PLATELET 5-HYDROXYTRYPTAMINE UPTAKE
IN AFFECTIVE DISORDERS

by

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SUMMARY

There is evidence that blood platelets accumulate, store and release 5-hydroxytryptamine (5-HT, serotonin) in a manner analogous to the central nervous system serotonergic synaptosome. The biogenic amine hypothesis of affective disorders states that depression is associated with a deficiency of brain 5-HT. Therefore, a study was carried out to compare the platelet 5-HT uptake characteristics of platelets from depressive patients and healthy control subjects.

The rate of platelet 5-HT uptake was lower in depressive patients than in age and sex matched control subjects. There was no correlation between severity of illness and rate of 5-HT uptake, and the results for a group of patients tested both when depressed and again after recovery were similar on the two occasions. It is suggested that a low rate of 5-HT uptake is a trait contributing to a predisposition to depressive illness.

A seasonal variation in 5-HT transport was observed in both controls and patients. The rate of 5-HT uptake was lowest in late spring and early summer, which is also the time of year of greatest incidence of depression and the peak in the suicide rate.

Inhibition of 5-HT uptake was observed in depressed patients treated with either antidepressants. The extent of inhibition of 5-HT transport was related to plasma drug concentrations, but did not correlate with clinical improvement.

Prophylactic treatment with lithium increased the rate of platelet 5-HT uptake towards normal values in both unipolar and bipolar patients.

The significance of these findings and their possible relationship to some other biochemical abnormalities observed in depressive patients are discussed.

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DEDICATION

for Robert
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The primary characteristic of the affective disorders is an alteration of mood to such an extent as to cause serious distress or disruption of normal life. The mood is elevated in mania and lowered in depression (Kraepelin, 1921).

An episode of depression is accompanied by one or more of the following symptoms. The patient complains of lack of energy and a loss of interest. The accustomed sleep pattern is disturbed. There is a loss of appetite, leading to loss of weight. The patient is self-reproachful and has a feeling of guilt. Anxiety is common, with irritability and a tendency to worry excessively over trivial matters. There is a loss of interest in sex and a decline in sexual activity. The patient may contemplate or attempt suicide.

Mania occurs less frequently than depression. The manic patient becomes increasingly active and is elated. He talks continually and loses the usual social inhibitions, becoming tactless, and excessively familiar with strangers. Although the patient is cheerful he is intolerant of frustration. There is a lack of insight. There is a reduction in sleep time, an increase in appetite, and excessive sexual activity (Hamilton, 1979).

The affective disorders are commonly recurrent. The first episode usually occurs in the fifth decade, and if it is not treated it will last for 6 to 12 months and then spontaneously remit. There then follows a period of affective normality for several years before a second episode of illness. With each subsequent period of illness—the length of time between each episode decreases, so that after several attacks the subject is suffering from affective illness for a considerable proportion (up to 50%) of his or her life (Coppen, 1974). A patient is described as unipolar if he or she has had three or more attacks of depressive illness. A bipolar patient is one who has had at least one episode of depressive illness and one episode of mania. A patient who has had only one or two attacks of depressive illness and has never had an attack of mania is described as monopolar (Coppen, 1970).
There is evidence that the affective disorders are of a familial nature (James and Chapman, 1975), and can be traced through several generations of the same family. The lifetime morbidity risk for affective disorders is higher in females than in males: Rawnsley (1967) quotes figures estimated for the population of London of 0.8 and 1.4 per cent of live births for males and females respectively. The morbidity risk for unipolar depression is about 12 per cent for first degree relatives of probands with unipolar depression. The morbidity risk for bipolar affective disorder in first degree relatives of bipolar probands is 16 per cent (Smeraldi, Negri and Melica, 1977; Trzebiatowska-Trzeciak, 1977). Leonhard (1962) suggested that unipolar and bipolar subjects are genetically distinct. This hypothesis is supported by several workers (Cadoret, Winokur and Clayton, 1970; Pardue, 1975; Perris, 1973).

THE BIOCHEMISTRY OF AFFECTIVE DISORDERS

(A) **INTRODUCTION**

There are two groups of biochemical factors to be considered in the study of the affective disorders. Firstly, it is necessary to investigate changes related to the onset and duration of the illness. Secondly, there may be abnormalities in these patients that may make them vulnerable to these changes. It is therefore essential that patients suffering from affective disorders should be studied both during a period of illness and after recovery from the illness. The results should be compared with those obtained from control subjects, representative of the normal healthy population (Coppen, 1974). The tissues usually studied are blood and urine, but cerebrospinal fluid (CSF) and post-mortem brain tissue have also been investigated. Another approach is to study the ways that drugs effective in treating affective disorders alter the biochemical functioning or composition of patients, normal control subjects, or experimental animals (Green and Costain, 1979).

There are several major difficulties which have to be considered when investigating the biochemistry of the affective disorders. There is still some controversy about the psychiatric classification of
depression, and it is difficult to measure clinical change quantitatively. There are difficulties in deciding whether any observed biochemical changes are a cause or a consequence of the mood change. Biochemical changes produced by a drug may be unrelated to its therapeutic action.

(B) **THE BIOGENIC AMINE HYPOTHESIS**

(i) **Indirect evidence from drug studies**

The biogenic amines act as neurotransmitters in both the central and peripheral nervous system. There is evidence that the biogenic amines are involved in the aetiology of the affective disorders. Reserpine, an alkaloid used for the treatment of hypertension, was observed to produce severe depression of mood, similar to endogenous depression, in about 15% of cases treated (Harris, 1957). Reserpine and related compounds produce a marked depletion of noradrenaline, dopamine and 5-hydroxytryptamine (5-HT, serotonin) from the mammalian central nervous system and from peripheral tissues by interfering with the intracellular vesicle storage mechanisms for these amines (Carlsson, Rosengreen, Bertler and Nilsson, 1957; Costa, Silber and Murphy, 1977). The drug-induced impairment of the amine-binding mechanisms allows the amines to diffuse from the storage vesicles into the cytoplasm, where the amines are metabolised by intraneuronal monoamine oxidase (MAO) (Iversen, 1973).

It was noted that the anti-tuberculosis drugs isoniazid and iproniazid had mood-elevating properties, and these drugs were found to be monoamine oxidase inhibitors (MAOI) (Zeller, 1955). Following the report by Crane (1956) of the antidepressant effect of iproniazid, several more potent and less toxic MAOI have been used as antidepressants.

These observations of the effects of reserpine and the MAOI led to the formulation of the biogenic amine hypothesis of affective disorders, according to which depression is associated with a deficiency of indoleamines or catecholamines, or both, and mania is associated with either a deficiency or an excess of biogenic amines (Pare and Sandler, 1959; Schildkraut, 1965; Coppen, 1971).
Pare (1965) measured the post-mortem levels of brain 5-HT in patients who had been given a MAOI for varying periods before they died, and found that the drug had to be administered for at least 2 weeks before the levels of 5-HT in the brain tissue began to rise. This period corresponds to the time taken for MAOI to exert their maximum antidepressant effect. In animals, the increase in brain 5-HT and catecholamines that occurs with the administration of a MAOI may be considerably enhanced by feeding the animal large doses of the amino acid which is the precursor of the monoamine. For example, a MAOI given with tryptophan will result in a large increase in the levels of 5-HT and tryptamine in rat brain (Hess and Doepfner, 1961). It has been reported that the antidepressant effect of a MAOI in man is enhanced when tryptophan is given with the MAOI (Coppen, Shaw and Farrell, 1963).

The MAOI are irreversible enzyme-inhibitors and can cause a near total inhibition of MAO activity in all tissues of the body, producing toxic side-effects. The inhibition of MAO in the gastrointestinal tract and liver exposes patients to the potentially toxic effects of dietary pressor amines such as tyramine and β-phenylethylamine, which are normally metabolised by monoamine oxidase and prevented from entering the general circulation. Therefore patients receiving MAOI have to observe certain dietary restrictions.

The tricyclic antidepressant drugs such as amitriptyline and imipramine are potent inhibitors of the reuptake of amines at synaptic terminals, and they therefore increase the effective concentrations of monoamines at the central receptor sites, even though the total brain content is not increased. Although the reuptake blocking activity of these compounds is at present believed to be their main mechanism of action, the degree of reuptake inhibition does not correlate with clinical effectiveness (Ghose and Coppen, 1977).

Apart from their reuptake blocking activity, the tricyclic antidepressants possess other pharmacological properties. They have anticholinergic and antihistaminic properties and cause sedation. It is possible that the tricyclics also act as weak MAO inhibitors: Sullivan, Dackis and Stanfield (1977) reported that blood platelet MAO activity was decreased by 40% in depressed patients after 3 weeks of treatment with either amitriptyline or imipramine. As with
the MAOI, there is some evidence that the clinical effect of tricyclic compounds is enhanced if given in conjunction with tryptophan (Wålinder, et al., 1976).

(ii) Direct evidence from studies of human tissues

(a) Neurotransmitters and their metabolites in brain

There is some evidence that 5-HT and 5-hydroxyindoleacetic acid (5-HIAA, metabolite of 5-HT) concentrations are lower in the brains of depressed patients who have committed suicide than in the brains of people dying from other causes (Shaw et al., 1966; Bourne et al., 1968; Lloyd, Farley, Deck and Hornykiewicz, 1974), although some workers have not been able to replicate these findings (Beskow, Gottfries, Roos and Winblad, 1976; Cochran, Robins and Grote, 1976).

Studies in which catecholamine levels in brain tissues were measured did not find any differences between suicides and controls in the levels of either noradrenaline or dopamine (Bourne et al., 1968; Pare, Young, Price and Stacey, 1969).

There are a number of factors which make it difficult to interpret the results of analysis of post-mortem brain tissues. Not all suicides are the result of depressive illness. Their nutritional state and the type and quantity of drugs taken before death are usually not known. It may be difficult to ascertain the time of day when suicide was committed, and it is known that many biochemical variates show circadian concentration variations. It has been shown that the storage time between death and biochemical assay can significantly affect the results obtained (Coppen, 1974; Beskow, Gottfries, Roos and Winblad, 1976).

(b) Neurotransmitters and their precursors and metabolites in cerebrospinal fluid

There have been many studies of the biochemistry of the cerebrospinal fluid (CSF) of patients with affective disorders. Tryptophan has been found by some groups to be reduced in the CSF of depressives compared with normal controls (Coppen, 1974; Bridges, et al., 1976), but others have reported normal CSF tryptophan levels in depressives (Ashcroft, et al., 1973). Several groups have reported
reduced CSF levels of 5-HIAA in depressed patients (Papeschi and McLure, 1971; Coppen, Prange, Whybrow and Noguera, 1972; Asberg, et al., 1975; Bridges, et al., 1976), but there are also reports of unchanged levels of CSF 5-HIAA (Vestergaard, et al., 1978). Asberg, Traskman and Thoren (1976) found a bimodal distribution of 5-HIAA levels in CSF samples from a group of 68 depressed patients. In the subgroup characterized by a low level of 5-HIAA in the CSF, there was a negative correlation between 5-HIAA concentration and the severity of depression. Also, the patients in this subgroup attempted suicide significantly more often than those in the subgroup with normal 5-HIAA levels, and they used more violent means.

The CSF concentration of homovanillic acid (HVA), a deaminated O-methylated metabolite of dopamine, has been found by some workers to be decreased in depression (Goodwin, Post, Dunner and Gordon, 1973; Papeschi and McLure, 1971; Ashcroft, et al., 1973), although others have reported elevated levels of HVA in depression (Vestergaard, et al., 1978).

Christensen and colleagues (1980) reported that the CSF concentration of adrenaline was significantly lower in depressed patients than in control subjects. When the patients were recovered the CSF adrenaline concentration was found to have returned to normal levels. No significant difference in CSF noradrenaline levels was found between depressive patients and control subjects.

Apart from the difficulties in evaluating this conflicting data, there is some dispute as to the relevance of neurotransmitter metabolite concentrations in lumbar CSF to neurotransmitter availability and function in the brain. It has been shown that in rats there is a significant correlation between brain tryptophan concentration and CSF tryptophan levels (Modigh, 1975; Young, Etienne and Sourkes, 1976). Sjostrom, Ekstedt and Anggard (1975) measured 5-HIAA, HVA, 3-methoxy-11-hydroxymandelic acid (VMA, a metabolite of noradrenaline) and 3-methoxy-4-hydroxyphenylglcol (MHPG, a metabolite of noradrenaline) in CSF from four different regions along the CSF system in 17 patients. They found a pronounced gradient of HAV concentration, with a ratio between the first and last fraction of 1:7. There was a slight increase along the system in 5-HIAA concentration, while MHPG and VMA showed no change. They concluded that lumbar CSF concentrations of HVA reflect dopaminergic activity in the brain, but lumbar CSF concentrations of 5-HIAA and
KMPG/VMA reflect the activity of 5-HT and noradrenaline secreting neurones in both brain and spinal cord.

The administration of probenecid has been shown to cause an increase in the concentration of free tryptophan in plasma by displacing tryptophan from its binding sites on plasma albumin. In rats probenecid causes an increase in brain tryptophan concentration (Tagliamonte, et al., 1971), but the administration of probenecid in human subjects has been shown to have no effect on CSF tryptophan concentration (Young, et al., 1975). However, probenecid does cause an increase in human CSF concentrations of 5-HIAA by blocking the transport of 5-HIAA out of the nervous system, and possibly also by causing an increase in the rate of synthesis of 5-HT, since the increase in free plasma tryptophan may cause an increase in brain tryptophan concentration which might be reflected in increased production of 5-HT rather than in increased brain tryptophan concentration alone. It has been shown that the increase in 5-HIAA after probenecid is much less in depressed patients than in control subjects, which may be due to a reduction in 5-HT synthesis in depression (Goodwin and Post, 1975).

(a) Amino acid precursors of neurotransmitters in blood

The catecholamines are synthesized from the amino acids L-tyrosine and L-phenylalanine, and 5-HT is synthesized from L-tryptophan. The monoamine neurotransmitters are synthesized intracellularly, predominantly at the nerve endings which have an active transport mechanism for the appropriate amino acids. The active transport of amino acids through the blood-brain barrier is saturable and stereospecific (Pardridge and Oldendorf, 1977). The hydroxylation of tryptophan is the rate-limiting step in 5-HT synthesis in the brain as tryptophan-5-hydroxylase is unsaturated with respect to its substrate (Curzon, 1975). Since the synthesis of the neurotransmitters is limited in the brain by amino acid availability, the rates of neurotransmitter synthesis will rise or fall with changes in the plasma concentration of precursor amino acids, and will therefore depend at least partly on nutritional status (Fernstrom, 1973; Curzon and Knott, 1974). Depression is a symptom in certain types of malnutrition, for example, pellagra (Krishnaswamy and Murthy, 1970; Dickerson and Wiryanti, 1978).

The large neutral amino acids compete with each other for
transport into the brain, so that variations in the proportions of the various plasma amino acids may affect brain neurotransmitter synthesis. Furthermore, tryptophan is bound to plasma albumin (McMenamy and Oncly, 1958), and the free fraction is only about 20% of the total plasma tryptophan. Some workers infer that only the free fraction is available for transport into the brain (Curzon and Knott, 1974). There are reports of lowered concentrations of free tryptophan in depression (Coppen, et al., 1973; Baumann, Schmocker, Reyero and Heimann, 1975; Kishimoto and Hama, 1976), although others have reported normal levels of free tryptophan in depression (Garfinkel, Warsh, Stancer and Sibony, 1976; Peet et al., 1976). Niskanen and colleagues reported higher plasma free tryptophan levels in depression (Niskanen, Huttunen, Tamminen and Jaaskelainen, 1976).

The only naturally occurring class of substances which has so far been shown to affect the binding of tryptophan to albumin is nonesterified fatty acids (NEFA). In the rat it has been found that plasma NEFA levels and free tryptophan concentrations are positively correlated (Curzon and Knott, 1974). Plasma NEFA levels are reduced by insulin (Manowitz, et al., 1977). As well as lowering blood glucose levels, insulin also causes a decrease in plasma levels of most amino acids, with the exception of tryptophan, which is increased (Fernstrom, 1973). Thus, the administration of insulin, or the consumption of a carbohydrate meal, causes an increase in the concentration of tryptophan in the rat brain (Fernstrom, Larin and Wurtman, 1973; Tagliamonte, et al., 1975). It is important, therefore, that food intake is controlled in laboratory animals used in experiments involving centrally-acting drugs.

The results obtained with human subjects vary in some instances from those obtained from animal studies. Lipsett observed that after oral glucose administration to fasting humans, the resulting release of insulin caused a decline in both plasma free tryptophan and plasma NEFA (Lipsett, Madras, Wurtman and Munro, 1973). Manowitz found that an injection of insulin administered to healthy fasting humans caused a decrease in plasma NEFA levels but had no effect on free tryptophan concentration, although there was a decrease in total tryptophan and an increase in other plasma amino acids. There was no correlation between free tryptophan and NEFA levels (Manowitz, et al., 1977). Coppen and Wood (1978) found that both free plasma tryptophan levels and plasma NEFA levels were significantly lower in depressed patients than in
control subjects, but they did not find any correlation between free plasma tryptophan levels or percentage free plasma tryptophan and NEFA levels.

It has been suggested that the constant of tryptophan binding to plasma albumin approximates the apparent $K_m$ of tryptophan transport through the blood-brain barrier, so that albumin-bound tryptophan may be stripped off the plasma protein as it traverses the cerebral capillary bed (Pardridge and Oldendorf, 1977). Thus, if the brain's affinity for tryptophan is greater than that of albumin, brain tryptophan levels would show a better correlation with the ratio of plasma total tryptophan to the sum of the competing neutral amino acids that with the ratio of plasma free tryptophan to the sum of its competitors. It has been shown in rats that a considerable fraction of albumin-bound tryptophan is stripped from albumin sites during a single capillary pass from blood to brain, that tryptophan uptake is concentration-dependent, and that amino acid competition for carrier sites is quantitatively the most important factor in regulating tryptophan uptake into brain (Yuwiler, Oldendorf, Geller and Braun, 1977). In a study of 29 control subjects, Young and colleagues found that the CSF tryptophan concentration correlated significantly and positively with the total serum tryptophan concentration, but not with the free serum tryptophan concentration (Young et al., 1975). There is general agreement that total plasma tryptophan concentrations are unaltered in depression (Coppen, 1974; Garfinkel, Warsh, Stancer and Sibony, 1976; Möller, Kirk and Fremming, 1976; Riley and Shaw, 1976; Niskanen, 1976), although there is at least one report of decreased total plasma tryptophan in depression (Baumann, Schmacker, Heyer and Heimann, 1975).

There are conflicting reports on the plasma levels of other amino acids in depression. Kishimoto, Hama and Nagasaki (1978) measured plasma amino acid concentrations in 17 depressed patients and 15 control subjects, and found that of the large neutral amino acids, four were significantly lower in depressives (leucine, isoleucine, phenylalanine, and tyrosine) while two were unchanged (valine and tryptophan). Benkert measured plasma tyrosine concentrations only and found significantly lower concentrations in a group of 38 endogenous depressed patients than in a control group of 26 healthy subjects, though the concentrations in a group of 14 neutotic depressives did not differ from control values.
These results, if substantiated, would refute the argument that competition from other large neutral amino acids is increased in depression.

Möller, Kirk and Fremming (1976) found no difference in concentration of any of the large neutral amino acids between 19 manic depressive patients (18 depressed and 1 manic at time of sample collection) and 25 healthy subjects. However, when the ratio of total tryptophan to the sum of the competing amino acids was examined, a small group of patients showed a low ratio, while the rest showed the same pattern as the controls. Those patients with a low ratio responded well to treatment with tryptophan, while those with normal ratios, with one exception, were resistant to tryptophan therapy. These results agree with those from animal experiments, which have shown the importance of the competition among amino acids for transport into the brain. Both tyrosine and phenylalanine at high plasma concentrations inhibit the passage of the other large neutral amino acids into rat brain (McKean, Boggs and Peterson, 1968). Smyth and colleagues reported that high plasma concentrations of tyrosine caused large reductions in noradrenaline, dopamine and 5-HT in rat brain (Smyth, Tong and D'Iorio, 1977). The increase in brain 5-HT concentration which follows treatments that raise brain tryptophan levels reflects an increase in the synthesis of 5-HT, and not a decrease in the release or metabolism of the monoamine, since brain levels of 5-HIAA, the major metabolite of 5-HT, also rise in parallel (Wurtman and Fernstrom, 1976).

(a) Enzymes of amine metabolism in blood cells

The biogenic amines are metabolised by MAO, dehydrogenase, and catechol-O-methyltransferase (COMT). Blood platelet MAO activity and red blood cell COMT activity have both been shown to be relatively stable characteristics of the individual which are genetically determined (Leckman, Gershon, Nichols and Murphy, 1977), and any changes which do occur are not related to clinical state (Murphy and Donnelly, 1974; Wyatt, et al., 1973; Shulman, Griffiths and Diewold, 1978).

Platelet MAO activity is higher in female than in male control subjects (Wyatt, Potkin, Gillin and Murphy, 1978), and in the majority of women there is a variation in platelet MAO activity through the menstrual cycle, with the nadir occurring 5 to 11 days after ovulation (Belmaker, Murphy, Wyatt and Lorieux, 1974; Wirz-Justice, Puhrisinger, Hole and Menzi,
These data may relate to the higher incidence of depression in women than men (Robinson, et al., 1971), and to the impairment of mood experienced by many women during the pre-menstrual phase (Smith, 1975).

Reports of MAO and COMT activity in patients with affective disorders are not consistent (Gershon, 1978). Platelet MAO activity has been reported as being higher (Murphy and Weiss, 1972; Landowski, Lysiak and Angielski, 1975) and lower (Dunner, Cohn, Gershon and Goodwin, 1971; Nies, Robinson, Harris and Lamborn, 1974) in bipolar patients. Similarly, erythrocyte COMT activity has been found to be both higher and lower in depressed patients (Shulman, Griffiths and Diekold, 1978). It has been reported that MAO activity is lower in the brains of suicides than in the brains of control subjects (Gottfries, Oreland, Wiberg and Winblad, 1974).

There are difficulties in determining the relationship between platelet-MAO and central-MAO activity, for it has been shown that for at least some of the factors affecting MAO activity, e.g. iron deficiency marked changes in peripheral-MAO activity are not reflected in central-MAO activity (Youdim and Green, 1977). It has been shown that the activity of MAO has to be very much inhibited (85% of the enzyme in rat brain) before there is any appreciable effect on monoamine metabolism (Green, Mitchell, Tordoff and Youdim, 1977). Although it is possible that behavioural and mood changes may occur before there are marked biochemical changes, it is also possible that observed natural variations in platelet-MAO activity are behaviourally irrelevant.

(e) Excretion of neurotransmitter metabolites in urine

Since the brain 5-HT pool is very small compared with the peripheral pool, it seems unlikely that meaningful information can be gained by studying urinary amine metabolite excretion. This view is reinforced by the conflicting data obtained on indoleamine metabolite excretion.

There have been several studies which suggest that MHPG (3-methoxy-4-hydroxyphenyl glycol) is the main noradrenaline metabolite in the brain (Schanberg, et al., 1968). It is difficult to assess what proportion of urinary MHPG arises from central noradrenaline metabolism, although Kopin and Ebert (1974) have suggested that the proportion may
be significant, and that the predominant peripheral metabolite is VMA. There are conflicting reports on the excretion of MHPG in depression: some workers have reported reduced MHPG excretion in depression (Greenspan, et al., 1970; Watson, Hartman and Schildkraut, 1972; Maas and Landis, 1968) but others have reported no difference between patients and controls (Bunney, et al., 1972; Coppen, et al., 1979).

(C) ELECTROLYTE BALANCE HYPOTHESIS

The interest in electrolyte balance in the biochemistry of the affective disorders is due to a number of factors. The resting and action potentials of nerve and muscle cells depend on inorganic ions having a different concentration inside the cell to the concentration in the extracellular fluid. The cell membrane is freely permeable to potassium and chloride, but only slightly permeable to sodium. An active system of transport of sodium, mediated by the enzyme Na\(^+\)/K\(^+\)-ATPase, maintains the sodium concentration within the cell at about one-tenth of its concentration in the extracellular fluid. The active uptake of neurotransmitters and their monoamine precursors is linked to the sodium/potassium transport system, and is therefore dependent on the concentration and balance of these inorganic ions. In addition, an inorganic ion, lithium, has been found to be effective in the treatment of the affective disorders.

Schottstaedt, Grace and Wolff (1956) were the first to attempt to correlate changes in sodium balance with changes in mood. They reported that periods of depression in normal subjects were accompanied by a decreased urinary excretion of sodium. Using the isotope dilution technique to measure exchangeable sodium and potassium, Gibbons (1960) found that depressive patients retain sodium while depressed, but they excrete more sodium than normal subjects during their recovery, so that the total body sodium decreased to below normal in recovered patients. These findings have been confirmed by other workers, who have also shown that total body potassium is reduced in depressive patients (Coppen, 1965; Baer, Platmen and Fieve, 1970; Cox, Pearson and Speight, 1971). Plasma concentrations of sodium and potassium are normal in depressed patients, but there are reports of shrinkage in volume of the extracellular space and of total body water (Coppen and Shaw, 1963; Hullin, et al., 1967; Cox, Pearson and Speight, 1971). Shaw, Frizel, Camps and White (1969) examined the fore-brains of depressive suicides, and found that, compared
to a control group, the depressed group brain tissue had a higher water content and a low concentration of sodium. The potassium concentration was also low, although not statistically significant from the control data.

There have been a few studies of magnesium and calcium in depression. Flach (1964) followed the urinary excretion of calcium in depressed patients, maintained on a constant intake of calcium and phosphate, before and during recovery from their episode of depressive illness. Those patients who recovered showed a significant decrease in the excretion of calcium. Plasma calcium levels have been reported to be normal in depressed patients (Gour and Chaudrey, 1957; Frizel, Coppen and Marks, 1969). Plasma magnesium has been reported to be both raised (Cade, 1964) and decreased (Frizel, Coppen and Marks, 1969) in depression.

The administration of lithium salts, which are effective prophylactic agents against the recurrence of depression and mania, cause a marked drop in exchangeable and residual sodium, but have no effect on total body potassium or on the urinary excretion of sodium or potassium (Coppen, 1967). Keynes and Swan (1959) have shown that during an action potential, when sodium normally enters the cell, lithium and sodium enter the cell with equal facility, but lithium is removed from the cell at only 4 to 10% the rate of sodium. Animal studies show that chronic lithium administration results in sodium depletion, which is greater in the CNS than the rest of the body (Baer, Kassir and Fieve, 1970).

The enzyme Na\(^+\)/K\(^+\)-ATPase is present in high concentrations in the brain and in tissues with a secretory function, such as the kidney. The activity of this enzyme depends on the presence of both sodium and potassium, and it drives the pump mechanism whereby sodium and potassium are differentially distributed across the cell membrane. The specific activity of Na\(^+\)/K\(^+\)-ATPase in erythrocyte membranes has been shown to be lower in depressed patients than control subjects (Hesketh, Glen and Reading, 1977). This finding may explain the observed disorders of sodium balance in depressed patients. Administration of lithium to depressive patients has been shown to cause an increase in erythrocyte Na\(^+\)/K\(^+\)-ATPase activity (Naylor, Dick, Dick and Moody, 1974; Sen, et al., 1976; Hokin-Neaverson, Burckhardt and Jefferson, 1976). This finding is supported by the reports of increased uptake of biogenic amines during lithium administration (Murphy, Colburn, Davis and Bunney,
Thus the biogenic amine hypothesis and the electrolyte balance hypothesis are not separate, but are rather two facets of the same problem.

THE BLOOD PLATELET AS A MODEL FOR THE SEROTONERGIC NEURONE

As human platelets transport and store biogenic amines, clinical investigators have searched for platelet abnormalities that might correlate with various neurologic and psychiatric syndromes. Patients with Down's syndrome have reduced levels of 5-HT in their platelets, and this has been shown to be largely due to a defect in the active transport of 5-HT at the platelet membrane (McCoy and Bayer, 1973). Phenylketonuria and histidinemia are associated with mental retardation and considerably diminished platelet 5-HT levels, the mechanism of which is not known. The levels of 5-HT are elevated in platelets from some patients with infantile autism. A platelet 5-HT releasing factor is thought to be present in plasma during migraine headaches, and it has been suggested that a drop in platelet 5-HT may play a role in the mechanism of migraine (Anthony, 1968). Krishnaswamy and Murthy (1970) found that platelet 5-HT levels were significantly lower in pellagrins with mental depression as compared to normal subjects, as well as to pellagrins whose mental status was apparently healthy. Niacin improved the clinical state, but caused a further reduction in platelet 5-HT levels. Possibly niacin facilitates the release of 5-HT and thereby promotes its action at the receptor sites.

No consistent change has been reported in platelet 5-HT levels of depressed patients, but the variations between "normal" values of different investigators is so great that small differences between patients and controls may easily have been hidden in several studies (Sneddon, 1973). Coppen, Turner, Rowsell and Padgham (1976) reported that the concentration of whole-blood 5-HT (i.e. effectively platelet 5-HT, since very little 5-HT is detectable in the blood outside the platelets) was significantly lower during depression, but returned to normal after recovery. Shaw and co-workers (1971) found no difference between depressed patients and healthy subjects in uptake and release of 5-HT by platelets. Hallstrom and colleagues (1976) measured the uptake of both 5-HT and dopamine by platelets from patients with various types of affective disorder. They found no difference in the uptake of either
amine in subjects with "neurotic" depression, but there was a significant reduction in the uptake of both amines in patients with "endogenous" depression. Tuomisto and Tukiainen (1976) reported a reduction in 5-HT uptake by platelets from patients with depression compared with platelets from control subjects.

Using blood platelets from rats, Drummond and Gordon (1975) have demonstrated the presence of three types of receptors for 5-HT on platelets. The binding of 5-HT to one of these sites is responsible for the stimulatory shape change that precedes platelet aggregation in the clotting of blood. This receptor has low structural specificity. A different receptor site is responsible for the uptake of 5-HT into the platelet, and this site has stringent structural requirements (Born, Juengjaroen and Michal, 1972). Bouillin has demonstrated the presence of at least two binding sites for 5-HT on human platelet membranes, one with a high affinity and low capacity, and one with a low affinity and high capacity (Bouillin et al., 1977). Differences between control subjects and depressive patients in platelet 5-HT uptake might be due to temporary or permanent changes in either binding affinity or capacity at the receptor sites on platelets from depressive patients.

**PURPOSE OF THIS THESIS**

In many ways blood platelets act like brain synaptosomes. If initial rates of uptake of 5-HT are measured, the kinetics and pharmacology of the transport mechanism closely resemble the uptake of 5-HT by serotonergic neurones. In this study, the platelet was used as a model for the central 5-hydroxytryptaminergic neurone.

Platelets were incubated for short periods with $^{14}C$-labelled 5-HT, and Michaelis - Menton kinetic analysis was used to determine the characteristics of the uptake process. The value of $K_m$ is an inverse measure of the affinity of 5-HT to bind to platelet membrane transport protein, and is defined as the concentration of 5-HT which is half that required to produce the maximal rate of transport, $V_{max}$. The actual rate of uptake under experimental conditions was expressed as $\bar{y}$, defined as the mean rate of uptake obtained with the different incubation concentrations of 5-HT. This method was also used to determine the characteristics of tryptophan uptake by platelets, although there is less evidence than for 5-HT for an analogy between this system and that
Blood platelet samples for the estimation of 5-HT and tryptophan uptake characteristics were obtained from drug-free depressed patients, drug-free recovered depressive patients and normal healthy control subjects. The results from the different groups were compared, with a view to discovering possible differences between controls and patients, and between patients before and after recovery from a depressive episode. A deficiency in the transport system of platelets from depressive patients, if assumed to reflect the state of the similar transport system in the brain, would be further evidence in support of the biogenic amine hypothesis of affective disorders, since such a deficiency might lead to a reduced neuronal 5-HT concentration.

Platelet samples were also obtained from depressive patients receiving drug therapy for the treatment or prevention of depressive illness, in order to determine the effect of drug treatment on 5-HT transport in vivo. It was hoped that the results may throw some light on the mode of action of some of the more widely used drugs, including a typical tricyclic antidepressant and lithium. Uptake results obtained before and during treatment for depression were examined for possible correlations with clinical state and clinical improvement. As tricyclic and related antidepressant drugs are thought to act by inhibiting the neuronal uptake of 5-HT and/or noradrenaline and dopamine, it was expected that the degree of inhibition of platelet 5-HT uptake would be correlated with clinical improvement.

Both naturally occurring and synthetic compounds (drugs) were added to platelet samples in vitro, before the addition of the $^{14}\text{C}$ labelled 5-HT, to study the effects of compounds of interest on platelet 5-HT transport. If the platelet 5-HT uptake characteristics were found to be changed in depression and/or related to clinical state, this in vitro test system may prove to be of value in the preliminary testing of potential new antidepressant drugs.

Tryptophan is the amino acid precursor of 5-HT. There are conflicting reports on plasma levels of total and free tryptophan in depressive patients. Tyrosine and phenylalanine are precursors of noradrenaline and dopamine. Tryptophan, tyrosine and phenylalanine are
all large neutral amino acids, and probably compete with each other for transport across the blood-brain barrier.

In this study, the plasma concentrations of tyrosine, phenylalanine and total and free tryptophan were measured in samples from normal control subjects, drug-free depressive patients before and after recovery, and patients receiving antidepressant drug therapy. The results were examined for possible evidence of reduced availability of any one or all of these precursor amino acids in the blood of depressive patients, and the effects of drug treatment on the plasma levels. If any changes were found to occur with drug treatment these were tested for correlation with clinical improvement. The ratios between the different amino acids were also examined for possible differences in patients, which might consequently vary the competition between them for the transport system across the blood-brain barrier. The plasma amino acid levels were examined for possible correlation with platelet 5-HT uptake characteristics, as it is possible that any or all of them may compete with 5-HT for transport into the platelet.

In addition, some of the sources of within-group variations in platelet 5-HT uptake characteristics were examined, including the effects of sex, age, whether or not the subject had had breakfast, variations during the menstrual cycle, and the time of year of the blood test. A difference in results between the sexes might contribute to explaining the 2:1 ratio of females to males among unipolar depressive patients. Similarly, a seasonal variation in platelet 5-HT uptake would be a possible cause of the observed seasonal variation in the incidence of depression.

It was hoped that the work described in this thesis would make a contribution towards the understanding of the cause of depressive illness, and that the measurement of platelet 5-HT uptake characteristics might prove to be of use in either the diagnosis or treatment of depression.
METHODS, AND RESULTS OF STUDIES OF CONTROL SUBJECTS

(A) BLOOD PLATELETS AS A NEURONAL MODEL

There is a severe limitation imposed on the investigator of the biochemistry of psychiatric disorders due to the inaccessibility of the brain in human subjects. As yet there are no entirely satisfactory animal models for depression. These problems have led to a search for a more accessible tissue which might serve as an appropriate model for amine-storing neurones.

It has been suggested that blood platelets may serve as a model for the uptake of biogenic amines by the synaptic apparatus in brain tissue. This proposal is attractive for several reasons. Platelets are easily and repeatedly obtainable in large numbers by routine venepuncture. They are readily separated from other blood cells by differential centrifugation. Most important of all, there is strong evidence that blood platelets accumulate, store and release 5-HT in a manner analogous to the CNS serotonergic synaptosomes, and that the same drugs in similar concentrations affect to the same extent the uptake, storage and release of 5-HT in both platelets and synaptosomes (Sneddon, 1973; Stahl, 1977; Maitre et al., 1980).

Human blood platelets take up and concentrate 5-HT from blood plasma in two ways (Stacey, 1961). An active transport mechanism works against a concentration gradient. This transport system is saturable, shows structural specificity, and is reduced by metabolic inhibitors (Pignatti and Cavalli-Sforza, 1975). A passive transport system becomes evident in the cold, and at high concentrations of 5-HT in the medium (Jerome and Kamoun, 1970). There are at least two subcellular transport sites for 5-HT: the platelet membrane and the intracellular storage granules, which serve to protect 5-HT from metabolism by platelet MAO. The binding of 5-HT to the platelet membrane occurs at specific receptor sites, and the transport of 5-HT across the membrane depends on the concentration of Na⁺ (Sneddon, 1969), and that of Cl⁻ (Lingjaerde, 1971). It also depends on Na⁺/K⁺ adenosine triphosphatase (ATPase) activity. Drummond and Gordon (1976) found that adenosine diphosphate (ADP) was a potent non-competitive inhibitor of 5-HT uptake by rat platelets. They concluded that ADP could inhibit 5-HT uptake by changing the Na⁺/K⁺
distribution across the cell membrane.

For this thesis a study was carried out to determine the characteristics of platelet 5-HT uptake in both normal control subjects and depressive patients. The results from the different groups were compared, with a view to discovering possible differences between controls and patients.

(B) **THE DETERMINATION OF 5-HT UPTAKE BY HUMAN BLOOD PLATELETS**

(i) **Methods and Materials**

Blood was collected by venepuncture between 8.30 and 10.00 a.m. after overnight fasting, and mixed with one-tenth its volume of a solution containing 27 mM disodium ethylenediaminetetra-acetic acid (EDTA), 120 mM sodium chloride and 6 mM glucose (Snaw et al., 1971). It was then centrifuged at 350 x g at room temperature for 10 min to obtain platelet-rich plasma (PRP). It was usually found to be necessary to centrifuge the blood samples a second time at 1,500 x g to collect extra platelet-poor plasma (PPP) to add to the PRP to obtain a sufficient volume of PRP. The concentration of platelets in the PRP was measured by diluting 50 μl of PRP with 5 ml of Lempbert-Kristenson's staining solution (Dacie, 1950), and counting the platelets in an aliquot of this mixture using an Improved Neubauer Haemacytometer.

Aliquots (0.5 ml) of PRP were preincubated for 10 min at either 37°C in a shaking water bath, or at 0°C in an ice-water bath, after which time 50 μl of 0.9% (w/v) saline containing 14C-labelled 5-HT (0.25, 0.5, 1.0, 2.0, and 4.0 μM, final concentration in PRP) (Radiochemical Centre, Amersham, U.K.) were added. The specific activity for all 14C-labelled 5-HT solutions was about 2.2 GBq/mmol. The platelets were incubated for a further 2 min, then the reaction was stopped by the addition to each aliquot of PRP of 2 ml of ice-cold 3% (w/v) formaldehyde in 0.9% (w/v) saline (Costa and Murphy, 1975). The platelet suspensions were centrifuged at 1,500 x g at 4°C for 10 min, the supernatant fractions were discarded, and the inside of each tube wiped dry with tissue paper. Aliquots (1 ml) of 1 M KOH were added to each tube. The tubes were sealed with aluminium foil and "Parafilm", and left overnight at 37°C. Aliquots (0.2 ml) of the platelet digests...
were added to 4 ml of scintillation fluid (NE 262; Nuclear Enterprises, Edinburgh, U.K.) and counted for radioactivity in an LKB Ulitrobeta Spectrometer for 30 min ($^{14}$C counting efficiency 90% using external standardization). The results were expressed as picomoles of 5-HT accumulated per $10^8$ platelets per minute. Except where stated otherwise, all chemicals were obtained from BDH.

(ii) Calculation of results

The values obtained for the uptake at $0^\circ$C represent the passive diffusion of 5-HT into the platelets, together with the small quantity of 5-HT trapped between the platelets in the pellet after centrifugation. These figures were subtracted from the values obtained for the total uptake at $37^\circ$C to yield the values for the active uptake of 5-HT by platelets. The results were then analysed in two ways. First, the overall uptake, $\bar{y}$, defined by Hallstom (1976) as the mean uptake over the five 5-HT concentrations, was calculated. This is a measure of the actual uptake of 5-HT by the platelets under these experimental conditions. Second, the $V_{\text{max}}$ (the theoretical maximum rate of uptake) and $K_m$ (the concentration of 5-HT at which the rate of uptake is half the maximum theoretical rate) were determined by Lineweaver - Burk plot (Lineweaver and Burk, 1934). The best fit lines for these plots were obtained by linear regression analysis.

The double reciprocal plot described by Lineweaver and Burk (1934) is the method of plotting which is most widely used (Dixon and Webb, 1964). Two other popular methods of plotting are those of Hanes (1952) ($\frac{s}{V}$ against $s$) and Hofstee (1959) ($V$ against $\frac{s}{V}$). Results for 10 subjects (4 control subjects and 6 depressive patients) were re-calculated using both the Hanes plot and the Hofstee plot, but the values obtained for $K_m$ and $V_{\text{max}}$ were not significantly different from those obtained using the Lineweaver - Burk plot (Table 2.1). The points gave slightly better straight lines for the Lineweaver - Burk and Hanes plots (mean correlations 0.997 and 0.999 respectively) than for the Hofstee plot (correlation 0.970).

(iii) Effects on results of varying incubation time and temperature

Lineweaver - Burk analysis requires the uptake measurements to
### Table 2.1
Comparison of values of $V_{\text{max}}$ and $K_m$ obtained by Lineweaver-Burk plot with those obtained by Hanes plot and Hofstee plot for a group of 10 subjects. Results shown as mean ± S.E.M. (coefficient of variance).

<table>
<thead>
<tr>
<th>Method of plotting</th>
<th>$K_m$</th>
<th>$V_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lineweaver-Burk</td>
<td>$0.53 \pm 0.07$ (43%)</td>
<td>$52.4 \pm 4.9$ (30%)</td>
</tr>
<tr>
<td>Hanes</td>
<td>$0.55 \pm 0.10$ (59%)</td>
<td>$51.9 \pm 4.4$ (27%)</td>
</tr>
<tr>
<td>Hofstee</td>
<td>$0.54 \pm 0.09$ (52%)</td>
<td>$52.0 \pm 4.6$ (28%)</td>
</tr>
</tbody>
</table>
be made during the initial stages of the reaction, when the rate of uptake is greatest, and the relationship between substrate concentration and rate of uptake is linear. Wielosz and co-workers (1976) reported that the uptake of 5-HT by human platelets was only linear for up to 8 min, and reached a steady state after about 15 min of incubation. Lingjaerde (1976), and Tuomisto and Tukiainen (1976) have used incubation times of 4 min to determine 5-HT uptake by human platelets. In the present study an incubation period of 2 min was chosen, as this was the shortest interval which still allowed sufficient time to add the 5-HT solutions to a set of five tubes "in circus". Also, errors in timing might become significant with incubation times of a minute or less.

In order to confirm that an incubation time of 2 min is short enough to satisfy the requirements of Michaelis-Menten analysis, samples of PRP were incubated with 0.5 μM 14C-labelled 5-HT for a range of time intervals (½, 1, 2, 4, 8 and 16 min) (Figure 2.1). Each point is the mean of seven determinations. The uptake of 5-HT appears to be linear for incubation times of up to 4 min. This result agrees with those reported by Wielosz and colleagues (1976).

The range of concentrations of 5-HT used was from approximately one-half to twice the K_m values reported recently for 5-HT uptake by platelets from human control subjects (Gordon and Overman, 1976; Lingjaerde, 1976; Tuomisto and Tukiainen, 1976; Wielosz et al., 1976). (See Table 2.2 for a comparison of results reported in the literature).

Platelet 5-HT uptake at different incubation temperatures (19, 25, 30, 34, 37 and 40°C.) was determined in one sample of PRP (Figure 2.2). Both K_m and V_max increased at an increasing rate with increasing incubation temperature. There was a two-fold increase in both K_m and -V_max with a rise in temperature from 37°C to 40°C: it is therefore important to strictly control the incubation temperature when measuring platelet 5-HT uptake.

In experiments to investigate the effects of drugs and naturally occurring substances of interest, the compound to be studied was added to the PRP in 20 μl of 0.9% (w/v) saline, 5 min before the addition of 14C-labelled 5-HT.
Figure 2.1 Variation in platelet 5-HT uptake with incubation time

5-HT uptake (pmol/10^8 platelets)

Incubation time (min)
Table 2.2
Characteristics of 5-HT uptake by platelets from control subjects reported in the literature

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (pmol/10$^8$ platelets/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gordon &amp; Olverman (1976)</td>
<td>?</td>
<td>1.0</td>
<td>120</td>
</tr>
<tr>
<td>Lingjaerde (1976)</td>
<td>?</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td>Tuomisto &amp; Tukiainen (1976)</td>
<td>10</td>
<td>0.4</td>
<td>92</td>
</tr>
<tr>
<td>Wielosz et al. (1976)</td>
<td>4</td>
<td>1.0</td>
<td>87</td>
</tr>
<tr>
<td>Rotman et al. (1979)</td>
<td>4</td>
<td>1.8</td>
<td>70</td>
</tr>
<tr>
<td>Tukiainen &amp; Leino (1980)</td>
<td>18</td>
<td>0.4</td>
<td>19</td>
</tr>
<tr>
<td>Malmgren et al. (1980)</td>
<td>10</td>
<td>1.0</td>
<td>145</td>
</tr>
<tr>
<td>Arora &amp; Meltzer (1982)</td>
<td>42</td>
<td>0.5</td>
<td>121</td>
</tr>
<tr>
<td>This thesis</td>
<td>81</td>
<td>0.5</td>
<td>44</td>
</tr>
</tbody>
</table>
Figure 2.2 Effect of incubation temperature on $K_m$ and $V_{max}$
In order to establish the normal range of values of platelet 5-HT uptake characteristics in a healthy population a group of 48 female and 33 male control subjects was studied. None of the control subjects had a history of psychiatric illness, and all were in good physical health and drug-free (with the exception of contraceptive hormonal preparations taken by some of the women) at the time of the test. The age range for the female subjects was from 17 to 66 years, and from 20 to 66 years for the male subjects. Blood was collected between 8.30 and 10.00 a.m. after overnight fasting.

There were no significant sex differences in age, platelet concentration or platelet 5-HT uptake characteristics in the control subjects, so the results obtained for the two sexes were combined (Table 2.3). There were no significant correlations between platelet concentration and age, $K_m$, $V_{max}$ or $\tilde{y}$. Significant correlations were found between age and $V_{max}$ ($r = -0.248$, $p < 0.05$) and between age and $\tilde{y}$ ($r = -0.250$, $p < 0.05$). It is therefore important that groups which are to be compared should be of similar age.

The values obtained for $V_{max}$ of platelet 5-HT uptake in control subjects were lower than those reported by other workers (see Table 2.2). However, the methods used by different groups vary in several respects, apart from variations in incubation time mentioned above. Gordon and Olverman (1976) and Wielosz et al. (1976) used tri-sodium citrate as anticoagulant, while other workers, including Lingjaerde (1976, 1979), used EDTA. Therefore a small study was carried out to compare the EDTA anticoagulant used throughout this project with tri-sodium citrate.

A 20 ml sample of blood was collected from each of 7 female and 8 male control subjects, aged between 20 and 56 years. 10 ml of each sample was mixed with 1 ml of the usual anticoagulant (see Section B above), and the remaining 10 ml was mixed with 1 ml of 3.8% (w/v)
Table 2.3
Platelet 5-HT uptake characteristics of control subjects
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (yr)</th>
<th>Platelet conc (x 10^6/ml)</th>
<th>K_m (µM)</th>
<th>V_max (pmol/10^8 platelets/min)</th>
<th>\bar{y}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>48</td>
<td>42.8 ± 1.9</td>
<td>2.76 ± 0.09</td>
<td>0.48 ± 0.02</td>
<td>42.7 ± 2.5</td>
<td>27.6 ± 1.6</td>
</tr>
<tr>
<td>Men</td>
<td>33</td>
<td>39.9 ± 2.4</td>
<td>2.54 ± 0.13</td>
<td>0.51 ± 0.03</td>
<td>45.4 ± 3.0</td>
<td>28.9 ± 1.8</td>
</tr>
<tr>
<td>Women &amp; men</td>
<td>81</td>
<td>41.6 ± 1.5</td>
<td>2.67 ± 0.07</td>
<td>0.49 ± 0.02</td>
<td>43.8 ± 1.9</td>
<td>28.1 ± 1.2</td>
</tr>
</tbody>
</table>
tri-sodium citrate. Apart from the type of anticoagulant used, the method of platelet separation and determination of 5-HT uptake characteristics was the same for all samples.

For all 15 subjects the concentration of platelets in PRP was lower in the citrated sample than in the EDTA sample (see Table 2.4). The anticoagulant made no difference to the value of $K_m$, but the citrated samples produced significantly higher values of $V_{\text{max}}$. It is possible that the extra platelets obtained using the EDTA anticoagulant are a sub-population with a reduced ability to take up 5-HT.

(iii) Comparison of fasting and non-fasting results

At the outset it had been decided to ask all subjects to fast overnight prior to venepuncture in order to reduce the possibility of variations in platelet 5-HT uptake caused by dietary differences between subjects. However, as not everybody was willing to forgo breakfast (or in some cases forgot to do so) it was decided to investigate the effects of "breakfast" on the platelet 5-HT uptake results.

A group of 11 control subjects (5 female, 6 male), aged between 20 and 60 years, were tested on two occasions, once when fasting (nothing to eat or drink from midnight until after the venepuncture at about 9.30 a.m.), and once when not fasting (subjects were requested to consume their usual breakfast). To eliminate the effects of seasonal variation the test-retest interval was restricted to 10 weeks or less, except in the case of one female subject for whom the test-retest interval was 54 weeks. The consumption of breakfast made no difference to the results (Table 2.5a).

It was considered necessary to confirm this result, so the test was repeated on a group of 22 subjects who had breakfast before testing and 22 subjects who fasted overnight. The subjects in the latter group were selected to match the non-fasting subjects as closely as possible for age, sex and month of test. Again, there were no significant differences between fasting and non-fasting subjects in platelet 5-HT uptake characteristics (Table 2.5b). However, the subjects who had breakfast did have a significantly higher concentration of
Table 2.4
Comparison of anticoagulants: blood samples from 15 control subjects

<table>
<thead>
<tr>
<th></th>
<th>EDTA</th>
<th>tri-sodium citrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet conc* (x 10^8/\text{ml})</td>
<td>2.81 ± 0.21</td>
<td>2.08** ± 0.17</td>
</tr>
<tr>
<td>(K_m) ((\mu\text{M}))</td>
<td>0.44 ± 0.03</td>
<td>0.44 ± 0.03</td>
</tr>
<tr>
<td>(V_{\text{max}}) (pmol/10^8 pl/min)</td>
<td>47.9 ± 2.6</td>
<td>58.7* ± 3.5</td>
</tr>
<tr>
<td>(\bar{V}) (pmol/10^8 pl/min)</td>
<td>31.7 ± 1.5</td>
<td>38.9** ± 2.2</td>
</tr>
</tbody>
</table>

Values significantly different are shown * when \(p < 0.01\) and ** when \(p < 0.001\)
Table 2.5a
Platelet 5-HT uptake in 11 control subjects tested with and without fasting
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Test condition</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (pmol/10^8 platelets/min)</th>
<th>$\bar{y}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>0.46 ± 0.03</td>
<td>47.1 ± 3.6</td>
<td>30.6 ± 2.1</td>
</tr>
<tr>
<td>Not fasting</td>
<td>0.48 ± 0.04</td>
<td>48.9 ± 3.8</td>
<td>31.8 ± 2.5</td>
</tr>
</tbody>
</table>
Table 2.5b
Platelet 5-HT uptake in control subjects tested either with or without fasting
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Fasting</th>
<th>Not fasting</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>43.7 ± 3.1</td>
<td>38.0 ± 2.5</td>
</tr>
<tr>
<td>Platelet conc (x 10^8/ml)</td>
<td>2.40 ± 0.13</td>
<td>3.06* ± 0.18</td>
</tr>
<tr>
<td>K_m (μM)</td>
<td>0.50 ± 0.03</td>
<td>0.45 ± 0.03</td>
</tr>
<tr>
<td>V_max (pmol/10^8 pl/min)</td>
<td>44.3 ± 3.3</td>
<td>45.8 ± 2.8</td>
</tr>
<tr>
<td>V (pmol/10^8 pl/min)</td>
<td>28.2 ± 2.2</td>
<td>30.3 ± 1.8</td>
</tr>
</tbody>
</table>

* significantly different p < 0.01
platelets in their platelet-rich plasma. This might be due to fasting subjects having a lower plasma protein concentration, and hence a lower plasma specific gravity, so that some platelets are spun down rather than remaining in the platelet-rich supernatant during the preparation of PRP.

(iv) Effect of delayed assay on platelet 5-HT uptake results

The incubation of PRP with $^{14}$C - labelled 5-HT at 37°C for the determination of active platelet 5-HT uptake was usually carried out between from $\frac{1}{2}$ to 1$\frac{1}{2}$ hours after venepuncture. Two small series of experiments were carried out to investigate the effect of delay. In one experiment a 20 ml sample of blood was collected from each of 3 female and 4 male subjects. 10 ml of blood was prepared as usual, with the PRP being incubated with $^{14}$C - 5-HT at 1 hour after the venepuncture. The second 10 ml of each sample was kept at room temperature and the preparation of PRP was commenced 1$\frac{1}{2}$ hour after the venepuncture. Similarly, 20 ml samples of blood from 3 female and 2 male control subjects were split, with the PRP preparation of the second half of each sample being delayed for 3 hours. The results (Table 2.6) showed that a delay in the preparation of PRP reduced the rate of 5-HT uptake. Moreover, the amplitude of the reduction increased with increasing delay, being 35% when PRP preparation was delayed for 1$\frac{1}{2}$ hour, and 54% when PRP preparation was delayed for 3 hours.

In the other experiment 25 ml samples of blood were collected from 2 female and 2 male control subjects, and PRP preparation carried out immediately after venepuncture as usual. The PRP was divided into 5 sets of 0.5 ml aliquots. One set was incubated with $^{14}$C - 5-HT at 37°C at 1 hour after venepuncture, and one set was used for incubation with $^{14}$C - 5-HT in an ice-water bath. The other 3 sets were kept at room temperature and incubated at 37°C with $^{14}$C - 5-HT at 1$\frac{1}{2}$, 3 and 4$\frac{1}{2}$ hours after the control set. The results showed (Figure 2.3) that delay reduced the values obtained for $V_{\text{max}}$ by 60 to 70%, and increased the values obtained for $k_m$ by up to 350%.

From the results of these experiments, it was clear that the active uptake of 5-HT by platelets should be determined as soon as possible after the collection of the blood sample, preferably with a
Table 2.6

Effect on platelet 5-HT uptake of delay in the preparation of PRP

Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (pmol/10⁸ platelets/min)</th>
<th>$\bar{y}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No delay</td>
<td>7</td>
<td>0.52 ± 0.05</td>
<td>61.1 ± 4.3</td>
<td>38.6 ± 2.3</td>
</tr>
<tr>
<td>1½ hr delay</td>
<td>7</td>
<td>0.37* ± 0.02</td>
<td>38.0** ± 3.0</td>
<td>26.3** ± 1.9</td>
</tr>
<tr>
<td>No delay</td>
<td>5</td>
<td>0.54 ± 0.08</td>
<td>61.2 ± 6.1</td>
<td>38.2 ± 3.0</td>
</tr>
<tr>
<td>3 hr delay</td>
<td>5</td>
<td>0.48 ± 0.05</td>
<td>27.3** ± 1.2</td>
<td>17.8† ± 1.6</td>
</tr>
</tbody>
</table>

Values significantly different are shown * when $p < 0.03$, ** when $p < 0.01$ and † when $p < 0.001$.
Legend to Figure 2.3

**Effect of delaying time of assay on results obtained for 5-HT uptake**

Points shown as mean with standard error bar

\[ K_m \]

\[ A \quad \text{higher than baseline, } p < 0.05 \]

\[ B \quad \text{higher than baseline, } p < 0.001, \text{ and higher than } 1 \frac{1}{2} \text{ h, } p < 0.01 \]

\[ V_{max} \]

\[ C \quad \text{lower than baseline, } p < 0.01 \]

\[ D \quad \text{lower than baseline, } p < 0.01, \text{ and lower than } 1 \frac{1}{2} \text{ h, } p < 0.001 \]

\[ \bar{y} \]

\[ E \quad \text{lower than baseline, } p < 0.001 \]

\[ F \quad \text{lower than baseline, } p < 0.001, \text{ and lower than } 1 \frac{1}{2} \text{ h, } p < 0.01 \]
Figure 2.3 Effect of delay of assay on platelet 5-HT uptake

- $K_m$ (μM)
- $V_{max}$ (pmol/10^8 pL/min)
- $\gamma$ (pmol/10^8 pL/min)

Blood drawn versus Delay (hours)
constant interval between venepuncture and incubation of PRP with $^{14}$C - 5-HT, so that the error due to the unavoidable delay caused by the time taken to prepare the PRP, divide it into aliquots, and preincubate for temperature equilibration, is constant for all samples.

(v) **Effect of a metabolic inhibitor**

A 20 ml sample of blood was collected from each of 5 control subjects. Half of the PRP obtained was used for a control determination of platelet 5-HT uptake, while the second half of each sample was used to determine the effect of ouabain, a metabolic inhibitor. Ouabain to a final concentration of $10^{-4}M$ was added to the aliquots of PRP 5 minutes before the addition of $^{14}$C - 5-HT. In all samples ouabain acted as a mixed inhibitor of platelet 5-HT uptake, causing a reduction in the values obtained for $V_{\text{max}}$ and $\bar{y}$, and an increase in the value obtained for $K_m$ (see Table 2.7). Metabolic inhibitors, such as ouabain, are believed to inhibit the active transport of 5-HT at the cell membrane (Pletscher, 1968). Ouabain added to PRP has been shown to cause an increase in sodium concentration and a decrease in potassium concentration in the platelet (Feinberg et al., 1977). Thus ouabain probably inhibits platelet 5-HT uptake by inhibiting platelet membrane $\text{Na}^+/\text{K}^+$ ATPase activity.

(vi) **Intraindividual variation in platelet 5-HT uptake: effect of the menstrual cycle**

Platelet 5-HT uptake was measured in 3 female and 4 male control subjects on four or more occasions. The results are shown in Table 2.8a, and the coefficient of variance for each subject's PRP platelet concentration and platelet 5-HT uptake characteristics are shown in Table 2.8b.

In female subjects it is possible that at least part of the observed variation in platelet 5-HT uptake is due to variations in hormonal status due to the menstrual cycle. The values of $K_m$, $V_{\text{max}}$, PRP platelet concentration, and basal body temperature (in degrees Fahrenheit, measured on awakening at approximately 7 a.m. each day), obtained from female 1 of Table 2.8 during one cycle of 29 days, were plotted against day of cycle (see Figure 2.4). Basal body temperature gives an approximate guide to hormonal status through the menstrual cycle: it is lowest at ovulation, then increases as plasma progesterone
Table 2.7
Effect of ouabain on platelet 5-HT uptake
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Test condition</th>
<th>n</th>
<th>( K_m )</th>
<th>( V_{max} )</th>
<th>( \bar{y} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0.46 ± 0.03</td>
<td>37.8 ± 3.6</td>
<td>24.9 ± 2.6</td>
</tr>
<tr>
<td>+ ouabain</td>
<td>5</td>
<td>0.76* ± 0.10</td>
<td>16.9** ± 1.3</td>
<td>9.9** ± 1.1</td>
</tr>
</tbody>
</table>

Results significantly different are shown * when \( p < 0.05 \) and ** when \( p < 0.01 \)
Table 2.8a
Intraindividual variation in platelet 5-HT uptake
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Test period (months)</th>
<th>No. of tests (n)</th>
<th>Platelet conc (\times 10^8 /\text{ml PRP})</th>
<th>(K_m) ((\mu\text{M}))</th>
<th>(V_{max}) (pmol/(10^8) platelets/min)</th>
<th>(\bar{y})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female 1</td>
<td>27</td>
<td>27</td>
<td>2.34 ± 0.07</td>
<td>0.41 ± 0.02</td>
<td>34.0 ± 2.0</td>
<td>22.9 ± 1.3</td>
</tr>
<tr>
<td>Female 2</td>
<td>16</td>
<td>5</td>
<td>3.23 ± 0.26</td>
<td>0.51 ± 0.06</td>
<td>44.9 ± 6.4</td>
<td>28.2 ± 3.5</td>
</tr>
<tr>
<td>Female 3</td>
<td>17</td>
<td>4</td>
<td>3.40 ± 0.37</td>
<td>0.43 ± 0.03</td>
<td>30.2 ± 3.4</td>
<td>20.1 ± 2.3</td>
</tr>
<tr>
<td>Male 1</td>
<td>31</td>
<td>9</td>
<td>3.15 ± 0.25</td>
<td>0.56 ± 0.07</td>
<td>36.7 ± 2.6</td>
<td>23.0 ± 1.9</td>
</tr>
<tr>
<td>Male 2</td>
<td>21</td>
<td>7</td>
<td>2.35 ± 0.51</td>
<td>0.50 ± 0.06</td>
<td>40.1 ± 5.4</td>
<td>25.5 ± 3.2</td>
</tr>
<tr>
<td>Male 3</td>
<td>11</td>
<td>5</td>
<td>2.24 ± 0.19</td>
<td>0.39 ± 0.04</td>
<td>38.3 ± 4.0</td>
<td>26.6 ± 3.1</td>
</tr>
<tr>
<td>Male 4</td>
<td>40</td>
<td>4</td>
<td>3.77 ± 0.38</td>
<td>0.47 ± 0.08</td>
<td>28.7 ± 6.7</td>
<td>18.5 ± 3.9</td>
</tr>
<tr>
<td>Subject</td>
<td>No. of tests</td>
<td>Platelet conc&lt;sup&gt;n&lt;/sup&gt;</td>
<td>$K_m$</td>
<td>$V_{max}$</td>
<td>$\bar{y}$</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>--------------</td>
<td>--------------------------</td>
<td>-------</td>
<td>-----------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>Female 1</td>
<td>27</td>
<td>16%</td>
<td>18%</td>
<td>31%</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td>Female 2</td>
<td>5</td>
<td>18%</td>
<td>26%</td>
<td>32%</td>
<td>28%</td>
<td></td>
</tr>
<tr>
<td>Female 3</td>
<td>4</td>
<td>22%</td>
<td>16%</td>
<td>23%</td>
<td>23%</td>
<td></td>
</tr>
<tr>
<td>Male 1</td>
<td>9</td>
<td>23%</td>
<td>36%</td>
<td>21%</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td>Male 2</td>
<td>7</td>
<td>58%</td>
<td>32%</td>
<td>36%</td>
<td>33%</td>
<td></td>
</tr>
<tr>
<td>Male 3</td>
<td>5</td>
<td>19%</td>
<td>25%</td>
<td>23%</td>
<td>26%</td>
<td></td>
</tr>
<tr>
<td>Male 4</td>
<td>4</td>
<td>20%</td>
<td>34%</td>
<td>47%</td>
<td>43%</td>
<td></td>
</tr>
</tbody>
</table>
uptake and basal body temperature during a menstrual cycle

PRP platelet concentration

$K_m$

$V_{max}$

Basal body temperature

Day of menstrual cycle
levels rise, to reach a peak a few days before the commencement of menstruation (Vollman, 1974).

The values obtained for $V_{\text{max}}$ seem to approximately follow the same cyclical pattern as basal body temperature. The correlation between $V_{\text{max}}$ and temperature is high, though not significant, at $r = +0.51$ ($n = 12$). The $V_{\text{max}}$ curve does not correspond to the pattern of secretion of any one of the female sex hormones during the menstrual cycle, and it is probably influenced by a number of interacting variables. Many other factors have been shown to vary according to predictable patterns through the menstrual cycle, including platelet MAO activity (Wirz-Justice et al., 1975), tyramine dose / pressor response test result (Ghose and Turner, 1977), plasma aldosterone and plasma renin activity (Katz and Romfh, 1972), plasma amino acids (Craft and Peters, 1971), perception (Henkin, 1974), amount of physical activity (Morris and Udry, 1970), and affective state (Herzberg, Johnson and Brown, 1970).

An in vitro study of the effects of a hormone of the oestrogen type on platelet 5-HT uptake was carried out. A 20 ml sample of blood was collected from each of five female control subjects. Half of each sample was used for a control determination of platelet 5-HT uptake, and the other half was preincubated with $10^{-8}$ M oestrone sulphate for 5 min before the addition of the $^{14}$C - 5-HT. Oestrone acted as a non-competitive inhibitor (Table 2.9) causing a reduction of about 50% in the value obtained for $V_{\text{max}}$.

(vii) Effect of factors affecting water balance

The possibility that an individual's water and electrolyte balance affects the platelet 5-HT transport system was tested by adding the antidiuretic hormone vasopressin to PRP samples from 4 female and 3 male control subjects, to a final concentration of 100 µUnits/ml. This hormone caused a non-competitive inhibition of 5-HT uptake, reducing $V_{\text{max}}$ by about 40% (Table 2.10a).

A baseline 10 ml sample of blood was drawn from each of 2 female and 3 male control subjects. Each subject then received an intravenous injection of 4 µg desmopressin (DDAVP, a synthetic compound with an effect similar to that of vasopressin). A second 10 ml sample
Table 2.9
Effect of $10^{-8}$ M oestrone on platelet 5-HT uptake in vitro
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (pmol/10^8 platelets/min)</th>
<th>$\bar{y}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0.42 ± 0.03</td>
<td>39.1 ± 5.1</td>
<td>26.3 ± 3.6</td>
</tr>
<tr>
<td>+ oestrone</td>
<td>5</td>
<td>0.68 ± 0.27</td>
<td>18.3* ± 2.8</td>
<td>12.1** ± 2.4</td>
</tr>
</tbody>
</table>

Results significantly different * when $p < 0.01$ and ** when $p < 0.001$
### Table 2.10

(a) Effect of vasopressin on platelet 5-HT uptake in vitro

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (pmol/10^8 platelets/min)</th>
<th>$\bar{y}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>0.56 ± 0.08</td>
<td>45.1 ± 7.8</td>
<td>28.0 ± 4.8</td>
</tr>
<tr>
<td>+ vasopressin</td>
<td>7</td>
<td>0.96 ± 0.41</td>
<td>26.8* ± 4.8</td>
<td>15.6* ± 3.3</td>
</tr>
</tbody>
</table>

* lower than own control values, p < 0.01

(b) Effect of DDAVP in vivo on platelet 5-HT uptake

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (pmol/10^8 platelets/min)</th>
<th>$\bar{y}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control baseline</td>
<td>5</td>
<td>0.45 ± 0.04</td>
<td>52.1 ± 8.3</td>
<td>33.9 ± 4.7</td>
</tr>
<tr>
<td>+ DDAVP</td>
<td>5</td>
<td>0.39 ± 0.03</td>
<td>50.3 ± 5.6</td>
<td>34.3 ± 3.2</td>
</tr>
</tbody>
</table>

(c) Effect on platelet 5-HT uptake of drinking 1 L water

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (pmol/10^8 platelets/min)</th>
<th>$\bar{y}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control baseline</td>
<td>6</td>
<td>0.38 ± 0.04</td>
<td>36.7 ± 4.4</td>
<td>25.5 ± 3.2</td>
</tr>
<tr>
<td>+ water</td>
<td>6</td>
<td>0.33 ± 0.02</td>
<td>42.3** ± 3.4</td>
<td>30.6** ± 2.4</td>
</tr>
</tbody>
</table>

** higher than own baseline values, p < 0.05
of blood was collected 3 hours after the injection of DDAVP, the subjects remaining fasting and thirsting until after the second venepuncture. The results of the platelet 5-HT uptake determinations on the two sets of samples are shown in Table 2.10b. Contrary to the results obtained when vasopressin was added in vitro, DDAVP had no effect on 5-HT transport.

A group of 3 female and 3 male control subjects gave a baseline 10 ml blood sample. Each subject then drank 1 litre of tap water, and gave a second 10 ml blood sample 1½ hours later, when the circulating levels of diuretic hormone were assumed to have reached their maximum following the stimulus. The platelet 5-HT uptake results are shown in Table 2.10c. There was a significant increase in the rate of 5-HT uptake of about 15%. Thus the observed effect of natural diuretic hormone ex vivo was contrary to the effect of antidiuretic hormone in vitro.

(viii) Effect of tryptophan, kynurenine, phenylalanine and tyrosine

The normal level of total tryptophan in human plasma is of the order of 10 µg/ml (about 50 µM). When the intake of tryptophan is increased as a form of antidepressant therapy (usual daily dose is 6 gm), then the total plasma concentration increases to 30 to 40 µg/ml. The effect of an additional 20 µg/ml PRP on platelet 5-HT uptake was determined in samples from 4 female and 2 male control subjects. The results are shown in Table 2.11. The extra tryptophan inhibited platelet 5-HT uptake, decreasing V max by about 30%.

Tryptophan is actively accumulated by blood platelets, although the affinity of the platelet transport system for tryptophan is much lower than the affinity for 5-HT: the K m for tryptophan uptake is 5 to 10 µM, while the K m for 5-HT uptake is only 0.5 to 1.0 µM (see this Chapter, section D). The changes in the K m and V max of the platelet 5-HT uptake caused by an additional 20 µg tryptophan/ml PRP are comparatively small, so it is unlikely that naturally occurring fluctuations in plasma tryptophan concentration caused by variations in diet and metabolism would significantly affect platelet 5-HT uptake in vivo.

Total plasma tryptophan concentrations (see this Chapter,
Table 2.11
Effect of tryptophan on platelet 5-HT uptake in vitro
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>K_m (μM)</th>
<th>V_max (μmol/10^8 platelets/min)</th>
<th>j</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>0.55 ± 0.11</td>
<td>43.2 ± 5.8</td>
<td>27.2 ± 4.0</td>
</tr>
<tr>
<td>+ tryptophan</td>
<td>6</td>
<td>0.84 ± 0.21</td>
<td>30.7 ± 5.7</td>
<td>18.1 ± 4.5</td>
</tr>
</tbody>
</table>

*lower than own control values, p < 0.01

Table 2.12
Effect of phenylalanine and tyrosine on platelet 5-HT uptake in vitro
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>K_m (μM)</th>
<th>V_max (μmol/10^8 platelets/min)</th>
<th>j</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>0.50 ± 0.07</td>
<td>53.6 ± 3.6</td>
<td>34.2 ± 1.3</td>
</tr>
<tr>
<td>+ phenylalanine</td>
<td>6</td>
<td>0.43 ± 0.04</td>
<td>33.0† ± 1.8</td>
<td>22.1† ± 1.4</td>
</tr>
<tr>
<td>+ tryrosine</td>
<td>6</td>
<td>0.48 ± 0.03</td>
<td>37.4† ± 3.6</td>
<td>24.0† ± 2.2</td>
</tr>
</tbody>
</table>

†lower than own control value, p < 0.001
section E) were available for 19 control subjects. There was no significant correlation of plasma tryptophan level with either $K_m$ or $V_{max}$ of platelet 5-HT uptake.

Kynurenine is a quantitatively important metabolite of tryptophan, formed by a tryptophan pyrrolase in the liver. It has been suggested that Kynurenine may inhibit brain tryptophan uptake (Green and Curzon, 1970). Platelets from one male control subject were preincubated with a range of concentrations of kynurenine sulphate to determine the effect, if any, of this compound on platelet 5-HT uptake. At the lower concentrations of kynurenine (5 and 10 μg/ml), $K_m$ was not affected (average change +3%) while $V_{max}$ was slightly decreased (average change - 12%). At 20 and 30 μg kynurenine/ml the decrease in $V_{max}$ was greater at -25%, while $K_m$ was decreased in 18%. Thus it is unlikely that fluctuations in kynurenine concentration in vivo will have any significant effects on 5-HT transport.

Phenylalanine and tyrosine, precursors of the neurotransmitter dopamine, are both large neutral amino acids which compete with tryptophan for transport across the blood-brain barrier. The effect on platelet 5-HT uptake of phenylalanine and of tyrosine (extra 100 μM added to PRP) was examined in one female and 5 male control subjects. The results are shown in Table 2.12. Both amino acids inhibited platelet 5-HT uptake. Phenylalanine reduced the value of $V_{max}$ by about 40%, and tyrosine reduced the value of $V_{max}$ by about 30%. However, since the normal plasma concentration of phenylalanine is about 70 μM, and the normal plasma concentration of tyrosine is about 80 μM, it is unlikely that normal variations in the levels of these amino acids have a significant effect of platelet 5-HT uptake.

(ix) Seasonal variation in platelet 5-HT uptake

In order to investigate the possible effect of seasonal variation on platelet 5-HT uptake, the results obtained for platelet 5-HT uptake in control subjects were divided into groups according to the time of year when the samples were collected. A two-monthly interval was decided on as the smallest interval which would still provide suitable numbers within each group to allow for statistical tests to be carried out. The intervals were arbitrarily chosen as
Table 2.12

Tryptophan uptake characteristics of platelets from
22 control subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>47.8 ± 2.7 yr</td>
</tr>
<tr>
<td>$K_m$</td>
<td>5.0 ± 0.5 μM</td>
</tr>
<tr>
<td>$V_{max}$</td>
<td>40.7 ± 4.7 pmol/10^8 platelets/min</td>
</tr>
<tr>
<td>$\bar{y}$</td>
<td>11.2 ± 1.0 pmol/10^8 platelets/min</td>
</tr>
</tbody>
</table>
Legend to Figure 2.5

Seasonal variation in platelet concentration and platelet 5-HT uptake in control subjects

Points shown as mean with standard error bar

platelet concentration  A lower than July + August group, p < 0.05

$\bar{V}$  B higher than May + June and November + December, p < 0.05, March + April, p < 0.01, July + August and September + October, p < 0.001

C lower than November + December, p < 0.02

$V_{\text{max}}$  D higher than May + June, p < 0.05, March + April and September + October, p < 0.01, and July + August, p < 0.001

E lower than November + December, p < 0.02

$K_{m}$  F lower than July + August, p < 0.02, September + October, p < 0.001, and November + December, p < 0.01
PRP platelet concentration

Months of test

J+F  M+A  M+J  J+A  S+O  N+D

n=15  n=13  n=19  n=13  n=34  n=22
January + February, March + April and so on. For those control subjects who were tested on more than one occasion, all the results were included in this analysis, except when this would mean that any one subject was represented more than once in any one two month group. In these cases, the most recent result was included and previous results omitted. In this way, a total of 116 (65 female and 51 male) platelet 5-HT uptake results were available.

The results are shown graphically in Figure 2.5. Platelet 5-HT uptake was found to vary according to the time of year, with the rate of uptake being greatest in the winter and lowest in the summer. Thus seasonal variation in results may account for at least part of the intraindividual variation in results described above. These findings also indicate that it is necessary to test concurrently groups of subjects which are to be compared.

(D) THE DETERMINATION OF TRYPTOPHAN UPTAKE BY HUMAN BLOOD PLATELETS IN VITRO

Platelet-rich plasma was prepared as described in the method for the determination of platelet 5-HT uptake. The levels of tryptophan usually found in plasma are high (about 50 μM) compared with the expected value of $K_m$ for the uptake of tryptophan by platelets, so it was necessary to carry out the determination of platelet tryptophan uptake with the platelets suspended in an artificial tryptophan-free medium, rather than in their autologous plasma.

The PRP obtained from 10 ml of blood was centrifuged at 2,300 x g at room temperature for 10 min, the supernatant fraction (platelet-poor plasma) was discarded, and the inside of the tube wiped dry with tissue paper. The platelets were resuspended by vortex mixing in a volume of artificial medium approximately equal to the original volume of the PRP. The artificial medium used contained 16.2 mM disodium phosphate, 3.8 mM monosodium phosphate, 2.7 mM disodium EDTA, 5.6 mM glucose, 11.0 mM sodium acetate, 104.5 mM sodium chloride and 4.0 mM potassium chloride, adjusted to pH 7.4 with 1 M sodium hydroxide (Kamoun, Lafourcade and Jerome, 1976). Aliquots (0.5 ml) of platelet suspension were preincubated for 10 min at either 37°C in a shaking water bath or at 0°C in an ice/water bath, after which time were added
50 μl of 0.9% (w/v) saline containing $^{14}$C-labeled tryptophan (0.5, 1.0, 2.0, 4.0 and 10.0 μM, final concentrations in platelet suspension) (Radiochemical Centre, Amersham, U.K.). The specific activity of the $^{14}$C-labeled tryptophan was approximately 60 μCi/μmole, the exact value varying from batch to batch of tryptophan. The reaction was stopped after 2 min by the addition to each tube of 2 ml of ice-cold 3% formaldehyde in 0.9% saline (both w/v). The platelets were separated from the incubation medium, digested and counted for radioactivity as described in the method for the determination of platelet 5-HT uptake.

A group of 11 female and 11 male control subjects was studied. The results are shown in Table 2.13. There was no significant difference between female and male subjects in any of the characteristics of platelet tryptophan uptake. There was no significant correlation between age and any of the platelet tryptophan uptake characteristics.

Platelet 5-HT uptake was also measured in this group of 22 control subjects, the blood being drawn as one sample with that for the tryptophan uptake determination. There was no significant correlation between the characteristics of platelet uptake of 5-HT with those of platelet uptake of tryptophan.

(E) THE DETERMINATION OF TOTAL AND FREE LEVELS OF TRYPHTOPHAN, PHENYLALANINE AND TYROSINE IN PLASMA

Tryptophan, the precursor of 5-HT, and phenylalanine and tyrosine, the precursors of dopamine, are all large neutral amino acids which probably compete with each other for transport across the blood-brain barrier. They may possibly also compete with 5-HT for transport into the blood platelet (see Section C above).

Blood plasma samples were collected after overnight fasting, at the same time that blood was taken for platelet uptake experiments. The determination of percentage-free values for tryptophan, phenylalanine and tyrosine were carried out within a few hours of sample collection. The rest of each plasma sample was stored frozen at -20°C until required for analysis.

Total plasma tryptophan levels were measured by a colleague.
Table 2.13
Tryptophan uptake characteristics of platelets from 22 control subjects
Results shown as mean ± S.E.M.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>47.8 ± 2.7 yr</td>
</tr>
<tr>
<td>$K_m$</td>
<td>5.0 ± 0.5 μM</td>
</tr>
<tr>
<td>$V_{max}$</td>
<td>40.7 ± 4.7 pmol/10^8 platelets/min</td>
</tr>
<tr>
<td>$\bar{y}$</td>
<td>11.2 ± 1.0 pmol/10^8 platelets/min</td>
</tr>
</tbody>
</table>
using the method of Denkla and Dewey (1967), with the modifications of Wood et al. (1977). The mean total plasma tryptophan concentration in a group of 129 control subjects was $58.3 \pm 0.8 \mu M$. Plasma phenylalanine was determined by the method of McCaman and Robins (1962), as described in Sigma Technical Bulletin No. 60-F (1974). The mean plasma concentration of phenylalanine in a group of 93 control subjects was $68.4 \pm 1.4 \mu M$. Plasma tyrosine was determined by the method of Wong, O'Flynn and Inouye (1964), as described in Sigma Technical Bulletin No. 70-F (1974). The mean plasma concentration of tyrosine in a group of 91 control subjects was $80.9 \pm 1.5 \mu M$.

Plasma percentage free tryptophan levels were determined by equilibrium dialysis, using a piece of apparatus similar to that described by Moe and Hammes (1974). This apparatus was manufactured in the M.R.C. Workshops, Carshalton, U.K. The chambers were separated by a single sheet of dialysis membrane, which was previously soaked for one hour in hot tap water, then rinsed three times in glass-distilled water (Visking Tubing; Scientific Supplies Ltd., London, U.K.). Into one side of each chamber was pipetted 100 μl of fresh plasma, and into the other side was pipetted 100 μl of a solution consisting of $^{14}C$-labelled tryptophan (2.22 MBq/μmol; approximately 2 nmol/ml; Radiochemical Centre, Amersham, U.K.) in 0.9% (w/v) saline. The apparatus was rotated at approximately 10 rev/min for 4 h at 37°C. After equilibration, 80 μl samples were withdrawn from each side of each chamber, and counted for radioactivity in 4 ml scintillation fluid (NE 262) in an LKB Ultrobeta 1210 spectrometer for 30 min ($^{14}C$ counting efficiency 90% using an external standardization). Radioactivity in the "saline" side of a chamber corresponds to free or "diffusible" tryptophan, and that in the "plasma" side of the chamber corresponds to free + protein-bound tryptophan. Calculation of the proportion of free and bound tryptophan was possible after the estimation of plasma total tryptophan.

It was found to be unnecessary to attempt to regulate the pH of the samples during dialysis. The pH of 36 plasma samples was determined using narrow range indicator paper as the aliquots were removed at the end of the dialysis period, and the mean pH value was $7.33 \pm 0.02$.

The length of time required at 37°C for the dialysis to reach equilibrium was determined using one sample of plasma. Aliquots were
Table 2.14

Percentage free plasma tryptophan values obtained after various dialysis periods at 37°C

Results shown as mean ± S.E.M.

Coefficients of variance shown in brackets

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>No. of chambers</th>
<th>Percentage free tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>$39.8 \pm 0.5$ (3%)</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>$27.8 \pm 0.3$ (3%)</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>$26.3 \pm 0.7$ (7%)</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>$25.4 \pm 0.3$ (3%)</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>$26.4 \pm 1.0$ (10%)</td>
</tr>
</tbody>
</table>
loaded into 31 chambers, and samples taken for counting from several chambers after 1, 2, 3, 4 and 5 hours. After examining the results obtained (shown in Table 2.14), a period of 4 h was chosen as a suitable and convenient dialysis time.

The effect of freezing on plasma percentage free tryptophan was examined in a group of 43 samples of plasma. The group was a mixed one, consisting of plasma samples from 14 control subjects, 7 depressed patients, 12 depressive patients on antidepressant drug therapy, and 10 drug-free recovered depressive patients. For each of these samples, the percentage free tryptophan was estimated by the equilibrium dialysis method on the day that the plasma was collected. The remainder of each plasma sample was stored frozen at \(-20^\circ\text{C}\) until the repeat determination was carried out. The storage time ranged from 3 days to 34 weeks, with a mean of 13.8 ± 1.8 weeks. The mean percentage free tryptophan was 27.5 ± 0.9 for the plasma samples when fresh, and 27.7 ± 0.9 for the same samples after they had been frozen. There was no significant difference between the two sets of results when examined by the paired t-test. There was no significant correlation between the difference in percentage free tryptophan between the two determinations and the length of time for which the samples had been stored frozen. There was a significant correlation between the results obtained using fresh plasma and the results obtained after the samples had been frozen (r = + 0.472, p < 0.01). Thus, if for any reason the equilibrium dialysis determination of percentage free tryptophan cannot be carried out on the same day that a plasma sample is collected, it is acceptable to store the sample at \(-20^\circ\text{C}\) for the estimation to be carried out at a more convenient date.

The equilibrium dialysis method was also used for the determination of plasma free tyrosine and plasma free phenylalanine levels. Percentage free tyrosine was determined in 14 samples of plasma, and was found to be 100% free in all cases. This result agrees with the generally held view that plasma amino acids (with the exception of tryptophan) are not appreciably bound to plasma proteins. Percentage free phenylalanine was determined in 27 plasma samples, and was found to be only 90.5 ± 0.4% free, i.e. about 10% of phenylalanine in these samples was bound to plasma proteins. This is contrary to the generally accepted
view, and is possibly worthy of further investigation.

The equilibrium dialysis method was also used to measure drug binding to plasma proteins in depressed patients treated with zimelidine, using $^3$H-labelled zimelidine and $^3$H-labelled norzimelidine (gifts of Astra Ltd.).
PLATELET UPTAKE OF 5-HT AND TRYPTOPHAN, AND PLASMA CONCENTRATION OF TRYPTOPHAN, TYROSINE AND PHENYLALANINE, IN DEPRESSIVE PATIENTS

(A) PATIENTS

Platelet 5-HT uptake characteristics were determined for 50 female and 27 male depressed patients. All were patients at the Clinical Investigation Ward of the MRC Neuropsychiatric Research Laboratory, West Park Hospital, and the majority were in-patients. All were diagnosed as suffering from primary depressive illness (Medical Research Council Clinical Psychiatry Committee, 1965), and none had a history of mania. The patients remained drug-free (with the exception of hypnotics for night sedation where necessary) and received supportive psychotherapy for 7 to 10 days before testing. At the end of this drug-free period the patients were assessed for the severity of their depressive illness using the first sixteen items of the Hamilton Rating Scale for depression (HRS; Hamilton, 1967). The HRS assessments were made by two independent assessors who showed a satisfactory concordance ($r = + 0.98$, $p < 0.001$), and the mean HRS score was recorded. A HRS score of 16 or more was considered to indicate moderate to severe depression, 7 to 15 mild depression, and patients scoring 6 or less were regarded as clinically recovered. Patients were also assessed by the Newcastle rating scale (Carney et al., 1965), on which a high score indicates an endogenous depression and a low score a non-endogenous (reactive or neurotic) depression.

At the end of the drug-free period, and when possible on the same day that HRS assessments were made, blood for the estimation of platelet 5-HT uptake characteristics was collected. The sample was obtained by venepuncture between 8 a.m. and 10 a.m., with the minimum of stasis, after overnight fasting.

Platelet 5-HT uptake was also determined for 24 female and 9 male recovered depressive patients. These patients had all been admitted to hospital suffering from primary depressive illness and had been treated either with an antidepressant drug (amitriptyline, cyclazindole or zimelidine) or by ECT. The medication was then withdrawn.
in those patients who had recovered, and after a further 14 days without antidepressant drug treatment their clinical state was assessed.

Those patients with a HRS score of 6 or less were regarded as recovered.

For comparison with the depressive patients, a group of 48 control subjects was selected from those described in Chapter 2, omitting results from those aged 35 years or less.

(B) RESULTS

(i) Platelet 5-HT uptake in depressive patients

The $V_{\max}$ of platelet 5-HT uptake was found to be significantly lower than control values in both depressed ($p < 0.05$) and recovered depressive patients ($p < 0.001$) (Table 3.1). The recovered patients had a lower $V_{\max}$ than the depressed patients ($p < 0.001$). The value of $K_{m}$ was almost the same for all three groups. Both groups of patients had a significantly higher concentration of platelets in the PRP than the control subjects ($p < 0.05$).

Hamilton rating scores were available for 65 of the depressed patients. The range was from 7 to 32. There was no significant correlation between HRS and age, PRP platelet concentration, $K_{m}$, $V_{\max}$ or $\bar{y}$.

Newcastle classification scores were available for 67 of the depressed patients. Since the Newcastle rating is an interval measure it cannot be used for product - moment correlations. Therefore, the results for these patients were divided into three groups corresponding to Newcastle ratings of 0 to 3, 4 to 6, and 7 to 11. There were no significant differences between the three groups in PRP platelet concentration, $K_{m}$, $V_{\max}$ or $\bar{y}$ (Table 3.2). The patients with a Newcastle rating between 7 and 11 were significantly older ($p < 0.02$) than those with a rating between 0 and 3, but this is probably due to older patients being more likely to have had a previous episode of depression, which adds 1 to the Newcastle rating. Also the average age of onset
Table 3.1
Platelet 5-HT uptake characteristics of control subjects, depressed patients and recovered depressive patients
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (yr)</th>
<th>Platelet conc (x 10^8/ml PRP)</th>
<th>K_m (µM)</th>
<th>V_max (pmol/10^8 pl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>48</td>
<td>51.0 ± 1.2</td>
<td>2.64 ± 0.10</td>
<td>0.50 ± 0.02</td>
<td>39.5 ± 2.4</td>
</tr>
<tr>
<td>Depressed</td>
<td>77</td>
<td>51.8 ± 1.6</td>
<td>2.93* ± 0.10</td>
<td>0.52 ± 0.03</td>
<td>33.2** ± 1.4</td>
</tr>
<tr>
<td>Recovered</td>
<td>33</td>
<td>52.2 ± 2.4</td>
<td>3.01* ± 0.14</td>
<td>0.54 ± 0.03</td>
<td>24.2*** ± 2.2</td>
</tr>
</tbody>
</table>

* higher than controls p < 0.05
** lower than controls p < 0.01
*** lower than controls and depressed p < 0.001
Table 3.2
Platelet 5-HT uptake of depressed patients divided according to Newcastle classification
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>0 to 3</th>
<th>4 to 6</th>
<th>7 to 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>21</td>
<td>28</td>
<td>18</td>
</tr>
<tr>
<td>Platelet conc (x $10^8$/ml)</td>
<td>$2.85 ± 0.22$</td>
<td>$2.87 ± 0.14$</td>
<td>$3.09 ± 0.20$</td>
</tr>
<tr>
<td>$K_m$ (μM)</td>
<td>$0.50 ± 0.04$</td>
<td>$0.47 ± 0.03$</td>
<td>$0.62 ± 0.09$</td>
</tr>
<tr>
<td>$V_{max}$ (pmol/$10^3$ pl/min)</td>
<td>$31.3 ± 2.3$</td>
<td>$30.3 ± 2.4$</td>
<td>$36.7 ± 3.0$</td>
</tr>
<tr>
<td>$\bar{y}$ (pmol/$10^3$ pl/min)</td>
<td>$20.3 ± 1.6$</td>
<td>$19.9 ± 1.5$</td>
<td>$22.6 ± 1.8$</td>
</tr>
</tbody>
</table>

* older than 0 to 3 group, p < 0.02
of neurotic depression is lower than that of endogenous depression.

In order to investigate further the possible relationship between clinical state and platelet 5-HT uptake, a group of 16 patients tested when severely depressed (HRS of 16 or more) were re-tested after recovery (HRS of 6 or less). Clinical state was found to have no effect on platelet 5-HT uptake (Table 3.3).

Since clinical state did not appear to affect platelet 5-HT uptake, results for depressed and recovered patients were combined for the purpose of analysis for seasonal variation. Any one patient was included only once in any 2-month sample: where depressed and recovered results were obtained within the same 2-month period, the recovered result was omitted. This was only the case for 2 patients, so that 108 results were available.

The PRP platelet concentration, and \( V_{\text{max}} \) and \( \tilde{y} \) of 5-HT uptake were found to vary according to the time of year of testing (Figure 3.1). Both \( V_{\text{max}} \) and \( \tilde{y} \) were lowest in May and June, which coincides with the time of year when the incidence of depression is greatest. There was no significant seasonal variation in \( K_m \).

The seasonal variation in \( V_{\text{max}} \) in depressive patients is similar to that found in control subjects (Chapter 2, Figure 2.5). However, the trough in the \( V_{\text{max}} \) curve for patients occurs in May and June, while the trough for controls occurs in July and August (Figure 3.2). Hence, the controls have significantly higher values of \( V_{\text{max}} \) than patients during Winter and Spring, but from July to October the two groups have very similar values of \( V_{\text{max}} \).

Since depressed patients often suffer from sleep disturbances, they frequently require or demand night sedation. When this project was commenced it was generally believed that the commonly administered hypnotics have no effect on neurotransmitter transport processes. Thus many of the depressed patients were receiving hypnotics when platelet 5-HT uptake estimations were made. By chance it was found that patients receiving nitrazepam in addition to lithium had significantly lower values of \( V_{\text{max}} \) than patients receiving only lithium (Chapter 5, Table 5.8). It proved difficult in many cases to find out retrospectively, in some
Table 3.3
Platelet 5-HT uptake in 16 patients tested before and after recovery from a depressive episode
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Depressed</th>
<th>Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet conc (x 10^8/ml)</td>
<td>2.78 ± 0.19</td>
<td>2.99 ± 0.19</td>
</tr>
<tr>
<td>K_m (μM)</td>
<td>0.52 ± 0.05</td>
<td>0.50 ± 0.04</td>
</tr>
<tr>
<td>V_max (pmol/10^8 pl/min)</td>
<td>26.0 ± 4.0</td>
<td>26.9 ± 3.7</td>
</tr>
<tr>
<td>y (pmol/10^8 pl/min)</td>
<td>16.6 ± 2.6</td>
<td>17.2 ± 2.4</td>
</tr>
</tbody>
</table>
**Legend to Figure 3.1**

**Seasonal variation in platelet concentration and platelet 5-HT uptake in depressive patients**

Points shown as mean with standard error bar

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Seasonal Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet concentration</td>
<td>A lower than January + February, July + August, September + October, and November + December ($p &lt; 0.05$)</td>
</tr>
<tr>
<td></td>
<td>B lower than July + August and November + December ($p &lt; 0.02$) and September + October ($p &lt; 0.01$)</td>
</tr>
<tr>
<td>$\bar{y}$</td>
<td>C lower than January + February ($p &lt; 0.05$)</td>
</tr>
<tr>
<td>$V_{max}$</td>
<td>July + August ($p &lt; 0.02$), September + October and November + December ($p &lt; 0.01$)</td>
</tr>
</tbody>
</table>
Figure 3.1 Seasonal variation in platelet 5-HT uptake in depressive patients

PRP platelet concentration

Month of test

<table>
<thead>
<tr>
<th></th>
<th>J+F</th>
<th>M+A</th>
<th>M+J</th>
<th>J+A</th>
<th>S+O</th>
<th>N+D</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>9</td>
<td>21</td>
<td>24</td>
<td>21</td>
<td>19</td>
</tr>
</tbody>
</table>

\( \text{V}_{\text{max}} \) (pmol/10^8 p1/min)

\( \text{K}_m \) (\( \mu \text{M} \))

\( \bar{y} \) (pmol/10^8 p1/min)
Figure 3.2 Seasonal variation in $V_{\text{max}}$ in control subjects and depressive patients

CONTROL SUBJECTS

DEPRESSIVE PATIENTS

Month of test

J+F  M+A  M+J  J+A  S+O  N+D

$n=15$  $n=13$  $n=19$  $n=13$  $n=34$  $n=22$

$p<0.001$  $p<0.01$  $p<0.05$
cases several years after testing, whether or not a depressed patient otherwise drug-free, was receiving night sedation. Definite information was available for 42 depressed patients, of whom 13 were completely drug-free, 5 were receiving chloral hydrate, 6 were on triazolam ("Halcion"), and 18 were on nitrazepam ("Mogadon"). Chloral hydrate and triazolam appeared to have no effect on platelet 5-HT uptake characteristics, but patients receiving nitrazepam had a lower $V_{\text{max}}$ (Table 3.4).

L-tryptophan, the precursor of 5-HT, has been used in the treatment of depression. In the doses usually used (3 to 8 gm/day), the plasma total tryptophan concentration is raised 3-fold, from about 50 µM to about 150 µM. Extra tryptophan, to a final concentration of 100 µM, was added to PRP samples from 6 patients, prior to the addition of $^{14}$C-5-HT. In vitro extra tryptophan was found to cause a small but significant ($p < 0.01$) reduction in $V_{\text{max}}$ and $\gamma$ (Table 3.5). This is similar to the result obtained when tryptophan was added in vitro to PRP from control subjects (Chapter 2, Table 2.10).

\[(ii) \text{ Plasma concentrations of tryptophan, phenylalanine and tyrosine in depressive patients.} \]

Plasma total tryptophan concentration (determined by a colleague), percent free tryptophan (determined by the equilibrium dialysis method), and plasma free tryptophan concentration were measured in plasma samples from 25 control subjects, 15 depressed patients and 19 recovered depressives. There were no significant differences between the groups in these comparatively small samples (Table 3.6).

Phenylalanine and tyrosine, both of which are large neutral amino acids which probably compete with tryptophan in transport systems, together with total tryptophan were determined in plasma samples from 50 control subjects, 43 depressed patients, and 27 recovered depressive patients. There were no significant differences between the groups in any of the three amino acid concentrations (Table 3.7). The ratios were calculated for each of these samples of tyrosine to tryptophan, phenylalanine to tryptophan, and phenylalanine to tyrosine; and of tryptophan + tyrosine to phenylalanine, tryptophan + phenylalanine to
Table 3.4
Effect of hypnotics on platelet 5-HT uptake in depressed patients
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (yr)</th>
<th>Hamilton Rating</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (pmol/10^8 pl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug-free</td>
<td>13</td>
<td>43.4 ± 3.8</td>
<td>17.6 ± 1.3</td>
<td>0.50 ± 0.06</td>
<td>34.8 ± 2.7</td>
</tr>
<tr>
<td>Chloral hydrate</td>
<td>5</td>
<td>53.8 ± 7.8</td>
<td>25.2 ± 1.6</td>
<td>0.50 ± 0.16</td>
<td>31.9 ± 3.9</td>
</tr>
<tr>
<td>Triazolam</td>
<td>6</td>
<td>62.3 ± 3.5</td>
<td>19.7 ± 2.5</td>
<td>0.47 ± 0.08</td>
<td>32.8 ± 5.0</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td>18</td>
<td>55.2 ± 2.4</td>
<td>19.3 ± 0.8</td>
<td>0.57 ± 0.06</td>
<td>22.4*** ± 2.4</td>
</tr>
</tbody>
</table>

* older than drug-free patients p < 0.01
** more depressed than drug-free patients and nitrazepam group p < 0.02
*** lower than drug-free patients p < 0.01
Table 3.5
Effect of tryptophan on 5-HT uptake by platelets from 6 depressive patients
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Patients' PRP</th>
<th>Patients' PRP + extra tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>K&lt;sub&gt;m&lt;/sub&gt; (μM)</strong></td>
<td>0.44 ± 0.03</td>
<td>0.51 ± 0.04</td>
</tr>
<tr>
<td><strong>V&lt;sub&gt;max&lt;/sub&gt; (pmol/10&lt;sup&gt;8&lt;/sup&gt; pl/min)</strong></td>
<td>19.6 ± 2.4</td>
<td>11.3 ± 0.8</td>
</tr>
<tr>
<td><strong>y (pmol/10&lt;sup&gt;8&lt;/sup&gt; pl/min)</strong></td>
<td>12.9 ± 1.5</td>
<td>7.2 * ± 0.4</td>
</tr>
</tbody>
</table>

* p < 0.01 (method of paired comparisons)
Table 3.6
Total, percent free and free plasma tryptophan (try) in control subjects, depressed patients, and recovered depressives
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Total try (µM)</th>
<th>% free</th>
<th>Free try* (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>23</td>
<td>55.3 ± 2.0</td>
<td>27.1 ± 1.3</td>
<td>14.8 ± 0.8</td>
</tr>
<tr>
<td>Depressed</td>
<td>15</td>
<td>52.4 ± 1.5</td>
<td>25.1 ± 1.5</td>
<td>13.0 ± 0.7</td>
</tr>
<tr>
<td>Recovered</td>
<td>19</td>
<td>53.9 ± 1.5</td>
<td>25.1 ± 1.5</td>
<td>13.4 ± 0.7</td>
</tr>
</tbody>
</table>

* Value calculated using total tryptophan concentrations and percent free tryptophan results.
Table 3.7
Concentrations of tryptophan, phenylalanine and tyrosine in plasma from control subjects, depressed patients and recovered depressive patients
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Tryptophan (µM)</th>
<th>Tyrosine (µM)</th>
<th>Phenylalanine (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>50</td>
<td>57 ± 1</td>
<td>80 ± 2</td>
<td>68 ± 2</td>
</tr>
<tr>
<td>Depressed</td>
<td>43</td>
<td>54 ± 1</td>
<td>77 ± 2</td>
<td>70 ± 2</td>
</tr>
<tr>
<td>Recovered</td>
<td>27</td>
<td>55 ± 1</td>
<td>77 ± 2</td>
<td>67 ± 3</td>
</tr>
</tbody>
</table>
tyrosine, and tyrosine + phenylalanine to tryptophan. There were no differences between the groups in any of the amino acid ratios.

Plasma total and free tryptophan concentrations determined in samples from 129 control subjects and 106 depressed patients were available for analysis for seasonal variation. The determinations were carried out by several colleagues, using an ultrafiltration technique for the determination of free tryptophan.

In both controls and patients there was significant seasonal variation in both total and free plasma tryptophan concentrations (Figure 3.3). Total tryptophan was lowest in January and February and in September and October in both groups; and was highest in March and April and in November and December in controls, and highest in May and June and November and December in depressed patients. Although total tryptophan concentrations were higher in the patients than in the controls at all times, the difference only reached significance ($p < 0.01$) in May and June.

Plasma free tryptophan showed two distinct peaks in controls in March and April and at the end of the year, whereas there was only one distinct peak in free tryptophan in the depressed patients, in May and June. The pattern of variation in the controls was the reverse of that in the patients, so that the controls had significantly higher levels of free tryptophan than the patients in the Spring and Autumn. Conversely, the free tryptophan levels in the two groups were almost identical in January, February and May and June. Investigations carried out during these months could be expected to fail to show any significant difference between depressives and controls. This may account for the lack of agreement in results presented by different groups. The troughs in the concentration of free plasma tryptophan in depressed patients that occur in Spring and Autumn correspond to the peaks in the seasonal variations that have been described in the incidence of affective disorders (Eastwood and Peacocke, 1976, Lester, 1979).

Plasma phenylalanine concentrations in 93 controls, 58 depressed patients and 36 recovered depressive patients were available for analysis for seasonal variation. In all three groups, the plasma concentration of phenylalanine was lowest in May and June. Only in the recovered depressives
**Legend to Figure 3.3**

**Seasonal variation in plasma tryptophan in control subjects and depressive patients**

Points shown as mean with standard error bar

Controls and patients significantly different where * $p < 0.01$, ** $p < 0.001$

<table>
<thead>
<tr>
<th>Total tryptophan: patients</th>
<th>A</th>
<th>lower than May + June and November + December ($p &lt; 0.05$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>lower than May + June and November + December ($p &lt; 0.001$)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total tryptophan: controls</th>
<th>C</th>
<th>lower than March + April ($p &lt; 0.02$) and November + December ($p &lt; 0.01$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D</td>
<td>lower than March + April ($p &lt; 0.01$) and November + December ($p &lt; 0.001$)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Free tryptophan: patients</th>
<th>E</th>
<th>lower than May + June ($p &lt; 0.05$)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Free tryptophan: controls</th>
<th>F</th>
<th>lower than January + February ($p &lt; 0.05$) and May + June ($p &lt; 0.01$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
<td>higher than January + February ($p &lt; 0.05$) and May + June ($p &lt; 0.01$)</td>
</tr>
</tbody>
</table>
Figure 3.3 Seasonal variation in plasma tryptophan concentrations in control subjects and depressive patients.

**TOTAL TRYPTOPHAN**

**FREE TRYPTOPHAN**

Month of test
was there any significant difference between the lowest level (56 μM) in May and June and the highest level (74.5 μM), which in this group occurred in November and December (p < 0.05).

Plasma tyrosine concentrations in 89 controls, 56 depressed patients, and 36 recovered depressive patients were available for seasonal analysis. In none of the groups was there any significant seasonal variation.

The ratio to the plasma phenylalanine concentration to plasma tyrosine concentration was calculated for 87 control subjects, 55 depressed patients, and 35 recovered depressive patients. In all three groups the ratio was low in May and June. In the recovered depressive patients there was a significant difference between the ratio in May and June (0.80) and the ratio in November and December (1.06) (p < 0.02). The lowest ratio in controls was obtained in November and December (0.77), and this was significantly lower than the peak (0.92) in September and October (p < 0.02). There was a significant difference in the ratio between the controls and the recovered depressive patients in November and December (p < 0.01).

(iii) **Platelet tryptophan uptake**

Platelet tryptophan uptake characteristics were determined for 20 depressed patients and 11 recovered depressives. There were no significant differences in K_m or V_max between the groups. The depressed patients had a significantly higher platelet tryptophan uptake than controls, as indicated by the value of y (p < 0.05) (Table 3.8).

(C) **DISCUSSION**

The rate of platelet 5-HT uptake was found to be significantly lower in depressed patients than in control subjects. This finding is in agreement with other reports (Tuomisto, Tukiainen and Ahlfors, 1979; Malmgren et al., 1983). The reduction in platelet 5-HT uptake in the patients was independent of clinical state. The rate of uptake of 5-HT was found to remain low in patients who had recovered from an episode of depressive illness. Therefore it is concluded that the low rate of platelet 5-HT uptake observed in depressive patients is a trait which contributes to a predisposition to depressive illness.
Table 3.8
Platelet tryptophan uptake characteristics of control subjects, depressed patients and recovered depressive patients
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (yr)</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (pmol/10⁸ pl/min)</th>
<th>$\bar{y}$ (pmol/10⁸ pl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>24</td>
<td>47.1 ± 2.7</td>
<td>4.67 ± 0.55</td>
<td>38.6 ± 4.5</td>
<td>11.2 ± 0.9</td>
</tr>
<tr>
<td>Depressed</td>
<td>20</td>
<td>50.9 ± 2.8</td>
<td>7.68 ± 1.34</td>
<td>54.3 ± 6.5</td>
<td>14.9* ± 1.5</td>
</tr>
<tr>
<td>Recovered</td>
<td>11</td>
<td>50.5 ± 3.3</td>
<td>4.88 ± 0.74</td>
<td>45.7 ± 10.3</td>
<td>12.7 ± 2.4</td>
</tr>
</tbody>
</table>

* higher than controls p < 0.05
When the platelet 5-HT uptake results obtained for the depressed patients were divided into three groups according to the Newcastle ratings of the patients, no significant differences were found between the groups. Thus both endogenous and non-endogenous depressed patients have a reduced rate of platelet 5-HT uptake. Therefore, this biochemical assay is of no assistance in the classification of depressive illness into the widely used categories of endogenous and non-endogenous or reactive.

A seasonal variation in the rate of platelet 5-HT uptake was observed in both control subjects and depressive patients. The rate of uptake was lowest in late spring and early summer, which is also the time of year when the incidence of depression and of suicides is greatest (Eastwood and Peacocke, 1976; Lester, 1979; Bazas et al., 1979). Thus a seasonal reduction in the rate of 5-HT uptake contributes to the observed increase in vulnerability to depressive illness. The finding of a seasonal variation in platelet 5-HT uptake also emphasises the necessity to test concurrently groups of subjects who are to be compared.

Although the addition to PRP of comparatively large amounts of tryptophan, kynurenine, phenylalanine and tyrosine caused inhibition of platelet 5-HT uptake, it is unlikely that natural variations in vivo have any significant effect on the rate of 5-HT transport. Tryptophan administered in therapeutic doses, which cause a 3-fold increase in plasma tryptophan concentration, may be expected to cause a slight degree of inhibition of 5-HT uptake if the results obtained in vivo reflect the effects observed in vitro. Thus in addition to increasing brain 5-HT synthesis tryptophan in therapeutic doses may also act in a similar way to the tricyclic antidepressants by inhibiting the re-uptake of 5-HT.

Depressed patients were shown to have a higher rate of uptake of tryptophan by platelets than control subjects. If this increased rate of tryptophan transport is reflected in the brain, and if the uptake is only of unbound tryptophan, this may indicate a compensating mechanism, since depressed patients have lower levels of plasma free tryptophan than control subjects. A higher rate of tryptophan uptake in depressed patients would lead to a restoration of the net accumulation
of tryptophan by the brain to normal.
THE EFFECTS OF AMITRIPTYLINE AND ZIMELIDINE ON PLATELET 5-HT UPTAKE IN DEPRESSED PATIENTS.

A clinical trial was carried out to compare the efficacy of a new drug, zimelidine, with that of a well-established tricyclic antidepressant, amitriptyline, in the treatment of depressive illness. In order to compare the effects of the two drugs as inhibitors of 5-HT uptake, the platelet 5-HT uptake characteristics of the patients were determined before and during treatment. Possible relationships between 5-HT uptake inhibition, clinical outcome and plasma drug concentrations were investigated for both amitriptyline and zimelidine.

(A) INTRODUCTION TO DRUGS STUDIED

(i) Amitriptyline

A comprehensive review by Morris and Beck (1974) showed that in 85 controlled trials comparing 93 treatment groups, tricyclic antidepressant drugs were superior to placebo in 60, and indistinguishable in 31. In no case was placebo superior in a controlled investigation. Thus, 65 to 70% of all depressed patients who receive a tricyclic drug are likely to benefit.

Amitriptyline, a typical tricyclic antidepressant, is a dimethylated tertiary amine with anticholinergic and antihistaminic actions. It also possesses mild tranquillising properties (Wade and Reynolds, 1977). Tricyclic antidepressant drugs are very potent inhibitors of the reuptake of biogenic amines at synaptic terminals. They therefore increase the effective concentrations of monoamines at central receptor sites, even though the brain amine content is not increased. This effect is thought to be the means by which they exert their antidepressant action. Amitriptyline is a potent inhibitor of 5-HT uptake (Kannengiesser, Hunt and Raynauld, 1973), while its desmethyl derivative, nortriptyline, strongly inhibits the uptake of noradrenaline (Carlsson et al., 1969). At higher concentrations, amitriptyline also acts as an α-adrenoreceptor blocker.
In blood, amitriptyline and nortriptyline bind to plasma proteins to a large extent (Borga and Lundi, 1970), so that renal excretion of the drug is slow. Only 30% of the ingested dose appears in the urine during the first 24 hours following an overdose (Gard et al., 1973). Hence, amitriptyline has a long plasma half-life of 25 to 40 hours (Braithwaite and Widdop, 1971; Jørgensen and Staehr, 1976). Amitriptyline has to be administered for from 3 to 4 weeks before the full therapeutic benefit is achieved. The optimum plasma levels which produce the best clinical response lie within the range 50 to 140 ng/ml: both very low and very high levels produce little or no therapeutic benefit (Asberg et al., 1971).

In vitro studies show that amitriptyline and nortriptyline are 90 - 95% bound to plasma albumin (Borga et al., 1969). During treatment with nortriptyline, the ratio of CSF nortriptyline to the total level in the plasma was found to be on average 7%, with a two-fold variation between individuals (Kragh-Sørensen et al., 1976): this figure is assumed to represent the proportion of unbound drug in the plasma, which is probably in equilibrium with the CSF. In a World Health Organization study involving 54 depressed patients, no correlation of importance was found between clinical improvement and plasma levels of amitriptyline or nortriptyline (Coppen et al., 1978). However, attempts to relate clinical and pharmacological effects of tricyclic drugs with their plasma levels in vivo have generally relied on measurements of total drug levels in the plasma. Since it is possible that only the free fraction is pharmacologically active, any correlations may lose significance because of variability in the degree of binding between individuals. The differences between individuals in the degree of protein binding of tricyclics has been shown to be partly under genetic control, partly subject to environmental influences (Alexanderson and Borga, 1972), and dependent on plasma albumin concentration (Borga et al., 1969).

(ii) **Zimelidine**

Zimelidine is a two ring compound derived from pheniramine. In rat brain slices both zimelidine and norzimelidine, its desmethylated secondary amine metabolite, have been shown to be powerful inhibitors of the neuronal reuptake of 5-HT, and to a very much lesser extent of noradrenaline (Ross and Renyi, 1977). After oral administration to rats,
zimelidine is about 7 times more potent than chlorimipramine, and about 12 times more potent than imipramine in inhibiting 5-HT uptake (Ross and Renyi, 1973). In studies in mice, zimelidine strongly potentiated the behavioural effects of 5-hydroxytryptophan but only weakly potentiated the effects of L-dopa: this is further evidence that zimelidine has a strong effect on the uptake of 5-HT, but only a weak effect on noradrenaline uptake (Ogren, 1973). In animal tests zimelidine showed only weak anticholinergic effects (Ogren, 1973).

On the basis of its effects in animals, it might be expected that zimelidine would have important antidepressant properties, but may lack the unpleasant anticholinergic side-effects commonly associated with tricyclic antidepressant drugs such as amitriptyline. Clinical studies have confirmed the pharmacological profile (Siwers et al., 1977), and the results of an open study suggested that zimelidine has an antidepressant action (Benkert et al., 1977).

(E) PATIENTS

The in-patients studied were all diagnosed to be suffering from primary depressive illness (Medical Research Council, 1965), and none had a history of mania. The patients remained drug-free and received matched placebo capsules and supportive psychotherapy for 7 to 10 days before active drugs were administered. No other medication apart from nitrazepam (maximum dose 10 mg daily) was allowed.

At the end of the drug-free period the patients were assessed for the severity of their symptoms using the Hamilton Rating Scale (HRS) for depression (Hamilton, 1967). Only those patients who scored 16 or more on the first 16 items of the HRS were included in the study. Nine male and 17 female patients, aged between 25 and 77 years, were randomly allocated to receive either amitriptyline or zimelidine. The sex ratio, mean age, and mean baseline Hamilton Rating score of both groups were similar (Table 4.1). The patients received either 75 mg amitriptyline or 100 mg zimelidine in a single evening dose for the initial 3 days. For the remainder of the trial period of 6 weeks they received an additional capsule in the morning. Thus, the patients received either 150 mg amitriptyline or 200 mg zimelidine per day. The trial was carried out using double-blind conditions, so that neither the patients, medical staff
Table 4.1
Details of patients treated with amitriptyline or zimelidine
Results shown as mean ± s.e.m.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Age (yr)</th>
<th>Baseline HRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitriptyline</td>
<td>13 (5M, 8F)</td>
<td>55.5 ± 2.5</td>
<td>20.3 ± 1.0</td>
</tr>
<tr>
<td>Zimelidine</td>
<td>13 (4M, 9F)</td>
<td>51.9 ± 2.9</td>
<td>19.5 ± 0.9</td>
</tr>
</tbody>
</table>
nor laboratory staff knew which drug any particular patient was receiving.

Severity of the patients' depression was assessed using the Hamilton rating scale at the end of the drug-free period (baseline) and after 2, 4 and 6 weeks of active drug treatment. Clinical change during active treatment was expressed in three ways:

1. The Hamilton rating score after 4 and 6 weeks of treatment;

2. The percentage improvement on the Hamilton score from the baseline to the 4 and 6 week scores;

3. The amelioration score, i.e. the change in Hamilton rating from the baseline to the 4 and 6 weeks scores.

The subjective side-effects of the drug treatments were assessed by asking the patients to complete a standardized side-effects inventory (Ghose, 1977) at baseline, and after 1, 2, 4 and 6 weeks of treatment. The subjective side-effects scores at baseline were deducted from the side-effects scores obtained on active medication to obtain a corrected side-effects score.

Plasma drug concentrations were determined at baseline, and after 1, 2, 4 and 6 weeks of treatment. Blood samples were drawn from the patients at about 9 a.m. after an overnight fast, i.e. about 13 hours after the last drug intake and before the morning dose. The plasma was separated and stored frozen until required for analysis. Amitriptyline and nortriptyline plasma levels were determined by a gas chromatographic method (Jørgensen, 1975). Plasma concentrations of zimelidine and norzimelidine were determined by high performance ion-pair partition chromatography (Westerlund, unpublished report, 1976). In 9 patients the non-bound (free) percentage of zimelidine and norzimelidine were determined by equilibrium dialysis. This was performed on fresh plasma after 4 weeks of treatment.

Blood samples for the estimation of platelet 5-HT and tryptophan uptake characteristics, and plasma levels of tryptophan, phenylalanine, and tyrosine, were collected at baseline and after 4 weeks of active drug treatment. Blood was obtained from the patients, who had fasted overnight, by venepuncture between 8.30 and 9.30 a.m., with the minimum of stasis.
RESULTS

(i) Clinical response and effect on platelet 5-HT uptake of amitriptyline and zimelidine.

There was no significant difference between amitriptyline and zimelidine in their antidepressant efficacy. Hamilton rating scores in the two treatment groups were similar at 2, 4 and 6 weeks (Table 4.2). However, while the amitriptyline group showed a relatively small positive corrected side-effects score, the zimelidine group showed a significant decrease from the baseline side-effects scores after 6 weeks. Thus, as was predicted from animal tests, the virtual absence of anticholinergic effects by zimelidine results in fewer unpleasant side-effects, so that in this respect zimelidine is a superior treatment when compared with typical antidepressants such as amitriptyline.

Both amitriptyline and zimelidine caused a large and significant reduction in platelet 5-HT uptake (Table 4.3). Both drugs acted as competitive inhibitors in 5-HT uptake, as they each caused an increase in $K_m$ but had no significant effect on the value of $V_{max}$. Zimelidine was a stronger inhibitor of 5-HT uptake than amitriptyline. The $K_m$ value obtained for patients receiving zimelidine was significantly higher than that obtained for patients receiving amitriptyline. The inhibition of uptake as indicated by the decrease in the value of $\tilde{y}$ was greater in patients on zimelidine (63% lower than baseline) than in patients on amitriptyline (56% lower than baseline).

No significant correlation was found between clinical response after 4 weeks of treatment and platelet 5-HT uptake in either treatment group. There was no correlation between 4 week values of $K_m$, $V_{max}$ and $\tilde{y}$ with Hamilton rating score, amelioration score or percentage improvement, neither was there any correlation between the change from baseline or the percentage change from baseline in platelet 5-HT uptake characteristics and any of the measures of clinical improvement. It is widely assumed that the mode of action of tricyclic antidepressants is directly related to the inhibition of the reuptake of released amines. It might therefore have been predicted not only that there would be a relationship between 5-HT uptake inhibition and clinical improvement, but also that zimelidine would be a more effective treatment than amitriptyline. This finding of
Table 4.2
Clinical progress of patients receiving either amitriptyline or zimelidine
Hamilton rating scores shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Stage of trial</th>
<th>Amitriptyline n = 15</th>
<th>Zimelidine n = 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline pre-entry</td>
<td>20.8 ± 0.9</td>
<td>19.7 ± 0.8</td>
</tr>
<tr>
<td>2 weeks</td>
<td>14.9 ± 0.8</td>
<td>14.8 ± 1.4</td>
</tr>
<tr>
<td>4 weeks</td>
<td>14.1 ± 1.5</td>
<td>12.7 ± 1.9</td>
</tr>
<tr>
<td>6 weeks</td>
<td>12.7 ± 1.9</td>
<td>12.2 ± 2.3</td>
</tr>
</tbody>
</table>
Table 4.3
Uptake of 5-HT by platelets from depressed patients and from patients receiving either amitriptyline or zimelidine
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (pmol/10$^8$ pl/min)</th>
<th>$\bar{y}$ (pmol/10$^8$ pl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitriptyline baseline</td>
<td>12</td>
<td>0.49 ± 0.05</td>
<td>19.8 ± 1.6</td>
<td>13.0 ± 1.0</td>
</tr>
<tr>
<td>Amitriptyline 4 weeks</td>
<td>12</td>
<td>2.63* ± 0.74</td>
<td>26.2 ± 3.0</td>
<td>8.3* ± 1.0</td>
</tr>
<tr>
<td>Zimelidine baseline</td>
<td>11</td>
<td>0.66 ± 0.07</td>
<td>27.0 ± 3.5</td>
<td>15.9 ± 2.0</td>
</tr>
<tr>
<td>Zimelidine 4 weeks</td>
<td>13</td>
<td>4.68** ± 0.74</td>
<td>27.7 ± 4.6</td>
<td>5.9** ± 0.8</td>
</tr>
</tbody>
</table>

$K_m$ higher and $\bar{y}$ lower in treated patients * $p < 0.02$  ** $p < 0.001$

† higher than amitriptyline treated $p < 0.05$
a complete lack of correlation between inhibition of 5-HT uptake and clinical outcome casts doubt on the amine hypothesis of depressive illness.

(ii) Plasma drug concentrations and platelet 5-HT uptake

Plasma drug concentrations reached steady-state levels during the second week of treatment. At 4 weeks the mean plasma concentrations of amitriptyline (378 ± 29 nM) and nortriptyline (393 ± 49 nM) were similar. Plasma drug levels in patients receiving zimelidine were higher than those in patients receiving amitriptyline, probably due to the larger dose. Also, the plasma concentrations of the metabolite norzimelidine were higher (868 ± 84 nM) than those of zimelidine (524 ± 97 nM). There were no significant correlations between plasma drug levels (amitriptyline, nortriptyline, amitriptyline + nortriptyline; zimelidine, norzimelidine, zimelidine + norzimelidine) and the therapeutic effect, whether assessed as raw Hamilton rating score, amelioration score, or percentage improvement in either of the two treatment groups.

The binding of zimelidine and norzimelidine to plasma proteins was measured by equilibrium dialysis in plasma samples from 9 patients who had received zimelidine for 4 weeks. It was found that zimelidine bound to plasma proteins to a greater extent (8.4 ± 1.1% free) than did its metabolite norzimelidine (20.7 ± 1.0% free). Since the plasma drug concentration and the percent free portion are both higher for norzimelidine, then plasma free norzimelidine is much higher than plasma free zimelidine.

The extent to which zimelidine and norzimelidine bind to plasma proteins was measured in plasma samples from 20 normal controls. All the subjects were completely drug-free: the ^3^H-labelled zimelidine or norzimelidine used for the estimation was the only drug present, so that the total drug levels in these samples was very low compared with the levels in the patients' samples. Very similar results were obtained for the control subjects: zimelidine was only 9.7 ± 0.6% free, and norzimelidine was 21.4 ± 0.5% free. Thus the extent to which zimelidine and norzimelidine bind to plasma proteins is remarkably constant.

Since there is very little variation between individuals in the extent to which zimelidine and norzimelidine bind to plasma proteins, the percentage free drug values did not correlate with total drug concentrations,
Using the percent free and total drug concentrations, the concentrations of free zimelidine and free norzimelidine were calculated for the patients. Plasma free zimelidine concentration correlated significantly with total zimelidine plasma concentration \( (r = 0.85; p < 0.01) \); free norzimelidine correlated significantly with total plasma norzimelidine concentration \( (r = 0.95; p < 0.001) \). There were no significant correlations between either free zimelidine or free norzimelidine and therapeutic outcome, although there was a trend towards a positive correlation between free norzimelidine and clinical improvement (free norzimelidine and Hamilton rating score at 4 weeks, \( r = 0.53 \)). Thus too high a plasma drug level may be associated with a poor clinical response.

In the patients who received amitriptyline there were significant correlation between plasma drug levels and the inhibition of platelet 5-HT uptake. The 4 week value of \( K_m \), the increase in \( K_m \) from the baseline value, and the percentage increase in \( K_m \) all correlated significantly with the plasma concentration of amitriptyline at 4 weeks (Table 4.4). These measures also correlated significantly with the total plasma level of amitriptyline plus nortriptyline, but not with the plasma levels of nortriptyline. Partial correlation confirmed that it is amitriptyline rather than its metabolite nortriptyline which inhibits platelet 5-HT uptake.

Similar effects on platelet 5-HT uptake were observed when amitriptyline and nortriptyline were added in vitro (Table 4.5). Amitriptyline acted as a competitive inhibitor, causing an increase in \( K_m \), but having no effect on \( V_{max} \). The extent of the increase in \( K_m \) was not so great in vitro as in vivo, and this is probably due to the fact that the concentration of amitriptyline added to the PRP in vitro (100 ng/ml) was lower than the concentration of amitriptyline found in vivo (119 ± 9 ng/ml, range 67 to 197 ng/ml). However, the effect of amitriptyline in vitro on 5-HT uptake by platelets from control subjects differs from its effect on platelets from depressed patients in that there is a reduction in \( V_{max} \) as well as an increase in \( K_m \). Thus in samples of PRP from controls amitriptyline acts as a mixed inhibitor. This difference may be simply explained by the fact that the drug-free value of \( V_{max} \) was higher in the control subjects than in the depressed patients, while the values of \( V_{max} \) obtained for the two groups after the addition of amitriptyline to the PRP in vitro were very similar. Conversely, it may be that there is some other difference between the samples of PRP from controls and those from depressed patients which causes the amitriptyline to behave differently in the
Table 4.4
Product–moment correlation coefficients between 5-HT uptake characteristics of patients on amitriptyline and plasma drug concentrations

<table>
<thead>
<tr>
<th></th>
<th>Amitriptyline</th>
<th>Nortriptyline</th>
<th>Ami + Nor</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 week $K_m$</td>
<td>+0.67*</td>
<td>+0.53</td>
<td>+0.67*</td>
</tr>
<tr>
<td>$\Delta K_m$</td>
<td>+0.77**</td>
<td>+0.53</td>
<td>+0.69*</td>
</tr>
<tr>
<td>% change $K_m$</td>
<td>+0.80**</td>
<td>+0.58</td>
<td>+0.74*</td>
</tr>
<tr>
<td>4 week $V_{max}$</td>
<td>+0.10</td>
<td>+0.39</td>
<td>+0.32</td>
</tr>
<tr>
<td>$\Delta V_{max}$</td>
<td>+0.41</td>
<td>+0.23</td>
<td>+0.33</td>
</tr>
<tr>
<td>% change $V_{max}$</td>
<td>+0.56</td>
<td>+0.33</td>
<td>+0.47</td>
</tr>
<tr>
<td>4 week $\bar{y}$</td>
<td>-0.43</td>
<td>-0.15</td>
<td>-0.30</td>
</tr>
<tr>
<td>$\Delta \bar{y}$</td>
<td>+0.04</td>
<td>-0.30</td>
<td>-0.19</td>
</tr>
<tr>
<td>% change $\bar{y}$</td>
<td>-0.20</td>
<td>-0.36</td>
<td>-0.34</td>
</tr>
</tbody>
</table>

n = 11 for 4 week values

n = 10 for $\Delta$ and % change values

* p < 0.05, ** p < 0.01

Partial correlation between $\Delta K_m$ (1) and amitriptyline concentration (2), eliminating effect of nortriptyline concentration (3) $r_{12.3} = +0.67$ p < 0.05

Partial correlation between $\Delta K_m$ (1) and nortriptyline concentration (3), eliminating effect of amitriptyline concentration (2) $r_{13.2} = +0.14$ not significant
Table 4.5
Effect of amitriptyline (100 ng/ml PRP) and of nortriptyline (100 ng/ml PRP) in vitro on 5-HT uptake by platelets from depressed patients and control subjects
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (pmol/10^8 pl/min)</th>
<th>$\bar{y}$ (pmol/10^8 pl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depressed patients</td>
<td>9</td>
<td>0.57 ± 0.09</td>
<td>19.5 ± 3.7</td>
<td>11.7 ± 1.6</td>
</tr>
<tr>
<td>Patients + amitriptyline</td>
<td>9</td>
<td>1.87† ± 0.37</td>
<td>16.8 ± 2.1</td>
<td>6.6‡‡ ± 1.0</td>
</tr>
<tr>
<td>Control subjects</td>
<td>9</td>
<td>0.39 ± 0.04</td>
<td>26.2 ± 4.5</td>
<td>16.6 ± 2.7</td>
</tr>
<tr>
<td>Controls and amitriptyline</td>
<td>9</td>
<td>1.46‡‡ ± 0.16</td>
<td>17.0† ± 2.8</td>
<td>7.2‡‡ ± 1.0</td>
</tr>
<tr>
<td>Control subjects</td>
<td>4</td>
<td>0.35 ± 0.03</td>
<td>24.0 ± 2.5</td>
<td>12.7 ± 2.8</td>
</tr>
<tr>
<td>Controls + nortriptyline</td>
<td>4</td>
<td>1.10‡‡ ± 0.14</td>
<td>13.4 ± 1.6</td>
<td>5.5‡‡ ± 0.7</td>
</tr>
</tbody>
</table>

Significant differences by paired tests: * p < 0.05, ** p < 0.02, † p < 0.01, ‡‡ p < 0.001
different samples. The effect of nortriptyline in vitro on the uptake of 5-HT by platelets from control subjects was very similar to the effect of amitriptyline: $K_m$ was increased and $V_{\text{max}}$ was reduced.

Correlations between $K_m$ changes and plasma drug levels were also found in patients receiving zimelidine, but in this group the only correlation which reached significance was that between norzimelidine concentration and the percentage change in $\bar{y}$ from baseline to 4 weeks (Table 4.6). The correlations between free drug plasma levels and platelet 5-HT uptake characteristics were greater than those obtained using total drug concentrations, though few reached significance (Table 4.7). The difference between the total and the free concentrations of zimelidine and norzimelidine with respect to their relationship to platelet 5-HT uptake would suggest that it is the non-bound portion of the drug that is biologically active. Also norzimelidine appears to have a greater effect than zimelidine, though this may be due to the fact that free norzimelidine levels are higher than free zimelidine levels.

Zimelidine added in vitro had a similar effect on platelets from depressed patients and control subjects: it caused a decrease in the total uptake of 5-HT, as indicated by the lowered value of $\bar{y}$, by causing a decrease in $V_{\text{max}}$ (Table 4.8). There was no change in the value of $K_m$, therefore zimelidine in vitro acts as a non-competitive inhibitor of platelet 5-HT uptake. This is in contrast to the results which were observed in vivo, where uptake of 5-HT was reduced by a large increase in $K_m$ and no effect on $V_{\text{max}}$ (Table 4.2). The concentration of zimelidine used for the experiments in vitro (200 ng/ml) was fairly close to the observed plasma concentration of zimelidine after 4 weeks of treatment with the drug (214 ± 39 ng/ml). There are several possible explanations for this discrepancy in the results. One is that the pre-incubation time of 5 minutes, the interval between the addition of zimelidine to the PRP and the addition of the $^{14}$C-labelled 5-HT, might have been too short to allow the zimelidine to bind to plasma proteins to the same extent as in vivo. This is unlikely as the same time interval has been used satisfactorily for other compounds. A more likely explanation is that the effect observed in vivo was due to the action of a metabolite of zimelidine rather than that of the parent compound. The observed correlation between platelet 5-HT uptake and free zimelidine concentrations might be explained by a linear relationship between the level of zimelidine
**Table 4.6**

Product - moment correlation coefficients between 5-HT uptake characteristics of patients on zimelidine and plasma drug concentrations

<table>
<thead>
<tr>
<th></th>
<th>Zimelidine</th>
<th>Norzimelidine</th>
<th>Zim + Norzim</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 week $K_m$</td>
<td>+0.34</td>
<td>+0.51</td>
<td>+0.44</td>
</tr>
<tr>
<td>$\Delta K_m$</td>
<td>+0.31</td>
<td>+0.47</td>
<td>+0.40</td>
</tr>
<tr>
<td>% change $K_m$</td>
<td>+0.13</td>
<td>+0.24</td>
<td>+0.22</td>
</tr>
<tr>
<td>4 week $V_{max}$</td>
<td>-0.03</td>
<td>+0.22</td>
<td>+0.09</td>
</tr>
<tr>
<td>$\Delta V_{max}$</td>
<td>+0.07</td>
<td>-0.11</td>
<td>-0.01</td>
</tr>
<tr>
<td>% change $V_{max}$</td>
<td>+0.07</td>
<td>-0.14</td>
<td>-0.06</td>
</tr>
<tr>
<td>4 week $\bar{y}$</td>
<td>-0.33</td>
<td>-0.06</td>
<td>-0.21</td>
</tr>
<tr>
<td>$\Delta \bar{y}$</td>
<td>+0.07</td>
<td>-0.41</td>
<td>-0.16</td>
</tr>
<tr>
<td>% change $\bar{y}$</td>
<td>-0.32</td>
<td>-0.58*</td>
<td>-0.47</td>
</tr>
</tbody>
</table>

$n = 15$ for 4 week values

$n = 13$ for $\Delta$ and % change values

$^*$ $p < 0.05$
Table 4.7
Product - moment correlation coefficients between 5-HT uptake characteristics of patients on zimelidine and plasma free drug concentrations

<table>
<thead>
<tr>
<th></th>
<th>Free zimelidine</th>
<th>Free norzimelidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 week $K_m$</td>
<td>+0.30</td>
<td>+0.68</td>
</tr>
<tr>
<td>$\Delta K_m$</td>
<td>+0.52</td>
<td>+0.69</td>
</tr>
<tr>
<td>% change $K_m$</td>
<td>+0.72*</td>
<td>+0.52</td>
</tr>
<tr>
<td>4 week $V_{max}$</td>
<td>-0.12</td>
<td>+0.48</td>
</tr>
<tr>
<td>$\Delta V_{max}$</td>
<td>+0.07</td>
<td>-0.36</td>
</tr>
<tr>
<td>% change $V_{max}$</td>
<td>-0.23</td>
<td>-0.59</td>
</tr>
<tr>
<td>4 week $\bar{y}$</td>
<td>-0.62</td>
<td>-0.15</td>
</tr>
<tr>
<td>$\Delta \bar{y}$</td>
<td>-0.32</td>
<td>-0.76*</td>
</tr>
<tr>
<td>% change $\bar{y}$</td>
<td>-0.62</td>
<td>-0.87**</td>
</tr>
</tbody>
</table>

n = 9 for 4 week values
n = 8 for $\Delta$ and % change values

* $p < 0.05$

** $p < 0.01$
Table 4.8

Effect of zimelidine (200 ng/ml PRP) and norzimelidine (200 ng/ml PRP) in vitro on the uptake of 5-HT by platelets from control subjects and depressed patients.

Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (pmol/10^8 platelets/min)</th>
<th>$\bar{y}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depressed patients</td>
<td>7</td>
<td>0.76 ± 0.16</td>
<td>34.7 ± 5.2</td>
<td>19.1 ± 1.9</td>
</tr>
<tr>
<td>Patients + zimelidine</td>
<td>7</td>
<td>0.75 ± 0.15</td>
<td>18.2* ± 1.4</td>
<td>10.5** ± 0.9</td>
</tr>
<tr>
<td>Control subjects</td>
<td>7</td>
<td>0.45 ± 0.05</td>
<td>32.4 ± 8.0</td>
<td>19.6 ± 5.3</td>
</tr>
<tr>
<td>Controls and zimelidine</td>
<td>7</td>
<td>0.60 ± 0.08</td>
<td>23.2* ± 5.4</td>
<td>13.3*** ± 3.7</td>
</tr>
<tr>
<td>Control subjects</td>
<td>5</td>
<td>0.36* ± 0.04</td>
<td>38.9 ± 9.1</td>
<td>21.7 ± 6.5</td>
</tr>
<tr>
<td>Controls and norzimelidine</td>
<td>5</td>
<td>4.68*** ± 0.63</td>
<td>26.0* ± 4.4</td>
<td>5.9*** ± 1.7</td>
</tr>
</tbody>
</table>

Significant differences by paired tests:

* $p < 0.05$,  ** $p < 0.02$,  *** $p < 0.01$
and the concentration of its more active metabolite. This hypothesis is supported by the results of the experiments in which norzimelididine, the primary metabolite of zimelidine, was added in vitro to samples of PRP from control subjects (Table 4.6).

Norzimelididine caused a large increase in the value of $K_m$, and a proportionately smaller, but still significant, decrease in $V_{max}$. These results are similar to those found for the effect of anitriptyline in vitro on platelets from control subjects (Table 4.5). The concentration of norzimelididine used for the experiments in vitro (200 ng/ml) was much lower than the plasma concentrations found in patients receiving zimelidine for 4 weeks (342 ± 33 ng/ml). Since the plasma concentration of norzimelididine at steady state conditions (342 ng/ml) was almost twice as great as that of zimelidine (214 ng/ml) it is possible that the metabolite exerts a greater effect than the parent compound. If the assumption that it is only the non-protein-bound portion of the drug that is biologically active is valid, then the discrepancy is increased, since zimelidine binds to plasma proteins to a greater extent (only 8.4 ± 1.1% free) than does norzimelididine (20.7 ± 1.0% free). The plasma concentration of free zimelidine was only 38.6 ± 7.1 ng/ml, while the plasma concentration of free norzimelididine was 189.3 ± 24.3 ng/ml.

(iii) Platelet tryptophan uptake and plasma levels of tryptophan, phenylalanine and tyrosine.

Tryptophan uptake characteristics were determined for platelet samples from 20 depressed patients at baseline, and after 4 weeks of treatment, from 8 patients receiving amitriptyline and from 10 patients receiving zimelidine. Neither amitriptyline nor zimelidine had any significant effect on platelet tryptophan uptake (Table 4.9).

Plasma percentage free tryptophan was determined by the equilibrium dialysis method for 5 patients before and during treatment with amitriptyline and for 9 patients before and during treatment with zimelidine. Neither treatment had any significant effect on percentage free tryptophan or free tryptophan concentration (Table 4.10). Both treatments caused a reduction in plasma total tryptophan concentration, though this only reached significance in the amitriptyline group.

Plasma concentrations of tryptophan, tyrosine and phenylalanine
Table 4.9
Platelet tryptophan uptake in depressed patients, and patients receiving either amitriptyline or zimelidine
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (pmol/10^8 pl/min)</th>
<th>$\bar{y}$ (pmol/10^8 pl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depressed</td>
<td>20</td>
<td>7.7 ± 1.3</td>
<td>54.3 ± 6.5</td>
<td>14.2 ± 1.5</td>
</tr>
<tr>
<td>On amitriptyline</td>
<td>8</td>
<td>9.2 ± 2.3</td>
<td>70.2 ± 18.3</td>
<td>14.1 ± 1.6</td>
</tr>
<tr>
<td>On zimelidine</td>
<td>10</td>
<td>6.5 ± 1.2</td>
<td>55.8 ± 15.8</td>
<td>14.3 ± 2.2</td>
</tr>
</tbody>
</table>
Table 4.10a  
Total, percent free and calculated free plasma tryptophan in 5 depressed patients before and during treatment with amitriptyline. Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>On amitriptyline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total tryptophan (µM)</td>
<td>52.4 ± 1.6</td>
<td>43.6* ± 2.9</td>
</tr>
<tr>
<td>% free tryptophan</td>
<td>23.1 ± 0.2</td>
<td>26.5 ± 2.9</td>
</tr>
<tr>
<td>Free tryptophan (µM)</td>
<td>12.1 ± 0.3</td>
<td>11.3 ± 0.8</td>
</tr>
</tbody>
</table>

* p < 0.05 (method of paired comparisons)

Table 4.10b  
Total, percent free and calculated free plasma tryptophan in 9 depressed patients before and during treatment with zimelidine. Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>On zimelidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total tryptophan (µM)</td>
<td>53.0 ± 2.4</td>
<td>48.5 ± 2.1</td>
</tr>
<tr>
<td>% free tryptophan</td>
<td>26.1 ± 2.6</td>
<td>25.8 ± 1.1</td>
</tr>
<tr>
<td>Free tryptophan (µM)</td>
<td>13.6 ± 1.1</td>
<td>12.8 ± 0.5</td>
</tr>
</tbody>
</table>
Table 4.11a
Plasma tryptophan, tyrosine and phenylalanine concentrations in 17 depressed patients before and during treatment with amitriptyline
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>On amitriptyline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan (µM)</td>
<td>54.0 ± 2.1</td>
<td>50.5 ± 2.2</td>
</tr>
<tr>
<td>Tyrosine (µM)</td>
<td>76.6 ± 3.1</td>
<td>79.6 ± 4.3</td>
</tr>
<tr>
<td>Phenylalanine (µM)</td>
<td>67.7 ± 2.9</td>
<td>63.6 ± 4.5</td>
</tr>
</tbody>
</table>

Table 4.11b
Plasma tryptophan, tyrosine and phenylalanine concentrations in 13 depressed patients before and during treatment with zimelidine
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>On zimelidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan (µM)</td>
<td>54.4 ± 2.6</td>
<td>47.4* ± 2.9</td>
</tr>
<tr>
<td>Tyrosine (µM)</td>
<td>75.3 ± 3.3</td>
<td>77.4 ± 3.9</td>
</tr>
<tr>
<td>Phenylalanine (µM)</td>
<td>68.8 ± 3.8</td>
<td>70.1 ± 4.3</td>
</tr>
</tbody>
</table>

* p < 0.05 (method of paired comparisons)
were measured in samples from 17 patients before and during treatment with amitriptyline, and from 13 patients before and during treatment with zimelidine. Both treatments caused a reduction in tryptophan concentration, though this only reached significance in the zimelidine group (Table 4.11). Neither treatment caused any significant change in the plasma concentration of tyrosine or phenylalanine.

(D) SUMMARY

In this trial the clinical effectiveness of zimelidine was shown to be of the same order as amitriptyline in the treatment of primary depressive illness. The incidence of unwanted subjective side-effects was found to be less, and probably reflects the weak anticholinergic activity of zimelidine. After 6 weeks of treatment with zimelidine the corrected side-effects score was negative, and since many of the side-effects are a reflection of depressive state, the decrease in the side-effects score reflects the clinical improvement of the patients. As is generally found with antidepressant drugs, there were no significant correlations between plasma drug concentrations and therapeutic effect.

Both amitriptyline and zimelidine in clinically effective doses caused highly significant inhibition of platelet 5-HT uptake. The lack of any correlation between the inhibition of 5-HT uptake caused by these drugs with clinical improvement casts doubt on their supposed mode of action.

THE EFFECTS OF MIANSERIN AND LITHIUM ON PLATELET 5-HT UPTAKE IN RECOVERED DEPRESSIVE PATIENTS.

(A) MIANSERIN

Mianserin hydrochloride has a tetracyclic piperazinoazepine structure and has been shown in controlled trials to have an antidepressant effect. Although classical animal pharmacological tests failed to predict any antidepressant activity for this compound, it was found that mianserin produces changes in the human electroencephalogram similar to those produced by the tricyclic antidepressants (Itil, 1973). The effects of mianserin have been compared with those of tricyclic antidepressant drugs, and it has been shown to have an antidepressant effect comparable with amitriptyline (Wheatley, 1975) and imipramine (Murphy, 1975), and to
be superior to placebo (Murphy, Donald and Moller, 1976). It has also
been found that, compared with most tricylic antidepressant drugs,
mianserin produces fewer and less severe unwanted side-effects as it
has less anticholinergic activity (Murphy, 1975; Coppen et al., 1976).

The plasma half-life of mianserin is 6 to 12 hours. Stable
steady state levels are reached after 2 weeks administration (Coppen
and Ghose, 1976). The drug is 90% to 95% protein bound in the plasma
at therapeutic concentrations (Kopera, 1975). Animal experiments have
shown that mianserin rapidly enters the brain and becomes evenly
distributed in the central nervous system.

It has been reported that, in contrast to tricylic antidepressant
drugs, mianserin in therapeutic concentrations in vivo does not block
the central noradrenaline reuptake mechanisms in rats (Leonard, 1974).
However, Raiteri, Angelini and Bertollini (1976) found that in vitro
mianserin behaved similarly to imipramine in blocking noradrenaline uptake
in rat hypothalamic synaptosomes. This group also investigated the effect
of mianserin on rat blood platelet uptake of 5-HT, using incubation times
of 10 minutes, and were unable to detect any inhibition of 5-HT uptake
with mianserin at a concentration of $2 \times 10^{-5}$ M.

Mianserin is of interest since it is the first antidepressant drug
to apparently increase noradrenaline release by an effect on the presynaptic
$\alpha_2$-receptors (Baumann and Maitre, 1975). It is only slightly active
as an inhibitor of the uptake of 5-HT and noradrenaline, and it has been
shown to be a 5-HT receptor antagonist (Saxena, Van Houwelingen and
Bonta, 1971).

A description of the medical use of lithium salts is given at the
beginning of Chapter 5.

(B) **PATIENTS**

A trial was conducted to compare the effectiveness of lithium
carbonate and of mianserin hydrochloride as prophylactic medication for
the prevention of recurrence of depression.

A group of 3 male and 15 female patients was studied. These
patients had all been admitted to hospital suffering from primary depressive illness, and had been treated with either antidepressant drugs (amitriptyline, cyclazimole or zimelidine) or ECT. The medication was withdrawn in those patients who had recovered, and after a further 14 days without antidepressant drug treatment their clinical state was assessed. Those patients with a Hamilton rating score of 6 or less were regarded as recovered, and were included in the study.

At the end of the drug-free period, a baseline blood sample was collected, after overnight fasting, for the estimation of platelet 5-HT and tryptophan uptake, and plasma concentrations of tryptophan, tyrosine and phenylalanine. The patients were then randomly allocated to receive either lithium carbonate or mianserin hydrochloride. For those patients who received lithium the dose was adjusted to produce a plasma concentration of about 1 mM, measured approximately 13 hours after the previous evening's dose. The patients on mianserin received 20 mg three times daily, i.e. 60 mg per day. The patients were treated on an out-patient basis during the course of the study. The treatment was continued for 6 weeks, after which time the patients returned to the out-patient clinic for a further blood sample to be taken for the estimation of plasma drug concentration, and for a repeat of the platelet 5-HT and tryptophan uptake measurements and determination of plasma concentrations of tryptophan, tyrosine and phenylalanine.

(C) RESULTS

(i) Platelet 5-HT uptake and plasma drug concentrations

Mianserin and lithium both caused an increase in platelet 5-HT uptake (Table 4.12) (The results obtained for the patients who received lithium are also presented in Chapter 5.). Both treatments caused a small increase in $K_m$, though this only reached significance in the mianserin group. Both treatments caused a significant increase in both $V_{max}$ and $\bar{y}$. Lithium had a greater effect on the rate of 5-HT uptake than did mianserin. Patients receiving lithium had a significantly higher value of $\bar{y}$ than did patients receiving mianserin. Thus, in contrast to antidepressant drugs such as amitriptyline and zimelidine, which inhibit platelet 5-HT uptake, both lithium and mianserin increase platelet 5-HT uptake in depressive patients towards normal control values.
Table 4.12
Uptake of 5-HT by platelets from recovered depressive patients, and patients receiving either mianserin or lithium
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (pmol/10^8 pl/min)</th>
<th>$\bar{y}$ (pmol/10^8 pl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovered depressives</td>
<td>18</td>
<td>0.61 ± 0.05</td>
<td>19.3 ± 2.1</td>
<td>11.3 ± 1.1</td>
</tr>
<tr>
<td>Patients on mianserin</td>
<td>10</td>
<td>0.71† ± 0.11</td>
<td>24.4* ± 4.1</td>
<td>12.8** ± 1.9</td>
</tr>
<tr>
<td>Patients on lithium</td>
<td>8</td>
<td>0.76 ± 0.19</td>
<td>34.4* ± 4.6</td>
<td>19.7††± ± 2.1</td>
</tr>
</tbody>
</table>

$K_m$, $V_{max}$ and $\bar{y}$ increased by treatment

* $p < 0.05$,  ** $p < 0.05$,  †† $p < 0.01$

Lithium higher than mianserin $p < 0.05$
The plasma mianserin concentration at 6 weeks was available for 7 patients. The mean was 39.1 ± 4.7 ng/ml. The plasma lithium concentration was available for 7 patients and the mean was 0.93 ± 0.05 mM (the eighth patient forgot to take her tablets on the evening prior to the 6 week appointment and had a plasma lithium level of only 0.06mM). There were no significant correlations between plasma drug concentration and platelet 5-HT uptake characteristics, or change in platelet 5-HT uptake from baseline, in either the mianserin or the lithium group.

Mianserin hydrochloride at a concentration of 50 ng/ml was added in vitro to platelet samples from 3 recovered depressive patients and 7 control subjects. In the samples from patients mianserin caused an increase in $K_m$ and a small decrease in $V_{max}$; these changes were not significant, probably due to the small sample size. In the samples from control subjects mianserin had no effect on $K_m$, but caused a significant decrease in $V_{max}$ (Table 4.13). It is possible that mianserin has a different effect on platelets from depressive patients than on platelets from control subjects, though for both groups the effects observed in vitro were different from the effects observed in vivo in depressive patients.

An analogue of mianserin, GC94, and two metabolites of mianserin, GF45 and OH46, were added in vitro to platelet samples from control subjects. The concentration used in each case was 50 ng/ml. None of these compounds had any effect of $K_m$, and all caused a significant reduction in $V_{max}$ (Table 4.14). These results fail to throw any light on the observed effects of mianserin in depressive patients.

(ii) **Platelet tryptophan uptake and plasma concentrations of tryptophan, phenylalanine and tyrosine.**

Platelet tryptophan uptake was measured in samples from 11 recovered depressive patients, 9 patients receiving mianserin and 8 patients receiving lithium. There were no significant differences between the groups, although the patients on lithium had a higher platelet tryptophan uptake than the drug-free patients and the patients on mianserin (Table 4.15).

Percentage free plasma tryptophan was determined by the equilibrium dialysis method in plasma samples from 19 recovered depressive patients,
### Table 4.13

Effect of mianserin (50 ng/ml) in vitro on 5-HT uptake by platelets from recovered depressive patients and control subjects

Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>(K_m) (µM)</th>
<th>(V_{max}) (pmol/10^8 pl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovered depressives</td>
<td>3</td>
<td>0.47 ± 0.08</td>
<td>10.5 ± 3.6</td>
</tr>
<tr>
<td>Recovered depressives + mianserin</td>
<td>3</td>
<td>0.71 ± 0.19</td>
<td>9.3 ± 1.7</td>
</tr>
<tr>
<td>Control subjects</td>
<td>7</td>
<td>0.65 ± 0.09</td>
<td>21.3 ± 3.6</td>
</tr>
<tr>
<td>Control subjects + mianserin</td>
<td>7</td>
<td>0.68 ± 0.09</td>
<td>16.7* ± 2.9</td>
</tr>
</tbody>
</table>

* lower than drug-free value (paired t-test, \(p < 0.01\)
Table 4.14
Effect of GC94, GF45 and OH46 on 5-HT uptake in vitro by platelets from control subjects
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>K_m (µM)</th>
<th>V_max (pmol/10^8 pl/min)</th>
<th>Y (pmol/10^8 pl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>5</td>
<td>0.47 ± 0.07</td>
<td>48.9 ± 7.0</td>
<td>32.0 ± 4.3</td>
</tr>
<tr>
<td>Controls + GC94</td>
<td>5</td>
<td>0.54 ± 0.09</td>
<td>31.6** ± 4.3</td>
<td>20.6*** ± 3.6</td>
</tr>
<tr>
<td>Control subjects</td>
<td>5</td>
<td>0.50 ± 0.03</td>
<td>38.8 ± 6.1</td>
<td>24.6 ± 3.6</td>
</tr>
<tr>
<td>Controls + GF45</td>
<td>5</td>
<td>0.50 ± 0.04</td>
<td>27.7** ± 3.8</td>
<td>17.7* ± 2.0</td>
</tr>
<tr>
<td>Control subjects</td>
<td>5</td>
<td>0.49 ± 0.04</td>
<td>39.2 ± 6.1</td>
<td>25.1 ± 3.5</td>
</tr>
<tr>
<td>Controls + OH46</td>
<td>5</td>
<td>0.63 ± 0.05</td>
<td>25.4* ± 2.1</td>
<td>15.3* ± 1.3</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.02, *** p < 0.01
9 patients on mianserin and 7 patients on lithium. There were no significant differences between the groups in percentage free tryptophan, total tryptophan concentration or calculated free tryptophan concentration (Table 4.16).

Plasma concentrations of tryptophan, tyrosine and phenylalanine were measured in samples from 27 recovered depressives, 10 patients on mianserin and 9 patients on lithium. There were no significant differences between the groups (Table 4.17).

(D) SUMMARY

Mianserin and lithium are both effective as prophylactic treatment for the prevention of recurrence of depressive illness, and both have fewer unwanted side-effects of the types caused by the anticholinergic activity of tricyclic antidepressant drugs. It is possible that both mianserin and lithium exert their prophylactic effect in depressive patients by increasing the rate of uptake of 5-HT towards normal values.
Lithium salts were introduced into medicine in the 1850's for the treatment of gout, following the demonstration that lithium urate was the most soluble of the urates (Garrod, 1859). The anti-manic properties of lithium were first described by Cade (1949). Subsequently a number of clinical trials demonstrated that lithium is an effective treatment for mania, and a powerful prophylactic against depressive swings in both bipolar and unipolar affective disorder (Schou et al., 1954; Maggs, 1963; Baastrup et al., 1970; Coppen et al., 1971; Hullin, McDonald and Allsop, 1972). Lithium has also been used with varying degrees of success in other psychiatric disorders, including schizophrenia, aggression, premenstrual tension and alcoholism (Schou, 1978). Originally lithium citrate was the preferred salt because of its solubility, but now the carbonate is preferred as it produces less alimentary disturbance.

At present, the mechanism by which lithium produces its therapeutic effects are unknown, although it has been shown that under some conditions of administration lithium produces alterations in the turnover and metabolism of biogenic amines (Knapp and Mandell, 1973; Collard, 1978). Haskovec and Rysanek (1969) found that lithium caused an increase in the excretion of 3-methoxy-4-hydroxymandelic acid (vanillylmandelic acid, or VMA, a metabolite of noradrenaline) in 10 healthy control subjects. Schildkraut (1974) reported an increase during lithium therapy of the excretion of 3-methoxy-4-hydroxyphenyl glycol (MHPG, a metabolite of noradrenaline largely derived from the brain). In rats given lithium for 3 days the turnover of 5-HT was increased (Perez-Cruet et al., 1971), but after about 20 days of lithium administration there was a decrease in 5-HT turnover (Corrodi and Schou, 1969). It has been suggested (Mandell and Knapp, 1977) that lithium first causes an increase in 5-HT turnover by increasing the high affinity uptake system for tryptophan into the nerve endings. In time a new steady-state is reached, with increased tryptophan uptake.
but decreased tryptophan hydroxylase activity and reduced 5-HT turnover.

Several investigators have studied the effect of lithium on platelet 5-HT uptake. Genefke (1972) found that in rats treated with lithium chloride platelet 5-HT uptake was not affected, but when added to the incubation medium lithium chloride inhibited 5-HT uptake by both rat and human platelets. Murphy and co-workers (1969, 1970) found that treatment with lithium carbonate caused a significant increase in platelet 5-HT uptake in a group of 10 patients with affective illness, whereas the addition of lithium chloride to the incubation medium had no effect on platelet 5-HT uptake. A similar finding was reported by Born, Grignani and Martin (1980). Coppen and co-workers (1976) reported that whole blood 5-HT concentrations were low in depressive patients, and that lithium therapy significantly increased the levels of whole blood 5-HT.

(B) THE EFFECT OF LITHIUM ON PLATELET 5-HT UPTAKE IN DEPRESSIVE PATIENTS

For the purpose of this thesis, the effect of lithium therapy in patients with affective disorder was investigated in two studies.

(i) Studies involving patients tested before and during lithium therapy

First, a small group of patients who had all recently recovered from an episode of depression were tested both before and during treatment with lithium carbonate. The patients studied were admitted to hospital suffering from primary depressive illness and had no history of mania or significant physical illness. After a drug-free period of at least 7 days, the patients were assessed for the severity of their symptoms by the Hamilton Rating Scale (HRS) for depression, and those patients who scored 16 or more on the first 16 items of the HRS were than treated for 6 weeks with a tricyclic antidepressant drug. The medication was then withdrawn in patients who had recovered, and after a further 14 days without drug treatment their clinical state was assessed. Those patients with a HRS score ≤ 6 were regarded as recovered.
Blood samples for estimation of platelet 5-HT uptake characteristics were collected from one male and seven female recovered patients. They were then treated with lithium carbonate (sustained-release tablets, Priadel), the dose being adjusted so that a plasma level of 0.8 to 1.0 mmol/l, measured approximately 12 hours after the last dose, was achieved. After six weeks' treatment a repeat blood sample was taken for platelet 5-HT uptake estimation.

The administration of lithium carbonate for 6 weeks caused a significant increase in the value of $V_{\text{max}}$ (Table 5.1), but had no effect on $K_m$, or on the number of platelets in the plasma. The mean plasma lithium concentration was $0.82 \pm 0.12$ mequiv/l.

A similar group of seven female recovered patients (including 4 subjects from the group described above) were investigated before and after one year of lithium therapy. Administration of lithium caused a significant increase in $V_{\text{max}}$ (Table 5.2), but had no effect on $K_m$. The number of platelets in the circulation was also increased. The mean plasma lithium concentration was $0.90 \pm 0.04$ mequiv/l.

(ii) Study of patients on long-term lithium therapy

Second, a group of 65 out-patients who attended the lithium clinic at Greenbank Ward, West Park Hospital, were tested on one or more occasions. At the time of testing all patients were being treated only with lithium carbonate; patients receiving any other form of medication in addition to lithium were excluded at this stage of the study. The patients had been receiving lithium for from 6 weeks to over 10 years. The group consisted of 30 female and 16 male unipolar patients, all of whom had suffered at least 3 episodes of depression without any history of mania; and 10 female and 9 male bipolar patients, all of whom had suffered at least one episode of mania, and many of whom had also had several episodes of depression. The sex ratios in the two diagnostic categories are similar to those usually found, and support the hypothesis that unipolar and bipolar illness are genetically distinct (Leonhard, Korff and Schulz, 1962).

There were no significant differences between the sexes in either unipolar or bipolar patients for platelet concentration, $K_m$ or $V_{\text{max}}$ (Table 5.3), so the results for males and females were combined. There were no significant differences between unipolar and bipolar
Table 5.1

Uptake of 5-HT by platelets from 8 recovered depressive patients before and after 6 weeks lithium therapy
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Drug-free</th>
<th>On lithium</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Platelet concentration</strong> (x 10^8/ml PRP)</td>
<td>3.21 ± 0.29</td>
<td>3.23 ± 0.42</td>
</tr>
<tr>
<td><strong>Kₘ (μM)</strong></td>
<td>0.73 ± 0.10</td>
<td>0.76 ± 0.19</td>
</tr>
<tr>
<td><strong>Vₘₐₓ</strong> (pmol/10^8 pl/min)</td>
<td>22.4 ± 3.4</td>
<td>34.4* ± 4.6</td>
</tr>
</tbody>
</table>

* p < 0.05, (method of paired comparisons)
Table 5.2
Uptake of 5-HT by platelets from 7 recovered depressive patients before and after one year's lithium therapy
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Drug-free</th>
<th>On lithium</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Platelet concentration</strong></td>
<td>2.91 ± 0.31</td>
<td>3.74* ± 0.62</td>
</tr>
<tr>
<td>(x 10^8/ml PRP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Km</strong></td>
<td>0.62 ± 0.09</td>
<td>0.61 ± 0.05</td>
</tr>
<tr>
<td>(µM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>V_max</strong></td>
<td>14.1 ± 2.3</td>
<td>28.6** ± 3.8</td>
</tr>
<tr>
<td>(pmol/10^8 pl/min)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05

** p < 0.01

(method of paired comparisons)
### Table 5.3
Platelet 5-HT uptake characteristics of patients receiving lithium therapy

Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (yr)</th>
<th>Platelet conc (^n) (x 10(^8)/ml PRP)</th>
<th>(K_m) (µM)</th>
<th>(V_{max}) (pmol/10(^8) pl/min)</th>
<th>Plasma Li (mequiv/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male unipolar</td>
<td>16</td>
<td>54.1 ± 2.0</td>
<td>3.31 ± 0.23</td>
<td>0.48 ± 0.04</td>
<td>33.7 ± 2.9</td>
<td>0.80 ± 0.05</td>
</tr>
<tr>
<td>Female unipolar</td>
<td>30</td>
<td>57.8 ± 1.8</td>
<td>3.52 ± 0.16</td>
<td>0.57 ± 0.05</td>
<td>40.0 ± 3.3</td>
<td>0.86 ± 0.03</td>
</tr>
<tr>
<td>Male bipolar</td>
<td>9</td>
<td>48.9 ± 3.7</td>
<td>2.80 ± 0.19</td>
<td>0.67 ± 0.12</td>
<td>40.8 ± 5.2</td>
<td>0.94 ± 0.06</td>
</tr>
<tr>
<td>Female bipolar</td>
<td>10</td>
<td>53.8 ± 4.9</td>
<td>3.08 ± 0.22</td>
<td>0.50 ± 0.05</td>
<td>48.2 ± 4.7</td>
<td>0.98 ± 0.08</td>
</tr>
</tbody>
</table>
patients, although $V_{\text{max}}$ was slightly higher in bipolar (mean 44.7 + 3.5 pmol/10^8 platelets/min) than in unipolar patients (mean 37.8 + 2.4).

The results for unipolar and bipolar patients were combined, and compared with those obtained for control subjects (as described in Chapter 2, but eliminating persons aged 35 years or less), depressed patients and recovered depressive patients (Table 5.4). There was no significant difference between any of the patient groups in age, platelet concentration or $K_m$. The control subjects were younger than the patients on lithium ($p < 0.05$), and also had a lower platelet concentration ($2.64 \pm 0.10 \times 10^8$/ml PRP) than the lithium patients ($3.26 \pm 0.11$) ($d = 4.168$, $p < 0.001$).

There was no significant correlation between platelet concentration and age, $K_m$ or $V_{\text{max}}$ in any of the groups. There was a significant correlation between age and $V_{\text{max}}$ ($r = -0.451$, $p < 0.01$) in the recovered depressive patients. In the unipolar patients receiving lithium there was a significant correlation between plasma lithium concentration and $K_m$ ($r = +0.356$, $p < 0.05$).

The effect on platelet 5-HT uptake of adding lithium carbonate in vitro was examined in PRP samples from 7 control subjects. The addition of lithium carbonate to a final concentration in the PRP of 1 mequiv/1 caused a significant reduction in the rate of 5-HT uptake (Table 5.5). This finding is in agreement with the report by Genefke (1972) that lithium chloride inhibited 5-HT uptake when added to the incubation medium.

Although all subjects were asked to fast overnight prior to venepuncture, it is possible that some of the lithium clinic out-patients may have neglected to do so. In order to confirm that, as found with control subjects (see Chapter 2), the consumption of "breakfast" has no effect on the platelet 5-HT uptake results, a group of 14 patients (9 female unipolar, 3 male unipolar, one female bipolar and one male bipolar), all of whom were known to be reliable, were tested once when fasting, and then again when not fasting at their subsequent visit to the clinic. The test-re-test interval
Table 5.1
Platelet 5-HT uptake characteristics of control subjects, depressed patients, recovered depressives, and depressive patients receiving lithium therapy
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age</th>
<th>$K_m$</th>
<th>$V_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(yr)</td>
<td>(µM)</td>
<td>(pmol/10^8 pi/min)</td>
</tr>
<tr>
<td>Control subjects</td>
<td>48</td>
<td>51.0 ± 1.2</td>
<td>0.50 ± 0.02</td>
<td>39.5 ± 2.4</td>
</tr>
<tr>
<td>Depressed patients</td>
<td>77</td>
<td>51.8 ± 1.6</td>
<td>0.52 ± 0.03</td>
<td>33.2*** ± 1.4</td>
</tr>
<tr>
<td>Recovered depressives</td>
<td>33</td>
<td>52.2 ± 2.4</td>
<td>0.54 ± 0.03</td>
<td>24.2*** ± 2.2</td>
</tr>
<tr>
<td>Patients on lithium</td>
<td>65</td>
<td>55.1* ± 1.4</td>
<td>0.55 ± 0.03</td>
<td>39.8 ± 2.0</td>
</tr>
</tbody>
</table>

* older than controls, $p < 0.05$

** lower than controls, $p < 0.05$, and lithium patients, $p < 0.01$

*** lower than controls, depressed patients, and lithium patients, $p < 0.001$
Table 5.5

Effect on platelet 5-HT uptake of lithium carbonate added in vitro to PRP from 7 control subjects

Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (pmol/10^8 pl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>0.61 ± 0.16</td>
<td>24.8 ± 5.0</td>
</tr>
<tr>
<td>Controls + Li$_2$CO$_3$</td>
<td>0.80 ± 0.32</td>
<td>16.3 * ± 4.9</td>
</tr>
</tbody>
</table>

* $p < 0.01$ (method of paired comparisons)
Table 5.6
Platelet 5-HT uptake in 14 patients on lithium tested twice: with and without fasting
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Fasting</th>
<th>Not fasting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet concentration (x 10⁸/ml PRP)</td>
<td>3.27 ± 0.29</td>
<td>3.23 ± 0.26</td>
</tr>
<tr>
<td>Kₘ (µM)</td>
<td>0.44 ± 0.03</td>
<td>0.49 ± 0.04</td>
</tr>
<tr>
<td>Vₘₐₓ (pmol/10⁸ platelets/min)</td>
<td>43.8 ± 3.6</td>
<td>49.3 ± 3.2</td>
</tr>
<tr>
<td>Plasma lithium concentration (mequiv/L)</td>
<td>0.75 ± 0.03</td>
<td>0.74 ± 0.06</td>
</tr>
</tbody>
</table>
was from 6 to 10 weeks, which was short enough to eliminate any possible effects of seasonal variation. It was found that consumption of "breakfast" had no significant effect on the results (Table 5.6).

Due to participation in various studies to compare the efficacy of lithium in conjunction with or instead of other treatments, a number of patients on lithium only were tested on several occasions. The results for a group of 9 patients (2 male unipolar, 4 female unipolar, and 3 female bipolar) who had been tested on four or more occasions were examined for intraindividual variation (Tables 5.7 and 5.8). The intraindividual variation, as indicated by the coefficients of variance, in both $K_m$ and $V_{max}$ was similar to that found in control subjects (Chapter 2, Tables 2.7a and 2.7b), with a mean coefficient of variance of about 30% for both $K_m$ and $V_{max}$ in both groups. However, the mean coefficient of variance of platelet concentration was lower (15%) in the lithium treated patients than in the controls (25%).

In order to investigate the possible effect of seasonal variation, the results obtained for platelet 5-HT uptake in patients receiving lithium therapy were divided into two-monthly groups, according to the system used for the control subjects (Chapter 2). A total of 132 platelet 5-HT uptake results were available.

As found in control subjects and drug-free depressive patients (Chapters 2 and 3), the rate of platelet 5-HT uptake in lithium-treated patients was greatest in the winter and lowest in the summer (Figure 5.1). This finding emphasises that it is essential to test concurrently groups of subjects which are to be compared.

A considerable proportion of the lithium clinic out-patients require hypnotics. In order to investigate the possible effects on platelet 5-HT uptake of nitrazepam (Mogadon), a commonly administered hypnotic, a group of 21 patients who were receiving both lithium and nitrazepam were tested, and the results compared with those for a group receiving lithium only, matched to the first group as closely as possible for age, sex, diagnosis and month of test.

It was found, as might be expected, that it was the older patients who were receiving nitrazepam, the mean age of the group receiving both lithium and nitrazepam being 61.8 ± 1.9 years. These
Table 5.7
Intraindividual variation in platelet 5-HT uptake and plasma lithium concentration in patients on lithium. Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Test period (months)</th>
<th>No. of tests (n)</th>
<th>Platelet conc&lt;sup&gt;n&lt;/sup&gt; (x 10&lt;sup&gt;8&lt;/sup&gt;/ml PRP)</th>
<th>K&lt;sub&gt;m&lt;/sub&gt; (µM)</th>
<th>V&lt;sub&gt;max&lt;/sub&gt; (pmol/10&lt;sup&gt;8&lt;/sup&gt; pl/min)</th>
<th>Li level (mequiv/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Unipolar 1</td>
<td>16</td>
<td>4</td>
<td>3.51 ± 0.64</td>
<td>0.45 ± 0.03</td>
<td>46.1 ± 4.4</td>
<td>0.63 ± 0.07</td>
</tr>
<tr>
<td>Male Unipolar 2</td>
<td>45</td>
<td>6</td>
<td>3.04 ± 0.14</td>
<td>0.48 ± 0.08</td>
<td>39.6 ± 3.2</td>
<td>0.90 ± 0.06</td>
</tr>
<tr>
<td>Female Unipolar 1</td>
<td>16</td>
<td>5</td>
<td>4.04 ± 0.24</td>
<td>0.41 ± 0.03</td>
<td>35.0 ± 2.0</td>
<td>0.85 ± 0.04</td>
</tr>
<tr>
<td>Female Unipolar 2</td>
<td>21</td>
<td>4</td>
<td>2.31 ± 0.05</td>
<td>0.39 ± 0.07</td>
<td>44.9 ± 17.4</td>
<td>0.70 ± 0.05</td>
</tr>
<tr>
<td>Female Unipolar 3</td>
<td>16</td>
<td>5</td>
<td>3.09 ± 0.15</td>
<td>0.40 ± 0.05</td>
<td>44.8 ± 5.7</td>
<td>0.80 ± 0.08</td>
</tr>
<tr>
<td>Female Unipolar 4</td>
<td>22</td>
<td>4</td>
<td>3.52 ± 0.26</td>
<td>0.46 ± 0.03</td>
<td>46.7 ± 6.3</td>
<td>0.89 ± 0.01</td>
</tr>
<tr>
<td>Female Bipolar 1</td>
<td>41</td>
<td>4</td>
<td>2.90 ± 0.13</td>
<td>0.53 ± 0.13</td>
<td>32.1 ± 5.9</td>
<td>0.87 ± 0.06</td>
</tr>
<tr>
<td>Female Bipolar 2</td>
<td>15</td>
<td>4</td>
<td>1.77 ± 0.10</td>
<td>0.54 ± 0.05</td>
<td>52.4 ± 9.5</td>
<td>0.89 ± 0.05</td>
</tr>
<tr>
<td>Female Bipolar 3</td>
<td>20</td>
<td>4</td>
<td>2.90 ± 0.34</td>
<td>0.50 ± 0.08</td>
<td>64.2 ± 17.0</td>
<td>0.87 ± 0.09</td>
</tr>
</tbody>
</table>
Table 5.8
Intraindividual variation: coefficient of variance of 5-HT uptake and plasma lithium concentration

<table>
<thead>
<tr>
<th>Subject</th>
<th>No. of tests</th>
<th>Platelet conc $^n$</th>
<th>$K_m$</th>
<th>$V_{max}$</th>
<th>Li level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Unipolar 1</td>
<td>4</td>
<td>37%</td>
<td>14%</td>
<td>19%</td>
<td>22%</td>
</tr>
<tr>
<td>Male Unipolar 2</td>
<td>6</td>
<td>11%</td>
<td>42%</td>
<td>20%</td>
<td>16%</td>
</tr>
<tr>
<td>Female Unipolar 1</td>
<td>5</td>
<td>13%</td>
<td>15%</td>
<td>13%</td>
<td>12%</td>
</tr>
<tr>
<td>Female Unipolar 2</td>
<td>4</td>
<td>14%</td>
<td>37%</td>
<td>78%</td>
<td>14%</td>
</tr>
<tr>
<td>Female Unipolar 3</td>
<td>5</td>
<td>11%</td>
<td>27%</td>
<td>28%</td>
<td>23%</td>
</tr>
<tr>
<td>Female Unipolar 4</td>
<td>4</td>
<td>15%</td>
<td>14%</td>
<td>27%</td>
<td>2%</td>
</tr>
<tr>
<td>Female Bipolar 1</td>
<td>4</td>
<td>9%</td>
<td>47%</td>
<td>37%</td>
<td>12%</td>
</tr>
<tr>
<td>Female Bipolar 2</td>
<td>4</td>
<td>11%</td>
<td>18%</td>
<td>26%</td>
<td>12%</td>
</tr>
<tr>
<td>Female Bipolar 3</td>
<td>4</td>
<td>23%</td>
<td>32%</td>
<td>53%</td>
<td>20%</td>
</tr>
</tbody>
</table>
Legend to Figure 5.1

Seasonal variation in platelet 5-HT uptake in depressive patients receiving lithium

Points shown as mean with standard error bar

\[
\begin{align*}
V_{\text{max}} & \quad \text{A lower than January + February, } p < 0.05, \text{ and March + April} \\
& \quad p < 0.02 \\
& \quad \text{B lower than January + February and March + April } p < 0.001 \\
K_{\text{m}} & \quad \text{C lower than March + April } p < 0.02 \text{ July + August } p < 0.05 \\
& \quad \text{and September + October } p < 0.001 \\
& \quad \text{D higher than January + February } p < 0.01
\end{align*}
\]
Figure 5.1 Seasonal variation in platelet 5-HT uptake in depressive patients receiving lithium.

- PRP platelet concentration
- \( V_{max} \)
- \( K_m \)

<table>
<thead>
<tr>
<th>Month of test</th>
<th>n=39</th>
<th>n=18</th>
<th>n=9</th>
<th>n=15</th>
<th>n=26</th>
<th>n=25</th>
</tr>
</thead>
<tbody>
<tr>
<td>J+F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+J</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J+A</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>S+O</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N+D</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5.9
Platelet 5-HT uptake in patients receiving lithium only, or lithium with nitrazepam
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Lithium</th>
<th>Lithium + Nitrazepam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>55.6 ± 2.6</td>
<td>61.8 ± 1.9</td>
</tr>
<tr>
<td>Platelet conc(^n) (x 10^8/ml PRP)</td>
<td>3.32 ± 0.27</td>
<td>3.33 ± 0.21</td>
</tr>
<tr>
<td>K(_m) (\mu M)</td>
<td>0.50 ± 0.03</td>
<td>0.49 ± 0.03</td>
</tr>
<tr>
<td>V(_{max}) (pmol/10^8 platelets/min)</td>
<td>42.7 ± 4.0</td>
<td>28.2* ± 2.7</td>
</tr>
<tr>
<td>Plasma Li conc(^n) (mequiv/L)</td>
<td>0.85 ± 0.04</td>
<td>0.95 ± 0.04</td>
</tr>
</tbody>
</table>

* p < 0.01
Table 5.10
Platelet 5-HT uptake in patients receiving either lithium only, or lithium with valium
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Lithium</th>
<th>Lithium + Valium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>49.4 ± 1.3</td>
<td>57.2 ± 3.5</td>
</tr>
<tr>
<td>Platelet concentration (x 10^8/ml PRP)</td>
<td>3.40 ± 0.31</td>
<td>3.05 ± 0.45</td>
</tr>
<tr>
<td>K_m (μM)</td>
<td>0.54 ± 0.07</td>
<td>0.44 ± 0.05</td>
</tr>
<tr>
<td>V_max (pmol/10^8 platelets/min)</td>
<td>37.2 ± 7.8</td>
<td>40.4 ± 6.8</td>
</tr>
<tr>
<td>Plasma lithium concentration (mequiv/L)</td>
<td>0.80 ± 0.03</td>
<td>0.87 ± 0.08</td>
</tr>
</tbody>
</table>
Table 5.11
Effect on platelet 5-HT uptake of diazepam added in vitro to PRP from 6 control subjects
Results shown as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (pmol/10^8 pl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>0.42 ± 0.03</td>
<td>42.0 ± 3.0</td>
</tr>
<tr>
<td>Controls + diazepam</td>
<td>0.38 ± 0.02</td>
<td>29.5* ± 2.6</td>
</tr>
</tbody>
</table>

* $p < 0.01$ (method of paired comparisons)
patients had a significantly lower rate of platelet 5-HT uptake than the patients on lithium only (Table 5.9). This finding is contrary to the widespread view that benzodiazepines have no effect on the transport of biogenic amines.

In order to investigate the possible effects of diazepam (valium), a commonly administered anxiolytic, a group of 5 patients who were receiving both lithium and diazepam were tested, and the results compared with those for a group receiving lithium only, matched to the diazepam group as closely as possible for age, sex, diagnosis and month of test. It was found that diazepam had no significant effect on platelet 5-HT uptake in patients receiving lithium therapy (Table 5.10).

Diazepam, final concentration 200 ng/ml, was added in vitro to PRP samples from six control subjects. It was found to cause a significant reduction in $V_{\text{max}}$ but had no effect on the $K_m$ of platelet 5-HT uptake (Table 5.11).

(C) SUMMARY

In depressive patients receiving prophylactic treatment with lithium carbonate the rate of uptake of 5-HT by platelets was found to be increased to control values. This finding is in agreement with other reports in the literature (Murphy et al., 1970; Born, Grignani and Martin, 1980; Meltzer, Arora and Goodnick, 1983), and is in accord with the observation that lithium therapy significantly increased the levels of whole blood 5-HT in depressive patients (Coppen et al., 1976).

There was found to be a seasonal variation in the rate of platelet 5-HT uptake in patients receiving lithium therapy. The pattern of variation was similar to that found for drug-free depressive patients, with the rate of uptake being greatest in the winter and lowest in late spring and early summer.
DISCUSSION OF RESULTS

(A) PLATELET 5-HT UPTAKE IS REDUCED IN DEPRESSIVE PATIENTS

The rate of platelet 5-HT uptake was lower in depressive patients than in age and sex matched control subjects. The defect in the patients' 5-HT transport system was independent of clinical state, as there was no correlation between severity of illness and rate of 5-HT uptake in depressed patients, and the results for a group of patients tested both when depressed and again after recovery were similar on the two occasions.

If the assumption that the kinetics of platelet 5-HT uptake are similar to the kinetics of 5-HT transport across synaptic membranes is valid for human tissues (the evidence for this assumption is based on results obtained on laboratory animals), then the results described here are consistent with the biogenic amine hypothesis of depressive illness by supplying evidence of a reduced availability or turnover of neuronal 5-HT. The reduced rate of 5-HT uptake in depressed patients leads to a low platelet 5-HT content (Coppen et al., 1976). A low rate of 5-HT transport in the brains of depressed patients may be related to the reduced 5-HT concentrations observed in the brains of people who have committed suicide (Shaw, 1966; Bourne et al., 1968; Lloyd et al., 1974).

Since the impairment of platelet 5-HT in depressive patients is apparently unrelated to clinical state, it is suggested that a low rate of 5-HT uptake is a trait contributing to a predisposition to depressive illness. Although the rate of 5-HT uptake is significantly lower in patients than in control subjects, there is a great deal of overlap between the groups, i.e. a high rate of uptake does not necessarily mean that the subject is immune to depressive illness or, since it is possible that some of the control subjects, although without a history of psychiatric illness, may suffer from depression in the future, a low rate of 5-HT uptake does not necessarily confirm that the subject is prone to depression. A reduced rate of 5-HT uptake thus probably contributes to, but is not essential for, vulnerability to the
Affective disorders.

Taken alone, the measurement of a patient's platelet 5-HT uptake characteristics will neither confirm nor disprove a diagnosis of depression. However, it may still be of diagnostic use if carried out in conjunction with other biological assays, e.g. the dexamethasone suppression test.

(4) Effect of membrane Na⁺/K⁺ ATPase activity on 5-HT transport

Platelets, like most cells, maintain a low internal sodium concentration by means of Mg²⁺-dependent Na⁺/K⁺-stimulated ATPase in the membrane, which transports Na⁺ outward and K⁺ inward against a concentration gradient. The active uptake of 5-HT by platelets shows an absolute dependence upon the extracellular Na⁺ concentration, and in the absence of Na⁺ there is no net 5-HT uptake (Lingjaerde, 1969). There are reports of decreased Na⁺/K⁺ ATPase activity in membrane preparations from depressed patients (Choi, Taylor and Abrams, 1977; Hesketh, Glen and Reading, 1977; Naylor et al., 1980), although there is one report of an increase in Na⁺/K⁺ ATPase activity in depressives (Sengupta, Datta and Sengupta, 1981). All used erythrocyte membranes rather than platelet membranes, but probably the membrane Na⁺/K⁺ ATPase activity is similar in both erythrocytes and platelets.

If the reports of decreased Na⁺/K⁺ ATPase activity in depressed patients are validated, then this could account for the reduced rate of 5-HT uptake by platelets. This is supported by a report of a correlation between platelet 5-HT uptake and platelet 5-HT content (Worz-Justice, Lichsteiner and Feer, 1977). One group measured not only the activity of the enzymes but also the number of sodium pump sites per erythrocyte, which was found to be similar in control subjects and depressed patients (Naylor et al., 1980). This might explain the similar Kₘ values for 5-HT uptake in controls and depressive patients. If, on the other hand, Na⁺/K⁺ ATPase activity is normal or increased in depressed patients, then the normal Kₘ and reduced Vₘₕ of depressive patients might be explained by a deficiency in the dense granules which store the accumulated 5-HT within the platelet. This possibility is supported by the lack of correlation between 5-HT uptake and 5-HT content in platelets from control subjects reported by Stahl, Ciaranello and Berger (1982).
(ii) **Imipramine binding sites: possible relationship to 5-HT uptake sites**

Recently there has been increasing interest in investigations into the characteristics of imipramine binding sites in both brain membrane preparations and on blood platelets. There are probably both high- and low-affinity binding sites (Reith et al., 1983). The high-affinity binding sites in brain tissue are associated with the neuronal uptake system for 5-HT (Reith et al., 1983; Rehavi, Skolnick and Paul, 1983). There are reports of reduced numbers of imipramine binding sites per platelet, though not in the affinity, in depressed patients compared with control subjects (Briley et al., 1980; Gay et al., 1983). A reduction in binding sites, but normal affinity, has also been reported for brains of suicides (Stanley, Virgilio and Gershon, 1982; Perry et al., 1983). If the imipramine binding site is closely associated with the 5-HT transport site, then these findings might be taken as validation of the use of the platelet as a neuronal model in humans.

(B) **INHIBITION OF PLATELET 5-HT UPTAKE BY ANTIDEPRESSANT DRUGS**

Platelet 5-HT uptake is inhibited by tricyclic and similar antidepressant drugs. Inhibition is caused by interference with 5-HT binding to the transport molecule, reducing the affinity and thereby causing an increase in the value of $K_m$ of 5-HT uptake. The majority of these drugs do not significantly affect $V_{max}$, an exception being fluvoxamine.

It could be argued that since the rate of 5-HT uptake is already reduced in depressive patients, it is illogical to treat depression with drugs which reduce 5-HT uptake even further away from control values. In addition, the degree of inhibition of 5-HT uptake does not correlate with clinical state or clinical improvement.

Since completing the work described in Chapter 4, it has been possible to assemble results from several clinical trials with 5-HT uptake inhibitors and these have been combined, since the correlations presented in Chapter 4 may have failed to show significance due to small sample size. Platelet 5-HT uptake was measured in samples from a total of 79 patients who had received an antidepressant drug for either 2 ($n = 24$) or 4 ($n = 55$ weeks). Baseline drug-free estimations were available.
Table 6.1
Hamilton rating scores and platelet 5-HT uptake characteristics of depressed patients before and during treatment with an antidepressant drug.

Results shown as mean ± S.E.M. with range of values in brackets.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>On treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>64</td>
<td>79</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>47.2 ± 1.6</td>
<td>(20 to 77)</td>
</tr>
<tr>
<td>Hamilton Rating Score</td>
<td>20.4 ± 0.4</td>
<td>11.6* ± 0.7</td>
</tr>
<tr>
<td></td>
<td>(15 to 31)</td>
<td>(1 to 28)</td>
</tr>
<tr>
<td>Kₘ (µM)</td>
<td>0.54 ± 0.03</td>
<td>4.06* ± 0.49</td>
</tr>
<tr>
<td></td>
<td>(0.27 to 1.15)</td>
<td>(0.72 to 29.11)</td>
</tr>
<tr>
<td>Vₘₐₓ (pmol/10⁸ pl/min)</td>
<td>35.5 ± 1.8</td>
<td>32.1 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>(11.0 to 81.6)</td>
<td>(5.1 to 120.4)</td>
</tr>
<tr>
<td>ȳ (pmol/10⁸ pl/min)</td>
<td>22.6 ± 1.1</td>
<td>8.8* ± 0.5</td>
</tr>
<tr>
<td></td>
<td>(6.0 to 45.3)</td>
<td>(1.1 to 20.3)</td>
</tr>
</tbody>
</table>

* different from baseline values, p < 0.0001

† Antidepressant drugs used were amitriptyline (n = 12), clomipramine (n = 11), Imipramine (n = 15), Fluvoxamine (n = 15), and zimelidine (n = 26).
Table 6.2

Product-moment correlation coefficients between clinical improvement and platelet 5-HT uptake characteristics in 79 patients treated with antidepressant drugs

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Baseline HRS</th>
<th>Treatment HRS</th>
<th>% Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline $K_m$</td>
<td>64</td>
<td>+0.209</td>
<td>+0.071</td>
<td>+0.028</td>
</tr>
<tr>
<td>Treatment $K_m$</td>
<td>79</td>
<td>+0.242*</td>
<td>+0.150</td>
<td>-0.039</td>
</tr>
<tr>
<td>% change $K_m$</td>
<td>64</td>
<td>+0.117</td>
<td>-0.005</td>
<td>+0.118</td>
</tr>
<tr>
<td>Baseline $V_{max}$</td>
<td>64</td>
<td>-0.089</td>
<td>-0.002</td>
<td>-0.048</td>
</tr>
<tr>
<td>Treatment $V_{max}$</td>
<td>79</td>
<td>+0.021</td>
<td>-0.044</td>
<td>+0.060</td>
</tr>
<tr>
<td>% change $V_{max}$</td>
<td>64</td>
<td>+0.054</td>
<td>-0.029</td>
<td>+0.076</td>
</tr>
<tr>
<td>Baseline $\bar{y}$</td>
<td>64</td>
<td>-0.131</td>
<td>-0.053</td>
<td>-0.010</td>
</tr>
<tr>
<td>Treatment $\bar{y}$</td>
<td>79</td>
<td>-0.167</td>
<td>-0.192</td>
<td>+0.159</td>
</tr>
<tr>
<td>% change $\bar{y}$</td>
<td>64</td>
<td>-0.091</td>
<td>-0.158</td>
<td>+0.158</td>
</tr>
</tbody>
</table>

* $p < 0.05$
for 64 of these patients (Table 6.1). The antidepressant drugs studied were amitriptyline, clomipramine, imipramine, fluvoxamine and zimelidine.

There were no significant correlations between any of the measures of clinical improvement (Hamilton rating at 4 weeks, change and percent change in Hamilton rating) and any of the values obtained during treatment, change from baseline or percent change in $K_m$, $V_{max}$ or $\gamma$ of platelet 5-HT uptake (Table 6.2). It could be argued, from the lack of significant correlations, that inhibition of 5-HT uptake is of no relevance in the treatment of depression.

In general, it is found that clinical improvement does not correlate with plasma levels of antidepressant drugs. This agrees with the results presented here, since 5-HT uptake inhibition was shown to be correlated with plasma drug-concentrations, but not with clinical improvement. It is possible, though unlikely, that brain concentrations of antidepressant drugs are unrelated to plasma drug levels.

It has been suggested that newer drugs, such as zimelidine, which inhibit specifically the uptake of a single neurotransmitter might prove more effective than the original non-specific inhibitors, such as amitriptyline. However, the efficacy of zimelidine has been shown to be the same as that of amitriptyline rather than being superior, in spite of zimelidine being a specific inhibitor of 5-HT uptake. The main differences between their actions of clinical significance are the much lower incidence of side-effects with zimelidine due to the absence of anticholinergic activity, lower cardiac toxicity, and lack of interaction with alcohol.

(C) LITHIUM RESTORES 5-HT UPTAKE TO NORMAL IN DEPRESSIVE PATIENTS

Both unipolar and bipolar patients receiving lithium carbonate as a prophylactic against further episodes of affective illness had platelet 5-HT uptake characteristics which did not differ significantly from control values. Although the mechanism by which lithium exerts its therapeutic effect is not known, it might be supposed that the restoration of the rate of 5-HT uptake to normal is a contributing factor.
Since it is a monovalent cation, lithium may influence 5-HT transport by substituting for either sodium or potassium and thereby affecting the activity of \( \text{Na}^+ / \text{K}^+ \text{ATPase} \). There are reports that lithium treatment increases \( \text{Na}^+ / \text{K}^+ \text{ATPase} \) activity in erythrocyte membranes (Hesketh, 1976; Naylor et al., 1977).

(D) COMPARISON OF RESULTS WITH THOSE OF OTHER LABORATORIES

There seems to be general agreement among the reports in the literature that platelet 5-HT uptake is reduced in depressed patients (Tuomisto, Tukiainen and Ahlfors, 1979; Malmgren et al., 1981; Meltzer et al., 1981; Stahl et al., 1983). Although most reported values of \( K_m \) are similar, there is a wider variation between reports of the value of \( V_{\max} \); the values being up to 3 times as high as those reported here. Some of the differences between the reports will be due to differences in methodology, including variations in incubation time, incubation medium, platelet concentration and 5-HT concentrations. It is also probable that the period over which samples were collected may have been only a few months, particularly in those reports on small numbers of patients. Due to seasonal variation, samples collected only during the winter months would be expected to be significantly higher than those collected only in the summer months or regularly over a whole year.

It is also possible that differences between the reports partly reflect differences between populations, both of depressed patients and of control subjects, in different parts of the world. The diagnosis of the affective disorders, and the classification of patients is still a subject for discussion among psychiatrists, with cultural variations. Since vulnerability to depression is in part genetically determined, it is reasonable to suppose that different populations will show differences both in the incidence and the type of affective disorder. Similarly, since platelet 5-HT uptake is dependent on enzyme activity, and therefore might be partly genetically determined, it is to be expected that populations will show different mean values of platelet 5-HT uptake characteristics.

Since the determination of platelet 5-HT uptake must be carried out within a few hours of venepuncture, it would not be possible to
carry out a multi-centre study using one laboratory to compare different populations. However, it would be possible to investigate the extent of genetic determination of 5-HT transport rates by studying groups of blood relatives.

Membrane samples for the determination of affinity and density of imipramine binding sites can be stored frozen before assay, hence a multicentre study would be possible. If it is proved that the imipramine binding site is related to 5-HT transport, then this might prove a means of an indirect comparison of 5-HT uptake between populations.

(E) SEASONAL VARIATION IN 5-HT UPTAKE

Control subjects, drug-free depressive patients, and patients receiving lithium all showed seasonal variation in the value of $V_{max}$ of platelet 5-HT uptake. All three groups showed a ratio of between 0.6 and 0.7 between their lowest and highest 2-monthly means. The timing of the peaks and troughs of the three groups did not coincide exactly, although this is probably not significant. The rate of 5-HT uptake is lowest in summer and highest in winter. Due to the observed seasonal variation it is of importance that groups which are to be compared with respect to their 5-HT uptake characteristics should be samples concurrently.

Lowest rates of platelet 5-HT uptake are observed in late spring and early summer. This coincides with the time of year of greatest incidence of depression and the peak in the suicide rate. Since the variation in day length or photoperiod throughout the year is the most regular environmental variable, it is suggested that seasonal variation in amount of natural light is a contributing factor in the cause of depression. More specifically, a prolonged period of reduced daylight hours as experiences in the winter months contributes to the greater incidence of depression in the spring: an episode of depressive illness characteristically develops over a period of several weeks or months.

This hypothesis is difficult to prove since it would require a prospective study of large populations for any variation in incidence of depression to be detected. It would require the use of full sunlight spectrum light bulbs so that the amount of natural-type light people
are exposed to could be artificially increased. Also the hypothesis does not explain the year-round incidence of depression.

Daylength is usually considered to be the circadian basis of seasonal biological rhythms (Pittendrigh and Daan, 1976). The severity of the symptoms of endogenous depression is generally recognised as showing a circadian variation, with the depression of mood being at its most severe in the morning and improving towards evening. A single experiment using blood samples from one male control subject showed no difference in 5-HT uptake between 9 a.m. and a 4 p.m. sample ($V_{\text{max}}$ and $K_m$ values both within $\pm 5\%$), it would be worth repeating with groups of controls and depressed patients. Wirz-Justice and Puhringer (1978) reported a diurnal rhythm in platelet 5-HT content in both control subjects and depressed patients, and a seasonal variation in the diurnal rhythm in both groups.

High affinity imipramine binding has been shown to undergo circadian variations in rat brain (Wirz-Justice et al., 1983). The suprachiasmatic nuclei of the anterior hypothalamus has highest imipramine binding at the end of the dark and lowest at the end of the light phase. A similar circadian rhythm for 5-HT uptake in this region has also been observed. A circadian variation in serotonergic turnover in the hypothalamus could explain the diurnal variation in mood observed in endogenous depression.

(F) **SUGGESTION FOR A POSSIBLE CLINICAL USE THE PLATELET 5-HT UPTAKE ASSAY**

Due to the extent of interindividual variation in platelet 5-HT uptake, and the overlap in values between controls and depressives, the determination of platelet 5-HT uptake characteristics cannot be used in the diagnosis of depression, nor for predicting clinical response to antidepressant drug therapy due to the lack of correlation between 5-HT uptake and clinical ratings. However, the inhibition of 5-HT uptake correlates well with plasma concentrations of drugs. Although inhibition of 5-HT uptake does not correlate with efficacy of drug treatment, the monitoring of 5-HT uptake may be of use in regulating drug dose, and in checking drug-taking compliance. Plasma drug levels generally do not correlate with clinical effect, but it is usually accepted that plasma drug concentrations which are either very low or very high are of little
benefit. The steady-state plasma concentration obtained with a fixed
dose regime of antidepressant drugs and their metabolites, which are often
also of therapeutic benefit, vary widely, up to 30-fold, between patients
due to variations in metabolism, excretion rates, and induction of
metabolism by other drugs. The method for the determination of platelet
5-HT uptake is simple and fairly inexpensive to perform, and results
can be available within 30 hours of blood sample collection (allowing
for overnight digestion of platelet pellet at 37°C prior to counting of
radioactivity: this period could be reduced by incubation for a shorter
time at a higher temperature).

(G) PLATELET HETEROGENEITY: THE PLATELET-RICH PLASMA VS TOTAL WHOLE
BLOOD PLATELET SAMPLE CONTROVERSY

One criticism of the method used here, and probably by all other
workers to date, for the determination of platelet 5-HT uptake
characteristics is the method of separating the platelets from the sample
of whole blood. The preparation of platelet-rich plasma by low speed
centrifugation does not necessarily yield a truly representative sample
of the whole blood platelet population, since it is possible that the
larger platelets are spun down and thus lost from the PRP.

Heavy platelets are up to 1.4 times larger than the smallest
platelets (Corash, Tan and Gralnick, 1977). The heaviest platelets
have the same number of mitochondria as the lightest platelets, but
have up to 3 times as many dense storage granules and are therefore
able to store more 5-HT. Thus large platelets might be expected to have
a higher rate of 5-HT uptake. Also, it has been shown, by the injection
of radiochromium labelled light and heavy platelets, that the large
platelets have a longer survival time in the circulation, and it is
concluded that the largest platelets are also the youngest and therefore
probably also the healthiest (Corash, 1980).

Platelets are produced by fragmentation of megakaryocytes, and
removed from the circulation by the spleen and liver. Their life span
in the blood is about 10 days in man. Conditions which affect either
the rate of production, the life span or the rate of removal of platelets
from the circulation can be expected to alter the size distribution in
the platelet population. Thus, it might be argued that since lithium
tends to increase the number of platelets in the circulation this might be achieved by stimulating their production, thereby increasing the proportion of large, young platelets. This could explain the increased rate of platelet 5-HT uptake observed in patients on lithium. It is worth noting that a seasonal variation was observed in PRP platelet concentration, the trough coinciding with the trough in platelet 5-HT uptake. However, it must be remembered the rate of uptake does not correlate with the concentration of platelets in PRP, at least within the normal population limits.

A second criticism of the method of obtaining the platelet sample is that it is possible that the PRP may be contaminated with white blood cells, especially if the pipette tip is allowed too near the interface when withdrawing the PRP. Although it is not generally considered that the white cells have a system for actively taking up 5-HT, they may possibly remove some from the plasma during incubation by passive diffusion.

It is therefore suggested that the active uptake of 5-HT by platelets should be further studied, either using whole blood, or by separating the platelets from the whole blood sample by a method which recovers better than 95% of the total population.

Preliminary experiments to measure 5-HT uptake by whole blood have already posed more questions than they have answered. The method used was as described in Chapter 2, except that whole blood was used instead of PRP, and the KOH digest was bleached by adding one part digest to two parts hydrogen peroxide (20 volumes) in order to prevent quenching. Compared with results of control 5-HT uptake carried out on PRP prepared from the same blood samples, the uptake was two to three times higher in whole blood. The rate of uptake was sufficiently great to cause significant reductions in the amount of free $^{14}$C-5-HT within the 2 minute incubation period. Subsequently, time course experiments showed that the rate of uptake in whole blood had declined significantly from the initial rate by 2 minutes. It is possible that incubation times of 30 seconds or less may be required for whole blood 5-HT uptake experiments. It will be interesting to study this further: is the difference in uptake rates between whole blood and PRP preparations real, and if so why: do other blood cells take up 5-HT? Although the
red and white cells are not able to store 5-HT as platelets do, they may still remove small amounts from the plasma, even if it is immediately released again. By sheer weight of numbers, the red cells would each only need to take up very little to make a large difference to the apparent results.

There are two fairly simple methods for removing a total platelet population from other whole blood constituents. One is by density gradient centrifugation, using a product such as Ficoll. The other which is marginally easier to perform (density gradient methods require a steady hand!), is a sequence of dilution/low speed centrifugations. Briefly, 5ml of blood is mixed with 2ml of phosphate buffered saline glucose solution, the mixture centrifuged at 600 x g for 3 minutes, and the supernatant portion removed. The volume of the residual red cell platelet is made up to 7ml with buffer, and then centrifuged again. After 5 washes the pooled supernatant fractions should contain at least 95% of the total platelet population (Corash, 1980).

It is possible that the observed differences in platelet 5-HT uptake rates between control subjects and depressive patients may be due to differences between the groups in their platelet population structure. Using the Corash method, 5-HT uptake determinations might be done on platelet samples which are accurate representatives of the total platelet population. Alternatively, the total platelet populations obtained might be sub-divided into three or four fractions according to size, and the proportions of larger and smaller platelets determined and their 5-HT uptake rates compared for the different groups.

(E) SUMMARY

The work described in this thesis has provided further evidence that an abnormality of 5-HT turnover is an important factor in the aetiology of depressive illness. The rate of platelet 5-HT uptake is significantly lower in depressive patients that control subjects. If the platelet is valid model for the serotonergic neurone, then depressive patients have an impairment of the transmission of 5-HT across the synapse.

The measurement of platelet 5-HT uptake characteristics has
been shown to be of use in the investigation of the effects of antidepressant drugs. The method can be used (1) to compare the potency of different drugs as 5-HT uptake inhibitors; (2) as a guide to plasma drug concentrations and allow thereby for dosage adjustment to maintain adequate drug levels; and (3) as a test of compliance in patients receiving 5-HT uptake inhibitors.

Platelet 5-HT uptake characteristics by themselves cannot be used to confirm a diagnosis of depressive illness, due to overlap between patient and control values. However, it might be of use in diagnosis if taken in conjunction with other biological measures, for example the dexamethasone suppression test (Carroll, 1982; Meltzer et al., 1983), or analysis of erythrocyte membrane polyunsaturated fatty acids (Fehily, 1980). Although the majority of cases of depressive illness do not require laboratory confirmation, there are a few cases which are difficult to diagnose on clinical data alone, and for these cases a confirmation of diagnosis would be useful as a guide to treatment. Biochemical confirmation of diagnosis is also of use in multicentre trials and investigations into depressive illness and its treatment, especially where results from different countries and cultures are to be combined.


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