, any relationship between anxiety and plasma prolactin would

more likely reflect an effect of anxiety on prolactin rather than vice versa.

The observed relationship between 'blues' and prolactin (ibid) may be associated with the increased proportion of breast-feeders amongst 'blues' cases seen in the present study. Although George and Wilson (1980) did control for the effects of breast feeding on prolactin by taking blood samples before the first breast feed of the day. No relationship was found between 'blues' and breast-feeding in that study (ibid). These results would suggest that the increased plasma prolactin seen amongst 'blues' cases may be unrelated to the increased number of breast-feeders amongst the 'blues' cases seen in the present study.

Halbreich and colleagues (1979) reported that diurnal patterns of prolactin release were altered in non-puerperal depressives with increased secretion of prolactin in the evening and early morning. A possible connection between diurnal prolactin secretion and diurnal mood change was postulated. However this study does not overcome the problem of the possible effects of mood change on prolactin secretion. The possibility of hyperprolactinaemia being causative of psychological changes is remote. Hyperprolactinaemia is a common cause of infertility in human females, however such cases of hyperprolactinaemia rarely, if ever, exhibit the psychological symptoms of either depression or 'blues' (Parsons, personal communication). Increased plasma prolactin has also been found in sufferers of premenstrual syndrome (Ghosh et al, 1981), again this may have been explained by the effect of the psychological symptoms on plasma prolactin, except that the dopamine agonist bromocryptine was reported to be effective both against the hyperprolactinaemia and the premenstrual syndrome.

Unfortunately, the full details of controls and placebo effects for this study are unavailable, therefore the importance of this finding is uncertain.

Oestrogens have been seen to produce an antidopaminergic effect on prolactin release, such that increased oestrogens produce increased prolactin release (Ferland et al., 1979). This could therefore mean that the elevated plasma prolactin reported to be concurrent with the hormonally related mood changes may reflect increased plasma oestrogens. However, there have been no reports of increased plasma oestrogens at these times of mood change.

Central 5HT is also involved in the control of prolactin secretion, however, the precise nature of this involvement is uncertain. Koenig and colleagues (1979) reported that prevention of 5-hydroxytryptaminergic transmission produced an increase in prolactin secretion. Pilotte and Porter (1979) however reported that administration of 5HT, either I.V. or S.C. to rats also increased prolactin secretion. Similarly, Pavasuthipaisit and colleagues (1980) reported that 5HT receptor blockade attenuated the release of prolactin which normally follows electrical stimulation of the medial basal hypothalamus of the rhesus monkey. From these reports therefore, it is not possible to speculate as to whether the increased prolactin secretion reported to occur in premenstrual syndrome and puerperal 'blues' might reflect either increased or decreased central 5HT activity.

It is possible that the findings of increased plasma prolactin concurrent with these psychological syndromes is an artefact of a disrupted diurnal rhythm as previously postulated by

Halbreich and colleagues (1979). Therefore, although the the test group of subjects and the control group may be being compared at the same time of day, it is possible that the differences in plasma prolactin are due to the two groups being at different stages of their diurnal cycles. Support for such a disruption of diurnal rhythms in depression comes from the work of Pflug and colleagues (1976) and Wirz-Justice (1978). In addition, anti-depressant drugs have been shown to modulate diurnal rhythms in rat plasma TP and rat brain TP and 5HT (Martin & Redfern, 1982).

Cyclic AMP.

Ballinger (1980) reported that subjects showing the greatest mood changes in the puerperium showed the greatest fall in urinary cAMP during the first week post-partum. Cyclic AMP is a second messenger for a number of neurotransmitters including the β action of noradrenaline and certain of the dopamine receptors. Therefore a fall in cAMP, concurrent with mood changes post-partum may reflect a decreased activity for one or more of the neurotransmitters. However, cAMP is also involved in muscular glycogen metabolism and in the action of several hormones, therefore it is unlikely that urinary cAMP excretion accurately reflects changes in transmitter activity in the brain (Weiss & Greenberg, 1975). Thomas (1982) reported that oestradiol is capable to producing a protein which inhibits cAMP formation. It was postulated that this action may be involved in the aetiology of the hormonally related mood changes (ibid). However, as cAMP excretion is high during pregnancy, a time of high plasma oestradiol, it is unlikely that an inhibition of cAMP formation by oestradiol is related to the changes reported by Ballinger (1980).

Plasma Progesterone.

Dalton (1971) postulated that the sudden fall in progesterone may be the cause of post-partum mood changes. However, no differences in any endocrinological factors were found between 'blues' cases and non cases (Nott et al., 1976). The present study found similar results in that there were no differences in antenatal or puerperal plasma progesterone, between either 'blues' cases and non cases or depression cases and non cases. Similarly, Dalton (1971) postulated that the fall in plasma progesterone may be causative of premenstrual syndrome. O'Brien and colleagues (1980) measured plasma progesterone throughout the menstrual cycle in premenstrual syndrome sufferers and non sufferers, sufferers of the syndrome were shown to have increased post-ovulation progesterone. However these workers concluded that the premenstrual mood changes occurred later than the changes in progesterone, therefore the possibility of a causal relationship was dismissed. From these studies, it therefore appears that there is no biochemical basis for the progesterone hypothesis of post-partum mood changes or premenstrual syndrome. However in uncontrolled studies, Dalton (1964 & 1982) reported that chronic progesterone supplements are beneficial in the prophyllaxis of both post-natal depression and premenstrual syndrome. These reports do not consider any possible placebo effects. Progesterone supplements have also been used in the treatment of post-partum psychosis (Schmidt, 1943). In connection with this, Cernik (1980) reported that post-partum psychiatric subjects had decreased plasma progesterone levels at the time of onset of the psychosis as compared with non-psychotic post-partum controls. In the case of post-partum psychosis, therefore, there may be a deficit of progesterone,

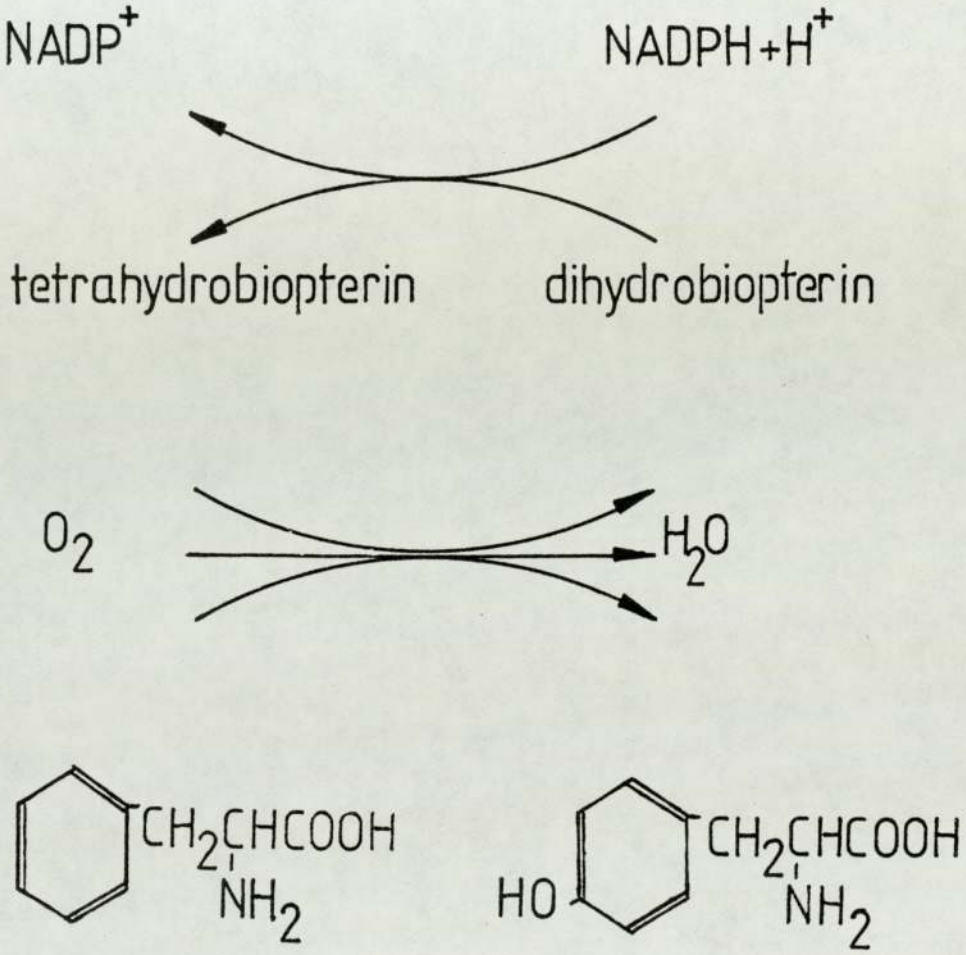
however, in the case of 'blues' and post-partum depression, this does not appear to be so.

Rather than mood changes being produced by decreased levels of progesterone, it is possible that there is some abnormal 'sensitivity' to progesterone which is associated with the hormonally-related mood changes. Huffer and colleagues (1970) postulated that the psychological side effects of oral contraceptive treatment may be produced by some abnormal sensitivity to oestrogens and progesterone. Similarly, Dalton (1971) postulated that premenstrual and post-partum mood changes may be related to some increased 'sensitivity' to sudden changes in plasma progesterone. There is however no speculation as to the nature of this progesterone 'sensitivity', nor is there nor evidence to support the hypothesis.

Plasma Dihydrobiopterin.

Plasma 7, 8-dihydrobiopterin has been seen to be increased in human females receiving tricyclic anti-depressants (Leeming et al., 1982), it was therefore postulated that this effect may be involved in the therapeutic actions of these drugs. Dihydrobiopterin is related to 5, 6, 7, 8-tetrahydrobiopterin (See Fig VIII) which is a natural cofactor in the hydroxylation of phenylalanine to tyrosine, and of tyrosine to dopa. This hydroxylation is rate limiting in the synthesis of dopamine and noradrenaline. Tetrahydrobiopterin is also required for the synthesis of 5HTP from TP (Hosoda & Glick, 1966). Results of the present study show that there is no difference in plasma dihydrobiopterin between either puerperal 'blues' cases and non cases, or post-partum depression cases and non cases. Dihydrobiopterin has previously been seen to

Fig VIII The relationship between dihydrobiopterin and tetrahydrobiopterin in the hydroxylation of phenylalanine to tyrosine



be slightly, but non significantly decreased in 'blues' cases (Handley and colleagues, unpublished data). This result does not preclude a dihydrobiopterin deficit in non-hormonally related depressions.

Urinary MHPG.

Urinary excretion of MHPG has previously been reported as being decreased in some depressive patients (Maas, 1968). In the present study, no difference was seen between 'blues' cases and non cases in terms of MHPG excretion. However subjects that went on to become depressed post-partum showed significantly increased excretion of MHPG per unit volume of urine, this difference was probably due to differences in urinary volume rather than due to differences in MHPG excretion. This result therefore suggests that subjects that go on to become depressed post-partum are not all of a subgroup that exhibit decreased urinary excretion of MHPG. This may indicate that post-partum depression is an entity separate from other forms of non-puerperal depression.

Cortisol.

Urinary excretion of cortisol has been seen to be increased in non-puerperal depressed patients (Board et al., 1957; Bunney et al., 1965). Handley and co-authors (1980) reported a significant correlation between antenatal plasma cortisol and puerperal 'blues'. The present study however, failed to replicate this finding either concerning puerperal 'blues' or post-partum depression. The reasons for the failure to replicate this result are unclear, but seasonal variations in plasma cortisol may be involved. In the study of Handley and colleagues (1980), plasma cortisol was seen to be signific-

antly greater in the period January to June that the period July to December. The incidence of 'blues' was also seen to be increased during the first half of the year. However, the present study took place between February and June, and therefore should not be so greatly affected by seasonal variations of plasma cortisol. It is however possible that changes in the environmental temperature may have had some effect on plasma cortisol such as to mask any differences between the psychiatric classifications.

Plasma Tryptophan.

Previous studies of the 'blues' have reported decreased plasma free TP in 'blues' cases (Stein et al., 1976; Handley et al., 1977), the present study however failed to find such a relationship. Many workers have investigated the relationship between plasma free TP and non puerperal depression, but free TP has been seen to be decreased (Coppen et al., 1972, 1973, 1978; Kishimoto & Hama, 1976; Aylward, 1976), unchanged (Garfinkel et al., 1976; Riley & Shaw., 1976) or increased (Niskanen et al., 1976) in depressive illness. The reason for this disparity of results is unclear. Handley and colleagues (1980) failed to replicate their earlier result of an association between plasma free TP and puerperal 'blues'. In this thesis, no association was found between plasma free TP and mood either immediately post=partum or perimenopausally. However free TP was found to be significantly decreased post menopausally, this is in agreement with the previous results of Aylward (1976) but contradictory to those of Coppen and Wood (1978) who reported no significant differences in plasma free TP between pre and post menopausal subjects, but decreased plasma

free TP in perimenopausal subjects. The reason for this disparity in unknown.

The decrease in plasma free TP at the time of the menopause was explained by both Aylward (1976) and Coppen and Wood (1978) as being due to a decrease in circulating oestrogens. Aylward (1976) stated that oestrogens compete with TP for binding to plasma albumin, therefore a decrease in plasma oestrogens would produce increased binding of TP to albumin, in support of this, a significant correlation has been seen between plasma oestrogens and plasma free TP (Thomson et al, 1977). The fact that there is no change in plasma total TP supports this hypothesis of a change in TP binding. In addition, Coppen and Wood (1978) reported that the decreased free TP could not be explained in terms of changes in plasma concentrations of albumin, NEFA or LNAA. However, Gould (1979) reported that oestrogens have no effect on TP binding to albumin <u>in vitro.</u> Therefore some factor other than decreased oestrogens, eg FSH, may be responsible for the decrease in plasma free TP seen post menopausally.

In the present study, no difference was seen in plasma free TP between perimenopausally depressed and non depressed subjects. This result is contradictory to the earlier work of Coppen and Wood (1978). This disparity may be explained by the heterogeneity of the subject population in the present study. No differentiation was made between reactive and endogenous depressions in the present study. Therefore if there were reactive depressions within the sample this may account for the lack of difference between depression cases and non cases in terms of plasma free TP. All previous differences

in plasma free TP have been seen between endogenous depression cases and non cases.

The finding of the present study that plasma free TP was significantly increased on the fifth day post-partum in subjects that subsequently went on to become depressed is difficult to explain. For this particular time point, the number of subjects is relatively small due to the fact that many subjects leave hospital and return home before the fifth day, especially multiparae, therefore this result is from a small population sample. However it may be indicative of a disturbed TP metabolism in subjects that are predisposed to depression post-partum. Such a finding has not previously been reported.

As mentioned previously(page303), some previous workers have found decreased plasma free TP in endogenously depressed patients. The present study was an investigation of subjects up to 6 months before the onset of the depressive symptoms. This study therefore shows that subjects are not pre-disposed to post-partum depression in terms of a chronically decreased plasma free TP.

With the exceptions of Handley and colleagues (1980) and Riley and Shaw (1976), previous studies have failed to find any relationship between depressed affect and plasma total TP. Riley and Shaw (1976) reported that plasma total TP was decreased in depressed patients, whilst Handley and colleagues (1980) reported that 'blues' cases exhibited abnormal plasma total TP dynamics immediately post-partum (See Introduction). Kelly et al.(1980) also reported that in the depression commonly seen in Cushing's syndrome, plasma total TP was decreased,

however plasma free TP and plasma LNAA were unchanged. The results of the present studies are in agreement with the majority of other workers (eg Coppen et al, 1973) in that no relationship was found between plasma total TP and depressed affect either perimenopausally or during the menstrual cycle. Similarly, subjects who went on to become depressed post-partum showed no abnormalities in their plasma total TP dynamics immediately post-partum. The results of the menstrual and perimenopausal studies therefore indicate that decreased plasma total TP is not associated with the affective changes seen at these times. It is not possible to make similar conclusions concerning post-partum depression since the plasma samples were obtained several months before the onset of the depressive illness. However, it is possible to conclude that subjects who go on to develop depression post-partum, are not previously predisposed to the depression in terms of a decreased plasma total TP.

Plasma total TP however may be involved in the aetiology of puerperal 'blues'. The mean plasma total TP on day 1 post-partum was found to be significantly decreased in 'blues' cases as compared with 'blues' non cases. A similar finding was previously reported by Handley and colleagues (1980), who found significantly decreased plasma total TP on post-partum days 1 and 2 amongst 'blues' cases. These workers explained the results in terms of two subgroups of subjects: those that showed a normal rise of plasma total TP immediately post-partum ('Risers') and those that failed to show such a rise ('Non Risers') (See Introduction). Their results showed that TP 'non risers' showed an increased incidence of puerperal 'blues', and also an increased incidence of visits to their

GP, with depressive symptoms, in the six months post-partum. In the present study, it was again possible to divide the subjects into TP 'risers' and 'non risers', but in this case there was no significant relationship between the 'riser' classification and the incidence of either 'blues' or post-partum depression. Therefore, although the present study replicated the earlier work of Handley and colleagues (1980) in that the mean plasma total TP of 'blues' cases was signficantly decreased immediately post-partum, not all 'blues' cases exhibited the delayed rise of plasma TP post-partum. Similarly, not all subjects with the delayed rise of plasma total TP post-partum were 'blues' cases, hence the relationship between the TP rise and 'blues' as reported by Handley and colleagues (1980)was not replicated in the present study. This incongruity between the results of the present study and those of Handley and colleagues (1980) can be explained in terms of the definition of 'riser' classification. 'Non risers' are defined as subjects whose plasma total TP does not *rise above* antenatal values by the second day post-partum. Therefore the criterion involves the antenatal total TP value. Hence not all subjects with low plasma total TP immediately post-partum are 'non risers', depending on their antenatal TP levels.

From the results of both the present study and that of Handley and colleagues (1980), there appears to be a deficit of plasma total TP one or two days prior to the presentation of the 'blues' syndrome. This deficit however is unlikely to be the direct cause of the 'blues' syndrome. Harris(1980) administered TP to puerperal subjects, and saw no effect on either symptoms or incidence of 'blues'. It is probable

therefore that the change in plasma total TP is a marker of some underlying change, rather than being the cause of the 'blues' syndrome per se.

Plasma NEFA.

Van Praag and Leijnse (1966) reported increased NEFA metabolism in endogenous depressive subjects. This result was not confirmed by Mueller and colleagues (1970). An increased NEFA response to mild stress in depression was later reported by Curzon and colleagues (1979). In the present study, no external stresses were used to test this finding of Curzon and colleagues (1979), this may therefore explain the lack of difference, in terms of plasma NEFA, seen between subjects suffering menstrual mood changes and subjects not suffering such changes. It is possible that if a mild stressing procedure had been used, some differences may have been seen.

Immediately post-partum however, subjects who later went on to become depressed showed an increased plasma NEFA, superimposed onto the normal decline in plasma NEFA seen post-partum. However, there was no such differences between 'blues' cases and non cases which again indicates that 'blues' and post-partum depression are separate entities with differing causative factors.

The finding of increased post-partum plasma NEFA in subjects tha subsequently become depressed, may be interpreted in the light of the earlier workof Curzon and colleagues (1979).

It is possible that the process of parturition acts as a stressor, and that those subjects 'predisposed' to depression respond to this stressor with a greater release of NEFA than subjects not 'predisposed' to depression. These results may

therefore extend the previous work of Curzon and colleagues (1979) in that, whereas previously, endogenously depressed patients were seen to exhibit this increased NEFA response to stress during a depressive episode, the present results indicate that subjects predisposed to post-partum depression show a similar response during the puerperium, several months prior to their illness. The mechanisms controlling this response are unknown, but it is possible that the effect is produced by an 'over-active' sympathetic nervous system (Curzon et al., 1979). If this is so, then these results may indicate that there is an 'over-active' sympathetic nervous system during periods of endogenous depression, and there may be a similarly over-active sympathetic nervous system several months prior to the onset of post-partum depression. Therefore, if post-partum and non-puerperal endogenous depression are similar in terms of the NEFA response to stress, these results may indicate that both illnesses may involve an 'over-active' sympathetic nervous system, and that this may be a chronic predisposing factor of the illness.

The release of NEFA is normally controlled by the action of adrenaline on β-adrenoceptors (Hims-Hagen, 1967) therefore the increased release of NEFA seen in depressive patients may indicate either increased amounts of noradrenaline at these receptors, or increased sensitivity of the β-receptors. In addition to this, chronic antidepressant treatment has been seen to result in a down regulation of cortical β-receptors (Bannerjee et al., 1977). These two findings therefore suggest a possibility of supersensitive β-receptors being involved in the aetiology of depressive illness. This conclusion is speculative in nature, since chronic antidepressant treatment

has also been seen to produce down regulation of dopamine receptors (Chiodo & Antelman, 1980) and α_2 receptors (Crews et al., 1978; McMillan et al.,1980). All of these effects may be non-specific effects of the drugs, unrelated to their antidepressant action, however the abnormal NEFA response to stress in depressive patients may indicate a chronic, predisposing supersensitivity of β-adrenoceptors in these patients.

A second explanation for the increased rise in NEFA following stress in depressive patients may be that depressive patients perceive the stress as being greater than that perceived by control patients. The increased rise in NEFA may therefore reflect the increased perception of stress. In support of this hypothesis, as previously described, patients that become depressed post-partum have been seen to score more highly on the EPI neuroticism scale during pregnancy (Elliott 1982).

Plasma LNAA.

Large neutral amino acids (LNAA) have also been implicated in the aetiology of depressive illness. LNAA compete with TP for transport across the blood brain barrier (Pardridge, 1977) hence an increase in the concentrations of the other LNAA relative to plasma TP would result in decreased brain penetration of TP. Such an increase in the LNAA has been reported to occur in depressed patients (De Mayer et al., 1981). The ratio has also been seen to decrease with age (Moller et al., 1976). The present study failed to show such an increase of the ratios in relation to either 'blues' cases or depression cases. These results therefore indicate that the effects of LNAA on TP entry into the brain are not related

to the aetiology of 'blues' symptoms and that there is not a chronic predisposing decreased TP:LNAA ratio in depressive patients prior to the depressive episodes. This supports the previous findings of De Meyer and colleagues (1980) who reported that the decreased TP : LNAA ratios in depressed patients returned to normal values on recovery from the depression.

Electrolytes and Water Retention.

Stein (1980) reported that the onset of puerperal blues coincided with the diuresis and sudden weight loss that occur post-partum. It was postulated that the sudden excretion of electrolytes may be related to the 'blues'. O'Brien and co-workers (1980) studied electrolyte excretion in relation to premenstrual syndrome. However, it was concluded that the peak excretion of electrolytes, which occurred immediately post ovulation was before the onset of any psychological symptoms, and was therefore unrelated (ibid). This factor was not investigated in any of the present studies.

Summary.

The present studies failed to demonstrate any relationship beween plasma total TP or NEFA and the mood changes of the menstrual cycle. Similarly, no relationship was found between either free or total TP and depression perimenopausally. However, plasma total TP was found to be decreased immediately prior to the onset of puerperal 'blues', this is possibly a marker of some underlying mechanism involved in the aetiology of 'blues'. The relationship between a TP 'riser'/'non riser' classification and the incidence of 'blues', as previously reported by Handley and colleagues (1980), was not replicated in the present study.

There was no chronic deficit of either plasma total or free TP during the puerperium prior to the onset of the depressive symptoms during the months following parturition. Therefore there is no chronic deficit of plasma TP predisposing post-partum depression. However, there is possibly a predisposing factor of a NEFA metabolism abnormality prior to the onset of post-partum depression, this may indicate that there is a chronically over-active sympathetic nervous system, possibly due to supersensitive β-adrenoceptors, prior to the onset of depressive illness.

Factors involved in the Aetiology of the TP 'Rise':

The present studies were designed not only to investigate possible biochemical correlates of mood change, but also to investigate the factors underlying the TP 'riser'/'non riser' dichotomy as described by Handley and colleagues (1980).

As previously described, Handley and colleagues (1980) reported a subgroup of patients which failed to show the normal rise in plasma total TP immediately post-partum. This, they found, was related to the incidence of psychological disturbances post-partum. The present study was again able to identify such a group of TP non risers, however, there was no significant relationship between the TP rise and the incidence of 'blues' or depression post-partum. Some possible mechanisms involved in the TP rise or non rise were investigated.

A plethora of factors may affect the plasma concentration of TP. For example, hormonal acceleration of the enzyme tryptophan oxygenase may lead to a diversion of TP along the Kynurenine pathway. Similarly, variations in the use of TP for the synthesis of proteins may affect circulating levels

of TP (See Introduction). Also, if tissue (eg brain and muscle) entry of TP is largely dependent on plasma free TP concentrations as suggested by the work of Curzon and colleagues (See Introduction) then factors affecting either TP entry into the tissues or TP binding to plasma albumin may also ultimately affect total plasma levels of TP (Madras et al., 1974). However even though all of these variables are simultaneously undergoing constant flux, the plasma levels of both total and free TP are maintained within fairly strict limits. This fact therefore suggests that there is some homeostatic mechanism controlling plasma TP dynamics. The precise nature of this homeostatic mechanism is, as yet, unknown. It is possible however, that TP is removed from the blood by the liver. This is the process by which plasma levels of the other amino acids are controlled (Bell et al., 1976).

Previous work by Handley and colleagues (1980) has shown that there is a subgroup of subjects which shows abnormal plasma TP dynamics immediately post-partum. It is possible therefore that in these subjects there is a temporary 'breakdown' in the TP homeostatic mechanisms. Furthermore, it was shown that members of this subgroup showed a higher incidence of 'blues' and depression post-partum (ibid). This relationship between the TP rise and the psychiatric symptoms was not replicated in the present study (See page 271). It is however possible that the manifestation of these psychiatric symptoms post-partum are related to the breakdown of some TP homeostatic mechanism at this time.

From Chapter 1 it is seen that a possible relationship exists between plasma total TP and plasma NEFA. This relationship appears to be disrupted immediately post-partum, the same time

that the abnormal plasma TP dynamics are seen in a small population subgroup. As described in the Introduction, plasma NEFA competes with TP for binding to plasma albumin hence an increase in plasma NEFA produces an increase in plasma free TP. This may promote tissue entry of TP and thus possibly lead to a lowering of plasma total TP (Gessa & Tagliamonte, 1974). Thus variations of plasma NEFA may ultimately produce variations in plasma total TP. It is therefore possible that plasma concentrations of NEFA are related to the homeostasis of plasma TP.

This hyptothesis was investigated by studying the relationship between plasma NEFA and plasma total TP in Human males and non-pregnant females. If plasma NEFA are to be implicated in the failure of TP homeostasis immediately post-partum it must be first demonstrated that under normal conditions, there is a fixed relationship between plasma NEFA and plasma TP. The results of such a study demonstrated that in Human females, whilst both plasma NEFA and plasma total TP vary throughout the menstrual cycle, there was no significant relationship between these two parameters. In Human males however, a significant positive relationship was seen between plasma total TP and NEFA. This latter finding is in a direction opposite to that predicted following the animal studies of Gessa and Tagliamonte (1974) and therefore supports the conclusion that plasma NEFA are not related to the homeostasis of plasma TP.

It is unlikely that plasma NEFA are involved in the aetiology of the total TP rise post-partum. TP non risers were found to exhibit decreased plasma NEFA on days 1 and 3 post-partum.

Such a decrease of plasma NEFA would normally be expected to decrease the proportion of the free TP and to increase plasma total TP (Gessa & Tagliamonte, 1974). There were no significant correlations between plasma NEFA and plasma total TP, it therefore is unlikely that the TP rise is related to any changes in plasma NEFA.

Similarly, TP non risers exhibited decreased plasma LNAA on days 1 and 2 post-partum. This suggests that whatever mechanism is producing the changes in the plasma TP produces similar changes in the plasma LNAA, although there was a significant correlation between TP and LNAA only at 38 weeks of gestation. The fact that TP non risers exhibit decreased plasma TP, NEFA and LNAA immediately post-partum suggests the possibility of a haemodilution effect producing the phenomenon. However, there were no differences in any of the endocrine or other factors between TP risers and non risers. Plasma concentrations of the endocrine factors rapidly decrease post-partum, these is also a marked diuresis post-partum, hence it is unlikely that plasma concentrations of these hormones are under strict homeostatic control at this time. Therefore the lack of difference in the plasma hormone concentrations between TP risers and non risers suggests that the riser phenomenon is not a result of haemodilution.

Wood *et al.* (1979) postulated that platelet uptake of TP may be important in the control of plasma free TP. However the effects of alterations of platelet TP uptake on plasma total TP dynamics are unknown. It is therefore unclear whether the TP riser phenomenon may be mediated via an alteration in platelet uptake of TP.

The mechanism underlying the TP riser phenomenon therefore remains unclear, except that the mechanism may also influence the other LNAA, although there was no significant correlation between total TP and LNAA post-partum. There were no consistently significant relationships between plasma TP (either total or free) and progesterone or oestradiol.

TP non risers also showed a lower proportion of breast feeders ($p = .02$). It is unclear whether this factor is involved in the aetiology of the TP rise. Similarly, TP non risers reported a greater incidence of previous menstrual irregularities ($p = .03$), however there was no significant differences with any other menstrual disorders. Therefore the meaning of this relationship between the TP rise and menstrual irregularity is unclear.

CHAPTER 5

CHAPTER 5

AN INVESTIGATION OF THE SEDATIVE EFFECTS OF NATURAL PROGESTERONE AND SYNTHETIC PROGESTOGENS IN MALE AND FEMALE MICE.

5.1 Introduction.

5.2 Results.

- 5.2.1 Progesterone Sedation in Female Mice.
- 5.2.2 Prolongation of Barbiturate Sleeping Time in Female Mice.
- 5.2.3 Prolongation of Barbiturate Sleeping Time in Male Mice.
- 5.2.4 Comparison of Male and Female Mice.

5.3 Summary and Comments.

Chapter 5

An Investigation of the Sedative Effects of Natural Progesterone and Synthetic Progestogens in Male and Female Mice.

5.1 INTRODUCTION.

It is known that progesterone has an anaesthetic effect in humans and animals (Selye, 1941; Merryman, 1954). This property was therefore utilised to study the central effects of progesterone and progestogens. The aim of the experiment being to investigate whether these hormones, at the doses and via the routes used subsequently in Chapters 6 and 7, are crossing the blood-brain barrier and having an effect on the CNS.

In addition to this, two further factors were investigated. Firstly, Merryman (1954) reported that there was a sex difference in the response of humans to progesterone, females being more susceptible to the anaesthesia than males. The extent of this sex difference was therefore studied in mice.

The second additional factor to be studied was the difference between natural progesterone and synthetic progestogens. This is of importance following the work of Dalton (1971) who reported that in the treatment of premenstrual syndrome, only natural progesterone supplements were beneficial. Synthetic progestogens were seen to be of no value in the treatment of this syndrome. The differential effects of natural and synthetic progestogens as measured by sedation in mice were therefore investigated.

5.2 RESULTS.

5.2.1 Progesterone Sedation in Female Mice.

Sexually mature female mice (25-35g) were housed together in groups of 5 in cages 30 x 13 x 11 cm. under the conditions described in Methods Section 7. Animals were housed under these conditions for 2 weeks prior to the beginning of the experiment in order to ensure the synchronisation of the oestrous cycles within groups.

This initial study was meant as a pilot study for further work, because of this, a very high dose of progesterone was used and no vehicle control group was run in parallel with the drug treatment group. The drug treatment group was compared with an untreated control group.

The mice received progesterone 30mg/kg in gum acacia suspension (5ml/kg) p.o. Drug administration took place between 12.45 - 12.50 hours. Following the injection the animals were placed into an observation arena and their behaviour was observed.

Five minutes after the injection all the mice were very still and they appeared sedated. After 20 minutes 8 of the 10 mice were asleep. All of the mice were asleep 30 minutes after the injection, however the sleep was not deep enough to allow the mice to be turned onto their backs. After 46 minutes, there was occasional movement, with the mice responding to environmental noise, there was also marked piloerection in all of the animals. After approximately one hour

(65.83 ± 2.16mins (n=10)) all of the animals were judged to have returned to their pre-injection state of arousal.

Non-treated control animals were seen to be still, however they were responsive to environmental noise, and movements such as grooming were frequent. None of the controls were judged to sleep during the experiment.

Comment

Although the progesterone treated mice did show sedation, it was not of a great enough degree to be measured by any other than subjective methods. It was therefore decided to measure the sedative effects of the drugs by using the prolongation of barbiturate sleeping time method.

5.2.2 Prolongation of Barbiturate Sleeping Time in Female Mice.

Female mice were housed together as in section 5.2.1 prior to experimentation. The barbiturate sleeping time experiment was performed as described in Methods section 2.4. The 1 hour hormone pretreatment was commenced at 1310 hours, and oestrous cycle determinations for each animal were performed at the end of the experiment, post mortem. (Methods 6.2).

The doses of hormones and the route of administration, ie progesterone 10mg/kg p.o. and dydrogesterone 5mg/kg p.o. were those used in chapters 6 and 7. The rationale for the choice of the doses is explained in these chapters.

The exceptions to these were norethisterone acetate 200 µg/kg and 20 µg/kg p.o. In chapter 6, these doses were given in ethanol/water s.c., however, in this present study, the drug was given in gum acacia suspension p.o. This allowed results for norethisterone acetate to be compared with those for progesterone and dydrogesterone.

Due to the directional hypothesis that the hormones would prolong the barbiturate anaesthesia, 1-tailed tests of significance were used.

The barbiturate sleeping time was seen to be significantly prolonged by all of the hormones : progesterone $p = .0099$, dydrogesterone $p = .0348$ and norethisterone acetate 20 and 200 µg/kg $p = .0206$ and $.0026$ respectively. At the doses used, progesterone was seen to produce the greatest increase in sleeping time, with the two doses of norethisterone acetate producing similar results. Dydrogesterone showed the least prolongation of sleeping time.

All animals were in the metaphase of the oestrous cycle. (See Table 5.1, Fig 5.1).

Table 5.1 : Effects of Natural and Synthetic Progestogens on Barbiturate Sleeping Time in Female Mice.

Treatment	Sleeping Time (minutes) mean	SE	N	students t	1-tailed significance
Gum Acacia Control p.o.	3.19	0.64	8	-	-
Progesterone 10mg/kg p.o.	11.50	3.91	6	2.6610	.0099
Dydrogesterone 5mg/kg p.o.	7.63	3.49	4	2.0125	.0348
Norethisterone Acetate 200µg/kg p.o.	9.40	1.72	10	3.2592	.0026
Norethisterone Acetate 20µg/kg p.o.	10.00	4.18	5	2.2884	.0206

5.2.3 Prolongation of Barbiturate Sleeping Time in Male Mice.

Mature male mice (30-35g) were housed in groups of 25 in cages (50 x 30 x 13 cm) in the experimental cabins for one week prior to experimentation (See Methods section 7). On the morning of the experiments, animals were divided into groups of 5 in cages 30 x 13 x 11 cm. The experiment was commenced with the 1 hour hormone pretreatment at 1300 hours. The experimental procedure for the prolongation of barbiturate sleeping time experiment was performed as described

in Methods section 2.4. The doses of drugs used and the route of administration were identical to those used in chapter 7, section 7.3. The doses were thus : progesterone 10mg/kg; dydrogesterone 5mg/kg; norethisterone acetate 1mg/kg and a combination of ethinyl/oestradiol 1μg/kg and norethisterone acetate 100μg/kg. All drugs were administered in gum acacia suspension in volumes of 5ml/kg. The rationale for the choice of these doses is given in chapter 7.

Unlike section 5.2.2, the non-directional nature of the hypothesis meant that 2-tailed tests of significance were appropriate. The results showed that at the doses used, none of the hormones significantly prolonged barbiturate sleeping time (see table 5.2, Fig. 5.2).

Table 5.2 : Effects of Natural and Synthetic Progestogens and an Oestrogen/Progestogen Combination on Barbiturate Sleeping Time in Male Mice.

Treatment	Sleeping Time (minutes) mean	SE	N	student's t	2-tailed significanc
Gum Acacia Control p.o.	4.46	1.03	14	-	-
Progesterone 10mg/kg p.o.	2.26	0.11	7	1.5369	.1376
Dydrogesterone 5mg/kg p.o.	2.15	0.32	10	1.9210	.0648
Norethisterone Acetate 1mg/kg p.o.	9.94	3.63	9	1.8378	.0772
Ethinyl/Oestradiol 1μg/kg + norethisterone acetate 100 μg/kg p.o.	3.38	0.92	4	0.5644	.6872

5.2.4 Comparison of Male and Female Mice.

This series of experiments was not designed to investigate sex differences in the effect of hormones on barbiturate sleeping time. However, the available results do permit an investigation of such sex differences.

Apart from the gum acacia control, two other drugs were administered to both male and female mice at the same dose, via the same route. These were progesterone 10mg/kg and dydrogesterone 5mg/kg p.o. Again, due to the directional hypothesis that females would show a greater response than males, a 1-tailed test of significance was appropriate.

When the results for the two groups were compared, it was seen that females showed a greater sleeping time than males for both progesterone ($p=.0082$) and for dydrogesterone ($p=.0059$) at the doses used. There was however no significant difference between males and females for the gum acacia control group. This fact shows that the sex difference is due to differences in the response to the hormones, and not differences in response to the barbiturate. Table 5.3, Fig 5.3.

Table 5.3 : Sex Differences in the Response to Progesterone and Dydrogesterone.

Treatment	Sleeping Time (minutes) ±SE		*t* value	1-tailed significance
	Male	Female		
Gum Acacia Control	4.46±1.03(n=14)	3.19±0.64(n=8)	0.9163	.3133
Progesterone 10mg/kg p.o.	2.29±0.11(n=7)	11.50±3.91(n=6)	2.8107	.0082
Dydrogesterone 5mg/kg p.o.	2.15±0.32(n=10)	7.63±3.49(n=4)	2.9486	.0059

5.3 SUMMARY AND COMMENTS.

The results of this series of experiments therefore show that hormones, at the doses, and via the routes used in chapters 6 and 7, are capable of producing an effect on the CNS. The effect measured being the prolongation of barbiturate sleeping time.

This effect however was sex dependant, females being more susceptible to prolongation of anaesthesia than males.

Fig 5·1 Effect of acute hormone treatment on thiopentone sleeping time in female mice

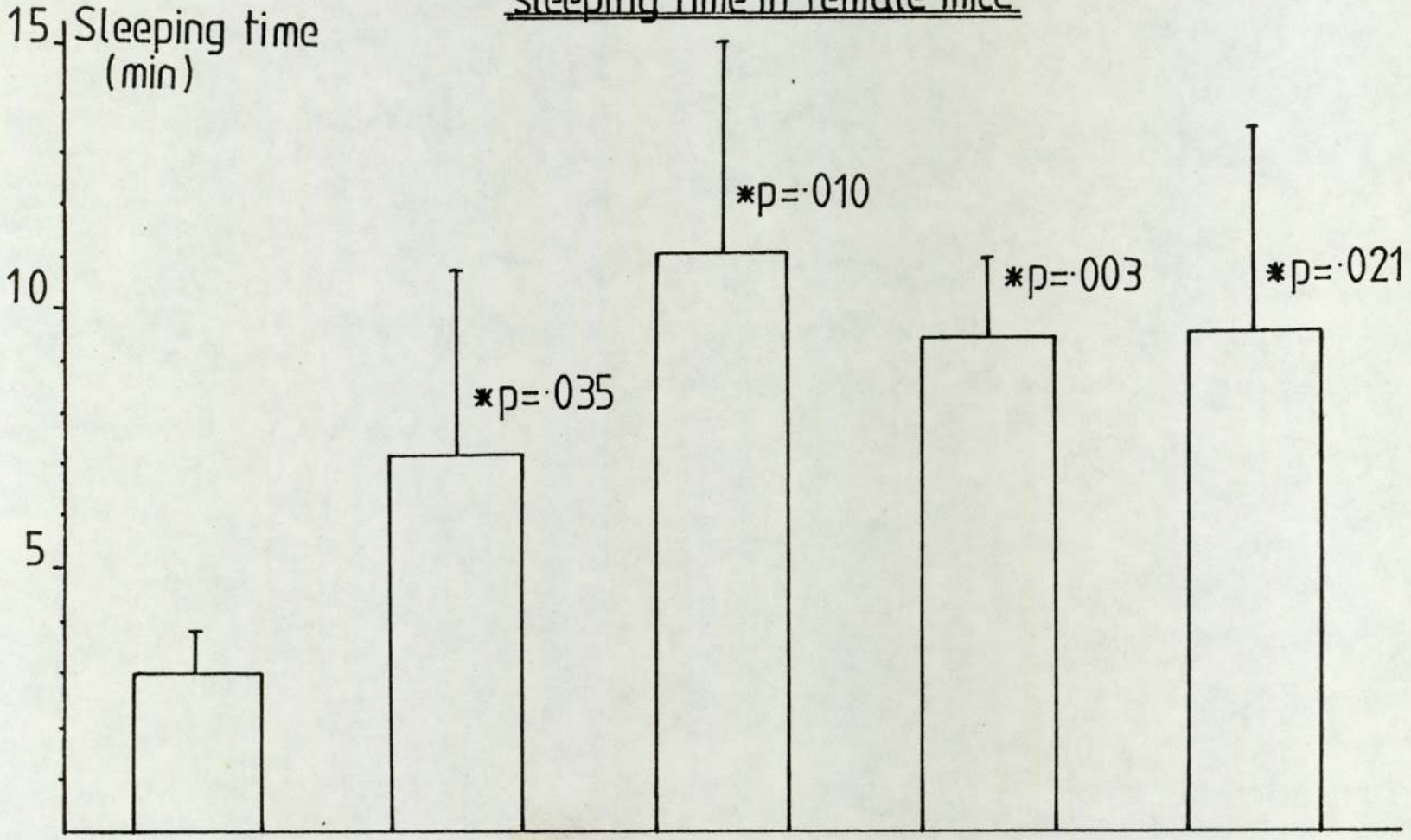


Fig 5·2 Effects of acute hormone treatment on thiopentone sleeping time in male mice

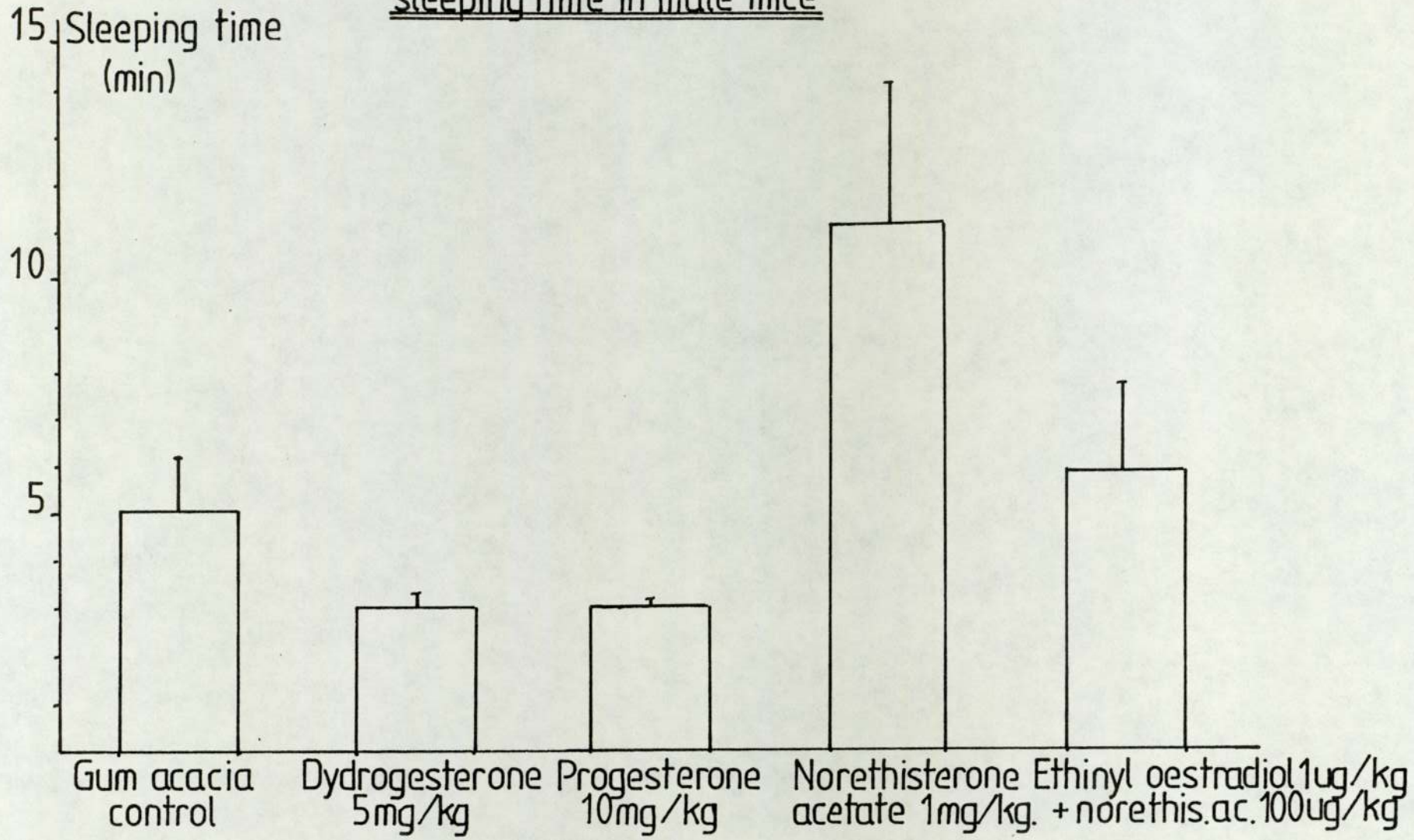
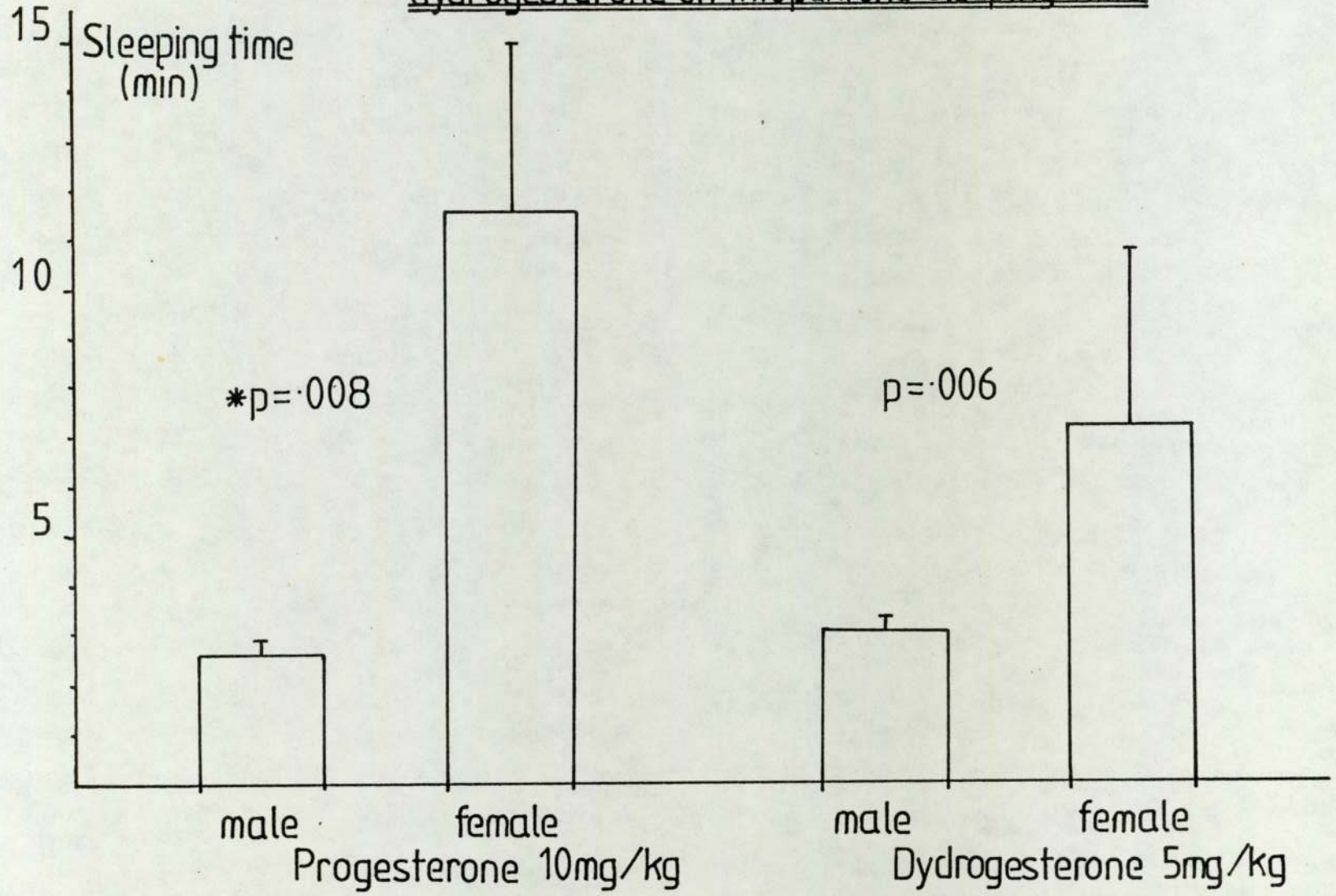


Fig 5·3 Sex differences in the effects of progesterone & dydrogesterone on thiopentone sleeping time



CHAPTER 6

CHAPTER 6

THE EFFECTS OF CHRONIC PROGESTERONE OR PROGESTOGEN ADMINISTRATION AND ACUTE WITHDRAWAL OF TREATMENT ON BEHAVIOUR, PLASMA TOTAL TRYPTOPHAN AND PLASMA NON ESTERIFIED FATTY ACIDS IN MALE AND FEMALE MICE.

Chapter 6

The Effects of Chronic Progesterone or Progestogen Administration and acute withdrawal of treatment on behaviour, plasma total tryptophan and plasma non-esterified fatty acids in male and female mice.

6.1 INTRODUCTION.

During human pregnancy, it has been shown that plasma levels of total TP decrease (Handley et al., 1980). These levels then rise acutely within the first few days post-partum (ibid). The puerperium is also a time of mood change and mood lability and it is recognised that approximately 50 per cent of mothers suffer from 'blues' during the initial puerperal period (Pitt, 1973). Handley and co-workers (1980) were able to show a direct relationship between the mood changes and plasma total TP. The relationship was that there was a subgroup of women who failed to show the increase of plasma total TP immediately post-partum, the rise being delayed for up to 2 days, and it was women of this subgroup which showed the greater incidence of 'blues'. However, it was not postulated that the 'blues' were caused by the TP deficit *per se*, a view supported by the fact that TP supplements did not prevent or cure the 'blues' (Harris, 1980), but rather the failure of the early TP rise was seen as a marker of some underlying abnormality. It is probably this basic, underlying abnormality which is causative of the 'blues'.

In addition to the studies of plasma TP post-partum and its relationship to mood changes (See Introduction), there has been speculation concerning the rôle of progesterone in mood changes (Dalton, 1971).

Nott and co-workers (1976) failed to show any difference in plasma progesterone levels either antepartum or post partum, between 'blues' cases and non-cases. However, Dalton (1971) speculated that the mood changes were caused not by an absolute deficit of progesterone, but by a sudden change in progesteronelevels; the mood change being caused by a failure to adapt to this sudden change.

Non puerperal depressed patients have been shown to exhibit an abnormal plasma NEFA response following mild stress (Curzon et al., 1979). NEFA also have been shown to be involved with the entry of plasma TP into the brain (See Introduction). It is therefore possible that NEFA may play some part in 'blues', either as a marker of an underlying problem, or by being directly involved in TP and 5HT metabolism and transport.

This study was therefore designed to investigate whether sudden changes in progesterone levels might produce any behavioural changes. Plasma total TP and NEFA were also investigated to see whether the changes in these chemicals that reportedly occur in puerperal and non-puerperal affective disorders might be produced by underlying changes in progesterone.

Design of the Study.

Progesterone and progestogens may be used in humans for the treatment of menstrual disorders. The natural hormone is seen to be poorly effective when given orally due to its rapid metabolism by the liver producing a great 'first pass' effect (Aufrere & Benson, 1976). The usual route of

administration is therefore by intramuscular injection in an oily suspension. This route however would be inappropriate for a study involving the measurement of fatty acids since the oil vehicle may produce profound effects on lipid metabolism. Progesterone is also insoluble in water. The vehicle therefore selected for progesterone administration was gum acacia suspension. This was administered orally. It was believed that chronic high doses of progesterone, with a possible depot effect of suspension in gum acacia would produce increased plasma levels of the hormone.

During pregnancy in humans plasma progesterone levels are seen to be raised to levels greater than ten times the normal menstrual levels, for the final three quarters of pregnancy. The dose selected for this animal study was therefore equivalent to ten times the dose normally given to humans during menstrual cycle treatments.

The dose and route selected was 10mg/kg given orally in gum acacia. As seen in Chapter 5, this dose and route is capable of producing effects on the CNS.

The normal length of gestation in the mouse is 22 days, the drug was therefore given daily for approximately 14 days in order to simulate the period of increased progesterone in human pregnancy, ie the latter half of pregnancy. Drug withdrawal was used to simulate parturition.

Following the work of Bond, 1979, a dose of norethisterone acetate 20μg/kg given s.c.in ethanol and saline is equivalent to the doses of the hormone used for contraception in humans. This dose and route was therefore utilised in this study. A dose ten times greater was also used for the reasons previously stated (See above). Again the high dose was given daily

for 14 days in order to simulate pregnancy. The low dose was given for 23 days in order to simulate long term use of contraceptive steroids.

6.2 PROCEDURE.

Animals were housed in groups of 25 in cages 50 x 30 x 15 cm. in an experimental cabin under conditions described in Methods section 7. Animals were given a period of 2 weeks in the cabin prior to the start of experimentation in order that they might adapt to the environment. This period also served to allow synchronisation of the oestrous cycles in the female mice.

Animals received either hormone treatment or the appropriate vehicle control daily, between 1500 - 1600 hours, for either 14 or 23 days. Treatment was then withdrawn acutely.

At various stages throughout the drug administration, and for the few days following withdrawal, behavioural tests were performed on groups of five animals. On the occasions of the behavioural tests, the plate crossing test (Methods 2.3) was performed between 0900 - 1000 hours. The Irwin profile (Methods 2.1) was then performed between 1000 - 1200 hours. Following the behavioural tests the animals were sacrificed and exsanguinated (Methods 8.3) between 1230 - 1300 hours.

Dark time locomotor activity (Methods 2.2) was also measured throughout the experiment. During drug administration the treatment and vehicle control groups were tested on alternate days. Following the drug withdrawal, the drug treatment group was tested daily, with the control group being tested

only on the penultimate day of drug withdrawal. This procedure was necessary as only one Animex recording device was available.

Drug Regimens Used.

Male Mice : Progesterone 10mg/kg (in gum acacia) daily p.o. for 14 days (10ml/kg).

Female Mice : Progesterone 10mg/kg (in gum acacia) daily p.o. for 13 days (10ml/kg).
Norethisterone acetate 20µg/kg (in ethanol/saline) daily s.c. for 24 days.
Norethisterone acetate 200µg/kg (in ethanol/saline) daily S.C. for 15 days.

Appropriate vehicle controls were carried throughout the study. Drug administration commenced on day 1, hence behavioural testing after one drug administration was performed on day 2.

6.3 BEHAVIOURAL RESULTS.

6.3.1 Effects of Progesterone 10mg/kg in Male Mice.

Irwin Profile.

No significant differences were seen for any of the variables measured either during drug administration or following drug withdrawal between the progesterone group and the vehicle control group (Mann-Whitney U Test).

Plate Crossing.

There are four measures obtained from plate crossing experiments. The total number of crossings in 90 seconds, and the latency to 1, 5 and 10 crosses.

There were no differences at any point during drug administration or drug withdrawal between the treatment and the vehicle control group with respect to the latency to 1 or 5 crosses. However, for the latency to 10 crosses, the progesterone group showed a significantly greater latency than the control group on the last day of drug administration, day 15. ($t = 2.57$; $p = .0320$).

The progesterone group also showed a significantly greater number of plate crossings in 90 seconds on day 8 of drug administration ($p = .05$, Mann - Whitney)

All other differences were non-significant (Figs 6.1, 6.2, 6.3 and 6.4).

Locomotor Activity.

Male mice exhibit three distinct peaks of locomotor activity during the evening and hours of darkness. (See Fig 6.5). These peaks are at approximately 1900 hrs, 2100 hrs (offset of lights) and at 0700 hrs (onset of lights).

Results for the first 6 days of drug administration were discarded and the mean peak heights for progesterone and vehicle control groups were compared for drug administration and drug withdrawal (Fig 6.6). The results showed that there were no significant differences between the groups during drug administration. Statistical analysis of data for drug withdrawal activity was not possible as the control group was only tested once following treatment withdrawal.

However, it was possible to compare the locomotor activity counts for the progesterone group during drug administration with the counts following drug withdrawal. When this was done, there was no significant difference in the locomotor activity between progesterone administration and progesterone withdrawal.

6.3.2 Effects of Progesterone 10mg/kg in Female Mice.

Irwin Profile.

There were no differences seen between the treatment group and the control group for any variable either during drug administration or following drug withdrawal (Mann-Whitney U Test).

Plate Crossing.

Again four measures were obtained (See 6.3.1). It was found that immediately following the commencement of drug administration (day 2), the progesterone group showed a significantly greater latency to the first plate crossing ($t=2.74$; $p=.0246$). Following drug withdrawal the progesterone group showed a significantly shorter latency to the first plate crossing (day 16) ($t=2.40$; $p=.0418$) (Fig 6.7).

There were no significant differences in the latency to 5 plate crossings, except for day 5 of drug withdrawal when the progesterone group showed a significantly shorter latency ($t=3.03$; $p=.0187$) (Fig 6.8).

This difference on day 5 of withdrawal was also present for latency to 10 plate crossings ($t=3.73$; $p=.0075$).

Total number of crossings was significantly increased in the progesterone group relative to vehicle control on the last day of drug administration and on the fifth day of drug withdrawal ($p=.02$ and .05 respectively Mann-Whitney U) Figs 6.9 and 6.10.

All other differences were non significant.

Locomotor Activity.

Female mice exhibit 4 peaks of locomotor activity. These peaks occur at 1900 hrs, 2100 hrs, 0200 hrs and 0700 hrs. (Fig 6.11). Results for the initial 6 days of drug administration were discarded, and the mean peak heights for progesterone and vehicle control groups for each activity peak were compared for drug administration and drug withdrawal (Fig 6.12)

The results showed that during drug administration, the progesterone group showed greater locomotor activity for peak two ($p=.0213$) and peak three ($p=.0180$). These differences were not apparently present following drug withdrawal, although statistical analysis of withdrawal data was not possible due to the small number of activity readings taken. When the locomotor activity counts during progesterone administration were compared with those for the progesterone group following drug withdrawal, it was found that peaks two and three were significantly decreased following progesterone withdrawal ($p=.0196$ & .0256 respectively).

6.3.3 Effects of Norethisterone Acetate 20 µg/kg in Female Mice.

Irwin Profile.

There were no differences seen between the treatment group and the control group for any variable, either during drug administration or during drug withdrawal (Mann-Whitney U Test).

Plate Crossing.

The data was treated as in section 6.3.1. There were no significant differences between the treatment group and the vehicle control group for any of the measures except for latency to first crossing on the second day following drug withdrawal, when the norethisterone acetate group showed a significantly shorter latency to the first cross ($t=3.92$; $p=.0047$) and the total number of crossings on day 3 of withdrawal were significantly decreased in the norethisterone acetate group ($p=.05$, Mann-Whitney) (Figs 6.13 - 6.16).

Locomotor Activity.

There were no significant differences between the treatment and the control group either during drug administration or following treatment withdrawal (Fig 6.17).

6.3.4 Effects of Norethisterone Acetate 200 µg/kg in Female Mice.

Due to the lack of consistent significant differences on behavioural measures for the treatments described

earlier in this chapter, the only behavioural test performed for this treatment was that of locomotor activity (Fig 6.18). No significant differences were seen between the control group and the treatment group during drug administration. Due to the lack of sufficient results, statistical analysis of locomotor activity following treatment withdrawal was not possible.

6.3.5 Comparison of Locomotor Activity in Untreated Mice and in Mice Receiving Chronic Gum Acacia.

The control mice from section 6.3.3 were compared with untreated mice, Fig 6.19. It was shown that there were no significant differences between the two groups during administration,

6.4 EFFECTS ON NON-ESTERIFIED FATTY ACIDS.

6.4.1 Progesterone 10mg/kg in Male Mice.

There were no significant differences between the progesterone group and the vehicle control group for any time point during drug administration. However on the fifth day following drug withdrawal (day 20) the progesterone group were found to have significantly increased plasma concentrations of NEFA ($p = .0217$, 2 tailed). Fig 6.20.

6.4.2 Progesterone 10mg/kg in Female Mice.

Progesterone treated mice were found to have significantly decreased plasma concentrations of NEFA on days 8 and 11 of drug administration ($p=0.0448$ & 0.0371 respectively).

There were no differences between the two groups at any time point following drug withdrawal Fig 6.21.

Plasma levels of NEFA for the progesterone and vehicle control groups were found to be in the order of 1.2 mEq/dm^3. (Figs 6.20, 6.21). Untreated male mice were found to have plasma NEFA concentrations of 0.767 $\pm$ 0.053 mEq/dm^3. It can therefore be seen that, in general, administration of either progesterone or vehicle control produced an increase in plasma NEFA concentrations.

Due to lack of consistent significant differences between the progesterone group and the controls, with respect to plasma NEFA concentrations, NEFA were not measured for the norethisterone acetate treatment groups.

6.5 EFFECTS ON PLASMA TOTAL TRYPTOPHAN.

6.5.1 Progesterone 10mg/kg in Male Mice.

No differences were found in plasma total TP between the progesterone group and the vehicle control group either during drug administration or following treatment withdrawal. Fig 6.22.

6.5.2 Progesterone 10mg/kg in Female Mice.

In an initial study, a trend of decreased plasma total TP during progesterone administration with increased plasma TP following treatment withdrawal, was seen. (Fig 6.23).

However, the experiment was repeated using Progesterone 10mg/kg, in gum acacia 5ml/kg, as opposed to 10ml/kg, p.o. For this second experiment animals were housed in groups of 5 in cages 30 x 13 x 11 cm. (previously animals were housed in groups of 25). Fourteen days were allowed for synchronisation of the oestrous cycles before drug administration began. Oestrous cycle determinations were performed post mortem. In this experiment no significant differences were seen between the progesterone group and the vehicle control group, except for the final day of progesterone administration, when the progesterone group showed significantly increased plasma TP ($p = .0055$), Fig 6.24.

6.5.3 Norethisterone acetate 20 µg/kg in Female Mice.

No differences were found in plasma total TP between the norethisterone acetate group and the vehicle control group, either during drug administration or following treatment withdrawal. Fig 6.25.

6.5.4 Norethisterone acetate 200 µg/kg in Female Mice.

No differences were found in plasma total TP between the norethisterone acetate group and the vehicle control group, either during drug administration or following treatment withdrawal. Fig 6.26.

6 5 5 Repeated Norethisterone acetate Treatment in Female Mice

Three groups of animals received norethisterone acetate 20 µg/kg for 24 days as in 6.5.3. Two weeks later the animals received norethisterone acetate 200 µg/kg daily as in 6.5.4. Parallel control groups of repeated gum acacia treatment were also run.

No significant differences were seen between these groups either during the second norethisterone administration or following the second treatment withdrawal.

6.5.6 Comparison of Untreated Mice and Chronic Gum Acacia in Female Mice.

Plasma total TP in female mice that had received gum acacia 5ml/kg p.o.daily for 15 days was compared with plasma total TP values from untreated female mice that had been housed under exactly the same conditions. The gum acacia mice were found to have a mean plasma total TP concentration of 35±1.97μg/ml(n=5) whilst the untreated group showed values of 24.78±1.99 μg/ml (n=5). This difference was statistically significant (p=.0038).

6.6 SUMMARY AND COMMENTS.

Summary.

Progesterone administration and withdrawal had no consistent significant effects on the behavioural parameters, plasma NEFA or plasma total TP in male mice. Neither progesterone nor norethisterone acetate consistently affected plasma NEFA or total TP in female mice, although progesterone did produce increased plasma total TP after 15 days administration.

Progesterone administration was also seen to produce increased locomotor activity in female mice.

Comments.

1) At the outset of these studies it was believed that housing of female mice together in groups of 25 animals would produce synchronisation of the oestrous cycles.

However, it was subsequently found that such synchronisation of oestrous cycles only occurs in small groups of animals (Bond, personal communication). Therefore for the repeat study of progesterone in female mice animals were housed in groups of 5. Oestrous cycle determinations were also performed. However the stage of the oestrous cycle did not appear to influence the results. (Fig 6.24).

2) Gum acacia administration was seen to lead to an increase in plasma TP and NEFA, as compared with untreated controls.

Progesterone treatment-male mice

Fig 6·1 Plate crossing: time to first crossing

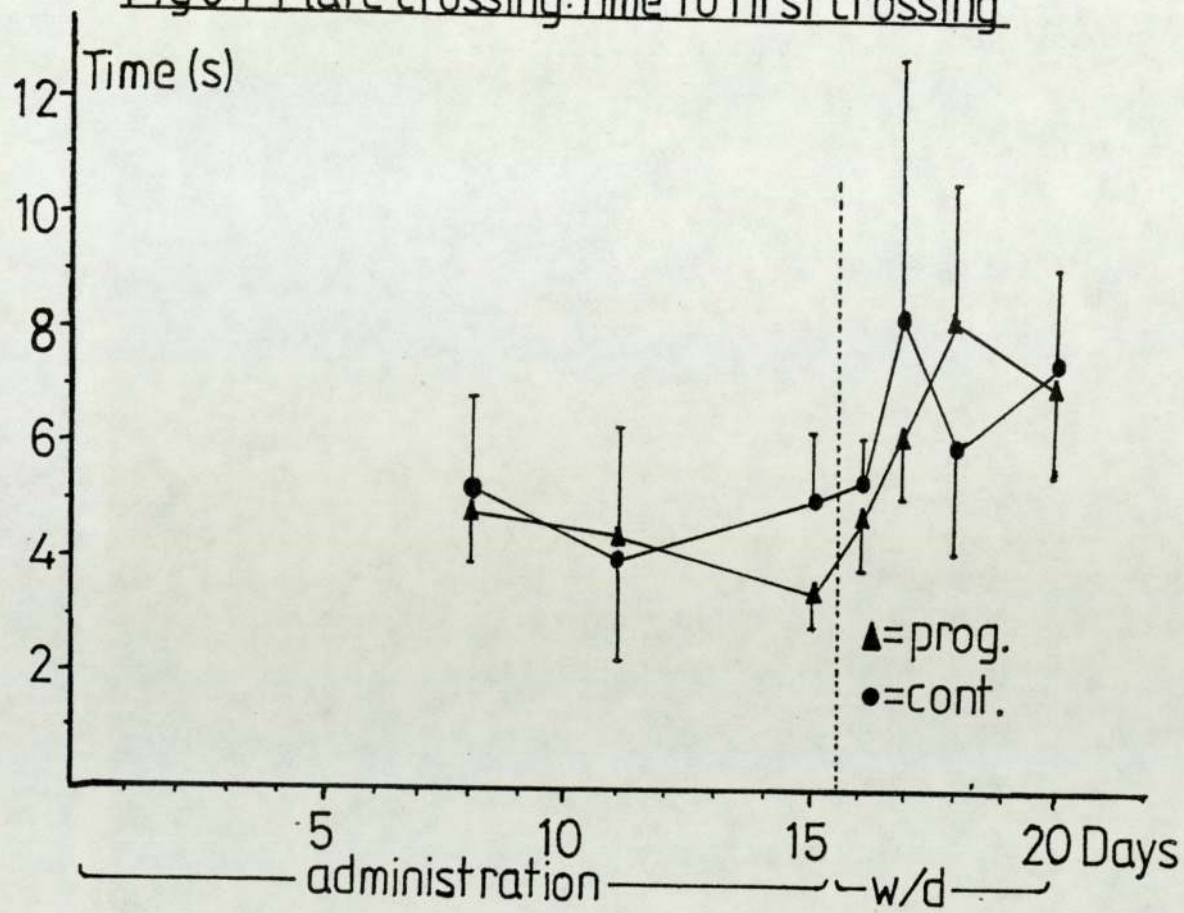
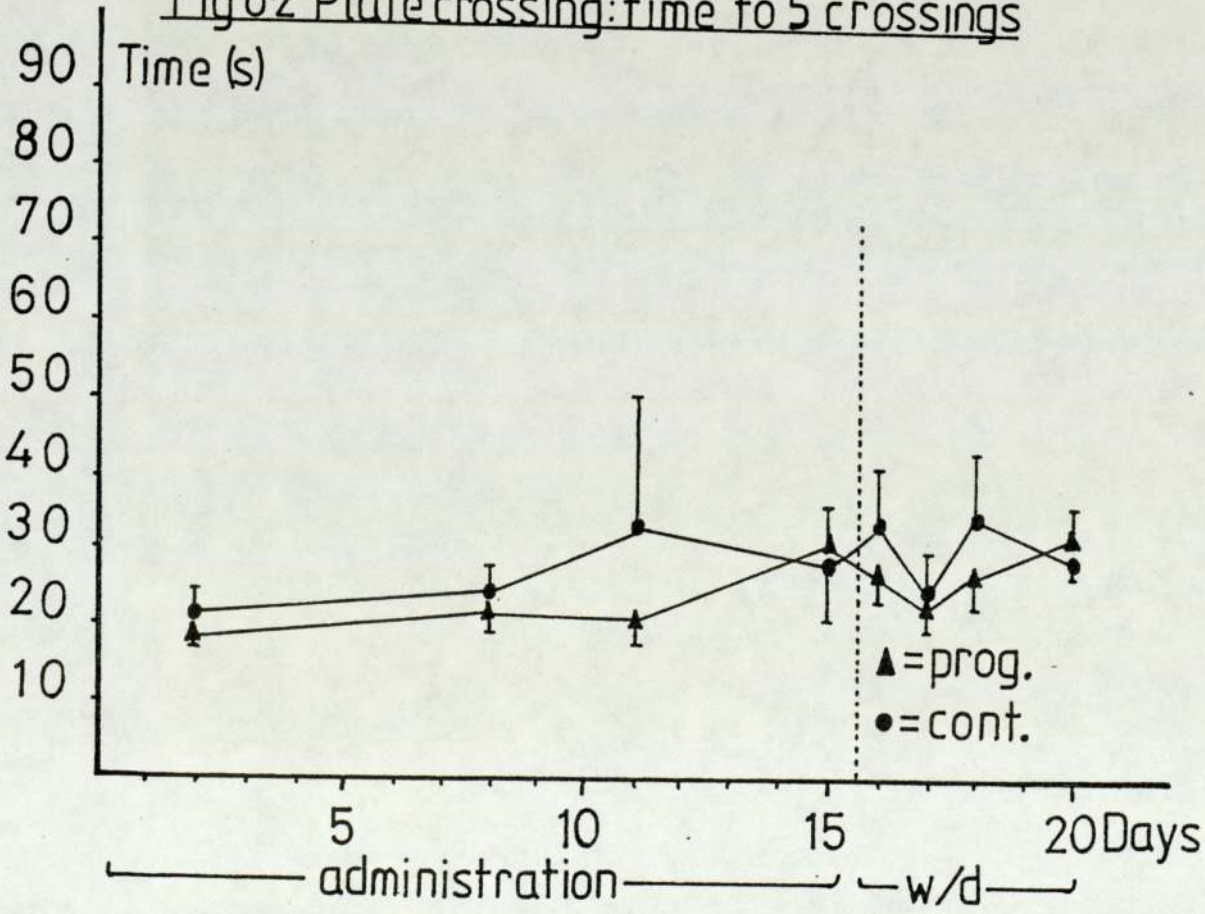


Fig 6·2 Plate crossing: time to 5 crossings



Progesterone treatment-male mice

Fig 6·3 Plate crossing: time to 10 crossings

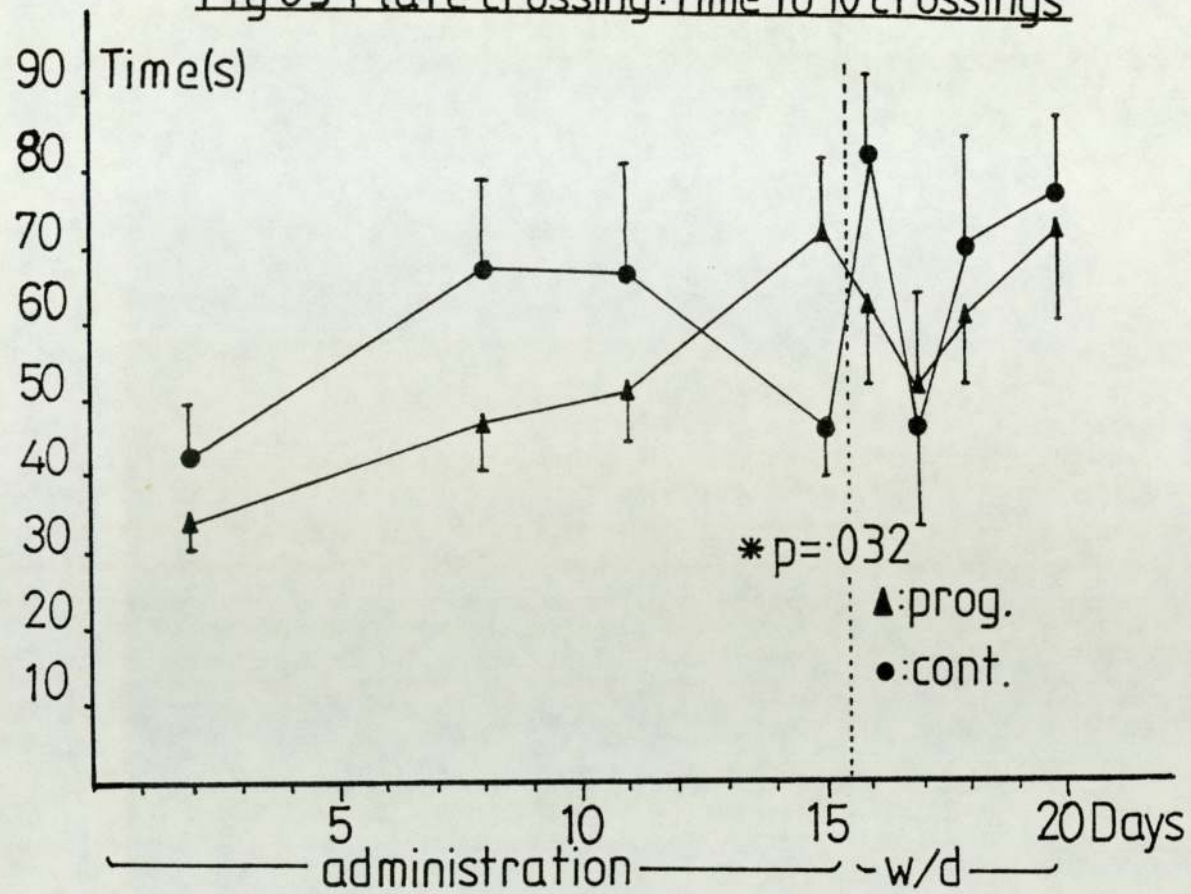


Fig 6·4 Plate crossing: total crossings in 90s

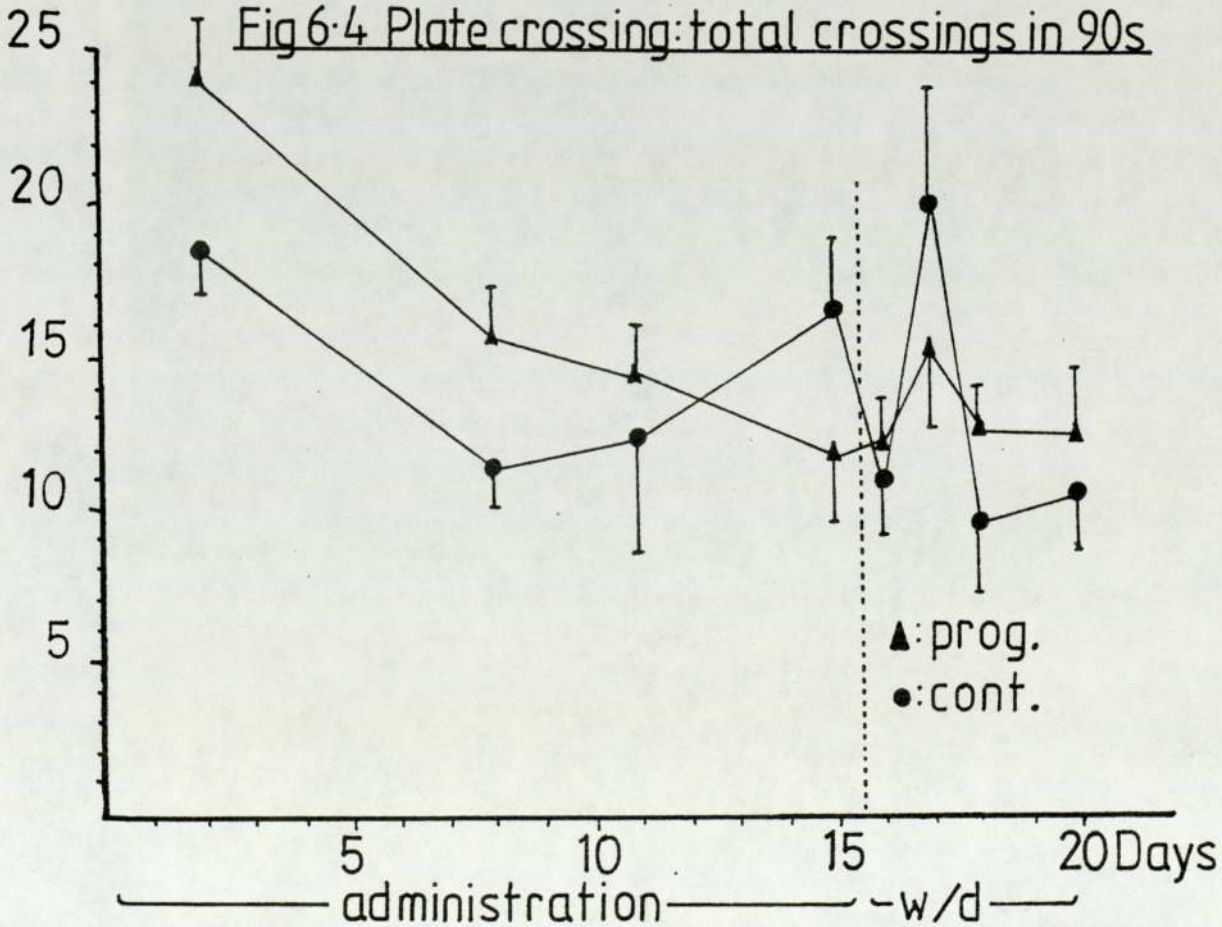
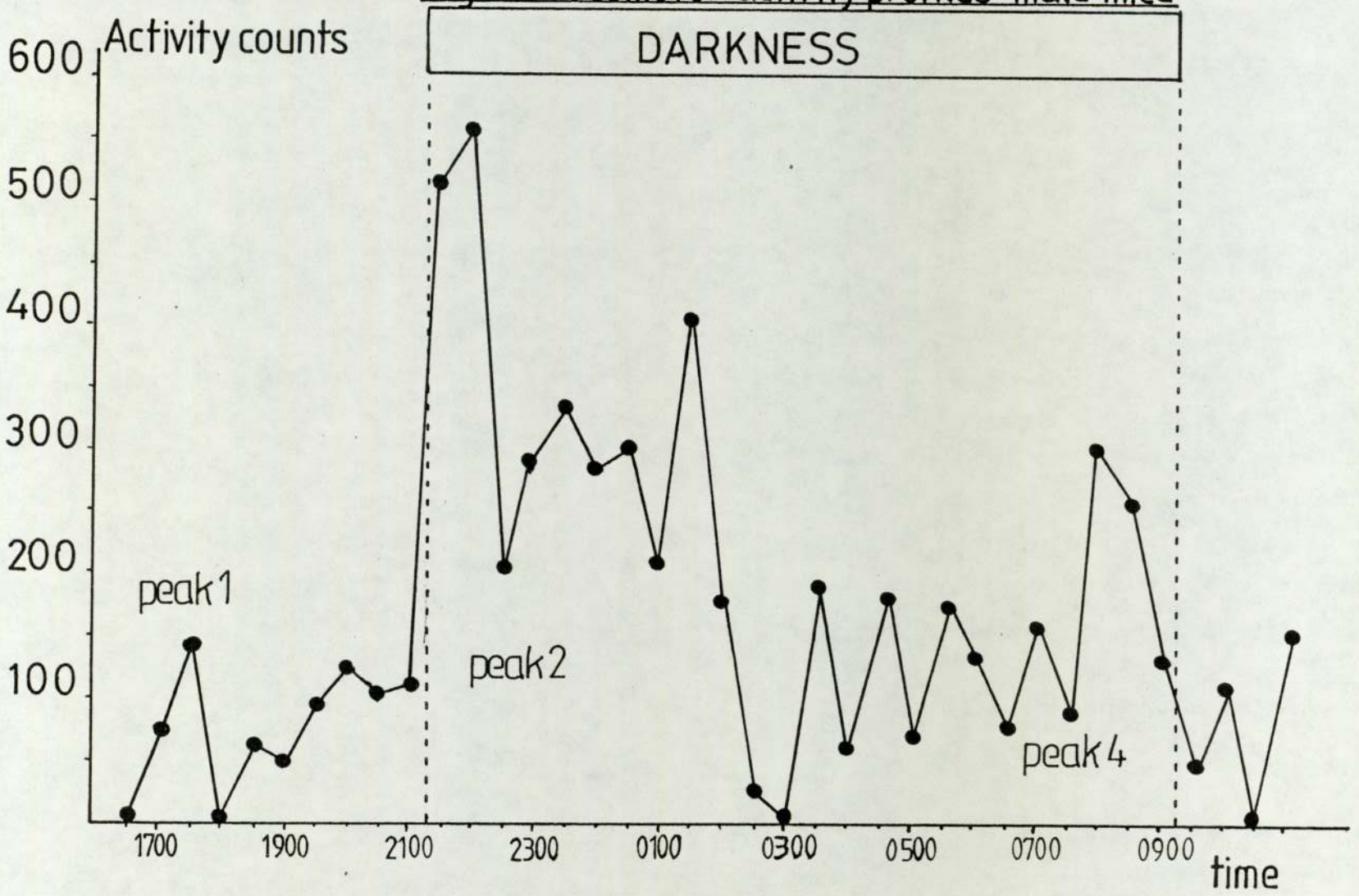


Fig 6·5 Locomotor activity profiles-male mice



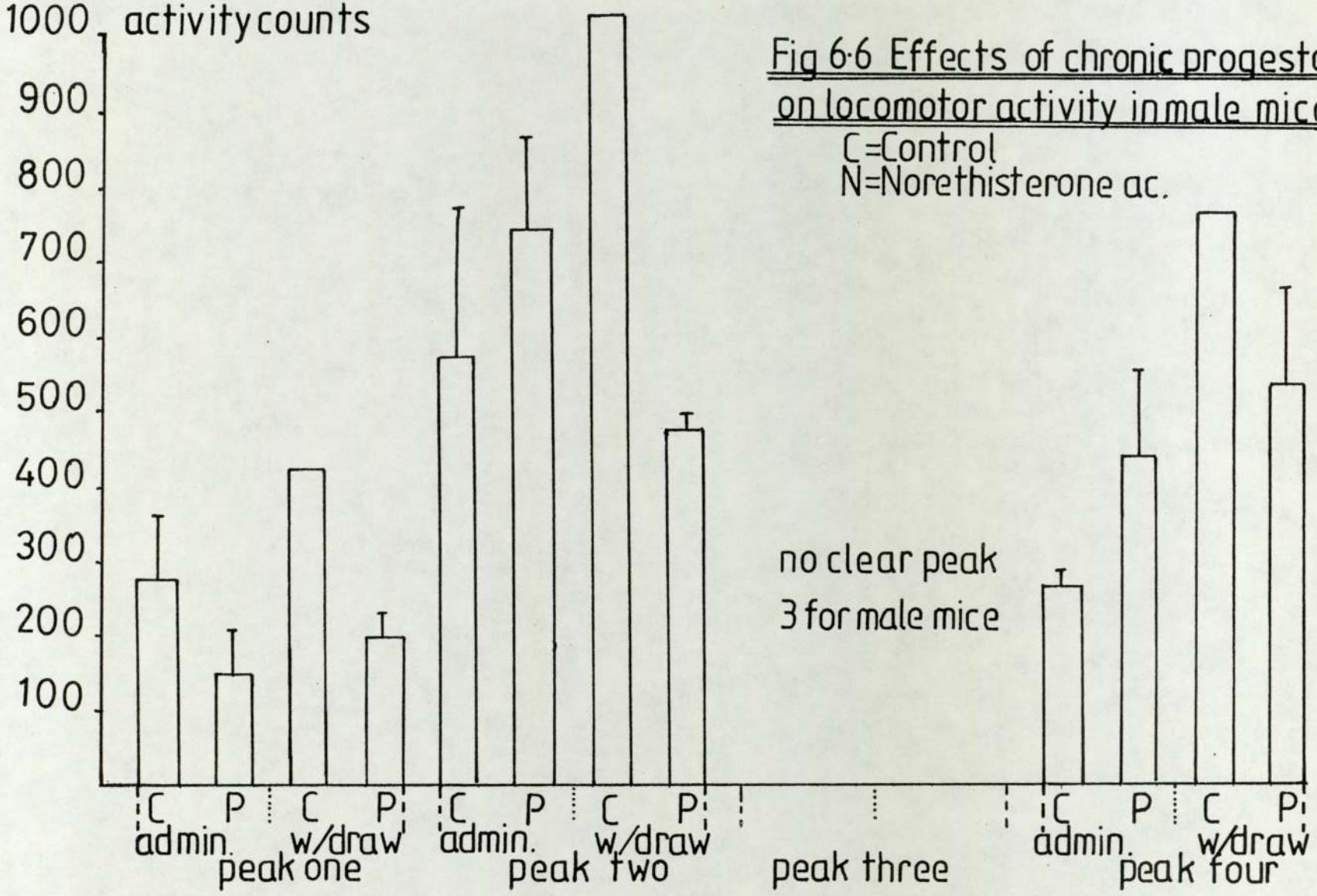


Fig 6·6 Effects of chronic progesterone on locomotor activity in male mice

C=Control
N=Norethisterone ac.

Progesterone treatment-female mice

Fig 6·7 Plate crossing: time to first crossing

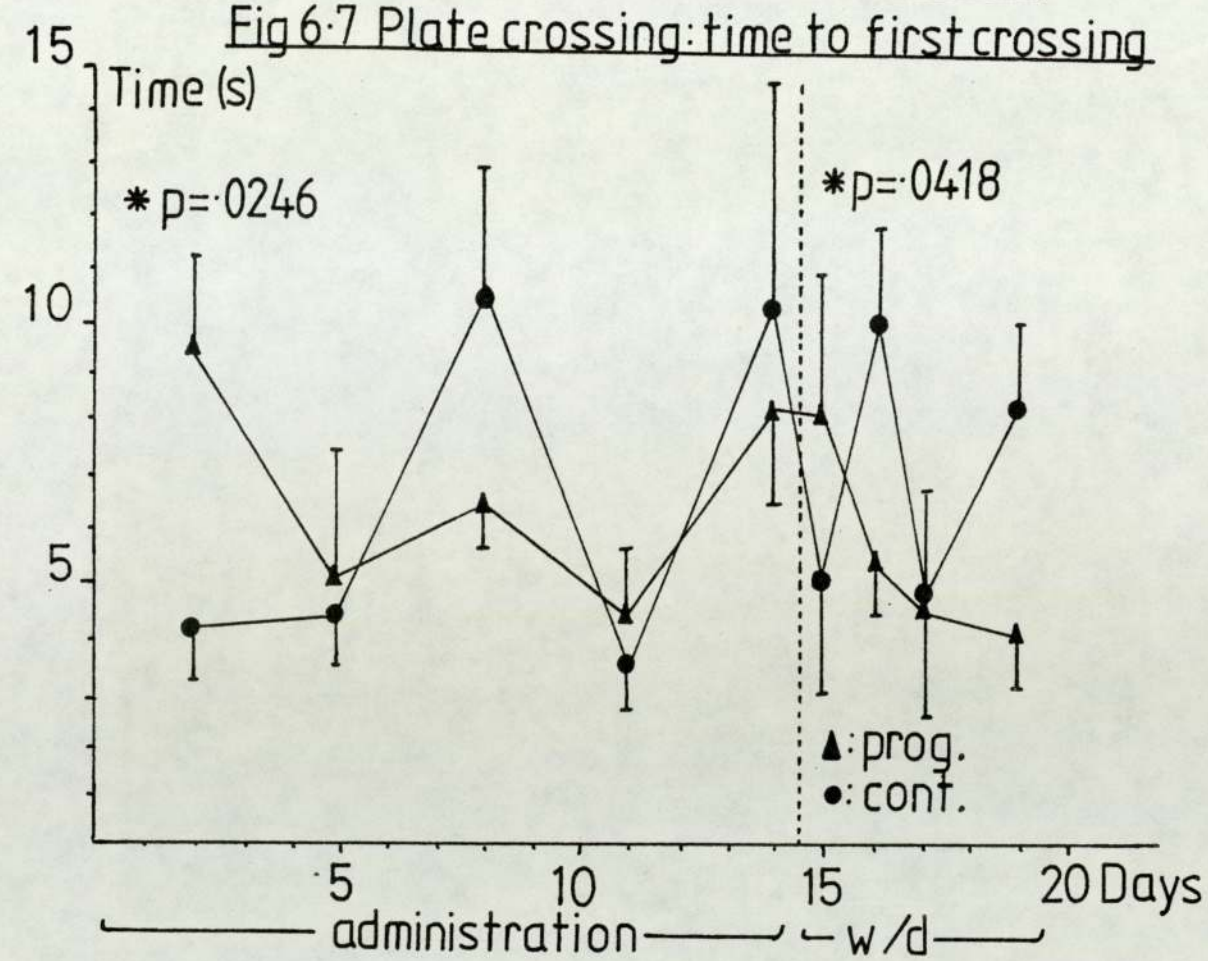
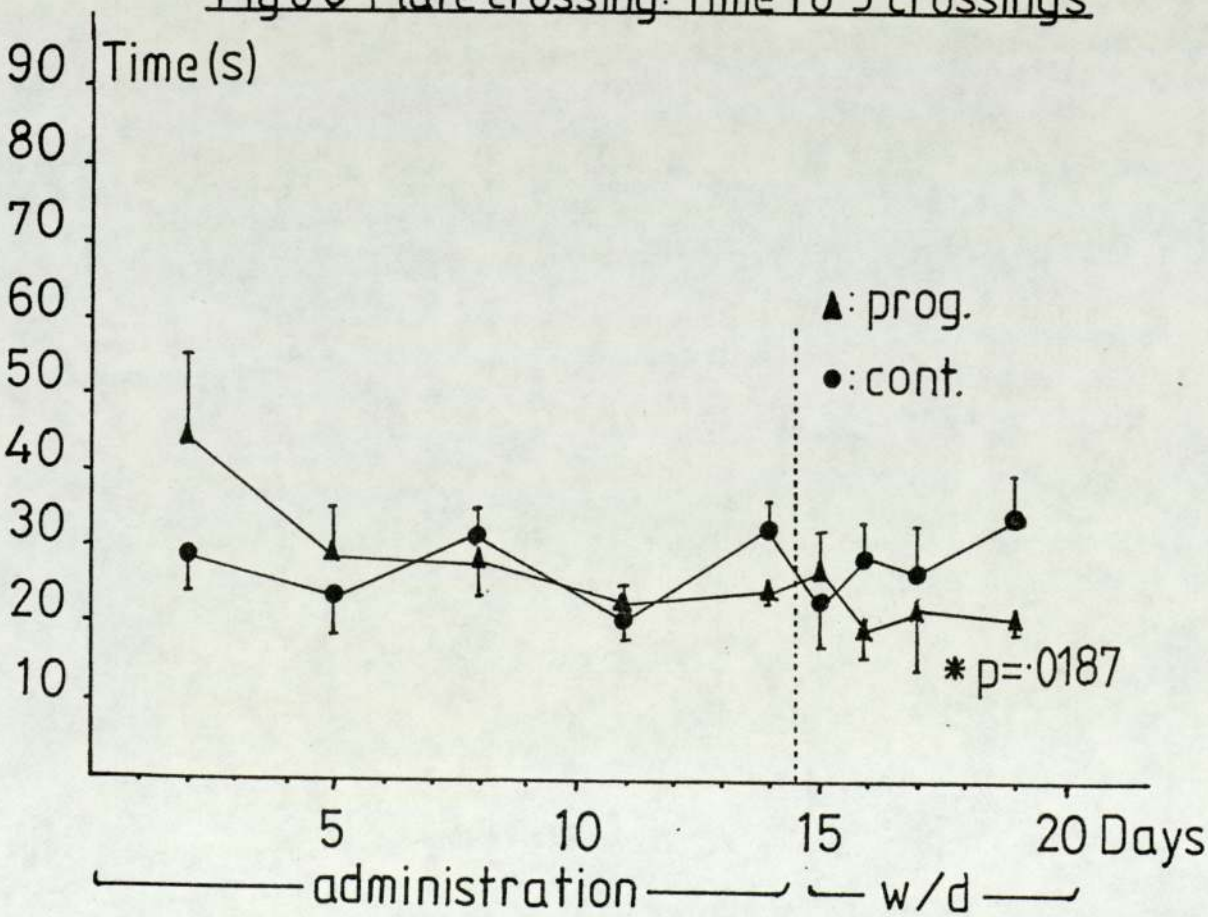


Fig 6·8 Plate crossing: time to 5 crossings



Progesterone treatment - female mice

Fig 6·9 Plate crossing: time to 10 crossings

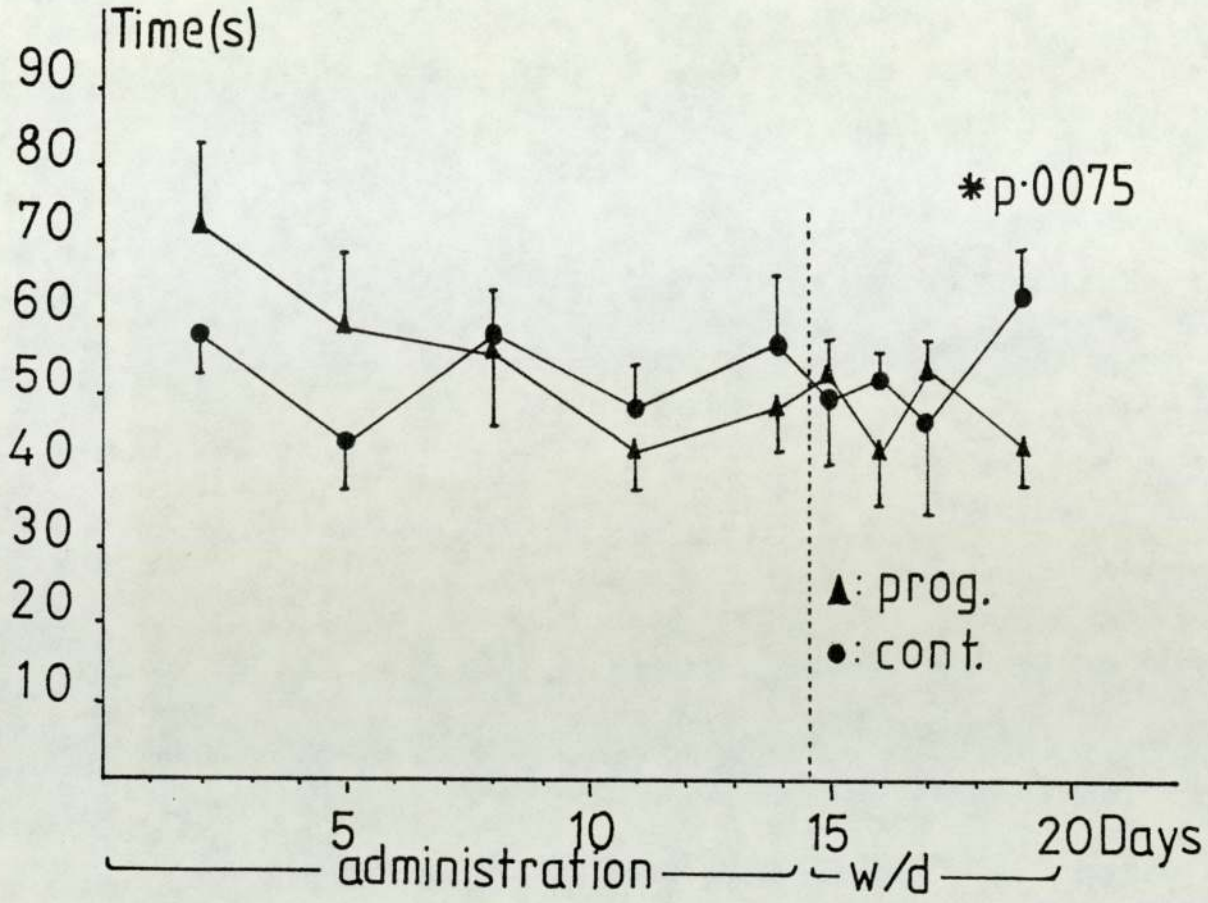


Fig 6·10 Plate crossing: total crossings in 90s.

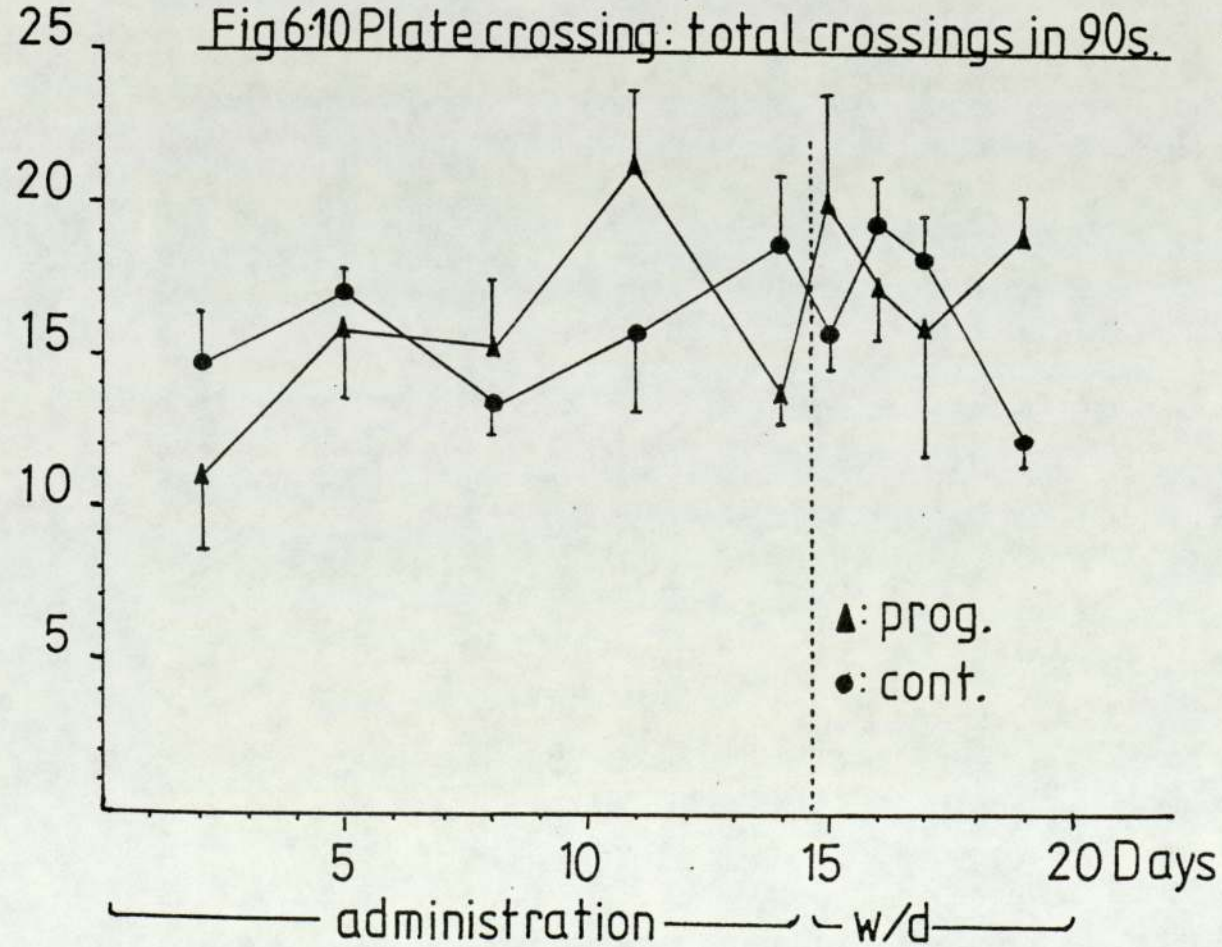


Fig 6·11 Locomotor activity profile – female mice

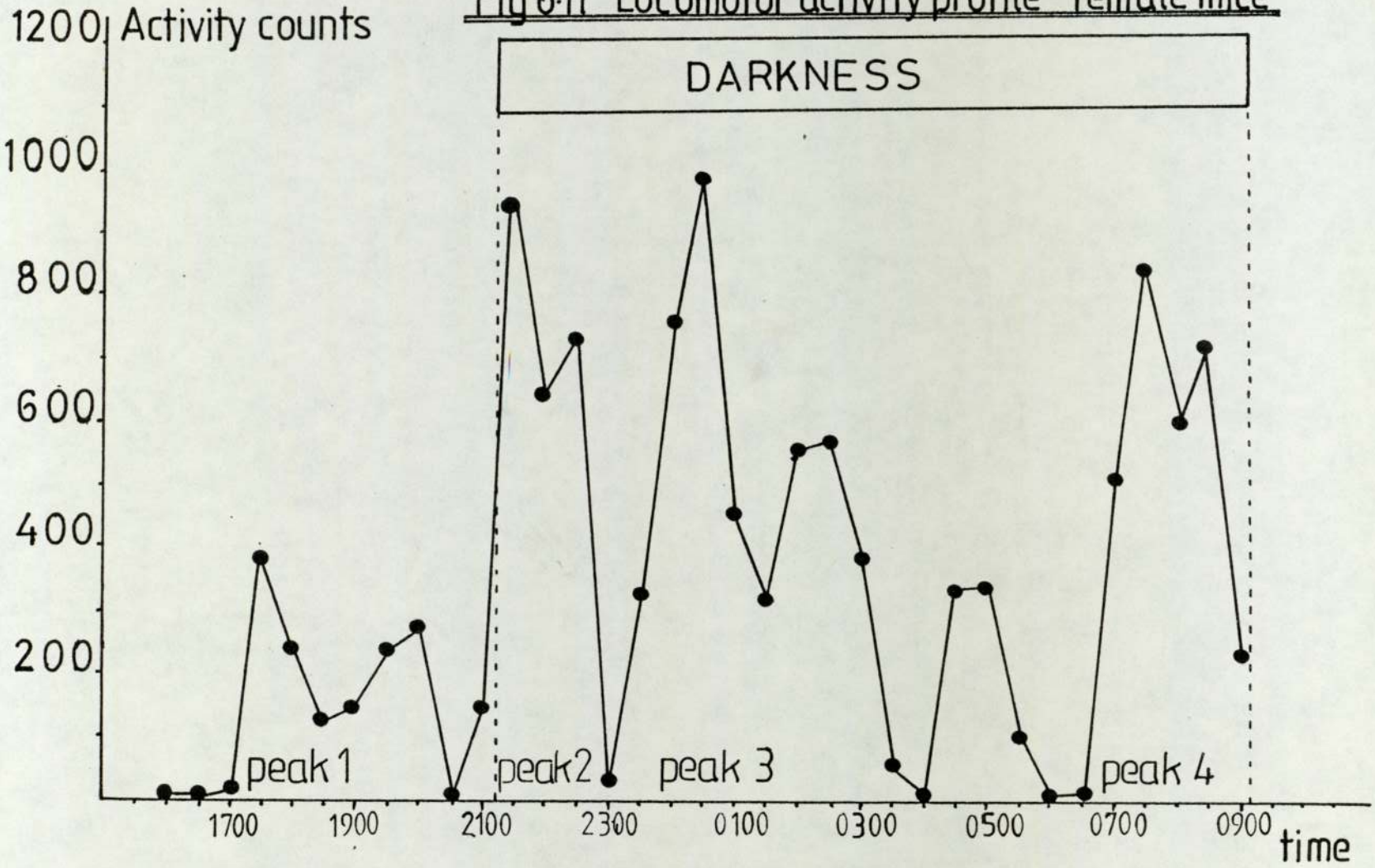
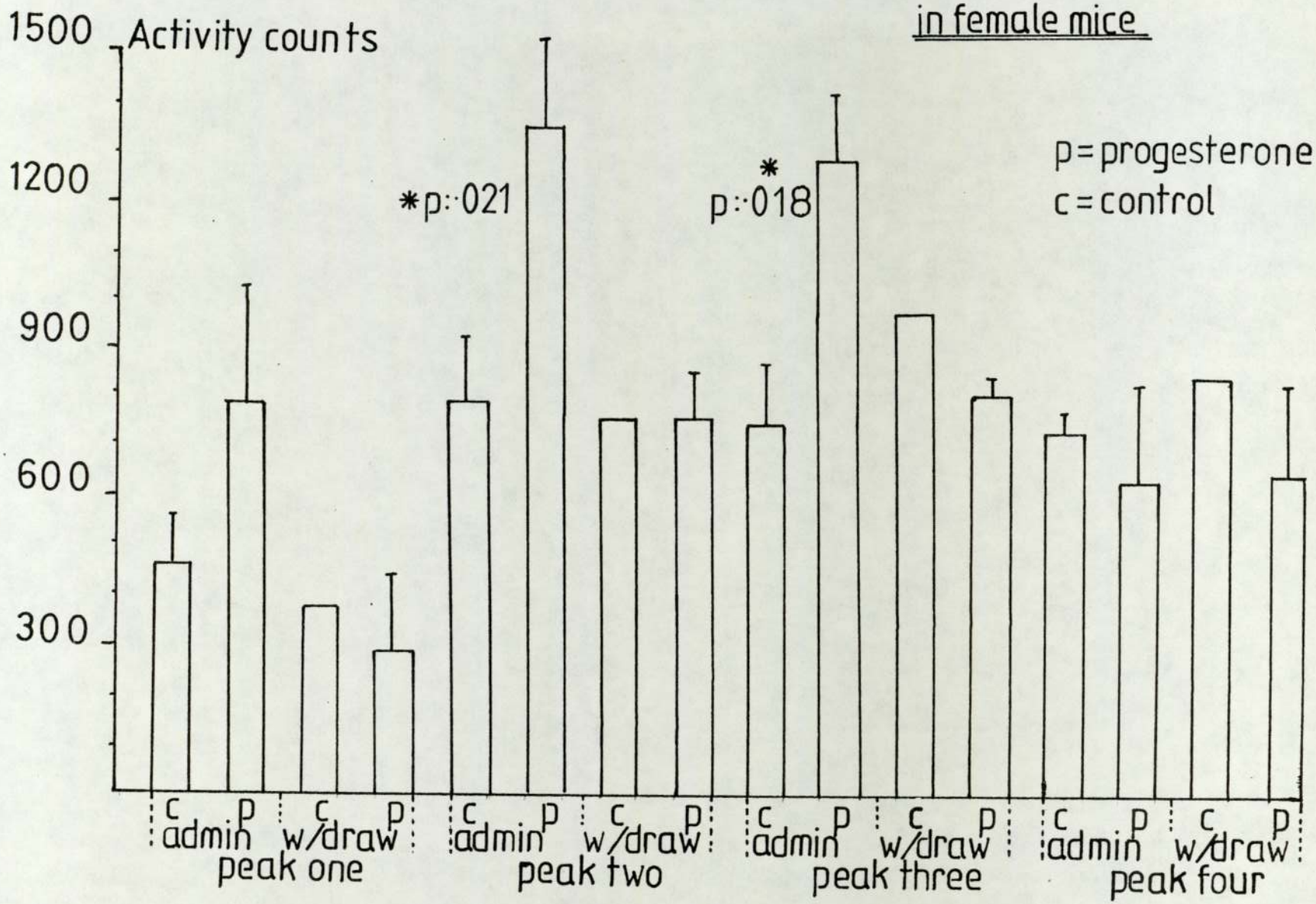


Fig 6·12 Effects of progesterone treatmenton locomotor activity in female mice



Norethisterone acetate (20ug/kg) – female mice

Fig 6·13 Plate crossing: time to first crossing

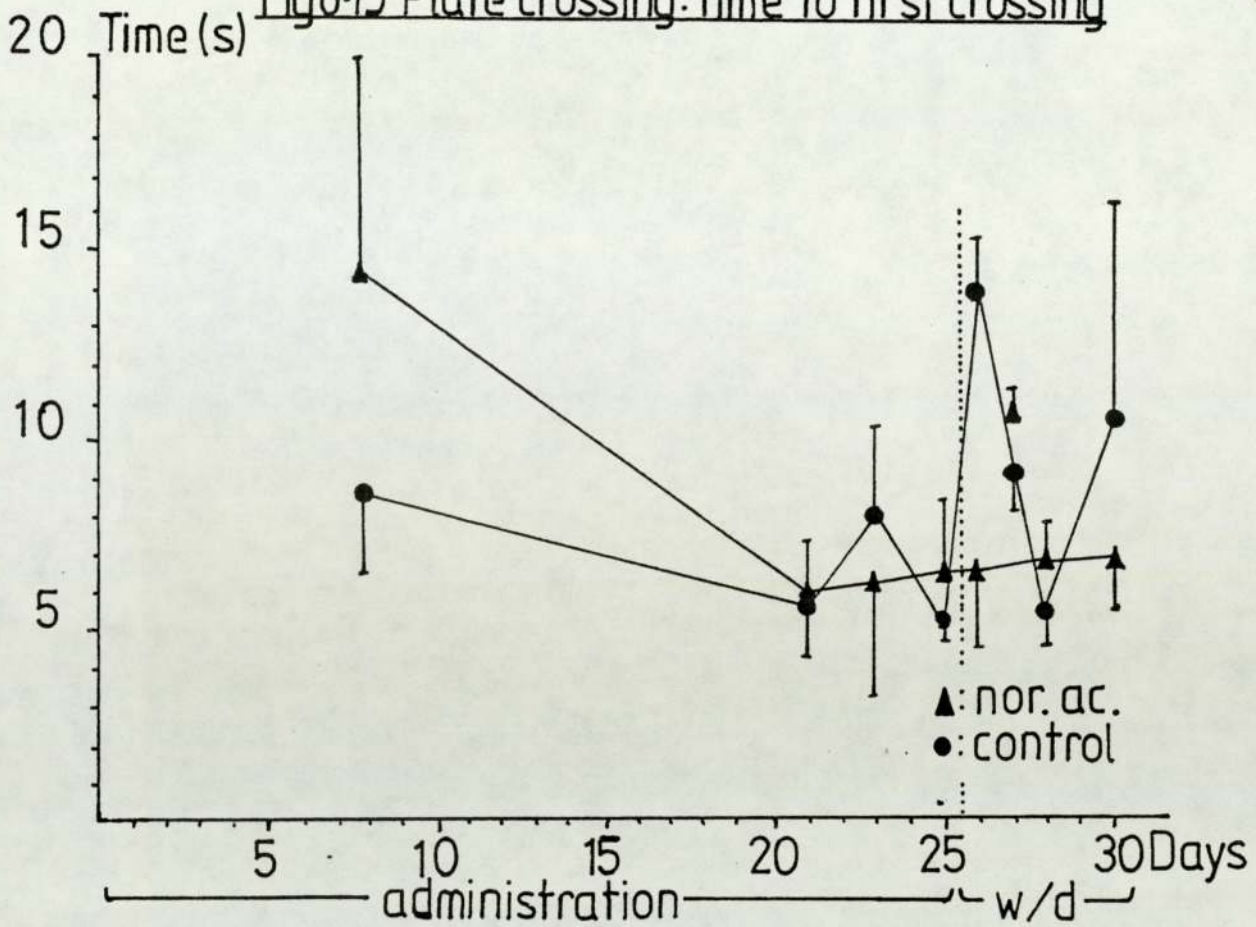
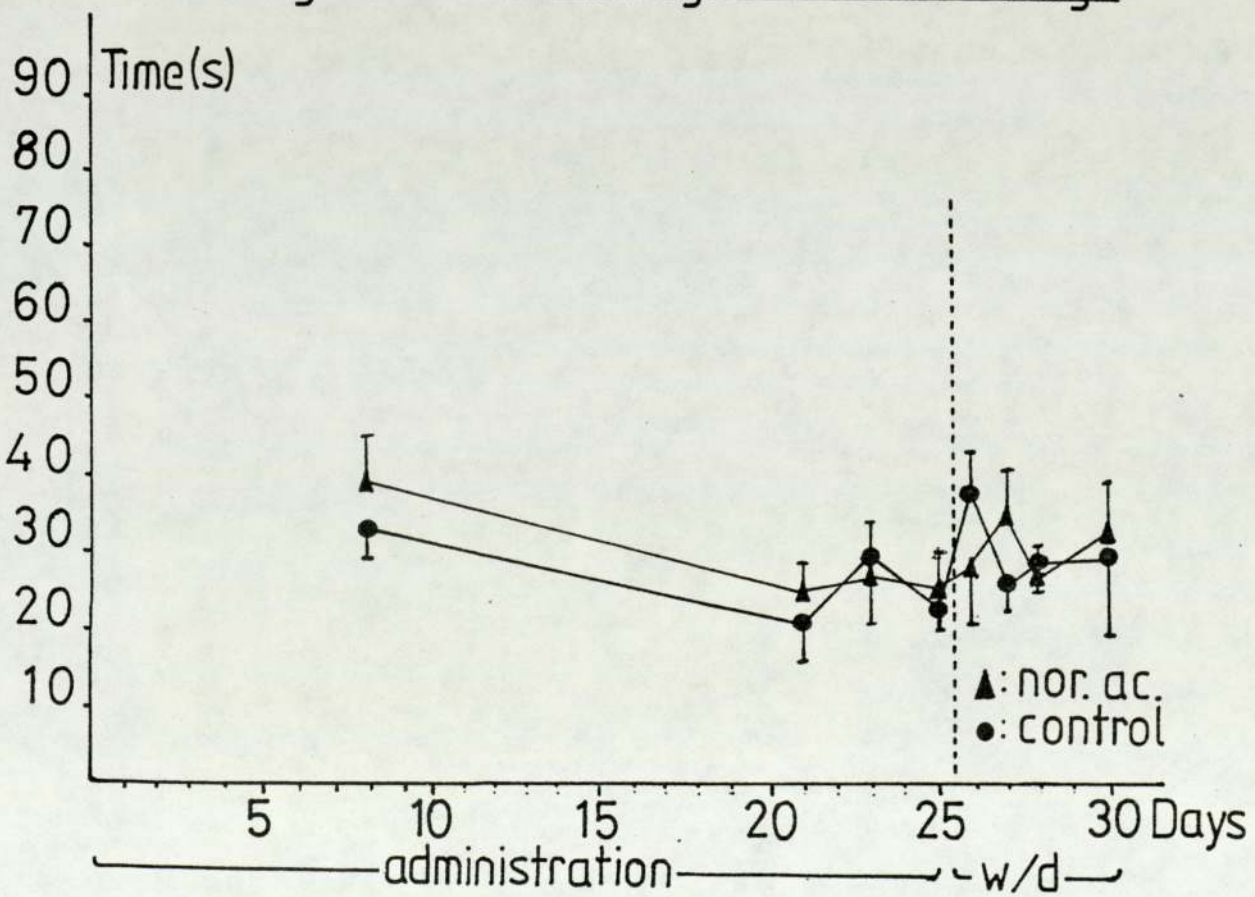


Fig 6·14 Plate crossing: time to 5 crossings



Norethisterone acetate (20ug/kg)-female mice

Fig 6·15 Plate crossing: time to 10 crossings

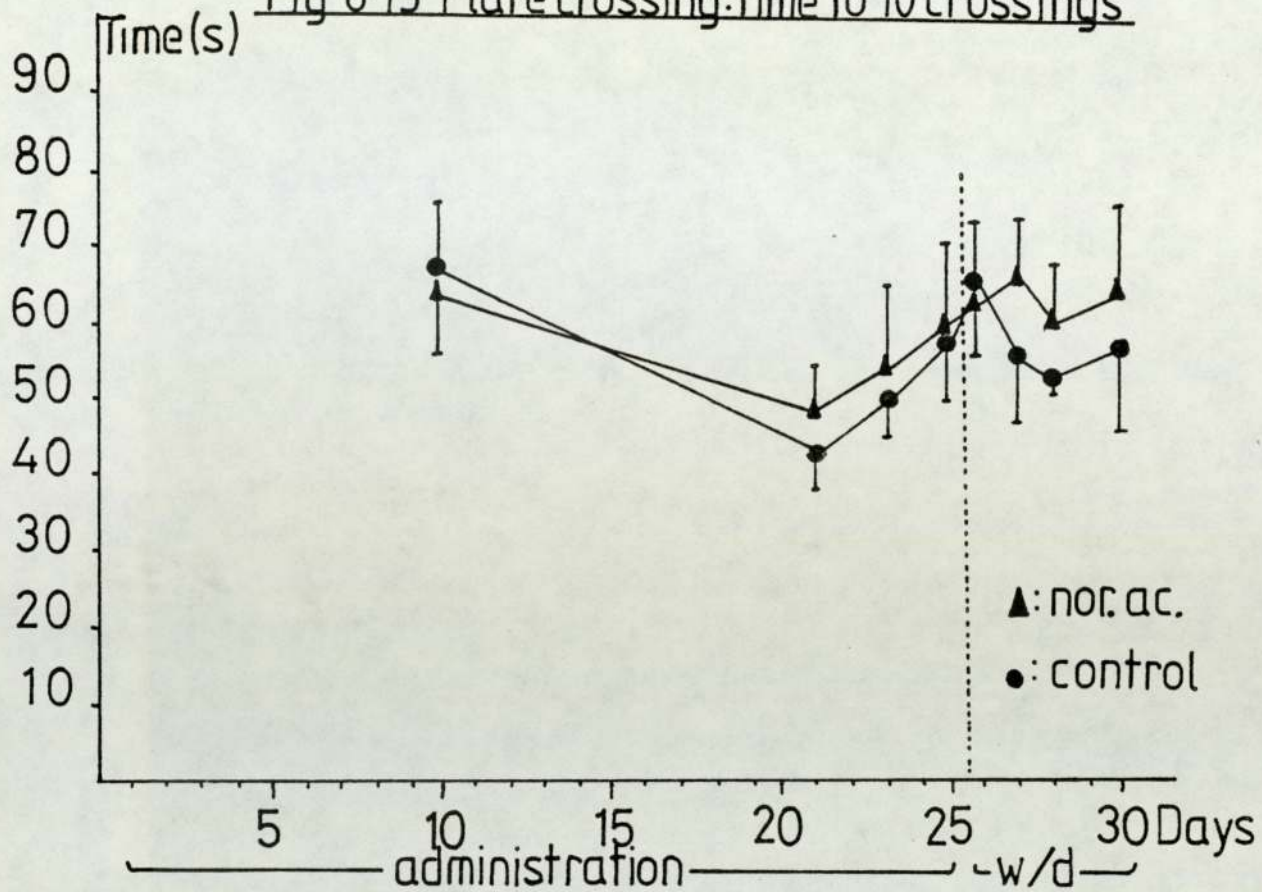


Fig 6·16 Plate crossing: total crossings in 90s.

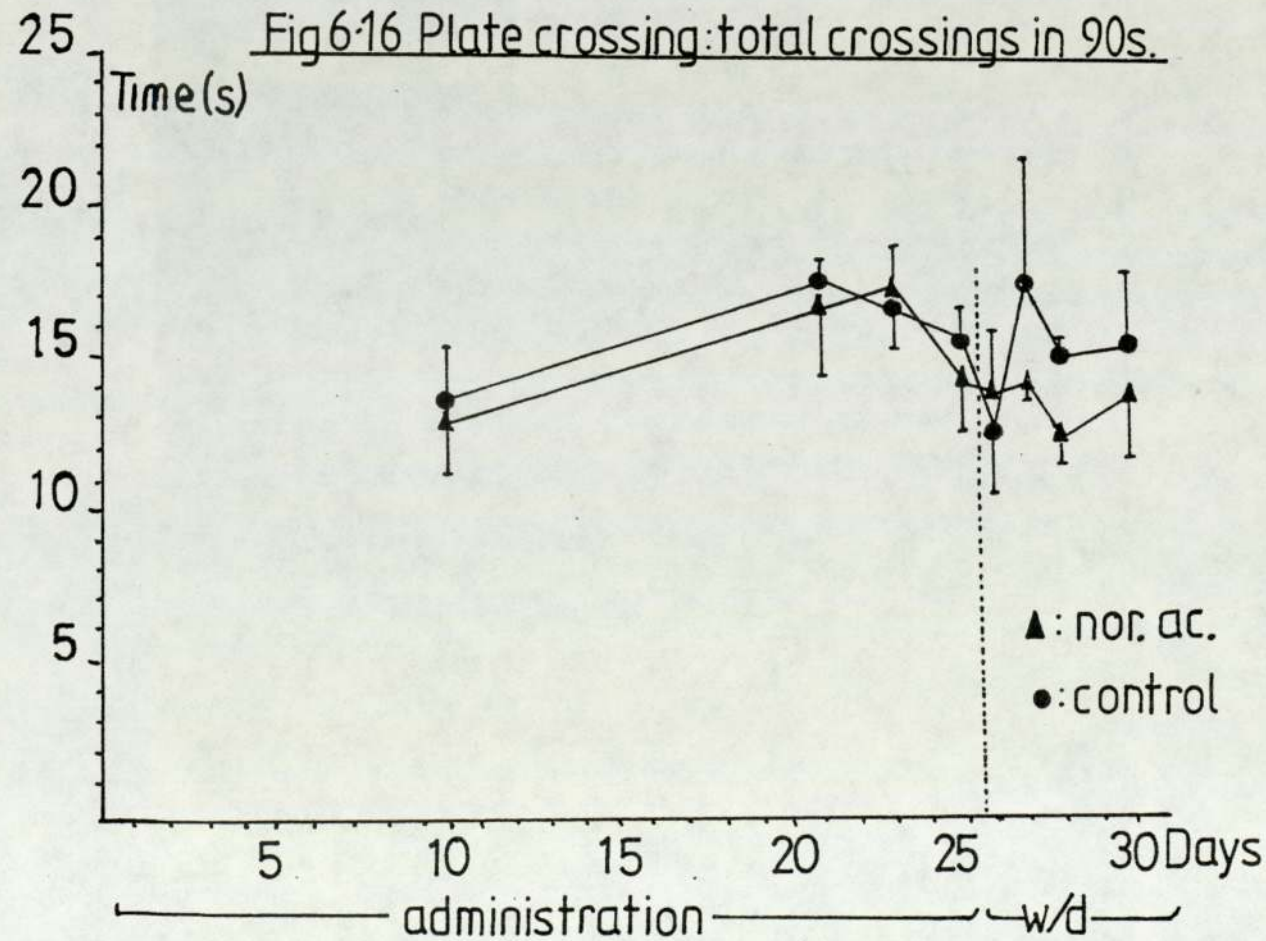


Fig 6·17 Effects of norethisterone acetate (20 ug/kg) on locomotor activity in female mice

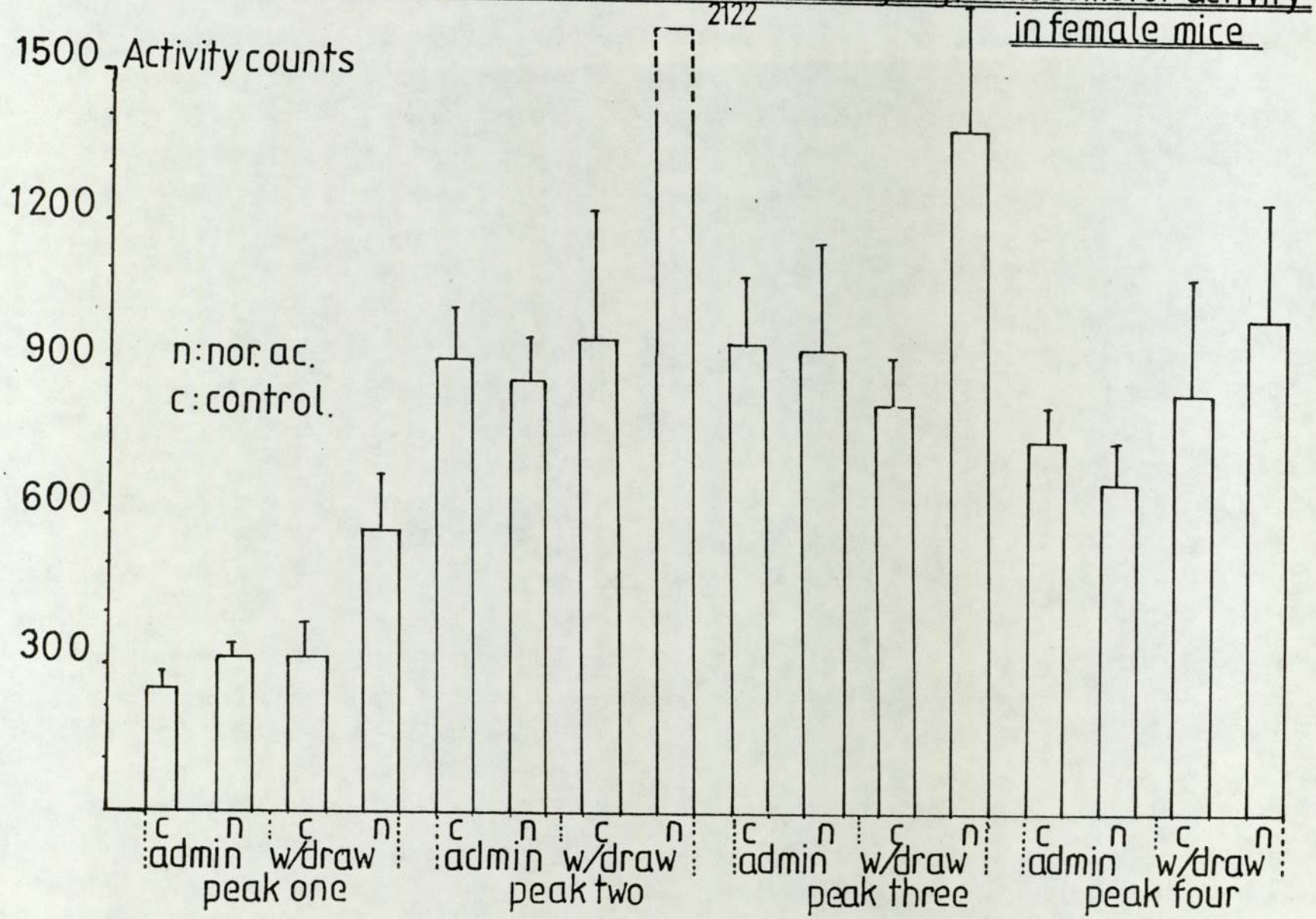


Fig 6·18 Effects of norethisterone acetate (200ug/kg) on locomotor activity in female mice

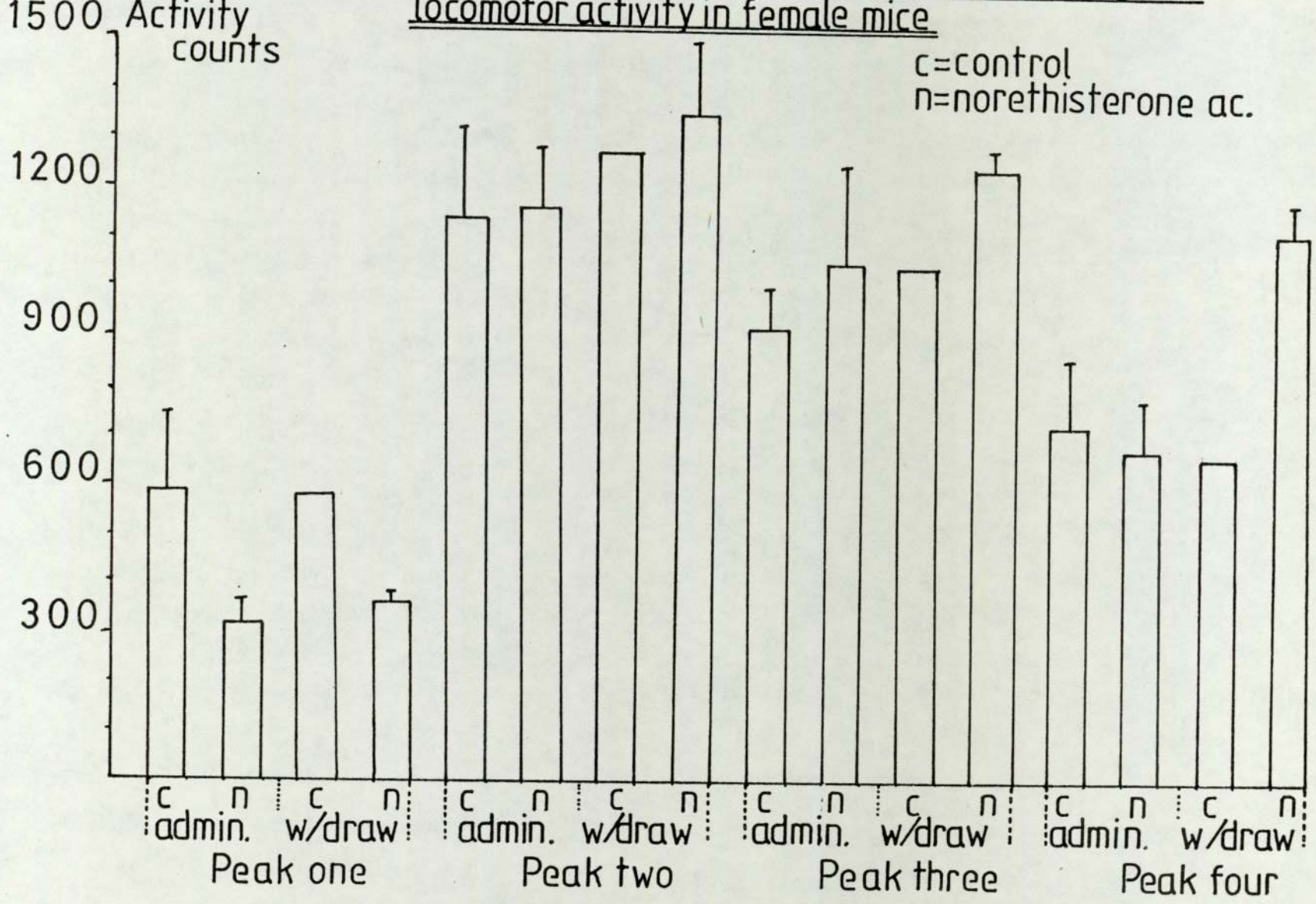


Fig 6·19 Comparison of locomotor activity in untreated mice and in mice receiving gum acacia

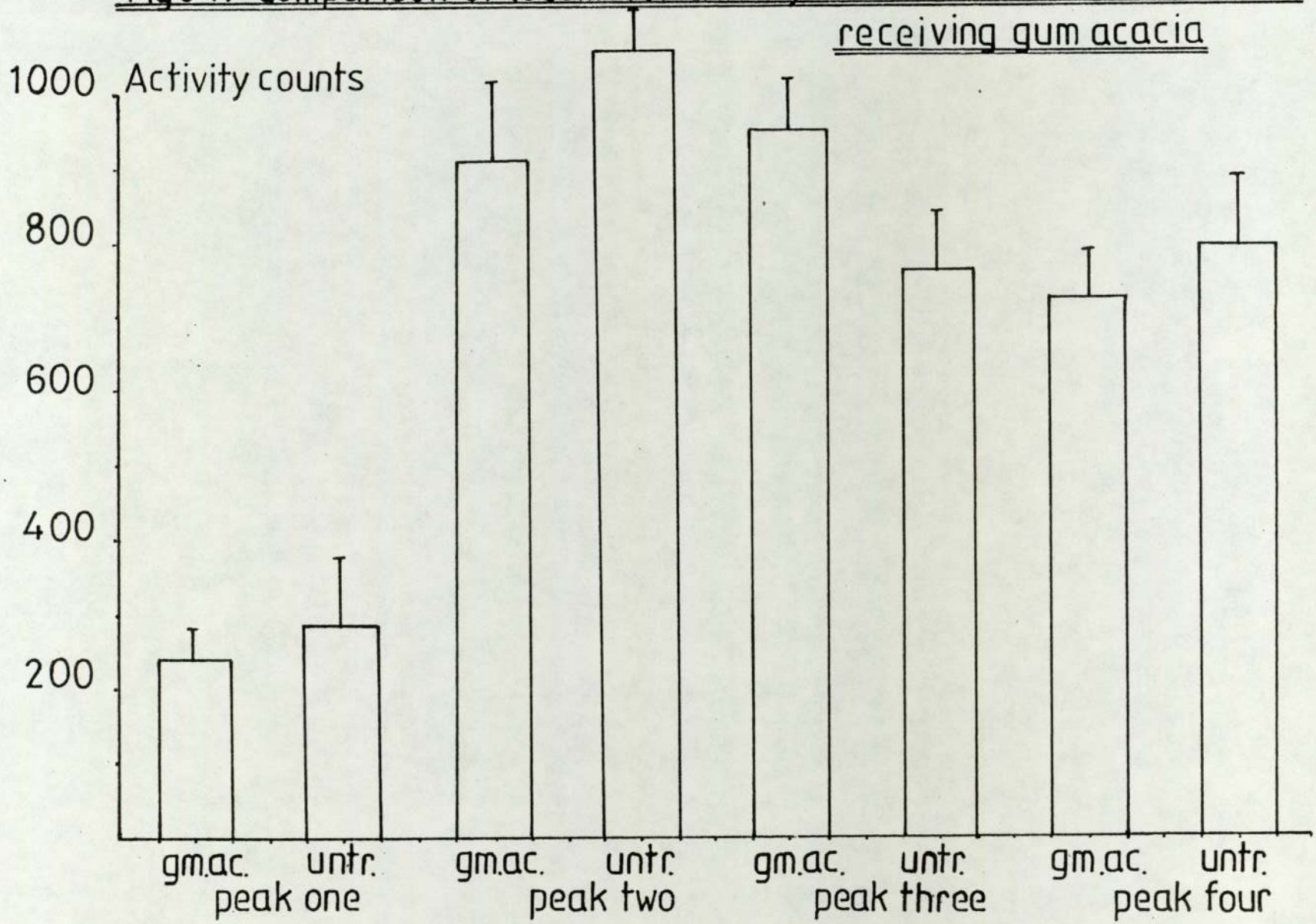
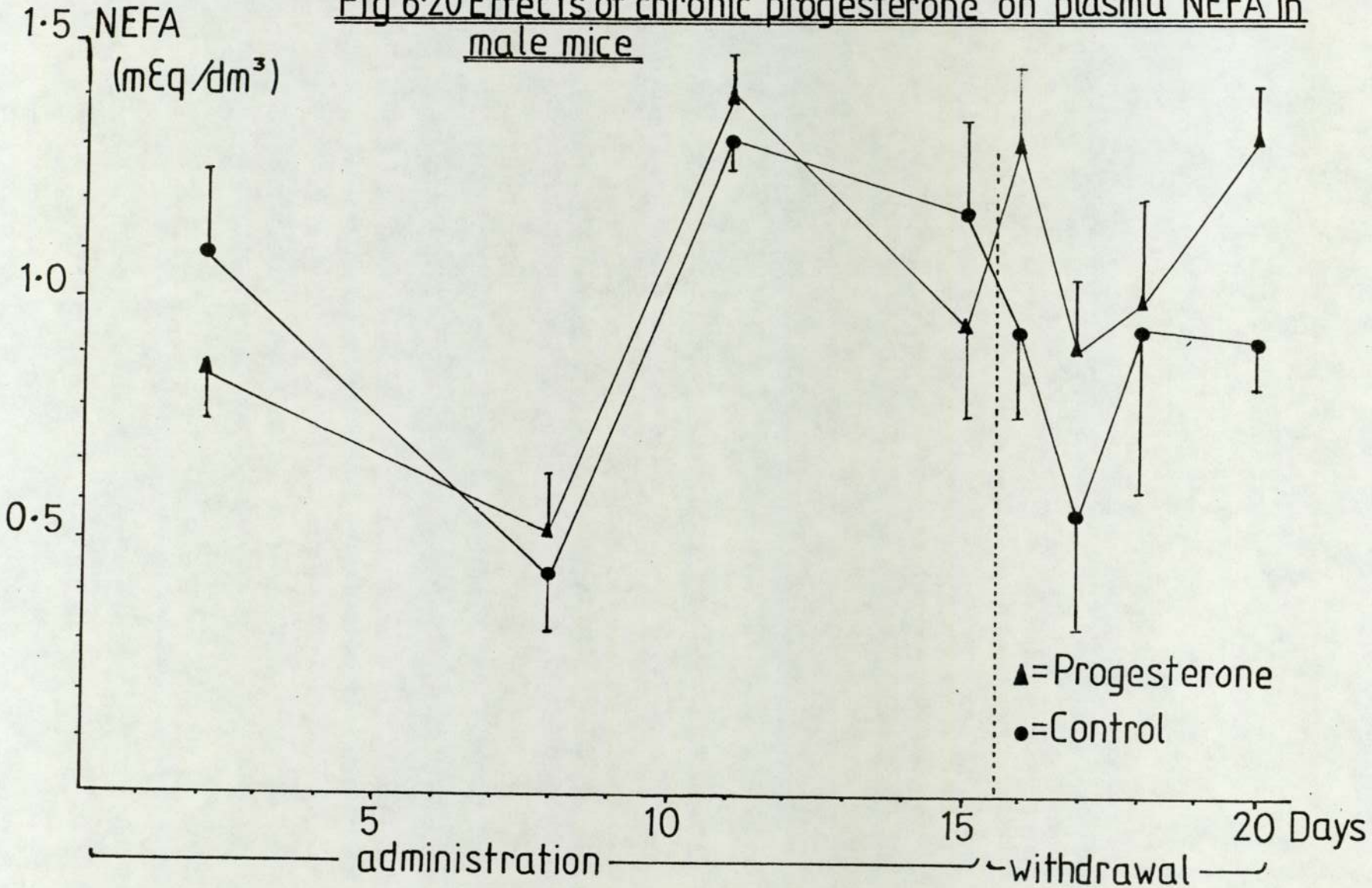


Fig 6·20 Effects of chronic progesterone on plasma NEFA in male mice



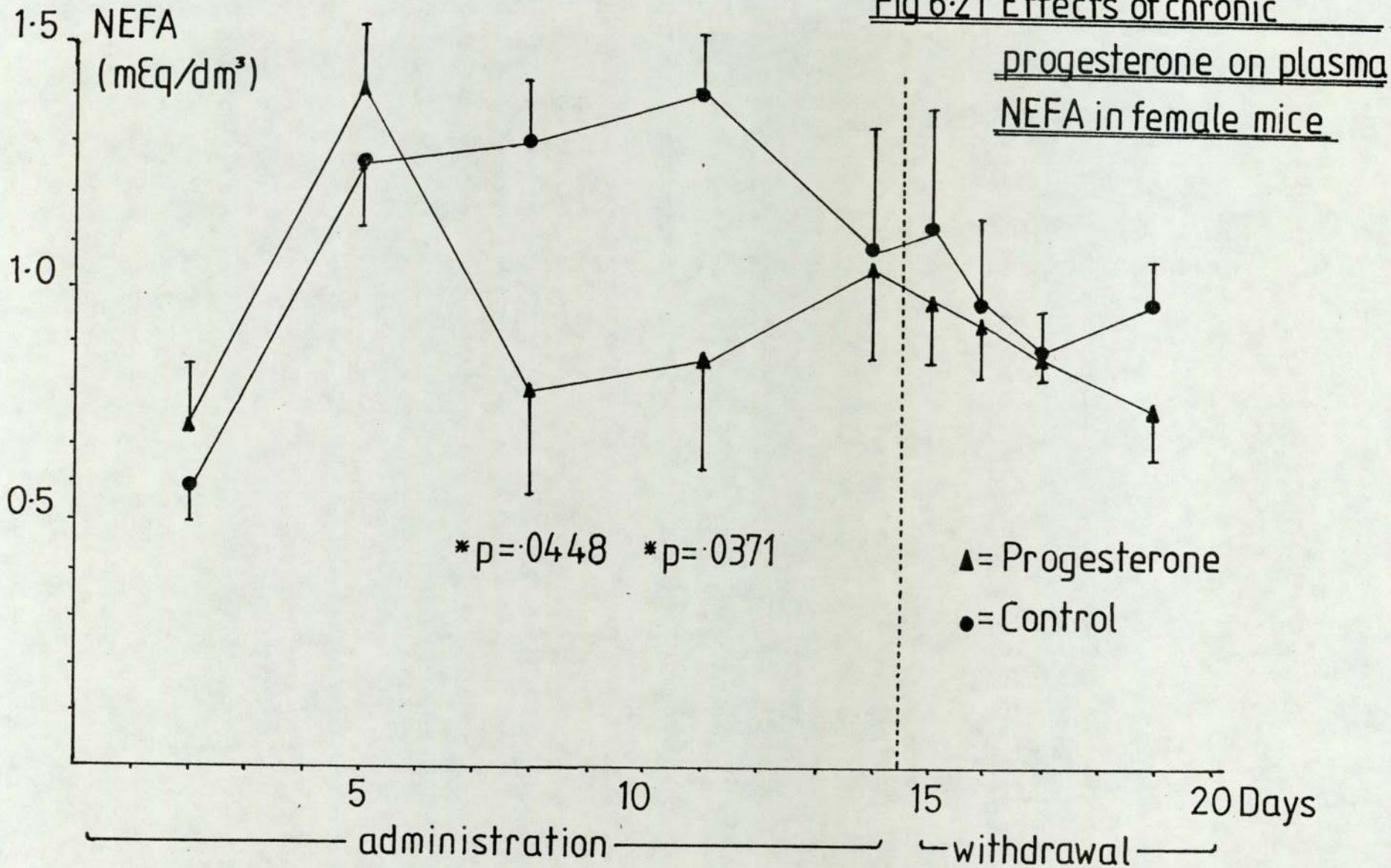


Fig 6·21 Effects of chronic progesterone on plasma NEFA in female mice

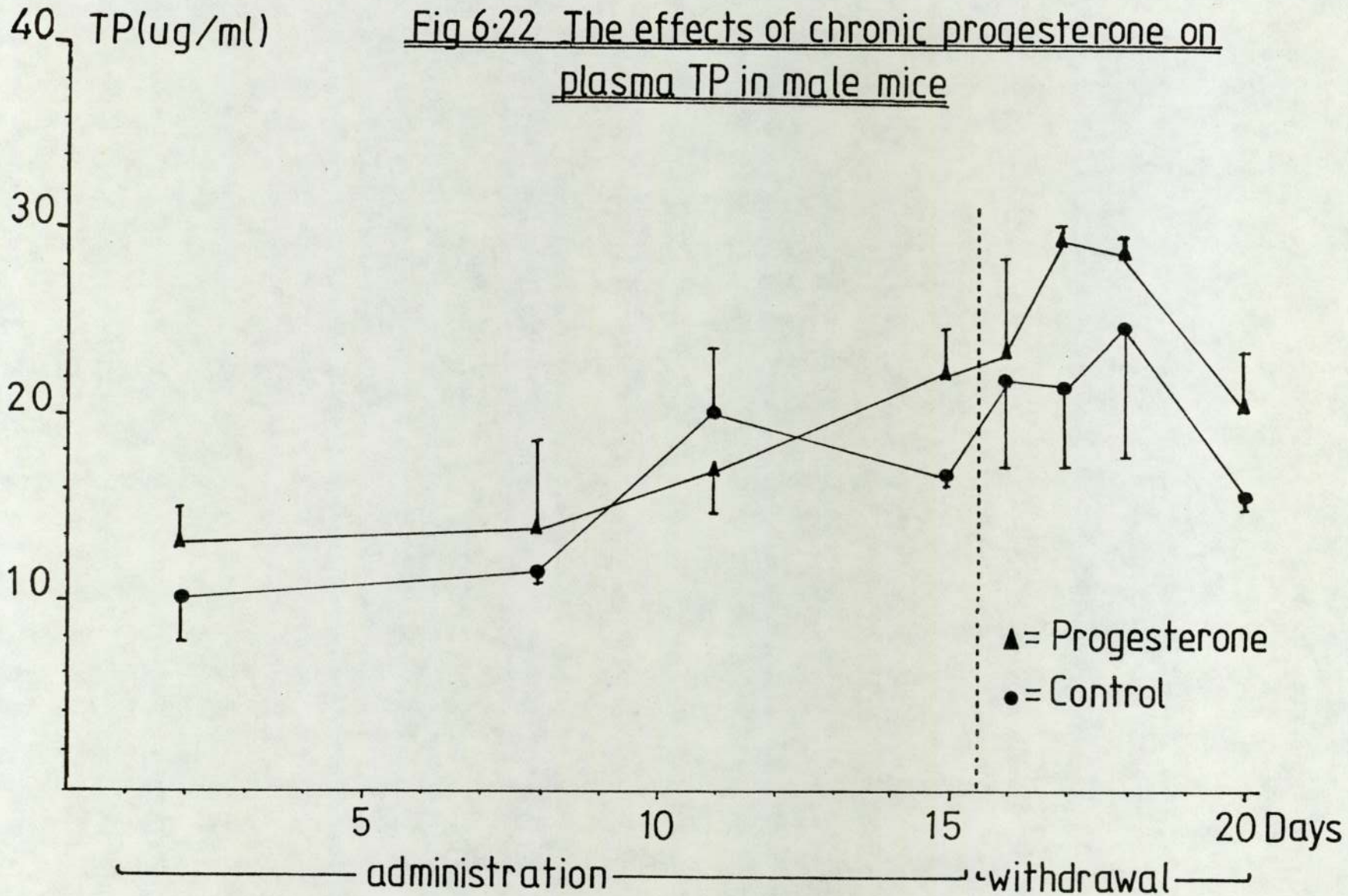


Fig 6·22 The effects of chronic progesterone on plasma TP in male mice

Fig 6·23 Effects of chronic progesterone on plasma TP in female mice

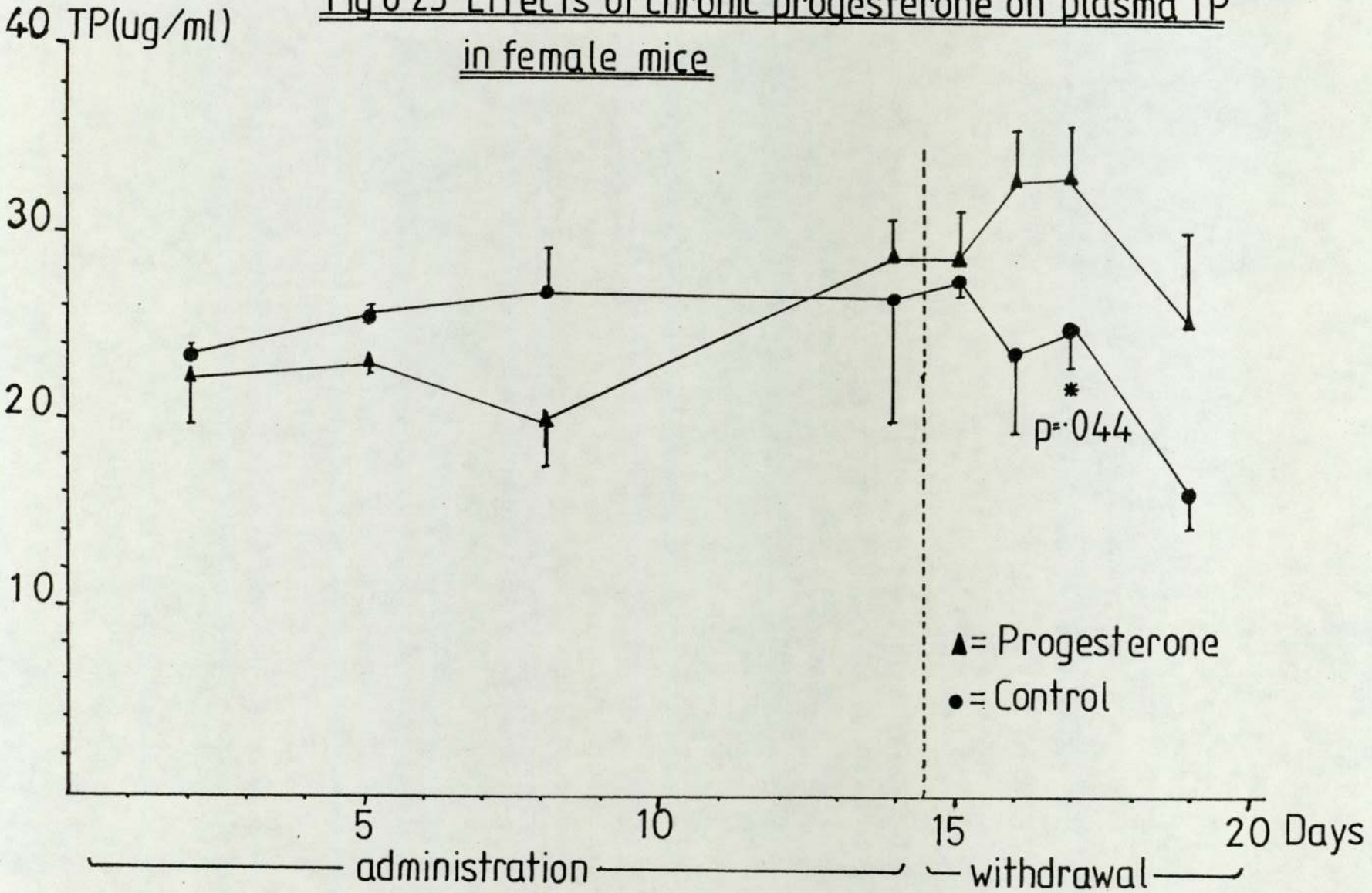


Fig 6·24 Effects of chronic progesterone on plasma TP in female mice (repeat study)

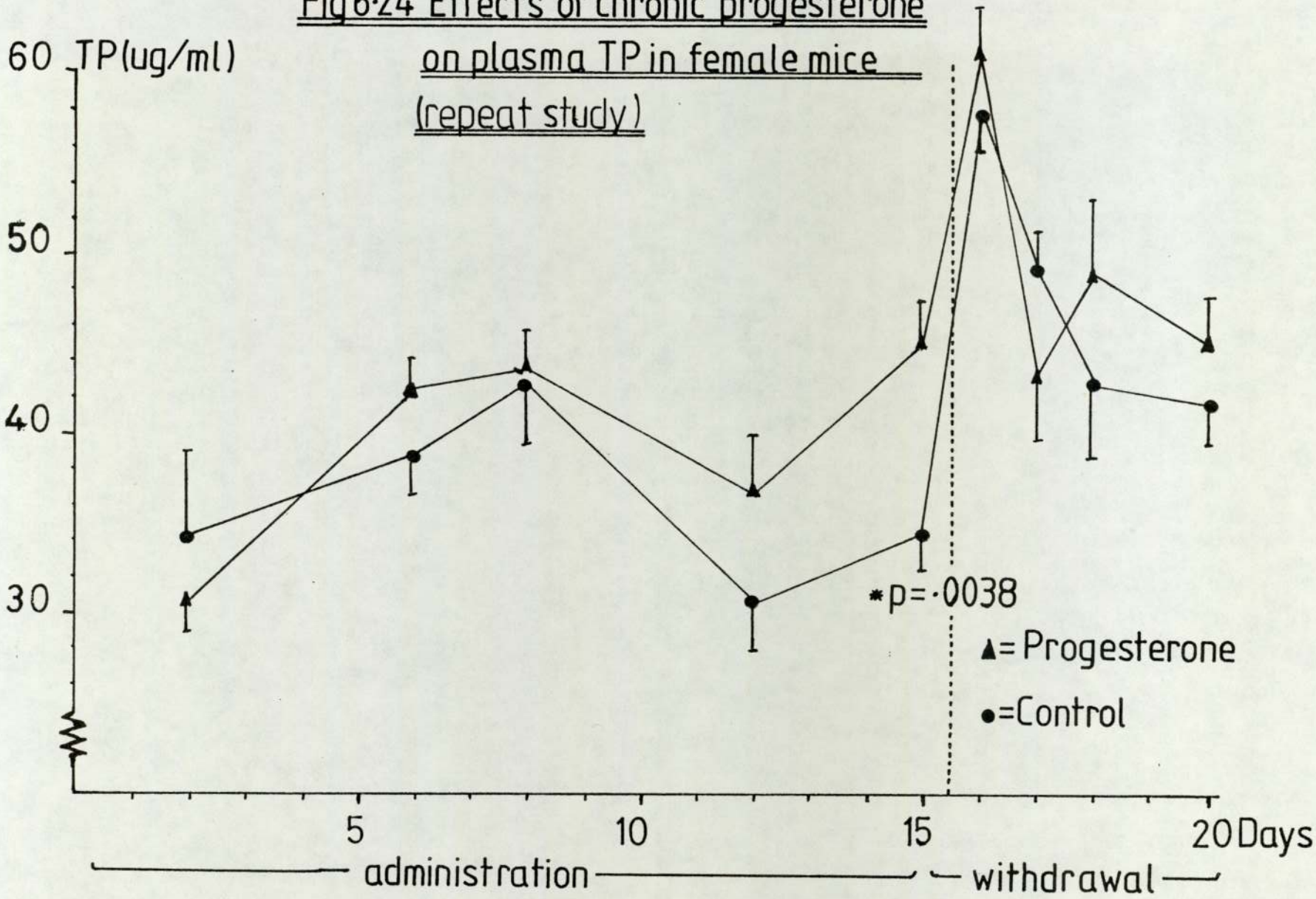


Fig 6·25 Effects of chronic norethisterone acetate (20ug/kg s.c.) on plasma TP in female mice

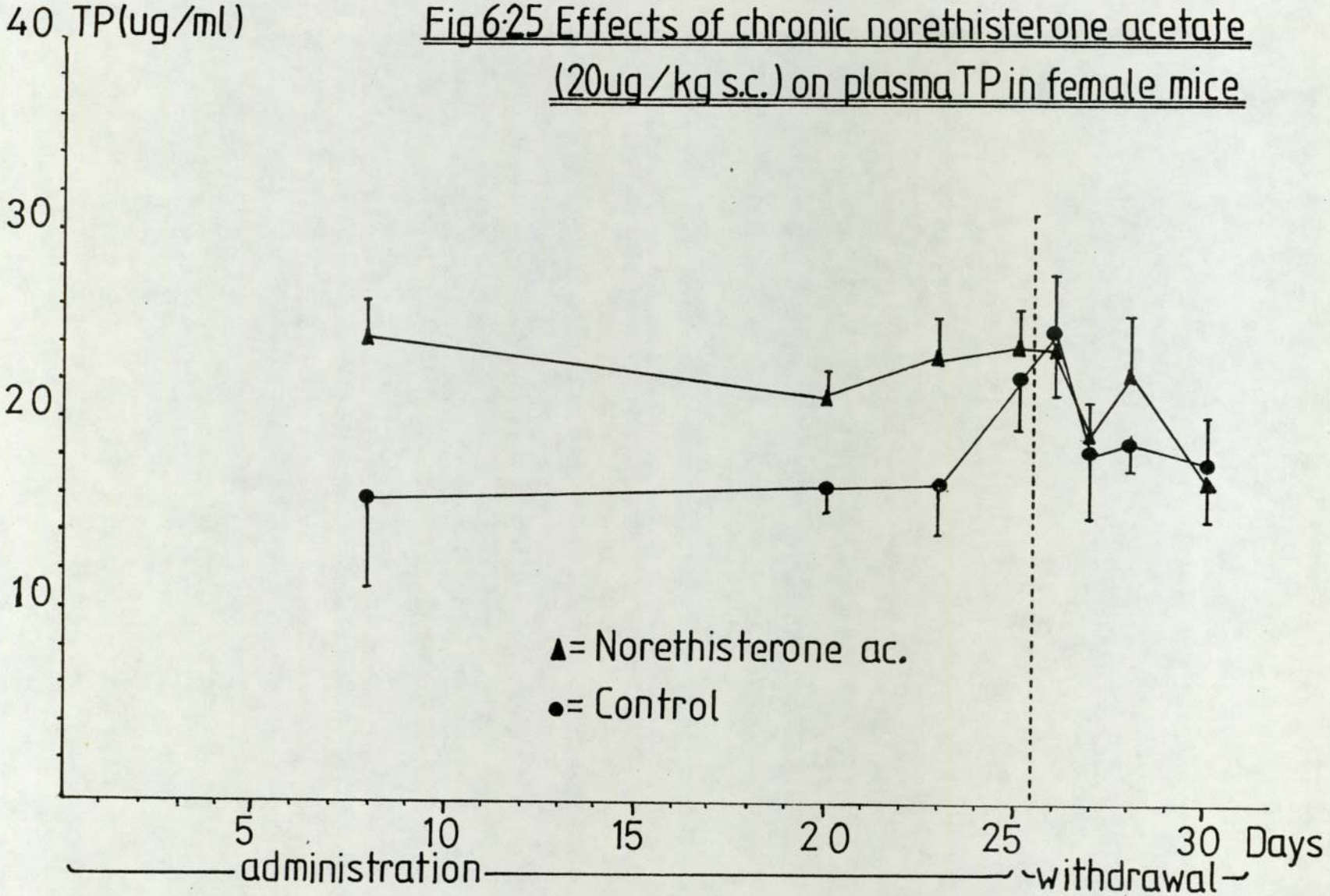
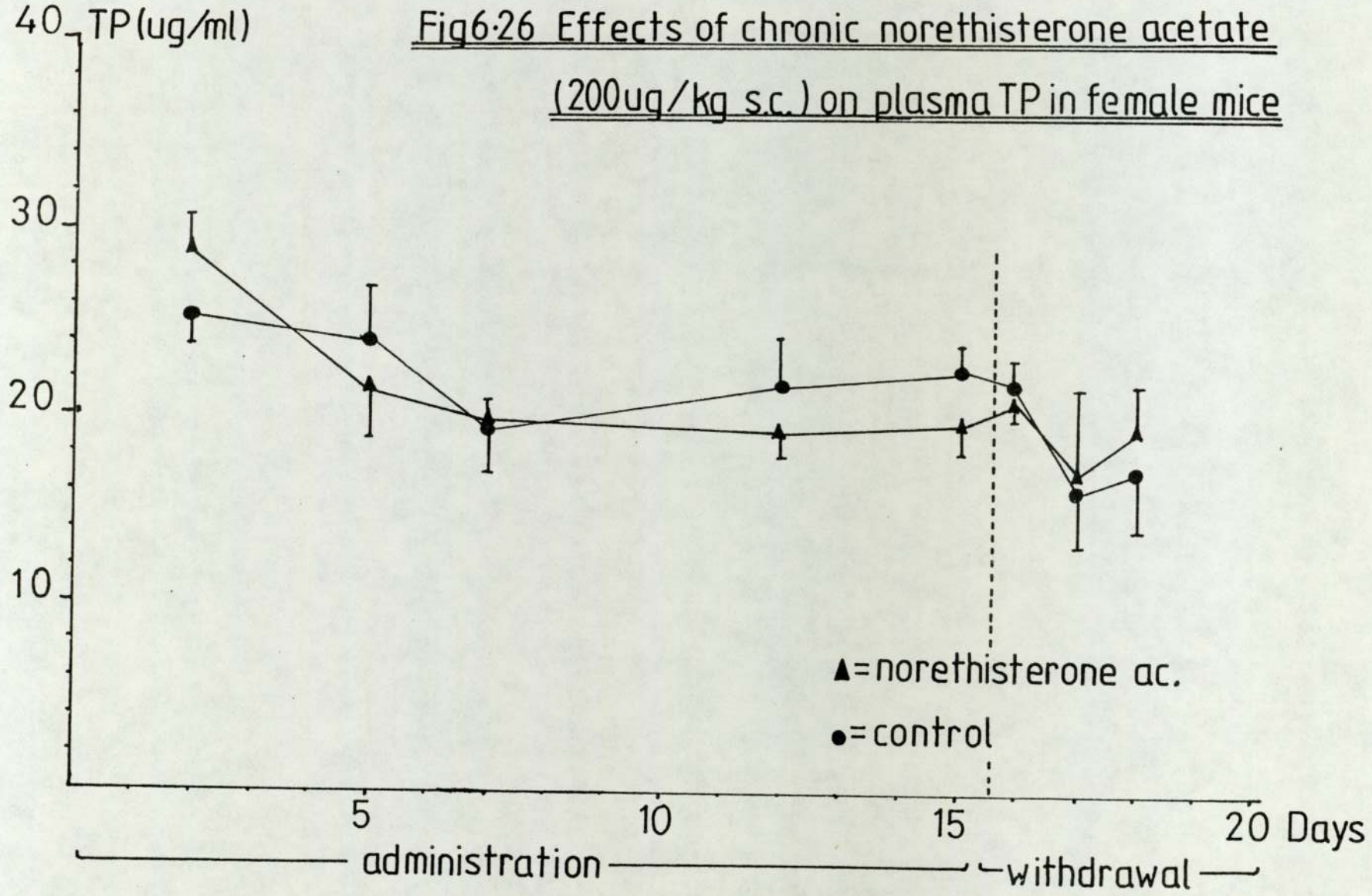


Fig6·26 Effects of chronic norethisterone acetate (200ug/kg s.c.) on plasma TP in female mice



Chapter 7

CHAPTER 7

THE EFFECTS OF CHRONIC ADMINISTRATION AND ACUTE WITHDRAWAL OF PROGESTERONE, SYNTHETIC PROGESTOGENS OR AN OESTROGEN/ PROGESTOGEN COMBINATION ON MOUSE BRAIN INDOLEAMINES

7.1 Chronic Administration of Progesterone in male mice

- 7.1.1 Effects on brain TP
- 7.1.2 Effects on brain 5HT
- 7.1.3 Effects on brain 5HIAA

7.2 Acute Withdrawal of Progesterone treatment in male mice

- 7.2.1 Effects on brain TP
- 7.2.2 Effects on brain 5HT
- 7.2.3 Effects on brain 5HIAA

7.3 Chronic Administration of either Progesterone, Dydrogesterone, Norethisterone Acetate or Ethinyl Oestradiol/Norethisterone Acetate combination in female mice

- 7.3.1 Effects on brain TP
- 7.3.2 Effects on brain 5HT
- 7.3.3 Effects on brain 5HIAA

7.4 Acute Withdrawal of either Progesterone, Dydrogesterone, Norethisterone Acetate or Ethinyl Oestradiol/Norethisterone Acetate combination in female mice.

- 7.4.1 Effects on brain TP
- 7.4.2 Effects on brain 5HT
- 7.4.3 Effects on brain 5HIAA

7.5 Effects of Hormone Administration and Withdrawal on 5HT/5HIAA Ratio

7.5.1 Male Mice

7.5.2 Female Mice

7.6 Effects of Hormone Administration on Oestrous cycle length

7.7 Summary and Comments

Chapter 7

The Effects of Chronic Administration and Acute Withdrawal of Progesterone, Synthetic Progestogens or an Oestrogen/Progestogen Combination on Mouse Brain Indoleamines.

7.1 CHRONIC ADMINISTRATION OF PROGESTERONE IN MALE MICE.

Male mice (30 - 35g) were housed in an experimental cabin, with a 12 hour light/dark cycle in cages (size 50 x 30 x 15 cm) of 25 animals. (See Methods section 7). Each animal received either 10mg/kg (10ml/kg) progesterone in gum acacia, or 10ml/kg gum acacia control p.o., daily between 3.00 - 4.00 pm. On days 2, 5, 8, 11 and 15 of the administration, groups of five animals were removed from the cages, sacrificed and the brains were removed and assayed for TP, 5HT and 5HIAA as described in Methods. Brains were always removed between 12.00 noon - 1.00pm. Drug administration commenced on Day 1 of the experiment.

7.1.1 Effects on Brain TP.

Progesterone was seen to significantly increase the brain concentrations of TP on day 5 of administration ($p = .035$, 2-tailed). However, there were no significant differences in brain TP at any other time point (See Table 7.1).

Table 7.1 : Effects of Chronic Progesterone Administration on Brain TP in Male Mice.

Time	Brain TP(ug/g brain matter)±SEM		2-tailed probability
	Control	Progesterone	
Day 2	3.448±.452 (n=5)	2.971±.324 (n=5)	p=.6319
Day 5	3.259±.402 (n=5)	5.018±.712 (n=4)	p=.0347
Day 8	8.963±1.762 (n=5)	9.785±1.383 (n=5)	p=.6930
Day 11	10.449±2.240 (n=5)	11.014±1.166 (n=4)	p=.8177
Day 15	5.304±1.933 (n=5)	4.73±.337 (n=5)	p=.5895

7.1.2 Effects on Brain 5HT.

Progesterone administration had no significant effect on brain concentrations of 5HT (See Table 7.2).

Table 7.2 : Effects of Chronic Progesterone Administration on brain 5HT in Male Mice.

Time	Brain 5HT(ng/g brain matter)± SEM		2-tailed probability
	Control	Progesterone	
Day 2	140.5±102.3 (n=3)	560.2±293.0 (n=5)	p=.2817
Day 8	943.7±4.95 (n=3)	1019.8±165.0 (n=4)	p=.8417
Day 15	160.06±24.6 (n=5)	140.12±20.37 (n=5)	p=.5084

7.1.3 Effects on Brain 5HIAA.

Progesterone administration was generally seen to decrease the brain concentration of 5HIAA, however this difference only achieved significance on day 11 ($p = .021$). (See Table 7.3)

Table 7.3 : Effects of Chronic Progesterone Administration on Brain 5HIAA in Male Mice.

Time	Brain 5HIAA(ng/g brain matter) ±SEM		2-tailed probability
	Control	Progesterone	
Day 1	605.8±89.6 (n=5)	504.8±46.7 (n=5)	p =.2971
Day 5	678.5±68.2 (n=5)	533.5±71.0 (n=5)	p =.1358
Day 11	1126.5±163.0 (n=5)	524.4±156.0 (n=4)	p=.0210
Day 15	620.8± 55.4 (n=5)	512.0±127.0 (n=5)	p =.5102

7.2 ACUTE WITHDRAWAL OF PROGESTERONE TREATMENT IN MALE MICE.

Male mice (30-35g) were housed and treated for 15 days as in section 7.1. On the fifteenth day, treatment was withdrawn, animals were then removed, sacrificed and their brains assayed for TP, 5HT and 5HIAA on days 1, 2, 3 and 5 following treatment withdrawal.

7.2.1 Effects on Brain TP.

Following treatment withdrawal, brain TP levels for both progesterone and control groups, were generally lower than levels seen during progesterone administration. There were however, no significant differences between progesterone and vehicle control groups. (See Table 7.4)

Table 7.4 : Effects of Acute Progesterone Treatment Withdrawal on Brain TP in Male Mice.

Time	TP(μg/g brain matter)±SEM		2-tailed probability
	Control	Progesterone	
Day 1 (45 hrs after last dose)	5.47±1.13 (n=5)	5.08±0.67(n=5)	p=.2755
Day 2	5.75±1.81(n=5)	6.93±1.19(n=5)	p=.5864
Day 3	2.75±1.28(n=4)	3.22±0.57(n=5)	p=.6898
Day 5	3.79±0.62(n=5)	4.99±0.86(n=5)	p=.2420

7.2.2 Effects on Brain 5HT.

As with brain TP levels, following treatment withdrawal concentrations of brain 5HT were seen to fall to levels generally below those seen during progesterone administration. However, there were no significant differences between progesterone and vehicle control groups (See Table 7.5).

Table 7.5 : Effects of Acute Progesterone Treatment Withdrawal on Brain 5HT in Male Mice.

Time after withdrawal	5HT(ng/g brain matter)±SEM		2-tailed probability
	Control	Progesterone	
Day 1	110.9±5.8(n=5)	121.9±6.2 (n=5)	p=.7448
Day 2	71.5±19.9(n=5)	139.5±35.5 (n=4)	p=.0814
Day 3	56.8±21.4(n=4)	64.5±10.2 (n=5)	p=.7025
Day 5	116.0± 9.7(n=5)	110.0±16.0 (n=5)	p=.6912

7.2.3 Effects on Brain 5HIAA.

As with TP and 5HT, following withdrawal of treatment, concentrations of brain 5HIAA were seen to fall to levels below those seen during progesterone or vehicle control administration. However, there were no significant differences between the progesterone group and the vehicle control group (See Table 7.6).

Table 7.6 : Effects of Acute Progesterone Treatment Withdrawal on Brain 5HIAA in Male Mice.

Time after Withdrawal	5HIAA(ng/g brain matter) ±SEM		2-tailed probability
	Control	Progesterone	
Day 1(45hrs after last dose)	591.9±38.2 (n=5)	663.5±65.8 (n=5)	p=.1602
Day 2	510.8±46.9 (n=4)	612.0±122.5 (n=5)	p=.1691
Day 5	514.0±82.0 (n=5)	616.0±32.0 (n=5)	p=.2295

7.3 Chronic Administration of Either Progesterone, Dydrogesterone, Norethisterone Acetate or Ethinyl Oestradiol/Norethisterone Acetate Combination in Female Mice.

Sexually mature female mice (30-35g) were housed in groups of 5 in cages 30 x 13 x 11 cm. under the conditions described in Methods section 7. Animals were housed under the conditions for 2 weeks prior to the beginning of the experiment in order to ensure the synchronisation of the oestrous cycles within groups. This was verified by the random checks of

oestrous cycle stages. Animals were also treated with the vehicle control for 1 week before the first drug administration in order to overcome possible problems of initial disruption of the oestrous cycle due to the stress of the drug-administration technique. Again this was checked randomly.

Following this initial 3 week period, the animals were given either progesterone 10mg/kg; dydrogesterone 5mg/kg; norethisterone acetate 1mg/kg, an ethinyl oestradiol (1µg/kg)/norethisterone acetate (100µg/kg) combination or vehicle control. All drugs were administered in gum acacia suspension p.o. 5ml/kg. Animals received the treatment daily (8.00-10.00am) for 43 days. On days 5, 8, 12, 15, 30 and 43 of drug administration, groups of animals were sacrificed and the brains assayed for TP, 5HT and 5HIAA. Brains were removed at the same time of day on each occasion (11.30 am - 12.30 pm). Oestrous cycle determinations were performed post mortem.

7.3.1 Effects on Brain TP.

Progesterone, dydrogesterone and the combination were seen to significantly increase brain TP on day 15 of treatment, this was not so for norethisterone acetate. There were no differences in any group however for days 30 and 43 of treatment. (Fig 7.1, 7.2, 7.3 and Table 7.7)

7.3.2 Effects on Brain 5HT.

All treatments were seen to produce a general increase in brain 5HT levels, however progesterone only attained significance on day 15 (p=.0062 2-tailed). Dydrogesterone produced little change in 5HT, however 5HT was significantly increased on day 15 (p=.0045, 2-tailed). Norethisterone acetate produced significantly increased 5HT on day 5 (p= .0279, 2-tailed). The norethisterone acetate/ethinyl oestradiol combination also significantly increased brain 5HT on day 5 (p=.0065, 2-tailed) and day 30 (p=.0418 2-tailed). See Table 7.8 and figures 7.4 - 7.9.

7.3.3 Effects on Brain 5HIAA.

Progesterone showed no significant effect on brain 5HIAA.Dydrogesterone significantly increased brain 5HIAA on day 15 (p=.0004, 2-tailed) and day 43 (p = .0147, 2-tailed). Norethisterone acetate initially significantly decreased brain 5HIAA on day 5 (p = .0005, 2-tailed), however 5HIAA levels were increased on day 43 (p = .0029, 2-tailed). Similarly the norethisterone acetate/ethinyl oestradiol combination significantly decreased brain 5HIAA on day 5 (p = .0001, 2-tailed) and day 8 (p = .0366, 2-tailed). See Table 7.9 and figures 7.10 - 7.15.

7.4 ACUTE WITHDRAWAL OF EITHER PROGESTERONE, DYDROGESTERONE, NORETHISTERONE ACETATE OR ETHINYL OESTRADIOL/NORETHISTERONE ACETATE COMBINATION TREATMENT IN FEMALE MICE.

Animals were treated as in section 7.3, except that they

received the drugs for just 15 days, treatment was then withdrawn and brain levels of TP, 5HT and 5HIAA were measured on day 1 to 3 following withdrawal. As before, brain samples were always taken between 11.30 am - 12.30 pm, and oestrous cycle determinations were performed post-mortem.

7.4.1 Effects on Brain TP.

The upward trend of brain TP over the first 15 days of drug administration continued for the first day following withdrawal, hence brain TP was significantly increased for progesterone ($p = .035$, 2-tailed), dydrogesterone ($p = .0038$, 2-tailed) and norethisterone acetate ($p = .0018$, 2-tailed), the combination was also increased, but not to a significant extent. This difference was not present on the second day following withdrawal, except that the brain TP of the progesterone group was significantly decreased. ($p = .0267$, 2-tailed). There were no significant differences for the third day following withdrawal, however all of the treatment groups had slightly decreased brain TP (See Table 7.10 and Figs 7.16 - 7.19).

7.4.2 Effects on Brain 5HT.

As with brain TP, the increased brain 5HT seen on the last day of drug administration (Day 15) were still present on the first day of drug withdrawal. Hence on the first day of drug withdrawal, brain 5HT of the drug treatment groups were increased above those for the control groups. These increases were statistically significant for progesterone ($p = .0104$, 2-tailed), norethisterone acetate ($p = .0072$, 2-tailed) and the oestrogen/progestogen

group (p = .0328, 2-tailed). The dydrogesterone group however did not achieve significance.

There was no difference between the control group and the treatment groups for the second day following treatment withdrawal. However on the third day, following withdrawal, the brain 5HT for the treatment groups were decreased as compared with vehicle controls. This difference was not significant for the dydrogesterone group, however statistical significance was achieved for progesterone (p = .0348, 2-tailed), norethisterone acetate (p = .0392, 2-tailed) and the oestrogen/progestogen combination (p = .0305, 2-tailed). (See Table 7.11 and Figs 7.20 - 7.23)

7.4.3 Effects on Brain 5HIAA.

On the final day of drug treatment, brain 5HIAA in the dydrogesterone group were significantly increased relative to the vehicle control group (p = .0004, 2-tailed). None of the other groups were significantly different from the vehicle control group. On the first day of drug withdrawal, the brain 5HIAA of the dydrogesterone group remained significantly increased relative to the vehicle control group (p = .0008, 2-tailed). The other drug treatment groups however tended to have brain 5HIAA levels below those of the vehicle control group, this difference was only significant for the norethisterone acetate group (p = .0338, 2-tailed).

By the second day of drug withdrawal, all of the drug treatment groups had brain 5HIAA decreased relative to the vehicle control group. The decrease was not

significant for the oestrogen/progestogen combination group, but the differences were significant for progesterone ($p = .0035$, 2-tailed), dydrogesterone ($p = .0045$, 2-tailed) and norethisterone acetate ($p = .0013$, 2-tailed). By the third day following withdrawal there were no differences between the drug treatment groups and the control group. (See Table 7.12 and Figs 7.24 - 7.27)

7.5 EFFECT OF HORMONE ADMINISTRATION AND WITHDRAWAL ON 5HT/5HIAA RATIO.

The 5HT/5HIAA ratio may be used as an indication of 5HT turnover (Bourgoin et al., 1978). Hence a decreased ratio would signify an increased turnover of 5HT.

Ratios were compared using the Mann-Whitney U Test as the distribution of ratios would not be normal.

7.5.1 Male Mice.

There were no significant differences in 5HT : 5HIAA ratio between the progesterone group and the vehicle control group, either during drug administration or drug withdrawal.

7.5.2 Female Mice.

Dydrogesterone produced a significantly decreased ratio on day 43 of drug administration ($p = .02$). This drug did not significantly alter the ratio, as compared with the vehicle control, at any other point during drug administration or withdrawal.

Progesterone did not significantly alter the ratio at any time during drug administration, however the ratio was significantly increased relative to vehicle control group, on the second day of drug

withdrawal ($p = .02$).

Norethisterone acetate significantly increased the ratio on day 5 of administration ($p = .02$) however on days 12 and 43 of administration the ratios were significantly decreased relative to vehicle controls ($p = .05$ and $.02$ respectively).

On days 2 and 3 of drug withdrawal, Norethisterone acetate significantly increased the ratios relative to controls ($p = .02$ and $.02$ respectively).

The Ethinyl estradiol/norethisterone acetate combination produced significantly increased ratios on day 5 of drug administration ($p = .02$). However all other differences during drug administration and withdrawal were not significantly different from the vehicle control.

7.6 EFFECTS OF HORMONE ADMINISTRATION ON OESTROUS CYCLE LENGTH.

Oestrous cycle stage determinations were performed daily on a single group of animals from each treatment group.

The gum acacia control group showed a oestrous cycle of six days duration. The metaphase and dioestrous lasted for 4 days with proestrous and oestrous lasting for 2 days.

The same pattern was seen for the dydrogesterone and progesterone groups. It should be stressed however that although the cycles were prolonged above the normal 4 day oestrous cycle, the animals showed a definite cycle.

The norethisterone acetate group showed a disruption of the oestrous cycle, all stages being prolonged, especially the dioestrous stage.

The norethisterone acetate/ethinyl oestradiol combination group showed the vaginal cytology of constant oestrous, however the number of cornified cells were fewer than that

seen in other mice at the oestrous stage.

7.7 SUMMARY AND COMMENTS.

Initial administration of progesterone, progestogens or a progestogen/oestrogen combination produced increased whole brain indoleamine concentrations relative to vehicle control. However after 30 or 43 days of administration this increase was no longer present.

Withdrawal of these drugs produced decreased whole brain indoleamine concentrations relative to vehicle control.

Norethisterone acetate was also seen to consistently affect 5HT : 5HIAA ratio.

This effect was sex dependant, progesterone produced little effect on brain indoleamine concentrations or 5HT : 5HIAA ratio in male mice, although other hormones were not tested.

Fig 7·1 Effects of chronic hormone treatment on brain TP in female mice after 15 days treatment

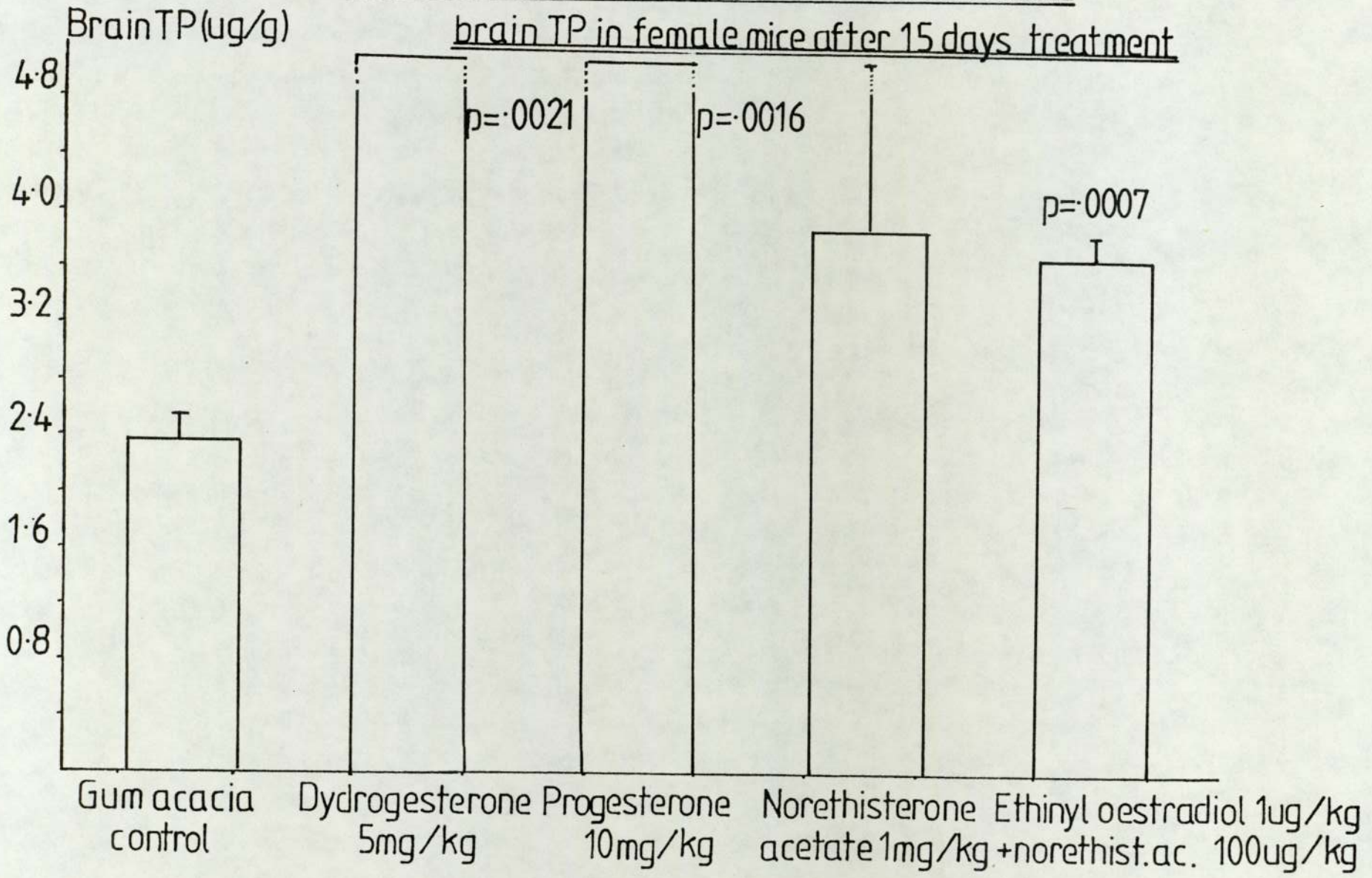
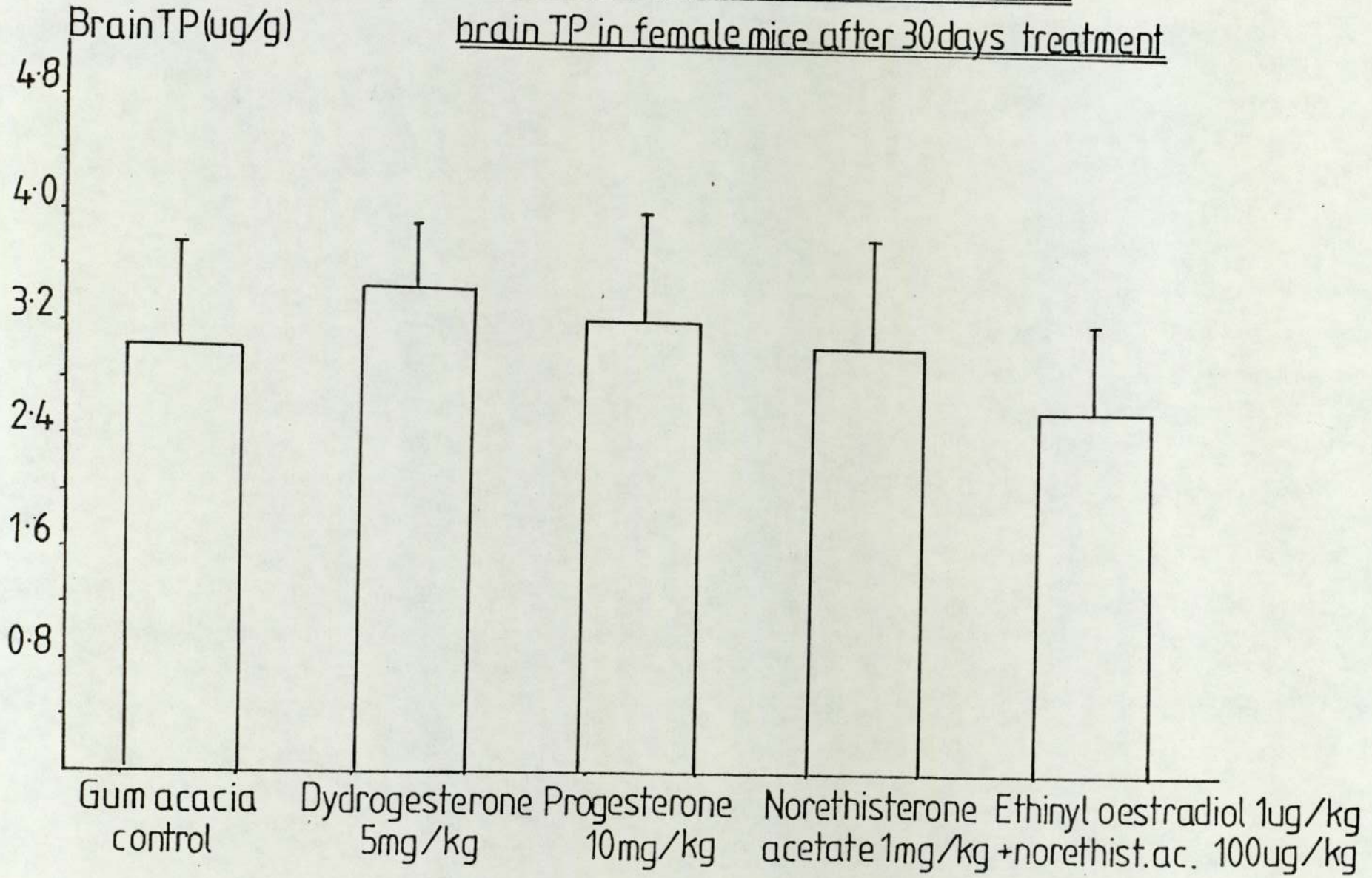


Fig 7·2 Effects of chronic hormone treatment on brain TP in female mice after 30 days treatment



Eig 7.3 Effects of chronic hormone treatment on brain TP in female mice after 43 days treatment

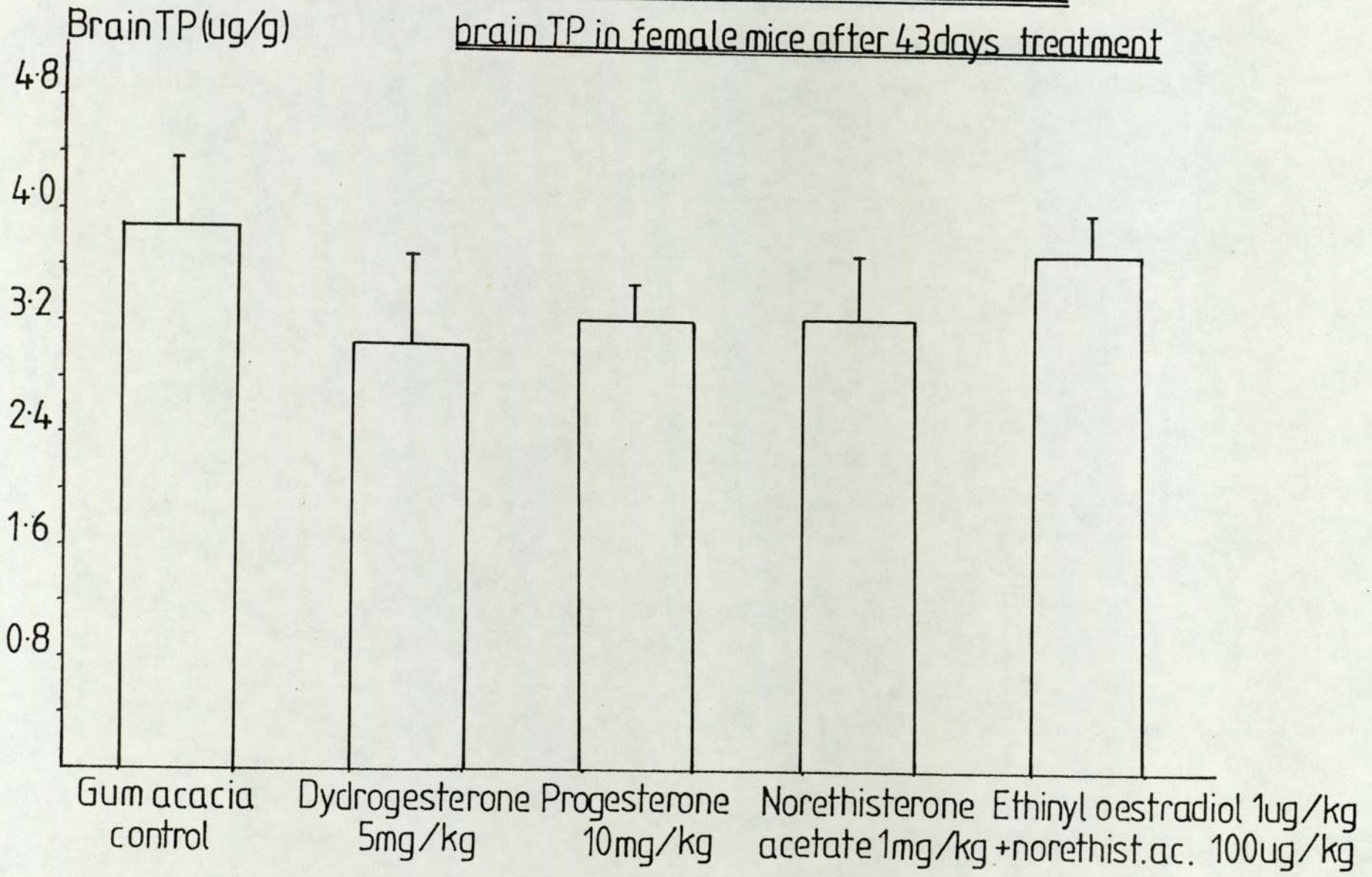


Fig 7·4 Effects of chronic hormone treatment on brain 5HT in female mice after 5 days treatment

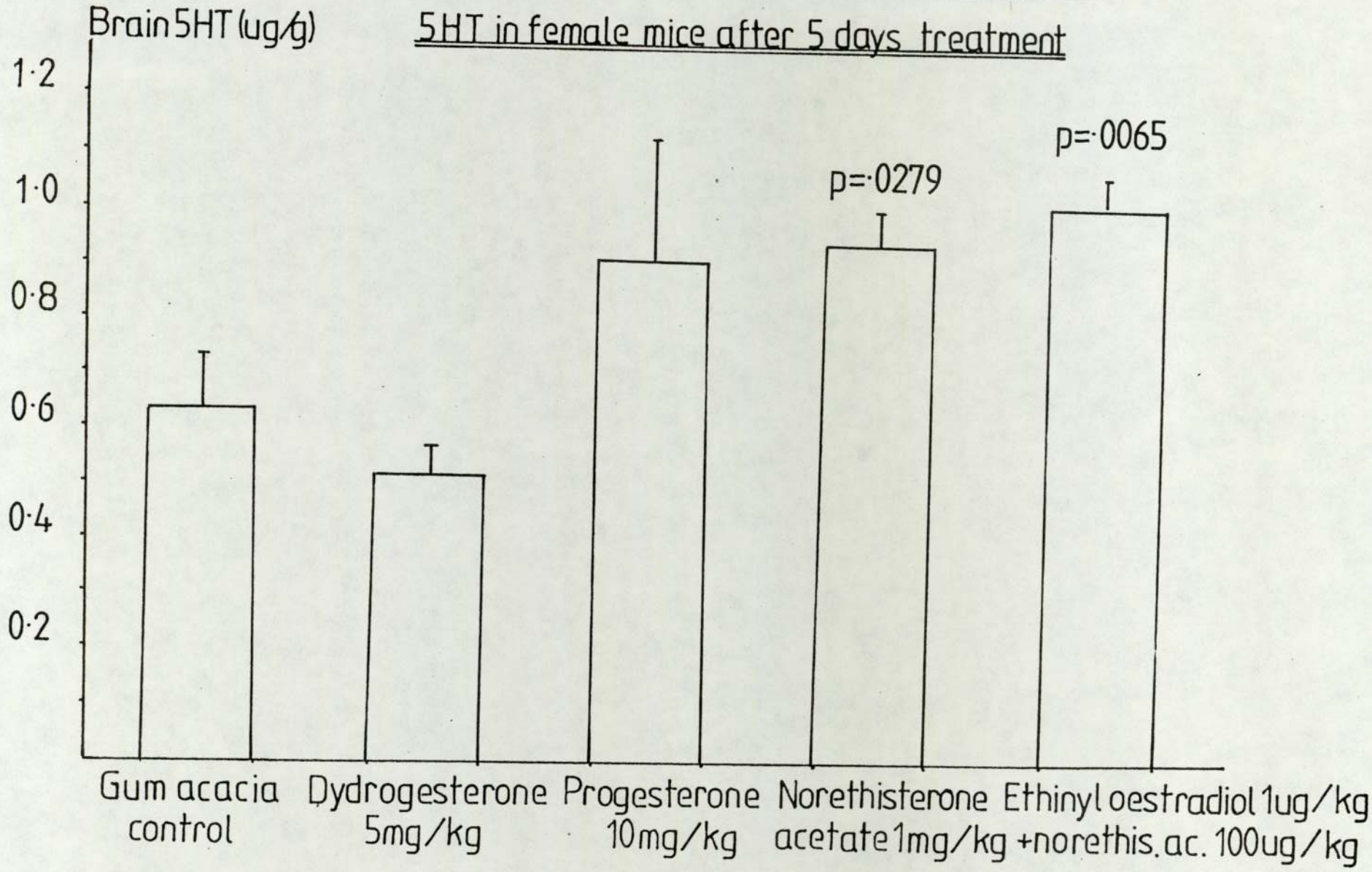


Fig 7·5 Effects of chronic hormone treatment on brain 5HT in female mice after 8 days treatment

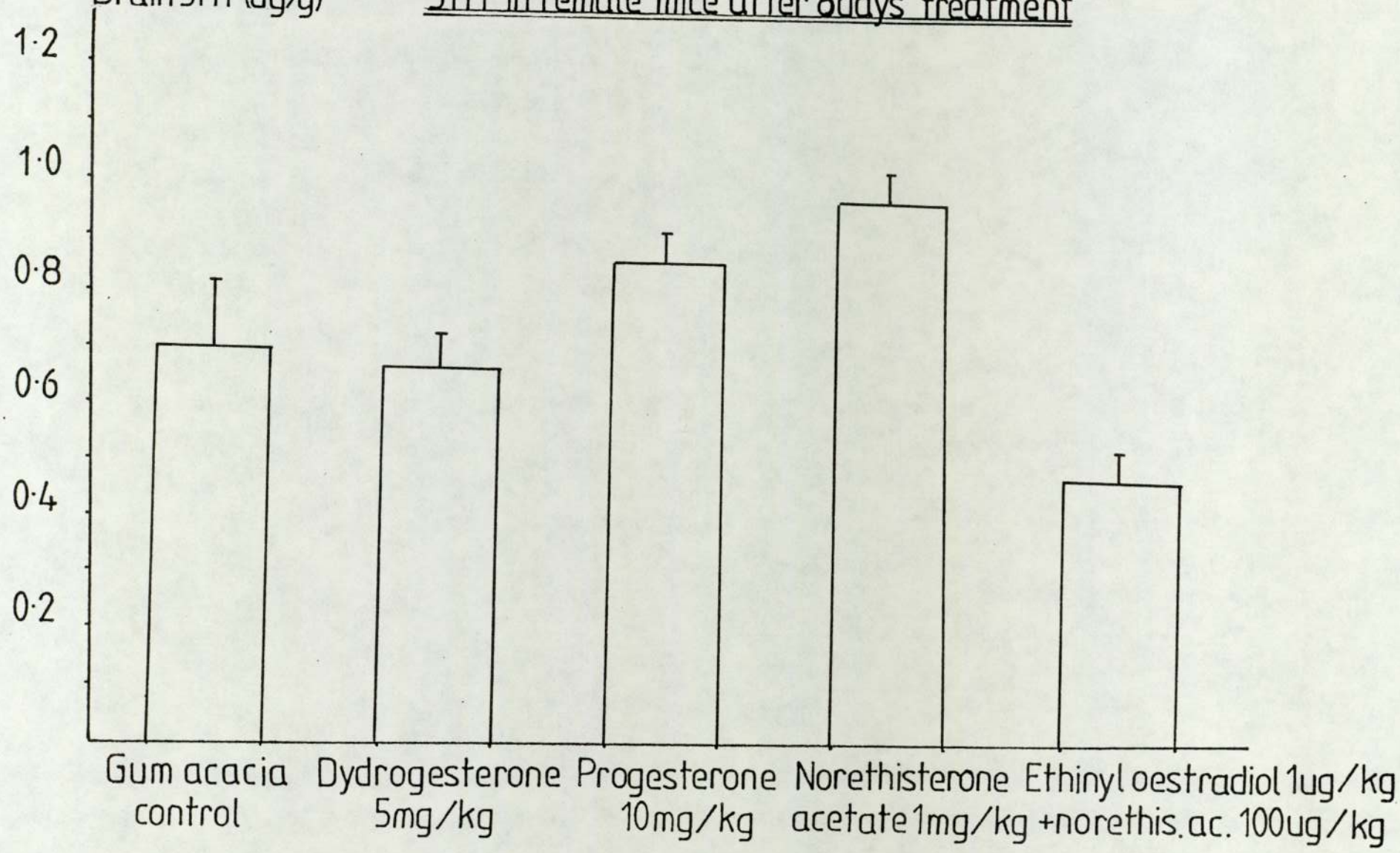


Fig 7·6 Effects of chronic hormone treatment on brain 5HT in female mice after 12 days treatment

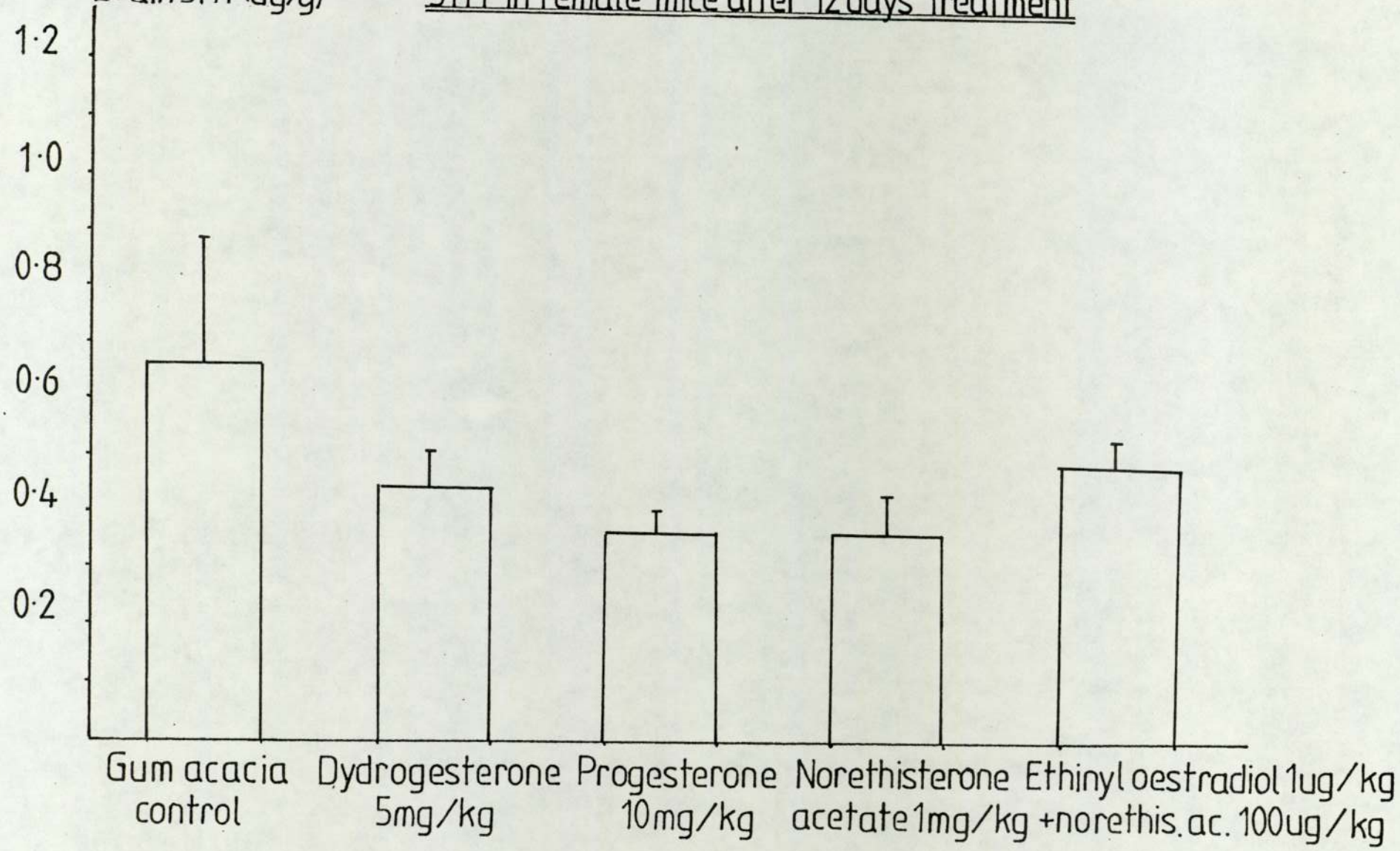


Fig 7·7 Effects of chronic hormone treatment on brain 5HT in female mice after 15 days treatment

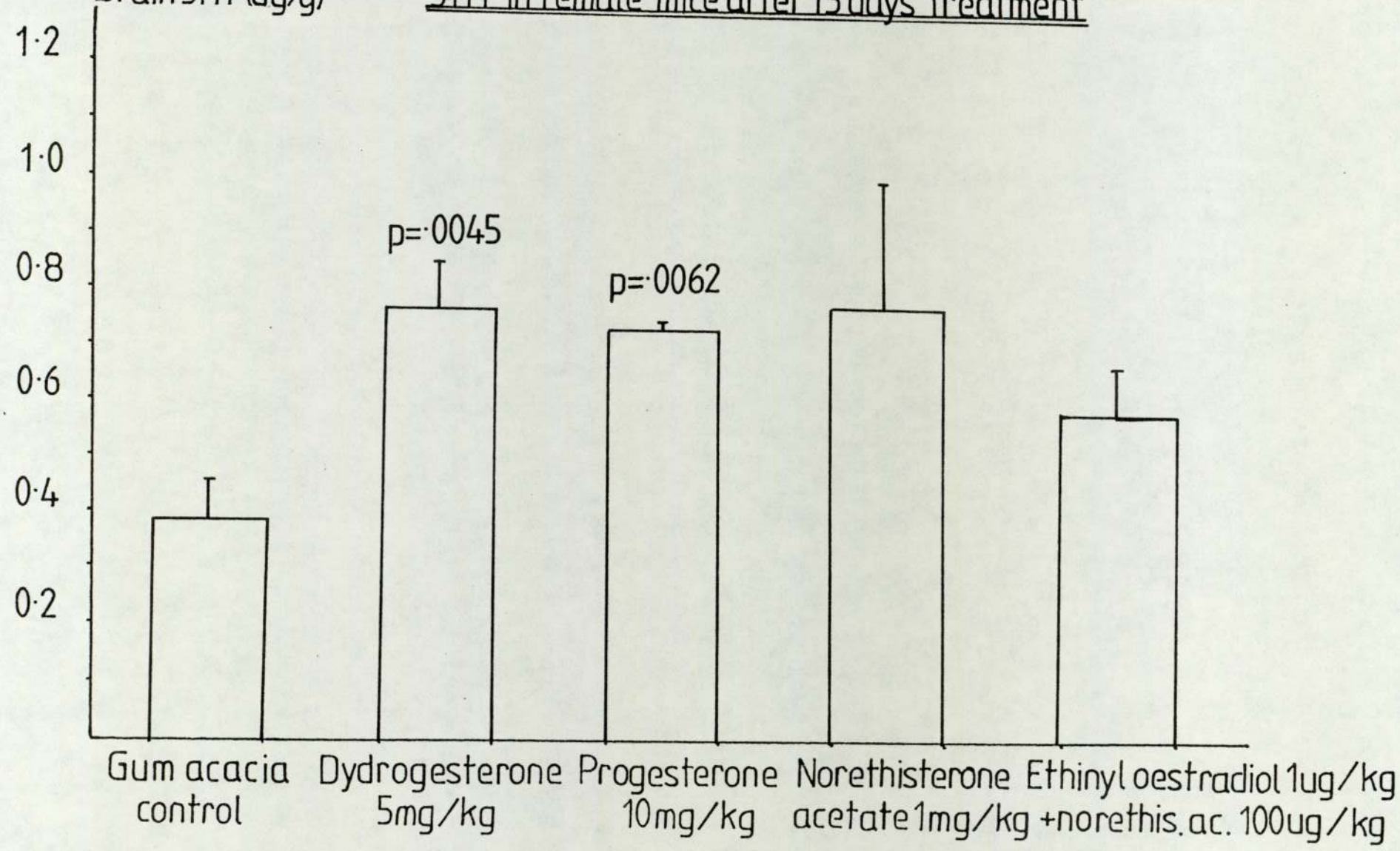


Fig 7·8 Effects of chronic hormone treatment on brain 5HT in female mice after 30 days treatment

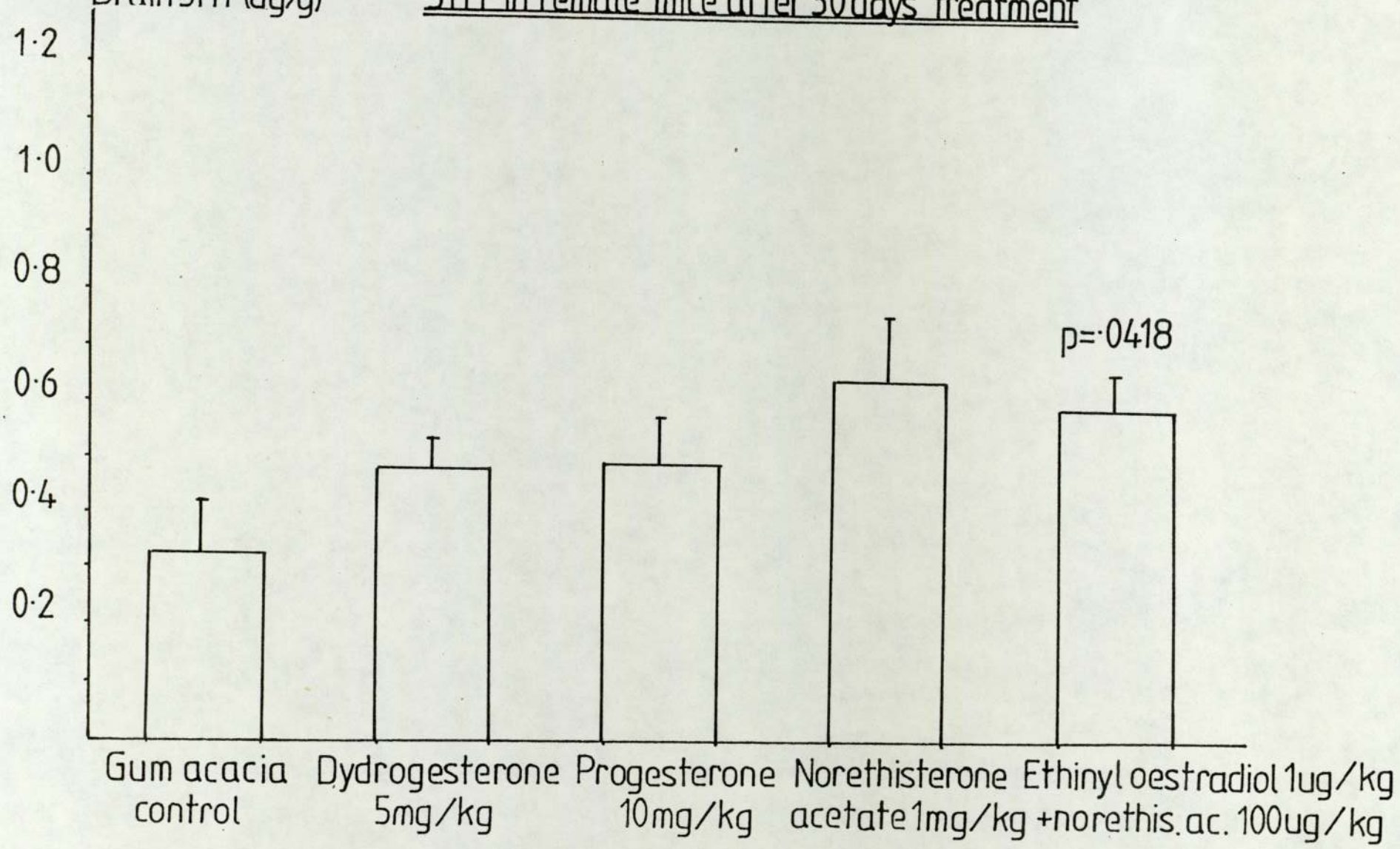


Fig7·9 Effects of chronic hormone treatment on brain 5HT in female mice after 43days treatment

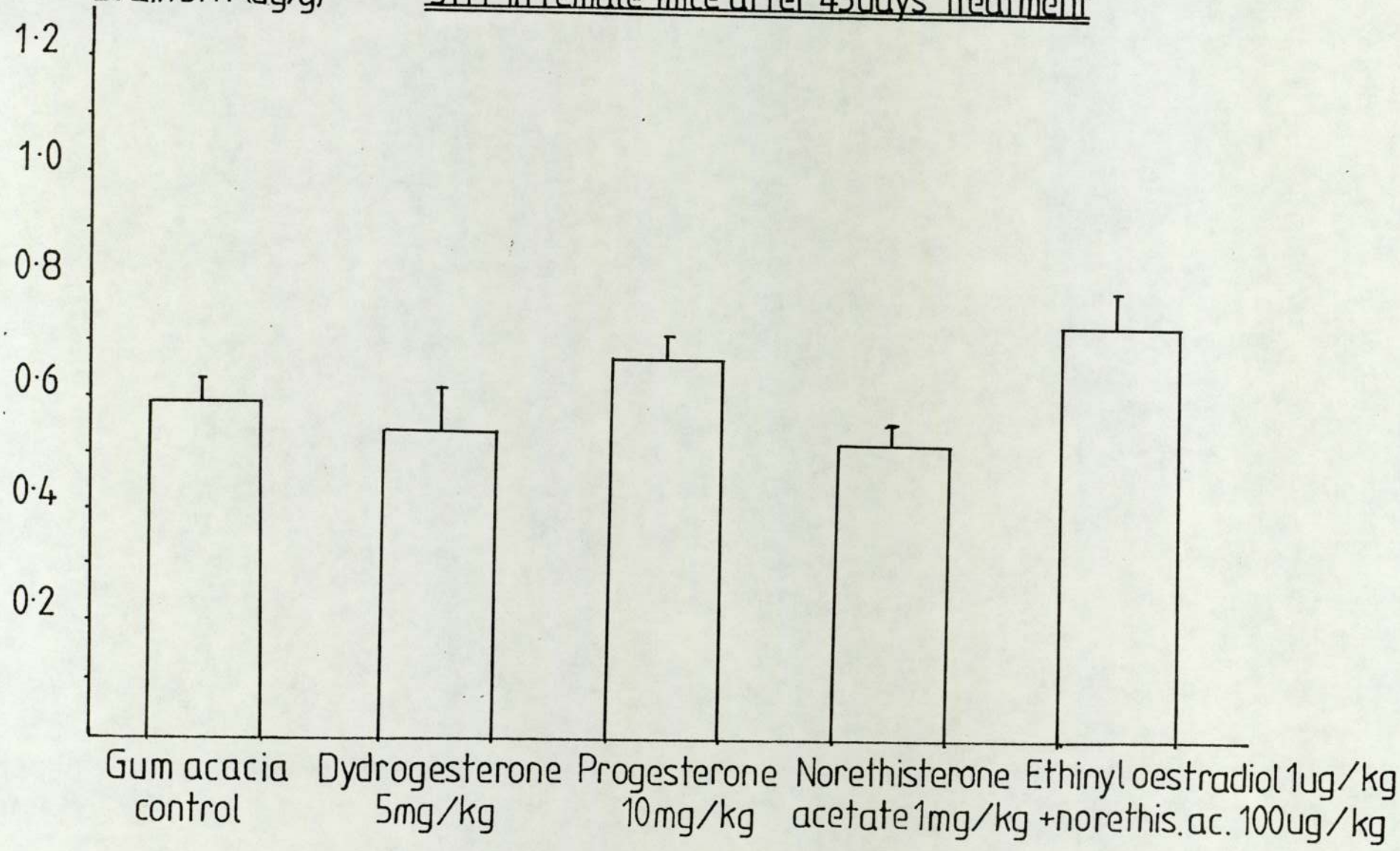


Fig 7·10 Effects of chronic hormone treatment on brain 5HIAA in female mice after 5 days treatment

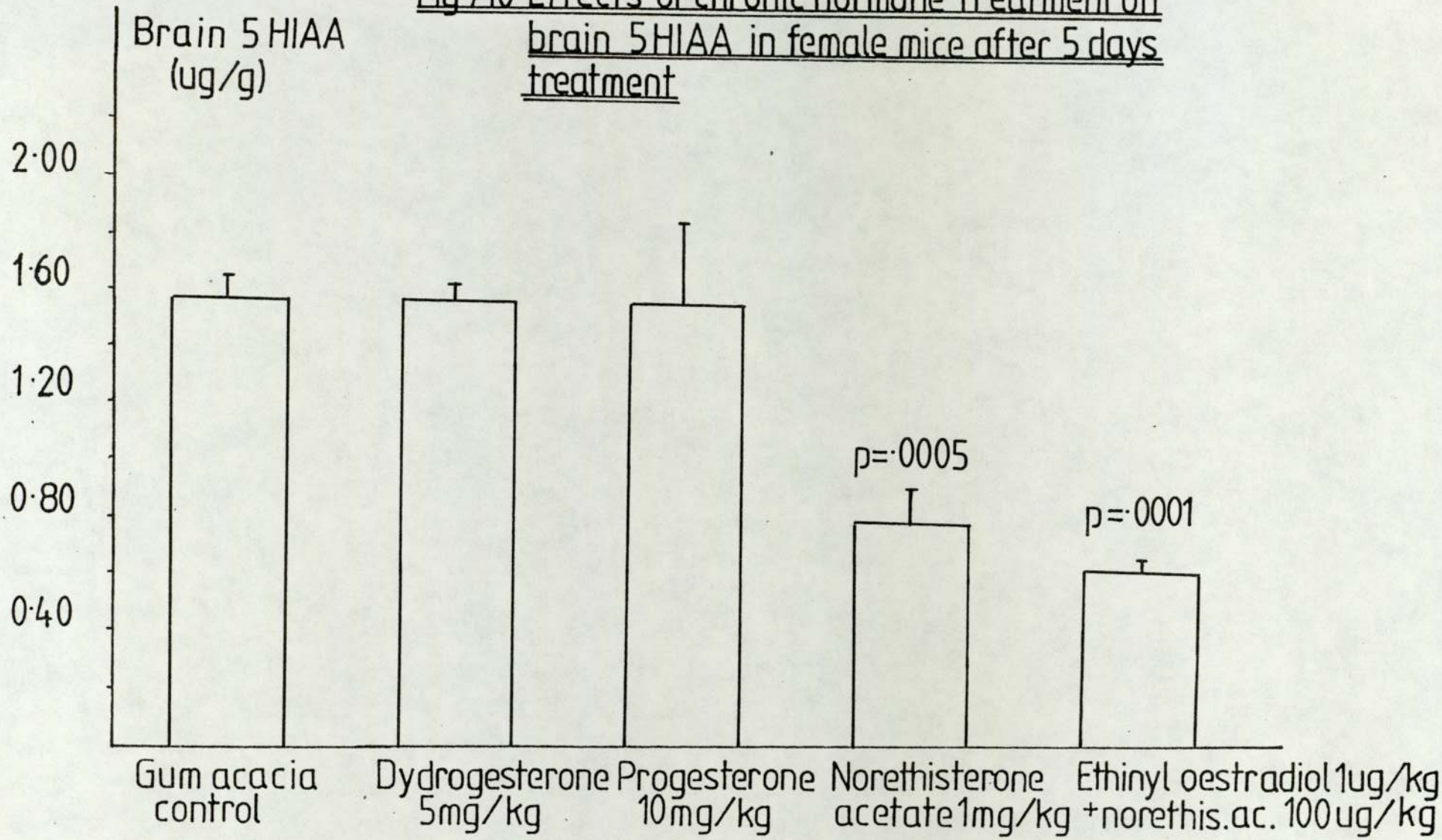


Fig 7·11 Effects of chronic hormone treatment on brain 5HIAA in female mice after 8 days treatment

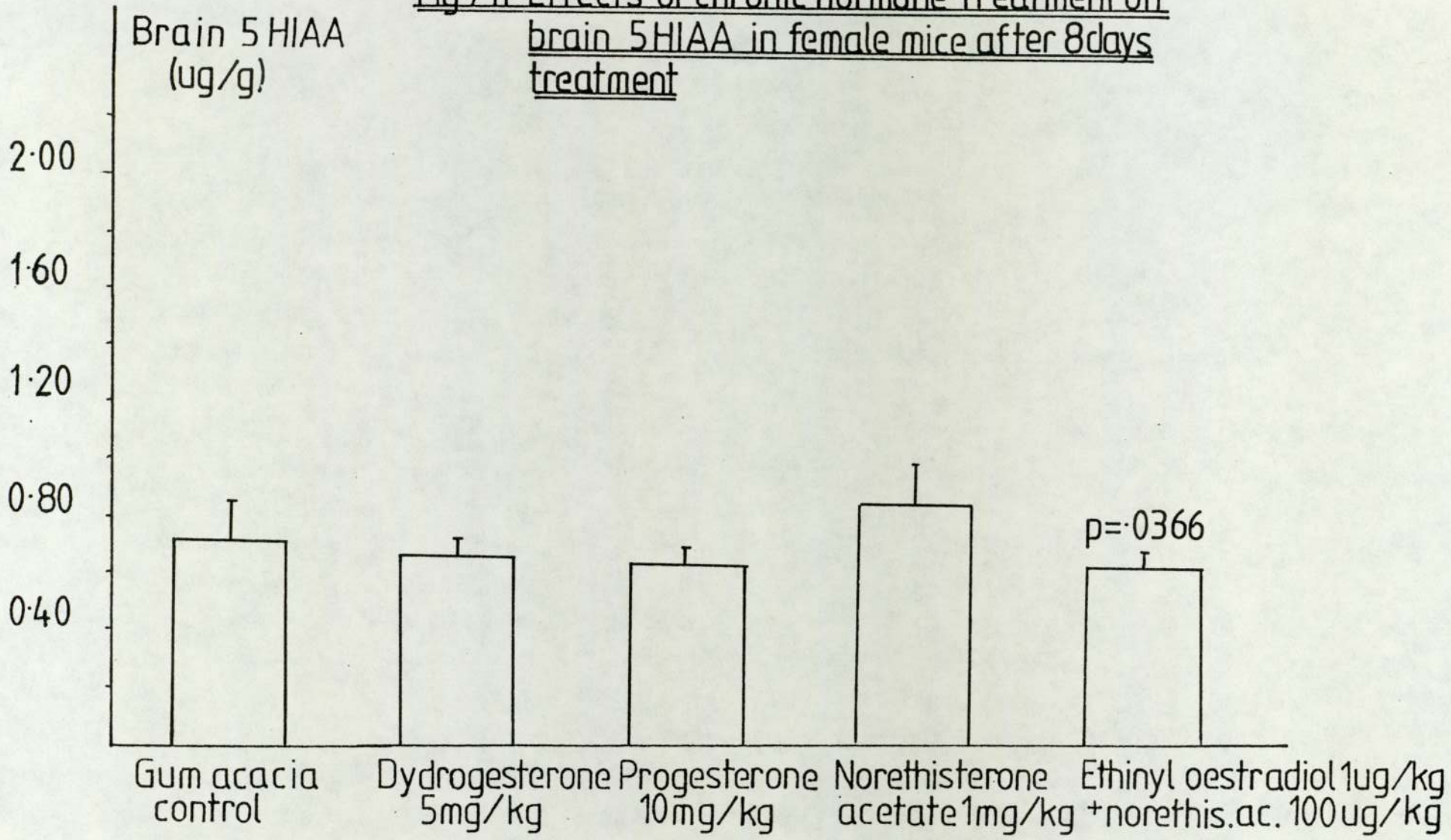


Fig 7·12 Effects of chronic hormone treatment on brain 5HIAA in female mice after 12 days treatment

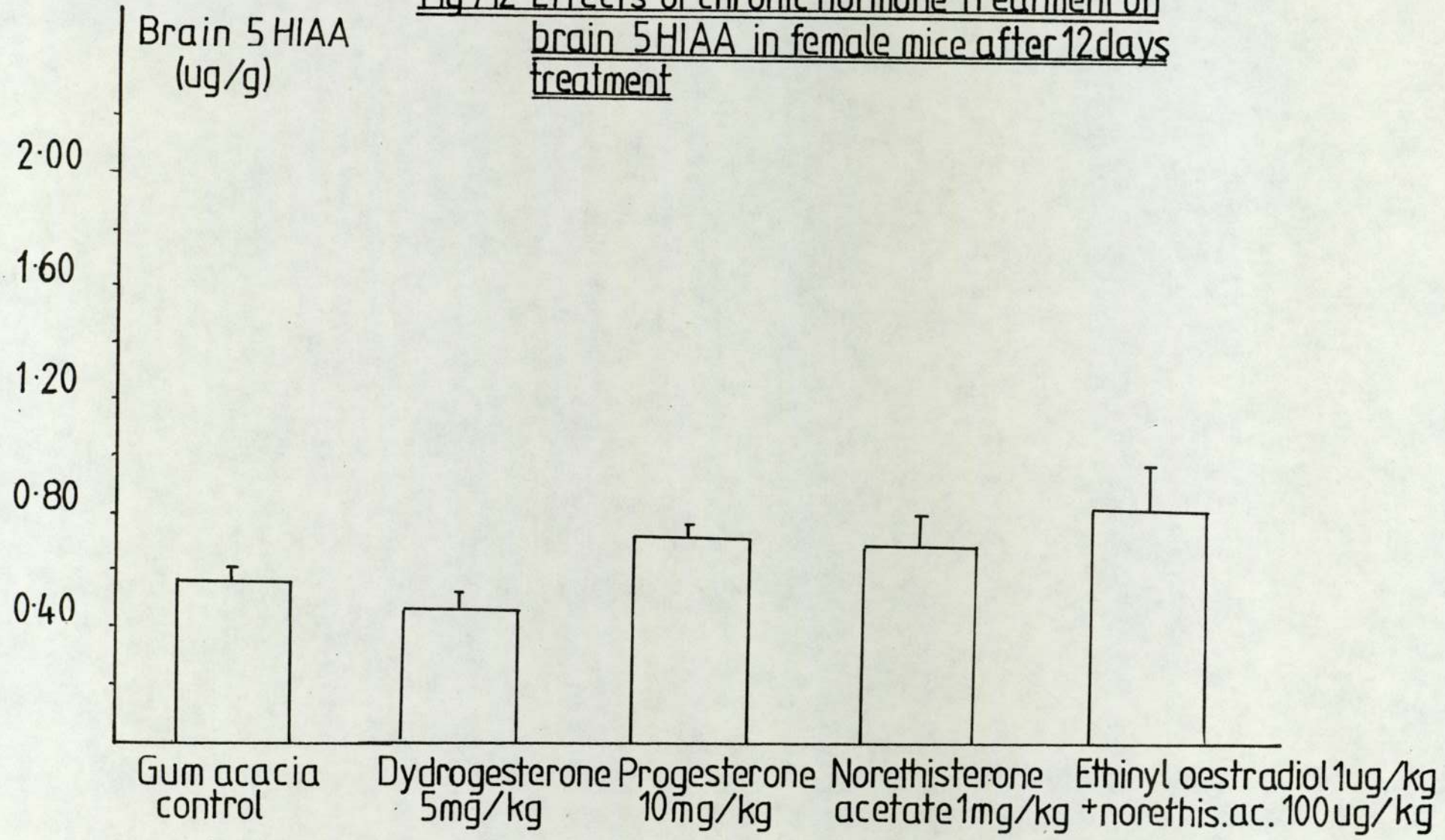


Fig 7·13 Effects of chronic hormone treatment on brain 5HIAA in female mice after 15 days treatment

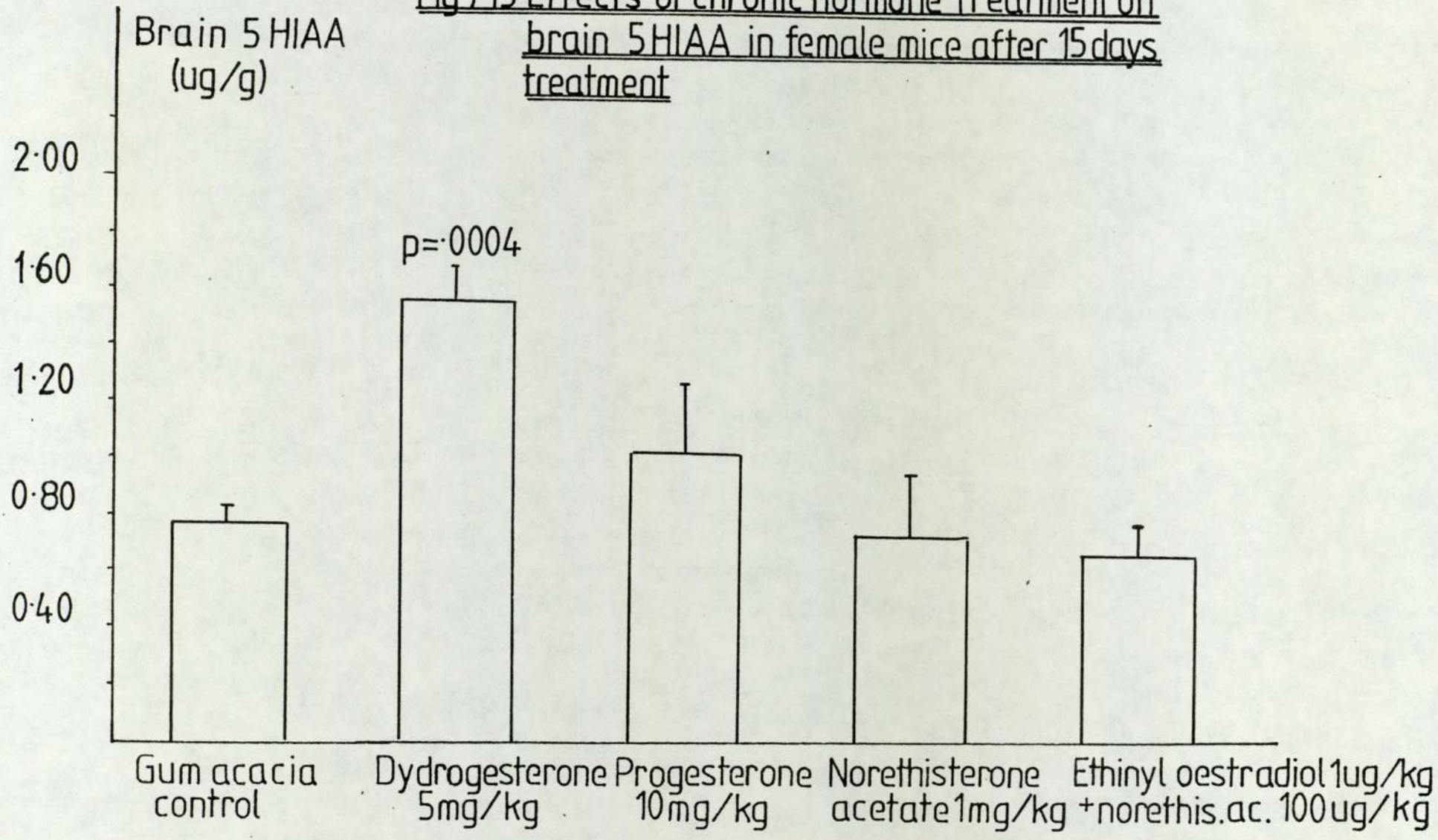


Fig 7·14 Effects of chronic hormone treatment on brain 5HIAA in female mice after 30 days treatment

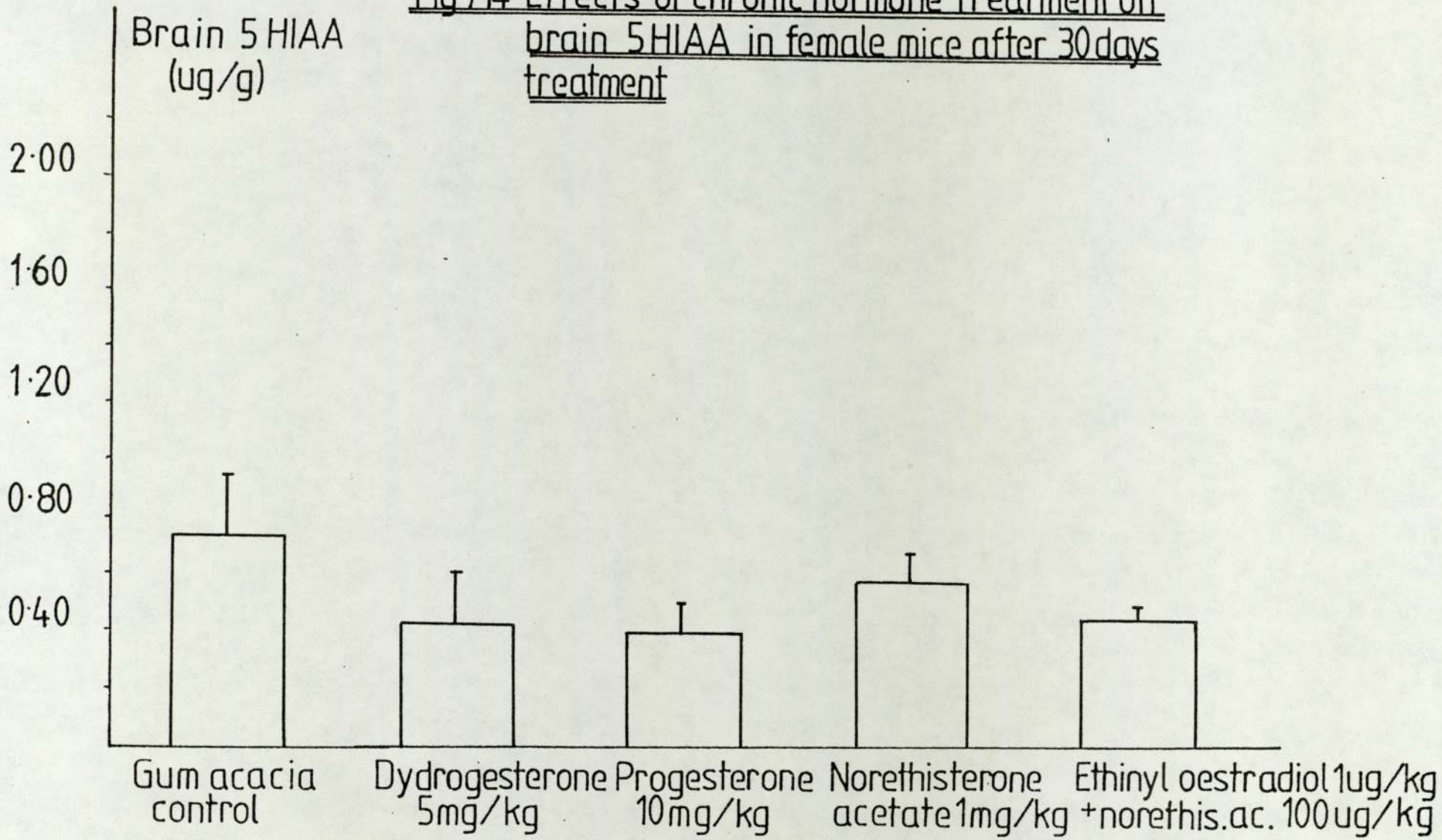


Fig 7·15 Effects of chronic hormone treatment on brain 5HIAA in female mice after 43 days treatment

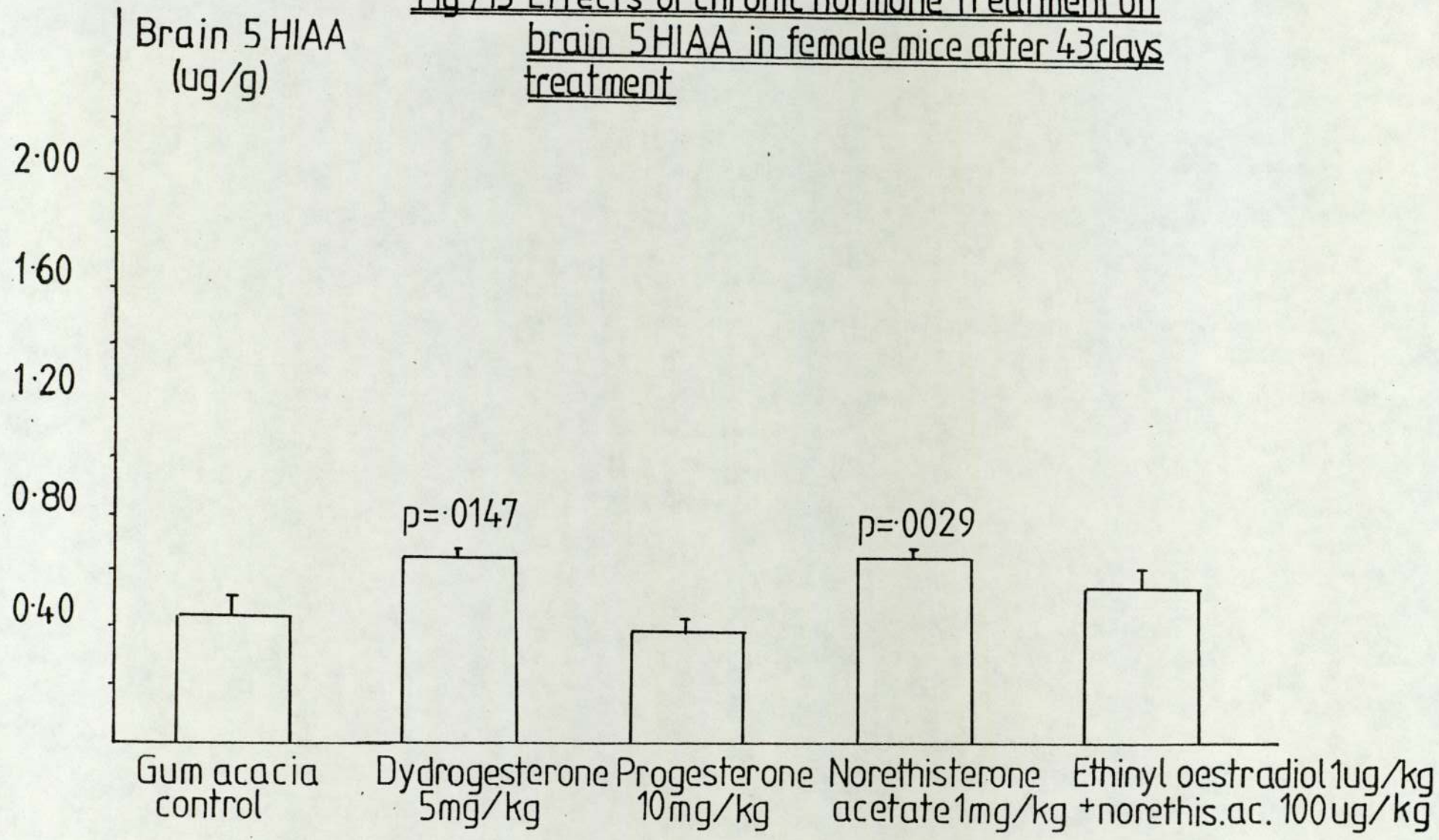


Fig 7·16 Effects of chronic hormone treatment on brain TP in female mice after 15 days treatment

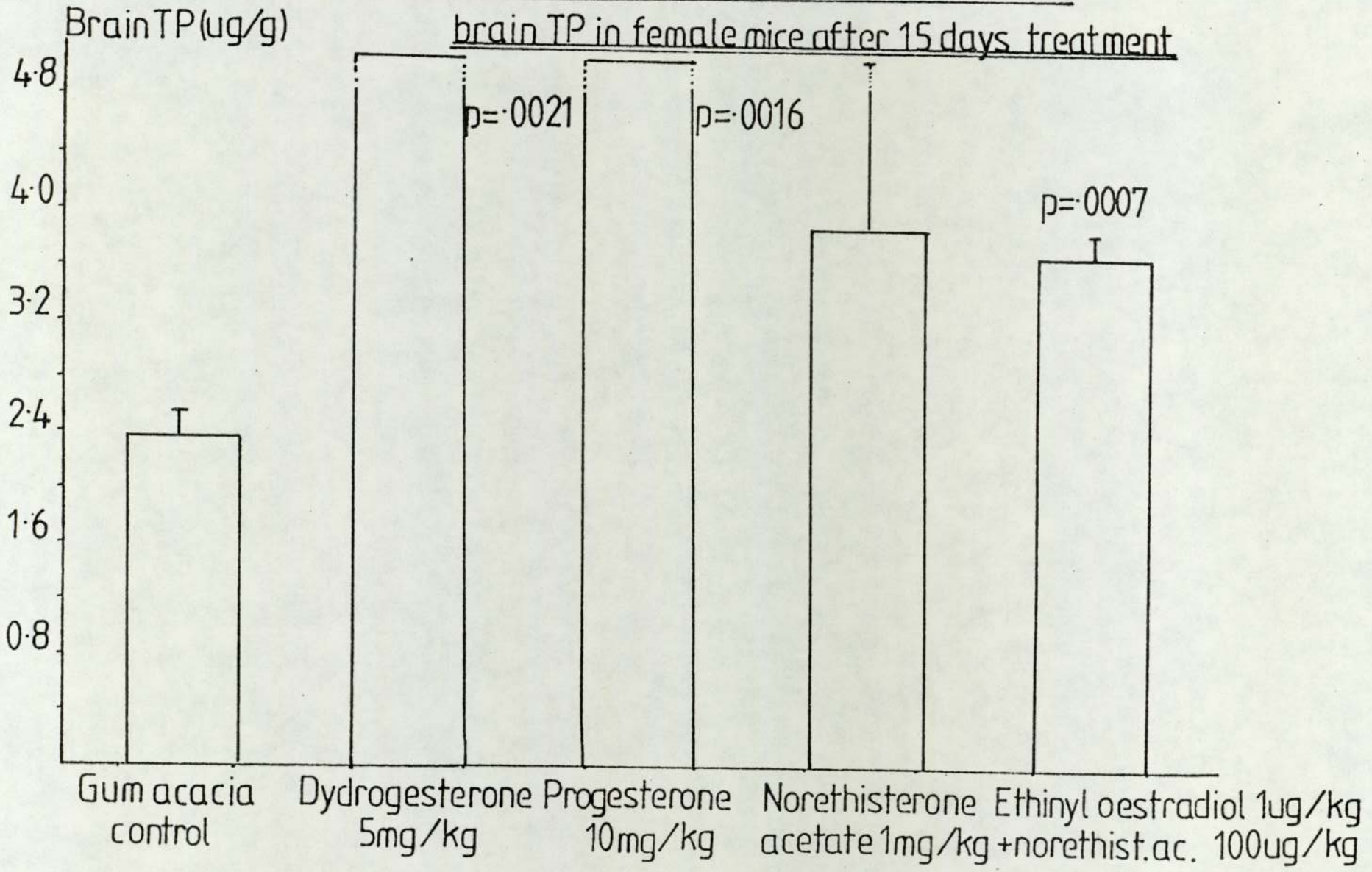


Fig 7.17 Effects of chronic hormone treatment on brain TP in female mice on day 1 of withdrawal after 15 days treatment

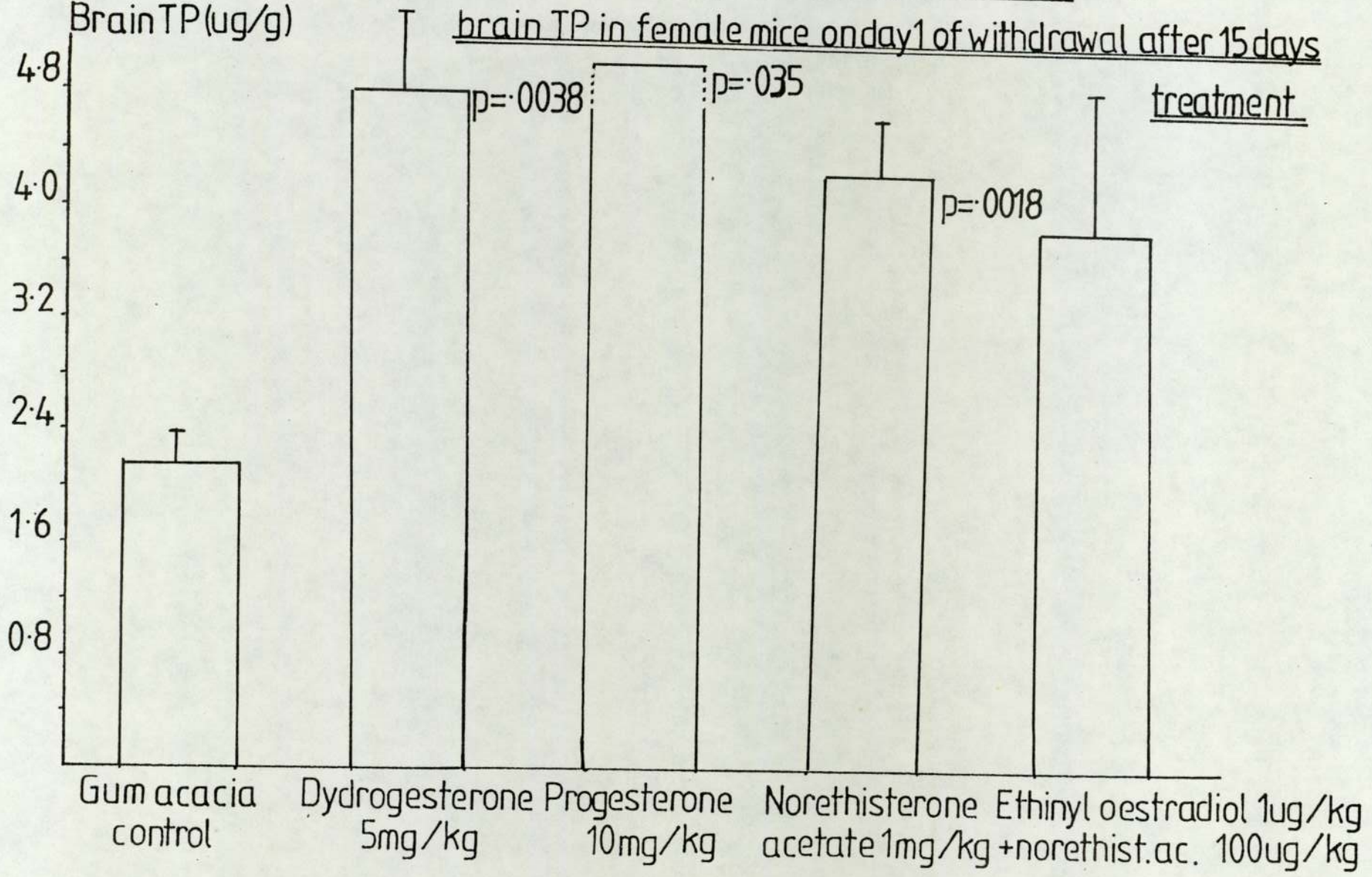


Fig 7·18 Effects of chronic hormone treatment on brain TP in female mice on day 2 of withdrawal after 15 days treatment

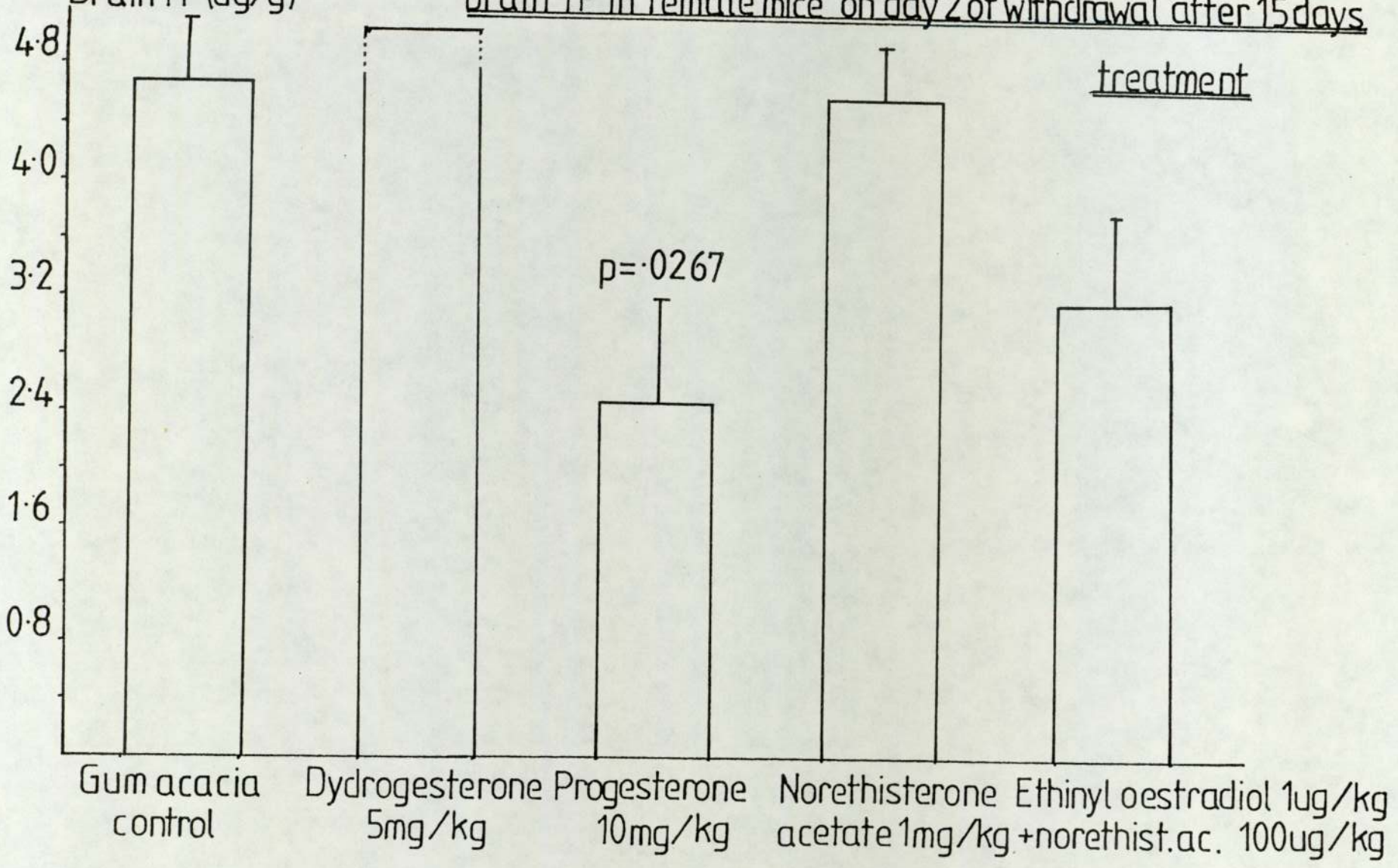


Fig 7·19 Effects of chronic hormone treatment on brain TP in female mice on day 3 of withdrawal after 15 days of treatment

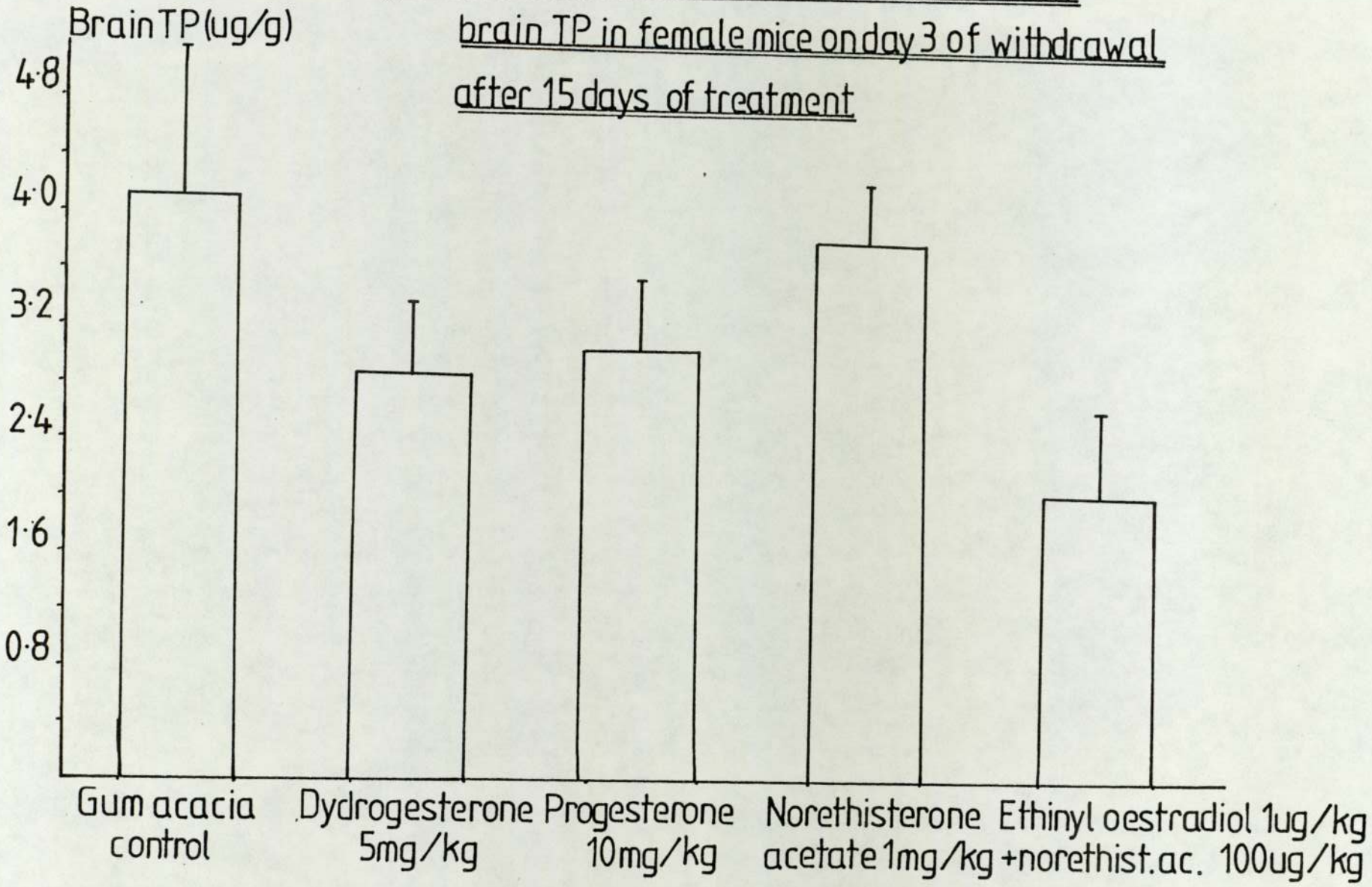


Fig 7.20 Effects of chronic hormone treatment on brain 5HT in female mice after 15 days treatment

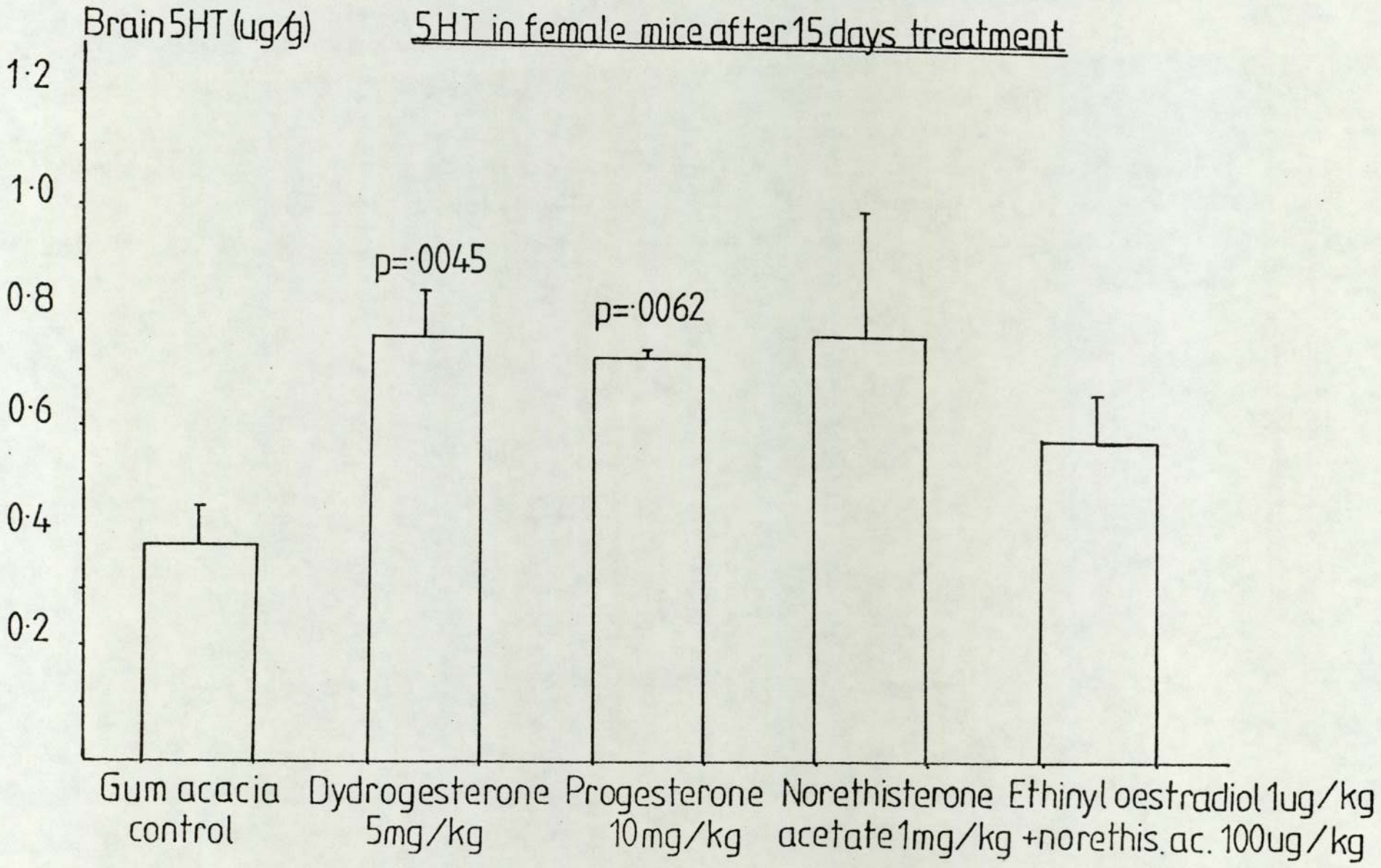


Fig 7·21 Effects of chronic hormone treatment on brain 5HT in female mice on day 1 of withdrawal after 15 days treatment

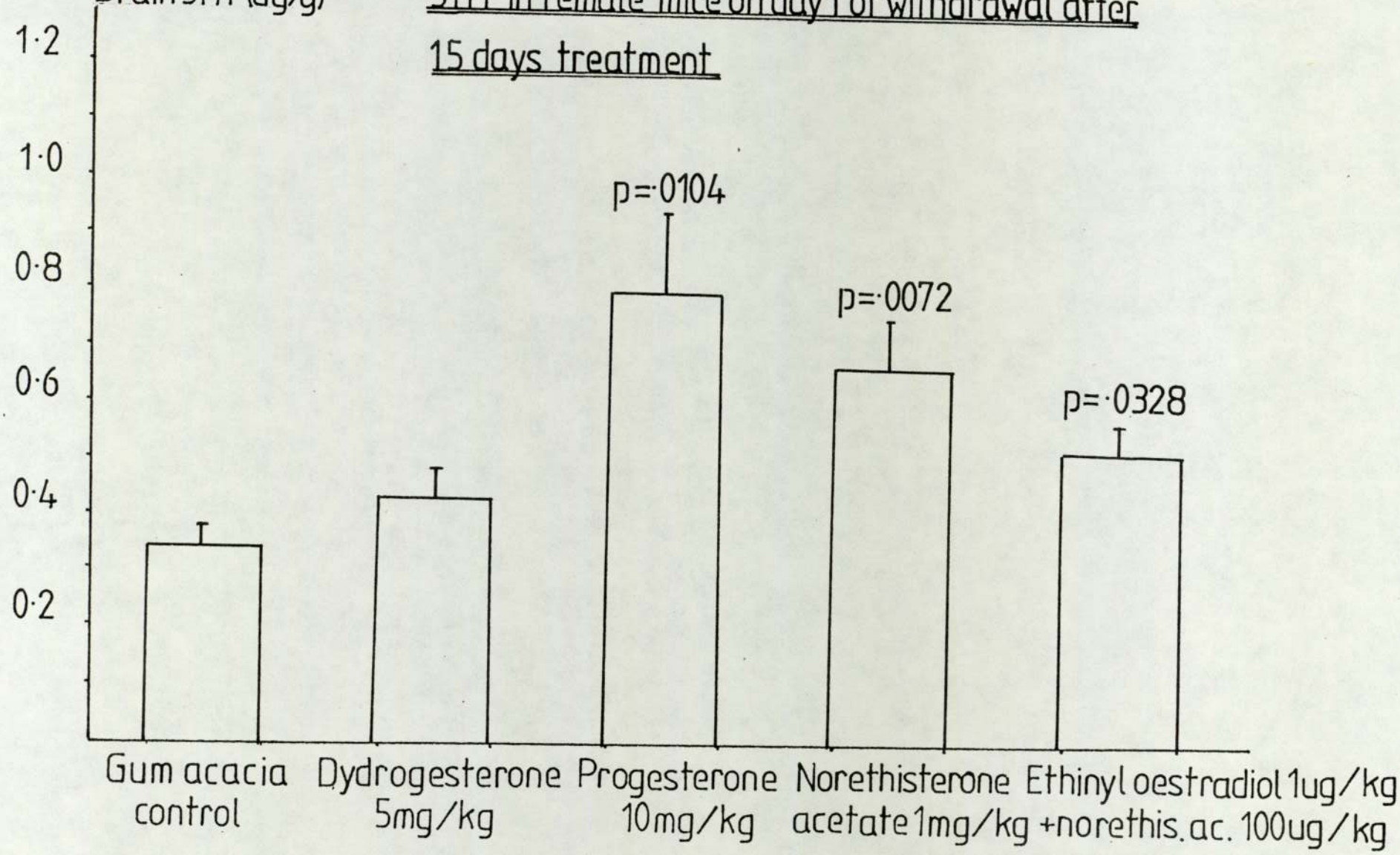


Fig 7·22 Effects of chronic hormone treatment on brain 5HT in female mice on day 2 of withdrawal after 15 days of treatment

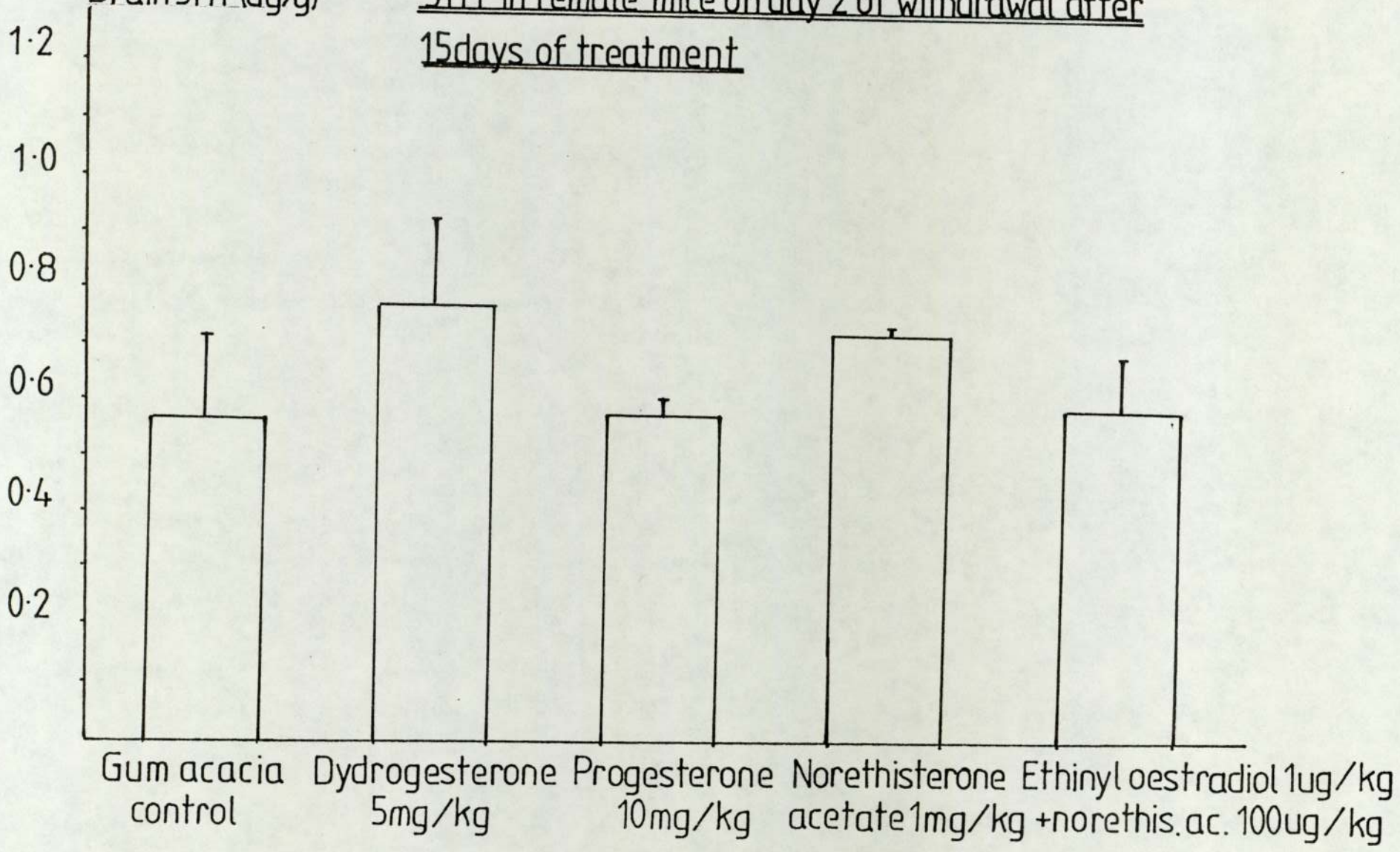


Fig 7·23 Effects of chronic hormone treatment on brain 5HT in female mice on day 3 of withdrawal after 15 days treatment

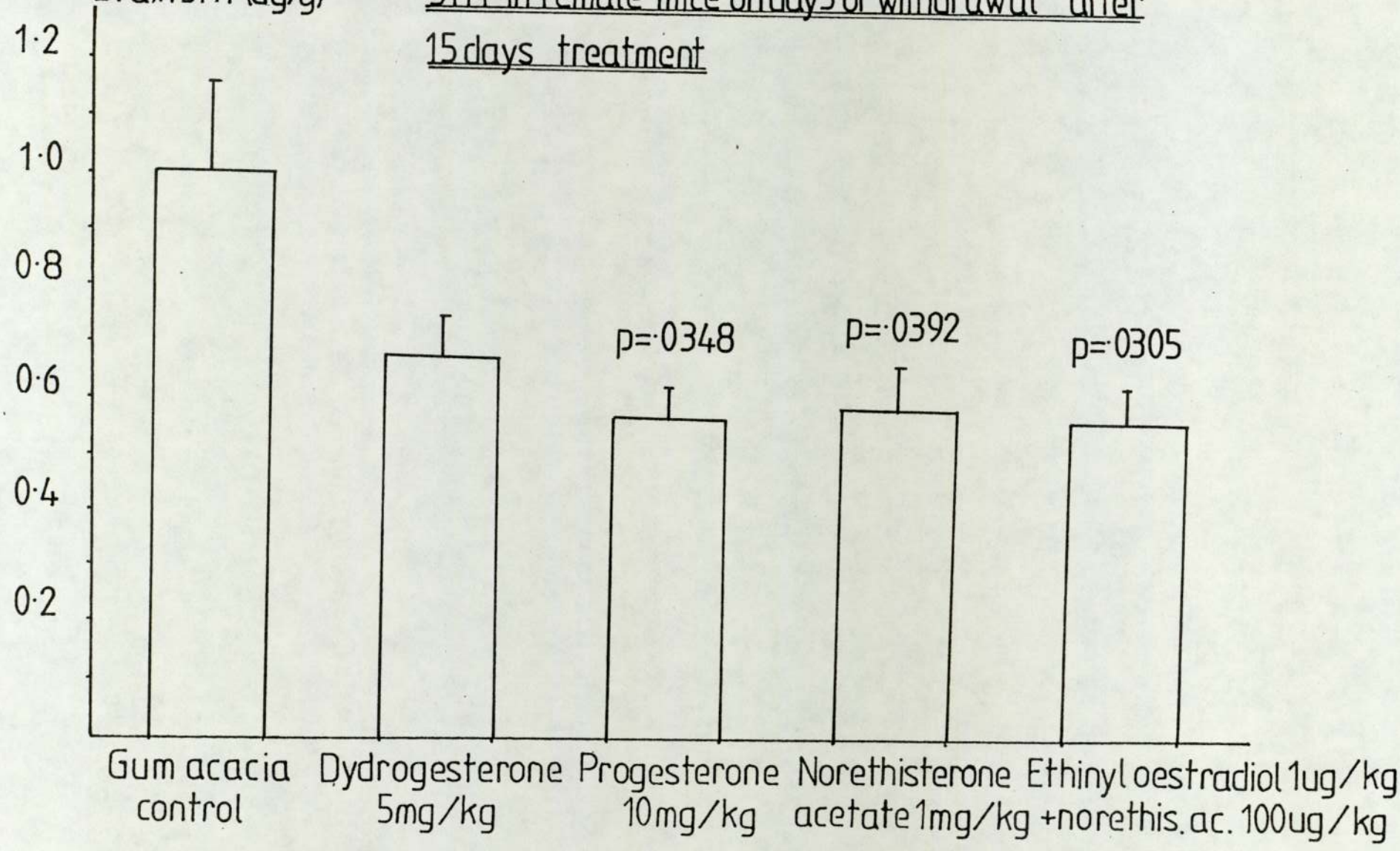


Fig 7·24 Effects of chronic hormone treatment on brain 5HIAA in female mice after 15 days treatment

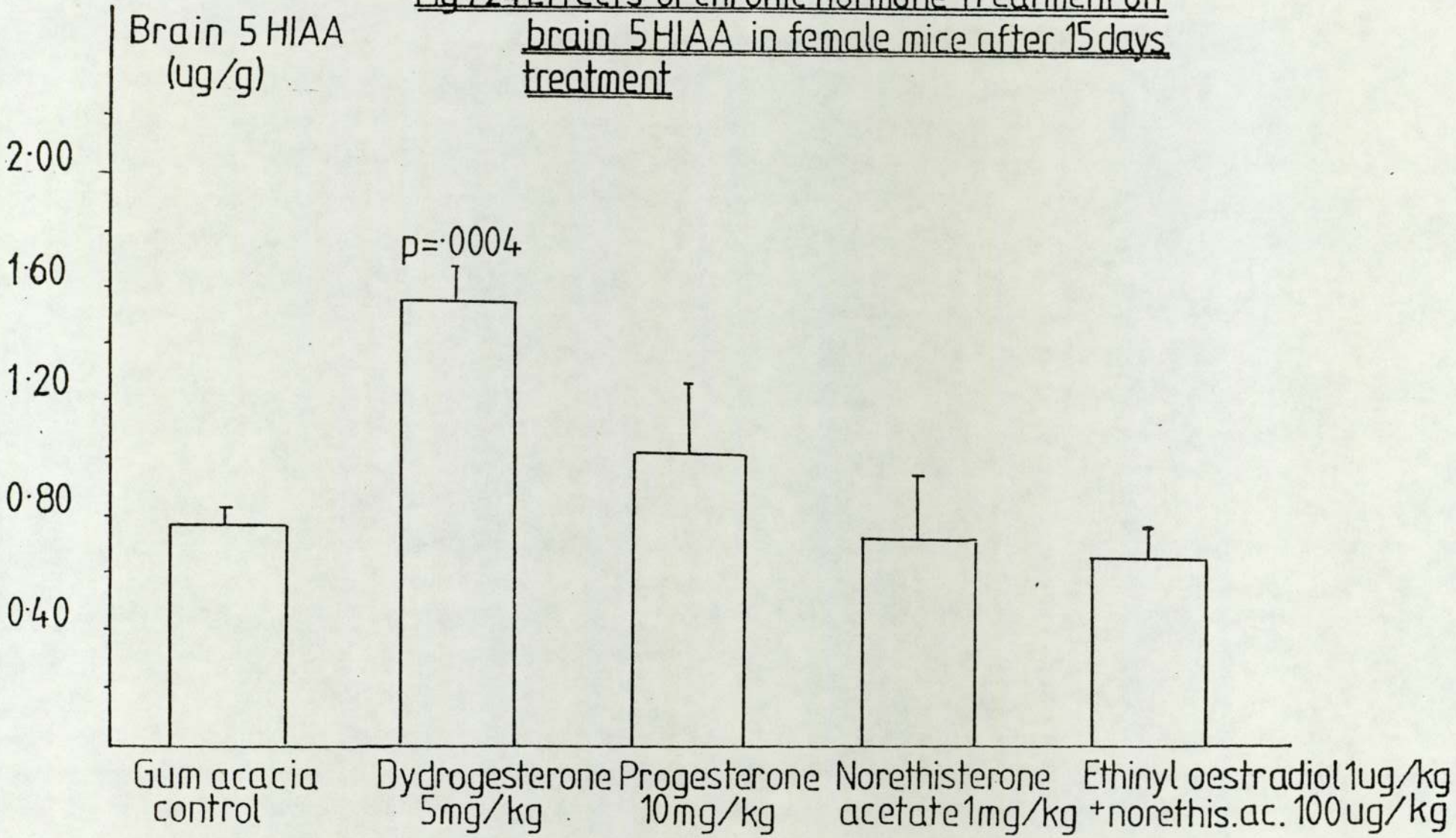


Fig 7.25 Effects of chronic hormone treatment on brain 5HIAA in female mice on day 1 of withdrawal after 15 days treatment

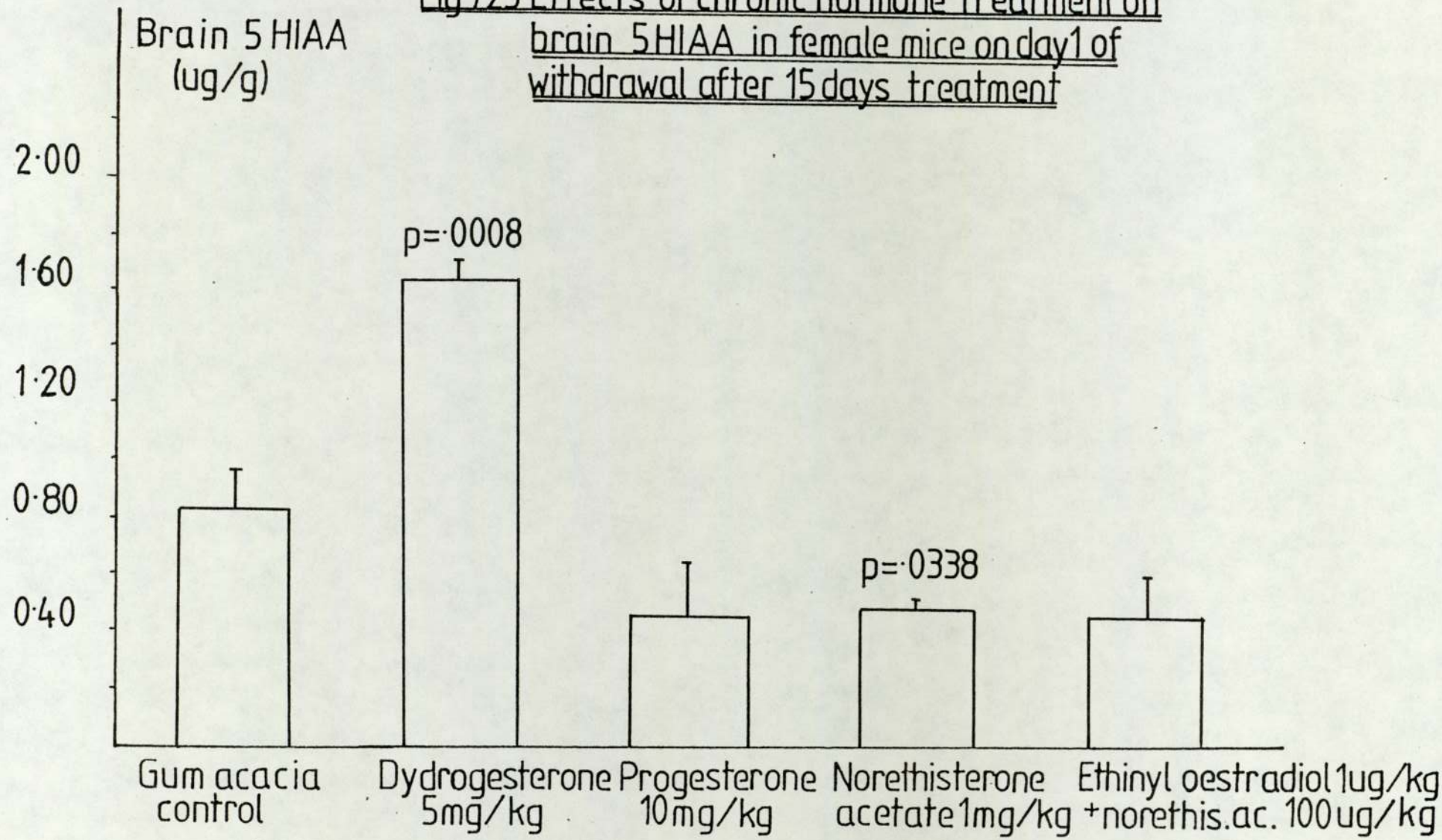


Fig 7·26 Effects of chronic hormone treatment on brain 5HIAA in female mice on day 2 of withdrawal after 15 days treatment

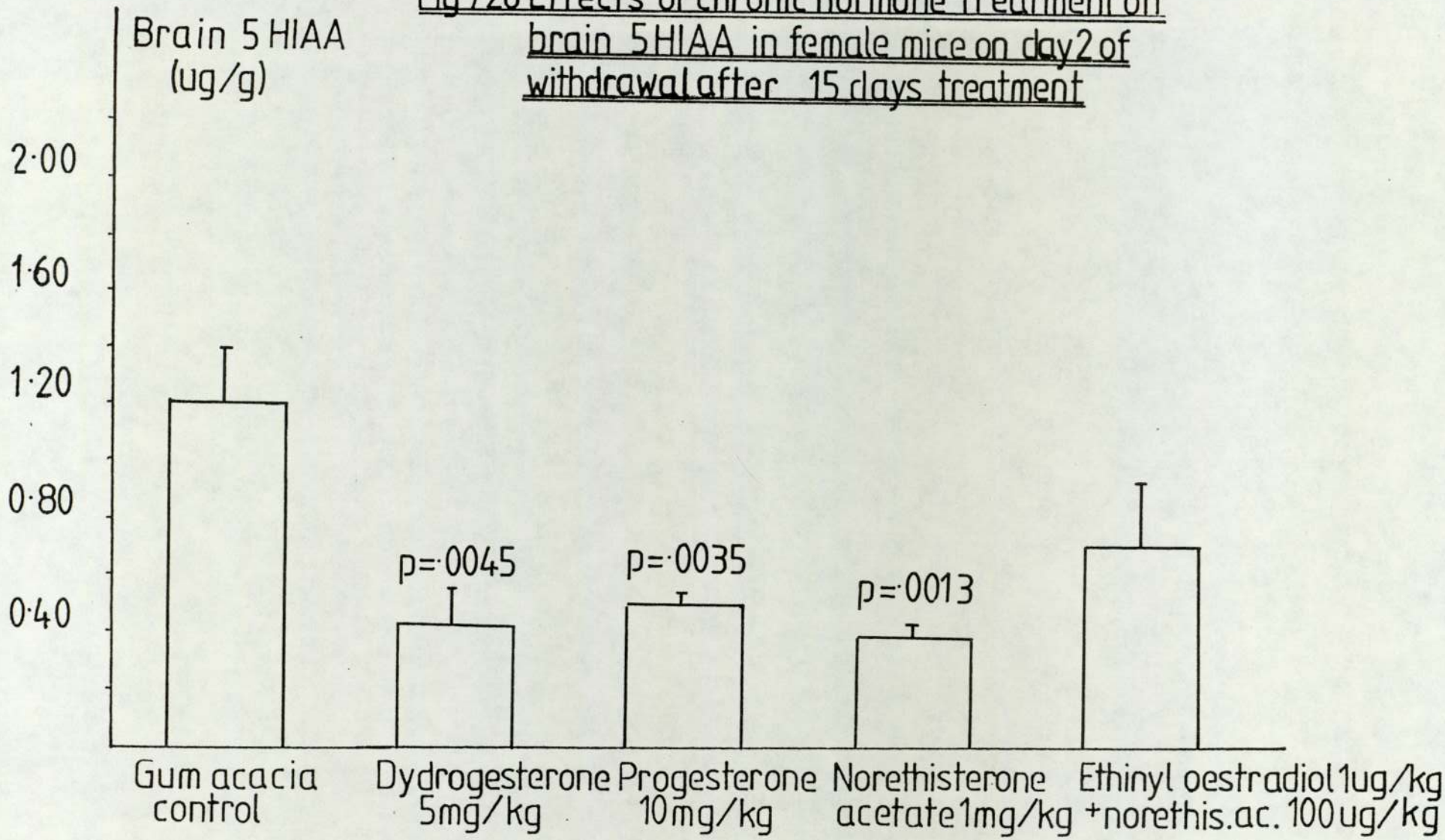


Fig 7·27 Effects of chronic hormone treatment on brain 5HIAA in female mice on day 3 of withdrawal after 15 days treatment

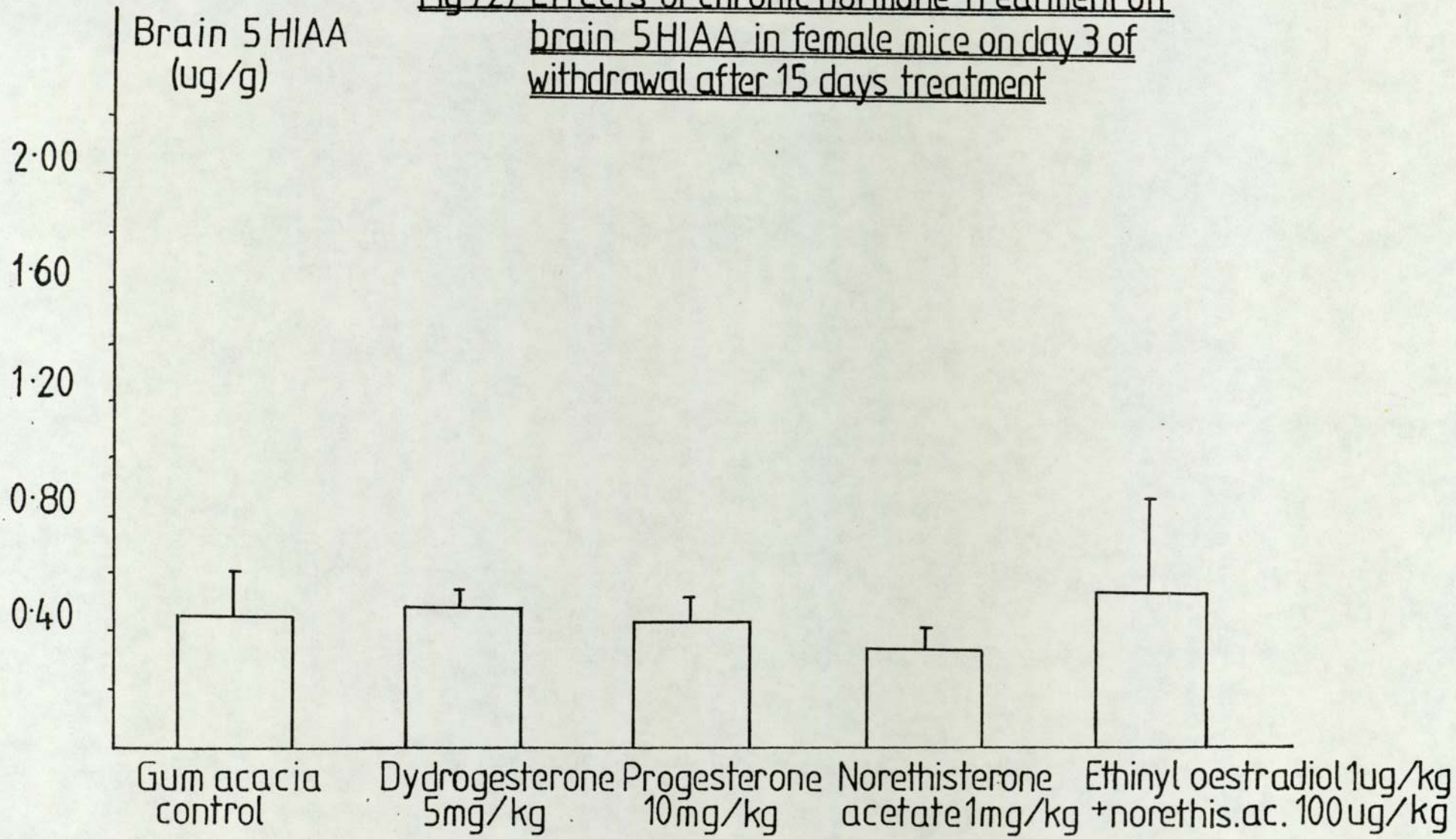


Table 7 7 : Effects of Chronic Treatment with Progesterone, Synthetic Progestogens or an Oestrogen/Progestogen Combination on Brain TP in Female Mice

Treatment / Time	Brain TP (µg/g brain matter) ± S E M				
	Gum acacia control 5ml/kg	Dydrogesterone 5mg/kg	Progesterone 10mg/kg	Norethisterone acetate 1mg/kg	Ethinyl oestradiol 1µg/kg+norethisterone acetate 100µg/kg
Day 15	2.35 ± 0.19 (n=5)	5.32 ± 0.17 (n=5) p = .0021	6.23 ± 0.87 (n=5) p = .0016	3.83 ± 1.92 (n=4) p = .3499	3.64 ± 0.17 (n=5) p = .0007
Day 30	2.93 ± 0.77 (n=3)	3.37 ± 0.47 (n=5) p = .5740	3.19 ± 0.78 (n=5) p = .7989	2.98 ± 0.78 (n=4) p = .9587	2.55 ± 0.65 (n=5) p = .6918
Day 43	3.88 ± 0.46 (n=8)	2.91 ± 0.68 (n=10) p = .2533	3.20 ± 0.28 (n=10) p = .1763	3.20 ± 0.45 (n=9) p = .2767	3.69 ± 0.32 (n=8) p = .7282

NB Normal Concentrations - 4.63 ± 0.73 µg/g

Table 7 8 : Effects of Chronic Treatment with Progesterone, Synthetic Progestogens or an Oestrogen/Progestogen Combination on Brain 5HT in Female Mice

Treatment / Time	Brain 5HT (ng/g brain matter) ± S E M				
	Gum acacia control 5ml/kg	Dydrogesterone 5mg/kg	Progesterone 10mg/kg	Norethisterone acetate 1mg/kg	Ethinyl oestradiol 1µg/kg+norethisterone acetate 100µg/kg
Day 5	632 ± 103 (n=5)	506 ± 56 (n=5) p=.2634	894 ± 235 (n=5) p=.2872	926 ± 68 (n=5) p=.0279	1022 ± 59 (n=5) p=.0065
Day 8	706 ± 136 (n=5)	668 ± 68 (n=5) p=.7822	854 ± 59 (n=5) p=.3048	960 ± 59 (n=5) p=.0899	465 ± 57 (n=5) p=.099
Day 12	676 ± 232 (n=5)	450 ± 65 (n=5) p=.3256	372 ± 45 (n=5) p=.1861	364 ± 75 (n=5) p=.1884	488 ± 41 (n=5) p=.5983
Day 15	388 ± 46 (n=5)	770 ± 85 (n=5) p=.0045	723 ± 15 (n=3) p=.0062	760 ± 23 (n=5) p=.1154	570 ± 89 (n=5) p=.1025
Day 30	333 ± 99 (n=5)	478 ± 60 (n=5) p=.1765	488 ± 89 (n=5) p=.2271	638 ± 12 (n=4) p=.0604	586 ± 68 (n=5) p=.0418
Day 43	595 ± 82 (n=10)	547 ± 82 (n=10) p=.6112	637 ± 41 (n=9) p=.2261	579 ± 38 (n=10) p=.2203	723 ± 61 (n=8) p=.0994

NB Normal Concentrations : 717 ± 141 ng/g

Table 7.9 : Effects of Chronic Treatment with Progesterone, Synthetic Progestogens or an Oestrogen/Progestogen Combination on Brain 5HIAA in Female Mice

Treatment / Time	Brain 5HIAA (ng/g brain matter) ± S E M				
	Gum acacia control 5ml/kg	Dydrogesterone 5mg/kg	Progesterone 10mg/kg	Norethisterone acetate 1mg/kg	Ethinyl oestradiol 1µg/kg+norethisterone acetate 100µg/kg
Day 5	1550 ± 96 (n=5)	1550 ± 96 (n=5) p=.9421	1550 ± 297 (n=5) p=.9763	768 ± 114 (n=5) p=.0005	718 ± 53 (n=5) p=.0001
Day 8	864 ± 85 (n=5)	1094 ± 113 (n=5) p=.1047	638 ± 76 (n=5) p=.0561	840 ± 163 (n=5) p=.8821	626 ± 65 (n=5) p=.0366
Day 12	550 ± 60 (n=5)	460 ± 65 (n=5) p=.3030	708 ± 44 (n=4) p=.0673	692 ± 108 (n=5) p=.2431	825 ± 165 (n=5) p=.0910
Day 15	734 ± 63 (n=5)	1544 ± 128 (n=5) p=.0004	1066 ± 260 (n=5) p=.2023	690 ± 230 (n=3) p=.7804	652 ± 118 (n=5) p=.5180
Day 30	724 ± 208 (n=5)	430 ± 187 (n=4) p=.2859	394 ± 110 (n=5) p=.1538	582 ± 105 (n=4) p=.5204	442 ± 47 (n=5) p=.1759
Day 43	404 ± 67 (n=10)	604 ± 41 (n=10) p=.0147	386 ± 53 (n=10) p=.8190	657 ± 37 (n=10) p=.0029	540 ± 72 (n=8) p=.1614

Normal Concentrations : 615 ± 103 ng/g

of an oestrogen/progestogen combination treatment on brain TP in Female Mice.

Treatment / Time	Brain TP (µg/g brain matter) ± S E M				
	Gum acacia control 5ml/kg	Dydrogesterone 5mg/kg	Progesterone 10mg/kg	Norethisterone acetate 1mg/kg	Ethinyl oestradiol 1µg/kg+norethisterone acetate 100µg/kg
Last Day of Drug Admin. Day 15	2.35 ± 0.19 (n=5)	5.32 ± 0.70 (n=5) p=.0021	6.23 ± 0.87 (n=5) p=.0061	3.82 ± 1.92 (n=4) p=.3499	3.64 ± 0.17 (n=5) p=.0007
Day 1 withdrawal(28 hr after last dose)	2.14 ± 0.25 (n=5)	4.75 ± 0.67 (n=5) p=.0038	5.28 ± 1.55 (n=4) p=.0350	4.18 ± 0.40 (n=5) p=.0018	3.81 ± 0.98 (n=4) p=.1019
Day 2 withdrawal	4.77 ± 0.57 (n=4)	6.24 ± 0.73 (n=3) p=.1131	2.47 ± 0.69 (n=5) p=.0267	4.60 ± 0.37 (n=5) p=.7724	3.18 ± 0.61 (n=5) p=.0715
Day 3 withdrawal	4.07 ± 1.06 (n=4)	2.83 ± 0.72 (n=4) p=.3075	2.93 ± 0.47 (n=5) p=.2638	3.73 ± 0.41 (n=3) p=.7718	2.10 ± 0.67 (n=3) p=.1555

N B - Normal Concentrations : 4.63 ± 0.73 µg/g

Table 7.11 : Effects of Acute Withdrawal of Progesterone, Synthetic Progestogen or an Oestrogen/Progestogen Combination Treatment on Brain 5HT in Female Mice

Treatment / Time	Brain 5HT (ng/g brain matter) ± S E M				
	Gum acacia control 5ml/kg	Dydrogesterone 5mg/kg	Progesterone 10mg/kg	Norethisterone acetate 1mg/kg	Ethinyl oestradiol 1µg/kg+norethisterone acetate 100µg/kg
Last Day of Drug Admin. Day 15	388 ± 67 (n=5)	770 ±85 (n=5) p=.0045	723 ± 15 (n=3) p=.0062	760 ± 230 (n=5) p=.1154	570 ± 89 (n=5) p=.1025
Day 1 withdrawal (28 hr after last dose)	348 ± 46 (n=5)	436 ± 53 (n=5) p=.1999	796 ± 144 (n=5) p=.0104	668 ± 88 (n=5) p=.0072	512 ± 055 (n=5) p=.0328
Day 2 withdrawal	578 ± 160 (n=5)	778 ± 160 (n=5) p=.3393	580 ± 28 (n=5) p=.9857	718 ± 18 (n=5) p=.3290	582 ± 93 (n=5) p=.9786
Day 3 withdrawal	998 ± 153 (n=5)	674 ± 75 (n=5) p=.0641	564 ± .055 (n=5) p=.0348	583 ± 75 (n=4) p=.0392	560 ± 66 (n=4) p=.0305

NB Normal Concentrations : 717 ± 141 ng/g

Table 7.12 : Effects of Acute Withdrawal of Progesterone, Synthetic Progestogens or an Oestrogen/Progestogen Combination Treatment on Brain 5HIAA in Female Mice.

Treatment / Time	Brain 5HIAA (ng/g brain matter) ± S E M				
	Gum acacia control 5ml/kg	Dydrogesterone 5mg/kg	Progesterone 10mg/kg	Norethisterone acetate 1mg/kg	Ethinyl oestradiol 1µg/kg+norethisterone acetate 100µg/kg
Last Day of Drug Admin. Day 15	734 ± 63 (n=5)	1544 ± 128 (n=5) p=.0004	1066 ± 260 (n=5) p=.2023	690 ± 230 (n=3) p=.7804	652 ± 118 (n=5) p = .5180
Day 1 withdrawal (28 hr after last dose)	816 ± 143 (n=5)	1612 ± 72 (n=5) p=.0008	466 ± 185 (n=5) p=.1303	472 ± 52 (n=5) p=.0338	446 ± 158 (n=5) p = .0861
Day 2 withdrawal	1210 ± 185 (n=5)	436 ± 121 (n=5) p=.0045	510 ± 47 (n=5) p=.0035	382 ± 7 (n=5) p=.0013	696 ± 230 (n=5) p = .0813
Day 3 withdrawal	470 ± 164 (n=5)	494 ± 55 (n=5) p=.8753	440 ± 95 (n=5) p=.8582	345 ± 75 (n=4) p=.5070	560 ± 328 (n=4) p = .7692

NB Normal Concentrations : 615 ± 103 ng/g

INTERPRETATION OF RESULTS FROM CHAPTER 7

Interpretation of Results from Chapter 7.

The possible interpretations of results from studies including the measurement of whole brain TP, 5HT and 5HIAA are multiple and complex. One frequently used method for the interpretation of such results is the use of the ratio of 5HT : 5HIAA as an indicator of 5HT 'turnover' (eg File & Velucci, 1978). There are several other more precise methods for the measurement of 5HT turnover such as the use of radiolabelled tracers (Wise et al, 1972) or the measurement of 5HIAA accumulation following probenecid (Friedman et al, 1975). Both of these methods are time consuming, involving repeated measurements, and expensive, both financially and in the number of animals required. Therefore the cheaper method of utilising the 5HT : 5HIAA ratios was utilised. Thus, in cases where 5HT turnover is increased, there would be an increase in brain 5HIAA relative to brain 5HT, due to the increased metabolism of 5HT to 5HIAA. This would result in a decrease in the 5HT : 5HIAA ratio. The opposite is true for a decrease in 5HT turnover.

This method however, has some inherent problems. Mathematically the results of such a computation of ratio are similar when the precise interpretations may differ. For example, a decrease in 5HIAA, with no change in 5HT would increase the 5HT : 5HIAA ratio. The same would be true for an increase in 5HT, with no change in 5HIAA. Both may be interpreted as decreased 5HT turnover.

The actual mechanisms underlying these two examples would be different. In the first case, (decreased 5HIAA,unchanged 5HT) the changes may have been produced by a decreased metabolism of 5HT by monoamine oxidase. This would therefore produce

the decreased 5HIAA. Such a decreased synthesis of 5HT could be brought about either by reduced monoamine oxidase activity, or by a decreased release of 5HT from presynaptic stores. Both of these mechanisms may produce an increase of brain 5HT due to a 'backing up' of unmetabolised or unreleased 5HT. Therefore if 5HT levels are unchanged it suggests that there may be a simultaneous decrease in 5HT synthesis perhaps due to decreased TP availability as TP availability is the rate-limiting step of 5HT synthesis. Hence this example may be the result of decreased 5HT metabolism and/or release, together with a decreased synthesis of 5HT.

The second case (increased 5HT, unchanged 5HIAA) may be produced via a different mechanism. The increased 5HT may reflect either an increased synthesis, possibly due to increased TP availability (Hamon et al., 1974), or a decreased release of 5HT, together with increased storage. The latter explanation however would be likely to produce a concurrent decrease in 5HIAA, as this is not present in the example, the former explanation is the more likely. Hence this example may be explained by an increased synthesis and storage of 5HT with unchanged release or metabolism of 5HT.

Because of the different possible interpretations of a similar effect on 5HT : 5HIAA ratio, this measure alone will not be used as an indicator of 5HT metabolism, but rather the whole brain concentrations of TP, 5HT and 5HIAA will all be used in an attempt to explain more precisely, the mechanisms underlying any changes in 5HT metabolism.

In addition to interpretation of such results in terms of 5HT metabolism, the functional importance of any such changes

must also be considered. Following the work of Grahame-Smith and colleagues (Grahame-Smith, 1974; Green & Grahame-Smith, 1976) many of the changes seen in 5HT metabolism may be irrelevant in terms of 5HT function. Increased TP availability produces an increased synthesis of 5HT, due to the normally unsaturated state of the rate limiting enzyme, tryptophan hydroxylase (See Introduction). However the behavioural effects of increased plasma TP do not reflect an increase in central 5HT activity (Grahame-Smith, 1974). This is possibly due to the fact that the newly formed 5HT does not enter the 'functional' 5HT stores. Green and Grahame-Smith (1976) suggested that there may be two pools of central 5HT, one being 'functional' ie used to produce an effect on the post-synaptic receptor, and one pool being 'non-functional'. Hence, newly formed 5HT may not necessarily enter the 'functional' pool of 5HT, but rather may become bound intraneuronally, and may undergo oxidative deamination without ever being 'functional'. Thus increases in brain 5HT or 5HIAA may not reflect alterations of 5HT function, but may indicate changes of 'non-functional' 5HT. This factor must therefore be considered in the interpretation of the results of any studies of brain 5HT. The results of chapter 7 will be interpreted in the light of these constraints. A fuller discussion of the implications of these results will be presented later.

All hormone treatments used in chapter 7 were administered orally in a gum acacia vehicle, and a parallel gum acacia vehicle control group was run against each hormone treatment group. This vehicle was selected in preference to the more common vehicle for progesterone, arachis oil, as the oil may

have produced profound effects on fatty acid metabolism. This in turn may influence TP metabolism (See Introduction). The results for each hormone treatment group are expressed relative to the vehicle control group. Therefore, any effects of the hormone treatments seen are in fact, superimposed onto any effects of the vehicle control.

The effects of the gum acacia treatment on brain TP, 5HT and 5HIAA will be considered first.

Effects of Gum Acacia in Male Mice.

1 Administration.

Whole brain levels of TP, 5HT and 5HIAA were all seen to rise after 11 days of chronic treatment. This result may have been produced by an increased synthesis and metabolism of 5HT. The increased synthesis of 5HT may have followed the increased availability of TP (Hamon et al., 1974). This effect was transient in that the levels of TP, 5HT and 5HIAA all returned to levels seen at the outset of the study by the fifteenth day of chronic treatment. This return of the increased levels to pre-treatment levels following 15 days' treatment suggests the possibility of some compensatory decrease in 5HT synthesis and metabolism. The mechanism of such a compensation is unclear except that an acute increase in brain TP has previously been seen to decrease tryptophan hydroxylase activity (Azmitia & McEwan, 1974). However, in contradiction to this possible mechanism of compensation, a chronic increase of brain TP has been seen to increase tryptophan hydroxylase activity (Diez et al., 1976).

2 Withdrawal.

At the end of the gum acacia administration, whole brain TP,

5HT and 5HIAA were all at pre-treatment levels. Following withdrawal of the gum acacia treatment, whole brain TP, 5HT and 5HIAA all fell to below pre-treatment values within 3 days of withdrawal. Levels returned to pre-treatment values by the fifth day following treatment withdrawal.

These results suggest that gum acacia may initially increase 5HT synthesis and metabolism, possibly via an increase in TP availability. However, by the fifteenth day of gum acacia administration, some compensatory mechanism had caused the 5HT synthesis and metabolism to return to pre-treatment levels. Immediately following gum acacia treatment withdrawal the effects of gum acacia were removed, leaving the compensatory mechanism to produce a decrease in 5HT synthesis and metabolism. This 'inappropriate' compensatory decrease in 5HT synthesis and metabolism was not present on the fifth day following gum acacia treatment withdrawal.

Effects of Chronic Gum Acacia In Female Mice.

1 Administration.

Within this vehicle control group, brain 5HIAA was found to be significantly increased during the metoestrous as compared with the dioestrous stages of the oestrous cycle. ($p = .0037$). There were no such changes seen in 5HT ($p = 0.70$) or in TP ($p = .060$). Therefore the effects of gum acacia are superimposed onto this effect of the oestrous cycle. Fortuitously, in later sections, the drug treatment groups and vehicle control groups were at similar stages of the oestrous cycle.

The gum acacia treatment did not affect brain 5HT, although 5HIAA levels were elevated following 5 days of chronic

treatment and brain TP levels were elevated after 43 days of chronic gum acacia administration. The elevated 5HIAA levels had returned to pre-treatment values by the eighth day of treatment and they then remained stable. These results suggest that there may be an increased metabolism of 5HT following 5 days of treatment, such an increase in 5HT metabolism possibly reflects an increased 5HT release. Since 5HT levels are not decreased by this effect, it is possible that there is a simultaneous increase in 5HT synthesis, possibly reflecting an increased TP availability. Brain TP levels were elevated following 43 days of gum acacia treatment. The changes in 5HIAA could not be explained by the variation of the oestrous cycle.

The increased 5HIAA seen after 5 days of treatment was not present on the eighth day of treatment, this suggests a possible compensatory decrease in 5HT metabolism by this time.

2 Withdrawal.

Following 15 days of chronic gum acacia treatment, brain TP, 5HT and 5HIAA were all at pre-treatment values. Following treatment withdrawal, TP and 5HIAA were both elevated on the second day, but fell to pre-treatment levels by the third day following treatment withdrawal. 5HT increased beyond pre-treatment values following withdrawal, and levels were still elevated on the third day following withdrawal. These results suggest that immediately following treatment withdrawal there may have been an increase in 5HT synthesis and metabolism resulting in the increased levels of 5HT and 5HIAA. By the third day, following drug withdrawal the 5HT metabolism may have returned to normal, whilst an increased synthesis and

storage of 5HT may have been responsible for the significantly increased 5HT levels. Again, these changes could not be explained in terms of oestrous cycle variations.

From these results, it can be seen that in both male and female mice, chronic gum acacia treatment initially produced signs of an increased synthesis and metabolism of 5HT. This effect was possibly compensated for by about the eighth day of treatment. Following treatment withdrawal there were signs of a possible decrease in 5HT metabolism in male mice, but in female mice, withdrawal produced a possible further increase in 5HT synthesis.

Gum acacia is a polysaccharide. It has previously been shown that ingestion of a carbohydrate diet produced decreased plasma NEFA and free TP, with increased plasma total TP and brain TP (Madras et al., 1974). Therefore, ingestion of a polysaccharide may produce increased brain TP. This in turn may produce increased brain 5HT (See Introduction). However, a similar increase in brain 5HT and 5HIAA has been reported for rats following 4 days chronic treatment with sesame oil vehicle control S.C.(Ladisich, 1974). This result suggests that the alterations of 5HT synthesis and metabolism seen following treatment with gum acacia may be due to some non-specific effects of the control treatment administration, rather than being due to the gum acacia itself.

Effects of Chronic Progesterone Treatment in Male Mice.

1 Administration.

Chronic progesterone administration was seen to increase brain TP after 5 days of treatment and decrease brain 5HIAA after 11 days of treatment, both relative to vehicle control. There

were no effects on 5HT relative to control. The increased brain TP, unchanged 5HT and decreased 5HIAA may be produced by a decreased release of 5HT, resulting in a decreased metabolism to 5HIAA. The increased TP may be the result of a 'backing-up' of TP due to its decreased utilisation of 5HT synthesis. Such an effect might be theoretically produced by a decrease of tryptophan hydroxylase activity. In support of this, decreased 5HT turnover has previously been shown to produce decreased tryptophan hydroxylase in rat brain (Zivkovic et al, 1974). An alternative explanation would be that the elevated brain TP was produced by a decreased utilisation of TP for some purpose other than 5HT, eg protein synthesis. There is no further evidence to support a decrease in protein synthesis following gum acacia treatment.

2 Withdrawal.

Chronic progesterone treatment produced signs of a decreased 5HT metabolism. Following progesterone withdrawal, this decreased metabolism immediately returned to vehicle control values, which were equivalent to pre-treatment values.

Effects of Chronic Progesterone Treatment in Female Mice.

1 Administration.

Chronic progesterone was seen to increase brain TP and 5HT relative to vehicle controls after 15 days of chronic treatment. However, this effect was not present after 30 or 43 days of treatment. In contrast, brain 5HIAA was not significantly different from vehicle controls at any point during progesterone administration. These results suggest that after 15 days of chronic progesterone treatment in

female mice, there is an increased availability of TP and an increased 5HT synthesis and storage. However, there is apparently no effect on 5HT utilisation or metabolism to 5HIAA. This effect disappears following treatment for longer than 15 days, possibly due to some homeostatic compensatory mechanism.

2 Withdrawal.

After 15 days of progesterone treatment, brain TP and 5HT were significantly increased relative to vehicle control. Following withdrawal of chronic progesterone treatment, brain TP, 5HT and 5HIAA were all significantly decreased relative to vehicle control, the 5HT : 5HIAA ratio was significantly increased relative to vehicle control. Brain TP, 5HT and 5HIAA were also decreased relative to the levels of the final day of drug administration before withdrawal. These results suggest that following drug withdrawal, there is possibly a decreased synthesis and storage of 5HT, possibly resulting from a decrease of the availability of TP in the brain. The increased 5HT : 5HIAA ratio also suggests a decreased release of 5HT and a decreased metabolism of 5HT to 5HIAA. The differences in the brain 5HIAA following drug withdrawal may be associated with the change of brain 5HIAA seen to occur at different stages of the oestrous cycle (See Previous).

Effects of Chronic Dydrogesterone Treatment in Female Mice.

1 Administration.

Chronic administration of dydrogesterone was seen to increase brain TP, 5HT and 5HIAA, relative to vehicle controls, after 15 days of chronic treatment. The ratio of 5HT : 5HIAA was

no different from vehicle control at this time. Brain 5HIAA was also significantly increased relative to vehicle control following 43 days of dydrogesterone administration, 5HT and TP were not significantly different from the vehicle control group at this time point. These differences in brain 5HIAA could not be explained in terms of the oestrous cycle stages.

These results suggest that after 15 days of chronic treatment with dydrogesterone, there may be increased TP availability, possibly due to increased brain entry of TP, 5HT synthesis may also have been increased, and 5HT metabolism to 5HIAA similarly increased. This effect was not present following 30 days of chronic dydrogesterone treatment, this indicating that some compensatory mechanism may be present beyond 15 days of chronic treatment.

The increased 5HIAA produced by 43 days of chronic dydrogesterone treatment, with unchanged 5HT or TP, suggests a possible increase in 5HT metabolism. As there is no corresponding decrease in brain 5HT concurrent with the increased metabolism of 5HT, there may be a simultaneous increase in 5HT synthesis.

2 Withdrawal.

Dydrogesterone withdrawal significantly decreased brain 5HIAA but did not affect brain 5HT or TP relative to the vehicle control, this was not related to changes during the oestrous cycle. Brain TP and 5HIAA fell below the level produced by dydrogesterone administration. These results suggest a possible decrease in 5HT metabolism following dydrogesterone withdrawal. As there is no increased storage of 5HT there may also be a concurrent decrease in 5HT synthesis.

Effects of Chronic Norethisterone Acetate Treatment in Female Mice.

1 Administration.

Chronic norethisterone acetate administration did not affect brain TP relative to vehicle control. Brain 5HT was significantly increased, and 5HIAA significantly decreased following 5 days of drug administration, unrelated to oestrous cycle stages. This suggests a possible decrease in the metabolism of 5HT. Such an effect may be produced by either decreased release of 5HT, with increased 5HT storage, or by a reduction in monoamine oxidase activity. However progesterone and progestogens have previously been seen to increase monoamine oxidase activity (Grant & Pryse-Davies, 1968; Parry & Rush, 1979). Therefore the former explanation is the more likely. Brain TP availability appeared to be unaffected, relative to vehicle control.

The decreased metabolism of 5HT was not present following dosing for longer than 5 days. This suggests that some homeostatic compensatory mechanism may be active. Following 43 days of norethisterone acetate treatment, brain 5HIAA was increased relative to vehicle control, 5HT and TP were unaffected. At this time point, the two groups were at different stages of the oestrous cycle. This suggests an increased metabolism of 5HT, as there is no concurrent decrease in 5HT stores, there may be a simultaneous increase in 5HT synthesis. This may be part of the compensatory mechanism postulated above.

Such an increase in brain 5HIAA, without there being an effect on 5HT or TP, may also occur if the efflux of 5HIAA

from the CNS were inhibited by a possible probenecid-like action of norethisterone acetate. This explanation is however unlikely as no such activity of norethisterone acetate has previously been reported. In addition, it is unlikely that a probenecid-like activity would be able to produce decreased 5HIAA following 5 days of drug treatment, with increased 5HIAA following 43 days of drug treatment.

2 Withdrawal.

There were no significant differences between the vehicle control group and the norethisterone group immediately prior to drug withdrawal. Withdrawal of the norethisterone acetate produced decreased brain 5HT and 5HIAA relative to vehicle control, unrelated to stage of oestrous cycle. TP was no different. 5HT : 5HIAA ratios were also significantly increased relative to vehicle controls. (There were no differences in the brain levels of TP, 5HT or 5HIAA between the final day of norethisterone acetate administration and days following drug withdrawal).

The decreased 5HT and 5HIAA levels following norethisterone acetate withdrawal relative to vehicle controls, suggest a possible decrease in the synthesis and metabolism of 5HT. Such decreased synthesis may reflect changes in tryptophan hydroxylase activity (Zivkovic et al., 1974), or changes in TP availability (Hamon et al., 1974).

Effects of Chronic Norethisterone Acetate/Ethinyl Oestradiol Treatment in Female Mice.

1 Administration.

Norethisterone acetate/ethinyl oestradiol combination administration produced increased brain TP. 5HT and 5HIAA. However there

appeared to be multiple mechanisms for the production of these effects. Following 5 days of chronic drug combination treatment 5HT and 5HIAA were significantly increased relative to vehicle control, the 5HT : 5HIAA ratio was also significantly increased. At this time point, the groups were at different stages of the oestrous cycle. Brain TP was unchanged at this time. These results suggest a possible increase in 5HT synthesis and storage with a relatively smaller increase in 5HT metabolism. Such an increase in 5HT synthesis may reflect an increased availability of TP (Hamon et al., 1974).

On the fifteenth day of drug combination administration, brain TP was significantly increased with 5HT and 5HIAA being unchanged, relative to vehicle control. Such an increase in brain TP would normally be expected to produce an increase in 5HT synthesis (Hamon et al., 1974). However increased brain TP with unchanged 5HT may occur in the presence of decreased tryptophan hydroxylase activity. Following the work of Zivkovic and colleagues (1974), it is possible that the decreased 5HT turnover seen following 5 days of drug combination treatment may have produced decreased tryptophan hydroxylase activity. An alternative explanation could be that the increased brain TP is due to decreased TP utilisation for something other than 5HT synthesis, eg protein synthesis. The resulting increase in brain TP may be unavailable for 5HT synthesis, ie it may be anatomically separate from the 5-Hydroxytryptaminergic neurons. There is however no evidence to support this explanation.

Following 30 days of norethisterone acetate/ethinyl oestradiol combination treatment, brain 5HT was significantly increased,

whilst brain TP and 5HIAA were unchanged relative to vehicle controls. Differences in the 5HT : 5HIAA ratio failed to achieve statistical significance. This result suggests either an increased synthesis and/or storage of 5HT or a decreased release and metabolism of 5HT. As 5HIAA was unchanged relative to vehicle control, the former is more likely. An increase in 5HT synthesis may reflect an increase in TP availability (Hamon et al., 1974).

2 Withdrawal.

Withdrawal of drug combination treatment produced decreased brain 5HT, with unchanged TP and 5HIAA relative to vehicle control. Differences in the 5HT : 5HIAA ratio failed to reach statistical significance. This suggests a possible decrease in the synthesis of 5HT, with the metabolism and release of 5HT being unchanged. Such a decrease in 5HT synthesis may reflect either decreased tryptophan hydroxylase activity or decreased TP availability. Brain TP was found to be lower following drug withdrawal than on the last day of drug administration.

Therefore all of the hormone treatments were seen to produce an increase in brain 5HT, whilst withdrawal of the hormone treatments produced a decrease in brain 5HT. However differing mechanisms appeared to underlie these effects. A summary of the possible mechanisms is presented in Table II.

Table II

Summary of the Possible Explanations for the Effects of Chronic Hormone Administration on Brain 5HT.

Treatment	Action
Progesterone (males)	↓5HT Synthesis; ↓ 5HT metabolism
Progesterone (females)	↑TP availability; ↑5HT synthesis; no change 5HT metabolism
Dydrogesterone (females)	↑TP availability; ↑5HT synthesis; ↑ 5HT metabolism
Norethisterone acetate (females)	Early : ↓5HT metabolism; no change 5HT synthesis Later : ↑TP availability; ↑5HT synthesis; ↑ 5HT metabolism
Norethisterone acetate/ ethinyl estradiol	Early : ↑TP availability; ↑5HT synthesis; ↑ 5HT metabolism Later : ↑TP, no change 5HT synthesis or metabolism Later : ↑TP availability; ↑5HT synthesis; no change 5HT metabolism

DISCUSSION OF CHAPTERS 5 - 7

Discussion of Chapters 5 - 7.

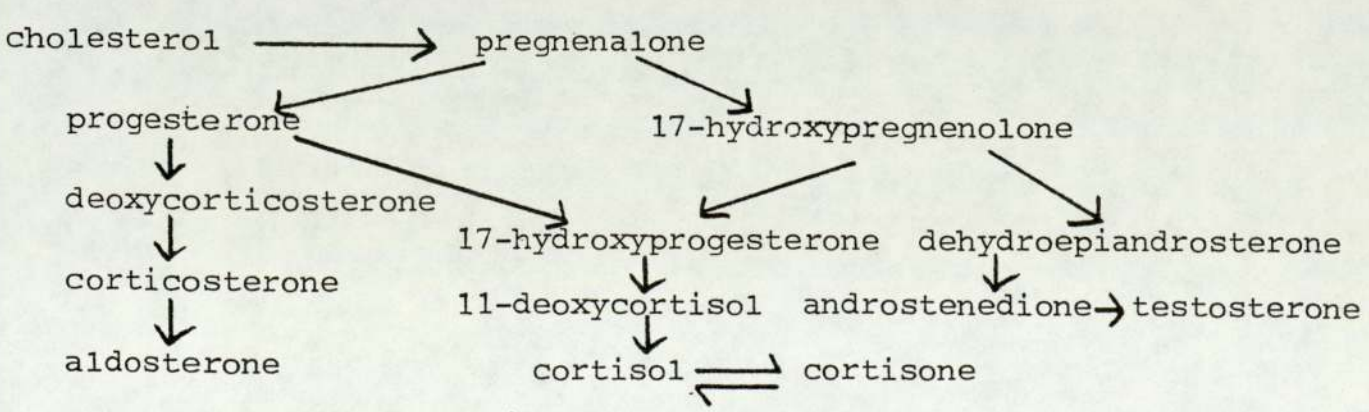
There has been much speculation concerning the rôle of progesterone in the aetiology of the symptoms of premenstrual syndrome and post natal depression (See Introduction), the major hypothesis being that a deficit of progesterone during the luteal phase of the menstrual cycle, or immediately post-partum is causative of the psychological symptoms. Partial support for this hypothesis was provided by the work of Backstrom (1974) who found that women suffering from pre-menstrual syndrome hadconsistently lower plasma progesterone than controls, during the luteal phase of the cycle. However, Munday (1977) reported that not all premenstrual syndrome sufferers had low luteal plasma progesterone. Nott and coauthors (1976) also reported that subjects suffering from post-partum 'blues' showed no differences in plasma progest-erone in comparison to non 'blues' sufferers.

Dalton (1971), suggested that an absolute deficit of prog-esterone may not be the cause of the psychological symptoms but rather the sudden changes in progesterone might produce the effects. Plasma progesterone falls considerably immediat-ely premenstrually (Bell et al., 1976) and immediately post-partum (Yonnone et al., 1968). Dalton therefore suggested that a failure to adapt to the rapidly changing plasma progesterone concentrations at these times resulted in the psychological symptoms. Some support for this hypothesis was provided by Nott and colleagues (1976) who reported that those subjects self-reporting the greatest severities of 'blues' also showed the greatest fall of plasma progesterone

antenatally to postnatally. This finding was not repeated in the present study.

The greatest evidence for the progesterone deficit hypothesis comes from the clinical use of progesterone and progestogens in the treatment of premenstrual syndrome. Dalton (1964) reported the use of progesterone in the treatment of this syndrome. The progesterone was administered either by intramuscular injection, or as suppositories, due to the fact that progesterone is not generally believed to be orally active. However in high doses, progesterone has been seen to be of use in hormone replacement therapy if given orally (Parsons, personal communication). Dalton (1971) also reported that the orally active synthetic progestogens (eg norethisterone acetate) were of no use in the treatment of premenstrual syndrome. The explanation offered for this finding was that, by definition, a progestogen is a substance with a progesterone-like activity on the endometrium. However other actions of progesterone are not considered. For instance, whereas progesterone may be converted to aldosterone, cortisol and testosterone, these conversions are not possible for the progestogens (ibid). See Figure IX.

Fig IX Pathways for Synthesis of Adrenal Steroids

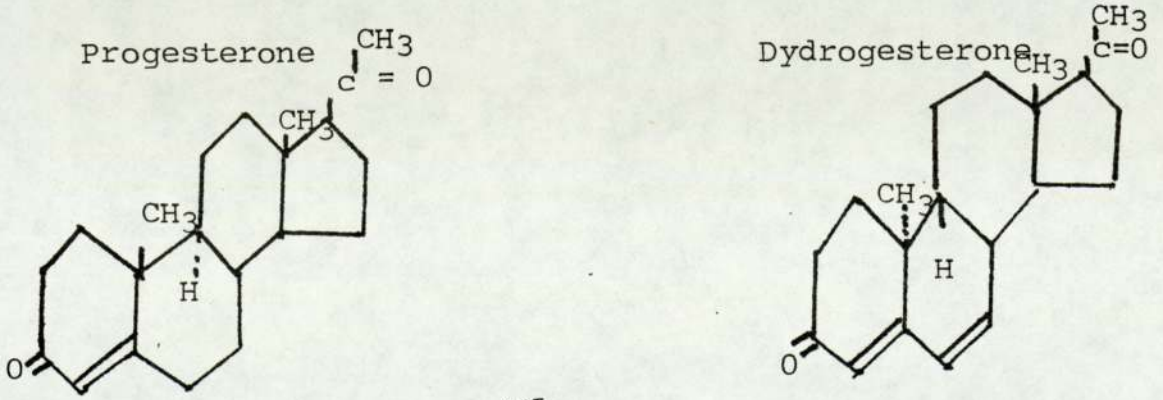


Progesterone also has a direct sedative action on the CNS, which is not considered in the definition of a progestogen.

In addition to its use in premenstrual syndrome, progesterone is reported to be of use in the prophylactic treatment of postnatal depression (Dalton, 1982); the postulated mode of action being the prevention of the abrupt drop in progesterone which occurs following the delivery of the placenta (ibid).

The recent development of the synthetic progestogen, dydrogesterone has lead to the production of further interesting results. Dydrogesterone is an orally active progestogen, chemically very similar to progesterone (Fig X). It is claimed to be over ten times more potent, weight for weight, than progesterone. (Goodman & Gilman, 1976) This progestogen is reported to be unlike other progestogens in that it is of use in the treatment of premenstrual syndrome (Taylor, 1977; Kerr et al., 1980). Up to 72 per cent of premenstrual syndrome sufferers reported some improvement in their symptoms following treatment with dydrogesterone (Kerr et al., 1980).Therefore in the treatment of premenstrual syndrome it appears that progesterone and dydrogesterone are similar in their action and that both are different from other progestogens. Hence it seems possible that the psychological symptoms seen in some subjects premenstrually and post-partum may be

Fig X Chemical Structures of Progesterone and Dydrogesterone



brought about by the rapid fall in plasma progesterone. The present studies were therefore performed to investigate the effect of a rapid fall in plasma progesterone on brain 5HT.

The results of the preceding chapters show that chronic elevation of plasma progesterone, to levels normally seen in the latter half of pregnancy, by the chronic oral administration of high doses of progesterone leads to an increase in mouse whole brain 5HT. A 15 day duration of treatment served as a simulation of the time course of a normal murine pregnancy.

Following acute hormone treatment withdrawal, ie simulated parturition, whole brain 5HT was found to be decreased. Therefore if it is possible to extrapolate results from animal studies to the case of humans, it is possible that the chronic high plasma levels of progesterone found in the latter half of human pregnancy may produce an increase in whole brain 5HT. The acute fall in plasma progesterone seen in humans following the delivery of the placenta at the time of parturition may produce decreased whole brain 5HT.

Similarly, Shetty and Gaitonde (1980) reported that chronic administration of either ethinyl oestradiol plus norgestrel, or norgestrel alone, to rats produced decreased brain 5HT. Again, if this result of animal work may be extrapolated to humans, chronic usage of oral contraceptives may produce decreased brain 5HT during administration.

As described in the Introduction, a decreased brain 5HT function has been postulated as being involved in the aetiology of depressive illness in humans. It is therefore possible that the depressive mood symptoms frequently reported by human females premenstrually, post-partum and to some extent, perimenopausally,

and following use of oral contraceptives, may be due to decreased brain 5HT activity at these times, produced by the changes in plasma progesterone.

The efficacy of progesterone supplements for the alleviation of these depressive mood changes may also involve changes in brain 5HT. As reported in Chapter 7, chronic administration of progestational hormones to mice, for a period of 15 days, produced increased brain 5HT concentrations. If the same happens in humans receiving these hormones, an increase in brain 5HT concentrations may be the mechanism by which the depressive symptoms are alleviated. However it is unclear as to whether any changes in the 5HT levels are changes in the 'functional' or 'non functional' 5HT pools. In addition, the different progestational hormones appear to increase brain 5HT levels via different mechanisms (See Table II page 429), these differences may be important in their therapeutic action. Note that the decreased rat brain 5HT concentrations seen following contraceptive steroid treatment (Shetty & Gaitonde, 1980), occurred after 3 months of chronic treatment; whilst in the present study increased mouse brain 5HT was seen within 15 days of chronic treatment.

This postulated mode of action of progesterone supplements on depressive mood does not concur with the report of Dalton (1977). Dalton stated that progesterone supplements were beneficial in the treatment of premenstrual syndrome, and more recently in the prophylactic treatment of post natal depression (Dalton, 1982), but that the synthetic progestogens were of no use in the treatment of these syndromes (Dalton, 1971). (NB Dydrogesterone has been marketed since the original Dalton publication).

The results of Chapter 7 indicate that both natural progesterone and the synthetic progestogens are capable of elevating brain 5HT concentrations in mice. This suggests that, if Dalton is correct in that synthetic progestogens are of no beneficial therapeutic use, the elevation of brain 5HT may not be the mechanism by which progesterone alleviates the depressive symptoms. Dalton's report that synthetic progestogens were of no beneficial value was based on anecdotal evidence, no controlled studies have been performed to validate this report.

Not all previous studies are in complete agree ment with the results of the present study. Gould (1979), using a subcutaneous dose of norethisterone, similar but a much higher dose of ethinyl oestradiol than the hormone combination group of Chapter 7, reported no effects on either whole brain 5HT or 5HT turnover after 14 day chronic treatment in mice. However, Bond (1979), using subcutaneous doses of a steroidal contraceptive combination similar to that of Chapter 7 (ie ethinyl oestradiol and norethisterone acetate)reported increased whole brain 5HT following 43 days chronic treatment in mice. Shetty and Gaitonde (1980) used contraceptive steroid (ethinyl oestradiol/ norgestrel or norgestrel alone) doses similar in magnitude to those of the present study, administered orally in arachis oil to female rats. In their study, no effects on brain 5HT were seen for the initial 2 months of chronic treatment, but decreased brain 5HT was seen after 3 months. Results from these and related studies are summarised in table III. Of the studies presented, this present study is the only one to investigate the effects of chronic treatment with high doses of progestational hormones,

Table III Effects of Oestrogens and Progestogens on Brain 5HT

Study	Oestrogen	Progestogen	Animal	Route	Duration	Effect
Wirz-justice & Hackman (1972)	Oestradiol 0.4mg/rat	Progesterone 0.4mg/rat	rat rat	- -	Acute Acute	↓ 5HT uptake ↑ 5HT uptake
Fuxe et al (1974)	Oestradiol 5µg 5µg	Progesterone 2mg	cast. rat cast. rat	- -	Acute Acute	↑ 5HTturn over Reduces effect of oestrogen
Present Study	Ethinyl/Oestradiol 100 µg/kg	Norethisterone acetate 1mg/kg Norethisterone acetate 1mg/kg Progesterone 10mg/kg Dydrogesterone 5mg/kg	mouse mouse mouse mouse	oral oral oral oral	5 days 5 days 15 days 15 days	↑ 5HT ↑ 5HT ↑ 5HT ↑ 5HT
	Ethinyl/Oestradiol 100 µg/kg	Norethisterone acetate 1mg/kg	mouse mouse	oral oral	15 days 15 days	no effect no effect
Gould (1979)	Oestradiol 100µg/kg	Norethisterone 200 µg/kg	mouse	S C	15 days	no effect
Bond (1979)	Ethinyl/Oestradiol 1 µg/kg	Norethisterone acetate 20µg/kg Norethisterone acetate 20µg/kg	mouse mouse	S C S C	43 days 43 days	↑ 5HT ↑ 5HT
Present Study	Ethinyl/Oestradiol 100 µg/kg	Norethisterone ac. 1mg/kg Norethisterone acetate 1mg/kg Progesterone 10mg/kg Dydrogesteron-e 5mg/kg	mouse mouse mouse mouse	oral oral oral oral	43 days 43 days 43 days 43 days	no effect (↑ 5HIAA) no effect no effect no effect (↑ 5HIAA)
Shetty & Gaitonde (1980)	Ethinyl/Oestradiol 1µg/kg)	Norgestrel 10µg/kg Norgestrel 10µg/kg	rat rat rat rat rat rat	oral oral oral oral oral oral	1 month 2 month 3 month 1 month 2 month 3 month	no effect no effect ↓ 5HT no effect no effect ↓ 5HT

most studies have concentrated on the effects of steroidal hormones at doses equivalent to those used in oral contraceptives. There are therefore no previous studies of the effects of increased progesterone, to the levels and for the duration seen during pregnancy, on brain 5HT synthesis and metabolism.

From Chapter 7, chronic administration of progestational hormones increased brain 5HT and acute withdrawal of such treatment decreased brain 5HT concentrations. It has been suggested previously that these effects may be important in the aetiology of the mood changes seen at times of changing progesterone levels in humans. As described in the Introduction, Handley and colleagues (1980) reported that plasma total TP rose rapidly from pregnancy levels to pre-pregnancy levels immediately post-partum. The mechanism underlying such a change in plasma total TP is not known. In addition, a subgroup of subjects who did not exhibit the usual plasma total TP dynamics immediately post-partum was identified (ibid). This subgroup showed an increased incidence of psychological disturbance post-partum. Such a relationship between TP 'non risers' and the incidence of post-partum psychological disturbances was not replicated in the present study, although 'blues' cases were seen to have significantly decreased plasma total TP on day 1 post-partum. Results of chapter 6 show that in male mice, chronic high doses of progesterone, followed by acute withdrawal of treatment did not affect plasma total TP relative to vehicle control. Similar results were found for the effects of norethisterone acetate administration and withdrawal in female mice. In female mice

receiving progesterone however, drug administration was seen to elevate plasma total TP relative to vehicle controls. There was no difference in plasma total TP between any of the treatment groups and the vehicle control groups following hormone withdrawal. These results therefore suggest that the decreased plasma total TP seen during human pregnancy, and the rapid rise of plasma total TP seen immediately post-partum are not related to alterations of plasma progesterone seen at these times. It is therefore unlikely that progesterone is involved in the delay of the plasma total TP rise immediately post-partum seen amongst the subgroup of subjects reported by Handley and colleagues (1980).

Curzon and colleagues (1979) reported that depressed subjects exhibited abnormal plasma NEFA responses to stress (See Introduction). In addition plasma NEFA was seen to be increased during human pregnancy and to decrease immediately post-partum (Burt, 1960). In the present study, the effects of chronic progesterone treatment on plasma NEFA was investigated. In male mice the progesterone administration had no effect on plasma NEFA, relative to vehicle controls. Following treatment withdrawal in male mice, plasma NEFA was increased relative to vehicle controls. In female mice, progesterone administration significantly decreased plasma NEFA, the NEFA returned to control values following progesterone treatment withdrawal.

In this study, the plasma NEFA concentrations found for both the progesterone groups and the gum acacia control groups of either sex were of the order of 1.2 mEq/dm^3. This value is much greater that the value of 0.7 mEq/dm^3 found for untreated male mice. This increased plasma NEFA may be

caused by an effect of gum acacia *per se*, or produced by the stress of the oral dosing technique. In humans, plasma NEFA is increased at times of stress (Cardon& Gordon, 1959), it is therefore possible that these increased plasma concentrations of NEFA in mice are a result of the stress of the experimental procedures involved. If this is so, it may be that the significantly decreased plasma NEFA seen in female mice following progesterone administration may be due to progesterone producing some protecting effect against the stress of the experimental procedures. A similar effect of progesterone 'protecting' against the effects of vehicle control administration techniques was reported by Ladisch (1974). The 'protecting' effect may be related to the sedative properties of progesterone at this dose (Chapter 5). The lack of such a 'protective' effect of progesterone in male mice could then be due to the sex dependent nature of the sedative effects of progesterone. (Chapter 5).

On the last day of progesterone administration to female mice, ie after 15 days of dosing, there was no difference in plasma NEFA between the progesterone group and the vehicle control group. From Fig 6.21, this effect is due to a decrease in the plasma NEFA of the vehicle control group, rather than an increase in the plasma NEFA of the progesterone group. This possibly reflects some habituation to the stress of the dosing and handling by the vehicle control group, this habituation being manifest in the decreased plasma NEFA.

Following progesterone treatment withdrawal in female mice, the plasma NEFA immediately returned to vehicle control values. This possibly reflects the short plasma half-life of progesterone (Aufrere & Benson, 1976).

In male mice plasma NEFA was significantly increased above vehicle controls on the fifth day following progesterone withdrawal. As progesterone has a plasma half-life of only a few minutes (ibid), it is unlikely that this effect would be due to a direct action of progesterone itself. Progesterone may therefore be having some effect on a secondary process which does not become apparent until after the progesterone has been metabolised. This effect was not seen in female mice, therefore the secondary effect of progesterone may be sex specific.

The decreased plasma NEFA seen in female mice during chronic progesterone treatment may explain the increased plasma total TP seen at this time. As stated in the Introduction, plasma TP is normally 70 % bound to plasma albumin, and it is the unbound portion of TP that is more available for utilisation by the brain or for tissue synthesis, etc. NEFA are also capable of displacing TP from albumin, therefore a decrease in plasma NEFA would lead to an increase in the bound portion of the plasma TP. This in turn lowers the availability of the plasma TP and therefore could lead to an increase in plasma total TP. Such an effect of decreased NEFA producing decreased free TP and increased total TP was previously seen by Madras and colleagues (1974).

In human pregnancy plasma NEFA increases by about 30% (Burt, 1960). The decrease of plasma NEFA in female mice following progesterone treatment therefore suggests that the increase in human plasma NEFA during pregnancy is independent of any changes in plasma progesterone.

Progesterone administration to both male and female mice was found to increase whole brain TP. This effect was not seen following chronic norethisterone acetate treatment to female mice. As stated previously, progesterone treatment in female mice increased plasma total TP and decreased plasma NEFA, therefore this increase in brain TP may have been due to an increase in plasma unbound TP. Madras and colleagues (1974) similarly reported that decreased NEFA produced increased plasma total TP and increased brain TP. As progesterone also produced increased brain TP in male mice, but without affecting plasma NEFA or total TP, the results suggest that brain TP is increased via some other mechanism than an increase in plasma total TP. There is no data to suggest the nature of the mechanism*.

The results of the preceding chapters therefore show that at times of chronic high plasma progesterone there is an increase in whole brain 5HT, whilst at times of rapidly decreasing plasma progesterone, there is decreased brain 5HT. As described in the Introduction, brain 5HT may be involved in the aetiology of depressed affect, therefore the decreased brain 5HT seen following progesterone withdrawal may be involved in the aetiology of the depressive symptoms frequently reported at times of sudden plasma progesterone decreases, whilst chronic progesterone or synthetic progestogen treatment may offer some protection against depressed affect related to decreased brain 5HT.

Studies of whole brain TP, 5HT and 5HIAA however may be mis-

* Plasma free TP was not measured in these studies as the experiments were designed to investigate the possible involvement of progesterone in the plasma total TP changes at parturition.

leading when the results are used as inferences to the central 5-hydroxytryptaminergic activity. Green and Grahame-Smith (1976) state that much of the central 5HT is intraneuronally bound, and is not available for release from the pre-synaptic site. These workers postulate that there are two or more 'pools' of 5HT within the brain, only one of which is 'functional' as a neurotransmitter. Therefore dietary induced changes in plasma TP, LNAA and NEFA may affect brain 5HT synthesis (See Introduction) without affecting 5HT transmission. The 'non functional' pools of 5HT may act as stores for the functional pool of 5HT.

Following from this, much of the 5HIAA produced by oxidative deamination of 5HT would be formed from non-functional stores of 5HT, therefore it is uncertain that changes in brain 5HIAA reflect changes in 5HT transmitter activity.

In addition to this, much of the brain TP is used for protein and tissue synthesis within the brain, therefore fluctuations in brain TP may not necessarily be related to changes in brain 5HT activity.

One way of inferring changes in central 5-hydroxytryptaminergic activity is to investigate behavioural changes. At the same hormone doses as were used in the studies of the brain TP, 5HT and 5HIAA; progesterone, norethisterone acetate and dydrogesterone were all seen to produce a potentiation of barbiturate sleeping time in female mice. However, there was no such effect in male mice. A similar sex difference in the sedative effect of progesterone was previously seen in humans by Merryman (1954). In this human study however,

there was also a slight sedative effect in males, although not to the same extent as that seen in females. A sex difference has also been reported for rats (Kato et al., 1971), the mechanism of this difference was found to be a sex difference in the hepatic metabolism of progesterone. Males were found to metabolise progesterone more rapidly than females via Δ^4- reductase, hence the decreased activity of progesterone in males was due to its increased rate of metabolism in the liver (ibid).

The precise mechanism by which progesterone potentiates the barbiturate sleeping time is unclear. One possible explanation would be that progesterone inhibits the metabolism of the barbiturate, thereby prolonging its activity. This explanation however is unlikely since the action of the particular barbiturate used is normally limited by the redistribution of the drug away from the CNS rather than by hepatic metabolism (Price et al., 1960). This therefore suggests that progesterone is producing its effects via a direct central action. Further support for this conclusion can be found in the fact that progesterone was seen to produce a slight sedative effect in females when administered alone.

No evidence of the sedative effects of progesterone was found in the studies of observed murine behaviour using the method of Irwin (1968). Thus, neither progesterone in males or females, or norethisterone acetate in females produced any differences in behaviour, when compared with vehicle controls, either during chronic hormone administration, or following acute withdrawal of treatment.

The lack of observed effect on mouse behaviour possibly reflects the relative crudity of the method used. Studies of locomotor activity in female mice receiving chronic doses of progesterone showed that this treatment produced an increase in locomotor activity relative to vehicle control. There was no such effect on locomotor activity during chronic norethisterone acetate administration to female mice, or during chronic progesterone administration to male mice.

This effect of progesterone on locomotor activity in female mice may be due to the sedative effect of progesterone. At highly sedative doses, a decrease in locomotor activity might be expected as a direct consequence of the sedation. It is possible however that at lower levels of sedation the progesterone produces a state similar to the euphoric/excitatory state seen at low doses of anaesthesia in humans. Such an excitatory state may explain the increased locomotor activity seen in female mice. An increase in locomotor activity has previously been reported following administration of low doses of anaesthetics (eg Hynes & Berkowitz, 1979; Waldeck, 1975).

Increased locomotor activity has previously been seen to be correlated with decreased brain 5-hydroxytryptaminergic activity (Neuberg & Thut, 1974; Grabowska & Michaluk, 1974). However, in the present study whole brain 5HT was seen to be increased with unchanged 5HT metabolism during chronic progesterone treatment to female mice. Therefore it is unlikely that the altered locomotor activity in female mice is related to the changes in brain 5HT, it is however possible that there may be changes in the 'functional' 5HT that are masked by changes in the 'non functional' pool, but it is not possible

to differentiate between the activities of the two pools from data on whole brain TP, 5HT and 5HIAA. It is also possible that progesterone may be producing an effect on locomotor activity via some neurotransmitter other than 5HT.

Plate crossing is reported to be a measure of anxiety-like behaviour in mice (Marriott & Smith, 1972; Brown & Handley, 1980). Hence, an increase in plate-crossing, or a decreased latency to crossing is seen as a reduction of anxiety-like behaviour, and a drug which increases plate-crossing may have an anxiolytic action.

In male mice, chronic progesterone administration produced an initial increase in plate-crossing, but after repeated dosing there was a decrease in plate-crossing in naive mice relative to naive vehicle controls. In female mice, chronic progesterone initially produced decreased plate-crossing relative to vehicle controls, however this difference disappeared following repeated hormone administration. Chronic norethisterone acetate administration had no effect in female mice. Plate crossing was not seen to be affected by the stage of the oestrous cycle.

The anxiolytic effect of drugs has been correlated with a decrease in central 5HT turnover (File & Velucci, 1978). Therefore if plate-crossing is a good measure of anxiety-like behaviour, it would be expected that an increase in plate-crossing may be correlated with a decreased 5HT activity. The results of Chapter 7 show that there is a decreased metabolism of 5HT in male mice receiving chronic progesterone but that there is no effect of chronic progesterone on brain 5HT metabolism in female mice. It is therefore unlikely

that the decreased plate-crossing in either male or female mice receiving progesterone was related to changes in central 5HT activity. From the data available, it is not possible to speculate as to the mechanisms producing the changes in plate-crossing.

The behavioural results of the present study are not in agreement with the previous work of Bond (1979). This worker reported that chronic administration of norethisterone acetate to female mice, at the same dose and via the same route as used in the present study, produced a decrease in locomotor activity. This disparity of results however is possibly explained by a difference in the methods used. Bond (1979) compared the locomotor activity counts of animals following chronic norethisterone acetate dosing with a value for locmotor activity obtained on day one of drug administration whilst the animals were at the oestrous stage of the oestrous cycle. Animals were found to remain at dioestrous following chronic norethisterone treatments (Bond, 1979). Therefore locomotor activity counts for animals at dioestrous were being compared for locomotor activity counts of oestrous controls. Locomotor activity is known to be significantly greater at oestrous than at dioestrous (ibid). This may therefore explain the findings of decreased locmotor activity in female mice receiving chronic norethisterone acetate.

In the present study animals were housed together in order to ensure synchronisation of the oestrous cycles. Locomotor activity counts for the treatment group were compared with activity counts from a parallel vehicle control group. The mean counts from each of eight days of chronic treatment were used in the statistical analysis, hence 2 complete oestrous

cycles were studied for each group. Using this method, chronic norethisterone acetate administration to female mice did not affect locomotor activity as compared with a parallel vehicle control group.

The effects of the withdrawal of chronic progestational hormone treatment in male and female mice was also investigated. No differences were seen in observed behaviour, relative to vehicle control, in male mice after progesterone withdrawal, or in female mice after the withdrawal of either progesterone or norethisterone acetate. Again this possibly reflects the relative crudity of the method.

Due to the availability of only one locomotor activity recording device, it was not possible to study the effects of hormone withdrawal in both a hormone group and a vehicle control group simultaneously. Therefore, as the experiment was designed to investigate the effects of the hormone withdrawal, locomotor activities following hormone withdrawal were compared with activity counts prior to hormone withdrawal (ie during hormone administration). Using this measure withdrawal of the vehicle control treatment did not affect the locomotor activity of these animals, however progesterone withdrawal significantly decreased locomotor activity in female mice, as compared with locomotor activity levels during progesterone administration. Norethisterone acetate withdrawal significantly increased locomotor activity in female mice, as compared with levels during drug administration. There was no effect of progesterone withdrawal in male mice. The fact that withdrawal of norethisterone acetate and progesterone had opposite effects in female mice, whilst

withdrawing of both these drugs produced decreased brain 5HT, suggesting that the changes in locomotor activity are unrelated to the brain 5HT.

Plate-crossing was measured for all groups following drug withdrawal. No effect on plate-crossing relative to vehicle control, was seen in male mice following progesterone withdrawal. Female mice exhibited an increase in plate-crossing relative to vehicle control, following withdrawal of both norethisterone acetate and progesterone. From Chapter 7, withdrawal of both progesterone and norethisterone acetate in female mice produced an increase in whole brain 5HT : 5HIAA ratios relative to vehicle control. This suggests that the'anxiolytic' action of withdrawal of both progesterone and norethisterone acetate may be related to a decreased metabolism of 5HT, however whether this is a decreased metabolism of 'non-functional' 5HT, or a decreased utilisation of 'functional' 5HT is unclear.

A summary of these conclusions drawn from the results of chapters 5 - 7 shows that sudden withdrawal of chronic administration of progestational hormones in femalemice produces decreased brain 5HT concentrations. As described in the Introduction, a decrease in central 5HT may be related to the depressed affect frequently seen at times of sudden decrease in plasma progesterone in human females, eg premenstrually and puerperally. Chronic administration of progestational hormones was also seen to produce increased brain 5HT via some mechanism other than an increase in plasma total TP. This effect of these drugs may be involved in the reported ability of progesterone and dydrogesterone to prevent

premenstrual syndrome and post-partum depression in humans. However, if the anecdotal reports of Dalton (1971) are accepted, only progesterone is active in the prevention of these syndromes, although dydrogesterone has recently been reported to be therapeutically active against premenstrual syndrome (Kerr et al., 1979). Within this thesis however, chronic administration of norethisterone acetate was also seen to increase brain 5HT in female mice. Hence it is possible that the increases of 5HT in mouse brains are unrelated to the therapeutic actions of these progestational agents in humans.

In addition to this, the results of behavioural tests performed during the hormone treatments show that the majority of the behavioural results are different from those that may be expected from increases in central 5HT activity. Therefore it is probable that the changes in brain 5HT seen following either chronic hormone administration, or acute hormone withdrawal are produced via actions on a 'non-functional' pool of 5HT and that there may be little or no effect on 'functional' 5HT utilisation. These results therefore suggest that in humans, the mood changes seen at times of progesterone decline, ie premenstrually or puerperally may not be related to progesterone-induced changes in brain 'functional' 5HT synthesis or metabolism.

The results of these chapters have also shown that alterations in plasma total TP and NEFA seen in humans during late pregnancy and the puerperium are probably independent of the plasma progesterone changes that occur at these times. The TP 'riser' and 'non-riser' subgroups of Handley and colleagues (1980) are also probably unrelated to plasma progesterone.

GENERAL DISCUSSION

General Discussion.

The aims of this thesis were to investigate some of the factors that may be involved in the aetiology of the hormone-related affective disorders eg premenstrual syndrome post-natal depression and perimenopausal depression. Whilst both sociological and biochemical factors were investigated, the emphasis of the thesis lay on biochemical factors, especially those biochemical factors which may affect 5HT metabolism in the CNS. In addition to this, some of the possible factors that may be involved in the TP 'riser'/'non riser' phenomenon of Handley and colleagues (1980), were studied in order to attempt to explain the observed link between this phenomenon and post-partum affective disorder. A general discussion of the studies and their implications will be presented here, a more detailed discussion of specific results has been presented previously.

Studies of this type have many problems associated with them. Some of these problems were recognised at the outset of the study, and therefore considered at the design stage of the experiments, other problems were seen only after the outset of the study. These problems will now be presented and some of the methods that may be used to overcome them will be discussed.

One problem in any area of psychiatric/psychological research is the diagnosis and definition of the illness or mood state under consideration. In the clinical situation, the majority of psychiatric diagnoses are made following an interview of the patient by a clinician. The information gained is added to a knowledge of the patient's medical history and a background

of comments made by friends and relatives of the patient. All of this gathered information is then analysed and compared with the clinician's personal concept of the patient's disease state concerned. This 'concept' of the illness is based largely on the clinician's previous experience, the final diagnosis is then made by comparing the patient's symptoms with those symptoms involved in the personal concept of the illness. For the majority of cases, this diagnostic process is of sufficient accuracy to allow for the treatment of the patient. However, in some situations, two independent clinicians may diagnose a different illness 'label' for the same list of symptoms. This method has therefore inherent problems in that each clinician works from his own personal criteria based on his own personal experience. Therefore, in a sense, each diagnosis is personally defined. The recent development of a standard set of psychiatric diagnostic categories, the Research Diagnostic Criteria (Spitzer et al, 1978), attempted to overcome this problem, however these standards are not yet used by all workers. It is of interest to note that there are no categories for premenstrual syndrome or 'blues' within these research diagnostic critera.

Another problem associated with interviews is that each interview situation is unique. Interactions between a male interviewer and a female interviewee are different from those between a male interviewer and a male interviewee (eg Harris, 1971). Similarly the personal appearance, facial expression, tone of voice, etc of the interviewer may all influence the patient's responses (eg Harris, 1971; Bell, 1978; Kendell, 1978). Most of the work mentioned above has been performed using

normal healthy subjects, therefore the effects of these variables on the behaviour of psychiatrically disturbed patients is unknown. All of these variables may therefore contribute to a different diagnosis of the same symptoms by two different clinicians.

The most common method used to overcome these problems of interviewer/interviewee interactions, and differing criteria is the use of mood questionnaires. Such questionnaires set a fixed format of questions, with fixed response sets, and used fixed scoring keys with fixed diagnostic criteria. Therefore the administration of such questionnaires removes many of the problems of interviews as listed above. In addition, it is possible for different clinicians to use the same questionnaires as long as the questionnaires are presented in a uniform manner, and the results from different research centres would thus be comparable. This uniformity offered by the use of such questionnaires makes them important tools for psychiatric research.

Mood questionnaires do, however have problems. A competent level of reading ability and comprehension is required on behalf of the patient. It would not be possible for an illiterate subject to complete a self report questionnaire. It is also possible for subjects to deliberately give incorrect responses (ie to lie) and therefore to invalidate the questionnaire. It would of course be similarly possible for a sufficiently adept actor to lie throughout the course of an interview. Another problem of mood questionnaires that arose during the research for this thesis was that subjects sometimes reported that none of the available responses adequately described their mood. In such cases, the subject's desired response had to be forced into one of the available response

categories. The final questionnaire score therefore missed some of the subtleties of symptom descriptions. Problems of this type may explain the recent report of Kearns and colleagues (1982). These workers reported that many psychiatric questionnaires, including the Beck Depression Inventory, were unable to sufficiently differentiate between the different severities of depressive illness. This might be explained by the restricted response choice. Another problem of mood questionnaires is that severe psychiatrically ill patients may be unable to complete them. However it is similarly impossible to rate severely depressed patients by interview due to the presence of such features as stupor or delusional ideation (Kearns et al., 1982).

Both methods of psychiatric assessment therefore have associated short falls. The study of post-partum mood changes presented within this thesis attempted to overcome these problems by using both mood questionnaires and semi-structured interviews for the assessment of mood. The final mood assessment was therefore made by considering both interview and mood questionniare data.

When considering the results of the present study, it is of interest to note the low scores of the BDI immediately post-partum. The 80th percentile score was 4 and the questionnaire was of little use in the differentiation of 'blues' cases and non cases.* This may reflect the inability of the BDI to measure mood of the severity present in 'blues'. An alternative explanation may be that 'blues' is a different entity from

*The form of the BDI used in this study was a shortened version of the standard version (See Methods). This partially explains the low scores obtained in the present study compared with scores obtained by other workers (eg Handley et al., 1980)

any other non-puerperal depressive mood changes. This latter explanation would indicate that standard depression rating scales are inappropriate in the measurement of 'blues'.

Another problem to emerge from this project was the definition of post-partum depression. The incidence of this disorder is generally reported as being about 10 per cent (Pitt, 1968; Dalton 1971; Kumar & Robson, 1978). The present study reports an incidence of 20 per cent. This higher figure is supported by the work of Nott (1982). The disparity appears to arise from two sources. Firstly the present study followed up mothers at 9 months post-partum, whilst Nott (1982) used a period of one year post-partum. In contrast, previous workers (eg Pitt, 1968; Kumar & Robson, 1978) used a follow-up of 3 months post-partum. These different definitions of the post-partum epoch may explain the different reported incidence rates of the syndrome. A uniform time period is therefore required. A second source of this disparity is that the present study, together with that of Nott (1982) report on cases exhibiting 'depressive symptoms', whilst Kumar and Robson (1978, 1982) discuss 'mild neurotic depressive disorders'. A more stringent criterion of the depression is therefore required.

Similarly, although not relevant to the results of this thesis, dispute exists concerning the timing of puerperal psychosis. It is unclear whether a psychosis is puerperal if it presents within one year of childbirth, or whether the illness must present within 1 week of delivery in order to be classified as puerperal (Brockington, 1982). Again a uniform standard is required.

Another problem encountered within this research project was that of extensive statististical analysis of experimental data and the resultant levels of probability. Throughout this thesis a finding was considered as being statistically significant if the probability of the result occurring by chance was less than 5%. However, this decision inherently meant that 5 out of every 100 statistical tests may produce significant results by chance. The number of chance results can be reduced by either decreasing the number of statistical tests performed, testing the data only for the purpose for which the study was designed, or by using a more stringent level of significance. Within this thesis, multiple tests were performed, many of which were irrelevant to the purpose of the study. This was due to the relative ease of computer programming for testing of all data rather than testing for selected data. However, only those comparisons designated by the design of the study were interpreted further. The 5 per cent level of significance was retained within this thesis as it was considered to be of greater value to isolate any possible correlates of mood change, accepting that some results would be 'false positives', than to 'miss' some possible correlates of mood change by the use of more stringent significance levels. Results from this thesis must now be verified by replication in further studies.

One point to emerge from these studies was the dissimilarity of the hormone-related mood changes to other psychiatric conditions. 'Blues' is generally regarded as being a period of transitory depression and tearfulness occurring immediately post-partum (Pitt 1973). Many of the subjects of the present study reported feelings of tearfulness, irritability and mood lability post-partum, this they recognised as being symptomatic

of 'blues'. Subjects frequently reported that although they felt tearful, they did not feel sad or depressed. Similar findings by other workers have led to the suggestions that irritability, rather than depression may be the predominant symptom of 'blues' (Stein, 1982). If this is so, then the use of depression rating scales for the assessment of 'blues' may be inappropriate. Many of the subjects also reported that the symptoms of 'blues' were unlike anything that they had previously experienced. But in spite of this, subjects were generally easily able to decide whether they had suffered from 'blues' or not. This indicates that there is a definite set of symptoms which subjects readily label as 'blues', whilst such symptoms are not readily labelled as depression or anxiety, etc. This suggests that 'blues' is a unique entity, and not a mild version of any other psychiatric condition.

There are anecdotal reports of a 'blues-like' syndrome occurring in other post-operative situations (Parsons - personal communication). However, no systematic studies of such a syndrome are known of. If post-operative 'blues' does exist, it would indicate that the psychological symptoms are associated with the operative procedures rather than any biochemical change associated with childbirth. There are however problems in testing such a hypothesis as in contrast to childbirth, the majority of surgical operations are associated with illness or abnormality and may be an emergency or life-threatening situation. Other forms of surgical procedures may involve some form of loss (eg hysterectomy). Therefore there is not an adequate operative control for the process of parturition without the attendant major biochmical

changes that are involved in pregnancy or parturition. If 'blues' is a syndrome not specific to parturition then this fact may indicate that the symptoms are brought on by some non-specific 'operative' stress, rather than any fluctuation of the female sex hormones.

Similarly, delirium has been noted post-operatively (Morse, 1970). Post-operative depression following leg amputation has also been reported (Ritchie, 1977), however, it is unclear whether such a response therefore is a sequel to the operation per se, or to the loss of a limb.

Just as 'blues' may be a unique syndrome, unrelated to any other psychiatric state, it is possible that the more serious post-partum depression may be an entity separate from other forms of non-puerperal depression. Pitt (1968) reported that post-partum depression was generally 'atypical' showing different features from those of 'classical' depression. These symptoms are reported to be similar to those of reactive or neurotic depression rather than endogenous depression (Sargant, 1961). It may therefore be that post-partum depression is different from other forms of 'classical' depression and therefore the underlying causative factors may be unrelated to any factors that may underlie 'classical' depression.

Similarly post-partum psychosis may be separate from other forms of psychoses. Within this thesis no studies have been conducted on psychosis, and none of the subjects became psychotic, however the evidence for the differentiation of post-partum psychoses from other psychoses will be presented.

From a cluster analysis of 147 schizophrenic patients, Hays (1982) was able to show that puerperal psychotics were different from other manic-depressive schizophreniform psychotics on several factors, although the illnesses were seen not to be immedately distinguishable clinically. The non puerperal psychotic patients were seen to have evidence of some genetic predisposition to the illness. However, the puerperal psychotics were seen to be a different group, with a family history of depression following oral contracept- and menopausal depression. Brockington and colleagues (1978) reported that puerperal psychoses generally exhibited a better prognosis than other similar non-puerperal psychoses. These factors would suggest that puerperal psychosis may be a separate entity with manic-depressive schizophrenic characteristics. However, there is no special category for puerperal psychosis within the research diagnostic criteria (Spitzer et al., 1978).

The depressive symptoms occurring perimenopausally may also be unique in nature. There may be a subgroup of menopausal depressed patients which respond to hormone replacement therapy, whilst the remainder must be treated by conventional anti-depressants or psychotherapy (Parsons - in preparation). It is therefore possible that the population of menopausally depressed patients contains a subgroup of patients suffering from a hormone related illness, and which may be treated by hormone replacement therapy, the remainder of the patients may be comprised of classical endogenous and reactive depressives. These putative subgroups cannot be readily differentiated in terms of symptomatology (ibid). This evidence therefore suggests that there may be a hormone-related perimenopausal depression which may be unrelated to other

forms of depression.

The final type of hormonally-related disorder to be considered is that of premenstrual syndrome. Research concerning this syndrome is vague, much of the early research utilising populations of female undergraduates (eg Sutherland & Stewart, 1965). However, recent work suggests that premenstrual syndrome is a major disorder, occurring mainly in parous women, aged 25 to 35 years (Sampson, personal communication). A strictly defined chronological cyclicity must now be established before the condition is labelled premenstrual syndrome (Sampson & Jenner, 1977). However, such cyclical mood changes are not always related to the premenstruum and symptoms may recurrently occur at other stages of the menstrual cycle (Sutherland & Stewart, 1965) or in patients with amenorrhoea (ibid). It is therefore possible that the cyclical symptoms, which are similar in character irrelevant of their time of presentation, may be produced by some cyclical changes other than the female sex hormones (ibid). The premenstruum is however the peak time of symptom reports with a set group of symtoms (see Introduction). Thus, premenstrual syndrome is a unique collection of both somatic and psychological symptoms, dissimilar to any other psychiatric condition as described in the research diagnostic criteria. Hence, it would appear that the premenstrual syndrome is a unique entity, although the term 'premenstrual' may be a misnomer.

From the preceding paragraphs therefore, it appears that the hormonally related affective disorders are symptomatologically distinct from the other forms of affective disorders and that they may possibly be produced by distinct aetiological factors. There is however still the attraction of the fact that

mood changes occur in human females at times of hormonal flux, and therefore the underlying causes of the mood changes may be the same for all of the hormone-related syndromes. This idea is contradicted by the results of this thesis, which indicate that there is no interrelationship between the various hormone-related mood syndromes. This suggests that there is no underlying predisposition common to all of these syndromes (See Discussion of Chapters 1 - 4). In addition, the differing symptomatologies of the hormone-related syndromes further suggest that each of these syndromes is itself unique, and possibly produced by unique factors.

These results suggest any findings concerning the biochemical aetiology of the hormonally-related depressive syndromes cannot be generalised to cover all of the hormonally-related syndromes, nor can the results be extrapolated to other forms of depression. However, the studies are of value in their own right in that the study of the causative factors underlying each of the unique hormonally-related syndromes may eventually lead to the effective treatment of that syndrome.

One of the aims of this thesis was to seek a possible biochemical explanation for the reported efficacy of progesterone supplements in premenstrual syndrome and more recently, the prophyllaxis of 'post natal depression' (Dalton, 1982). Sampson (1977) failed to find any difference between progesterone and placebo in the treatment of premenstrual syndrome, but further support for the work of Dalton was produced by Kerr and colleagues (1982) who reported the efficacious use of dydrogesterone in premenstrual syndrome. The possible mode

of action of such progesterone replacement is unclear.

No differences in plasma progesterone have been seen in premenstrual syndrome sufferers (O'Brien et al., 1979) or post-partum 'blues' or post-partum depression sufferers (Nott et al., 1976; Chapter 4, this thesis). Therefore a simple hormone replacement mode of action seems unlikely. However, progesterone 'withdrawal' was seen to produce decreased brain 5HT concentrations in mice, with chronic high dose progesterone producing increased brain 5HT levels in mice (Chapter 7). Hence, possibly a relative fall in progesterone decreases brain 5HT concentrations, whilst progesterone supplements may increase brain 5HT concentrations. As brain 5HT has been implicated in depressed affect (see introduction), it is possible that changes in plasma progesterone may affect mood via an effect on brain 5HT. However, the results of the behavioural studies within this thesis suggest that any progesterone-induced changes in brain 5HT concentrations are non-functional. This conclusion assumes that the tests used would have been able to show the changes in brain 5HT. In addition, progesterone has been shown to be unrelated to the plasma total TP rise phenomenon that was reported to be associated with the incidence of 'blues' and depression post-partum (Handley et al., 1980). This conclusion was possible as there were no differences in plasma progesterone between TP 'risers' and 'non risers', also progesterone, when administered to mice, did not produce the same changes in plasma TP that is seen in human pregnancy and puerperium (Chapters 4 & 6). The mode of action of progesterone in the treatment of these conditions therefore remains unclear.

Another aim of this thesis was to investigate some of the possible biochemical correlates of the hormone-related psychological syndromes. The results of these studies have been discussed previously. The results of greatest interest were a decreased plasma total TP immediately post-partum in 'blues' cases, and elevated post-partum plasma NEFA in subjects who go on to become depressed post-partum. As described previously, the decreased total TP in 'blues' cases may be related to the finding of a subgroup of TP 'non risers' by Handley and colleagues (1980). Also this subgroup of TP 'non-risers' may be produced by some mechanism acting on plasma LNAA, the precise details of which are unknown (See Discussion - Chapters 1 to 4).

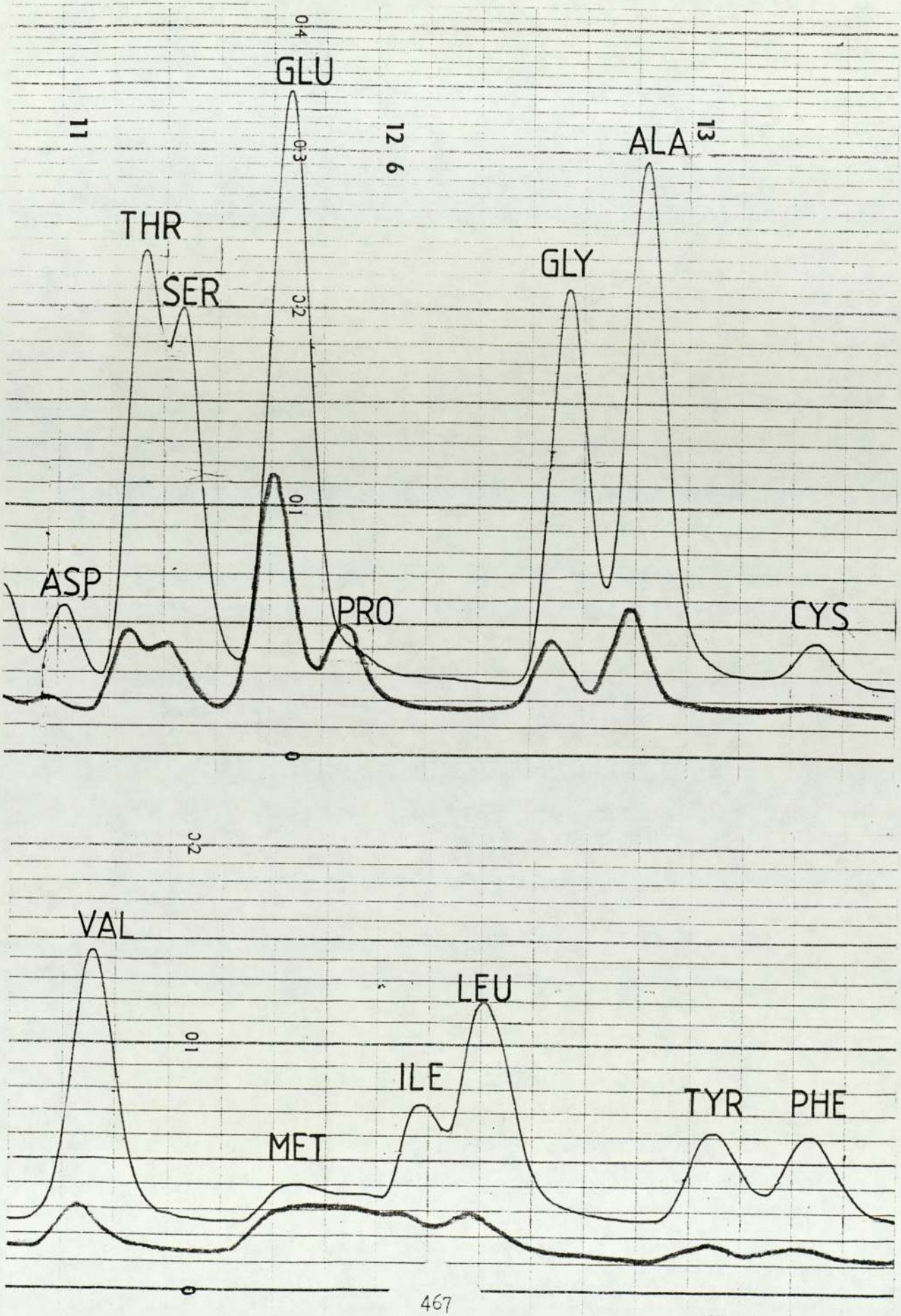
It might therefore be of interest to investigate the mechanisms involved in the control of plasma TP further. The relationship between any such factors and the aetiology of the puerperal mood changes should also be investigated. One possible direction that this research may take would be a study of membrane transport of TP. Woods and colleagues (1979) reported that platelet uptake of TP was increased amongst endogenous depressives. This finding supported the possibility of some membrane transport dysfunction in depression, as previously postulated by Sandler and colleagues (1975). Hence it would be of value to study any changes in membrane transport that occur during pregnancy and the puerperium, and to investigate any relationship between these changes and mood changes.

Similarly, the finding of increased plasma NEFA immediately post-partum, amongst those subjects who became depressed within 9 months of parturition merits further investigation. Plasma NEFA is released by the action of adrenaline on

β-adrenoceptors (Himms-Hagen, 1967), this result therefore suggests a possible chronic supersensitivity of peripheral β- adrenoceptors amongst subjects who are predisposed to depression. In addition, chronic anti-depressant treatment in rats has been shown to produce a down regulation of cortico1β- adrenoceptors (Banerjee et al., 1977). It therefore is possible thatβ- adrenoceptors are involved in the aetiology of depressive illness. It would therefore be of interest to study central and peripheralβ- adrenoceptors during pregnancy and the puerperium in animals, in man and to compareβ- adrenoceptor sensitivity between depressed patients and normal controls.

APPENDICES

Appendix I Sample amino acid autoanalysis printout



Appendix II 'Blues' inventory

ate ..... Record No .....

ay ..... Sample No .....

esterday I felt	much better than usual	0
	better than usual	0
	about the same as usual	0
	more unhappy than usual	1
	much more urhappy than usual	2
esterday I felt	much the same all day	0
	"up and down" in my mood	1
esterday	I felt like crying but did not cry	1
	I cried once or twice	2
	Cried on and off for a long time	3
ast night	I slept as well as possible under the circumstances	0
	I did not sleep as well as possible	1
esterday	I felt as calm as usual	0
	I felt more worried than usual	1

you were upset at all yesterday - can you write below
y reason for this?

Maximum possible score: 8

Appendix III Interview schedules

ANTENATAL INTERVIEW

Primagravidae/Multigravidae

Primiparae/Multiparae

Planning - (ex-infertile)

Previous menstrual history - PMT?

How has the pregnancy been - Physically: 1st, 2nd, 3rd } Trimester

Emotionally: Mood lability
Depressed
Elated
Anxious

Crying epidosdes (inc. reason)

Particular anxieties: Labour, Hospitals, morbid preoccupation with normality of baby?

- specific reasons
- does this cause sleepless nights?

Broodiness, loss of sexuality/attractiveness

Relationship with husband/and other children:

- mutual planning
- husband helpful and considerate
- sexual activity

Preparation: Literature/Maternal/Sibling

Talkativeness/Hostility

DAILY INTERVIEW

Subjective mood:

- Much better
- Better
- Same
- Worse
- Much worse

Objective mood (inc. talketiveness)

Crying episodes: How many?
Time of day?

What emotions have been felt?

- Misery (Dep)
- Anger
- Anxiety
- Elation

- Has mood been labile?
- Do little things upset you?
- Are your anxieties about the baby?
  or other:

Have you been irritable

- who with?
- when?
- at visiting time?

How are you sleeping?

- sleeping tablets?

How's your appetite?

Breakfast

- satisfactory
- unsatisfactory —— Fasting
  \ wrong or excessive

Can you concentrate as usual?

How is the baby feeding -+(mode)

DAY FIVE INTERVIEW

Observer rating of Blues YES/NO Severity: Nurses (Kardex) comments:

Have you heard of 'baby blues' YES/NO

Do you think that you have had the blues with this baby?

YES/DON'T KNOW/NO

Severity:

Have you ever had the blues with any previous babies?

YES/NO/IRRELEVANT

Can you describe the blues in your own words?

Is the blues like any other feeling you have had at a time when you have not had a baby?

YES/NO

If so, when?

Do you ever get PMT?

Post natal follow-up

Visits to GP < Baby / Mother

What for?

General health since birth < Mother / Baby

Baby's sleeping habits — What time go to bed and wake up?

Baby's feeding habits — any problems?

Mothers sleeping — insomnia?

Mothers appetite.

Mothers feelings since birth

Depression
Anxiety
Irritability

When and for how long.

Relationship with husband — Better/ worse

Sexual activity

Social life

Does husband help with baby? — including getting up at night?

Does husband play with baby

Is there any jealousy amongst other children

How does mother react to other children and other mothers?

Does mother go out with baby very often?

Does mother ever feel lonely at home?

Does mother mix with other young mothers?

Overall has life changed for better or worse?

Would mother gladly go through pregnancy and birth again?

Does mother want to go on to have any more children?

What has been the most difficult time since the delivery?
Why?

Appendix IV Menstrual diary

Menstrual Chart

Indicate on the chart the days on which symptoms trouble you using the appropriate letter or letters from the key below.

Fill in months (eg. May.)	Days of month. 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
1st Month _____	
2nd Month _____	
3rd Month _____	

Key to symptoms:
D depression. P pain – backache or headache. T tension or irritability. F fatigue. B bloated feeling. M menstruation.

Appendix V Subject history coding forms

Record No. [ ][ ][ ] (1 2 3)

Basic Background Information

1. Age (years)..... [4]

2. Nationality
   British 1
   Other .....2 ....
   (Specify) [6]

3. Marital Status
   Single 1
   Married 2
   Widowed .....3 ...
   Divorced 4
   Separated 5
   Cohabiting 6
   [7]

4. Social Class
   1-5 ..... [8]
   (Specify husbands occupation)

5. Age of Menarche (years) ..... [9]

6. Menstrual Difficulties
   (If Nil write 0 in each box)
   Irregular .....1]... [11]
   Dysmenorrhea .....1]... [12]
   Other .....1]... [13]
   (Specify)

7. Past Medical History
   Non Relevant 0
   Moderate .....1 ...
   Severe 2
   [14]
   (Specify)

8. Past Psychiatric History (Depression)

None 0
G.P. only ..... 1 ... [15]
Psychiatric Treatment 2

9. Past Psychiatric History (Non affective)

None 0
G.P. only ..... 1 ... [16]
Psychiatric Treatment 2

10. Family Medical History

(If Nil write 0 in each box)

Physical ..... 1 ... [17]
Mental (other than affective) ..... 1 ... [18]
Affective ..... 1 ... [19]

(Specify)

20 → 78 = Δ Card No. [0][1] (79 80)

Record No. [1 2 3]

Antenatal Interview

1. Week of pregnancy ..... [4]

---

2. Planning of pregnancy

Planned 1
No Precautions 2
Inadequate precautions 3
Other 4

..... [6]

(Specify:

---

3. Oral Contraception

Never 0
Stopped > 6 months before conception 1
Stopped < 6 months before conception 2
Stopped after conception 3

..... [7]

---

4. Reason for cessation of Oral Contraception

Not Applicable 0
Side Effects 1

(Specify:

Planned pregnancy 2
Other 3

(Specify:

..... [8]

---

5. Switch to other form of contraception

Not Applicable 0
No 1
Yes 2

..... [9]

(Specify:

---

6. Todays Diet

Fasting 1
Satisfactory 2
Excessive 3

..... [10]

Record No. _____

Obstetric Data - Mother

1. Parity

	Gravida	11
	Para	13
No. of:	Live births	15
	Still births	17
	Terminations	19
	Abortions	21
	Multiple	23

2. Current Obstetric Problems

Presence (1) or absence (0) of hyperemesis	25
Duration of any antenatal admission (weeks)	26

Specify cause:-

3. Duration of Pregnancy

Weeks	28

4. Date of Delivery

DDMMYY	29

5. Hour of Delivery

/24	35

6. Induction

	Yes 1	37
	No 0	
Method:	ARM 1	38
	Oxytocin 2	

7. Forceps

Yes 1	39
No 0	

(Specify reason and type:-

8. Post-partum Haemoglobin and Haematocrit

40

Haemoglobin (g/100ml) ..... | | | . | |

44

Haematocrit ..... | | | . | |

---

9. Blood Transfusion

48

Total ml ..... | | | | |

(Specify day(s) p.n.

---

10. Intravenous Infusion

52

Total ml ..... | | | | |

(Specify type, and day(s) p.n.

Record No. _____

Obstetric Data - Infant

1. Number ..... [56]

2. Sex (M=1; F=2) ..... [57]

3. Apgar score ..... [58]

4. Birthweight (grams) ..... [60]

5. Light for dates

Light	1
Normal	2
Heavy	3

..... [64]

6. Maturity

Prem	1
Mature	2
Post	3

..... [65]

Repeat for twins (if none, write 0 in each box)

7. Sex (M=1; F=2) ..... [66]

8. Apgar score ..... [67]

9. Birthweight ..... [69]

10. Light for dates

Light	1
Normal	2
Heavy	3

..... [73]

74	75	76	77	78		
Δ	Δ	Δ	Δ	Δ	0	2

Card No.

Appendix VI MEH consent form

TOWER HAMLETS HEALTH DISTRICT

CONSENT BY PATIENT OR VOLUNTEER TO CLINICAL RESEARCH INVESTIGATION

I, ...

of .....

.....

hereby fully and freely consent to undergo the procedures involved in the clinical research investigation entitled:-

.....

.....

I understand and acknowledge that the investigation is a contribution to medical knowledge and does not form part of any medical treatment I may currently be receiving.

I note that I may withdraw my consent at any stage in the investigation and I acknowledge that the nature, purpose and risks of the procedures have been explained to me by

.....

and that I have had an opportunity to discuss them with him/her. I have received a written explanation of these procedures, a copy of which is attached to this form.

Signed .....

Witness to signature of patient/volunteer and to fact that he/she has read the document and freely given his/her consent. (Witness must not be a member of project team.)

Signed .....

I confirm that I have explained to the patient/volunteer the nature and effect of these procedures.

Signed .....

(Member of project team acting on behalf of Physician/Surgeon responsible for project).

Date .....

Appendix VII BMH consent form

BIRMINGHAM MATERNITY HOSPITAL

CONSENT BY PATIENT OR VOLUNTEER TO CLINICAL RESEARCH INVESTIGATION

I, ...

of .....

.....

hereby fully and freely consent to undergo the procedures involved in the clinical research investigation entitled:-

.....

.....

I understand and acknowledge that the investigation is a contribution to medical knowledge and does not form part of any medical treatment I may currently be receiving.

I note that I may withdraw my consent at any stage in the investigation and I acknowledge that the nature, purpose and risks of the procedures have been explained to me by

.....

and that I have had an opportunity to discuss them with him/her. I have received a written explanation of these procedures.

Signed: .....

I confirm that I have explained to the patient/volunteer the nature and effect of these procedures.

Signed: .....

(Member of project team acting on behalf of Physician/Surgeon responsible for project).

Date: .....

Appendix VIII Postal follow-up questionnaire

Thank you for taking part in our research study, the final part of the project to find out how you have been since your baby was born. In order to do this could please complete the following questionnaire by circling the answer most appropte to the way that you have felt. If there is not a suitable answer please feel e to add any comment of your own. May I remind you that all information that you e us is totally confidential.

POST-NATAL FOLLOW-UP Subject No._____

e you been to see your doctor for any reason at all ce your baby was born? Yes No

yes, when, and what for? .....

e you taken your baby to the doctors for any reason? Yes No

yes, when, and what for? .....

e you, at any time since your baby was born, felt any of the following?

f yes, please state when.

ressed	Definitely not	Not more than before I was pregnant
	Yes sometimes	Yes severely

If yes, - how often? One episode Two or more episodes
How long did the feelings last?

Less than 1 day
1 day - 1 week
1 week - 4 weeks
more than 4 weeks

When did the feeling start? [ ] month after the birth

Compared with other people, do you think that you feel miserable

more frequently	the same	less frequently

Have you been to see the doctor about your feelings?

Yes
I went to the doctors about other things but really wanted to tell him how 'down' I felt
I thought about it, but didn't go in the end
No

If yes, could you please give details of advice or treatment given by the doctor? .....

able Yes No When?

ed about anything Yes No When? What about?

ertired So that you fall asleep during the day | Yes | No | When?

at time does your baby go to sleep? .....

at time does he/she wake up? .....

es he/she wake during the night? | Yes | No | how often? .....

not disturbed by the baby, do you ever have trouble getting off to sleep? Yes No
you ever find yourself waking very early? | Yes | No |

es your husband help with the baby | A lot | Sometimes | Never |

s your baby affected your relationship with your husband?
| Put it under strain | No change | Improved |

s the physical side of your relationship changed?
| Decreased | No change | Increased |

s your appetite changed since before your pregnancy?
| Decreased | No change | Increased |

your weight changed since before your pregnancy?
| Decreased | No change | Increased |

you feel physically back to normal | Yes | No |

your life changed since your baby was born? | Better | No change | Worse |

your social life changed? | Better | No change | Worse |

often do you go out during the day? (eg Shopping etc.)
| Once per week | Twice per week | Daily |

ou ever feel lonely at home? | Yes | No |

there any other young mothers or friends that live near you, that you mix with?
| None | 1 or 2 | lots |

ing back, would you gladly go through the pregnancy and birth again?
| Yes | No |

ou want any more children? | Yes | No |

there any other comments that you would like to make about the last nine months
e your baby was born?

Thank you

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