ABSTRACT

A study of lipids in the plasma and erythrocyte membrane of patients suffering from depressive illness, patients with other psychiatric disorders and women using oral contraceptive agents is reported. It shows that there are differences in the concentrations of long chain polyunsaturated fatty acids (LCP) and linoleic acid (18:2\(\omega_6\)) in the plasma choline phosphoglycerides (CPG) and erythrocyte phospholipids of patients suffering from endogenous depression, patients suffering from postpartum depression and women using oestrogen and progestogen combined oral contraceptive agents, compared with those of matched controls. The expected effects of these differences on membrane function and the synthesis of prostaglandins and related compounds are discussed.

One of the aims of the study was to ascertain whether measurement of the LCP composition of the plasma CPG would be of value as a diagnostic tool for endogenous depression: although the difference from matched controls is highly significant (\(p < 0.001\)), it is concluded to be insufficient to be used as the sole diagnostic criterion.

The concentration of docosahexaenoic acid (22:6\(\omega_3\)) correlates with the severity of depression in the endogenous group, but not in the reactive group.

Total plasma cholesterol is low in the endogenous group compared with controls, but there is no abnormality in the free: esterified ratio. There is no difference in the total plasma CPG, the relative proportions of erythrocyte phospholipids or the ratio of cholesterol: phospholipid in erythrocytes between any of the groups of psychiatric patients and matched controls.
The phospholipid LCP concentrations normalise with clinical improvement in the endogenous group, but are unaltered in the reactive group. Patients receiving prophylactic lithium treatment have similar concentrations of LCP in their plasma CPG to matched controls.

Possible reasons for the differences in phospholipid LCP concentrations are investigated and discussed.

Finally, some suggestions for future research are made.
ACKNOWLEDGEMENTS

I am especially grateful to Professor J.W.T. Dickerson, Dr B.W. Meade and the late Dr F.R. Ellis for their invaluable help and supervision.

I would also like to thank the following people: Dr Olga Bowey for the selection and assessment of psychiatric patients; Dr Judy Kane and Dr Jo Boxer for their co-operation in obtaining patients with early symptoms of depressive illness; Dr A. Coppen for plasma samples from patients receiving prophylactic lithium treatment; Dr T.A.B. Sanders for his advice and technical assistance; John Daly, Peter Bridle, Norman Harling, David Simms, John Sheldon and other members of the Pathology Department of Kingston Hospital for their technical assistance; Peter Trumper for the administration of drugs and heart puncture of experimental animals; members of the Pathology Department of St Luke's Hospital, Guildford for the fasting serum insulin determinations; Joan Dawson for her secretarial assistance; Dawn Crees for typing this thesis; Andrew Jones for the artwork (Figures 1-19); and finally all the subjects who have taken part in the study.

This study was financially supported by a grant to Dr F.R. Ellis and Professor J.W.T. Dickerson from the De-Centralised Research Fund of the South West Thames Regional Health Authority.
To the memory of Dr Frey Ellis,
without whom this work would never have been carried out.
<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAPTER ONE</td>
<td>6 - 53</td>
</tr>
<tr>
<td>Introduction</td>
<td></td>
</tr>
<tr>
<td>CHAPTER TWO</td>
<td>54 - 71</td>
</tr>
<tr>
<td>Subjects, Materials and Methods</td>
<td></td>
</tr>
<tr>
<td>CHAPTER THREE</td>
<td>72 - 105</td>
</tr>
<tr>
<td>Some Blood lipid measurements in patients suffering from depressive illness and in patients with other psychiatric disorders.</td>
<td></td>
</tr>
<tr>
<td>CHAPTER FOUR</td>
<td>106 - 125</td>
</tr>
<tr>
<td>The effect of treatment on the LCP composition of the plasma choline phosphoglycerides and erythrocyte phosphoglycerides.</td>
<td></td>
</tr>
<tr>
<td>CHAPTER FIVE</td>
<td>126 - 141</td>
</tr>
<tr>
<td>The fatty acid composition of the plasma choline phosphoglycerides and erythrocyte phospholipids of oral contraceptive users.</td>
<td></td>
</tr>
<tr>
<td>CHAPTER SIX</td>
<td>142 - 157</td>
</tr>
<tr>
<td>Factors affecting phospholipid LCP composition.</td>
<td></td>
</tr>
<tr>
<td>CHAPTER SEVEN</td>
<td>158 - 163</td>
</tr>
<tr>
<td>Discussion</td>
<td></td>
</tr>
<tr>
<td>REFERENCES</td>
<td>164 - 183</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>i - vii</td>
</tr>
</tbody>
</table>
INTRODUCTION

Depression, defined as "the lowering of mental and physical vitality to the point of distress" (Watts, 1973), is the commonest of mental illnesses. It affects two to three times as many women as men (Weissman and Klerman, 1977; Callan, 1979) and episodes occur more frequently in Spring and Autumn than at other times of the year (Eastwood and Peacocke, 1976).

Due to the heterogeneity of the disorder numerous attempts have been made to produce a suitable classification system and this has resulted in one of the greatest controversies in psychiatry today. The endogenous/reactive dichotomy is the most widely used, the argument being whether they constitute distinct categories (Kiloh and Garside, 1963; Mendels and Cochrane, 1968; Kiloh et al., 1972; Spicer et al., 1973; Cochrane, 1977; Checkley, 1979) or are opposite ends of a continuum (Kendell, 1968; 1972; 1976). Endogenous depression is said to be due to internal factors such as a metabolic abnormality not yet elucidated and has a strong hereditary element, whereas reactive depression occurs in response to environmental factors in vulnerable personalities. Endogenous depression can be subdivided into bipolar (alternating mania and depression) and unipolar (depression only) illness. The clinical characteristics of endogenous and reactive depression are compared in Table 1. However, the dividing line is not clear in all cases: according to Kendell (1972) patients with mixed symptoms are more common than those with pure symptoms of either group. Also it has been pointed out that attacks of endogenous depression may be precipitated by adverse environmental influences (Kiloh and Garside, 1963). It is important to accurately
distinguish between the two types in order to decide upon suitable treatment (Carney and Sheffield, 1973; Bieds, 1976; Davidson et al., 1977; Royal College of Psychiatrists, 1977; Freeman, 1979). If an endogenous depressive is incorrectly diagnosed, months or even years of invalidism may result or the patient may end it all by suicide.

**TABLE 1**

Clinical Characteristics of Endogenous and Reactive Depression

<table>
<thead>
<tr>
<th>Endogenous Depression</th>
<th>Reactive Depression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: 40 yrs or older.</td>
<td>Age: usually under 40 yrs.</td>
</tr>
<tr>
<td>Early awakening.</td>
<td>Difficulty in going off to sleep and further interruptions.</td>
</tr>
<tr>
<td>Depression worse in morning, gradually improving throughout the day.</td>
<td>Depression worse, if at all, in the evening and when alone.</td>
</tr>
<tr>
<td>General slowing up in thinking and activity (psychomotor retardation). Energy and libido markedly reduced.</td>
<td>Anxiety and irritability.</td>
</tr>
<tr>
<td>Agitation usually present.</td>
<td>Energy variably reduced.</td>
</tr>
<tr>
<td>Marked feelings of inferiority, uselessness and hopelessness.</td>
<td>Agitation not present.</td>
</tr>
<tr>
<td>Delusions and hallucinations may be present.</td>
<td>Not marked, but other variations in personality may be present.</td>
</tr>
<tr>
<td>Reduced appetite and loss of weight.</td>
<td>Delusions and hallucinations do not occur.</td>
</tr>
<tr>
<td></td>
<td>Usually little change in appetite and weight.</td>
</tr>
</tbody>
</table>

*Summarised from Dominion (1976).*

Clearly the classification system is in urgent need of revision.

Alternative systems have been proposed (Pollitt, 1965; Overall et al., 1966; Blinder, 1966; Paykel, 1971; Kerry and Orme, 1975) but none have been widely accepted.
To ascertain whether there is a genetic component in endogenous depression both twin and family studies were examined. Data from twin studies showed an overall concordance rate of 76 percent for monozygotic (MZ, identical) twins and 19 percent for dizygotic (DZ, fraternal) twins (Tsuang, 1975). The concordance rates for MZ twins varied between 33 percent and 93 percent compared with 0 to 39 percent for DZ twins (Perris, 1976; Mendlewicz, 1977). Although concordance was significantly higher for MZ than for DZ twins it was still well below the expected 100 percent which would have proven more conclusively the involvement of genetics. The interpretation of the results is complicated by the fact that environmental influences tend to be more similar for MZ than for DZ twins. Only twelve pairs of MZ twins who had been reared apart since childhood have been studied: eight pairs (67 percent) showed concordance (Price, 1968), a rate similar to that reported by Tsuang (1975). These results suggest the presence of a genetic factor in endogenous depression.

A re-analysis of twin data based on the unipolar/bipolar classification revealed that 86 percent of MZ twins were concordant for one or other form of the illness (Mendlewicz, 1977). This suggests that unipolar and bipolar depression might be genetically separate.

Data from family studies showed that there was a significantly greater rate of endogenous depression in first-degree relatives (parents, siblings and children) of endogenous depressives than in the general population (Angst, 1974; Tsuang, 1975; Perris, 1976; Mendlewicz, 1977; Trzebiatowska-Trezeciak, 1977) suggesting a genetic component. Trzebiatowska-Trezeciak (1977) found a lower morbidity risk for second-degree relatives than for first-degree relatives and also found different
morbidity risks for unipolar and bipolar depression, suggesting that the two illnesses are distinct entities.

The precise mode of inheritance is unknown but different models have been proposed: a single dominant or polygenic type (Tsuang, 1975; Perris, 1976; Mendlewicz, 1977). An X-linked factor has been found in some bipolar families but not in those with unipolar illness (Cadoret, 1976). Unipolar depression has been suggested to consist of three or more separate illnesses each with its own mode of inheritance (Schlesser et al., 1979).

BIOLOGICAL STUDIES OF DEPRESSION

Since the beginning of this century the biological aspects of depression have received considerable attention. Virtually all the known constituents of blood, urine and cerebrospinal fluid (CSF) have been measured and pathological studies of brain and other organs conducted. However, many positive findings have been contradicted in the course of time and even in the more recent literature, after the introduction of antidepressant drugs in the 1950's, a great deal of controversy was seen. Inadequate control of factors such as age, sex, body weight, diet and administration of drugs other than antidepressants undoubtedly contributed to this.

The main areas of research have been biogenic amines, electrolytes and hormones. In reviewing the literature biological systems will be discussed individually for convenience but it must be remembered that they interact with each other in a complex manner and that changes in one may lead to changes in others.
Interest in the association between biogenic amines and emotion stemmed from Cannon's discovery in the early 1900's that animals secreted increased amounts of adrenalin when exposed to situations inducing rage or fear. Further stimulus to the investigations came from two accidental observations. Firstly, a small but significant number of patients treated with reserpine for hypertension developed a condition clinically similar to endogenous depression (Harris, 1957) and secondly, tubercular patients being treated with iproniazid responded with a much brighter mood than would be expected from clinical improvement (Pare and Sandler, 1959). Pharmacological studies indicated that reserpine depleted noradrenalin, dopamine and serotonin (5-hydroxytryptamine, 5HT) within the central nervous system (CNS) (Shore et al., 1955; Carlsson et al., 1957) and that iproniazid increased these amines (Spector et al., 1958). These findings led to the formulation of the biogenic amine hypothesis of affective disorders (Pare and Sandler, 1959; Prange, 1964; Schildkraut, 1965; Bunney and Davis, 1965; Lapin and Oxenkrug, 1969; Ashcroft et al., 1972). Briefly this suggests that depression is associated with a deficiency of noradrenalin and/or serotonin at functionally important receptor sites in the brain whereas mania is associated with an excess of the amine(s). More recently it has been postulated that cholinergic factors are also involved in the regulation of affect (Janowsky et al., 1972; Davis, 1975). Both depression and mania were said to represent an imbalance of the adrenergic-cholinergic system, depression being the result of cholinergic predominance and mania the result of adrenergic predominance. In support of this hypothesis, Tamminga et al. (1976) noted the occurrence of depression after treatment with oral choline.

Noradrenalin, dopamine and serotonin together with acetylcholine
function as transmitter substances within the peripheral nervous system and there is general agreement that they play a similar role in the CNS although the individual synapses have not been elucidated. The area of the brain concerned with emotion is the limbic system of which the hypothalamus is part. Noradrenalin and serotonin are present in many areas of the brain but dopamine is found almost exclusively in the basal ganglia (Schildkraut and Kety, 1967). The highest concentrations of noradrenalin and serotonin have been said to occur in the hypothalamus (Schildkraut and Kety, 1967) yet serotonin concentrations four times greater than those of the hypothalamus have been reported in certain raphe nuclei of the pons-medulla of the lower brainstem (Lloyd et al., 1974). Centres controlling eating, drinking and sleeping are situated in the hypothalamus and there may be a link between the biogenic amines and these functions: the relationship between serotonin and sleep is well known (Koella, 1974).

Noradrenalin and dopamine are synthesised from the amino acid L-tyrosine which in turn can be formed from L-phenylalanine. Serotonin is synthesised from the amino acid L-tryptophan. Catecholamine formation, catecholamine catabolism and serotonin metabolism are shown in Figures 1, 2 and 3 respectively.

(i) Blood Studies

Early studies failed to differentiate between depressed and normal subjects on the basis of plasma catecholamine concentrations (Manger et al., 1957; Reilly and Regan, 1957). However the methods available at that time were neither sensitive nor specific enough to measure catecholamine values reliably. In a more recent study Wyatt
Figure 1

Biosynthesis of Catecholamines

TYROSINE  
\[ \text{Tyrosine Hydroxylase} \]

3,4 - DIHYDROXYPHENYLALANINE (DOPA)

DOPAMINE  
\[ \text{Dopamine-\(\beta\)-Hydroxylase} \]

NORADRENALIN  
\[ \text{Phenylethanolamine-N-Methyl Transferase} \]

ADRENALIN
Catabolism of noradrenalin and adrenalin

Noradrenalin

\[ \text{NORADRENALIN} \xrightarrow{\text{COMT}} \text{NORMETANEPHRINE} \xrightarrow{\text{MAO}} \text{CONJUGATED NORMETANEPHRINE} \xrightarrow{\text{Aldehyde Dehydrogenase}} \text{3-METHOXY-4-HYDROXY-MANDELIC ALDEHYDE} \xrightarrow{\text{Aldehyde Reductase}} \text{3-METHOXY-4-HYDROXY-PHENYLGLYCOL (MHPG)} \]

Adrenalin

\[ \text{ADRENALIN} \xrightarrow{\text{COMT}} \text{METANEPHRINE} \xrightarrow{\text{MAO}} \text{CONJUGATED METANEPHRINE} \]

MAO = monoamine oxidase
COMT = catechol-O-methyltransferase
The 5-hydroxyindole pathway

TRYPTOPHAN

Tryptophan Hydroxylase

5-HYDROXYTRYPTOPHAN (5HTP)

5-Hydroxytryptophan Decarboxylase

5-HYDROXYTRYPTAMINE (5HT, SEROTONIN)

Monoamine Oxidase

5-HYDROXYINDOLE ACETALDEHYDE

Aldehyde Dehydrogenase

5-HYDROXYINDOLE ACETIC ACID (5HIAA)
et al. (1971), contrary to the biogenic amine hypothesis, found catecholamines significantly elevated in drug-free depressed patients compared with normal controls. The values decreased with amelioration of the depression.

Serotonin concentrations have been shown to be normal in serum (Kaneko et al., 1975) but reduced in platelets (Coppen et al., 1976) of patients suffering from depressive illness. Takahashi (1976) found that platelet serotonin concentrations were lower in patients with bipolar depression than in those with unipolar depression or in controls. Other workers have reported reduced serotonin uptake by platelets of depressed patients (Hallstrom et al., 1976; Tuomisto and Tukiainen, 1976; Coppen et al., 1978; Coppen et al., 1980), indicating an abnormality in membrane transport of serotonin. Oxenkrug et al. (1978) demonstrated a circadian rhythm for serotonin uptake by platelets and found that this was altered in depressive illness.

Monoamines are unable to enter the brain from the vascular system unlike their precursor amino acids. Tryptophan differs from other amino acids in that it exists in the plasma mainly bound to albumin (McMenamy and Oncley, 1958). Some investigators have suggested that the level of brain tryptophan and hence serotonin synthesis is related to the concentration of free tryptophan in the plasma (Fernstrom and Wurtman, 1971; Knott and Curzon, 1972; Tagliamonte et al., 1973), whereas others have reported that total plasma tryptophan predicts brain tryptophan concentrations (Fernstrom et al., 1976; Wiryanti, 1980).

No difference has been found in the total plasma tryptophan concentrations of depressed patients compared with controls (Coppen et al., 1972a; Coppen et al., 1973a; Aylward and Maddock, 1973; Niskanen et al., 1976; Coppen and Wood, 1978). Reduced plasma free tryptophan has been
reported in depressive illness by some (Coppen et al., 1972a; Coppen et al., 1973a; Aylward and Maddock, 1973; Coppen and Wood, 1978) but not all investigators (Niskanen et al., 1976; Riley and Shaw, 1976; Garfinkel et al., 1976). Reasons for this discrepancy were discussed by Coppen and Wood (1978): the heterogeneity of the illness, differences in methodology and differences in the antidepressant-free period prior to the studies (shorter drug-free periods resulting in higher free tryptophan levels), appear to be the most likely explanations. Differences in diet were not thought to be a factor as this would have resulted in altered total plasma tryptophan concentrations. Free tryptophan levels have been reported to increase on recovery (Coppen et al., 1973a; Coppen and Wood, 1978) but this has been contradicted (Peet et al., 1976).

The concentrations of other plasma neutral amino acids (Phenylalanine, tyrosine, leucine, isoleucine and valine) may also affect brain tryptophan concentrations as they compete with tryptophan for the same transport system (Fernstrom and Wurtman, 1972). Möller and Amdisen (1979) reported a reduced ratio of tryptophan to other neutral amino acids in the plasma of a single bipolar depressed patient compared with controls. Administration of a tryptophan load led to increased plasma concentrations of the competing amino acids in the patient compared with a decrease in the control subjects. The authors concluded that there is a dysfunction of processes mediating active transport of tryptophan and other neutral amino acids to the brain and tissues. Their results are however of questionable value since only one depressed patient was studied.

Takahashi et al. (1968) measured fasting plasma tyrosine levels of patients suffering from manic-depressive illness and found no difference from controls. Benkert et al. (1971) investigated 24-hour plasma tyrosine concentrations and reported reduced 11 a.m. values in patients with endogenous depression. This was disputed by a more recent study
Rosenblatt et al. (1979) measured twenty amino acids in the plasma and erythrocytes of patients suffering from endogenous depression and controls. There was no differences in the plasma amino acid concentrations of the two groups but patients with endogenous depression had significantly greater amounts of glycine in their erythrocytes. This was unaltered by electroconvulsive therapy (ECT) or by clinical improvement.

There have also been several studies of the activity of enzymes involved in the formation and catabolism of catecholamines and serotonin but these have failed to show any consistent abnormality.

Shopsin et al. (1972) measured serum dopamine-β-hydroxylase activity (the enzyme catalysing the conversion of dopamine to noradrenalin, see Figure 1) in patients with bipolar depression, unipolar depression, schizophrenia and in patients with character disorders, but were unable to differentiate between any of the groups. Van Cauter and Mendlewicz (1978) established a circadian rhythm for the enzyme and found that this was altered in endogenous depression.

Monoamine oxidase (MAO) is involved in the catabolism of both catecholamines (Figure 2) and serotonin (Figure 3). Its activity tends to be higher in females than in males (Murphy and Costa, 1975) and to increase with age (Robinson et al., 1972). Feldstein et al. (1964) studied the conversion of $^{14}$C-serotonin to $^{14}$C-5-hydroxyindole acetic acid (5HIAA) as a quantitative index of MAO activity and found no difference between depressed patients and controls. More recently platelet MAO activity has been investigated. Nies et al. (1971) found higher platelet MAO activity in depressed patients than in controls. Bipolar depressed patients have been reported to have low (Murphy and Weiss,
high (Belmaker et al., 1976) and normal (Takahashi, 1977; Edwards et al., 1978) MAO activity compared to those with unipolar depression and controls. Leckman et al. (1977) reported low MAO activity in first-degree relatives of bipolar depressives but were unable to distinguish well from ill members of the families. They suggested that this may be an indicator of increased familial vulnerability to endogenous depression. The inconsistencies between these studies may have been due to the use of different substrates for MAO and to differences in sample size.

Catechol-O-methyl transferase (COMT) is involved in the catabolism of catecholamines (Figure 2). Erythrocyte COMT activity has been reported to be low in endogenous depression by some investigators (Cohn et al., 1970; Dunner et al., 1971) but normal by others (White et al., 1976; Dunner et al., 1977). The discrepancy is probably due to differences in methodology. The earlier studies used a poorer COMT substrate (noradrenalin) than the later studies (3,4-dihydroxy benzoic acid) and employed a very low concentration of the methyl donor, S-adenosyl-L-methionine (SAM), so that enzyme activities were obtained under conditions of limited substrate concentration (White et al., 1976). Differences may also have been due to the heterogeneity of the disorder since Shulman et al. (1978) reported that among patients suffering from endogenous depression, those with agitation had COMT activities significantly greater than controls whereas those with retardation had lower COMT activities than controls. Davidson et al. (1976) found that patients with reduced COMT activity responded well to treatment with imipramine.

Andreoli et al. (1978) measured the blood SAM concentrations of depressed patients and controls but found no difference between the two groups.
Urine Studies

The measurement of urinary amine metabolites has been used to explore the relationship between biogenic amines and depressive illness. Unfortunately it is impossible to conclude much about brain function from these studies since only about 5 per cent of each metabolite is of central origin, with the exception of 3-methoxy-4-hydroxy phenylglycol (MHPG) (Maas and Landis, 1968; Ebert and Kopin, 1975). There is a diurnal variation in catecholamine excretion and also a multitude of other factors which influence urinary levels. These include body weight, diet, alcohol, nicotine, stress, muscular work, anxiety, postural changes, motor activity, urinary volume and pH and administration of certain drugs such as chloral hydrate and meprobamate (Shopsin et al., 1974).

Considering the lack of control for some or all of these factors in the literature, it is hardly surprising that results have been very inconsistent. Noradrenalin excretion has been found to be reduced (Strom-Olsen and Weil-Malherbe, 1958), normal (Bergsman, 1959) and increased (Curtis et al., 1960; Bunney et al., 1967) in depressed patients. Bunney and his co-workers (1967) reported that patients suffering from psychotic (endogenous) depression excreted greater amounts of noradrenalin, 3-methoxy-4-hydroxy mandelic acid (VMA), metanephrine and 3,4-dihydroxy mandelic acid than those suffering from neurotic (reactive) depression. More recently, Deleon-Jones et al. (1975) were unable to find any difference between subgroups of depressed patients and controls in the urinary excretion of normetanephrine, metanephrine or VMA.

MHPG excretion has been found to be reduced in some depressed patients and raised in others (Maas et al., 1968; Greenspan et al., 1970;
Bond et al., 1972; Schildkraut, 1973; Deleon-Jones et al., 1975; Pickar et al., 1978; Taube et al., 1978; Garfinkel et al., 1979). It has been suggested that the excretion of this metabolite may provide a useful predictor of response to tricyclic drug therapy in unipolar but not bipolar depressed patients: low pretreatment levels of MHPG indicating favourable response to imipramine and high levels indicating favourable response to amitriptyline (Fawcett et al., 1972; Schildkraut, 1973; Mendels et al., 1976). MHPG excretion is unaffected by physical activity (Pickar et al., 1978). It is however affected by diet in depressed patients but not in controls (Muscettola et al., 1977). This suggests that normal controls may not be suitable for evaluating the contribution of some potential sources of variance in amine metabolism in depressive illness. Wehr et al. (1980) demonstrated a circadian rhythm for MHPG excretion and reported that this was abnormal in bipolar depressive illness.

Serotonin excretion has been reported to be 50 percent lower during depressive illness than on recovery or in normal subjects (Coppen et al., 1965; Coppen, 1972). Cazzullo et al. (1966) found that 5HIAA excretion was normal in depressed patients, but Amamoto and Sarai (1976) reported low values after an oral load of 5-hydroxytryptophan.

Recent studies have shown decreased urinary levels of trace amines, tyramine and octopamine, and their metabolites (see figure 4) in depressive illness (Bonham Carter et al., 1978; Sandler et al., 1979a). However, as the authors pointed out, a substantial proportion of these metabolites may derive from the diet or gut flora.

(iii) CSF Studies

CSF became the focus for the study of amine metabolism due to its
Figure 4

Metabolism of tyramine and octopamine

L-Phenylalanine

\[ \downarrow \]

Phenylethylamine $\rightarrow$ Phenylacetic acid

\[ \downarrow \]

\[ p\text{-Tyramine} \quad \text{MAO} \quad p\text{-Hydroxyphenylacetaldehyde} \]

\[ \downarrow \]

\[ p\text{-Octopamine} \quad p\text{-Hydroxyphenylacetic acid} \]

\[ \downarrow \]

\[ p\text{-Hydroxymandelic acid} \]

\[ \text{MAO} = \text{monoamine oxidase} \]
contiguity to brain and partial isolation from the periphery. Nevertheless, there are problems associated with the interpretation of such studies and as with blood and urine investigations findings have been inconsistent.

Dencker et al. (1966a) reported noradrenalin levels three times greater in depressed patients than in controls but this has not been confirmed (Post et al., 1978). The latter group did however find higher levels in patients with high anxiety compared to those with low anxiety. Reduced levels of MHPG have been reported in endogenous depression by some investigators (Gordon and Oliver, 1971; Post et al., 1973a) but not by others (Shopsin et al., 1973; Vestergaard et al., 1978). Homovanillic acid (HVA), a metabolite of dopamine, has been shown to be low (Papeschi and McClure, 1971) and high (Vestergaard et al., 1978) in endogenous depression.

5HIAA levels have been reported to be low in depressive illness by some investigators (Ashcroft and Sharman, 1960; Ashcroft et al., 1966; Dencker et al., 1966b; Coppen, 1972) but not by others (Bowers et al., 1969; Papeschi and McClure, 1971; Vestergaard et al., 1978). Tryptophan has also been found to be low in the CSF in depressive illness (Coppen et al., 1972b).

Sandler et al (1979b) measured phenylacetic acid (see Figure 4) in the CSF of patients with depressive illness and in controls. Depressed patients had lower levels, indicating a possible deficiency of trace amine (tyramine and octopamine) production.

GABA (γ-amino-butyric acid), a major inhibitory neurotransmitter, has been shown to be reduced in the CSF of patients suffering from depressive illness (Gold et al., 1980). The biological significance of this is unknown but may relate to the proposed noradrenergic mechanisms

The variability between studies may be the result of differences in the procedure used to obtain spinal fluid, the volume of fluid used and variations in physical activity of the subjects (Post and Goodwin, 1975). Also several of the fluorimetric methods used to assay HVA and 5HIAA are not very reliable when used to measure the low basal concentrations of these metabolites found in lumbar fluid (Wilk and Green, 1972). Interpretation of the results is complicated by the fact that although most HVA is derived from the brain (Papeschi et al., 1971; Post et al., 1973b) significant amounts of 5HIAA and MHPG are also derived from sources in the spinal cord (Bulat and Zivkovic, 1971; Post et al., 1973b; Weir et al., 1973) and by the fact that there is active transport of metabolites out of the CSF (Moir et al., 1970). Thus changes in lumbar concentrations of metabolites may reflect changes in transport out of the CSF rather than changes in turnover in the CNS.

In an attempt to obtain more consistent results, several investigators studied the accumulation of metabolites in lumbar fluid after the administration of probenecid. Probenecid blocks active transport of 5HIAA and HVA from the CSF, thereby increasing the reliability of the fluorimetric assay and providing a more accurate reflection of ventricular levels. Low levels of 5HIAA and HVA have been found in endogenous depression (Van Praag and Korf, 1971; Goodwin et al., 1973; Van Praag et al., 1973; Post and Goodwin, 1975). Bowers (1974) showed that levels of 5HIAA and HVA were lower in bipolar depression than in unipolar depression: patients with unipolar depression had normal 5HIAA levels but increased VMA levels. Noradrenalin levels have been reported to be normal in depressive illness (Post et al., 1978).
As pointed out by Mendels et al. (1976) the probenecid technique is not without its problems: there may be variations in probenecid absorption with consequent differences in CSF probenecid concentration and degree of blockade of transport obtained and also possible effects of probenecid itself on brain tryptophan concentration and serotonin turnover. Nevertheless, more consistent results have been reported using this technique.

The influence of dietary factors on CSF amine metabolite concentrations cannot be excluded: Botez et al. (1979) have shown that reduced 5HIAA concentrations occur in the CSF of folic acid deficient subjects.

(iv) Post-Mortem Studies

Serotonin (Shaw et al., 1967; Pare et al., 1969) and 5HIAA (Bourne et al., 1968) concentrations have been found to be significantly lower in the brains of subjects who had committed suicide compared to those of subjects who had died of other causes. There was no significant difference in noradrenalin (Bourne et al., 1968; Pare et al., 1969) or dopamine (Pare et al., 1969) concentrations. Lloyd et al. (1974) studied discrete brain areas and found that serotonin levels were significantly reduced in the raphe nuclei dorsalis and centralis inferior. Normal concentrations of serotonin were found in the higher brainstem and telencephalon, but 5HIAA was increased in the mammillary bodies.

There are several problems associated with the interpretation of post-mortem studies. It may be difficult to obtain reliable data concerning diagnosis, diet and drugs taken by the patient. It is also difficult to control for losses of brain amines after death. The brain should be removed
from the skull and deep-frozen immediately after death, otherwise large losses of serotonin occur (Joyce, 1962). Even storage itself may have an effect on brain serotonin and 5HIAA content; according to Dowson (1969) there is a significant fall in serotonin and 5HIAA concentrations between one and twenty six days storage, resulting in a loss of 34 percent of the serotonin and 44 percent of the 5HIAA. The decay process may also be influenced by changes in serotonin metabolism associated with depression. Clearly, further study is required in this area.

(b) Electrolytes

There are many reports in the literature suggesting that disturbances of electrolyte metabolism occur in depression. These are of importance since electrolytes play a central role in several aspects of neuronal function: Maintenance of the resting potential; carrying the current required for the action potential; synthesis, storage, release and inactivation of neurotransmitters; and in carrying the current responsible for depolarization of postsynaptic membranes (Bogdonski and Brodie, 1966; Bogdonski et al., 1968; Wright, 1971; Molinoff and Axelrod, 1971; Jimerson et al., 1979).

A critical factor in the maintenance of the resting potential is that neuronal cytoplasm is low in sodium and chloride but rich in potassium, with the reverse occurring in the extracellular fluid. The cell membrane is freely permeable to potassium and chloride but relatively impermeable to sodium: sodium which leaks into the cell is extruded by an active transport mechanism, the "sodium pump". Stimulation of a nerve fibre produces a transient increase in membrane permeability to sodium and this sets off an action potential which is propagated along the fibre.
Electrolyte studies were prompted by the discovery of Schottstaedt 
et al. (1956) that periods of depression in normal subjects were 
accompanied by a reduction in urinary excretion of sodium. Investigations 
have suggested that clinical depression is associated with a retention 
of sodium which is reduced on recovery (Anderson and Dawson, 1963; 
Coppen and Shaw, 1963; Baer et al., 1969; Bjorum et al., 1972). The 
original observation by Gibbons (1960) of a reduction in exchangeable 
sodium on recovery from depressive illness was not confirmed by Coppen 
et al. (1962) nor by the balance studies of Russell (1960). It has been 
suggested that the sodium retained results in increased residual sodium 
(intracellular plus some bone sodium) (Coppen and Shaw, 1963; Cox et al., 
1971).

Another approach to the study of sodium metabolism in depressive 
illness has been the measurement of the rate of entry of radioactive 
sodium into the CSF. Coppen (1960) reported a decrease in the rate of 
entry of $^{24}$Na from blood into CSF in a group of thirty one depressed patients 
which returned to normal on recovery. This was not confirmed (Fotherby 
et al., 1963; Carroll et al., 1969). Fotherby et al. (1963) discovered 
that exercise significantly increased the transfer rate of $^{24}$Na and 
therefore decreased physical activity in depressed patients could account 
for the reduced transfer rate reported by Coppen (1960).

Plasma and erythrocyte sodium concentrations have been determined 
in depressive illness. Plasma sodium has been shown to be reduced 
(Sharma et al., 1970), normal (Bjorum, 1972) and increased (Ramsey et al., 
1979) during the illness, with no alteration on recovery (Coppen and Shaw, 
1963; Bjorum, 1972). Erythrocyte sodium levels of patients with 
endogenous depression have been found to be normal (Naylor et al., 1970a;
Ramsey et al., 1979) and low (Mendels et al., 1972) compared with controls; a reduction (Naylor et al., 1971), no alteration (Mendels et al., 1972), and an increase (Mendels and Frazer, 1975) have been reported with clinical improvement.

Total body potassium (TBK) has been shown to be low (Shaw and Coppen, 1966) and normal (Platman et al., 1970) and intracellular potassium (ICK) to be low (Shaw and Coppen, 1966; Abe and Coppen, 1969) in depressed patients compared with controls. The control group of Shaw and Coppen (1966) was unfortunately compiled from another study. No alteration has been reported in exchangeable potassium (Gibbons, 1960), ICK (Shaw and Coppen, 1966; Abe and Coppen, 1969) or TBK (Coppen and Shaw, 1963; Shaw and Coppen, 1966; Baer et al., 1970; Platman et al., 1970) on recovery from depressive illness.

Plasma potassium has been found to be high (Sharraa et al., 1970) and low (Bjorum, 1972) in depressive illness with no alteration on recovery (Coppen and Shaw, 1963). There was no difference in erythrocyte potassium concentrations in patients suffering from depressive illness compared with controls (Naylor et al., 1970a; Mendels et al., 1972) or on recovery (Naylor et al., 1971; Mendels et al., 1972). Urinary excretion of potassium was normal in depressive illness (Sharma et al., 1970) but increased significantly with clinical improvement (Bjorum et al., 1972). Paschalis et al. (1977) examined sweat electrolytes of bipolar depressed patients and found reduced potassium levels compared with controls.

Extracellular fluid (ECF) volume has been shown to be low in depressive illness (Shaw and Coppen, 1966). Total body water (TBW) (Coppen and Shaw, 1963; Coppen and Shaw, 1967; Abe and Coppen, 1969), ECF (Coppen and Shaw, 1963; Brown et al., 1963) and intracellular water (ICW) (Coppen and Shaw, 1967; Abe and Coppen, 1969) have been reported to increase with clinical
improvement. Shaw and Coppen (1966) however, found no change in ECF volume on recovery.

The first direct test of an electrolyte hypothesis of depression using human brain tissue was carried out by Shaw and his colleagues (1969). Brains of subjects who had committed suicide contained more water and less sodium (expressed as mEq/100g fat free weight) than brains of subjects who had died of natural causes. The potassium content was similar in both groups. They interpreted their results to indicate that in depression the brain extracellular space may be contracted and the intracellular space enlarged, resulting in a reduction of the intracellular potassium concentration.

These conflicting reports of sodium and potassium levels in depressive illness could have arisen from differences in diagnosis, failure to control diet by some investigators, differences in "metabolic" control and in choice of control groups. There may even be differences between depressives and controls in their attitudes to admission to a metabolic ward. The use of patients as their own controls i.e. depressed and recovered avoids some of these difficulties but gives more limited information. The discrepancy between the results of Sharma et al. (1970) and Bjorum (1972) of plasma sodium and potassium levels in depressive illness appear to be due to differences in sample size (Bjorum's sample being much larger) and in the constancy of the changes observed.

A reduction in active transport of sodium across erythrocyte membranes has been found in endogenous depression by some (Naylor et al., 1970b; Hesketh et al., 1977; Choi et al., 1977; Scott and Reading, 1978) but not all investigators (Naylor et al., 1973; Frazer et al., 1978). Here again, differences may have arisen from differences in diagnosis and in selection of controls.
A reduction in intracellular potassium and increase in intracellular sodium would tend to increase the excitability of cell membranes (Coppen, 1967). It has been suggested that neurones are hyperexcitable in depressive illness (Whatmore, 1966; Whybrow and Mendels, 1969) and that receptors are supersensitive (Shaw et al., 1977; Friedman, 1978).

(ii) Calcium and Magnesium

Studies of calcium and magnesium have been more limited than those of sodium and potassium and have yielded more conflicting results.

Serum calcium has been reported to be high (Hakim et al., 1975a), normal (Naylor et al., 1972) and low (Frizel et al., 1969; Bjorum, 1972) in depressive illness and to decrease following treatment (Bjorum, 1972; Carman et al., 1977). Urinary excretion of calcium has been found to be low (Sharma et al., 1970) and normal (Bjorum et al., 1972) in endogenous depression and to decrease with clinical improvement (Flach, 1964). CSF calcium has been shown to be high (Hakim et al., 1975b) and normal (Jimerson et al., 1979) in endogenous depression and to decrease on recovery (Carman et al., 1977; Jimerson et al., 1979; Carman and Wyatt, 1979).

Serum magnesium has been reported to be high (Cade, 1964; Bjorum, 1972; Hakim et al., 1975a), normal (Naylor et al., 1972) and low (Frizel et al., 1969) in depressive illness and to increase on recovery (Cade, 1964; Frizel et al., 1969). Goodwin et al. (1968) found raised plasma magnesium levels in bipolar but not unipolar depression. Urinary excretion of magnesium was low in endogenous depression and increased on recovery (Bjorum et al., 1972). CSF magnesium levels were higher in patients with endogenous depression than in those with reactive
depression or controls (Hakim et al., 1975b).

The most likely explanation for these conflicting results of calcium and magnesium levels in depressive illness is differences in methodology: the studies of Cade (1964) and Hakim et al. (1975a,b) used colorimetric methods; Frizel et al. (1969) used an ion exchange strip; Bjorum and others used atomic absorption spectrophotometry. The latter is the most accurate for the determination of calcium and magnesium. As with sodium and potassium studies, inconsistencies may have also arisen from differences in diagnosis, failure to control diet by some investigators, differences in "metabolic" control and in selection of control subjects.

(c) Hormones

Most of the research concerning hormones in depressive illness has concentrated on the corticosteroids although growth hormone, luteinizing hormone, prolactin and thyroid hormone have been studied.

(i) Corticosteroids

There have been a vast number of studies producing evidence of increased adrenocortical activity in depressive illness. Plasma corticosteroid levels were shown to be raised, particularly in the morning and there was a greater degree of diurnal variation in depressed patients compared with controls (Board et al., 1957; Gibbons and McHugh, 1962; Doig et al., 1966; Bridges and Jones, 1966; McClure, 1966a; Hullin et al., 1967; Butler and Besser, 1968; Fullerton et al., 1968; Sachar et al., 1973a; Sachar, 1975). Plasma levels correlated with the
severity of the illness (Gibbons and McHugh, 1962; McClure, 1966a)
and were found to decrease on recovery by most (Board et al., 1957;
Gibbons and McHugh, 1962; Bridges and Jones, 1966; Doig et al., 1966;
McClure, 1966b; Brooksbank and Coppen, 1967; Hullin et al., 1967)
but not all investigators (Fullerton et al., 1968). Studies of plasma
cortisol-binding capacity showed that this was normal in depressive
illness (King, 1973; King, 1975).

Raised twenty-four hour urinary corticosteroid excretion has
frequently been reported in depressive illness (Kurland, 1964;
Jakobson et al., 1966; Coppen et al., 1967a; Butler and Besser, 1968;
Fullerton et al., 1968; Mendels, 1969; Baer et al., 1969), the elevation
occurring more frequently in unipolar than in bipolar depression
(Dunner et al., 1972). Urinary 17-hydroxycorticosteroids (17-OHCS)
have been found to be low (Curtis et al., 1960) and normal (Kurland,
1964) in depressive illness and to increase (Stenback et al., 1966)
and decrease (Gibbons, 1966) with clinical improvement. Bunney and his
colleagues (1969) reported elevated 17-OHCS levels in subjects who
subsequently committed suicide or made a serious suicide attempt.
Urinary 17-ketosteroids (17-KS) were raised in depressed patients
compared with controls (Kurland, 1964; Mendels, 1969) and correlated with
the severity of the illness (Kurland, 1964). Mendels (1969) discovered
a greater increase in the 11-oxy-17-KS fraction in eight male
depressed patients compared with the 11-deoxy-17-KS fraction. There was
also an increase in urinary androsterone (an 11-deoxy-17-KS) in two of
the patients compared to control values. The diurnal rhythm of
corticosteroid excretion has been shown to be abnormal in depressive
illness (Butler and Besser, 1968; Fullerton et al., 1968).

Cortisol may be elevated in the CSF (Carroll, 1972) but this is
uncertain (Coppen et al., 1971a).
Corticosteroid secretion rates were found to be elevated during the illness (Gibbons, 1964; Sachar et al., 1971a; Carpenter and Bunney, 1971; Sachar et al., 1973a; Sachar, 1975). Sachar (1975) noted active secretion of cortisol during the night when secretion is normally minimal. The number of secretory episodes over twenty-four hours was increased from nine to twelve and the plasma cortisol concentration was markedly elevated both at the beginning and end of episodes. Secretion normalised on recovery (Gibbons, 1964; Gibbons, 1966; Sachar et al., 1973a; Sachar, 1975).

In conflict with the above studies, corticosteroids were found to be lower in brain tissue of persons who died of suicide compared with those who died of other causes (Brooksbank et al., 1972; Brooksbank et al., 1973).

Another approach to the study of corticosteroid metabolism in depressive illness has been determination of the extent of its suppression after dexamethasone administration. Dexamethasone inhibits adrenocorticotropic hormone (ACTH) secretion. Suppression has been reported to be normal (Shopsin and Gershon, 1971; Carpenter and Bunney, 1971) and reduced (Butler and Besser, 1968; Carroll and Davies, 1970; Carroll et al., 1976; Brown et al., 1979) in endogenous depression. Brown et al. (1980) suggested that the dexamethasone suppression test identifies two subgroups of depressed patients each of which responds preferentially to a specific type of antidepressant. Resistance to dexamethasone suppression also occurs in Cushing's disease (hyperadrenalism) (Butler and Besser, 1968) in which depression is a frequent complication (Prange et al., 1977).

Checkley and Crammer (1977) examined the corticosteroid response to methylamphetamine and found a lower response in depressive illness.
than on recovery. They suggested that their findings may indicate a functional deficiency of noradrenalin at α-adrenergic receptors in depressive illness.

Data concerning corticosteroids is difficult to interpret due to inadequate control of several factors. For example, corticosteroid excretion is in part dependent upon age, sex and body weight (Sachar, 1967). Many of the studies were conducted during the hospital admission period when plasma and urinary corticosteroids are elevated (Mason et al., 1965). The increase varies according to the type of ward to which the patient is admitted: plasma 17-OHCS levels are significantly greater in those admitted to a psychiatric ward than in those admitted to a metabolic ward (Anderson and Dawson, 1965). The value of the data is further reduced by the frequent use of non-hospitalised controls. Some of the drugs commonly administered to psychiatric patients may lead to spurious findings. Barbiturates, used in several of the studies, block ACTH secretion at the hypothalamic level, altering the circadian rhythm (Krieger and Krieger, 1967) and may also affect the metabolism of cortisol (Conney et al., 1965). Phenothiazines, imipramine and desipramine interfere with some of the methods used to determine 17-OHCS, resulting in false low readings (Sachar, 1967). Thus the assumption that a fall in corticosteroid levels is due to clinical improvement may be false if patients are treated with these drugs or if pretreatment levels were determined during the hospital admission period. Increased adrenocortical activity may therefore be the result of nonspecific factors such as the stress of the illness, anxiety or hospitalisation. However, this does not provide an adequate explanation for the abnormality of dexamethasone suppression which occurs in depressives but not in schizophrenics with equivalent psychosis ratings (Carroll, 1976) nor for the increased cortisol secretion during the night when both stress
and anxiety are presumably at a minimum.

Increased adrenocortical activity results in reduced serotonin synthesis (Curzon, 1969) due to the induction of tryptophan pyrrolase, the enzyme catalysing the rate-limiting step in the conversion of tryptophan to nicotinic acid. Lack of serotonin is associated with reduced sleep (Koella, 1974) and therefore the high morning plasma cortisol concentrations observed may be a factor in producing the early awakening characteristic of endogenous depression. The conversion of noradrenalin to adrenalin is enhanced by corticosteroids due to the induction of phenylethanolamine-N-methyl transferase (Harrison et al., 1968). Hydrocortisone has been shown to increase the noradrenalin uptake by rat cerebral cortex (Maas and Mednieks, 1971).

The increased adrenocortical activity may reflect an abnormality in the hypothalamic control of pituitary function and the subsequent release of ACTH. Mendels et al. (1976) suggested that there may be a failure to inhibit the mechanism which normally stops the release of corticotrophin releasing factor in depressive illness and that this is due to a reduction in activity of noradrenergic neurones which normally inhibit ACTH release. If this explanation is correct it would support the biogenic amine hypothesis.

(ii) Growth Hormone

A potent stimulus to growth hormone (GH) secretion is a fall in blood glucose. There have been several reports of reduced GH response to insulin-induced hypoglycaemia in depressive illness (Mueller et al., 1969; Sachar et al., 1971b; Sachar et al., 1973b; Heninger et al., 1975; Sachar 1975). Patients with unipolar depression were found to
secrete significantly less GH than those with bipolar depression or controls in the same age range (Sachar et al., 1973b). However the groups were unequally distributed with regard to sex: most of the unipolar patients were postmenopausal women. This is of importance since GH response is related to circulating oestrogen levels (Sachar, 1975; Gold et al., 1976), the response being greatest in premenopausal women, intermediate in men and lowest in postmenopausal women.

Sachar (1975) studied postmenopausal depressed women and found reduced GH responses to insulin-induced hypoglycaemia compared with age-matched postmenopausal female controls.

It has been suggested that patients suffering from depressive illness are insensitive to insulin and have a reduced rate of glucose utilisation (Mueller et al., 1969; Carroll, 1969; Heninger et al., 1975; Wright et al., 1978). Thus it is possible that the degree of hypoglycaemia produced in some studies may have been insufficient to cause adequate GH release. Nevertheless in Sachar's study (1975) the hypoglycaemic responses were identical in the two groups both in terms of absolute glucose drop and percent drop from the baseline.

L-dopa, the immediate precursor of dopamine, also stimulates GH secretion (Boyd et al., 1970; Kansal et al., 1972). Sachar et al. (1973b) reported lower responses to L-dopa in patients with unipolar depression than in those with bipolar depression or controls, but the groups were not matched for sex. A later study (Sachar et al., 1975) showed no difference between any of the groups. Others have found greater GH responses to L-dopa in bipolar depressed patients than in those with unipolar depression or controls (Gold et al., 1976; Gold and Goodwin, 1977). The difference between these studies and that of Sachar et al. (1975) was the use of a controlled monoamine diet by the former.
depressives and controls respond differently to a monoaminergic diet (Muscettola et al., 1977; Langer and Sachar, 1977). Nevertheless, Mendlewicz et al. (1977), using a controlled monoamine diet, found that GH responses in patients with bipolar depression were similar to those of patients with unipolar depression and to those of controls.

The GH response to hypoglycaemia is mediated by biogenic amines: the response is abolished or inhibited by catecholamine (Frantz et al., 1973) and serotonin (Bivens et al., 1973) depletors and blockers. The GH response to L-dopa is also mediated by biogenic amines (Martin, 1973). Sachar (1975) suggested two possible reasons for the abnormal GH response of depressed women to hypoglycaemia but not to L-dopa: firstly the GH response to L-dopa depends on the brain conversion of exogenous amine precursor, while the GH response to hypoglycaemia calls upon endogenous brain amines; and secondly the two stimuli may act by different neurochemical pathways.

Apomorphine stimulates dopamine receptors directly and does not affect noradrenaline receptors or serotonin metabolism (Lal et al., 1972; Frazer, 1975). Administration of apomorphine has been demonstrated to stimulate GH release in normal subjects (Lal et al., 1972). Frazer (1975) was unable to find any consistent abnormality in GH response to apomorphine in unipolar or bipolar depression, suggesting that dopamine is probably not involved in their aetiology.

(iii) Luteinizing Hormone

Plasma luteinizing hormone (LH) concentrations have been found to be lower in postmenopausal depressed women than in postmenopausal female controls (Altman et al., 1975).
Plasma LH increases postmenopausally due to lack of feedback inhibition by oestrogen. This increase can be blocked by catecholamine depletors (Ojeda and McCann, 1974). Thus the lower plasma LH in the depressed group lends support for the hypothesis of diminished noradrenergic activity in depressive illness. Nevertheless, the possibility of a difference in oestradiol secretion between the two groups cannot be excluded.

The response of LH to LHRH (LH-releasing hormone) has been shown to be normal in unipolar depressed men (Ettigi et al., 1979).

(iv) Prolactin

Prolactin is involved in lactation but has other functions. It promotes renal water, sodium and potassium retention and calcium excretion (Horrobin et al., 1976). These authors suggested that prolactin may be capable of stimulating sodium movement into erythrocytes and an outward movement of some osmotically active solute. Prolactin also exerts an effect on progesterone secretion: normal levels stimulate progesterone secretion whereas high levels inhibit its secretion (Horrobin et al., 1976; McNeilly, 1980; Veldhuis and Hammond, 1980). Veldhuis and Hammond (1980) discovered that oestradiol treatment can modify the action of prolactin on progesterone secretion.

Plasma prolactin has been reported to be raised (Sachar et al., 1973b; Horrobin et al., 1976) and normal (Gold et al., 1976; Cole et al., 1976; Mendlewicz et al., 1977) in depressive illness. There is a circadian rhythm for prolactin in normal subjects (Nokin et al., 1972; Sassin et al., 1972; Osterman and Wide, 1975): serum levels are raised during sleep and diminish rapidly just before waking. This rhythm was
shown to be abnormal in depressed patients (Halbreich et al., 1979) with increased prolactin levels during the evening, several hours before sleep.

Sachar et al. (1973b) found a normal prolactin response to L-dopa in unipolar and bipolar depression but others have reported suppression of prolactin secretion in bipolar depression but not unipolar depression (Gold et al., 1976; Gold and Goodwin, 1977; Mendlewicz et al., 1977).

Prolactin secretion is suppressed by prolactin-inhibiting factor (PIF), which increases the activity of dopaminergic neurones (Sachar et al., 1973b; Horrobin et al., 1976), and is elevated by TSH-releasing hormone (TRH) and prolactin-releasing hormone (PRH). These excitatory factors may be regulated by noradrenergic and/or serotonergic neurones (Horrobin et al., 1976).

(v) Thyroid Hormone

Depression is frequently found in hypothyroid patients but not in those suffering from hyperthyroidism (Whybrow et al., 1969). Thus it is possible that thyroid function may be reduced in depressive illness.

Serum thyroxine (T₄) has been reported to be low (Rybakowski and Sowinski, 1973; Rinieris et al., 1978a) normal (Salvadorini and Saba, 1973; Amsterdam et al., 1979) and high (Kirkegaard et al., 1974) in depressed patients compared with controls. Free thyroxine index (FTI) has also been found to be low (Rybakowski and Sowinski, 1973; Rinieris et al., 1978a) normal (Salvadorini and Saba, 1973; Kirkegaard et al., 1978a) and high (Whybrow et al., 1972; Kirkegaard et al., 1974) in
depressive illness? Serum tri-iodothyronine (T₃) concentrations (Rybakowski and Sowinski, 1973; Kirkegaard et al., 1978a; Amsterdam et al., 1979) and T₃¹²⁵I uptake (Rinieris et al., 1978a) were normal in depressive illness.

The inconsistencies between these studies may have been due to differences in the type and severity of depression. For example, Kolakowska (1977) stated that raised serum FTI was associated with agitation and/or high anxiety and occurred in patients suffering from psychotic or recurrent depression. However, Rinieris et al. (1978b) found lower FTI values in patients with psychotic depression than in those with neurotic depression or controls. There may also have been differences in the degree of alcohol abuse of depressed patients which could have contributed to the inconsistencies between studies: alcohol abuse significantly reduces the FTI (Kolakowska, 1977).

A reduced response of TSH to TRH has been widely reported in depressive illness (Kastin et al., 1972; Prange et al., 1972; Coppen et al., 1974; Kirkegaard et al., 1974; Kirkegaard and Smith, 1978; Kirkegaard et al., 1978a; Mendlewicz et al., 1979; Amsterdam et al., 1979; Bjorum and Kirkegaard, 1979; Kirkegaard and Bjorum, 1980) but this has been disputed (Ettigi et al., 1979). Some investigators found a lower response in unipolar than in bipolar depression (Gold et al., 1977; Gold et al., 1979), but this has also been contradicted (Bjorum and Kirkegaard, 1979; Mendlewicz et al., 1979). The response normalised with clinical improvement (Kirkegaard et al., 1974; Kirkegaard and Smith, 1978; Kirkegaard and Bjorum, 1980).

The TSH response is influenced by circulating thyroid hormones, the response being reduced when circulating thyroid hormone levels are raised. This contradicts the hypothesis of reduced thyroid function
in depressive illness. Prange et al. (1977) suggested that the reduced TSH response to TRH may be due to a reduction in the releasable pool of TSH or to an impairment in the TSH release mechanism.

(d) Conclusion

The review of the biological studies of depressive illness has revealed the abundance of inconsistencies. Hence, despite the vast amount of research, the aetiology of the illness remains a mystery. The main hypothesis is that there is a deficiency of biogenic amine(s) in the CNS, but this has so far neither been accepted nor rejected. Differences in electrolyte and hormone metabolism could be secondary to this proposed abnormality, although changes in these systems can produce alterations in biogenic amine metabolism.

Major support for the biogenic amine hypothesis came from the effects of antidepressant drugs on amine metabolism (Carlsson, 1976): both monoamine oxidase inhibitors (MAOI) and the tricyclics increase the amount of physiologically active amine at functionally important receptor sites in the brain. The tricyclics inhibit amine reuptake into the presynaptic neurone whereas the MAOI prevent amine catabolism, but may also inhibit amine reuptake (Hendley and Snyder, 1968; Escobar et al., 1974).

The tricyclic compounds are either secondary or tertiary amines. The tertiary amines (eg. imipramine and amitriptyline) strongly inhibit serotonin uptake but are less effective inhibitors of noradrenalin uptake. On the other hand, the secondary amines (eg. desipramine and nortriptyline) are potent inhibitors of noradrenalin uptake but have less effect on serotonin uptake (Carlsson et al., 1969a,b;
Todrick and Tait, 1969; Salama et al., 1971). Thus there may be a subgroup of depressed patients with a disorder of serotonin metabolism (Asberg et al., 1976; Goodwin et al., 1977) and a subgroup with a disorder of noradrenalin metabolism (Goodwin et al., 1977; Maas, 1978).

Iprindole, chemically related to the tricyclics and as effective an antidepressant as imipramine, has no ability to block amine reuptake (Fann et al., 1972). This suggests that tricyclics may exert their effect through another mechanism.

Lithium has been shown to increase serotonin biosynthesis (Sheard and Aghajanian, 1970; Perez-Cruet et al., 1971), platelet serotonin levels (Coppen et al., 1976) and the rate of serotonin transport in vivo but not in vitro (Coppen et al., 1980). Lithium has also been shown to reduce the rate of disappearance of labelled serotonin from brain (Schildkraut et al., 1969a) but to increase the rate of disappearance of labelled noradrenalin (Schildkraut et al., 1969b).

Although antidepressant drugs appear to increase the amount of amine at receptor sites this does not necessarily mean that depression is due to an amine deficiency. These drugs may be activating amine systems which compensate for a deficit in other systems. The biogenic amine hypothesis has been criticised by Baldessarini (1975) as being too simple, focusing on only a few amines and considering the amines as static rather than dynamic systems. Support for the involvement of trace amines comes from studies showing that MAOI increase octopamine concentrations in brain and heart and elevate levels of octopamine and its metabolite p-hydroxy mandelic acid, in urine (Molinoff and Axelrod, 1972; Kakimoto and Armstrong, 1962). Dietary factors can influence the synthesis of neurotransmitters (Fernstrom and Wurtman, 1974; Growdon and Wurtman, 1979; Sahaikian et al., 1979) necessitating more carefully
controlled studies. Also the biogenic amine hypothesis is not specific for depression: biogenic amines may play a role in altering schizophrenic symptoms (Ashcroft et al., 1966; Bowers et al., 1969; Bowers, 1978).

POSTPARTUM DEPRESSION

Postpartum depression is no longer believed to be a distinct condition since Vislie (1956) and Foundeur et al. (1957) demonstrated that both the clinical features and prognosis were similar to those of endogenous depression. As a result, interest in the concept waned and few studies concerned specifically with depressive illness in the postpartum period have been carried out. Nevertheless, there is good evidence that childbirth is followed by a sharp rise in the incidence of depressive illness (Pugh et al., 1963; Paffenberger, 1964; Kendell et al., 1976). It has been suggested that 50 percent of women experience mild disturbances of mood in the first ten days postpartum (Yalom et al., 1968; Pitt, 1968; Pitt, 1973). Estimates of the occurrence of depression of sufficient severity to require treatment vary. Some investigators have reported an incidence of 3 percent within six months postpartum (Ryle, 1961; Tod, 1964) while others have found an incidence of 6 percent (Baker et al., 1971), 7 percent (Dalton, 1971) or 11 percent (Pitt, 1968).

It has been suggested that women diagnosed as having an effective disorder independent of the postpartum period have an increased risk of developing a postpartum affective illness (Bratfos and Haug, 1966; Winokur and Ruangtrakool, 1966; Reich and Winokur, 1970), although postpartum illnesses appear to occur less frequently in patients with
unipolar affective disorder than in those with bipolar affective disorder (Bratfos and Haug, 1966; Winokur and Ruangtrakool, 1966; Protheroe, 1969; Reich and Winokur, 1970; Baker et al., 1971). It is also possible that women suffering from postpartum affective illness have an increased risk of later development of non-postpartum affective illness (Thornton, 1977; Uddenberg and Engelsson, 1978).

The aetiology of the disorder is unknown, but as with endogenous depression genetic factors have been suggested to be involved (Thuwe, 1974). Several theories have been put forward concerning the biochemical abnormality responsible for the illness, the role of the dramatic hormonal changes that occur in the few days after childbirth and with the onset of lactation being a recurrent theme. The main hypotheses concern progesterone (Malleson, 1953; Yalom et al., 1968; Dalton, 1971; Welburn, 1980): plasma progesterone levels before delivery may be excessively high in women who develop affective illness; the rate of fall after delivery may be too fast; or there may be an abnormal ratio of oestrogen to progesterone. Other hypotheses relate the affective illness to low corticosteroid levels (Bower and Altschule, 1956), to prolactin release (Oppenheim, 1962) and to abnormalities in thyroid function (Ballarchy et al., 1958). Nott et al. (1976) measured plasma LH, FSH (follicle stimulating hormone), total oestrogen, progesterone and prolactin in pregnant women before parturition and on sixteen days in the six weeks following delivery, but failed to produce any strong evidence that hormones are related to mood at this time. However, the emotional disturbances reported were neither severe nor prolonged.

Stein et al. (1976) measured free and total plasma tryptophan on the sixth day postpartum in eighteen women. Those with severe depression were found to have significantly reduced concentrations of free tryptophan. Values were similar to those of patients suffering from endogenous
depression and correlated negatively with the severity of the illness. Handley et al. (1977) studied free and total tryptophan and cortisol in the plasma of eighteen women, daily from day two to five postpartum. Over this period plasma free tryptophan concentrations tended to rise and plasma cortisol concentrations to decline. There was a positive correlation between plasma free tryptophan concentrations and elevation of mood and a negative correlation with depression. Plasma cortisol concentrations also correlated positively with elation, but there was no significant correlation between cortisol and either free or total tryptophan concentrations. Reduced concentrations of plasma free tryptophan may result in decreased brain serotonin synthesis (Fernstrom and Wurtman, 1971; Knott and Curzon, 1972; Tagliamonte et al., 1973), but this is uncertain (Fernstrom et al., 1976; Wiryanti, 1980).

Vitamin B₆ is an essential cofactor for the decarboxylase involved in the formation of serotonin from tryptophan (Buxton and Sinclair, 1956) and hence a deficiency of vitamin B₆ might contribute to the production of postpartum depression. Although vitamin B₆ deficiency is associated with the depression occurring in pregnant women (Pulkkinen et al., 1978) and in those using oral contraceptives (Adams et al., 1973; Wynn, 1975; Drug and Therapeutics Bulletin, 1978; Donald and Bossé, 1979; Bossé and Donald, 1979), Livingston et al. (1978) found no evidence of vitamin B₆ deficiency in women suffering from postpartum depression. Thornton (1977) described a case history of depression in the postpartum period which was due to folic acid deficiency. This is of interest since a deficiency of folic acid may also reduce the formation of serotonin (Botez et al., 1979).

Ballinger et al. (1979) measured twenty-four hour urinary excretion of cyclic AMP (adenosine 3'5' cyclic monophosphate) in a group of women on two occasions in the week following childbirth and again two to three
months later. Cyclic AMP concentrations were higher in the week following childbirth than two to three months later. Those women showing the most emotional disturbance on the third day after delivery and women indicating most mood change in the direction of becoming elated had the highest levels of cyclic AMP excretion on that day. This is in agreement with previous work in mania and depression (Abdullah and Hamadah, 1970).

In conclusion, it appears that biochemical abnormalities in women with postpartum depression are similar to those in patients with endogenous depression. In addition, environmental factors may play a role in the genesis of depressive illness in some postpartum women (Welburn, 1980).

BACKGROUND TO THE PRESENT STUDY

In the normal healthy mammal, there are two major series of long chain polyunsaturated fatty acids (LCP) derived from two short chain polyunsaturated fatty acids (SCP), linoleic acid (18:2ω6) and α-linolenic acid (18:3ω3). These two parent fatty acids cannot be synthesised de novo (Holman, 1970a,b; Brenner, 1974) and are therefore obtained from the diet. Linoleic acid gives rise to the ω6 series of fatty acids: γ-dihomolinolenic acid (20:3ω6) and arachidonic acid (20:4ω6) and α-linolenic acid gives rise to the ω3 series:

* Fatty acid nomenclature as follows: the number before the colon represents the number of carbon atoms. The number after the colon represents the number of cis double bonds. The number following the omega represents the position of the first double bond measured from the methyl end of the molecule. It is assumed that all of the double bonds are methylene interrupted.
Eicosapentaenoic acid (20:5ω3) and docosahexaenoic acid (22:6ω3) (see Figure 5). Interconversion between the two series does not appear to occur (Holman, 1970a,b). In addition to these two series there is a third series of LCP, the ω9 series, derived from oleic acid (18:1ω9) (see Figure 5) which can be synthesised de novo.

These pathways were elucidated in the rat by several groups of investigators using a combination of radioisotope techniques and gas-liquid chromatography (Steinberg et al., 1957; Fulco and Mead, 1959; Klenk and Mohrhauer, 1960; Mead, 1961).

Competitive inhibition occurs between fatty acids for the chain elongating and desaturating enzyme systems (Brenner and Peluffo, 1966; Alfin-Slater and Aftergood, 1968; Brenner, 1974). For any given chain length the more unsaturated the fatty acid the greater is its affinity for the enzyme system. Hence 18:3ω3 is desaturated in preference to 18:2ω6 which is desaturated in preference to 18:1ω9. Increasing the relative proportion of the less unsaturated fatty acid will reverse the preference, for example when the concentration of 18:2ω6 is high relative to that of 18:3ω3, 18:2ω6 is desaturated in preference to 18:3ω3. Also there is some evidence that LCP can inhibit their own production from SCP: both 22:6ω3 (Brenner and Peluffo, 1967; Christiansen et al., 1968; Actis Dato and Brenner, 1970) and 22:5ω6 (Actis Dato and Brenner, 1970) can inhibit their own production from 18:3ω3 and 18:2ω6 respectively.

The rate-limiting step in the synthesis of LCP is the first desaturation, catalysed by Δ6 desaturase. This enzyme requires CoA (Coenzyme A), ATP (adenosine triphosphate), Mg$^{2+}$ and NADH (Brenner and Peluffo, 1966; 1969; Castuma et al., 1972; Cook, 1978) and a protein factor, as yet unidentified (Catalá et al., 1975; Catalá et al., 1977). Δ6 desaturase activity is affected by a number of
Figure 5

Biosynthesis of long chain polyunsaturated fatty acids

\[
\begin{align*}
18:3\omega3 & \xrightarrow{\Delta6} 18:4\omega3 & 18:4\omega3 & \xrightarrow{\Delta5} 20:4\omega3 & 20:4\omega3 & \xrightarrow{\Delta4} 20:5\omega3 & 20:5\omega3 & \xrightarrow{\Delta5} 22:5\omega3 & 22:5\omega3 & \xrightarrow{\Delta4} 22:6\omega3 \\
\text{\(\alpha\)-linolenic acid} & & & & & & & & & \\
18:2\omega6 & \xrightarrow{\Delta6} 18:3\omega6 & 18:3\omega6 & \xrightarrow{\Delta5} 20:3\omega6 & 20:3\omega6 & \xrightarrow{\Delta4} 20:4\omega6 & 20:4\omega6 & \xrightarrow{\Delta5} 22:4\omega6 & 22:4\omega6 & \xrightarrow{\Delta4} 22:5\omega6 \\
\text{linoleic acid} & & & & & & & & & \\
18:1\omega9 & \xrightarrow{\Delta6} 18:2\omega9 & 18:2\omega9 & \xrightarrow{\Delta5} 20:2\omega9 & 20:2\omega9 & \xrightarrow{\Delta4} 20:3\omega9 & 20:3\omega9 & \xrightarrow{\Delta5} 22:3\omega9 & 22:3\omega9 & \xrightarrow{\Delta4} 22:4\omega9 \\
\text{oleic acid} & & & & & & & & & \\
\end{align*}
\]

Double arrow indicates desaturation step.
dietary and hormonal factors (Brenner, 1974; Brenner, 1977) which will be discussed in more detail in Chapter 6.

LCP can also be obtained directly from the diet as they are present in animal products (Crawford and Sinclair, 1972; Paul and Southgate, 1978).

The brain contains considerable amounts of LCP (mainly 20:4ω6 and 22:6ω3) which are either derived from in situ conversion of 18:2ω5 and 18:3ω3 respectively (Mead et al., 1977) or arrive at the brain as preformed compounds (Galli et al., 1977). Fatty acids produced in the liver can be transported to the brain via the plasma, 18:2ω6 and 18:3ω3 being transported preferentially as free fatty acids (Dhopeshwarkar and Mead, 1973) and LCP being transported primarily in the choline phosphoglycerides (Ansell and Spanner, 1972; Sinclair, 1975; Crawford et al., 1976).

Little is known about the function of LCP. In the brain they are associated with membrane phospholipids (Cotman et al., 1969) and hence may affect membrane function. Changes in the fatty acid composition of structural lipids in brain membranes have been reported to modify Na⁺-K⁺-ATPase activity in synaptosomes (Sun and Sun, 1976) and ω6 and ω3 fatty acids are considered important for the activity of some other membrane-bound enzymes (Bernsohn and Spitz, 1974). Moreover, alterations in the fatty acid composition of membrane lipids affect the permeability of the membrane (van Deenan, 1971; McElhaney, 1974).

LCP are also important functional components of the photoreceptor membranes of the retina, 22:6ω3 being the predominant fatty acid of the rod outer segments (Anderson and Maude, 1970; Borggreven et al., 1970; Nielson et al., 1970). When the content of LCP is reduced, there is an
alteration in the electrical response of the photoreceptor membranes (Benolken et al., 1973; Wheeler et al., 1975; Anderson et al., 1977).

The ω3 series affect electroretinogram amplitudes to a greater extent than the ω6 series, indicating a selective functional role in the visual system for ω3 fatty acids.

When rats (Holman, 1970a,b) human infants (Söderhjelm et al., 1970) or adults (Collins et al., 1971; Holman, 1973) consume a fat-free diet they develop a generalised scaliness of the skin which can only be reversed by 18:2ω6 or its metabolite 20:4ω6. Thus 18:2ω6 is said to be an essential fatty acid (EFA). Diets deficient in 18:2ω6 and 18:3ω3 (Paoletti and Galli, 1972; Galli et al., 1975a,b; Lamptey and Walker, 1978), or diets with different 18:2ω6/18:3ω3 ratios (Lamptey and Walker, 1976) produce modifications in the learning behaviour of animals.

Some LCP (20:3ω6, 20:4ω6, and 20:5ω3) are precursors of biologically active substances, the prostaglandins and related compounds (Alfin-Slater and Aftergood, 1968; Galli et al., 1977; Galli et al., 1978). The system which forms prostaglandins from 20:4ω6 has been extensively studied in several laboratories. The formation of an endoperoxide intermediate in the synthesis of prostaglandins and thromboxanes has been described by Hamberg and Samuelsson (1973; 1974). The rate-limiting step in the overall formation of active products from 20:4ω6 is the release of the precursor from phospholipids (Horton, 1975; Flower and Blackwell, 1976). This step involves activation of a phospholipase A2 acting on the 2 position of the phosphoglycerides. It is not known whether a specific phospholipid is the donor of 20:4ω6 for prostaglandin synthesis, although release of 20:4ω6 mainly from phosphatidyl inositol has been reported in stimulated platelets (Schoene and Iacono, 1976; Bills et al., 1976).

Within the CNS, prostaglandins alter the effects of neurotransmitters at pre- and postsynaptic sites (Horton, 1973; Hedqvist, 1974; Hedqvist,
The plasma choline phosphoglycerides (CPG) account for 70 percent of the total plasma phospholipids in man (Phillips and Dodge, 1967) and have been shown to be good indicators of polyunsaturated fatty acid status (Rivers et al., 1975). Nevertheless, the fatty acid composition of the erythrocyte phospholipids probably gives a better indication of tissue polyunsaturated fatty acid status (Sanders and Naismith, 1979).

The lipids of erythrocytes are confined to the membrane (Cooper, 1970). In human red cells the major lipids are cholesterol and the phospholipids. There are four main phospholipids, each having its own characteristic fatty acid composition. The ethanolamine (EPG) and serine phosphoglycerides (SPG) contain large amounts of LCP, the choline phosphoglycerides (CPG) contain lesser amounts of LCP and greater amounts of 18:2ω6 and short chain saturated fatty acids and the sphingomyelin fraction contains considerable amounts of long chain saturated (23:0 and 24:0) and monounsaturated (24:1) fatty acids (Dodge and Phillips, 1967). The chemical structures of these phospholipids are shown in Figure 6. The R' position in the phosphoglycerides is almost invariably occupied by saturated fatty acids whereas the R" position contains all the C_{18}, C_{20} and C_{22} polyunsaturated fatty acids (Christie, 1973). The R' position occasionally contains a vinyl ether (plasmalogen) residue (Farquhar, 1962; Christie, 1973). The EPG, which constitute about 30 percent of the erythrocyte phospholipids (Dodge and Phillips, 1967), have been used as indicators of LCP status (Crawford and Sinclair, 1972).

A preliminary study of six patients suffering from endogenous depression (Ellis and Sanders, 1977) revealed that there was a higher proportion of LCP, in particular 22:6ω3 and 20:5ω3, in their plasma CPG than in those of age-sex-matched controls or patients suffering
The structures of the four major erythrocyte phospholipids

- **Ethanolamine Phosphoglyceride**
  - \[ \text{RCOO} - \text{CH} - \text{O} - \text{CH}_2 - \text{P} - \text{O} - \text{CH}_2 \text{NH}_3^+ \]

- **Serine Phosphoglyceride**
  - \[ \text{RCOO} - \text{CH} - \text{O} - \text{CH}_2 - \text{COOH} \]

- **Choline Phosphoglyceride**
  - \[ \text{RCOO} - \text{CH} - \text{O} - \text{CH}_2 - \text{N}^+ \text{CH}_3 \]

- **Sphingomyelin**
  - \[ \text{CH}_3 \text{(CH}_2)_2 \text{CH} = \text{CHOHCHCH}_2 - \text{O} - \text{P} - \text{O} - \text{CH}_2 \text{NH}_3^+ \text{CH}_3 \]
from other psychiatric disorders. Differences in the erythrocyte EPG were in the same direction but were not significant. The present study was undertaken to repeat and to extend these investigations, since it is possible that a higher proportion of LCP may affect membrane function (van Deenan, 1971; McElhaney, 1974; Lenaz, 1979) and hence help to explain the proposed abnormality in membrane transport of cations (Mendels and Frazer, 1973; Mendels and Frazer, 1974; Hesketh et al., 1977; Choi et al., 1977) and serotonin (Coppen et al., 1978; Coppen et al., 1980) in some patients with depressive illness.

There were only a few other studies in the literature concerning lipid metabolism in patients with depressive illness. Total serum lipids have been reported to be low in depressed patients (Shaheen et al., 1971) due to their disturbed appetite and to increase (Shaheen et al., 1971) and decrease (Hullin and Court, 1970) on recovery. Serum triglyceride concentrations were also found to be low in patients suffering from endogenous depression but to decrease further with clinical improvement (Shaheen et al., 1971). Three possible reasons for this were suggested: disturbed synthesis of triglycerides; inhibition of lipogenesis; and/or disturbed transmethylation processes. Finally, plasma free fatty acid concentrations have been shown to be raised in depressed patients (Cardon and Mueller, 1966; Mueller et al., 1970). Mueller et al. suggested that this may be related to the reduced glucose utilisation of depressed patients.

**AIMS OF THE PRESENT STUDY**

The present study was undertaken in order to repeat and extend the investigations of Ellis and Sanders (1977) as these related to only...
six patients.

The aims were as follows:

1) To ascertain whether determination of the LCP composition of the plasma CPG would be of value as a diagnostic tool for endogenous depression.

2) To ascertain whether the higher LCP concentrations are reflected in the erythrocyte membrane phospholipids.

3) To discover if the LCP concentrations return to normal with clinical improvement.

4) To determine whether the abnormality occurs in postpartum depression.

5) To ascertain whether the use of oral contraceptive agents affects phospholipid LCP composition.

6) To examine factors affecting the LCP composition of the plasma CPG, in order to discover the aetiology of the observed differences.
CHAPTER TWO

SUBJECTS, MATERIALS AND METHODS.

SUBJECTS

Patients suffering from depressive illness or from other psychiatric disorders were in-patients at Kingston Hospital. Patients with early symptoms of depressive illness were contacted through general practitioners (Drs. J. Kane and J. Boxer, 11 Birkenhead Avenue, Kingston upon Thames). Control subjects were apparently healthy hospital staff, their relatives and friends and were matched to the patients by age and sex.

BLOOD SAMPLES

Blood samples were taken by venepuncture with stasis at 9:30 a.m. after an overnight fast and sent to the laboratory immediately. Serum was used for the estimation of thyroid function and fasting insulin concentrations but for other analyses blood was anticoagulated with trisodium ethylenediamine tetraacetate (EDTA). Samples were analysed 'blind' i.e. the diagnoses and other details of the patients were known only to the psychiatrist until after the results were obtained.

CHEMICALS

Most of the chemicals used were purchased from BDH Chemicals Ltd. (Poole, Dorset) and were Analar grade. Toluene was Aristar grade. Standards for thin-layer chromatography (TLC) were obtained from Sigma Chemical Company (Poole, Dorset), and those for gas-liquid chromatography (GLC) from Sigma Chemical Company, Field Instruments (Twickenham, Middlesex), N.E.N. Chemicals (Dyson Instruments, Newton Hall, Durham).
Uniscience Ltd. (Cambridge) and Nu-Chek Prep (Elysian, Minnesota, U.S.A.). Packing materials for GLC columns were purchased from the following firms: Silar 10CP (100%-3-cyanopropyl silicone) coated on chromasorb W AW/DMCS, 100-120 mesh, from N.E.N. Chemicals; 10% EGSS-Y coated on chromasorb W AW/DMCS, 100-120 mesh, from Perkin-Elmer Ltd. (Beaconsfield, Bucks).

**ANALYTICAL METHODS**

**General Precautions Taken in Lipid Analyses**

Plastic apparatus and containers other than teflon were avoided. Analyses (except the preparation of methyl esters) were carried out in all-glass apparatus. Methyl esters were prepared in glass tubes with teflon-lined screw caps. Glassware was cleaned by soaking in chromic acid for at least 24 hours, rinsed thoroughly in tap water, twice in distilled water and finally dried in an oven prior to use. Teflon-lined screw caps were cleaned by boiling in methanol.

In order to minimise auto-oxidation of polyunsaturated fatty acids (Dodge and Phillips, 1966) the antioxidant 2, 6-di-tert-butyl-p-cresol (butylated hydroxytoluene, BHT) was added at a level of 50mg/l to all solvents and spray reagents, except hexane (Christie, 1973) and 500mg/l to all TLC solvents (Dodge and Phillips, 1967). BHT was twice recrystallised from methanol prior to use.

Lipid extracts were stored in chloroform under nitrogen at -20°C and methyl esters in hexane under nitrogen at -20°C. Solvents were evaporated prior to TLC or GLC using a stream of nitrogen and TLC plates were also dried with nitrogen.

Blood was chilled to 4°C and then centrifuged at 3,000 g for ten minutes. Plasma was removed and stored at -20°C. Under these conditions
there is no alteration in the fatty acid composition of the choline phosphoglycerides for at least a year (Sanders, 1977). Erythrocyte lipids were extracted immediately.

Preparation of Lipid Extracts

(a) Erythrocytes

The residual packed cells were washed three times with an equal volume of 0.15 M (8.9 g/l) saline, centrifuged and the supernatant and buffy coat discarded.

Erythrocyte lipids were extracted using chloroform-isopropanol 7:11 (v/v) (Rose and Ocklander, 1965) for subsequent fatty acid and aldehyde analysis or using chloroform-methanol 1:1 (v/v) (Dodge and Phillips, 1967) for subsequent lipid phosphorus and free cholesterol determinations. The chloroform-isopropanol procedure is better suited for the preparation of an extract for fatty acid and aldehyde analysis since it uses much smaller volumes of solvent thereby reducing the possibility of contamination of the sample, whereas the chloroform-methanol extraction is preferred when lipid phosphorus is to be determined since inorganic phosphorus is not removed by the former method (Sanders, 1977).

(b) Plasma

Plasma lipids were extracted as described by Folch, Lees and Sloane-Stanley (1957) using chloroform-methanol 2:1 (v/v) and finally washing the extract with one-fifth its volume of water containing 0.29% sodium chloride in order to remove non-lipid contaminants.

Isolation and Separation of Phospholipids

Phospholipids were isolated from other lipids and then separated into
individual phospholipid classes by TLC on a 0.5mm layer of silica gel HR.

TLC plates were prepared with water (neutral plates): 90 mls to 40g silica gel, and the slurry applied to 20 x 20 cm glass plated using the Unoplan spreader and plate leveller (Shandon Southern Instruments, Camberley, Surrey). After spreading the gel was allowed to dry horizontally in air until the sheen on the surface was replaced by a dull matt appearance (approximately one hour). The plates were then activated by heating at 110°C for one hour. Unused plates were reactivated in the same manner prior to use.

Lipid extracts were dried under nitrogen and then redissolved in a small volume of chloroform. An equivalent of 0.5 ml of plasma or packed red cells was applied to the plate as a 2cm band, 1.5 - 2 cm from the bottom. The plate was then developed with acetone-hexane 1:3 (v/v) at 4°C (Skipski and Barclay, 1969). The chromatography tank was lined with blotting paper which dipped into the solvent. This produced a solvent-saturated atmosphere inside the tank which helped to maintain reproducibility from plate to plate, assisted even running and helped to increase the speed of running. In this solvent system the phospholipids remained at the origin whereas less polar neutral lipids such as cholesterol and the triglycerides migrated up the plate. When the solvent reached the top (1/2 - 2 hour), the plate was dried under nitrogen and redeveloped in the same direction with chloroform-methanol-acetic acid-water, 25:15:4:2 (v/v) (Skipski, Peterson and Barclay, 1964) under conditions of tank saturation at 4°C as above. This system separated the individual phospholipids. The plate was removed from the tank when the solvent front was about 2 cm from the top, dried under nitrogen and then sprayed with 2',7'-dichlorofluorescein reagent (0.1% solution in 95% methanol and washed with hexane (Parker and Peterson, 1965) before use). Bands were visualised under ultraviolet light and individual phospholipids identified by comparison.
Determination of the Phospholipid Fatty Acid and Aldehyde Composition

(i) Preparation of Methyl Esters and Dimethyl Acetals

Bands pertaining to the individual phospholipids were circled with a fine needle and each transferred to a centrifuge tube fitted with a Teflon-lined screw cap. 1 ml of methanol was added followed by 1 ml of 14% w/v boron trifluoride in methanol. The tubes were flushed with nitrogen, sealed and heated at 100°C for time periods specified by Morrison and Smith (1964).

(ii) Extraction of Methyl Esters and Dimethyl Acetals

(a) Plasma - Having allowed the tubes to cool to room temperature, 4 mls of hexane were added followed by 2 mls of water. The tubes were then mixed thoroughly and centrifuged; the 2',7'-dichlorofluorescein dye remained in the lower aqueous phase. The upper phase containing the methyl esters was collected and stored under nitrogen at -20°C.

(b) Erythrocytes - 4 mls of hexane were added followed by 2 mls of 5M sodium hydroxide, dropwise with stirring, at 0°C. After thorough mixing the tubes were centrifuged and the upper phase containing the methyl esters and dimethyl acetals collected. The base was added to prevent reversion of the dimethyl acetals (Morrison and Smith, 1964). A second extraction with 2 mls of hexane was carried out to ensure recovery of the dimethyl acetals. This was pooled with the first and stored under nitrogen at -20°C.

(iii) Purification of Methyl Esters and Dimethyl Acetals

This was carried out by TLC on a 0.5 mm layer of silica gel G. Samples were dried under nitrogen, redissolved in 50 μl of hexane and applied to
the plate as bands. The plate was developed with toluene at 4°C (Dodge and Phillips, 1967). After drying the plate under nitrogen, bands were located by spraying with 0.1% dichlorofluorescein reagent and visualised in ultraviolet light. Methyl esters and dimethyl acetalts were identified by comparison of their migration with authentic standards. The relevant bands were then removed from the plate and transferred to a glass-stoppered centrifuge tube. Elution from the gel by 2 ml of methanol containing 1% acetic acid (Dudley and Anderson, 1975) was followed by a hexane and water extraction. After centrifugation the upper phase was removed and stored under nitrogen at -20°C.

(iv) Analysis of Methyl Esters and Dimethyl Acetals

Samples were dried over anhydrous sodium sulphate and the hexane evaporated under nitrogen. Methyl esters and dimethyl acetalts were then taken up in 50 μl of hexane and analysed by GLC on a Perkin Elmer F11 gas chromatograph fitted with dual flame ionisation detectors and recorded on a Perkin Elmer 159 recorder. Analyses were carried out using a 2 m x 7 mm o.d. all glass column packed with 10% Silar 10CP on chromosorb W AW/DMCS 100-120 mesh at 190°C with a nitrogen flow of 50 ml/min and confirmed on a similar column packed with 10% EGSS-Y on chromosorb W AW/DMCS 100-120 mesh at 190°C with a nitrogen flow of 25 ml/min. Methyl esters were identified by comparison of their retention characteristics with those of authentic standards and secondary reference standards (Ackman and Burgher, 1965). Components were quantified by taking the retention time multiplied by the peak height as proportional to the weight of the component, (Christie, 1973), and the results expressed in mg/g total fatty acid methyl esters detected. Quantification was checked regularly using authentic standards of known composition.
One blood sample was taken through the whole procedure six times and coefficients of variation calculated for each fatty acid of all phospholipids analysed to establish the precision of the methods (Tables 2 – 4). Thereafter all analyses were carried out in duplicate.

These methods have previously been examined in detail (Sanders, 1977) and will not be discussed further here.
TABLE 2

Precision of the determination of the fatty acid composition
of the plasma choline phosphoglycerides

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Mean (mg/g)</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>298</td>
<td>1.7</td>
</tr>
<tr>
<td>18:0</td>
<td>128</td>
<td>2.0</td>
</tr>
<tr>
<td>18:1ω9</td>
<td>112</td>
<td>2.4</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>246</td>
<td>1.8</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>21</td>
<td>3.7</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>103</td>
<td>2.6</td>
</tr>
<tr>
<td>20:5ω3 + 24:1ω9</td>
<td>11</td>
<td>3.0</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>14</td>
<td>4.9</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>45</td>
<td>3.0</td>
</tr>
<tr>
<td>Methyl Esters and Dimethyl Acetals (DMA)</td>
<td>Ethanolamine phosphoglycerides</td>
<td>Serine phosphoglycerides</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-------------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td></td>
<td>Mean (mg/g)</td>
<td>Coefficient of variation (%)</td>
</tr>
<tr>
<td>16:0 + 16:0 DMA</td>
<td>132</td>
<td>1.4</td>
</tr>
<tr>
<td>18:0 + 18:0 DMA</td>
<td>141</td>
<td>1.4</td>
</tr>
<tr>
<td>18:1ω9 + 18:1 DMA</td>
<td>138</td>
<td>1.6</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>63</td>
<td>2.2</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>17</td>
<td>4.4</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>253</td>
<td>2.9</td>
</tr>
<tr>
<td>20:5ω3 + 24:1ω9</td>
<td>17</td>
<td>1.6</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>56</td>
<td>5.1</td>
</tr>
<tr>
<td>22:5ω6</td>
<td>6</td>
<td>5.4</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>62</td>
<td>3.0</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>104</td>
<td>2.3</td>
</tr>
</tbody>
</table>
TABLE 4

Precision of the determination of the fatty acid composition of the erythrocyte sphingomyelin

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Mean (mg/g)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>210</td>
<td>1.7</td>
</tr>
<tr>
<td>18:0</td>
<td>63</td>
<td>2.5</td>
</tr>
<tr>
<td>18:1ω9</td>
<td>9</td>
<td>4.1</td>
</tr>
<tr>
<td>20:0</td>
<td>20</td>
<td>2.9</td>
</tr>
<tr>
<td>22:0</td>
<td>95</td>
<td>1.6</td>
</tr>
<tr>
<td>23:0 + 20:4ω6</td>
<td>33</td>
<td>3.2</td>
</tr>
<tr>
<td>24:0 + 20:4ω3</td>
<td>257</td>
<td>1.4</td>
</tr>
<tr>
<td>24:1 + 20:5ω3</td>
<td>278</td>
<td>1.4</td>
</tr>
<tr>
<td>24:2ω6 + 24:2ω9</td>
<td>28</td>
<td>3.7</td>
</tr>
<tr>
<td>26:0</td>
<td>9</td>
<td>4.6</td>
</tr>
<tr>
<td>26:1ω9 + 24:4ω6</td>
<td>6</td>
<td>6.5</td>
</tr>
</tbody>
</table>
**Determination of Total Plasma Choline Phosphoglycerides (Lecithin)**

To determine total plasma lecithin an internal standard (50 µg of pentadecanoic acid, C15:0) was added at the stage of preparation of methyl esters of plasma choline phosphoglyceride fatty acids (Christie *et al.*, 1970). Total plasma lecithin was then calculated in the following way:

\[
\text{Total Lecithin} = \text{Area} \times 100 \times 1.371 \text{ (mg/l)}
\]

The coefficient of variation (c.o.v.) was 4.6%. The recovery of standard lecithin was (mean ± S.E.) 90.3 ± 2.8%.

**Determination of Total Lipid Phosphorus and the Relative Proportions of Phospholipids in Erythrocytes**

Aliquots of the total erythrocyte lipid extract were digested with 0.4 ml of 70% perchloric acid and phosphorus determined in triplicate using a modification of the method of Bartlett (1959) as described by Dittmer and Wells (1969). Optical density was measured in a Unicam SP-600 spectrophotometer at 830 nm. The standard curve is shown in Figure 7. The coefficient of variation was 5.4%.

To determine the relative proportions of phospholipids in the erythrocytes, phospholipids were separated by TLC as described above, the relevant bands removed from the plate and digested with 1 ml of 70% perchloric acid. The larger amount of perchloric acid was required to ensure sufficient acidity of the reaction mixture as perchloric acid is neutralised by silica gel: insufficient acidity results in artifically increased absorption (Dodge and Phillips, 1967). The phosphorus content of each band was determined and expressed as a percentage of the total phosphorus after TLC (max c.o.v. 5.2%). The recovery of phosphorus...
Figure 7

Standard curve for phosphorus

Concentration of phosphorus (μmol per tube)
Determination of Total and Free Cholesterol

Total plasma cholesterol was estimated by the ferric chloride - sulphuric acid reaction as described by Tietz (1976). Optical density was measured in a Unicam SP-600 spectrophotometer at 560 nm. The standard curve is shown in Figure 8. The coefficient of variation was 2.8%.

The ratio of free:esterified cholesterol in plasma was determined in the following way: free and esterified cholesterol were separated by TLC of the plasma lipid extract using hexane - diethyl ether - glacial acetic acid, 80:20:1 (v/v) as the developing solvent. Bands were located using iodine vapour and identified by comparison of their migration with authentic standards. Bands corresponding to free cholesterol and to esterified cholesterol were removed from the plate, extracted once with 2 mls of chloroform and three times with 1 ml of chloroform (Dodge and Phillips, 1967). The solvent was then evaporated to dryness under nitrogen and the cholesterol content of each band determined by the ferric chloride - sulphuric acid reaction. Free and esterified cholesterol were expressed as a percentage of the total cholesterol after TLC (max. c.o.v. 3.5%).

BHT was not added to any of the solvents used for the determination of the relative proportions of free and esterified cholesterol in plasma as it interferes with the ferric chloride - sulphuric acid reaction, producing a brownish product that absorbs at 560 nm (Dodge and Phillips, 1967).

Erythrocyte free cholesterol was determined in the same way as the plasma free cholesterol. The coefficient of variation was 4.3%. The presence of BHT in the lipid extract was not a problem as in the solvent
Figure 8

Standard curve for cholesterol

Cholesterol concentration (mmol/l)

Absorbance ($\lambda = 560$ nm)
system used it migrates just behind the cholesterol esters but with considerable overlap (Dodge and Phillips, 1967). Free cholesterol standards were subjected to TLC and the standard curve drawn from their optical densities after TLC, thereby making correction for recovery from the plate unnecessary. Recovery of the standards (mean ± S.E.) was 90.2 ± 2.5%.

ANCILLIARY INVESTIGATIONS

Blood counts were determined on a Coulter model S senior counter and blood films examined by the duty haematologist. Plasma viscosity was measured using a Coulter Viscometer (Harkness type). Thyroid function and fasting serum insulin were determined by radioimmunoassay techniques in use at Kingston Hospital and St. Luke's Hospital, Guildford, respectively. Blood glucose was estimated by an enzymic method (glucose oxidase) in use at Kingston Hospital. Plasma 17-β-oestradiol was determined by radioimmunoassay using the kit supplied by Serono U.K. Ltd. (Welwyn Garden City, Herts). The standard curve is shown in Figure 9. Plasma cyclic AMP (cyclic 3'5'-adenosine monophosphate) was determined by the radioimmunoassay technique described by Tovey et al. (1974) ("Cyclic AMP Assay Kit", Radiochemical Centre, Amersham). The standard curve is shown in Figure 10. Dietary information was collected by 24-hour recall and by dietary history (Mann et al., 1962).

STATISTICAL METHODS

The two-tailed student t-test was used for testing differences between means when samples were not paired. When samples were paired, either Wilcoxon's test for pair differences or a two-tailed paired sample t-test was used. Differences in the frequency of certain observations between
groups were tested using a $\chi^2$ 2 x 2 contingency table. The correlation coefficient ($r$) used was the coefficient of product-moment correlation. Statistical tests were performed as described by Campbell (1975). A value of $p < 0.05$ was taken to indicate statistical significance.
Figure 9

Standard curve for oestradiol

I indicates range

$\frac{B}{B_0}$

pg/mL
Figure 10
Standard curve for cyclic AMP
CHAPTER THREE

SOME BLOOD LIPID MEASUREMENTS IN PATIENTS SUFFERING FROM DEPRESSIVE ILLNESS AND IN PATIENTS WITH OTHER PSYCHIATRIC DISORDERS

Introduction

Erythrocyte membranes contain lipids (mainly cholesterol and phospholipids), proteins and carbohydrates. The latter are minor components and are usually bound to lipids, forming glycolipids or to proteins, forming glycoproteins. The lipid components are responsible for the structural integrity of the membrane (Lodish and Rothman, 1979). Each lipid molecule is amphipathic, that is one part is hydrophilic and another part is hydrophobic. The fatty acid substituents of the phospholipids are hydrophobic whereas the region containing the phosphate and amino groups is hydrophilic. The hydrophilic group in cholesterol is the hydroxyl group. Because the two parts of the molecule have incompatible solubilities the membrane is thought to be organised in the form of a bilayer, the two hydrophilic surfaces separated by a hydrophobic core (see figure 11). The bilayer is not static: the molecules are free to diffuse laterally within each monolayer but movement from one monolayer to the other (flip-flop) is rare (Lodish and Rothman, 1979). This concept of membrane structure was proposed by Danielli and Davson (1935) and has been both criticised (Korn, 1956) and defended (Stoekenius and Engelman, 1969). The theory is therefore viewed as possible but not established.

Alterations in the composition of the membrane affect its function. Changes in the fatty acid composition of membrane lipids affect the permeability of the membrane (van Deenan, 1971; McElhaney, 1974; Lenaz, 1979) and the activity of certain membrane-bound enzymes (Bernsohn and Spitz, 1974; Sun and Sun, 1976; Lenaz, 1979). The relative amounts of the
Figure 11
Structure of Membrane

Carbohydrate
Integral Protein
Lipid bilayer
Phospholipid
Cholesterol

Hydrophilic Region
Hydrophobic Region
Integral Protein
Peripheral Protein

CYTOPLASM

Taken from Lodish and Rothman (1979).
various phospholipids can also influence the permeability of the membrane (Cooper et al., 1977) and affect the activity of some membrane-bound enzymes (Kimmelberg and Papahadjopoulos, 1972; Coleman, 1973; Cooper et al., 1977). The ratio of cholesterol:phospholipid is another important determinant of membrane function (Cooper, 1970; Vanderkooi et al., 1974; Cooper et al., 1975; Shinitzky and Inbar, 1976; Cooper, 1978; Cooper et al., 1978; Lenaz, 1979). These factors were investigated in patients suffering from depressive illness and in those suffering from other psychiatric disorders.

**Patients**

Twenty-six patients suffering from endogenous depression, twenty-three patients suffering from reactive depression, six patients suffering from postpartum depression, eleven patients suffering from other psychiatric disorders and age-sex-matched apparently healthy controls were studied. Of the patients suffering from endogenous depression, seven were bipolar and nineteen were unipolar. The mean age was 52 years, ranging from 21 to 74 years. The mean age of patients with reactive depression was 38 years, ranging from 22 to 65 years. Of the patients suffering from postpartum depression, three had given birth to their first baby and three to their second baby; the mean length of time postpartum was 20 weeks, ranging from 8 to 32 weeks. The mean age of these patients was 30 years, ranging from 25 to 34 years. Of the patients with other psychiatric disorders, six were schizophrenics and five had personality disorders. The mean age was 35 years, ranging from 19 to 59 years. The ages of the controls were ±5 years those of the patients, although most were ±2 years. Patients suffering from postpartum depression were also matched for parity and length of time postpartum. All the patients and controls were caucasian.
Diagnosis of endogenous or reactive depression was based on the clinical interview: patients of doubtful classification were excluded from the study. The severity of depression was assessed by the Beck Inventory (Beck et al., 1961).

Fourteen of the patients suffering from endogenous depression (54 percent), fifteen of the patients suffering from reactive depression (65 percent), four of the patients suffering from postpartum depression (67 percent) and five of the other psychiatric patients (46 percent) were drug-free for at least two weeks before the study. The remainder were receiving a hypnotic and/or a tranquilliser. Two of the schizophrenic patients were receiving a neuroleptic. There was no difference in the phospholipid fatty acid composition of patients taking these drugs compared with drug-free patients in the same group.

RESULTS

(a) Studies of Patients Suffering from Endogenous Depression, Reactive Depression and Other Psychiatric Disorders

The plasma CPG of patients suffering from endogenous depression (Table 5) contained a higher proportion of LCP \( p < 0.001 \), in particular \( 22:6 \omega 3 \) \( p < 0.001 \) and \( 20:5 \omega 3 \) \( p < 0.05 \) and a reduced proportion of \( 18:2 \omega 6 \) \( p < 0.001 \) compared with those of age-sex-matched controls. The plasma CPG fatty acid composition of patients with reactive depression (Table 6) and of those with other psychiatric disorders (Table 7) did not differ from those of matched controls.

Of the patients suffering from endogenous depression, 81 percent had a \( 22:6 \omega 3 \) concentration of more than 54 mg/g total fatty acid methyl esters detected compared with only 19 percent of matched controls and a

* See Appendix
similar proportion of patients with reactive depression or with other psychiatric disorders (Table 8). Regarding the total LCP, 69 percent of the patients suffering from endogenous depression had a concentration of more than 244 mg/g total fatty acid methyl esters detected (Table 8) compared with only about 20 percent of matched controls and of patients in other groups.

In the endogenous group, six of the patients were referred from general practitioners before beginning treatment. These patients had similar LCP concentrations to those of in-patients suffering from endogenous depression (Table 9), indicating that the abnormality was present in the early stages of the illness.

The Beck Inventory scores of fifteen patients suffering from endogenous depression and of fifteen patients suffering from reactive depression (Figure 12) revealed that the severity of depression was similar in both groups. The 22:6ω3 concentrations in the plasma CPG of patients with endogenous depression correlated with the severity of the illness (Figure 13, $r = 0.80, p < 0.01$) whereas those of patients with reactive depression did not.

There were no significant differences in the total plasma CPG between any of the groups (Figure 14).

Total plasma cholesterol concentrations were lower in patients suffering from endogenous depression than in age-sex-matched controls (Table 10, $p < 0.05$). Patients suffering from reactive depression or from other psychiatric disorders did not differ from matched controls. The percentages of free and esterified cholesterol were similar in all groups (Table 10).

Changes in the fatty acid composition of the erythrocyte
**TABLE 5**

The fatty acid composition of the plasma choline phosphoglycerides of patients suffering from endogenous depression

Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters detected.

Figures in parenthesis indicate the number of subjects studied.

<table>
<thead>
<tr>
<th>Methyl Esters Endogenous Depressives</th>
<th>Age-sex-matched Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(26)</td>
</tr>
<tr>
<td>16:0</td>
<td>265 ± 4.5</td>
</tr>
<tr>
<td>18:0</td>
<td>136 ± 3.6</td>
</tr>
<tr>
<td>18:1ω9</td>
<td>134 ± 3.4</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>199 ± 7.3***</td>
</tr>
<tr>
<td>20:0</td>
<td>4 ± 0.4</td>
</tr>
<tr>
<td>18:3ω3 + 20:1ω9</td>
<td>5 ± 0.3</td>
</tr>
<tr>
<td>20:2ω6</td>
<td>4 ± 0.3</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>34 ± 1.4</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>112 ± 4.9</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>20 ± 1.0*</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>3 ± 0.3</td>
</tr>
<tr>
<td>22:5ω6</td>
<td>3 ± 0.2</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>16 ± 0.8</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>64 ± 2.5***</td>
</tr>
<tr>
<td>Total LCP</td>
<td>260 ± 7.2***</td>
</tr>
</tbody>
</table>

* \( p < 0.05 \)

**\( p < 0.001 \)

*Statistical test employed: paired sample t-test.*
## TABLE 6

The fatty acid composition of the plasma choline phosphoglycerides of patients suffering from reactive depression

Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters detected.

Figures in parenthesis indicate the number of subjects studied.

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Reactive Depressives (23)</th>
<th>Age-sex-matched Controls (23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>262 ± 4.7</td>
<td>258 ± 4.0</td>
</tr>
<tr>
<td>18:0</td>
<td>138 ± 3.7</td>
<td>142 ± 3.4</td>
</tr>
<tr>
<td>18:1ω9</td>
<td>135 ± 4.3</td>
<td>132 ± 4.6</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>236 ± 6.3</td>
<td>242 ± 6.0</td>
</tr>
<tr>
<td>20:0</td>
<td>3 ± 0.4</td>
<td>2 ± 0.2</td>
</tr>
<tr>
<td>18:3ω3 + 20:1ω9</td>
<td>5 ± 0.4</td>
<td>5 ± 0.3</td>
</tr>
<tr>
<td>20:2ω6</td>
<td>4 ± 0.3</td>
<td>4 ± 0.2</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>33 ± 1.3</td>
<td>32 ± 1.0</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>106 ± 3.2</td>
<td>102 ± 3.5</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>14 ± 1.0</td>
<td>14 ± 0.8</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>4 ± 0.3</td>
<td>3 ± 0.2</td>
</tr>
<tr>
<td>22:5ω6</td>
<td>3 ± 0.2</td>
<td>3 ± 0.2</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>14 ± 0.7</td>
<td>13 ± 0.6</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>43 ± 1.6</td>
<td>45 ± 1.9</td>
</tr>
</tbody>
</table>

Total LCP       | 224 ± 6.1                 | 218 ± 6.1                     |

There are no significant differences between the two groups.

Statistical test employed: paired sample t-test.
TABLE 7

The fatty acid composition of the plasma choline phosphoglycerides of patients suffering from non-depressive psychiatric disorders

Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters detected.

Figures in parenthesis indicate the number of subjects studied.

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Non-depressive Psychiatric Patients</th>
<th>Age-sex-matched Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(11)</td>
<td>(11)</td>
</tr>
<tr>
<td>16:0</td>
<td>260 ± 3.2</td>
<td>262 ± 4.0</td>
</tr>
<tr>
<td>18:0</td>
<td>143 ± 4.6</td>
<td>137 ± 5.5</td>
</tr>
<tr>
<td>18:1ω9</td>
<td>133 ± 3.8</td>
<td>131 ± 4.6</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>240 ± 6.4</td>
<td>245 ± 6.5</td>
</tr>
<tr>
<td>20:0</td>
<td>4 ± 0.3</td>
<td>3 ± 0.3</td>
</tr>
<tr>
<td>18:3ω3 + 20:1ω9</td>
<td>4 ± 0.3</td>
<td>4 ± 0.3</td>
</tr>
<tr>
<td>20:2ω6</td>
<td>5 ± 0.4</td>
<td>4 ± 0.2</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>33 ± 2.7</td>
<td>32 ± 1.2</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>99 ± 3.4</td>
<td>101 ± 3.1</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>14 ± 1.4</td>
<td>14 ± 1.1</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>4 ± 0.3</td>
<td>4 ± 0.2</td>
</tr>
<tr>
<td>22:5ω6</td>
<td>3 ± 0.3</td>
<td>3 ± 0.2</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>14 ± 0.8</td>
<td>14 ± 0.7</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>42 ± 2.8</td>
<td>44 ± 2.5</td>
</tr>
</tbody>
</table>

Total LCP             | 218 ± 6.6                         | 219 ± 4.9                |

There are no significant differences between the two groups.

Statistical test employed: paired sample t-test.
Comparison of the frequency of the observed differences in the plasma choline phosphoglyceride fatty acid composition of patients with endogenous depression, reactive depression, other psychiatric disorders and controls

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Number of Endogenous Depressives</th>
<th>Number of Reactive Depressives</th>
<th>Number of Other Psychiatric Patients</th>
<th>Number of Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2ω6 less than 220 mg/g</td>
<td>19(73%)*</td>
<td>6(26%)</td>
<td>3(27%)</td>
<td>8(31%)</td>
</tr>
<tr>
<td>20:5ω3 greater than 18 mg/g</td>
<td>10(39%)*</td>
<td>2(9%)</td>
<td>1(9%)</td>
<td>2(8%)</td>
</tr>
<tr>
<td>22:6ω3 greater than 54 mg/g</td>
<td>21(81%)*</td>
<td>4(17%)</td>
<td>2(18%)</td>
<td>5(19%)</td>
</tr>
<tr>
<td>Total LCP greater than 244 mg/g</td>
<td>18(69%)*</td>
<td>5(22%)</td>
<td>2(18%)</td>
<td>5(19%)</td>
</tr>
</tbody>
</table>

The controls are age-sex-matched to the patients with endogenous depression.

* p < 0.001

Statistical test employed: χ² 2x2 contingency table.
TABLE 9
The fatty acid composition of the plasma choline phosphoglycerides of patients with early symptoms of endogenous depression compared with that of age-sex-matched in-patients suffering from endogenous depression and age-sex-matched controls.

Figures in parenthesis indicate the number of subjects studied.
Results expressed as mean ± S.E. in mg/g total fatty acid methyl esters detected.

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Patients with early symptoms of endogenous depression (6)</th>
<th>In-patients with endogenous depression (6)</th>
<th>Age-sex-matched Controls (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>251 ± 4.0</td>
<td>263 ± 4.6</td>
<td>249 ± 5.0</td>
</tr>
<tr>
<td>18:0</td>
<td>136 ± 2.2</td>
<td>137 ± 3.6</td>
<td>144 ± 7.1</td>
</tr>
<tr>
<td>18:1ω9</td>
<td>133 ± 6.1</td>
<td>131 ± 6.5</td>
<td>138 ± 8.6</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>218 ± 10.3**</td>
<td>193 ± 8.6**</td>
<td>250 ± 14.1</td>
</tr>
<tr>
<td>20:0</td>
<td>3 ± 0.4</td>
<td>4 ± 0.3</td>
<td>2 ± 0.5</td>
</tr>
<tr>
<td>18:3ω3 + 20:1ω9</td>
<td>3 ± 0.4</td>
<td>5 ± 0.5</td>
<td>3 ± 0.4</td>
</tr>
<tr>
<td>20:2ω6</td>
<td>3 ± 0.3</td>
<td>4 ± 0.4</td>
<td>3 ± 0.4</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>33 ± 1.5</td>
<td>32 ± 4.4</td>
<td>34 ± 2.1</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>107 ± 2.8</td>
<td>115 ± 8.4</td>
<td>98 ± 5.1</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>19 ± 2.4</td>
<td>21 ± 1.5*</td>
<td>14 ± 1.0</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>4 ± 0.3</td>
<td>3 ± 0.4</td>
<td>4 ± 0.3</td>
</tr>
<tr>
<td>22:5ω6</td>
<td>4 ± 0.3</td>
<td>4 ± 0.4</td>
<td>4 ± 0.3</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>18 ± 1.2***</td>
<td>17 ± 1.5***</td>
<td>14 ± 1.2</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>67 ± 4.3**</td>
<td>70 ± 4.3**</td>
<td>42 ± 1.9</td>
</tr>
<tr>
<td>Total LCP</td>
<td>258 ± 6.1***</td>
<td>270 ± 10.8***</td>
<td>215 ± 7.2</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01; *** p < 0.001 compared to control group.

Statistical test employed: paired sample t-test.
A comparison of the Beck Inventory scores of 15 patients suffering from endogenous depression with those of 15 patients suffering from reactive depression.
Figure 13
Plot of the 22:6ω3 content of the plasma choline phosphoglycerides of patients suffering from depressive illness against their Beck Inventory scores

\[ y = 1.26x + 35.19 \]
\[ r = 0.80 \]
\[ p < 0.01 \]
phospholipids were in the same direction as those of the plasma but were less marked. The erythrocyte EPG of patients with endogenous depression (Table 11) contained significantly higher levels of LCP (p < 0.05), in particular 22:6w3 (p < 0.05), than those of matched controls. Patients with reactive depression and those with other psychiatric disorders had similar concentrations to matched controls.

The erythrocyte SPG of patients with endogenous depression (Table 12) contained significantly higher levels of 20:5w3 (p < 0.05) than those of matched controls. Again, patients with reactive depression and those with other psychiatric disorders did not differ from matched controls.

In the erythrocyte CPG (Table 13) the concentration of 18:2w6 was lower in patients with endogenous depression than in matched controls (p < 0.05). As above, patients with reactive depression and those with other psychiatric disorders did not differ from matched controls. The fatty acid composition of the erythrocyte sphingomyelin was similar in all groups (Table 14).

Control values for both the plasma and erythrocyte phospholipid fatty acids were similar to those reported by other workers (Phillips and Dodge, 1967; Dodge and Phillips, 1967; Sanders, 1977). The results of the determination of the relative proportions of erythrocyte phospholipids revealed that there were no significant differences between patients with endogenous depression, patients with reactive depression, patients with other psychiatric disorders and controls (Table 15). Similarly, there was no difference in the cholesterol:phospholipid ratio between any of the groups (Table 16).

The results of the haematological investigations are shown in Table 17. Patients with endogenous depression (p < 0.05) and those with

*The fatty acid composition of erythrocyte phospholipids of patients with reactive depression and of patients with other psychiatric disorders are compared with those of age-sex-matched controls in the Appendix.
Figure 1

Total plasma choline phosphoglycerides

---

- Mean

- Endogenous Depressives
- Reactive Depressives
- Other Psychiatric Patients
- Controls
TABLE 10
Plasma cholesterol concentrations of psychiatric patients

Results expressed as mean values ±S.E.

<table>
<thead>
<tr>
<th></th>
<th>TOTAL (mmol/l)</th>
<th>FREE (%)</th>
<th>ESTERIFIED (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENDODGENOUS DEPRESSIVES (26)</td>
<td>4.4±0.2 *</td>
<td>26.2±1.5</td>
<td>73.8±1.5</td>
</tr>
<tr>
<td>REACTIVE DEPRESSIVES (23)</td>
<td>5.2±0.2</td>
<td>25.7±1.5</td>
<td>74.3±1.5</td>
</tr>
<tr>
<td>OTHER PSYCHIATRIC PATIENTS (11)</td>
<td>5.6±0.4</td>
<td>25.8±2.0</td>
<td>74.2±2.0</td>
</tr>
<tr>
<td>CONTROLS (26)</td>
<td>5.8±0.2</td>
<td>25.0±2.4</td>
<td>75.0±2.4</td>
</tr>
</tbody>
</table>

Figures in parenthesis indicate the number of subjects on whom the investigation was carried out.

The control group shown are age-sex-matched to the endogenous depressives.

* Significant difference from controls (p < 0.05).

Statistical test employed: Wilcoxon's test for pair differences.
TABLE 11

The fatty acid and aldehyde composition of the erythrocyte ethanolamine phosphoglycerides

Figures in parenthesis indicate the number of subjects on whom the investigation was carried out.

Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters and dimethyl acetalts detected.

<table>
<thead>
<tr>
<th>Methyl Esters and Dimethyl Acetals (DMA)</th>
<th>Endogenous Depressives (26)</th>
<th>Reactive Depressives (23)</th>
<th>Other psychiatric patients (11)</th>
<th>Controls (26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 + 16:0 DMA</td>
<td>135 ± 4.9</td>
<td>144 ± 4.7</td>
<td>144 ± 6.4</td>
<td>142 ± 5.8</td>
</tr>
<tr>
<td>18:0 + 18:0 DMA</td>
<td>107 ± 6.7</td>
<td>111 ± 5.3</td>
<td>114 ± 7.2</td>
<td>114 ± 7.5</td>
</tr>
<tr>
<td>18:1ω9 + 18:1 DMA</td>
<td>141 ± 3.4</td>
<td>143 ± 3.6</td>
<td>138 ± 3.6</td>
<td>138 ± 4.8</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>65 ± 3.6</td>
<td>69 ± 3.4</td>
<td>69 ± 5.0</td>
<td>69 ± 4.1</td>
</tr>
<tr>
<td>20:0</td>
<td>4 ± 0.4</td>
<td>4 ± 0.4</td>
<td>5 ± 0.4</td>
<td>3 ± 0.4</td>
</tr>
<tr>
<td>18:3ω3 + 20:1ω9</td>
<td>3 ± 0.4</td>
<td>4 ± 0.4</td>
<td>4 ± 0.3</td>
<td>4 ± 0.3</td>
</tr>
<tr>
<td>20:2ω6</td>
<td>3 ± 0.3</td>
<td>4 ± 0.4</td>
<td>4 ± 0.3</td>
<td>3 ± 0.4</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>14 ± 1.1</td>
<td>14 ± 1.0</td>
<td>15 ± 1.5</td>
<td>14 ± 1.0</td>
</tr>
<tr>
<td>20:4ω5</td>
<td>255 ± 5.3</td>
<td>259 ± 5.7</td>
<td>260 ± 8.0</td>
<td>246 ± 6.6</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>21 ± 2.0</td>
<td>19 ± 1.4</td>
<td>17 ± 2.0</td>
<td>17 ± 1.5</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>69 ± 3.6</td>
<td>62 ± 4.0</td>
<td>63 ± 5.0</td>
<td>63 ± 2.2</td>
</tr>
<tr>
<td>22:5ω6</td>
<td>6 ± 0.4</td>
<td>8 ± 0.8</td>
<td>6 ± 0.9</td>
<td>6 ± 0.7</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>64 ± 3.0*</td>
<td>62 ± 3.5</td>
<td>58 ± 4.0</td>
<td>62 ± 2.9</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>113 ± 2.1</td>
<td>90 ± 3.1</td>
<td>95 ± 6.5</td>
<td>99 ± 3.3</td>
</tr>
</tbody>
</table>

Total LCP 549 ± 10.6* 522 ± 13.0 523 ± 18.9 573 ± 12.2

* p < 0.05. Statistical test employed: paired sample t-test.

Control group shown are age-sex-matched to the endogenous group.
**TABLE 12**

The fatty acid and aldehyde composition of the erythrocyte serine phosphoglycerides

Figures in parenthesis indicate the number of subjects on whom the investigation was carried out. Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters and dimethyl acetal detected.

<table>
<thead>
<tr>
<th>Methyl Esters and Dimethyl Acetals (DMA)</th>
<th>Endogenous Depressives (26)</th>
<th>Reactive Depressives (23)</th>
<th>Other Psychiatric Patients (11)</th>
<th>Controls (26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 + 16:0 DMA</td>
<td>65 ± 5.8</td>
<td>72 ± 6.9</td>
<td>70 ± 7.0</td>
<td>68 ± 5.9</td>
</tr>
<tr>
<td>18:0 + 18:0 DMA</td>
<td>302 ± 6.9</td>
<td>304 ± 6.2</td>
<td>305 ± 6.9</td>
<td>304 ± 6.4</td>
</tr>
<tr>
<td>18:1ω9 + 18:1 DMA</td>
<td>99 ± 3.5</td>
<td>95 ± 5.4</td>
<td>100 ± 7.6</td>
<td>98 ± 2.1</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>35 ± 3.5</td>
<td>40 ± 3.8</td>
<td>37 ± 4.2</td>
<td>37 ± 4.4</td>
</tr>
<tr>
<td>20:0</td>
<td>3 ± 0.3</td>
<td>3 ± 0.3</td>
<td>3 ± 0.3</td>
<td>4 ± 0.4</td>
</tr>
<tr>
<td>18:3ω3 + 20:1ω9</td>
<td>3 ± 0.2</td>
<td>5 ± 0.5</td>
<td>3 ± 0.2</td>
<td>4 ± 0.3</td>
</tr>
<tr>
<td>20:2ω6</td>
<td>3 ± 0.2</td>
<td>4 ± 0.5</td>
<td>3 ± 0.4</td>
<td>4 ± 0.4</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>18 ± 1.2</td>
<td>19 ± 1.5</td>
<td>19 ± 0.6</td>
<td>20 ± 1.2</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>260 ± 5.9</td>
<td>250 ± 6.8</td>
<td>259 ± 4.9</td>
<td>255 ± 6.7</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>15 ± 1.5*</td>
<td>11 ± 0.8</td>
<td>9 ± 0.9</td>
<td>10 ± 0.6</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>36 ± 2.1</td>
<td>37 ± 2.1</td>
<td>39 ± 4.7</td>
<td>38 ± 2.6</td>
</tr>
<tr>
<td>22:5ω6</td>
<td>9 ± 0.5</td>
<td>9 ± 0.6</td>
<td>8 ± 0.6</td>
<td>9 ± 0.8</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>47 ± 2.1</td>
<td>48 ± 2.2</td>
<td>46 ± 3.2</td>
<td>44 ± 2.0</td>
</tr>
<tr>
<td>Total LCP</td>
<td>504 ± 5.8</td>
<td>481 ± 7.6</td>
<td>481 ± 8.1</td>
<td>488 ± 11.4</td>
</tr>
</tbody>
</table>

* p < 0.05.

Statistical test employed: paired sample t-test.

Control group shown are age-sex-matched to the endogenous group.
TABLE 13
The fatty acid and aldehyde composition of the erythrocyte choline phosphoglycerides

Figures in parenthesis indicate the number of subjects on whom the investigation was carried out.
Results expressed as mean ± S.E. in mg/g total fatty acid methyl esters and dimethyl acetalts detected.

<table>
<thead>
<tr>
<th>Methyl Esters and Dimethyl Acetals (DMA)</th>
<th>Endogenous Depressives (26)</th>
<th>Reactive Depressives (23)</th>
<th>Other Psychiatric Patients (11)</th>
<th>Controls (26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 + 16:0 DMA</td>
<td>271 ± 3.3</td>
<td>266 ± 8.7</td>
<td>270 ± 8.5</td>
<td>268 ± 6.9</td>
</tr>
<tr>
<td>18:0 + 18:0 DMA</td>
<td>140 ± 5.7</td>
<td>137 ± 5.4</td>
<td>140 ± 5.2</td>
<td>139 ± 3.6</td>
</tr>
<tr>
<td>18:1ω9 + 18:1 DMA</td>
<td>190 ± 5.7</td>
<td>185 ± 4.4</td>
<td>180 ± 4.8</td>
<td>179 ± 4.4</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>172 ± 8.0*</td>
<td>205 ± 8.4</td>
<td>203 ± 7.6</td>
<td>200 ± 8.5</td>
</tr>
<tr>
<td>20:0</td>
<td>3 ± 0.3</td>
<td>3 ± 0.4</td>
<td>2 ± 0.3</td>
<td>3 ± 0.4</td>
</tr>
<tr>
<td>18:3ω3 + 20:1ω9</td>
<td>5 ± 0.2</td>
<td>5 ± 0.4</td>
<td>4 ± 0.3</td>
<td>4 ± 0.3</td>
</tr>
<tr>
<td>20:2ω6</td>
<td>5 ± 0.4</td>
<td>5 ± 0.4</td>
<td>4 ± 0.3</td>
<td>4 ± 0.3</td>
</tr>
<tr>
<td>20:3ω5</td>
<td>23 ± 0.8</td>
<td>26 ± 1.4</td>
<td>25 ± 1.5</td>
<td>24 ± 1.4</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>102 ± 3.6</td>
<td>99 ± 3.8</td>
<td>98 ± 5.1</td>
<td>102 ± 4.2</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>15 ± 0.9</td>
<td>11 ± 0.9</td>
<td>12 ± 1.2</td>
<td>12 ± 1.0</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>6 ± 0.3</td>
<td>6 ± 0.7</td>
<td>6 ± 0.5</td>
<td>6 ± 0.6</td>
</tr>
<tr>
<td>22:5ω5</td>
<td>3 ± 0.3</td>
<td>3 ± 0.5</td>
<td>5 ± 0.4</td>
<td>4 ± 0.4</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>15 ± 0.7</td>
<td>12 ± 0.8</td>
<td>12 ± 1.5</td>
<td>14 ± 1.3</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>47 ± 1.9</td>
<td>35 ± 1.7</td>
<td>37 ± 3.2</td>
<td>40 ± 2.7</td>
</tr>
<tr>
<td>Total LCP</td>
<td>219 ± 5.9</td>
<td>200 ± 8.9</td>
<td>201 ± 9.2</td>
<td>209 ± 7.8</td>
</tr>
</tbody>
</table>

* p < 0.05.

Statistical test employed: paired sample t-test.
Control group shown are age-sex-matched to the endogenous group.
TABLE 14

The fatty acid composition of the erythrocyte sphingomyelin

*Figures in parenthesis indicate the number of subjects on whom the investigation was carried out.*

Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters detected.

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Endogenous Depressives (26)</th>
<th>Reactive Depressives (23)</th>
<th>Other Psychiatric Patients (11)</th>
<th>Controls (26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>238 ± 5.6</td>
<td>229 ± 5.3</td>
<td>230 ± 6.5</td>
<td>232 ± 6.4</td>
</tr>
<tr>
<td>18:0</td>
<td>54 ± 4.2</td>
<td>59 ± 4.1</td>
<td>48 ± 4.9</td>
<td>49 ± 4.4</td>
</tr>
<tr>
<td>20:0</td>
<td>19 ± 2.0</td>
<td>18 ± 1.9</td>
<td>15 ± 1.8</td>
<td>21 ± 1.6</td>
</tr>
<tr>
<td>22:0</td>
<td>95 ± 5.8</td>
<td>104 ± 5.2</td>
<td>94 ± 4.9</td>
<td>99 ± 6.3</td>
</tr>
<tr>
<td>23:0 + 20:4ω6</td>
<td>34 ± 2.5</td>
<td>36 ± 2.9</td>
<td>30 ± 3.1</td>
<td>31 ± 2.2</td>
</tr>
<tr>
<td>24:0</td>
<td>231 ± 4.9</td>
<td>233 ± 5.3</td>
<td>238 ± 5.6</td>
<td>229 ± 6.1</td>
</tr>
<tr>
<td>24:1ω9 + 20:5ω3</td>
<td>253 ± 5.4</td>
<td>259 ± 4.8</td>
<td>247 ± 5.0</td>
<td>260 ± 5.7</td>
</tr>
<tr>
<td>24:2ω6</td>
<td>35 ± 1.9</td>
<td>32 ± 2.3</td>
<td>37 ± 2.5</td>
<td>31 ± 2.8</td>
</tr>
</tbody>
</table>

*There are no significant differences between any of the groups and matched controls.*

*Statistical test employed: paired sample t-test.*

*Control group shown are age-sex-matched to the endogenous group.*
### TABLE 15

The relative proportions of erythrocyte phospholipids

Results expressed as % of the total phospholipids (mean ± S.E.).

<table>
<thead>
<tr>
<th></th>
<th>Ethanolamine Phosphoglycerides</th>
<th>Serine Phosphoglycerides</th>
<th>Choline Phosphoglycerides</th>
<th>Sphingomyelin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endogenous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressives (21)</td>
<td>27.2 ± 0.7</td>
<td>17.4 ± 1.0</td>
<td>28.7 ± 0.9</td>
<td>26.7 ± 1.0</td>
</tr>
<tr>
<td>Reactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressives (20)</td>
<td>26.7 ± 1.0</td>
<td>15.7 ± 1.1</td>
<td>31.2 ± 1.0</td>
<td>26.4 ± 1.1</td>
</tr>
<tr>
<td>Other Psychiatric</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients (9)</td>
<td>25.2 ± 1.0</td>
<td>14.2 ± 1.3</td>
<td>33.4 ± 0.9</td>
<td>27.2 ± 1.0</td>
</tr>
<tr>
<td>Controls (21)</td>
<td>25.8 ± 0.7</td>
<td>17.3 ± 0.9</td>
<td>30.5 ± 0.8</td>
<td>26.4 ± 0.5</td>
</tr>
</tbody>
</table>

Figures in parenthesis indicate the number of subjects on whom the investigation was carried out.

There are no significant differences between any of the groups.

Statistical test employed: student t-test.
**TABLE 16**

**Erythrocyte lipid phosphorus and free cholesterol concentrations**

*Results expressed as mean ±S.E. in mmol/ml packed cells.*

<table>
<thead>
<tr>
<th></th>
<th>Free Cholesterol</th>
<th>Lipid Phosphorus</th>
<th>Molar Ratio Cholesterol:Phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endogenous Depressives (21)</td>
<td>3.84 ± 0.15</td>
<td>4.02 ± 0.13</td>
<td>0.96 ± 0.02</td>
</tr>
<tr>
<td>Reactive Depressives (20)</td>
<td>3.90 ± 0.13</td>
<td>4.00 ± 0.14</td>
<td>0.98 ± 0.02</td>
</tr>
<tr>
<td>Other Psychiatric Patients (9)</td>
<td>3.95 ± 0.20</td>
<td>3.96 ± 0.18</td>
<td>1.00 ± 0.04</td>
</tr>
<tr>
<td>Controls (21)</td>
<td>3.97 ± 0.10</td>
<td>3.93 ± 0.12</td>
<td>1.01 ± 0.02</td>
</tr>
</tbody>
</table>

*Figures in parenthesis indicate the number of subjects on whom the investigation was carried out.*

*There are no significant differences between any of the groups.*

*Statistical test employed: student t-test.*
### TABLE 17

**Haematological investigations of female patients with endogenous depression**

**Female patients with reactive depression and female controls**

*Results expressed as mean values ±S.E.*

<table>
<thead>
<tr>
<th></th>
<th>White blood cell count</th>
<th>Red blood cell count</th>
<th>Haemoglobin concn in dl</th>
<th>Packed cell volume</th>
<th>Mean corpuscular volume in f1</th>
<th>Mean corpuscular haemoglobin in pg</th>
<th>Mean corpuscular haemoglobin concn in g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endogenous Depressives (20)</strong></td>
<td>6.81 ± 0.48</td>
<td>4.68 ± 0.10</td>
<td>14.4 ± 0.3</td>
<td>0.43 ± 0.009</td>
<td>93 ± 1.1</td>
<td>30.9 ± 0.3</td>
<td>33.6 ± 0.2</td>
</tr>
<tr>
<td><strong>Controls (20)</strong></td>
<td>6.76 ± 0.41</td>
<td>4.70 ± 0.09</td>
<td>14.1 ± 0.2</td>
<td>0.42 ± 0.006</td>
<td>89 ± 1.2</td>
<td>29.9 ± 0.5</td>
<td>33.4 ± 0.3</td>
</tr>
<tr>
<td><strong>Reactive Depressives (20)</strong></td>
<td>8.08 ± 0.56</td>
<td>4.72 ± 0.09</td>
<td>14.6 ± 0.3</td>
<td>0.43 ± 0.009</td>
<td>93 ± 1.2</td>
<td>31.3 ± 0.3</td>
<td>33.6 ± 0.3</td>
</tr>
</tbody>
</table>

*Figures in parenthesis indicate the number of subjects studied.*

*Statistically significant, p < 0.05 compared to control value.*

*Statistical test employed: student t-test.*
reactive depression \( (p < 0.05) \) were found to have greater mean corpuscular volumes (MCV) than controls. The mean corpuscular haemoglobin (MCH) was also greater in patients with reactive depression \( (p < 0.05) \) but did not reach significance in the endogenous group. No abnormalities in the shape of the erythrocytes were detected visually in any of the groups when blood films were examined.

(b) The Fatty Acid Composition of the Plasma CPG and Erythrocyte Phospholipids of Patients Suffering From Postpartum Depression

The plasma CPG of patients suffering from postpartum depression (Table 18) contained a higher proportion of LCP \( (p < 0.05) \), in particular 22:6w3 \( (p < 0.05) \), compared to those of postpartum subjects who were not depressed. In addition, the concentration of 18:2w6 was lower in the depressed group \( (p < 0.05) \).

The fatty acid and aldehyde composition of the erythrocyte EPG, SPG and CPG are shown in Table 19. There were no significant differences between the two groups. Similarly, there were no differences in the fatty acid composition of the sphingomyelin fraction (Table 20). These findings may be due to the small number of patients available for the study.
TABLE 18
The fatty acid composition of the plasma choline phosphoglycerides
of patients suffering from postpartum depression

Figures in parenthesis indicate the number of subjects studied.
Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters detected.

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Patients (6)</th>
<th>Matched Controls (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>264 ± 7.9</td>
<td>266 ± 6.7</td>
</tr>
<tr>
<td>18:0</td>
<td>137 ± 6.8</td>
<td>140 ± 7.1</td>
</tr>
<tr>
<td>18:1ω9</td>
<td>124 ± 4.2</td>
<td>130 ± 7.6</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>223 ± 8.2*</td>
<td>259 ± 8.7</td>
</tr>
<tr>
<td>20:0</td>
<td>3 ± 0.4</td>
<td>3 ± 0.3</td>
</tr>
<tr>
<td>18:3ω3 + 20:1ω9</td>
<td>4 ± 0.3</td>
<td>3 ± 0.4</td>
</tr>
<tr>
<td>20:2ω6</td>
<td>4 ± 0.3</td>
<td>2 ± 0.5</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>34 ± 4.6</td>
<td>27 ± 1.7</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>114 ± 6.1</td>
<td>99 ± 7.6</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>16 ± 1.3</td>
<td>15 ± 1.6</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>3 ± 0.4</td>
<td>3 ± 0.4</td>
</tr>
<tr>
<td>22:5ω6</td>
<td>3 ± 0.4</td>
<td>3 ± 0.4</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>14 ± 1.7*</td>
<td>11 ± 1.0</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>56 ± 4.6*</td>
<td>39 ± 3.3</td>
</tr>
<tr>
<td>Total LCP</td>
<td>247 ± 11.8*</td>
<td>202 ± 12.8</td>
</tr>
</tbody>
</table>

*p < 0.05

Statistical test employed: paired sample t-test.
### TABLE 19

The fatty acid and aldehyde composition of the erythrocyte phosphoglycerides of patients suffering from postpartum depression

Figures in parenthesis indicate the number of subjects studied.

Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters and dimethyl acetal detected.

<table>
<thead>
<tr>
<th>Methyl Esters and Dimethyl Acetals (DMA)</th>
<th>Ethanolamine Phosphoglycerides</th>
<th>Serine Phosphoglycerides</th>
<th>Choline Phosphoglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 + 16:0 DMA</td>
<td>144 ± 7.2</td>
<td>151 ± 6.9</td>
<td>81 ± 8.5</td>
</tr>
<tr>
<td>18:0 + 18:0 DMA</td>
<td>106 ± 6.9</td>
<td>110 ± 6.7</td>
<td>307 ± 8.5</td>
</tr>
<tr>
<td>18:1 + 18:1 DMA</td>
<td>147 ± 7.4</td>
<td>150 ± 6.2</td>
<td>105 ± 7.7</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>58 ± 4.0</td>
<td>68 ± 3.8</td>
<td>29 ± 4.0</td>
</tr>
<tr>
<td>20:0</td>
<td>4 ± 0.4</td>
<td>4 ± 0.4</td>
<td>4 ± 0.5</td>
</tr>
<tr>
<td>18:3ω3 + 20:1ω9</td>
<td>3 ± 0.4</td>
<td>4 ± 0.4</td>
<td>3 ± 0.5</td>
</tr>
<tr>
<td>20:2ω6</td>
<td>4 ± 0.4</td>
<td>5 ± 0.4</td>
<td>4 ± 0.4</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>15 ± 1.2</td>
<td>14 ± 1.1</td>
<td>17 ± 1.5</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>259 ± 8.2</td>
<td>248 ± 7.5</td>
<td>257 ± 8.5</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>16 ± 2.9</td>
<td>15 ± 2.4</td>
<td>13 ± 1.9</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>65 ± 4.5</td>
<td>68 ± 4.1</td>
<td>39 ± 2.4</td>
</tr>
<tr>
<td>22:5ω5</td>
<td>6 ± 0.7</td>
<td>9 ± 0.5</td>
<td>9 ± 2.0</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>65 ± 7.3</td>
<td>61 ± 6.4</td>
<td>46 ± 5.4</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>105 ± 5.9</td>
<td>90 ± 5.2</td>
<td>95 ± 7.0</td>
</tr>
<tr>
<td>Total LCP</td>
<td>539 ± 16.7</td>
<td>574 ± 15.4</td>
<td>473 ± 16.0</td>
</tr>
</tbody>
</table>

There are no significant differences between the two groups.

Statistical test employed: paired sample t-test.
TABLE 20

The fatty acid composition of the erythrocyte sphingomyelin of patients suffering from postpartum depression.

Figures in parenthesis indicate the number of subjects studied.

Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters detected.

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Patients(6)</th>
<th>Controls(6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>235 ± 6.9</td>
<td>243 ± 6.4</td>
</tr>
<tr>
<td>18:0</td>
<td>58 ± 5.3</td>
<td>50 ± 4.9</td>
</tr>
<tr>
<td>20:0</td>
<td>17 ± 2.4</td>
<td>20 ± 2.2</td>
</tr>
<tr>
<td>22:0</td>
<td>90 ± 6.5</td>
<td>96 ± 6.3</td>
</tr>
<tr>
<td>23:0 + 20:4ω6</td>
<td>31 ± 2.9</td>
<td>35 ± 2.7</td>
</tr>
<tr>
<td>24:0</td>
<td>229 ± 5.8</td>
<td>234 ± 5.4</td>
</tr>
<tr>
<td>24:1ω9 + 20:5ω3</td>
<td>256 ± 6.3</td>
<td>249 ± 5.9</td>
</tr>
<tr>
<td>24:2ω6</td>
<td>37 ± 2.4</td>
<td>33 ± 2.1</td>
</tr>
</tbody>
</table>

There are no significant differences between the two groups.

Statistical test employed: paired sample t-test.
Discussion

The preliminary findings of Ellis and Sanders (1977) of a higher proportion of LCP, in particular 22:6ω3 and 20:5ω3, in the plasma CPG of patients suffering from endogenous depression have been confirmed in a larger series of patients. In addition a reduction in the concentration of 18:2ω6 was observed in this group. One of the aims of the study was to discover whether the measurement of LCP, or more specifically of the 22:6ω3 concentration, would be of value in the diagnosis of endogenous depression: the raised 22:6ω3 concentration was present in the plasma CPG of a subgroup with early symptoms of the illness and of the whole group of patients with endogenous depression, 81 percent had a 22:6ω3 concentration of more than 54 mg/g total fatty acid methyl esters detected. This compares with 19 percent of matched controls and a similar proportion of patients in the other groups. Although the difference is highly significant ($p < 0.001$) it is insufficient to be of value as the sole criterion for diagnosis, but may be of use in conjunction with other methods. Nevertheless it must be pointed out that all the depressed patients may not have been correctly classified as endogenous or reactive using existing clinical criteria. Also it is possible that the high 22:6ω3 concentrations observed in a proportion of apparently healthy controls indicates vulnerability to the illness since higher concentrations were found in older subjects in whom monoamine oxidase (MAO) activity is higher (Robinson et al., 1972), thereby tending to produce a deficiency of serotonin and catecholamines.

High concentrations of LCP, in particular 22:6ω3 were also observed in patients with postpartum depression. This is of interest since it has been suggested that women who suffer from postpartum depression tend to have later developments of non-postpartum depression (Thornton, 1977).
The 22:6ω3 concentration of the plasma CPG of patients with endogenous depression but not that of patients with reactive depression, correlated with the severity of the illness \((p < 0.01)\). The severity of depression was similar in both groups.

The lower plasma cholesterol concentrations of the patients suffering from endogenous depression was probably due to a reduction in appetite, as this occurs more frequently in these patients than in those suffering from reactive depression (Dominian, 1976). However it could also be the result of an increase in thyroid function (Mathe and Chevalier, 1976) reported by some investigators (Kirkegaard et al., 1974; Kolakowska, 1977).

The higher MCV and MCH in the blood of depressed patients suggests that their alcohol intake might be greater than that of controls.

Analyses of the fatty acid composition of the erythrocyte phospholipids showed changes in the same direction as those of the plasma. These differences may be of importance since they could influence membrane function, for example the permeability and the activity of membrane-bound enzymes (van Deenan, 1971; McElhaney, 1974; Lenaz, 1979). Increasing the degree of unsaturation of the phospholipid fatty acid weakens the hydrophobic and London-van der Waals cohesive forces acting between the hydrocarbon chains of adjacent lipid molecules, resulting in a more loosely packed, more fluid bilayer. On the other hand, increasing the chain length of the fatty acid substituents has the reverse effect. Double bonds of cis configuration however produce a bending of the carbon atom chain thereby shortening the distance between the methyl and carboxyl ends of the molecule (Wojtczak, 1976). Hence an increase in LCP would tend to increase the fluidity of the membrane. An increase in membrane fluidity results in increased passive permeability. The
permeability of artificial membranes to water (Graziani and Livne, 1972; Fettiplace, 1978) and of erythrocyte and artificial membranes to non-electrolytes such as glycerol and erythritol (van Deenan, 1971) and glucose (Demel et al., 1968; Pilch et al., 1980) has been shown to increase with increasing unsaturation of the membrane fatty acids. Furthermore, the fatty acid composition of the erythrocyte phospholipids may affect the rate of other transport processes. The valinomycin induced Rb⁺ leak from liposomes is determined by the degree of unsaturation of the membrane phospholipid fatty acids (van Deenan, 1971). Rb⁺ is transported via the same mechanism as K⁺ in tissues and in cultured cells (Chen et al., 1978). Chan and Fishman (1978) reported that polyunsaturated fatty acids induced oedema in slices of rat brain cortex. The intracellular sodium concentration was increased while that of potassium was reduced. The authors concluded that these fatty acids may have affected membrane permeability. Furthermore, Lenaz (1979) suggested that the exceptionally high degree of unsaturation of the retinal rod membrane (with up to 45 percent 22:6ω3) may be responsible for the extremely high permeability to Na⁺ of liposomes derived from rod outer segment lipids, even when compared with total retinal liposomes.

The interpretation of the results of the present study is complicated by the fact that the erythrocyte phospholipids are distributed asymmetrically within the bilayer. The CPG and sphingomyelin are mostly contained in the outer leaflet whereas the EPG and SPG are localised in the inner leaflet (Trams, 1977). This suggests that in normal subjects the inner leaflet is more permeable than the outer leaflet. In patients suffering from endogenous depression, the total LCP and 22:6ω3 concentrations were increased in the EPG and the concentration of 20:5ω3 was increased in the SPG, thereby increasing the fluidity of the inner leaflet. The elevation of LCP in the CPG did not reach significance.
The activity of certain membrane-bound enzymes is affected by alterations in the phospholipid fatty acid composition. Na\(^{+}\)-K\(^{+}\)-ATPase activity is localised in the inner leaflet of the membrane (Trams, 1977) and is closely related to the active transport system of sodium and potassium in human red cells (Bonting and Caravaggio, 1963). There is controversy concerning whether this enzyme requires a specific phospholipid (SPG) for its activity (Kimelberg and Papahadjopoulos, 1972; Coleman, 1973) or simply a fluid bilayer (de Pont and Bonting, 1977). If the latter is correct, Na\(^{+}\)-K\(^{+}\)-ATPase activity would tend to increase in endogenous depression. Na\(^{+}\)-K\(^{+}\)-ATPase activity has been shown to be increased by prostaglandin E\(_1\) (formed from 20:3\(\omega_6\)) and prostaglandin E\(_2\) (formed from 20:4\(\omega_6\)) (Johnson and Ramwell, 1973), by chronic ethanol administration (Israel et al., 1970) and by EFA deficiency (Sun and Sun 1974; Lin et al., 1979).

The activities of brain MAO and 5'-mononucleotidase have been found to be decreased and that of glucose-6-phosphatase to be increased in rats after a period of lipid deprivation (Bernsohn and Spitz, 1974). On refeeding with either 18:2\(\omega_6\) or 18:3\(\omega_3\), MAO levels returned to normal and glucose-6-phosphatase levels decreased to below normal activity. Only 18:3\(\omega_3\) was effective in restoring 5'-mononucleotidase activities, indicating that 18:3\(\omega_3\) may have a biochemical function distinct from 18:2\(\omega_6\) in 5'-mononucleotidase metabolism. Bernsohn and Spitz (1974) suggested that the rate of synthesis of the enzyme could be dependent on a member of the \(\omega_3\) fatty acid series either directly (20:5\(\omega_3\) or 22:6\(\omega_3\)) or via a prostaglandin mechanism since 20:5\(\omega_3\) is the precursor of prostaglandin E\(_3\) and prostaglandin F\(_{3\alpha}\). Nevertheless it is unlikely that the increase in LCP in patients with endogenous depression will significantly alter the activity of 5'-mononucleotidase or MAO since both these enzymes are associated with the outer leaflet of membranes.
As far as can be ascertained from the literature, the specific function of 22:6\(\omega3\) is unknown. Babcock et al. (1976) studied the incorporation of \(^{14}\text{C}-22:6\omega3\) into the lipids of rats. In liver the radioactivity appeared most rapidly in the EPG and later in the CPG, unlike 18:2\(\omega6\) and 20:4\(\omega6\) which were incorporated most quickly into the CPG. This led Babcock et al. to suggest that the function of 22:6\(\omega3\) is different from those of other fatty acids. Galli et al. (1977) proposed that the \(\omega3\) series of fatty acids may play a special role in the CNS as high concentrations of 22:6\(\omega3\) are found in brain in comparison with other tissues.

Other factors affecting membrane permeability and the activity of membrane-bound enzymes are the relative proportions of erythrocyte phospholipids and the cholesterol:phospholipid ratio, but no abnormality was found in these parameters in patients suffering from endogenous depression. An increase in sphingomyelin (Fettiplace, 1978) or in the sphingomyelin:CPG ratio (Cooper et al., 1977) reduces membrane fluidity whereas a decrease in the sphingomyelin:CPG ratio (Borochov et al., 1977) results in an elevation of membrane fluidity. These changes could be the result of differences in the fatty acid composition of the phospholipids although the polar groups may play a role in transport processes (Robertson and Thompson, 1977). Alterations in the cholesterol:phospholipid ratio are concomitant with changes in the shape of the erythrocytes (Cooper, 1970; Cooper et al., 1975): no abnormalities were observed when blood films were examined. There is some question about the distribution of cholesterol within the membrane. Some data indicate that the sterol is evenly distributed between the two halves of the membrane (Lenard and Rothman, 1976) but Fisher (1976) suggested a partial
enrichment of the outer leaflet (about 2:1). The presence of cholesterol in membranes decreases their fluidity (Cooper, 1970; Vanderkooi et al., 1974; Shinitzky and Inbar, 1976; Cooper, 1978; Lenaz, 1979) and appears to limit the penetration of glycerol and erythritol (van Deenan, 1971), the diffusion of glucose (Demel et al., 1968) and also to reduce the permeability to Rb⁺ (van Deenan, 1971), and Na⁺ (Owen and McIntyre, 1978). Conversely, a decrease in membrane cholesterol results in increased permeability to glycerol (Bruckdorfer et al., 1969), acetate, erythritol and water (Grunze and Deuticke, 1974; Deuticke and Ruska, 1976), Rb⁺ (Chen et al., 1978) and K⁺ (Le Grimellec and Leblanc, 1978).

Studies of cation transport across red cell membranes have already led several investigators to suggest that there is an abnormality of cell membrane function in manic-depressive illness (Glen et al., 1968; Mendels and Frazer, 1973; Mendels and Frazer, 1974, Mendels and Frazer, 1975; Dorus et al., 1979). Lithium ratios (RBC:plasma) were reported to be higher in patients suffering from manic-depressive illness than in controls (Lyttkens et al., 1973; Ostrow et al., 1978) and depressed patients not responding to treatment with lithium carbonate (Mendels and Frazer, 1973; Mendels and Frazer, 1974) and to be higher in patients with a higher baseline erythrocyte sodium concentration (Mendels and Frazer, 1974). Since lithium is not actively removed from red blood cells by Na⁺-K⁺-ATPase (Maizels, 1968; Duhm and Becker, 1977) Mendels and Frazer suggested that the greater lithium ratios in these patients may be due to a greater leak of lithium into the cell rather than to reduced active transport out of the cell. This is consistent with the observed differences in fatty acid composition in the present investigation. However, Frazer et al. (1978) found no difference in passive transport of sodium across red cell membranes of patients with manic-depressive illness compared to that of controls. There is also disagreement as to whether
there is an abnormality of active transport in endogenous depression. This has been found to be normal by some investigators (Naylor et al., 1973; Frazer et al., 1978) but reduced by others (Naylor et al., 1970b; Hesketh et al., 1977; Choi et al., 1977). Thus it is possible that the observed differences in the erythrocyte phospholipid fatty acids may be insufficient to produce a significant alteration in membrane function.

Mendels and Frazer (1974; 1975) studied lithium ratios in two groups of sheep, one group having red blood cells with high potassium concentrations and low sodium concentrations (HKsheep) and the other group having red blood cells with low potassium concentrations and high sodium concentrations (LKsheep). There is a greater rate of passive diffusion of cations across erythrocyte membranes of LK sheep than HK sheep. In addition, erythrocyte membranes of LK sheep have lower Na\(^+\)-K\(^+\)-ATPase activity than those of HK sheep. Lithium ratios were found to be higher in LK sheep, in accordance with their studies of human erythrocytes.

Nelson (1967a) investigated the lipid composition of erythrocytes from these two groups of sheep. In agreement with the present investigation of human erythrocytes in endogenous depression, no difference was found in the relative proportions of phospholipids or in the cholesterol:phospholipid ratio. However, in contrast to the present investigation, a later study (Nelson, 1969) of the phospholipid fatty acid composition did not reveal any differences between the two groups of sheep. This anomaly may be due to the fact that sheep erythrocytes contain very little LCP (Nelson, 1969) and therefore reliable detection of abnormalities is difficult. There are also species variations in the relative proportions of erythrocyte phospholipids (de Gier and van Deenan, 1961; Nelson, 1967b), the CFG being absent from sheep erythrocytes.
The erythrocyte has been used by many investigators as a neuronal model since there are qualitative similarities in cation transport in erythrocytes and nerve cells (Mendels and Frazer, 1974). Quantitatively, both passive and active transport of cations occur at greater rates in nerve cells (Mendels and Frazer, 1974). This may be related to their greater concentrations of LCP. Changes in the proportions of LCP in rat erythrocytes have been shown to be reflected in other tissues, including liver and brain (Mohrhauer and Holman, 1963a,b,c) although the changes were less marked in the brain. Therefore, while it is likely that the higher proportions of LCP observed in the plasma CPG and erythrocyte phospholipids of patients with endogenous depression and postpartum depression are reflected in other tissues, they may not be of sufficient magnitude to affect brain function.
CHAPTER FOUR

THE EFFECT OF TREATMENT ON THE LCP COMPOSITION OF THE PLASMA CHOLINE PHOSPHOGLYCERIDES AND ERYTHROCYTE PHOSPHOGLYCERIDES

Introduction

There are several methods available for the treatment of depressive illness: ECT, tricyclic antidepressants, MAOI, lithium, L-tryptophan and psychotherapy.

ECT, introduced in the 1930's, is effective in the treatment of severe depression of the endogenous type (MRC, 1965; Carney and Sheffield, 1973; Shaw, 1977; Royal College of Psychiatrists, 1977; Fink, 1977; Freeman, 1979; Avery and Lubrano, 1979). It has been shown to be superior to simulated ECT (Freeman et al., 1978), placebo or MAOI (MRC, 1965; Royal College of Psychiatrists, 1977; Freeman, 1979) and to be more rapidly acting and as good (MRC, 1965; Royal College of Psychiatrists, 1977) or better (Heshe and Roeder, 1976; Avery and Lubrano, 1979; Freeman, 1979) than tricyclic antidepressants. Bilateral ECT may be more effective than unilateral ECT (Abrams and Taylor, 1976) but this is uncertain (d'Elia and Raotma, 1975). The treatment is however not without risk. There are an average of 3.0 deaths per year from ECT in England and Wales and a number of non-fatal complications associated with the treatment (Smith, 1977; Freeman, 1979). Nevertheless, Avery and Winokur (1976) found that the mortality rate was lower in patients given ECT than in those treated with antidepressant drugs or in untreated depressed patients due to the higher rates of suicide in the latter groups. Furthermore, the use of muscle paralysing agents, sedation and oxygenation have reduced the complications of the treatment (Fink, 1977).
The mechanism of the antidepressant action of ECT is unknown. Administration of electroconvulsive shocks to rats has been shown to increase the release and turnover of noradrenalin (Kety et al., 1967; Thierry et al., 1968; Ebert et al., 1973; Modigh, 1976) and to decrease noradrenalin uptake (Hendley and Welch, 1975); to have no effect on dopamine and serotonin turnover (Modigh, 1976); to increase the concentrations of serotonin (Gandolfi et al., 1978) and GABA (Green et al., 1978) and decrease the concentration of acetylcholine (Singh et al., 1979) in the brain; to reduce RNA (ribonucleic acid) synthesis (Wynter, 1979); to increase CSF cyclic AMP (3'5' cyclic adenosine monophosphate) concentrations (Clarenbach et al., 1978); and to increase the permeability of the blood-brain barrier (Bolwig et al., 1977a). Grahame-Smith et al. (1978) reported increased behavioural sensitivity to agents mimicking the actions of brain monoamines, which might be accounted for by increased receptor responses. Other workers found that electroconvulsive shock reduced the sensitivity of β-adrenergic receptors (Bergstrom and Kellar, 1979; Pandey et al., 1979) but had no effect on the sensitivity of α-adrenergic or serotonergic receptors (Bergstrom and Kellar, 1979). It has also been suggested that electroconvulsive shock may produce an increase in the number of synaptic vesicles (Jorgenssen and Bolwig, 1979). In man, ECT has been reported to have no effect on the CSF concentrations of HVA, 5HIAA or tryptophan (Abrams et al., 1976); to increase prolactin secretin (Ohman et al., 1976; O'Dea et al., 1978; Klimes et al., 1978); stimulate ACTH and cortisol production (Ylikorala et al., 1976; Delitala et al., 1977); to have no effect on serum TSH (Ryan et al., 1970; Ylikorala et al., 1976; Delitala et al., 1977); to decrease CSF calcium concentrations (Carman et al., 1977); and to increase the permeability of the blood-brain barrier (Bolwig et al., 1977b). In addition, plasma and urinary levels of cyclic AMP may be increased by
ECT (Hamadah et al., 1972; Holmes, 1975), but Ylikorala et al. (1976) failed to find any consistent change in plasma cyclic AMP concentrations after ECT. Measurements of the concentrations of FSH, LH and growth hormone in serum before and after ECT have also yielded conflicting results (Ryan et al., 1970; Ylikorala et al., 1976; Delitala et al., 1977).

The MAOI and tricyclic antidepressants were introduced in the 1950's. The former have been relegated to a lesser role in the treatment of depressive illness as they are less effective and more toxic than the tricyclics (Mielke, 1976).

The MAOI prevent amine catabolism by inhibiting monoamine oxidase and the tricyclics prevent amine inactivation by inhibiting amine reuptake into presynaptic neurones (Carlsson, 1976; Carlsson and Lindquist, 1978). The therapeutic effect of both these groups of drugs is thought to be due to the resultant increase in noradrenalin and/or serotonin availability at functionally important receptor sites (Carlsson, 1976; Carlsson and Lindquist, 1978; de Montigny and Aghajanian, 1978; Sangdee and Franz, 1979). Tricyclics also inhibit acetylcholine synthesis (Halaris and Karbowski, 1979). Some may increase prolactin secretion (Cole et al., 1976; Francis et al., 1976; Slater et al., 1977; Mendelwicz and Youdim, 1977) but this has been contradicted (Meltzer et al., 1977); some have been found to increase serum TSH concentrations (Linnoila et al., 1977); and to reduce plasma cortisol concentrations (McClure, 1966b).

Lithium was first introduced in 1949 for the control of mania and is now used to control both unipolar and bipolar depressive illness. It is an effective prophylactic agent (Coppen et al., 1971b; Lancet, 1972; Coppen et al., 1973b; Schou, 1979), reducing the mean percentage of patients becoming ill within one year of commencing treatment from approximately 70 percent to 20 percent, compared with a reduction to 35 percent when tricyclics were used (Schou, 1979).
There have been a large number of investigations of the metabolic effects of lithium. Briefly, these include effects on noradrenalin metabolism (Colburn et al., 1967; Stern et al., 1969; Schildkraut et al., 1969b; Gershon, 1972), dopamine metabolism (Corrodi et al., 1969; Friedman and Gershon, 1973), serotonin metabolism (Schildkraut et al., 1969a; Sheard and Aghajanian, 1970; Perez-cruet et al., 1971; Collard, 1978), acetylcholine metabolism (Yizi, 1975; Jope, 1979), serum electrolyte concentrations (Christiansen et al., 1978; Srinivasan et al., 1978; Hariharasubramanian et al., 1978), membrane transport (Glenn and Reading, 1973; Lingsch and Martin, 1976; Hesketh, 1976; Martin, 1978; Dick et al., 1978; Glen, 1978; Beaugé, 1978; Coppen et al., 1980), hormone-stimulated prostaglandin synthesis (Horrobin et al., 1978), carbohydrate metabolism (Lazarus, 1978; Plenge, 1978; Vendsborg, 1978), thyroid function (Bagchi et al., 1978), serum LH (Sheard et al., 1977) and cyclic AMP formation (Krutlick, 1977; Geisler et al., 1978; Geisler and Klysner, 1978; Zatz, 1979).

Studies of the effect of tryptophan on the recovery of depressed patients have yielded conflicting results (Coppen et al., 1967b; Broadhurst, 1970; Coppen and Noguera, 1970; Carroll et al., 1970; Coppen et al., 1972c; Dunner and Goodwin, 1972; Herrington et al., 1974, 1976) but a number of workers have reported that tryptophan potentiates the effect of MAOI (Coppen et al., 1963; Pare, 1963; Coppen et al., 1967b; Glassman and Platman, 1969) and tricyclics (Walinder et al., 1976). The efficacy of ECT was however unaffected by the addition of tryptophan (d'Elia et al., 1977; Kirkegaard et al., 1978b). Other monoamine precursors have not been found to affect the recovery of depressed patients (Carroll, 1971; Nahunek et al., 1972) with the possible exception of phenylalanine (Fischer et al., 1975).
Psychotherapy is of value in the treatment of reactive depression (Luborsky et al., 1975) but is frequently used as an adjunct to pharmacological treatment.

The review of the literature has shown that ECT and the pharmacological agents used in the treatment of depressive illness alter the metabolism of biogenic amines, electrolytes and hormones and may also affect membrane function. For example, tricyclic antidepressants inhibit amine reuptake into presynaptic neurones. It was suggested in Chapter three that the high LCP concentrations in patients suffering from endogenous depression may increase membrane permeability. It is possible that a decrease in these values during treatment would have the reverse effect and perhaps explain the mode of action of the tricyclics. Therefore the LCP composition of the plasma CPG and erythrocyte phosphoglycerides were determined during treatment of depressive illness.

Patients and Methods

Twelve patients suffering from endogenous depression and six patients suffering from reactive depression were studied. The mean age of patients with endogenous depression was 49 years, ranging from 21 to 72 years. The mean age of patients with reactive depression was 34 years, ranging from 18 to 48 years. All the patients in the endogenous group suffered from unipolar illness. Eight of the patients with endogenous depression (67 percent) and four of those with reactive depression (67 percent) were drug-free for at least two weeks before the beginning of the study. The remainder were receiving a hypnotic.

Blood samples were taken before treatment commenced and at the following times thereafter: one week, two weeks, four weeks and at twelve weeks. The severity of depression was assessed by the Beck Inventory
before treatment and at four weeks and twelve weeks after the beginning of treatment.

Of the patients suffering from endogenous depression, two were given ECT and ten were treated with tricyclic antidepressants. Of the patients with reactive depression, two received tricyclic antidepressants, one received a tranquilliser and three received psychotherapy.

In addition, the fatty acid composition of the plasma choline phosphoglycerides of fifteen patients receiving prophylactic lithium treatment was determined. Ten of the patients were classified as unipolar and five as bipolar, but none were depressed at the time of study. The mean age was 53 years, ranging from 35 to 64 years. Thirteen of the patients had received lithium for several years and two had received lithium for one year. Only one patient received other drugs (codeine and aspirin) in the two weeks prior to the study.

Results

The fatty acid composition of the plasma CPG of patients suffering from endogenous depression is shown in Table 21. Compared to the values before treatment, the total LCP concentration was significantly lower at two weeks ($p < 0.02$), four weeks ($p < 0.001$) and twelve weeks ($p < 0.001$) after treatment commenced. Regarding the individual fatty acids, the concentration of $22:6\omega3$ was significantly lower at two weeks ($p < 0.05$), four weeks ($p < 0.001$) and twelve weeks ($p < 0.001$); the concentration of $20:4\omega6$ was significantly lower at four weeks ($p < 0.01$) and twelve weeks ($p < 0.01$); the concentration of $20:5\omega3$ was significantly lower at four weeks ($p < 0.01$) and twelve weeks ($p < 0.01$); and the concentration of $22:5\omega3$ was significantly lower at four weeks ($p < 0.05$) after treatment commenced. Compared to the values at two weeks after the beginning of
TABLE 21

Effect of treatment of 12 patients suffering from endogenous depression on the concentrations of LCP in their plasma choline phosphoglycerides.

Results expressed as mean ± S.E. in mg/g total fatty acid and methyl esters detected.

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Before Treatment</th>
<th>1 week after commencing treatment</th>
<th>2 weeks after commencing treatment</th>
<th>4 weeks after commencing treatment</th>
<th>12 weeks after commencing treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2ω6</td>
<td>210 ± 7.6</td>
<td>197 ± 7.8</td>
<td>220 ± 7.5</td>
<td>231 ± 7.3^b</td>
<td>232 ± 6.8^b</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>32 ± 1.4</td>
<td>30 ± 1.5</td>
<td>33 ± 1.3</td>
<td>32 ± 1.2</td>
<td>32 ± 1.4</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>116 ± 4.5</td>
<td>115 ± 4.4</td>
<td>110 ± 4.6</td>
<td>99 ± 4.3^c,e</td>
<td>97 ± 4.4^c,e</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>21 ± 1.5</td>
<td>19 ± 1.5</td>
<td>18 ± 1.4</td>
<td>15 ± 1.2^c</td>
<td>16 ± 1.4^c</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>18 ± 1.2</td>
<td>18 ± 1.4</td>
<td>16 ± 1.3</td>
<td>15 ± 1.2^a</td>
<td>16 ± 1.1</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>67 ± 2.8</td>
<td>65 ± 3.0</td>
<td>60 ± 2.9^a</td>
<td>46 ± 2.8^d,h</td>
<td>52 ± 2.9^d,f</td>
</tr>
<tr>
<td>Total LCP</td>
<td>266 ± 7.1</td>
<td>267 ± 7.3</td>
<td>244 ± 7.0^b</td>
<td>219 ± 6.9^d,g</td>
<td>222 ± 6.6^d,g</td>
</tr>
</tbody>
</table>

a  p < 0.05  
b  p < 0.02  
c  p < 0.01  
d  p < 0.001  
e  p < 0.05  
f  p < 0.02  
g  p < 0.01  
h  p < 0.001

compared to values before treatment and at 1 week after commencing treatment.

compared to values at 2 weeks after commencing treatment.

Statistical test employed: paired sample t-test.
treatment, the total LCP concentration was lower at four weeks (p < 0.01) and twelve weeks (p < 0.01); the concentration of 22:6ω3 was lower at four weeks (p < 0.001) and twelve weeks (p < 0.02); and the concentration of 20:4ω6 was lower at four weeks (p < 0.05) and twelve weeks (p < 0.05). In addition, the concentration of 18:2ω6 was higher at four weeks (p < 0.02) and twelve weeks (p < 0.02) after treatment commenced compared with the concentration before treatment. The 22:6ω3 concentration at twelve weeks was higher than at four weeks, but this was not significant.

The fatty acid composition of the plasma CPG of patients suffering from reactive depression is shown in Table 22. There was no significant alteration during treatment. LCP concentrations in both endogenous and reactive patients before treatment were similar to those reported in chapter three.

In the erythrocyte EPG of patients with endogenous depression (Table 23), the concentration of 22:6ω3 was lower at four weeks (p < 0.05) and twelve weeks (p < 0.05) and the total LCP concentration was lower at twelve weeks (p < 0.05) after treatment commenced compared to the values before treatment. In contrast, there was no significant alteration in the fatty acid composition of the erythrocyte EPG of patients being treated for reactive depression (Table 24).

In the erythrocyte SPG of patients with endogenous depression (Table 25), the total LCP concentration was lower at twelve weeks after the beginning of treatment (p < 0.05) compared to the concentration before treatment. As above, there was no alteration in the fatty acid composition of the erythrocyte SPG of patients being treated for reactive depression (Table 26).

The erythrocyte CPG of patients with endogenous depression (Table 27) showed a significant decrease in total LCP (p < 0.05) and increase
TABLE 22

Effect of treatment of 6 patients suffering from reactive depression on the concentrations of LCP in their plasma choline phosphoglycerides

Results expressed as mean ± S.E. in mg/g total fatty acid methyl esters detected.

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Before treatment</th>
<th>1 week after commencing treatment</th>
<th>2 weeks after commencing treatment</th>
<th>4 weeks after commencing treatment</th>
<th>12 weeks after commencing treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2ω6</td>
<td>239 ± 8.2</td>
<td>244 ± 8.7</td>
<td>250 ± 8.3</td>
<td>248 ± 8.8</td>
<td>245 ± 9.2</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>30 ± 2.4</td>
<td>29 ± 2.1</td>
<td>33 ± 2.5</td>
<td>33 ± 2.0</td>
<td>30 ± 2.2</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>96 ± 6.6</td>
<td>95 ± 5.9</td>
<td>91 ± 6.1</td>
<td>89 ± 5.6</td>
<td>93 ± 5.8</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>13 ± 1.4</td>
<td>12 ± 1.5</td>
<td>12 ± 1.4</td>
<td>14 ± 1.1</td>
<td>12 ± 1.5</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>14 ± 1.7</td>
<td>11 ± 1.6</td>
<td>12 ± 1.7</td>
<td>14 ± 1.0</td>
<td>12 ± 1.4</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>41 ± 2.4</td>
<td>39 ± 2.2</td>
<td>42 ± 2.6</td>
<td>37 ± 2.8</td>
<td>37 ± 2.4</td>
</tr>
<tr>
<td>Total LCP</td>
<td>206 ± 9.7</td>
<td>200 ± 9.1</td>
<td>204 ± 9.5</td>
<td>199 ± 8.0</td>
<td>196 ± 8.5</td>
</tr>
</tbody>
</table>

There is no significant alteration during treatment.

Statistical test employed: paired sample t-test.
TABLE 23

Effect of treatment of 12 patients suffering from endogenous depression on the concentrations of LCP in their erythrocyte ethanolamine phosphoglycerides

Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters and dimethyl acetals detected.

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Before Treatment</th>
<th>1 week after commencing treatment</th>
<th>2 weeks after commencing treatment</th>
<th>4 weeks after commencing treatment</th>
<th>12 weeks after commencing treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2ω6</td>
<td>62 ± 4.1</td>
<td>61 ± 3.9</td>
<td>67 ± 3.7</td>
<td>65 ± 3.5</td>
<td>66 ± 3.9</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>14 ± 1.5</td>
<td>15 ± 1.8</td>
<td>16 ± 1.6</td>
<td>15 ± 1.7</td>
<td>16 ± 1.6</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>258 ± 6.4</td>
<td>255 ± 6.3</td>
<td>252 ± 6.8</td>
<td>248 ± 6.5</td>
<td>255 ± 6.9</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>23 ± 2.3</td>
<td>23 ± 2.0</td>
<td>21 ± 2.2</td>
<td>25 ± 2.4</td>
<td>25 ± 1.9</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>63 ± 4.1</td>
<td>65 ± 3.9</td>
<td>67 ± 4.3</td>
<td>64 ± 3.7</td>
<td>58 ± 3.5</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>67 ± 2.4</td>
<td>67 ± 2.5</td>
<td>64 ± 2.7</td>
<td>63 ± 2.6</td>
<td>60 ± 2.5</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>118 ± 3.5</td>
<td>114 ± 3.7</td>
<td>112 ± 4.0</td>
<td>107 ± 3.9a</td>
<td>106 ± 3.7a</td>
</tr>
<tr>
<td>Total LCP</td>
<td>555 ± 10.5</td>
<td>550 ± 10.3</td>
<td>543 ± 11.0</td>
<td>533 ± 10.7</td>
<td>520 ± 10.8a</td>
</tr>
</tbody>
</table>

a p < 0.05 compared to values before treatment.

Statistical test employed: paired sample t-test.
TABLE 24

Effect of treatment of 6 patients suffering from reactive depression on the concentrations of LCP in their erythrocyte ethanolamine phosphoglycerides

Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters and dimethyl acetalis detected.

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Before treatment</th>
<th>1 week after commencing treatment</th>
<th>2 weeks after commencing treatment</th>
<th>4 weeks after commencing treatment</th>
<th>12 weeks after commencing treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2ω6</td>
<td>68 ± 3.9</td>
<td>68 ± 4.2</td>
<td>69 ± 4.5</td>
<td>72 ± 3.8</td>
<td>73 ± 3.7</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>14 ± 1.9</td>
<td>16 ± 1.8</td>
<td>14 ± 2.1</td>
<td>15 ± 1.8</td>
<td>13 ± 1.6</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>261 ± 6.7</td>
<td>257 ± 6.5</td>
<td>258 ± 6.8</td>
<td>256 ± 6.1</td>
<td>259 ± 6.3</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>21 ± 2.2</td>
<td>19 ± 1.9</td>
<td>19 ± 1.8</td>
<td>22 ± 2.3</td>
<td>21 ± 1.9</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>62 ± 4.9</td>
<td>63 ± 4.5</td>
<td>64 ± 5.1</td>
<td>64 ± 4.3</td>
<td>60 ± 4.1</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>63 ± 3.5</td>
<td>61 ± 3.1</td>
<td>63 ± 3.8</td>
<td>64 ± 3.6</td>
<td>67 ± 3.3</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>90 ± 3.8</td>
<td>96 ± 3.4</td>
<td>90 ± 4.0</td>
<td>93 ± 3.6</td>
<td>94 ± 3.7</td>
</tr>
<tr>
<td>Total LCP</td>
<td>527 ± 12.1</td>
<td>534 ± 11.8</td>
<td>524 ± 14.3</td>
<td>529 ± 11.5</td>
<td>526 ± 11.6</td>
</tr>
</tbody>
</table>

There is no significant alteration during treatment.

Statistical test employed: paired sample t-test.
TABLE 25

Effect of treatment of 12 patients suffering from endogenous depression on the concentrations of LCP in their erythrocyte serine phosphoglycerides

Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters and dimethyl acetalts detected.

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Before treatment</th>
<th>1 week after commencing treatment</th>
<th>2 weeks after commencing treatment</th>
<th>4 weeks after commencing treatment</th>
<th>12 weeks after commencing treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2ω6</td>
<td>40 ± 3.9</td>
<td>39 ± 4.3</td>
<td>42 ± 4.5</td>
<td>45 ± 4.0</td>
<td>40 ± 4.1</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>17 ± 1.4</td>
<td>16 ± 1.6</td>
<td>18 ± 2.0</td>
<td>15 ± 1.7</td>
<td>18 ± 1.6</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>258 ± 6.4</td>
<td>255 ± 6.8</td>
<td>255 ± 7.2</td>
<td>253 ± 7.0</td>
<td>252 ± 6.9</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>14 ± 1.5</td>
<td>16 ± 1.7</td>
<td>16 ± 1.9</td>
<td>15 ± 1.7</td>
<td>13 ± 1.8</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>39 ± 2.5</td>
<td>38 ± 2.7</td>
<td>36 ± 3.0</td>
<td>34 ± 2.8</td>
<td>35 ± 2.9</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>49 ± 3.1</td>
<td>57 ± 3.6</td>
<td>52 ± 3.8</td>
<td>46 ± 3.2</td>
<td>44 ± 3.4</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>114 ± 3.9</td>
<td>111 ± 4.1</td>
<td>107 ± 4.5</td>
<td>105 ± 4.3</td>
<td>105 ± 4.2</td>
</tr>
<tr>
<td>Total LCP</td>
<td>509 ± 8.9</td>
<td>506 ± 9.1</td>
<td>503 ± 9.7</td>
<td>490 ± 9.5</td>
<td>484 ± 9.3</td>
</tr>
</tbody>
</table>

\( a \) \ p < 0.05 compared to values before treatment.

Statistical test employed: paired sample t-test.
**TABLE 26**

Effect of treatment of 6 patients suffering from reactive depression on the concentrations of LCP in their erythrocyte serine phosphoglycerides

*Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters and dimethyl acetals detected.*

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Before treatment</th>
<th>1 week after commencing treatment</th>
<th>2 weeks after commencing treatment</th>
<th>4 weeks after commencing treatment</th>
<th>12 weeks after commencing treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2ω6</td>
<td>37 ± 4.2</td>
<td>41 ± 4.5</td>
<td>45 ± 4.1</td>
<td>42 ± 4.9</td>
<td>38 ± 4.3</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>20 ± 2.0</td>
<td>23 ± 2.1</td>
<td>22 ± 1.5</td>
<td>19 ± 1.9</td>
<td>21 ± 1.8</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>249 ± 4.2</td>
<td>246 ± 4.5</td>
<td>248 ± 4.0</td>
<td>250 ± 3.6</td>
<td>245 ± 4.7</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>12 ± 0.7</td>
<td>14 ± 0.9</td>
<td>11 ± 1.0</td>
<td>13 ± 0.8</td>
<td>14 ± 1.0</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>38 ± 2.5</td>
<td>38 ± 2.6</td>
<td>40 ± 2.3</td>
<td>40 ± 2.1</td>
<td>37 ± 2.7</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>46 ± 2.4</td>
<td>45 ± 2.9</td>
<td>44 ± 2.5</td>
<td>48 ± 2.8</td>
<td>45 ± 2.8</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>93 ± 4.3</td>
<td>93 ± 4.9</td>
<td>89 ± 4.2</td>
<td>88 ± 5.1</td>
<td>92 ± 5.0</td>
</tr>
<tr>
<td>Total LCP</td>
<td>475 ± 6.1</td>
<td>474 ± 7.1</td>
<td>487 ± 6.5</td>
<td>484 ± 6.9</td>
<td>479 ± 7.5</td>
</tr>
</tbody>
</table>

*There is no significant alteration during treatment.*

*Statistical test employed: paired sample t-test.*
**TABLE 27**

Effect of treatment of 12 patients suffering from endogenous depression on the concentrations of LCP in their erythrocyte choline phosphoglycerides

Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters and dimethyl acetalis detected.

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Before treatment</th>
<th>1 week after commencing treatment</th>
<th>2 weeks after commencing treatment</th>
<th>4 weeks after commencing treatment</th>
<th>12 weeks after commencing treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2ω6</td>
<td>180 ± 8.1</td>
<td>184 ± 8.4</td>
<td>190 ± 8.5</td>
<td>195 ± 8.3</td>
<td>201 ± 7.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>26 ± 1.1</td>
<td>25 ± 1.3</td>
<td>25 ± 1.2</td>
<td>24 ± 1.5</td>
<td>24 ± 1.4</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>105 ± 4.3</td>
<td>104 ± 4.4</td>
<td>100 ± 4.2</td>
<td>96 ± 4.6</td>
<td>94 ± 4.7</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>16 ± 1.5</td>
<td>15 ± 1.6</td>
<td>14 ± 1.4</td>
<td>17 ± 1.8</td>
<td>16 ± 1.7</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>14 ± 0.8</td>
<td>13 ± 1.1</td>
<td>14 ± 0.9</td>
<td>13 ± 1.2</td>
<td>14 ± 1.1</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>46 ± 3.4</td>
<td>44 ± 2.8</td>
<td>40 ± 2.9</td>
<td>41 ± 3.2</td>
<td>38 ± 3.0</td>
</tr>
<tr>
<td>Total LCP</td>
<td>219 ± 7.9</td>
<td>213 ± 8.3</td>
<td>204 ± 8.1</td>
<td>202 ± 8.5</td>
<td>198 ± 8.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> p < 0.05 compared to values before treatment.

Statistical test employed: paired sample t-test.
in 18:2ω6 concentrations twelve weeks after the beginning of treatment. Again, there was no alteration in the fatty acid composition of the erythrocyte CPG of patients being treated for reactive depression (Table 28).

Of the patients suffering from endogenous depression, the mean score on the Beck Inventory before treatment was 26, ranging from 18 to 38. After four weeks treatment the mean score was 12, ranging from 3 to 18; a decrease of 54 percent. The mean score at twelve weeks after the beginning of treatment was 8, ranging from 0 to 14; a decrease of 69 percent compared to the pretreatment scores. Of the patients suffering from reactive depression, the mean score on the Beck Inventory before treatment was 24, ranging from 15 to 27. After four weeks treatment the mean score was 15, ranging from 0 to 20; a decrease of 37 percent. The mean score at twelve weeks after the beginning of treatment was 7, ranging from 0 to 15; a decrease of 71 percent compared to the pretreatment scores.

The 22:6ω3 content of the plasma CPG of patients with endogenous depression correlated with the Beck Inventory scores before treatment (see chapter three). However there was no correlation at 4 weeks or at 12 weeks after treatment commenced.

The LCP composition of the plasma CPG of patients receiving prophylactic lithium treatment is shown in Table 29. Concentrations were not significantly different from those of age-sex-matched controls, although the concentration of 22:6ω3 tended to be higher.

Discussion

Analysis of the Beck Inventory scores revealed that there was a substantial clinical improvement in both endogenous and reactive patients
TABLE 28

Effect of treatment of 6 patients suffering from reactive depression on the concentrations of LCP in their erythrocyte choline phosphoglycerides

Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters and dimethyl acetals detected.

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Before treatment</th>
<th>1 week after commencing treatment</th>
<th>2 weeks after commencing treatment</th>
<th>4 weeks after commencing treatment</th>
<th>12 weeks after commencing treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2ω6</td>
<td>202 ± 8.8</td>
<td>200 ± 8.5</td>
<td>203 ± 8.9</td>
<td>207 ± 8.3</td>
<td>205 ± 8.0</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>25 ± 0.8</td>
<td>29 ± 1.0</td>
<td>28 ± 0.9</td>
<td>26 ± 1.2</td>
<td>24 ± 1.3</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>99 ± 5.0</td>
<td>93 ± 5.3</td>
<td>95 ± 5.6</td>
<td>98 ± 4.9</td>
<td>94 ± 5.7</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>10 ± 0.7</td>
<td>10 ± 1.3</td>
<td>9 ± 0.9</td>
<td>10 ± 1.2</td>
<td>11 ± 1.4</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>12 ± 1.5</td>
<td>9 ± 1.6</td>
<td>11 ± 1.4</td>
<td>14 ± 1.2</td>
<td>12 ± 1.5</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>32 ± 1.5</td>
<td>33 ± 1.7</td>
<td>38 ± 1.9</td>
<td>34 ± 1.8</td>
<td>36 ± 1.9</td>
</tr>
<tr>
<td>Total LCP</td>
<td>206 ± 10.9</td>
<td>192 ± 11.3</td>
<td>199 ± 11.6</td>
<td>190 ± 10.5</td>
<td>186 ± 11.1</td>
</tr>
</tbody>
</table>

There is no significant alteration during treatment.

Statistical test employed: paired sample t-test.
TABLE 29

The LCP composition of the plasma choline phosphoglycerides of patients receiving prophylactic lithium treatment

Results expressed as mean ± S.E. in mg/g total fatty acid methyl esters detected.

Figures in parenthesis indicate the number of subjects studied.

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Patients (15)</th>
<th>Age-sex-matched controls (15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2ω6</td>
<td>226 ± 8.5</td>
<td>237 ± 8.2</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>30 ± 1.0</td>
<td>32 ± 1.4</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>96 ± 3.2</td>
<td>104 ± 3.8</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>16 ± 1.1</td>
<td>15 ± 1.0</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>18 ± 0.7</td>
<td>14 ± 0.7</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>58 ± 1.9</td>
<td>50 ± 2.7</td>
</tr>
<tr>
<td>Total LCP</td>
<td>228 ± 4.6</td>
<td>227 ± 5.9</td>
</tr>
</tbody>
</table>

There are no significant differences between the two groups.

Statistical test employed: paired sample t-test.
during treatment. This was accompanied by a decrease in the concentrations of LCP and increase in the concentration of 18:2ω6 in the plasma CPG and erythrocyte phosphoglycerides of patients being treated for endogenous depression, but not in those of patients being treated for reactive depression. The lack of correlation of the 22:6ω3 concentrations in the endogenous group with the Beck Inventory scores at 4 weeks and at 12 weeks after the beginning of treatment may have been due to the low depression ratings. In the plasma CPG of the endogenous group, the concentrations of total LCP and 22:6ω3 were unchanged one week after treatment commenced but had decreased slightly by two weeks. This is in agreement with the fact that antidepressant drugs (with which most of the patients were treated) require two to three weeks administration before a clinical response is observed (Mitchell, 1975; Segal et al., 1976).

The concentrations of total LCP, 22:6ω3, 20:5ω3 and 18:2ω6 had reached normal values by four weeks. There were also decreases in the concentrations of 20:4ω6 and 22:5ω3 even though the concentrations of these fatty acids were not significantly elevated before treatment. These changes were reflected in the fatty acid composition of the erythrocyte phosphoglycerides but were less marked, only reaching significance at twelve weeks after the beginning of treatment.

The concentration of 22:6ω3 in the plasma CPG of the endogenous group increased slightly between four weeks and twelve weeks after treatment commenced and patients who had received prophylactic lithium treatment for at least a year tended to have higher 22:6ω3 concentrations than matched controls. Although neither of these differences were statistically significant, they suggest that the effect of treatment on phosphopholid fatty acid composition may be only temporary.
The decrease in phospholipid LCP concentrations would tend to reduce membrane permeability and may therefore explain the inhibitory effect of tricyclic antidepressants on amine reuptake.

Bazan and his co-workers (Bazan, 1970; Bazan and Rakowski, 1970; Bazan, 1971) showed that free fatty acid levels, especially 20:4ω6, increased in the brains of rats after electroconvulsive shock. They suggested that this was due to the activation of phospholipase A₂ (Bazan, 1970; Bazan and Rakowski, 1970) possibly by noradrenalin (Bazan et al., 1971). This would tend to increase prostaglandin synthesis (Horton, 1975; Flower and Blackwell, 1976). In fact, prostaglandins have been found in increased amounts in human CSF following epileptic seizures (Wolfe et al., 1977). Electroshock has also been reported to reduce the incorporation of labelled free 20:4ω6 into lipids (Rodriguez de Turco et al., 1977), although the effect was only temporary. Therefore it is possible that the observed decrease in the concentrations of prostaglandin precursors (20:4ω6 and 20:5ω3) was due either to increased release of these fatty acids from phospholipids or to their reduced incorporation into phospholipids. The former hypothesis is the most likely since biogenic amines, which are increased at functionally important receptor sites after electroconvulsive shock (Modigh, 1976) or antidepressant drug treatment (Carlsson, 1976), increase the synthesis of prostaglandin F₂α (Wolfe et al., 1977). Gross et al. (1977) found a slight increase in the synthesis of prostaglandins of the F series with chronic lithium treatment, but L-tryptophan had the reverse effect. Prostaglandin F₂α may enhance the release of neurotransmitters (Horton, 1973). On the other hand, prostaglandins of the E series exert negative feedback on the presynaptic neurone to prevent transmitter release (Horton, 1973; Craig, 1975; Craig, 1976). Prostaglandins of the E series also promote transmitter reuptake into the presynaptic neurone (Hedqvist, 1974; Hedqvist, 1976).
an action which is antagonised by tricyclic antidepressants (Carlsson, 1976; Manku and Horrobin, 1976).

In conclusion, from published work it seems likely that the observed decrease in phospholipid LCP concentrations was due to increased release of these fatty acids from phospholipids, with a subsequent increase in prostaglandin $F_{2\alpha}$ synthesis. However, the present study suggests that the effect may be only temporary.
CHAPTER FIVE

THE FATTY ACID COMPOSITION OF THE PLASMA CHOLINE PHOSPHOGLYCERIDES

AND ERYTHROCYTE PHOSPHOLIPIDS OF ORAL CONTRACEPTIVE USERS

Introduction

Some studies have shown that depression is a side-effect of oral contraceptive use (Nilsson and Almgren, 1968; Lewis and Hoghughi, 1969; Herzberg and Coppen, 1970; Herzberg et al., 1970; Parry and Rush, 1979; Warnes and Fitzpatrick, 1979) and that it is a common cause of women stopping the pill (Dennis and Jeffrey, 1968; Parviz and Behrmann, 1976), but others found no evidence for this (Zell and Crisp, 1964; Goldzieher et al., 1971; Kutner and Brown, 1972a; b; Fleming and Seager, 1978). Goldzieher et al. (1971) found no increase in the overall incidence of depression among three hundred and ninety-eight women in a cross-over design involving a placebo and one of four oral contraceptive agents. In fact it has been suggested that a proportion of women using oral contraceptive agents experience an increased sense of well-being (Moos, 1968) and an improvement in sexual adaptation (Zell and Crisp, 1964; Nilsson et al., 1967). Moos (1968) found that 10 percent of the women studied reported an increase in well-being while another 10 percent reported a decrease. Herzberg and Coppen (1970) demonstrated that dysmenorrhea and premenstrual depression and irritability were relieved by oral contraceptive agents. The incidence of depression induced by oral contraceptive agents in this study was found to be between 5 percent and 10 percent.

The symptoms of depression induced by oral contraceptive agents differ from those of endogenous and reactive depression: pessimism, dissatisfaction, crying and tension being predominant, whereas sleep
disturbance and appetite disorders are rare (Herzberg et al., 1970).

Grant and Pryse-Davies (1968) reported that the incidence of depression was lowest among women using sequential formulations (fourteen to sixteen days of oestrogen alone, followed by seven to five days of oestrogen and progestogen combined). In a more recent study (Allen, 1974), the incidence of depression was found to be lowest among women using a very low dose combined pill (30μg of oestrogen plus 150μg of progestogen).

The occurrence of depression has usually been blamed on the progestogen component of the pill (Grant and Pryse-Davies, 1968; Lewis and Hoghughi, 1969) but the oestrogen component has also been held responsible (Cullberg, 1972a). However, Waxman (1968) claimed to have relieved depression by the administration of progesterone and Leeton (1973) stated that the oestrogen-progestogen balance was unimportant. Other workers have suggested that individual susceptibility may be more important than the composition of the pill in the development of depression (Herzberg and Coppen, 1970; Herzberg et al., 1970; Grounds et al., 1970; Fortin et al., 1972; Cullberg, 1972b). Women who developed depression as a side effect of oral contraceptive use were found to have higher neuroticism scores (using the MMPI\(^*\)) or to have more premorbid psychiatric symptoms (Herzberg and Coppen, 1970; Herzberg et al., 1970) or to have negative expectations about oral contraceptive agents (Grounds et al., 1970; Fortin et al., 1972; Cullberg, 1972b). Women with a history of psychiatric disorder, particularly depression (Nilsson et al., 1967; Lewis and Hoghughi, 1969) and especially those with a history of psychiatric disturbances during pregnancy (Nilsson and Almgren, 1968) or in the postpartum period (Marcotte et al., 1970) have been found to be at risk of developing depression during oral contraceptive use.

*Minnesota Multiphasic Personality Inventory.*
Rose (1966a, b) discovered that women who were using an oestrogen and progestogen combined oral contraceptive agent had a greatly increased urinary xanthurenic acid excretion after a loading dose of tryptophan. Subsequent studies confirmed this observation and showed that the urinary excretion of kynurenine, 3-hydroxy-kynurenine and to a lesser extent 3-hydroxyanthranilic acid (see Figure 15) were also raised in combined pill users after a loading dose of tryptophan (Price et al., 1967; Rose, 1972; Ahmed et al., 1975; Horwitt et al., 1975; Haspels et al., 1975; Green, 1978; Donald and Bossé, 1979). This was thought to be due to the induction of tryptophan pyrrolase, the rate-limiting enzyme in the tryptophan-nicotinic acid pathway, by oestrogen (Rose, 1966b; Rose and Braidman, 1970). The enhanced metabolism of tryptophan via this pathway means that less will be available for serotonin synthesis. However, Green (1978) reported that although oral contraceptive users excreted greater amounts of tryptophan metabolites of the kynurenine pathway, the excretion of 5HIAA was normal. He also suggested that since the plasma half-life and plasma clearance of tryptophan and the area under the plasma tryptophan curve were similar in oral contraceptive users and non-users, tryptophan pyrrolase activity may be normal in women using oral contraceptives. Total (Coppen et al., 1973c; Moller, 1979) and free (Coppen et al., 1973c) plasma tryptophan concentrations were found to be normal in oral contraceptive users but plasma tyrosine concentrations were low (Potera and Rose, 1978; Moller, 1979) indicating reduced formation of catecholamines.

It can be seen from Figure 15 that several of the enzymic reactions in the tryptophan-nicotinic acid pathway require pyridoxal phosphate (a form of vitamin B₆) as coenzyme. The enhanced metabolism of tryptophan via this pathway therefore increases the requirement for vitamin B₆. A further increase in the requirement arises from the fact that oestrogen
THE KYNURENE PATHWAY

TRYPTOPHAN

\[
\text{tryptophan pyrrolase} \quad \rightarrow \quad \begin{array}{c}
\text{NH}_2 \\
\text{CH}_2\text{CHCOOH}
\end{array}
\]

\[
\text{tryptophan pyrrolase} \quad \rightarrow \quad \begin{array}{c}
\text{NH}_2 \\
\text{CH}_2\text{CHCOOH}
\end{array}
\]

N-FORMYL KYNURENE

\[
\text{foramidase} \quad \rightarrow \quad \begin{array}{c}
\text{NH}_2 \\
\text{CH}_2\text{CHCOOH}
\end{array}
\]

ANTHRANILIC ACID

KYNURENE

\[
\text{kynurenine hydroxylase} \quad \rightarrow \quad \begin{array}{c}
\text{OH} \\
\text{NH}_2 \\
\text{CH}_2\text{CHCOOH}
\end{array}
\]

KYNURENIC ACID

3-HYDROXYKYNURENE

\[
\text{kynureninase} \quad \rightarrow \quad \begin{array}{c}
\text{COOH} \\
\text{OH} \\
\text{NH}_2
\end{array}
\]

3-HYDROXYANTHRANILIC ACID

XANTHURENIC ACID

8-HYDROXYQUINALDIC ACID

NICOTINIC ACID
conjugates compete for pyridoxal phosphate binding sites on the apoenzyme (Wynn, 1975). The level of plasma pyridoxal phosphate may be reduced in oral contraceptive users (Lumeng et al., 1974; Prasad et al., 1975). In addition, evidence of tissue depletion of vitamin B₆ has been shown by reduced activity and saturation with coenzyme in vitro of pyridoxal phosphate dependent red cell aminotransferases (Rose et al., 1972; Driskell et al., 1976; Bamji, 1978; Bossé and Donald, 1979).

A deficiency of vitamin B₆ in combined pill users could account for the occurrence of depression and of impaired glucose tolerance in these women (Wynn, 1975). Adams et al. (1973; 1974) studied depressed women whose symptoms were judged to be due to the effects of oral contraceptive agents and found biochemical evidence of vitamin B₆ deficiency in 50 percent of these. In a cross-over double-blind trial, the administration of pyridoxine hydrochloride (40mg daily) produced a significant improvement in the B₆ deficient women. No improvement occurred in the non-deficient women, or with placebo in either group. The abnormal tryptophan metabolism (Rose, 1966; Price et al., 1967; Adams et al., 1973; Haspels et al., 1975; Donald and Bossé, 1979) and impaired glucose tolerance (Wynn, 1975) have also been demonstrated to improve with vitamin B₆ supplementation.

It is possible that the incidence of depression in oral contraceptive users is also related to folic acid deficiency since this would reduce serotonin synthesis (Botez et al., 1979). There are many reports in the literature of low serum and red cell folic acid concentrations and raised urinary FIGLU (foriminoglutamic acid) excretion after a histidine load in women using oral contraceptive agents (Shojania et al., 1968; Shojania et al., 1971; Shojania et al., 1975; Smith et al.,
Da Costa and Rothenberg (1974) identified a macromolecular factor in leucocyte lysates of oral contraceptive users which binds unreduced folates and dihydrofolate.

Oral contraceptive agents have also been demonstrated to adversely affect the body's economy of other vitamins: riboflavin (Lancet, 1975; Wynn, 1975; Ahmed et al., 1975; Ahmed and Bamji, 1976; Bamji, 1978; Newman et al., 1978), vitamin B$_{12}$ (Lancet, 1975; Wynn, 1975; Journal of the American Medical Association, 1976), vitamin C (Lancet, 1975; Wynn, 1975) and vitamin A (Lancet, 1975; Wynn, 1975; Ahmed et al., 1975; Bamji, 1978; Bamji and Ahmed, 1978; Vahlqvist et al., 1979).

Oral contraceptive agents have many other more serious adverse effects. These include the frequently publicised effects on the cardiovascular system: deep vein thrombosis, pulmonary embolism, hypertension, stroke and acute myocardial infarction (Lancet, 1977; Royal College of General Practitioners, 1977a,b; Vessey et al., 1977; British Medical Journal, 1977; Jaffe, 1977; Kaplan, 1978; Vessey, 1978; Lancet, 1979; McEwen, 1979), which are thought to be due to the oestrogen component of the pill (Poller, 1978; McQueen, 1978; Shapiro et al., 1979).

There is evidence that the fatty acid composition of the plasma CPG and erythrocyte phospholipids may be affected by a deficiency of vitamin B$_6$ (Dussault and Lepage, 1975; Delorme and Lupien, 1976a) or vitamin B$_{12}$ (Peifer and Lewis, 1979) and may also reflect risk of cardiovascular disease (Dyerberg et al., 1978). Therefore these were determined in women using oestrogen and progestogen combined oral contraceptive agents and in women using progestogen-only oral contraceptive
**Subjects**

Twenty women using oestrogen and progestogen combined and six women using progestogen-only oral contraceptive agents were studied. The mean age of the combined pill users was 26 years, ranging from 19 to 37 years. The mean age of the progestogen-only pill users was also 26 years, ranging from 18 to 40 years. The combined pill group had been using oral contraceptives for an average of 27 months, ranging from 6 to 65 months. The progestogen-only group had been using them for an average of 12 months, ranging from 3 to 24 months. Of the combined pill users, fifteen were using a pill containing 30μg of oestrogen, 2 were using a pill containing 35μg of oestrogen and 3 were using a pill containing 50μg of oestrogen. None of the subjects were depressed at the time of the study, probably due to the low steroid doses used. Controls were women who had never used oral contraceptives or who had not used oral contraceptives for at least six months prior to the study.

**Results**

The fatty acid composition of the plasma CPG is shown in table 30. The combined pill group had higher concentrations of 16:0 (p < 0.05), and 18:2ω6 (p < 0.05) and lower concentrations of 18:0 (p < 0.05), 20:4ω6 (p < 0.05), 20:5ω3 (p < 0.05), 22:5ω3 (p < 0.01) and total LCP (p < 0.05) than matched controls. The progestogen-only group did not differ from matched controls in respect of any of the fatty acids.

Similar differences were observed in the erythrocyte phospholipids, significant differences occurring only in the combined pill group. Their
EPG (table 31) contained a higher concentration of 18:2\textomega{6} (p < 0.05) and lower concentrations of 20:4\textomega{6} (p < 0.05), 20:5\textomega{3} (p < 0.05), 22:5\textomega{3} (p < 0.01) and total LCP (p < 0.05) than matched controls.

The erythrocyte SPG (table 32) of combined pill users contained a higher concentration of 18:2\textomega{6} (p < 0.05) and lower concentrations of 22:5\textomega{3} (p < 0.01) and total LCP (p < 0.05) than matched controls.

The erythrocyte CPG (table 33) of combined pill users contained a higher concentration of 18:2\textomega{6} (p < 0.05) and lower concentrations of 18:0 (p < 0.05) and 22:5\textomega{3} (p < 0.01) than matched controls.

The fatty acid composition of the erythrocyte sphingomyelin was similar in all three groups (table 34).

Discussion

Analyses of the fatty acid composition of the plasma CPG and erythrocyte phospholipids showed that women using oestrogen and progestogen combined oral contraceptive agents had higher concentrations of 18:2\textomega{6} and lower concentrations of LCP than matched controls. Women using progestogen-only oral contraceptive agents had similar concentrations to matched controls, indicating that the oestrogen component was responsible for the differences. Vitamin B\textsubscript{6} deficiency in rats has been reported to increase the concentration of 18:2\textomega{6} and decrease the concentration of 20:4\textomega{6} in tissue lipids (Mueller and Iacono, 1963; Dussault and Lepage, 1975; Delorme and Lupien, 1976a). The reverse occurred with vitamin B\textsubscript{6} supplementation (Scheier and Williams, 1964; Delorme and Lupien, 1976b). Therefore it is likely that the higher 18:2\textomega{6} and lower 20:4\textomega{6} and total LCP concentrations in combined pill users were due to a reduced vitamin B\textsubscript{6} status. The lower concentration
TABLE 30

The fatty acid composition of the plasma choline phosphoglycerides of oral contraceptive users

Results expressed as mean ± S.E. in mg/g total fatty acid methyl esters detected.
Figures in parenthesis indicate the number of subjects on whom the investigation was carried out.

<table>
<thead>
<tr>
<th>Methyl Esters</th>
<th>Users of oestrogen and progestogen combined pill (20)</th>
<th>Users of progestogen only pill (6)</th>
<th>Matched controls (20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>282 ± 5.1*</td>
<td>270 ± 4.5</td>
<td>264 ± 4.0</td>
</tr>
<tr>
<td>18:0</td>
<td>126 ± 3.0*</td>
<td>138 ± 4.3</td>
<td>145 ± 4.2</td>
</tr>
<tr>
<td>18:1ω9</td>
<td>127 ± 4.6*</td>
<td>123 ± 4.2</td>
<td>126 ± 4.4</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>264 ± 5.0*</td>
<td>254 ± 6.6</td>
<td>243 ± 5.3</td>
</tr>
<tr>
<td>20:0</td>
<td>3 ± 0.3</td>
<td>3 ± 0.3</td>
<td>2 ± 0.4</td>
</tr>
<tr>
<td>18:3ω3 + 20:1ω9</td>
<td>4 ± 0.2</td>
<td>4 ± 0.3</td>
<td>3 ± 0.3</td>
</tr>
<tr>
<td>20:2ω6</td>
<td>5 ± 0.4</td>
<td>3 ± 0.3</td>
<td>3 ± 0.3</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>32 ± 1.1*</td>
<td>30 ± 2.6</td>
<td>31 ± 1.1</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>94 ± 2.4*</td>
<td>108 ± 4.8</td>
<td>107 ± 3.6</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>9 ± 0.7*</td>
<td>11 ± 1.2</td>
<td>14 ± 0.8</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>3 ± 0.3</td>
<td>3 ± 0.3</td>
<td>3 ± 0.2</td>
</tr>
<tr>
<td>22:5ω6</td>
<td>3 ± 0.2</td>
<td>2 ± 0.2</td>
<td>2 ± 0.2</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>7 ± 0.5**</td>
<td>12 ± 0.7</td>
<td>13 ± 0.7</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>39 ± 1.1*</td>
<td>38 ± 2.5</td>
<td>44 ± 1.6</td>
</tr>
<tr>
<td>Total LCP</td>
<td>195 ± 5.6*</td>
<td>210 ± 8.5</td>
<td>219 ± 5.6</td>
</tr>
</tbody>
</table>

* p < 0.05
** p < 0.01

compared to controls

Statistical test employed: paired sample t-test.
TABLE 31

The fatty acid and aldehyde composition of the erythrocyte ethanolamine phosphoglycerides of
oral contraceptive users

Results expressed as mean ± S.E. in mg/g total fatty acid methyl esters and dimethyl acetalts detected.
Figures in parenthesis indicate the number of subjects on whom the investigation was carried out.

<table>
<thead>
<tr>
<th>Methyl esters and dimethyl acetals (DMA)</th>
<th>Users of oestrogen and progestogen combined pill (20)</th>
<th>Users of progestogen only pill (6)</th>
<th>Controls (20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 + 18:0 DMA</td>
<td>156 ± 5.4</td>
<td>150 ± 6.8</td>
<td>145 ± 5.6</td>
</tr>
<tr>
<td>18:0 + 18:0 DMA</td>
<td>117 ± 5.5</td>
<td>115 ± 6.2</td>
<td>112 ± 5.9</td>
</tr>
<tr>
<td>18:1ω9 + 18:1 DMA</td>
<td>143 ± 6.1†</td>
<td>138 ± 7.1</td>
<td>141 ± 6.0</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>80 ± 4.9†</td>
<td>71 ± 6.0</td>
<td>65 ± 4.2</td>
</tr>
<tr>
<td>20:0</td>
<td>5 ± 0.4</td>
<td>5 ± 0.4</td>
<td>4 ± 0.3</td>
</tr>
<tr>
<td>18:3ω3 + 20:1ω9</td>
<td>4 ± 0.3</td>
<td>4 ± 0.4</td>
<td>3 ± 0.4</td>
</tr>
<tr>
<td>20:2ω6</td>
<td>16 ± 1.3†</td>
<td>15 ± 1.4</td>
<td>14 ± 1.2</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>234 ± 6.1†</td>
<td>240 ± 5.8</td>
<td>258 ± 5.3</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>14 ± 1.3†</td>
<td>19 ± 1.5</td>
<td>20 ± 1.9</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>64 ± 3.0</td>
<td>65 ± 4.4</td>
<td>61 ± 4.3</td>
</tr>
<tr>
<td>22:5ω6</td>
<td>8 ± 0.7†</td>
<td>8 ± 0.8</td>
<td>7 ± 0.6</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>47 ± 3.1†</td>
<td>70 ± 3.8</td>
<td>65 ± 3.9</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>96 ± 5.7†</td>
<td>94 ± 5.8</td>
<td>98 ± 5.4</td>
</tr>
<tr>
<td>Total LCP</td>
<td>486 ± 14.0†</td>
<td>520 ± 15.4</td>
<td>529 ± 10.5</td>
</tr>
</tbody>
</table>

* p < 0.05
** p < 0.01

Statistical test employed: paired sample t-test.
TABLE 32
The fatty acid and aldehyde composition of the erythrocyte serine phosphoglycerides of oral contraceptive users

Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters and dimethyl acetal detected.
Figures in parenthesis indicate the number of subjects on whom the investigation was carried out.

<table>
<thead>
<tr>
<th>Methyl esters and Dimethyl acetal (DMA)</th>
<th>Users of oestrogen and progestogen combined pill</th>
<th>Users of progestogen only pill</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(20)</td>
<td>(6)</td>
<td>(20)</td>
</tr>
<tr>
<td>16:0 + 16:0 DMA</td>
<td>80 ± 6.9</td>
<td>82 ± 6.5</td>
<td>67 ± 5.7</td>
</tr>
<tr>
<td>18:0 + 18:0 DMA</td>
<td>305 ± 5.0</td>
<td>306 ± 5.8</td>
<td>302 ± 5.4</td>
</tr>
<tr>
<td>18:1ω9 + 18:1 DMA</td>
<td>96 ± 5.9</td>
<td>98 ± 6.2</td>
<td>94 ± 6.5</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>47 ± 3.8</td>
<td>44 ± 4.9</td>
<td>34 ± 4.5</td>
</tr>
<tr>
<td>20:0</td>
<td>4 ± 0.3</td>
<td>4 ± 0.3</td>
<td>4 ± 0.3</td>
</tr>
<tr>
<td>18:3ω3 + 20:1ω9</td>
<td>5 ± 0.4</td>
<td>5 ± 0.3</td>
<td>3 ± 0.2</td>
</tr>
<tr>
<td>20:2ω6</td>
<td>3 ± 0.3</td>
<td>20 ± 2.4</td>
<td>22 ± 1.6</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>24 ± 2.0</td>
<td>240 ± 8.2</td>
<td>256 ± 5.4</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>24 ± 7.6</td>
<td>10 ± 0.8</td>
<td>11 ± 1.2</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>8 ± 0.6</td>
<td>36 ± 2.4</td>
<td>40 ± 3.9</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>37 ± 2.2</td>
<td>9 ± 1.5</td>
<td>11 ± 1.4</td>
</tr>
<tr>
<td>22:5ω6</td>
<td>9 ± 1.3</td>
<td>42 ± 2.5</td>
<td>42 ± 1.6</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>30 ± 2.3</td>
<td>99 ± 5.0</td>
<td>106 ± 5.1</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>103 ± 4.7</td>
<td>463 ± 14.2</td>
<td>495 ± 7.6</td>
</tr>
<tr>
<td>Total LCP</td>
<td>460 ± 11.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\* \( p < 0.05 \) compared to controls.  
\** \( p < 0.01 \) 

Statistical test employed: paired sample t-test.
TABLE 33
The fatty acid and aldehyde composition of the erythrocyte choline phosphoglycerides of oral contraceptive users

<table>
<thead>
<tr>
<th>Methyl esters and dimethyl acetal (DMA)</th>
<th>Users of oestrogen and progestogen combined pill (20)</th>
<th>Users of progestogen only pill (6)</th>
<th>Controls (20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 + 16:0 DMA</td>
<td>272 ± 5.7 *</td>
<td>267 ± 6.0</td>
<td>264 ± 5.9</td>
</tr>
<tr>
<td>18:0 + 18:0 DMA</td>
<td>123 ± 4.2</td>
<td>125 ± 4.3</td>
<td>139 ± 3.9</td>
</tr>
<tr>
<td>18:1ω9 + 18:1 DMA</td>
<td>176 ± 3.9 *</td>
<td>175 ± 4.8</td>
<td>182 ± 2.9</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>236 ± 7.4</td>
<td>236 ± 8.8</td>
<td>211 ± 8.5</td>
</tr>
<tr>
<td>20:0</td>
<td>3 ± 0.4</td>
<td>3 ± 0.4</td>
<td>2 ± 0.3</td>
</tr>
<tr>
<td>18:3ω3 + 20:1ω9</td>
<td>4 ± 0.4</td>
<td>4 ± 0.4</td>
<td>4 ± 0.3</td>
</tr>
<tr>
<td>20:2ω6</td>
<td>4 ± 0.4</td>
<td>3 ± 0.4</td>
<td>4 ± 0.3</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>27 ± 0.7</td>
<td>22 ± 1.2</td>
<td>24 ± 0.9</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>93 ± 5.3</td>
<td>95 ± 6.1</td>
<td>99 ± 3.7</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>8 ± 0.8</td>
<td>8 ± 0.6</td>
<td>11 ± 0.9</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>6 ± 0.7</td>
<td>8 ± 0.5</td>
<td>7 ± 0.6</td>
</tr>
<tr>
<td>22:5ω6</td>
<td>3 ± 0.4*</td>
<td>3 ± 0.4</td>
<td>3 ± 0.4</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>7 ± 0.9</td>
<td>12 ± 1.3</td>
<td>12 ± 1.2</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>32 ± 1.7</td>
<td>38 ± 2.3</td>
<td>35 ± 3.1</td>
</tr>
<tr>
<td>Total LCP</td>
<td>185 ± 10.3</td>
<td>192 ± 12.5</td>
<td>198 ± 9.3</td>
</tr>
</tbody>
</table>

\* \textit{p < 0.05} \quad \text{compared to controls.}

\textit{p < 0.01}

Statistical test employed: paired sample t-test.
### TABLE 34
The fatty acid composition of the erythrocyte sphingomyelin of oral contraceptive users

Results expressed as mean ± S.E. in mg/g total fatty acid methyl esters detected.

Figures in parenthesis indicate the number of subjects on whom the investigation was carried out.

<table>
<thead>
<tr>
<th>Methyl esters</th>
<th>Users of oestrogen and progestogen combined pill</th>
<th>Users of progestogen only pill</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(20)</td>
<td>(6)</td>
<td>(20)</td>
</tr>
<tr>
<td>16:0</td>
<td>241 ± 5.7</td>
<td>238 ± 6.3</td>
<td>230 ± 6.0</td>
</tr>
<tr>
<td>18:0</td>
<td>45 ± 4.4</td>
<td>48 ± 5.0</td>
<td>51 ± 4.6</td>
</tr>
<tr>
<td>20:0</td>
<td>17 ± 1.4</td>
<td>18 ± 1.7</td>
<td>19 ± 1.5</td>
</tr>
<tr>
<td>22:0</td>
<td>99 ± 5.7</td>
<td>94 ± 6.1</td>
<td>95 ± 5.4</td>
</tr>
<tr>
<td>23:0 + 20:4ω6</td>
<td>30 ± 3.2</td>
<td>31 ± 3.7</td>
<td>33 ± 3.1</td>
</tr>
<tr>
<td>24:0</td>
<td>226 ± 5.4</td>
<td>228 ± 6.3</td>
<td>231 ± 5.0</td>
</tr>
<tr>
<td>24:1ω9 + 20:5ω3</td>
<td>245 ± 5.2</td>
<td>253 ± 5.9</td>
<td>250 ± 4.7</td>
</tr>
<tr>
<td>24:2ω6</td>
<td>29 ± 2.7</td>
<td>34 ± 3.1</td>
<td>31 ± 2.5</td>
</tr>
</tbody>
</table>

There are no significant differences between any of the groups.

Statistical test employed: paired sample t-test.
of 22:5ω3 in these women may also be a result of vitamin B₆ deficiency, since Goswami and Coniglio (1966) found that vitamin B₆ supplementation produced an increase in the concentration of 22:5ω3 in rat testicular lipids.

Deficiency of vitamin B₆ does not reduce the synthesis of 20:4ω6 from 18:2ω6 (Dussault and Lepage, 1975). Dussault and Lepage (1975) found that the incorporation of 20:4ω6 into liver lipids was reduced in vitamin B₆ deficient rats, but in a later study (Dussault and Lepage, 1979) they showed that the incorporation of 20:4ω6 into liver lipids was normal but that the incorporation of 16:0 and 18:2ω6 was increased. Delorme and Lupien (1976a) postulated that in vitamin B₆ deficiency there may be an alteration in the relative importance of the two pathways by which the CPG are synthesised: a relative decrease in the methylation of EPG (which produces CPG with a high LCP and low 18:2ω6 content) and increase in the CDP-choline (cytidine disphosphocholine) pathway (which produces CPG with a low LCP and high 18:2ω6 content). If this is correct, it would explain the findings of the present study.

Deficiency of thiamin (Williams et al., 1967) or of vitamin B₁₂ (Peifer and Lewis, 1979) affects fatty acid metabolism in the same way as vitamin B₆ deficiency. Thiamin deficiency does not occur in women using oestrogen and progestogen combined oral contraceptive agents (Ahmed et al., 1975), but vitamin B₁₂ deficiency has been reported (Lancet, 1975; Wynn, 1975; Journal of the American Medical Association, 1976). The latter may therefore be partly responsible for the observed differences in phospholipid fatty acid composition in these women.

It has been suggested that the high concentration of 20:5ω3 in the lipids of Eskimos protects them against thrombosis (Dyerberg et al., 1975; Dyerberg et al., 1978) as this fatty acid does not induce platelet aggregation.
in human platelet-rich plasma. This was thought to be due to the formation of thromboxane $A_3$ which does not have platelet aggregating properties and also to the synthesis of an anti-aggregating agent, a prostacyclin of the "3" series (Dyerberg et al., 1978). Thus it is possible that the low concentration of 20:5ω3 in the plasma CPG and erythrocyte phosphoglycerides of women using oestrogen and progestogen combined oral contraceptive agents may relate to their increased risk of thrombosis. According to Dyerberg et al. (1978) the ratio of 20:4ω6 : 20:5ω3 is important, higher ratios indicating higher risk. In the plasma CPG of combined pill users the ratio was 10, compared with 7 in the controls. Even higher values would be expected in women taking higher doses of these steroids.

The low concentrations of LCP in the erythrocyte phosphoglycerides of combined pill users may reduce the permeability of the membrane and affect the activity of certain membrane-bound enzymes (van Deenan, 1971; McElhaney, 1974). ATPase activities have been reported to be normal in combined pill users (Kaplay and Ramanadham, 1978) but Kamyab et al. (1978) found a lower urinary excretion of potassium. They suggested that this may be due to a retention of potassium within cells. Kaplay and Ramanadham (1978) showed that erythrocyte potassium concentrations tended to be higher in combined pill users but were not significantly different from those of controls.

In conclusion, it appears that the differences in phospholipid fatty acid composition observed in women using oestrogen and progestogen combined oral contraceptive agents were a result of their reduced vitamin $B_6$ and $B_{12}$ status. Unfortunately none of these women were depressed at the time of the study. This was probably due to the low steroid doses used (Allen, 1974): women using oral contraceptive agents containing
more than 50μg of oestrogen were not available for study. The low concentrations of LCP and high concentration of 18:2\omega6 found in combined pill users were the opposite of those observed in patients suffering from endogenous depression (chapter three).
Introduction

The aetiology of the high LCP concentrations in the plasma CPG, and to a lesser extent in the erythrocyte phospholipids, of patients suffering from endogenous depression is not clear. The abnormality could be due to increased dietary intake or to increased production of these fatty acids. Alternatively, the high LCP concentrations could be the result of a greater rate of incorporation of these fatty acids into lipids or a low phospholipase A2 activity.

LCP are found in animal products (Crawford and Sinclair, 1972), the main sources of 22:6ω3 being brain, liver, fish, chicken, turkey and egg (Paul and Southgate, 1978).

Δ6 desaturase is the rate limiting enzyme in the synthesis of LCP (Castuma et al., 1972; Brenner, 1974; Brenner, 1977) and requires a number of cofactors (CoA, ATP, Mg2+ and NADH) for optimum activity (Cook, 1978). There are also several other factors - dietary and hormonal (de Gomez Dumm et al., 1970; Peluffo et al., 1971; Faas et al., 1972; Brenner, 1974; Peluffo et al., 1976; de Gomez Dumm et al., 1976; de Gomez Dumm et al., 1977a,b; Faas et al., 1977; Brenner, 1977) - which affect the activity of this enzyme (see Table 35). There is controversy concerning the effect of thyroxine on Δ6 desaturase: in vitro studies have shown an increase in Δ6 desaturase activity (Faas et al., 1972; Faas et al., 1977) but in vivo studies have shown that thyroxine depresses Δ6 desaturase activity (de Gomez Dumm et al., 1977b). The latter workers measured the desaturation reaction through the conversion of labelled 18:2ω6 to 18:3ω6 in rat microsomes whereas in the studies of Faas et al.,
TABLE 35
Factors influencing Δ6 desaturase activity

<table>
<thead>
<tr>
<th>Factor</th>
<th>Induction of Enzyme</th>
<th>Depression of Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Diet</td>
<td>EFA deficiency</td>
<td>Fasting</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>Carbohydrates</td>
</tr>
<tr>
<td>2. Hormones</td>
<td>Insulin</td>
<td>Glucagon</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adrenalin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thyroxine</td>
</tr>
<tr>
<td>3.</td>
<td>ATP</td>
<td>Cyclic AMP</td>
</tr>
<tr>
<td>4. Environmental</td>
<td>Reduction</td>
<td>Increase</td>
</tr>
<tr>
<td>temperature</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(1972; 1977) only indirect conclusions were obtained. Thus it seems more likely that thyroxine depresses Δ6 desaturase activity.

The results of chapter five suggest that oestrogen may also affect phospholipid LCP composition. Furthermore, many of the patients studied were receiving a hypnotic and/or tranquilliser so that the effects of these require evaluation.

The effects of drugs, diet and hormones on phospholipid LCP composition were therefore investigated. It was not possible to measure the rate of incorporation of LCP into lipids or phospholipase \( \Delta_2 \) activity due to ethical considerations in humans and the difficulty of producing the syndrome of endogenous depression in experimental animals.

**Methods and Results**

**(a) Effect of Drugs**

The effects of nitrazepam (Mogadon), flurezepam (Dalmane), diazepam (Valium) and chlormethiazole edisylate (Heminevrin) on the fatty acid composition of the plasma CPG were investigated experimentally using adult female Wistar albino rats. Each drug was mixed with distilled water and administered with the aid of an oral dosing needle to a group of five animals for a period of four weeks. Per kilogram body weight, four times the human dose of each drug was used as rats have a much greater ability to metabolise drugs. The control group received distilled water.

The results are shown in table 36. None of the drugs investigated affected the concentration of \( 20:5\omega3 \) or \( 22:6\omega3 \) but both nitrazepam \( (p < 0.01) \) and diazepam \( (p < 0.02) \) reduced the concentration of \( 18:2\omega6 \). All four drugs increased the concentration of \( 16:0 \) (nitrazepam, flurezepam
and diazepam: \( p < 0.02 \); chlormethiazole edisylate: \( p < 0.01 \).

\( (b) \) Effect of Diet

Dietary information was collected by 24-hour recall and dietary history (Mann et al., 1962) from fifteen patients suffering from endogenous depression, fifteen patients suffering from reactive depression and fifteen controls. Weighed intakes were not feasible due to the commencement of treatment on the day after admission.

Dietary histories revealed that the frequency of eating foods which contain considerable amounts of 22:6\( \omega3 \) was similar in all three groups: liver, fish and chicken were eaten approximately once a week and egg two to three times per week. Total energy intakes (calculated from 24-hour recall) of patients with endogenous depression (\( p < 0.001 \)) and of those with reactive depression (\( p < 0.001 \)) were significantly lower than those of controls (Table 37). However there were no differences in the proportions of energy derived from protein, fat or carbohydrate (Table 37).

Sanders (1977) showed that there was a correlation between protein intake and plasma viscosity. The plasma viscosities of patients suffering from endogenous or reactive depression were slightly lower than those of controls (Table 38) but the differences were not statistically significant.

\( (c) \) Effect of Hormones

Fasting serum insulin, fasting blood glucose, serum thyroxine, plasma cyclic AMP and plasma 17-\( \beta \)-oestradiol concentrations were determined in psychiatric patients and controls. Plasma cyclic AMP and 17-\( \beta \)-oestradiol were only measured in men and postmenopausal women as they vary with the menstrual cycle (Holmes, 1975; Wright, 1971).
TABLE 36

The effects of drugs on the fatty acid composition of rat plasma choline phosphoglycerides

Results expressed as mean ± S.E. in mg/g total fatty acid methyl esters detected.

<table>
<thead>
<tr>
<th>Methyl esters</th>
<th>Control group</th>
<th>Nitrazepam group</th>
<th>Flurazepam group</th>
<th>Diazepam group</th>
<th>Chlormethiazole edisylate group</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>155 ± 4.3</td>
<td>170 ± 3.4</td>
<td>174 ± 4.0</td>
<td>176 ± 4.7</td>
<td>198 ± 4.6</td>
</tr>
<tr>
<td>18:0</td>
<td>250 ± 4.6</td>
<td>249 ± 3.7</td>
<td>252 ± 3.5</td>
<td>240 ± 4.9</td>
<td>242 ± 4.3</td>
</tr>
<tr>
<td>18:1ω9</td>
<td>63 ± 1.7</td>
<td>59 ± 2.9</td>
<td>67 ± 3.5</td>
<td>68 ± 4.0</td>
<td>65 ± 2.0</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>175 ± 4.5</td>
<td>150 ± 3.4</td>
<td>177 ± 4.1</td>
<td>156 ± 3.7</td>
<td>177 ± 4.0</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>226 ± 5.4</td>
<td>235 ± 4.7</td>
<td>222 ± 5.2</td>
<td>231 ± 5.6</td>
<td>221 ± 5.2</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>8 ± 0.8</td>
<td>8 ± 0.9</td>
<td>7 ± 0.6</td>
<td>7 ± 0.5</td>
<td>8 ± 0.7</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>63 ± 4.5</td>
<td>65 ± 3.1</td>
<td>62 ± 1.8</td>
<td>61 ± 1.9</td>
<td>54 ± 1.8</td>
</tr>
</tbody>
</table>

*p < 0.02

**p < 0.01

compared to control group.

Statistical test employed: paired sample t-test.
### TABLE 37

**Nutrient intakes of depressed patients**

Results expressed as mean \(\pm S.E\).

Figures in parenthesis indicate the number of subjects studied.

<table>
<thead>
<tr>
<th></th>
<th>Total Energy</th>
<th>Protein</th>
<th>Fat</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kcals</td>
<td>MJ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endogenous Depressives (15)</td>
<td>1239*</td>
<td>5.20*</td>
<td>13</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>(\pm) 101</td>
<td>0.42</td>
<td>1.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Reactive Depressives (15)</td>
<td>1486*</td>
<td>6.26*</td>
<td>14</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>(\pm) 166</td>
<td>0.70</td>
<td>1.1</td>
<td>3.6</td>
</tr>
<tr>
<td>Controls (15)</td>
<td>2081</td>
<td>8.75</td>
<td>14</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>(\pm) 110</td>
<td>0.46</td>
<td>1.0</td>
<td>3.2</td>
</tr>
</tbody>
</table>

* Indicates significant differences from controls (\(p < 0.001\)).

Statistical test employed: student's t-test.
### TABLE 38
Plasma viscosity of patients suffering from depressive illness

*Results expressed as mean ± S.E.*

<table>
<thead>
<tr>
<th></th>
<th>Plasma viscosity (cp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endogenous depressives (22)</td>
<td>1.62 ± 0.02</td>
</tr>
<tr>
<td>Reactive depressives (20)</td>
<td>1.64 ± 0.02</td>
</tr>
<tr>
<td>Controls (22)</td>
<td>1.67 ± 0.02</td>
</tr>
</tbody>
</table>

*Figures in parenthesis indicate the number of subjects studied.*

*Differences between groups are not statistically significant.*

*Statistical test employed: student t-test.*
Fasting serum insulin (Table 39), fasting blood glucose (Table 39) and serum thyroxine concentrations (Figure 16) of patients suffering from endogenous depression were similar to those of patients suffering from reactive depression, patients with other psychiatric disorders and controls. Plasma cyclic AMP concentrations (Figure 17) were significantly lower ($p < 0.05$) in patients suffering from endogenous depression than in matched controls. Values for both patients and controls were similar to those reported by Lykouras et al. (1978). Plasma 17-β-oestradiol concentrations of patients suffering from endogenous depression did not differ significantly from those of matched controls (Figure 18).

Fasting serum insulin, the ratio of insulin to glucose, serum thyroxine and plasma 17-β-oestradiol did not correlate with the concentration of 22:6ω3 in the plasma CPG. However a negative correlation was found between plasma cyclic AMP and the 22:6ω3 concentration in the plasma CPG ($r = -0.81, p < 0.01$; Figure 19).
### TABLE 39
Fasting serum insulin and fasting blood glucose concentrations

*Results expressed as mean ±S.E.*

<table>
<thead>
<tr>
<th></th>
<th>Insulin (μU/l)</th>
<th>Glucose (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endogenous depressives (16)</td>
<td>7 ± 0.9</td>
<td>4.4 ± 0.08</td>
</tr>
<tr>
<td>Reactive depressives (15)</td>
<td>8 ± 1.1</td>
<td>4.2 ± 0.10</td>
</tr>
<tr>
<td>Other psychiatric patients (5)</td>
<td>8 ± 1.9</td>
<td>4.0 ± 0.12</td>
</tr>
<tr>
<td>Controls (16)</td>
<td>7 ± 0.9</td>
<td>4.4 ± 0.13</td>
</tr>
</tbody>
</table>

*Figures in parenthesis indicate the number of subjects studied.*

*There are no significant differences between any of the groups.*

*Statistical test employed: student t-test.*
Figure 16
Serum thyroxine concentrations

- = mean

---

Endogenous Depressives
Reactive Depressives
Other Psychiatric Patients
Healthy Controls

(nmol/l)
Figure 17
Plasma cyclic AMP concentrations

--- = mean
p < 0.05

Endogenous Depressives
Matched Controls
Figure 18

Plasma 17-β-oestradiol concentrations

--- = mean

17-β-oestradiol (pg/ml)

Endogenous Depressives

Matched Controls
phosphoglycerides against the plasma cyclic AMP concentrations

\[ y = -1.79x + 87.35 \]
\[ r = -0.81 \]
\[ p < 0.01 \]
Discussion

The effects of drugs, diet and hormones on phospholipid LCP composition have been examined in an attempt to discover the cause of the high LCP concentrations observed in patients suffering from endogenous depression.

Administration to adult female rats, of drugs taken by some of the patients in the study showed that they had no effect on the concentration of 20:5ω3 or 22:6ω3 in the plasma CPG. Although the results of animal experiments are not necessarily applicable to the human situation, they suggest that the high LCP concentrations in patients with endogenous depression are not an artifact of their chemotherapy. Both nitrazepam and diazepam reduced the concentration of 18:2ω6 and therefore use of these drugs may be responsible, at least in part, for the low concentrations of this fatty acid in patients with endogenous depression. However, drug-free patients suffering from endogenous depression (for example those referred from general practitioners) also had lower 18:2ω6 concentrations than matched controls (chapter three), suggesting that other factors may also be involved.

Dietary histories revealed that patients suffering from endogenous depression did not consume greater quantities of foods containing 22:6ω3 than those suffering from reactive depression or controls and analysis of the 24-hour recalls showed that although the total energy intake of both groups of depressed patients was lower than that of controls, the proportions of energy derived from protein, carbohydrate and fat were similar in all three groups. This rules out the possibility of an increase in Δ6 desaturase activity produced by a high protein or low carbohydrate diet. The other dietary factor which increases Δ6 desaturase activity is essential fatty acid deficiency. This would however result
in increased concentrations of 18:1ω9 and 20:3ω9 and in reduced concentrations of 20:4ω6 as well as 18:2ω6. These differences were not observed in patients suffering from endogenous depression (see chapter three). Thus, although the dietary information is of limited value, it seems unlikely that the high concentrations of LCP observed in patients with endogenous depression are due to differences in diet.

Plasma cyclic AMP concentrations were significantly lower in patients suffering from endogenous depression than in controls (p < 0.05). This is in agreement with reports by other workers of low plasma (Holmes, 1975; Lykouras et al., 1978) and urinary (Abdullah and Hamadah, 1970; Paul et al., 1970; Paul et al., 1971; Naylor et al., 1974; Holmes, 1975; Sinanan et al., 1975; Moyes and Moyes, 1976) cyclic AMP concentrations in patients with endogenous depression. Cyclic AMP depresses Δ6 desaturase activity and therefore LCP synthesis will be increased when cyclic AMP is reduced. In the present study a negative correlation was found between plasma cyclic AMP and the concentration of 22:6ω3 in the plasma CPG (r = -0.81, p < 0.01), indicating that the high LCP concentrations in patients with endogenous depression may be a result of their low cyclic AMP concentrations. Plasma cyclic AMP has been shown to be increased by vigorous exercise (Holmes, 1975; Lin, 1978). However, the increase is transient (Holmes, 1975) and therefore differences in activity between patients and controls are unlikely to be responsible for the difference in plasma cyclic AMP concentrations.

ATP, from which cyclic AMP is formed, is a cofactor for Δ6 desaturase (Cook, 1978) and has been reported to induce the enzyme (Brenner, 1977). However a difference in ATP concentrations is unlikely to be responsible for the high LCP concentrations since blood levels of ATP have been found to be low (Hansen, 1972; Hansen and Dimitrakoudi, 1974) or normal (Naylor et al., 1976) in endogenous depression. Magnesium, another
cofactor of Δ6 desaturase (Cook, 1978) has been found to be low (Frizel et al., 1969) and normal (Naylor et al., 1972) in plasma and high (Bjorum, 1972; Hakim et al., 1975a) in serum in endogenous depression. Hence, no conclusion can be reached as to whether the high LCP concentrations are related to an abnormality of magnesium metabolism.

Hormones which alter the activity of Δ6 desaturase exert their effect via cyclic AMP. The formation of cyclic AMP from ATP is catalysed by adenyl cyclase (Rall and Sutherland, 1962; Robison et al., 1971). Thyroxine, glucagon and the catecholamines increase adenyl cyclase activity and thus increase cyclic AMP synthesis, whereas insulin has the opposite effect (Robison et al., 1971; de Gomez Dumm et al., 1977b). In the present study, no abnormality was found in fasting serum insulin, fasting blood glucose or serum thyroxine concentrations. Thus, in the light of the biogenic amine hypothesis of affective disorders (Schildkraut, 1965; Bunney and Davis, 1965), it is possible that the low cyclic AMP concentrations in patients with endogenous depression could be the result of a functional deficiency of catecholamines.

In conclusion, the high LCP concentrations in patients suffering from endogenous depression are thought to be due to an increase in Δ6 desaturase activity produced by a decrease in cyclic AMP. However, there could also be an increase in the rate of incorporation of LCP into lipids and/or a decrease in phospholipase A₂ in these patients.
The preliminary observations of Ellis and Sanders (1977) of high LCP concentrations in the plasma CPG in endogenous depression have been confirmed in a larger series of patients. The present study showed that patients suffering from endogenous depression and those suffering from postpartum depression had higher concentrations of LCP and lower concentrations of 18:2ω6 in their plasma CPG than matched controls, whereas women using oestrogen and progestogen combined oral contraceptive agents had lower LCP concentrations and higher concentrations of 18:2ω6 than matched controls. Patients suffering from reactive depression, those suffering from other psychiatric disorders and women using progestogen-only oral contraceptive agents did not differ from matched controls in respect of any of the fatty acids. Differences in the fatty acid composition of the erythrocyte membrane phospholipids were in the same direction as those of the plasma CPG, but were less marked. It is likely that these differences are reflected in other tissues (Mohrhauer and Holman, 1963a,b,c), for example the liver and the brain.

Because of the difficulty of classifying some depressed patients as "endogenous" or "reactive" using existing clinical criteria, the possibility that measurement of the plasma CPG LCP concentrations might be of value as a diagnostic tool was investigated. Although the difference in LCP, in particular 22:6ω3, between endogenous patients and matched controls was highly significant (p < 0.001), it was insufficient to be used as the sole diagnostic criterion (see chapter three). However, it may be of value in conjunction with other methods.

The differences in the fatty acid composition of the erythrocyte phospholipids may affect membrane function (van Deenan, 1971; McElhaney,
An increase in the LCP content would tend to increase the fluidity of
the membrane and hence increase its permeability and the activity of
certain membrane-bound enzymes, for example \( \text{Na}^+\text{K}^+\text{ATPase} \). A decrease in
the LCP content would have the opposite effect. Mendels and Frazer
(1973; 1974; 1975) suggested that the high lithium ratios (RBC:plasma)
in patients with manic-depressive illness, compared with those of controls,
were due to a greater leak of lithium into the cell rather than to reduced
active transport out of the cell, since lithium is not removed from
the cell by \( \text{Na}^+\text{K}^+\text{ATPase} \) (Maizels, 1968; Duhm and Becker, 1977). However,
studies of passive transport in patients suffering from manic-depressive
illness have shown that this is normal (Frazer et al., 1978) or low (Naylor
et al., 1970b). Studies of active transport have also been contradictory:
both normal (Naylor et al., 1973; Frazer et al., 1978) and low (Naylor
et al., 1970b; Choi et al., 1977; Hesketh et al., 1977) values have been
reported. Passive transport does not appear to have been studied in
women using oestrogen and progestogen combined oral contraceptive agents.
Active transport has been found to be normal (Kaplay and Ramanadham, 1978).
If the results of Frazer et al. (1978) and Kaplay and Ramanadham (1978)
are correct that is, there is no abnormality in either passive or active
transport in patients with manic-depressive illness or in active transport
in combined pill users, then it must be concluded that the observed
differences in erythrocyte phospholipid fatty acid composition may be of
insufficient magnitude to affect membrane function. However, if there is
no abnormality in either passive or active transport in manic-depressive
illness, how do the high lithium ratios occur in these patients?

Other factors which affect membrane function are the relative
proportions of phospholipids (Kimelberg and Papahadjopoulos, 1972;
de Pont and Bonting, 1977; Cooper et al., 1977; Borochov et al., 1977)
and the ratio of cholesterol:phospholipid (Cooper, 1970; Cooper, 1978; Cooper et al., 1978; Le Grimellec and Leblanc, 1978; Owen and MacIntyre, 1978; Chen et al., 1978) but these were normal in patients with endogenous depression. The protein content of the membrane may also affect its function (Lenaz, 1979) and this is a possibility for future study.

The fact that some LCP (20:3\(\Delta^6\), 20:4\(\Delta^6\) and 30:5\(\Delta^3\)) are precursors of prostaglandins and related compounds (Alfin-Slater and Aftergood, 1968; Galli et al., 1977) suggests that there may be abnormalities in the synthesis of these compounds in patients with endogenous depression, patients with postpartum depression and women using oestrogen and progestogen combined oral contraceptive agents. The rate of synthesis of prostaglandins is dependent upon the rate of release of the fatty acids from phospholipids, controlled by phospholipase \(A_2\) (Horton, 1975; Schoene and Iacono, 1976; Flower and Blackwell, 1976). It is not possible to state with certainty the direction in which prostaglandin synthesis would be altered in each group because the activities of phospholipase \(A_2\) are unknown.

Phospholipids of women using oestrogen and progestogen combined oral contraceptive agents contained low concentrations of 20:4\(\Delta^6\) and 20:5\(\Delta^3\). High concentrations of 20:5\(\Delta^3\) in lipids have been suggested to protect against thrombosis (Dyerberg et al., 1978) and therefore the low concentrations in combined pill users may be related to their risk of thrombosis. The phospholipids of patients with endogenous depression and of those with postpartum depression contained high concentrations of 20:5\(\Delta^3\). Treatment of endogenous depression by ECT or tricyclic antidepressants was associated with a decrease in the concentration of this fatty acid and in other LCP. Studies of the effect of electroconvulsive shock in rats (Bazán, 1970; Bazán and Rakowski, 1970; Bazán, 1971) showed that the treatment produced an increase in brain free fatty acid concentrations, in particular 20:4\(\Delta^6\). This was thought to be due to an
increased release of fatty acids from phospholipids. Hence it is possible that phospholipase A_2 activity may be low in endogenous depression with a resultant decrease in the synthesis of prostaglandins and related compounds. Brandrup and Randrup (1967) suggested that arteriosclerosis occurs more frequently in patients with manic-depressive illness than in controls. This does not seem to have been considered by more recent studies, but if correct may relate to the proposed low rate of release of 20:5ω3 from phospholipids.

The importance of the high concentration of 22:6ω3 in patients suffering from endogenous depression and in those suffering from postpartum depression remains to be discovered. This fatty acid occurs in high concentrations in membranes of the brain (Svennerholm, 1968; Crawford and Sinclair, 1972) and in the retina of the eye (Anderson and Maude, 1970; Borggreven et al., 1970; Nielsen et al., 1970) but its specific function is unknown.

The low total LCP concentration observed in women using oestrogen and progestogen combined oral contraceptive agents is of interest because this is the opposite of that observed in patients with endogenous depression and in those with postpartum depression. The low LCP was due to the oestrogen component of the pill since women using progestogen-only preparations had normal total LCP concentrations. Low concentrations of oestrogen and progesterone may play a role in the development of postpartum depression (Dalton, 1971) and therefore this suggests that a deficiency of oestrogen may be involved in the development of endogenous depression. However, the plasma 17-β-oestradiol concentrations of patients with endogenous depression were found to be similar to those of matched controls (see chapter six). Although the total LCP concentrations in patients with endogenous depression or postpartum depression were the opposite of those in combined pill users, there were differences in the individual fatty
acids which were affected. For example, the concentration of 22:6ω3 was high in patients with endogenous depression and in those with postpartum depression, but was normal in women using oestrogen and progestogen combined oral contraceptive agents; the concentration of 22:5ω3 was low in the combined pill group, but was normal in patients with endogenous depression and in those with postpartum depression. Hence other factors must have been responsible for the high LCP in endogenous depression and postpartum depression.

The most likely reason for the high LCP concentrations in patients with endogenous depression or postpartum depression is a high Δ6 desaturase activity. Factors affecting the activity of this enzyme were investigated in chapter six. Diet and drugs taken by some of the patients were unlikely to have produced the high LCP concentrations but hormonal factors may have been responsible. Plasma cyclic AMP concentrations were found to be significantly lower in patients with endogenous depression than in matched controls, in agreement with other studies (Holmes, 1975; Lykouras et al., 1978). A negative correlation was discovered between the plasma cyclic AMP concentrations and the concentrations of 22:6ω3 in the plasma CPG. Thus, the high LCP concentrations were probably due to the low cyclic AMP concentrations, which in turn may have been the result of a functional deficiency of catecholamines (Schildkraut, 1965; Bunney and Davis, 1965; Kunitada et al., 1978). It is interesting to note that Δ6 desaturase activity is lowest in summer (Peluffo and Brenner, 1974) when the incidence of depression is also low (Eastwood and Peacocke, 1976).

In addition to the proposed increase in Δ6 desaturase activity in endogenous and postpartum depression, there may be an increase in the rate of incorporation of LCP into lipids and/or a decrease in phospholipase A₂ activity. The latter could be produced by a deficiency or noradrenalin
Further studies of passive transport are required in order to establish whether this is abnormal in patients suffering from endogenous depression, patients with postpartum depression and in women using oestrogen and progestogen combined oral contraceptive agents. Studies could also be undertaken to combine analyses of the lipid and protein components of the erythrocyte membrane with measurements of both passive and active transport in these three groups.
REFERENCES

Ahmed F. and Bamji M.S. (1976) Contraception 14, 297-308
Aylward M. and Maddock J. (1973) Lancet 1, 936
Bjorum N. and Kirkegaard C. (1979) Lancet 2, 694
Cambridge University press.
Carlsson A. (1976) Pharmakopsychiatrie Neuropsychopharmakol. 9, 2-10
In "Psychotropic Drugs". Ed. by S. Garattini and V. Ghetti. Elsevier; Amsterdam p.363-370
Biol. Psychiat. 12, 5-17
Carney M.W.P. and Sheffield B.F. (1973) Lancet 1, 1505-1506
Carroll B.J. and Davies B.M. (1970) B.M.J. 1, 789-791
Cauter E. van, and Mendelwicz J. (1978) Life Sci. 22, 147-156
Checkley S.A. (1979) Lancet 1, 1081
Colburn R.W., Goodwin F.K., Bunney W.E. Jr. & Davis J.M. (1967)
Nature 215, 1395-1397
Craig G.M. (1976) B.M.J. 2 , 44
Dencker S.J., Häggendal J., and Malm U. (1966a) Lancet 2 , 754
Dennis K.J. and Jeffrey J.D. (1968) Lancet 2 , 454-455
Drug and Therapeutics Bulletin (1978) 16 , 86-87
Friedman E. and Gershon S. (1973) Nature 243 , 4
Fulco A.J. and Mead J.F. (1959) J. Biol. Chem. 234 , 1411-1416
J. Lipid Res. 11, 96-101
J. Lipid Res. 17, 616-621
Gómez Dumm C.L.A. de and Tacconi de Alaniz M.J. (1977a)
Mol. Cell. Biochem. 18, 15-20
Goodwin F.K., Murphy D.L. and Bunney W.E. (1968)
Amer. J. Psychiat. 130, 73-79
J. Nutr. 89, 210-216
Lancet 1, 254-256
Green A.R. (1978) Lancet 1, 661-666
Biol. Psychiat. 12, 347-357
Hakim A.H., Bomb B.S., Garg A.R. and Singh S.V. (1975a)
J. Assoc. Physicians India 23, 513-517
Hakim A.H., Bomb B.S., Pandey S.K. and Singh S.V. (1975b)
J. Assoc. Physicians India 23, 311-315
Halbreich U., Grunhaus L., and Ben-David M. (1979)
Arch. Gen. Psychiat. 36, 1183-1186
Postgrad. Med. J. 52, (Suppl. 3), 40-44
B.M.J. 2, 18-20
Harris T.H. (1957) Amer. J. Psychiat. 113, 950
Hendley E.P. and Welch B.L. (1975) Life Sci. 16, 45-54
Jorgensen O.S. and Bolwig T.G. (1979) Science 205, 705-707
Journal of the American Medical Association (1976) 68, 419-420
King D.J. (1973) Psychol. Med. 3, 53-65
King D.J. (1975) Psychol Med. 5, 273-275
Kirkegaard C. and Bjorum N. (1980) Lancet 1, 152
Lancet (1972) Lancet 2, 864-865
Lancet (1975) 1, 558
Lancet (1977) 2, 747-748
Lancet(1979) 1, 1063
Ramsey T.A., Frazer A. and Mendels J. (1979) Neuropsychobiol. 5, 1-10
Rose D.P. (1966a) Nature 210, 196-197
Rose H.G. and Oklander M. (1965) J. Lipid Res. 6, 428-431
Royal College of General Practitioners (1977a) Lancet 2, 727-731
Royal College of General Practitioners(1977b) B.M.J. 2, 947
Rybakowski J. and Sowinski J. (1973) Lancet 1, 889
Salvadorini F. and Saba P. (1973) Lancet 2, 964
Sangdee C. and Franz D.N. (1979) Psychopharmacologia (Berlin) 62, 9-16
Schildkraut J.J. (1973) Amer. J. Psychiat. 130, 695-698
Shojania A.M., Pornadyl G. and Barnes P.H. (1968) Lancet 1, 1376

Tod E.D.M. (1964) Lancet 2, 1264-1266


Vessey M.P. (1978) BMJ 2, 721-722


Waxman D. (1968) BMJ 4, 188


Wynn V. (1975) Lancet 1, 561-564
THE BECK INVENTORY

This is a questionnaire in which there are several groups of statements. We want you to circle the number of the one statement in each group which most accurately describes how you feel today - that is now.

If you feel that there are two or more statements in a group that describe how you feel, then circle the statement with the higher number.

If there are two statements that you consider are equally applicable to you, then circle the one that best describes how you feel at present.

Be sure to read all the statements in each group before making your choice.

NAME........................................ DATE.................
A. 0. I do not feel sad.
   1. I feel sad.
   2a I am sad all the time and I can't snap out of it.
   2b I am so sad or unhappy that it is quite painful.
   3. I am so sad or unhappy that I can't stand it.

B. 0. I am not particularly pessimistic or discouraged about the future.
   1a I feel discouraged about the future.
   2a I feel I have nothing to look forward to.
   2b I feel that I won't ever get over my troubles.
   3. I feel that the future is hopeless and that things cannot improve.

C. 0. I do not feel like a failure.
   1. I feel I have failed more than the average person.
   2a I feel I have accomplished very little that is worthwhile or that
      means anything.
   2b As I look back on my life all I can see is a lot of failures.
   3. I feel I am a complete failure as a person (parent, husband, wife).

D. 0. I am not particularly dissatisfied.
   1a I feel bored most of the time.
   1b I don't enjoy things the way I used to.
   2. I don't get satisfaction out of anything any more.
   3. I am dissatisfied with everything.

E. 0. I don't feel particularly guilty.
   1. I feel bad or unworthy a good part of the time.
   2a I feel quite guilty.
   2b I feel bad or unworthy practically all the time now.
   3. I feel as though I am very bad or worthless.

F. 0. I don't feel I am being punished.
   1. I have a feeling that something bad may happen to me.
   2. I feel I am being punished or will be punished.
   3a I feel I deserve to be punished.
   3b I want to be punished.

G. 0. I don't feel disappointed in myself.
   1a I am disappointed in myself.
   1b I don't like myself.
   2. I am disgusted with myself.
   3. I hate myself.

H. 0. I don't feel I am any worse than anybody else.
   1. I am critical of myself for my weaknesses or mistakes.
   2. I blame myself for my faults.
   3. I blame myself for everything bad that happens.

I. 0. I don't have any thoughts of harming myself.
   1. I have thoughts of harming myself but I would not carry them out.
   2a I feel I would be better off dead.
   2b I feel my family would be better off if I were dead.
   3a I have definite plans about committing suicide.
   3b I would kill myself if I could.
J. 0. I don't cry any more than usual.
1. I cry more now than I used to.
2. I cry all the time now, I can't stop.
3. I used to be able to cry but now I can't cry at all even though I want to.

K. 0. I am more irritated now than I ever was.
1. I get annoyed or irritated more easily than I used to.
2. I feel irritated all the time.
3. I don't get irritated at all at the things that used to irritate me.

L. 0. I have not lost interest in other people.
1. I am less interested in other people now than I used to be.
2. I have lost most of my interest in other people and have little feeling for them.
3. I have lost all my interest in other people and don't care about them at all.

M. 0. I make decisions about as well as ever.
1. I try to put off making decisions.
2. I have great difficulty in making decisions.
3. I can't make any decisions at all any more.

N. 0. I don't feel I look any worse than I used to.
1. I am worried that I am looking old or unattractive.
2. I feel that there are permanent changes in my appearance and they make me look unattractive.

O. 0. I can work about as well as before.
1a. It takes extra effort to get started at doing something.
1b. I don't work as well as I used to.
2. I have to push myself very hard to do anything.
3. I can't do any work at all.

P. 0. I can sleep as well as usual.
1. I wake up more tired in the morning than I used to.
2. I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
3. I wake up early every day and can't get more than 5 hours sleep.

R. 0. My appetite is no worse than usual.
1. My appetite is not as good as it used to be.
2. My appetite is much worse now.
3. I have no appetite at all any more.

S. 0. I have not lost much weight, if any, lately.
1. I have lost more than 5 pounds.
2. I have lost more than 10 pounds.
3. I have lost more than 15 pounds.

T. 0. I am no more concerned about my health than usual.
1. I am concerned about aches and pains or upset stomach or constipation.
2. I am so concerned with how I feel or what I feel that it is hard to think of much else.
3. I am completely absorbed in what I feel.

U. 0. I have not noticed any recent change in my interest in sex.
1. I am less interested in sex than I used to be.
2. I am much less interested in sex now.
3. I have lost interest in sex completely.
The fatty acid and aldehyde composition of the erythrocyte ethanolamine phosphoglycerides of patients with reactive depression and of those with other psychiatric disorders

Results expressed as mean ± S.E. in mg/g total fatty acid methyl esters and dimethyl acetalts detected.

<table>
<thead>
<tr>
<th>Methyl esters and dimethyl acetalts (DMA)</th>
<th>Reactive Depressives</th>
<th>Age-sex-matched controls</th>
<th>Other psychiatric patients</th>
<th>Age-sex-matched controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 + 16:0 DMA</td>
<td>144 ± 4.7</td>
<td>148 ± 5.5</td>
<td>144 ± 6.4</td>
<td>145 ± 5.3</td>
</tr>
<tr>
<td>18:0 + 18:0 DMA</td>
<td>111 ± 5.3</td>
<td>116 ± 5.0</td>
<td>114 ± 7.2</td>
<td>118 ± 6.0</td>
</tr>
<tr>
<td>18:1ω9 + 18:1 DMA</td>
<td>143 ± 3.6</td>
<td>139 ± 4.7</td>
<td>138 ± 3.6</td>
<td>142 ± 6.6</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>69 ± 3.4</td>
<td>67 ± 3.6</td>
<td>69 ± 5.0</td>
<td>69 ± 5.4</td>
</tr>
<tr>
<td>20:0</td>
<td>4 ± 0.4</td>
<td>4 ± 0.4</td>
<td>5 ± 0.4</td>
<td>4 ± 0.2</td>
</tr>
<tr>
<td>18:3ω3 + 20:1ω9</td>
<td>4 ± 0.4</td>
<td>3 ± 0.4</td>
<td>4 ± 0.3</td>
<td>3 ± 0.3</td>
</tr>
<tr>
<td>20:2ω6</td>
<td>14 ± 1.0</td>
<td>15 ± 1.1</td>
<td>15 ± 1.5</td>
<td>14 ± 1.3</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>259 ± 5.7</td>
<td>254 ± 5.6</td>
<td>260 ± 8.0</td>
<td>254 ± 7.5</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>19 ± 1.4</td>
<td>18 ± 1.5</td>
<td>17 ± 2.0</td>
<td>18 ± 2.1</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>62 ± 4.0</td>
<td>63 ± 4.0</td>
<td>63 ± 5.0</td>
<td>60 ± 4.3</td>
</tr>
<tr>
<td>22:5ω6</td>
<td>8 ± 0.8</td>
<td>7 ± 0.9</td>
<td>6 ± 0.9</td>
<td>7 ± 0.9</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>62 ± 3.5</td>
<td>63 ± 3.3</td>
<td>58 ± 4.0</td>
<td>60 ± 3.8</td>
</tr>
<tr>
<td>Total LCP</td>
<td>522 ± 13.0</td>
<td>522 ± 13.1</td>
<td>523 ± 18.9</td>
<td>518 ± 18.3</td>
</tr>
</tbody>
</table>

There are no significant differences between patients and matched controls.

Statistical test employed: paired sample t-test.
The fatty acid and aldehyde composition of the erythrocyte serine phosphoglycerides of patients with reactive depression and of those with other psychiatric disorders.

Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters and dimethyl acetals detected.

<table>
<thead>
<tr>
<th>Methyl esters and dimethyl acetals (DMA)</th>
<th>Reactive depressives</th>
<th>Age-sex-matched controls</th>
<th>Other psychiatric patients</th>
<th>Age-sex-matched controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 + 16:0 DMA</td>
<td>72 ± 6.9</td>
<td>66 ± 5.5</td>
<td>70 ± 7.0</td>
<td>69 ± 6.6</td>
</tr>
<tr>
<td>18:0 + 18:0 DMA</td>
<td>304 ± 6.2</td>
<td>306 ± 6.0</td>
<td>305 ± 6.9</td>
<td>302 ± 6.5</td>
</tr>
<tr>
<td>18:1ω9 + 18:1ωDMA</td>
<td>95 ± 5.4</td>
<td>97 ± 5.7</td>
<td>100 ± 6.6</td>
<td>102 ± 7.0</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>40 ± 3.8</td>
<td>35 ± 4.7</td>
<td>37 ± 4.2</td>
<td>37 ± 4.9</td>
</tr>
<tr>
<td>20:0</td>
<td>3 ± 0.3</td>
<td>3 ± 0.3</td>
<td>3 ± 0.3</td>
<td>3 ± 0.3</td>
</tr>
<tr>
<td>18:3ω3 + 20:1ω9</td>
<td>5 ± 0.5</td>
<td>3 ± 0.4</td>
<td>3 ± 0.2</td>
<td>3 ± 0.3</td>
</tr>
<tr>
<td>20:2ω6</td>
<td>4 ± 0.5</td>
<td>3 ± 0.3</td>
<td>3 ± 0.4</td>
<td>3 ± 0.3</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>19 ± 1.5</td>
<td>20 ± 1.1</td>
<td>19 ± 0.6</td>
<td>21 ± 1.3</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>250 ± 6.8</td>
<td>252 ± 4.4</td>
<td>259 ± 4.9</td>
<td>247 ± 6.7</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>11 ± 0.8</td>
<td>11 ± 0.9</td>
<td>9 ± 0.9</td>
<td>9 ± 1.0</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>37 ± 2.1</td>
<td>39 ± 3.1</td>
<td>39 ± 4.7</td>
<td>37 ± 4.4</td>
</tr>
<tr>
<td>22:5ω6</td>
<td>9 ± 0.6</td>
<td>10 ± 0.9</td>
<td>8 ± 0.6</td>
<td>10 ± 1.3</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>48 ± 2.2</td>
<td>49 ± 1.6</td>
<td>46 ± 3.2</td>
<td>45 ± 2.2</td>
</tr>
<tr>
<td>Total LCP</td>
<td>481 ± 7.6</td>
<td>490 ± 9.1</td>
<td>481 ± 8.1</td>
<td>482 ± 11.3</td>
</tr>
</tbody>
</table>

There are no significant differences between patients and matched controls.

Statistical test employed: paired sample t-test.
The fatty acid and aldehyde composition of the erythrocyte choline phosphoglycerides of patients with reactive depression and of those with other psychiatric disorders

Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters and dimethyl acetal detected.

<table>
<thead>
<tr>
<th>Methyl esters and dimethyl acetals (DMA)</th>
<th>Reactive depressives</th>
<th>Age-sex-matched controls</th>
<th>Other psychiatric patients</th>
<th>Age-sex-matched controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0 + 16:0 DMA</td>
<td>266 ± 8.7</td>
<td>266 ± 8.6</td>
<td>270 ± 8.5</td>
<td>269 ± 7.1</td>
</tr>
<tr>
<td>18:0 + 18:0 DMA</td>
<td>137 ± 5.4</td>
<td>139 ± 3.8</td>
<td>140 ± 5.2</td>
<td>138 ± 5.0</td>
</tr>
<tr>
<td>18:1ω9 + 18:1 DMA</td>
<td>185 ± 4.4</td>
<td>182 ± 3.9</td>
<td>180 ± 4.8</td>
<td>175 ± 3.8</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>205 ± 8.4</td>
<td>200 ± 8.1</td>
<td>203 ± 7.6</td>
<td>208 ± 6.1</td>
</tr>
<tr>
<td>20:0</td>
<td>3 ± 0.4</td>
<td>2 ± 0.2</td>
<td>2 ± 0.3</td>
<td>3 ± 0.4</td>
</tr>
<tr>
<td>18:3ω3 + 20:1ω9</td>
<td>5 ± 0.4</td>
<td>4 ± 0.3</td>
<td>4 ± 0.3</td>
<td>4 ± 0.3</td>
</tr>
<tr>
<td>20:2ω5</td>
<td>5 ± 0.4</td>
<td>4 ± 0.3</td>
<td>4 ± 0.3</td>
<td>5 ± 0.4</td>
</tr>
<tr>
<td>20:3ω6</td>
<td>26 ± 1.4</td>
<td>25 ± 1.2</td>
<td>25 ± 1.5</td>
<td>25 ± 1.1</td>
</tr>
<tr>
<td>20:4ω6</td>
<td>99 ± 3.8</td>
<td>102 ± 4.8</td>
<td>98 ± 5.1</td>
<td>101 ± 5.2</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>11 ± 0.9</td>
<td>11 ± 0.8</td>
<td>12 ± 1.2</td>
<td>11 ± 1.4</td>
</tr>
<tr>
<td>22:4ω6</td>
<td>6 ± 0.7</td>
<td>6 ± 0.9</td>
<td>6 ± 0.5</td>
<td>5 ± 0.6</td>
</tr>
<tr>
<td>22:5ω6</td>
<td>3 ± 0.5</td>
<td>5 ± 0.5</td>
<td>5 ± 0.4</td>
<td>4 ± 0.4</td>
</tr>
<tr>
<td>22:5ω3</td>
<td>12 ± 0.8</td>
<td>14 ± 1.2</td>
<td>12 ± 1.5</td>
<td>12 ± 1.0</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>35 ± 1.7</td>
<td>37 ± 2.7</td>
<td>37 ± 3.2</td>
<td>37 ± 3.8</td>
</tr>
<tr>
<td>Total LCP</td>
<td>200 ± 8.9</td>
<td>206 ± 8.7</td>
<td>201 ± 9.2</td>
<td>203 ± 9.0</td>
</tr>
</tbody>
</table>

There are no significant differences between patients and matched controls.

Statistical test employed: paired sample t-test.
The fatty acid composition of the erythrocyte sphingomyelin of patients with reactive depression and of those with other psychiatric disorders

Results expressed as mean ±S.E. in mg/g total fatty acid methyl esters detected.

<table>
<thead>
<tr>
<th>Methyl esters</th>
<th>Reactive depressives</th>
<th>Age-sex-matched controls</th>
<th>Other psychiatric patients</th>
<th>Age-sex-matched controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>229 ± 5.3</td>
<td>235 ± 5.8</td>
<td>230 ± 6.5</td>
<td>233 ± 6.1</td>
</tr>
<tr>
<td>18:0</td>
<td>59 ± 4.1</td>
<td>54 ± 4.0</td>
<td>48 ± 4.9</td>
<td>50 ± 4.5</td>
</tr>
<tr>
<td>20:0</td>
<td>18 ± 1.9</td>
<td>20 ± 1.5</td>
<td>15 ± 1.8</td>
<td>18 ± 1.7</td>
</tr>
<tr>
<td>22:0</td>
<td>104 ± 5.2</td>
<td>98 ± 5.7</td>
<td>94 ± 4.9</td>
<td>101 ± 5.4</td>
</tr>
<tr>
<td>23:0 + 20:4ω6</td>
<td>36 ± 2.9</td>
<td>32 ± 2.5</td>
<td>30 ± 3.1</td>
<td>35 ± 3.2</td>
</tr>
<tr>
<td>24:0</td>
<td>233 ± 5.3</td>
<td>226 ± 5.9</td>
<td>238 ± 5.6</td>
<td>231 ± 5.5</td>
</tr>
<tr>
<td>24:1ω9 + 20:5ω3</td>
<td>259 ± 4.8</td>
<td>254 ± 5.4</td>
<td>247 ± 5.0</td>
<td>250 ± 4.9</td>
</tr>
<tr>
<td>24:2ω6</td>
<td>32 ± 2.3</td>
<td>33 ± 2.5</td>
<td>37 ± 2.5</td>
<td>34 ± 2.7</td>
</tr>
</tbody>
</table>

There are no significant differences between patients and matched controls.

Statistical test employed: paired sample t-test.