REVIEW OF LITERATURE
The iminobibenzyl nucleus, also known as 5H-dibenz(b,f) azepine is known in the scientific literature since the turn of the century when in 1899, Thiele and Holzinger first synthesized this heterocyclic compound in which a seven membered ring containing nitrogen as the hetero atom is flanked by two benzene rings. Fig. I shows the basic skeleton of the iminodibenzyl nucleus. It was in 1958 when Hafliger synthesized a number of heterocyclic compounds, including iminodibenzyl derivatives, as potential antihistaminic analgesic and antiparkinson agents. Because of a similarity in the chemical structural and pharmacological properties to the antipsychotic agents phenothiazines, interest was developed in the iminodibenzyis and initial clinical trials with 5-(3-dimethylaminopropyl)-10, 11-dihydro-5H-dibenz(b,f) azepine (imipramine) (Fig.II) were carried out in schizophrenic and other psychotic patients. In 1957, R.Kuhn observed that though there was little antipsychotic action, an elevation of mood in depressed patients was evident.
The successful clinical trial of imipramine, an iminodibenzyl derivative, in depressed patients stimulated a renewed and vigorous interest in the syntheses of iminodibenzyl derivatives and structurally related compounds. This resulted in the synthesis of a large number of dibenzazepine and dibenzocycloheptene class of compounds, encompassing classical type of antidepressants, and the synthesis and screening of a range of new structurally unrelated nontricyclic compounds also known as the nonclassical type or second generation antidepressant. However, an up to date popular class in the field of psychoactive drugs with antidepressant effects and that there has been a great deal of research on these structures in attempts to separate the documented therapeutic activity from the side effects, which are for the most part associated with their anticholinergic and cardiotoxic activities.

The nomenclature of seven and higher membered heterocyclic system has been based on the Hantzsch-Widman system with the following stems denoting the ring size: -epine, 7; -ocine, 8; -onine, 9; -ecine, 10 etc. (Fig. III, IV and V). These are
used with a suitable elided prefix such as az-, diaz-, ox- or thi- to indicate the number and nature of the hetero atom(s). The names, thus formed are those of the parent compounds with the maximum number of non-cumulative double bonds. For seven membered rings containing nitrogen it is necessary to indicate the position of unsaturation by a locant designating a hydrogen atom. The approved (chemical Abstract) numbering of the ring positions of 5H-dibenz (b,f) azepine is show below:

Many structural analogues with various hetero atoms in the above ring system are known, namely, dibenzo(b,f) oxepin, dibenzo (b,f) thiepin, dibenzo(b,f) silepins etc. and their 10, 11-dihydro derivatives (Fig. V). Synthesis of dibenzo(b,f) metalepins of the
group IV elements, for example, 10, 11-dihydrodibenzo (b,f) silepin, -germepin, -stannepin, plumbepin have been reported by Corey et al. (1972). Salts of iodepiniumcation have also been reported by Beringer et al. (1965).

In the following pages methods of synthesis for the class of compounds which form leading antidepressants have been illustrated.

**Synthesis of Tricyclic Antidepressants of Dibenz (b,f) azepine class.**

The synthesis of 10, 11-dihydoribenz(b,f) azepins is achieved mainly by Wurtz Coupling of 0-nitrobenzyl chloride (VI) or by base catalyzed coupling of 0-nitrotoluene (VII) and subsequent reduction of the resulting 0, 0'-dinitrobenzyl (VIII) which is then cyclized to dibenz(b,f) azepine (IX) also known as iminodibenzy1. Sodium ethoxide-isoamyl nitrite is commonly used as the basic catalyst for the coupling of nitrotoluene.
Russell et al (1962, 1967, 1968) have extensively studied the base catalyzed coupling of nitrotoluenes and showed that the coupling proceeds via an intermediate charge-transfer complex (X), which reacts with an electron acceptor (an unionized nitrotoluene molecule or oxygen) to form the dibenzyl (IX).

O₂N
\[ \text{CH}_3 \]
\[ \text{NO}_2 \]
\[ \text{CH}_2^- \]
\[ \text{NO}_2 \]

0, 0′-diaminodibenzyls (XI) can also be cyclized to iminodibenzyls by heating the diamine bis-(methanosulfonate) above 295°.
This route to iminodibenzyls has been commercially utilised. Polyphosphoric acid may also be used as the cyclization agent for 0, 0'-diaminodibenzyls.

\[ \text{Reaction Scheme:} \]

The derivatives of iminodibenzyl may be prepared by dehydrochlorination of 0-amino-0'-chlorodibenzyls (XII) using potassium carbonate and copper powder in refluxing dimethyl formamide.
Bergman (1968) have successfully employed the internal coupling of N-acetyl-2-2'-di(bromomethyl) diphenylamine (XIV) which was obtained by brominating the N-acetyl-2, 2'-dimethyl diphenylamine (XIII), using phenyllithium in the preparation of iminodibenzyls.

\[ \text{CH}_2\text{Br} \]
\[ \text{CH}_2\text{Br} \]

\[ \text{XIII} \rightarrow \text{XIV} \]

\[ \text{X} = \text{NCOCH}_3 \]

Bergman, Rabinovitz and Bromberg (1968) used Wagner-Meerwein rearrangement of 9-hydroxymethyl-9, 10-dihydroacridine (XV) as a route to 5H-dibenz(b,f) azepine ring system and especially armatic ring and ethano bridge substituted derivatives.
Aultz et al. (1977) described the synthesis of dibenzo(b,f)oxepins. This was achieved by brominating ethyl-0-toluatate with N-bromosuccinamide to give ethyl-0-bromo-0-toluate (XVI). Ethyl-hydroxyphenylacetate on condensation with II in butanone afforded the crude diester which was hydrolysed to the diacid XVII. Cyclization of the diacid (XVII) using phosphorous pentoxide-ethanol was found to give poor results. A doubling of the quantity of the reagent and addition of sulfolane gave good results.
The synthesis of dibenzo (b,e) thiepins was also effected by Aultz et al (1977) utilizing the reaction of 4-mercaptophenylacetate (XVIII) with ethyl-\(\text{CH}_2\text{CO}_2\text{C}_2\text{H}_5\)-bromo-o-toluate and subsequent (alkaline) hydrolysis gave the diacid (XIX) which was cyclized with the help of polyphosphoric acid-acetic acid mixture to give the desired product (XX).

\[
\begin{align*}
\text{XVIII} & \quad \text{CH}_2\text{CO}_2\text{C}_2\text{H}_5 + \quad \text{CH}_2\text{Br} \\
\text{XIX} & \quad \text{CO}_2\text{H} \quad \text{CH}_2\text{COOH} \\
\text{XX} & \quad \text{CH}_2\text{COOH}
\end{align*}
\]

A newer method for the synthesis of dibenzothiepins has been given by Yamabe and Fujimoto (1978). They treated 2-(p-
(p-fluorophenylthio)5-bromophenyl lactic acid with polyphosphoric acid. The resulting compound was refluxed with $\text{N}_2\text{H}_4\text{H}_2\text{O}$ and 

![Diagram XXI](XXI)

stirred with NaOH in diethylene glycol to give substituted 10, 11-dihydropyridone(b,f) thiepines (XXI).

Bergman et al (1968) have earlier reported a method for the preparation of dibenz(b,f) oxepins (XXII). They made use of the internal coupling of N-acetyl-2,2'-di(bromomethyl) diphenylamine (XXII) with phenyl lithium (Analogously dibenzoxepins may be prepared from 0-ditoly1 ether).

![Diagram XXII](XXII)
The Synthesis of Dibenzocycloheptadienes

The highly similar structural features of dibenzocycloheptadienes to those of dibenzazepines have led many workers to prepare and test these compounds as possible psychotropic agents. Different workers have reported different routes for the synthesis of dibenzo(a,d) cycloheptadienes. Treibs and Klinkhammer (1950) were among the first to synthesize the dibenzo(a,d) (1,4) cycloheptadien-5-one. They started with benzalphthalide which was reduced to 2-(2-phenylethyl)benzoic acid (XXIV) with zinc and KOH. This on heating up to 200°C with in a polyphosphoric acid medium gave the desired dibenzocycloheptadiene-5-one (XXV).
Later Cope and Fenton (1951) described the synthesis of dibenzo (a,d) cycloheptadienones and dibenzo(a,d)cycloheptadines via a somewhat modified route. They also started with benzalphthalide which was reduced to 2-(2-phenylethyl) benzoic acid in good yield with hydriodic acid.

Winthrop and Coworkers (1962) synthesized a number of dibenzo (a,d) (1,4)-cycloheptadiene derivatives using a combination of various reported methods. In their hands, catalytic hydrogenation of benzalphthalide, contrary to the reports of Treibs and Klinkhammer, gave the desired 2-(phenethyl) benzoic acid and in satisfactory yield. Winthrop et al preferred this method to that of Cope and Fenton (1951) utilizing hydriodic acid for the reduction of benzalphthalide. The desired dibenzo (a,d) cycloheptatrien-5-ones were obtained by cyclodehydration of 2-(phenethyl) benzoic acid followed by bromination and subsequent dehydrobromination. The ketones, thus, obtained are usually alkylated with dialkylamino-alkylmagnesium chlorides to give the tertiary carbinols (XXVI). Compounds containing a basic side chain with a functional group incompatible with the Grignard reagent could not be prepared by the usual method, and an indirect route has been employed. Thus, ketone was alkylated with 3-benzyloxypropylmagnesium bromide or chloride to give the tertiary carbinol (XXVI), which after treatment with HBr, was aminated to give (XXVIII).
Shephard and Brenner (1981) recently reported a new synthetic route to benzocycloheptapridines. Cyclocondensation of trans-4-(α-methylstyryl) nicotinic acid in polyphosphoric acid afforded cycloheptapyridinone (XXIX), which underwent consecutive imination, tosylation and methylation to give (tosylamino) cycloheptapyridine. Base catalyzed cyclization and detosylation gave (XXX):
In a search for more rigid analogues, Carnnalm et al (1974) prepared a series of spiro derivatives of dibenz (a,d) cycloheptenes by applying the Robinson annelation reaction to the aldehyde (XXXII) obtained from the carbinol (XXXI) which gave the spiro ketone (XXXIII). Catalytic hydrogenation of the ketone gave the cyclohexanone which were converted to the desired amines (XXXIV) as is shown here:
Recently Meloni et al. (1979) synthesized a series of azetidine derivatives of dibenzocycloheptenes dibenzocyclooxepins and dibenzothiepins. They reacted the amine (XXXV) in CH$_3$OH with eipchlorohydrin at room temperature and then the product, after purification, was dissolved in DMF and to the reaction mixture K$_2$CO$_3$ was added to yield the azetidinols (XXXVI). Methanesulfonyl chloride was added to the azetidinol (XXXVI) in pyridine. The resulting sulfonate (mesylate) was dissolved in D$_2$MF and a large excess of amine was added. The reaction mixture, after an overnight heating, was poured into water and extracted with ethyl acetate to give the desired azetidines.

\[ A = \text{CH}_2\text{CH}_2, \text{CH}_2\text{O}, \text{CH}_2\text{S}, \text{CH} = \text{CH} \]
Lo and Taylor (1984) have very recently reported the synthesis of pyrido [1,4] benzodiazepine compounds having antidepressant properties. The reaction was carried out by condensing 2-chloro-3-nitropyridine with $\text{BzC}_6\text{H}_4\text{NH}_2\cdot 2$ to give nitropyridinylaminobenzene (XXXVIII). This was cyclized to pyrido [1,4] benzodiazepine (XXXIX) and similar compounds.
Non-tricyclic Structures with Antidepressant Activity:

Molecular manipulations in the structure of the lead compound, iminodibenzyl, have resulted in the syntheses of many new compounds and have led to the preparation of quite new and structurally different compounds, which are equally good and sometimes superior to the classical tricyclic antidepressants of iminodibenzyl class of compounds. Though many of them are still in the clinical trial stage, some have already found, after approval in clinical trials, their way to the market in Europe and the U.S.A. For example, trazodone, inkasan, amoxapine, amineptine and quinupramine etc. are the trade names of a few drugs recently introduced into the market.

In such an effort, Asselin et al (1979) prepared a series of substituted 4-oxa-10b-azafluranthenes. Condensation of tryptophol with 2-acetylbutyrolactone (XL) using p-toluenesulfonic acid as catalyst yielded pyranoindolylfuranone (XLI) which was reacted with dimethylamine to give the hydroxyamide (XLII). This hydroxyamide was cyclized with sodium hydride in dimethylformamide which on reduction with diisobutylaluminium hydride in hexane afforded the desired amines (XLIII).
Bernier (1977, 1980) inspired by the concept of drug receptor relationship, designed a lead synthesis of pyrimido-pyrimidine class of antidepressant compounds. The design is based on a simple condensation of a substituted aminouracil with a primary amine in presence of formaldehyde to give a substituted pyrimido (4,5-d)pyrimidine. Thus, for example:

\[
\text{H}_3\text{C} \quad \text{N} \quad \text{O} \quad \text{N} \quad \text{NH}_2 \\
\text{O} \quad \text{N} \quad \text{N} \quad \text{C} \quad \text{H}_3
\]

+ R-\text{NH}_2 + \text{H-CHO} \rightarrow

(XLIV)

\[
\text{H}_3\text{C} \quad \text{N} \quad \text{O} \quad \text{N} \quad \text{R} \\
\text{O} \quad \text{N} \quad \text{N} \quad \text{C} \quad \text{H}_3 \quad \text{H}
\]

(XLV)

\[ R = \text{CH}_2\text{Ph} \]

reaction of 1,3-dimethyl-6-aminouracil (XLIV) with an amine in presence of formaldehyde gives 1,3-dimethyl-2,4-benzyl-1, 2, 3, 4, 5, 6, 7, 8-octahydropyrimido(4,5-d)pyrimidine(XLV).
General consideration

The iminodibenzyls and the compounds of the related system, better known as tricyclic antidepressants, have been known for the last twenty years and are in clinical use for at least ten years. They are a group of potent compounds having an established role in the psychopharmacological treatment of depression. Despite a long history of existence, the first iminodibenzyl compound imipramine, was assessed only in 1948 for its pharmacological properties as antihistamine, sedative and antiparkinson agent. These studies were not pursued until the importance of chlorpromazine, which imipramine closely resembles chemically, was realized in the early 1950s. It was Kuhn (1958) who, in the hope of getting effects similar to those of chlorpromazine, used imipramine as an antipsychotic agent and found it to be beneficial in depression rather than in schizophrenia.

Depression is the most fundamental psychosomatic reaction, the etiology of which is varied. Primary disorders of mood or affect, characterized by abnormal emotions are called manic-depressive disorders. An elevation of mood in the case of mania and a lowering in depression are the central features of illness. Kraepelin (1921) defined manic-depressive disease as a mental disorder centrally featuring a change of mood, an elevation in the case of mania and a lowering in depressions. He also divided affective disorders into two main categories. Manic-depressive illness and dementia
praecox (schizophrenia). All the severe mood disorders with or without excitement alternating with depression belong to the first type of illness. The minor types of the illness categorized as "neurotic", "reactive", or "situational" are reactions to a stressful situation or event and remission can occur spontaneously. The major (endogenous) types of depression are characterized by somatic and physical symptom. Loss of appetite and hence loss of weight, insomnia, restless nights and sometimes constipation, dry mouth, aches and headache are the physical symptoms of the major (endogenous) depression.

Endogenous affective disorders have been further subdivided into several groups and subgroups by Leonhard (1962). The most important groups are the unipolar and the bipolar manic-depressive illness. The distinction cannot as yet be made absolutely. Any patient who is suffering from unipolar depression may, in due course, develop an attack of mania. Different forms of depression respond to different types of medication, for example, tricyclic antidepressants are superior in the treatment of unipolar depression whereas bipolar depression responds very well to lithium therapy. Monoamine oxidase inhibitors and electroshock therapy are good for unipolar depression.

The discovery of 5-hydroxytryptamine (5-HT) in mammalian brain tissue and the effects and structural similarity of LSD on 5-HT led Woolley and Shaw (1954) to propose that 5-HT might be involved in the regulation of mood. Later on reserpin, known for its anti-hypertensive effects, was found to produce a depressive episode by depleting the monoamines (Carlsson et al, 1957; Bunney and Davis, 1965). At about the same time the antitubercular drugs
isoniazid and iproniazid were noted to have mood elevating effects and in 1958, Zeller and Co-workers discovered these drugs to inhibit monoamine oxidase. Hence it was conceived that defect in monoamine metabolism and function is responsible for a mood change and that the changes are central to the pathology of depressive illness. Based on these findings there has further developed a pharmacological approach to therapy and drugs effective in the metabolism and function of monoamines have been developed. And further research advanced along two lines: in the first one the biochemical changes due to depression were examined; the second line of approach was based on the study of biochemical changes occurring in the brain of animals treated with drugs effective in depression (Green & Costa, 1980). But both approaches are subject to some difficulties inherent in considering depressive illness and there are controversies over the psychiatric classification of depression (Kendell, 1976). The biochemical difficulties lie in deciding which changes might be responsible for mood change and which are the results of mood change. It is also possible that the biochemical changes produced by a drug may be unrelated to its therapeutic action.

Norepinephrine and 5-hydroxytryptamine, respectively representing the catecholamine and indoleamine systems have been implicated in the depressive illness. These amines cannot normally pass from the periphery into the brain and monoamine transmitters are synthesized intracellularly from the appropriate amino acid and this occurs predominantly at the nerve endings which has an active transport mechanism for aminoacids (Grahame-Smith and Prafitt, 1970).
5-Hydroxytryptamine is synthesized in two steps from the amino acid tryptophan. The first step, the hydroxylation of tryptophan by tryptophan 5-hydroxylase is the rate limiting step in 5-HT synthesis (because tryptophan hydroxylase is unsaturated with respect to its substrate). Hence the rate of 5-HT synthesis is dependent on the brain tryptophan concentration which in turn is dependent on plasma tryptophan concentration (Young and Sourkes, 1977). Here the majority of tryptophan is bound to plasma albumin and little is present in the free form. Theories were developed suggesting one of the two forms of plasma tryptophan, the bound or the free (which is about 20%) form, influences the brain tryptophan levels (Wurtman and Fernstrom, 1976; Curzon and Knott, 1974). It has recently been suggested that these theories are compatible in many ways (Green, 1978). Yet free tryptophan appears to play a major role in controlling the brain 5-HT metabolism. Very little tryptophan is known to enter the brain from the periphery (Hagen and Cohen, 1966). Tryptophan, hydrocortisone or corticosterone increases the activity of tryptophan metabolizing hepatic enzyme tryptophan pyrrolosine (Curzon 1969; Lapin and OxenKrug, 1969) thereby decreasing plasma tryptophan and probably decreasing brain tryptophan levels. (Green et al, 1975a). It is apparent from several studies that a simple deficiency of tryptophan availability is not the cause of depression otherwise tryptophan would have been an effective antidepressant.

Several studies have been reported on the 5-HT metabolite, 5-hydroxyindole acetic acid (5-HIAA) concentration in brain and cerebrospinal fluid (CSF). Some studies found decreased 5-HIAA levels in brain and CSF while others reported normal values.
(Aschroft et al, 1966; Van Proag, 1970; Conpen, 1973; Llyod et al, 1974, Bourne et al, 1968, Pare et al, 1969). Asberg et al, (1976) recently reported the observation that 5-HIAA concentration in CSF follow a bimodal distribution pattern, one group having normal values, the other low concentrations. The help of probenecid to block 5-HIAA egress from CSF so as to measure the 5-HIAA accumulation rate was sought and developed by several workers to study 5-HT turnover in depression. Apart from difficulties in evaluating the conflicting data there are problems in interpretation of monoamine metabolite measurements. Since a portion of 5-HIAA is presumably being produced due to intraneural metabolism of 5-HT and therefore, only a portion of 5-HIAA being produced has been functionally active, hence amine metabolite concentration do not necessarily reflect changes in monoamine function (Green and Graehame-Smith, 1975a, 1976). There is also some argument as to the significance of CSF 5-HIAA levels. Many workers have reported little decrease in cerebrospinal fluid 5-HIAA levels in patients with a block in CSF flow, suggesting a possible spinal origin for the 5-HIAA. (Garelis and Sourkes, 1973; Young et al, 1973). The converse has been argued equally well (Aschroft, 1973; Weir et al, 1973). It should also be pointed out that Wilk (1973) has cast doubts about the methods used for determining cerebrospinal fluid 5-HIAA. Brain and platelet 5-HT studies have been suggested to be important models due to the fact that platelets are known to store and transport 5-HT in a manner similar to that of nerve endings (Paasonen 1968). However, few changes have been observed and reports in the literature are conflicting. Shaw et al (1971) in their study observed that there was no change in 5-HT uptake by platelets
from depressives while Ehsanullah (1980), reported a decreased platelet uptake of 5-HT in depressed patients. Total 5-HT is a combination of non-active processes and an active, energy requiring process. Tuomisto and Tukianen (1976) have been able to find out the active uptake of 5-HT by subtracting the portion accumulate due to nonactive process from the total and reported that the active uptake of 5-HT by platelets of depressed patients was about half that of control subjects. They observed that there were fewer transport molecules or amine carriers or that those present were in an inactive form. It is not known whether such a change in any way reflects a similar change in the brain or is merely secondary reflecting some compensatory mechanism to the depression.

The catecholamine precursor, tyrosine, in plasma has been reported to remain unaltered (Takahashi et al, 1968). Even the administration of phenylalanine (which is converted to tyrosine in the liver) or of tyrosine does not alter catecholamine synthesis (Levitt et al, 1965) and it is therefore, unlikely that plasma tyrosine would reflect changes in cerebral metabolism. Homovanillic acid (HVA), the acid metabolite of dopamine is known to reflect brain dopamine metabolism. There are few nerve endings in the spinal cord (Commissiong et al, 1977) and block of subarachnoid space, either partially (Curzon et al, 1971; Carelis and Sourkes 1973) or totally (Young et al, 1973) results respectively in a decrease and disappearance of HVA from the CSF below the block. Carelis et al, (1974) have suggested that the HVA originates mainly from the caudate nucleus. The origin of norepinephrine metabolite 3-methoxy-4-C hydroxyphenylglycol (MHPC) is less clear.
and unlike HVA, there is no gradient down the cord (Sourkes, 1973; Chase et al, 1973).

MHPG on the other hand has been reported to be decreased (Gordon and Oliver, 1971; Post et al, 1973; Jimerson et al, 1975) or normal (Shaw et al, 1973; Shopsin et al, 1973). Since MHPG occur both free and as sulfate ester and in these studies only total changes were measured, it is difficult to analyze the results of these studies. Jimerson et al, (1975) have noted a decreased VMA level in depression. While Mendels et al, (1972) reported an increased level of HVA on recovery which go against the observation of Coppen et al, (1972) and Aschroft (1973) who found none of the two to rise following the improvements of mood.

In their studies on post-mortem brain indoleamine and catecholamine changes, Bourne et al, (1968) and Pare et al, (1968) found no change in hindbrain norepinephrine. Pare et al, also found normal dopamine levels in caudate nucleus of depressive suicides.

Platelet-MAO activity seems to be a good indicator of the depressive metabolic episode and attention has been focused on it. Murphy and Weiss (1972) found a decrease in platelet-MAO activity. Schild-Krawt and Coworkers (1977) in an attempt to correlate the platelet-MAO activity with MHPG excretion suggested a subgroup of depressed patients with bizarre antisocial behaviour to have a relatively low MHPG excretion and higher platelet-MAO activity. Furthermore there are difficulties in determining the relationship between control MAO and platelet-MAO activity. For example iron deficiency causes marked changes in peripheral-MAO activity but little change in central-MAO activity and mono-
amine metabolism (Youdim 1975, Youdim and Green, 1977). Though the activity of MAO appears to have markedly inhibited in the brain before there is much effect on monoamine metabolism (Green et al, 1977), fine behavioural and mood changes could be occurring long before there are marked biochemical changes (Green and Costain, 1979). The majority of drugs used to treat depression have been in clinical use for more than twenty years. Until about four years ago there were a clear, although unproven, hypothesis of their mode of action. Recently, the advent of newer antidepressants, the so-called atypical or second generation antidepressants has led to a sharp increase in research as they do not markedly affect the monoamines. It also led to the realization that the existing monoamine hypothesis of depression is not supported by much of the current experimental evidence. There is no explanation for the time lag between the time of onset of antidepressant action and the speed with which the drugs inhibited monoamine uptake. These observations led to a re-evaluation of the monoamine hypothesis of depression. (Luchins, 1976; Murphy et al, 1978). However there are so many observations and experimental results in favour of this hypothesis that it cannot be rejected out of hand. The study of the effects of reserpine, the mode of action of antidepressants and the metabolite of antidepressants are the main arguments in favour of the monoamine hypothesis.

Biochemical And Neurophysiological Effects of Antidepressants

Drugs used for the clinical treatment of depression include inhibitors of monoamine oxidase (MAO), secondary and tertiary
amines of tricyclic antidepressants, and a number of other drugs which affect monoaminergic system in brain (e.g., maprotiline, mianserine, iprindole, nomifensine).

Severe side and toxic effects have seriously restricted the clinical use of MAO inhibitors as antidepressants. However, multiple form of MAOIs, with different substrate specificity, offer, the possibility of synthesizing more specific and safer MAO inhibitors. Based on the biphasic inhibition pattern of the metabolism of tyramine by clorgyline, Johnston (1968) proposed two forms of MAO, type A and type B; type A being selectively inhibited by clorgyline. 5-HT being prefrentially metabolized by type A MAO while tyramine and tryptamine appear to be substrate for both types. Norepinephrine, in addition to 5-HT is also selectively metabolized by the A form (Goridis and Neff, 1971) while phenylethylamine (PEA) (Yang and Neff, 1974) and benzylamine (Hall et al, 1969) are substrates for type B MAO. Dopamine (DA), like tryptamine and tyramine, is metabolized by both forms (Hall et al, 1969). Harmaline (Fuller, 1972) and Lilly 51641 (Fuller, 1968) are the other selective inhibitor of the A form; deprenyl appears to be a selective inhibitor of B form (Knoll and Magyar, 1972). However, the concept of multiple forms of MAO has been questioned recently (Houslay et al, 1976; Jain, 1977). And although the nature of multiplicity is unknown, in vivo studies support the concept of functionally different types of MAO. For example, in preparations containing brain mitochondria from rats pretreated with type A inhibitors, the metabolism of 5-HT was found to be selectively inhibited whereas pretreatment with deprenyl prefrentially inhibited the metabolism of PEA and
benzylamine (Christmas et al, 1972; Yang and Neff, 1974).

Although the endogenous level of 5-HT, NE, and DA have been reported to increase after clorgyline, the concentration of 5-hydroxy-indoleacetic acid (5-HIAA) and 3,4-dihydroxyphenylacetic acid (Yang and Neff, 1974). While these studies indicate that DA is a substrate for both A and B form of MAO, in accord with in vitro evidence, in vivo work indicates that DA is a preferential substrate for A form. The inhibition of monoamine oxidase in gastrointestinal tract and liver, however, exposes patients to the potentially toxic effects of dietary amines such as tyramine and $\alpha$-phenylethylamine, which are normally degraded by monoamine oxidase and prevented from entering the general circulation. The undesirable side-effect (the wine and cheese syndrome), together with the authoritative Medical Research Council trial (1965) indicate that the monoamine-oxidase inhibitor phenelzine is inferior to placebo in the treatment of a group of severely depressed.

The increased availability of physiologically active (intraneuronal) amines at their corresponding receptor sites following MAO inhibition leads to a decrease in the synthesis of catecholamines apparently due to feedback inhibition of tyrosine hydroxylase (Neff and Costa, 1966, Nagi et al, 1968). The work of Mandell and Knapp (1978) also indicates the possibility of the regulation of amine biosynthesis through changes in the rate of synthesis and disposition of monofolate pterin. Early studies have shown that MAO inhibitors effect the uptake and release of neurotransmitters but it does not appear to be a common property of all MAO inhibitors. For example, tranylcypromine and deprenyl
decrease the uptake of \( ^3 \)H NE into brain tissues while nargyline and nialamide have no effect (Knoll and Magyor, 1972). In their study, Hendley and Snyder (1968) found that blockade of uptake of tritiated metaraminol by MAO inhibitors correlated better with their antidepressant efficacy than did their ability to block the activity of MAO. Clinical evidence suggests that (-) tranylcypromine is a more effective antidepressant than the corresponding (+)-enantiomer (Escobar et al, 1974). This is of interest since the (+)-isomer is a more potent MAO inhibitor than the (-)-isomer (Zirkle et al, 1962) while the (-)-isomer is a more potent inhibitor of catecholamine uptake into brain synaptosomes (Horn and Snyder, 1972). MAO inhibitors under certain conditions can decrease the release of NE associated with the nerve stimulation (Davey et al, 1963) and the efflux of tritiated NE accumulated in heart tissue (Axelrod et al, 1961).

Tricyclic antidepressants have been predicted to effect the MAO activity. In vitro studies indicate that many of the tricyclic antidepressants can inhibit MAO (Gabay and Valcourt, 1968; Roth and Gillis, 1975 a,b.). Tricyclic antidepressants (TCAs) are also known to inhibit human platelet MAO in vitro (Edwards and Burns, 1974) and in vivo (Sullivan et al, 1977). But in vivo studies do not confirm this effect and there are conflicting reports in the literature. Mosnaim et al, (1974) reported that the administration if imipramine increases the level of PEA in brain while iprindole failed to significantly alter the PEA level. Von Voigtlander and Losey (1976) found imipramine, amitrinyltyline and imprindole not to block the disappearance of \( \text{C}^{14} \) PEA.
Failure of TCAs to antagonize the reserpine-like syndrome in rats whose brains have been selectively depleted of catecholamines (Sulser et al, 1964; Scheckel & Boff, 1964) and the inhibition of NE uptake by these drugs (Glowinski & Axelrod, 1965) formed the experimental basis of the clinically relevant catecholamine hypothesis of depression (Schild-Krant, 1965; Bunney and Davis, 1965). This hypothesis, based on studies of acute biochemical effects by a number of TCAs, does not take into account the discrepancy between the biochemical and pharmacological effects elicited by TCAs within minutes and the time required for the onset of therapeutic effects which spans over a period of several weeks.

Segal et al, (1974) were the first to report the adaptive changes in the activity of tyrosine hydroxylase following chronic administration of reserpine or TCAs. For example, desipramine given daily for eight days is known to significantly decrease tyrosine hydroxylase activity in the locus coeruleus and hippocampal area, but only a marginal decrease in the activity of the enzyme in the caudate nucleus, a predominantly dopaminergic area. No significant change in the activity of the enzyme was observed twenty-four hours following the desipramine administration. Such adaptive changes in the biosynthetic capacity can explain the decrease in the level of NE observed following chronic (Schild-Krant, 1970, 1971; Roffler-Tarlov et al, 1973) but not acute administration of TCAs (Sulser et al, 1964). A decreased activity of tyrosine hydroxylase is also compatible with the finding of a decreased turnover of NE following chronic administration of TCAs (Ros-Loff, 1975; Nielsen and Braestrup, 1977).
3-hydroxy-4-methoxyphenylglycol sulfate (MHPG) has been reported to increase after chronic administration and to decrease after acute administration of iminrimine or desipramine (Roffman et al, 1977). This suggests that chronic administration of these drugs may enhance the turnover of NE. Since amitriptyline, nortriptyline and many other TCAs do not show similar effects on the turnover rate of NE, these effects do not appear to have mechanistic implications for the delayed therapeutic activity of tricyclics.

Tricyclic antidepressants generally show a similarity of effects in the peripheral and anticholinergic properties with the structurally related phenothiazines and antihistaminics (Halliwell et al, 1964). Biochemically, desipramine has been reported to decrease the "bound" acetylcholine fraction in the brain, reserpine, significantly increased the amount of 'bound' acetylcholine in whole brain (Hardina and Ling, 1973). Many of the autonomic side effects of TCAs such as dry mouth and difficulty in accommodation can be explained on the basis of anticholinergic properties of these drugs, the contribution of anticholinergic properties to the antidepressant efficacy of TCAs is not known. Though TCAs block histamine H2 receptors in brain (Green and Maayani, 1977) and some of the pharmacological effects of TCAs may be related to blockade of H2 receptors, the antihistamine potency of these drugs do not seem to parallel their clinical efficacy.

Tricyclic antidepressants have also been shown to inhibit dopamine-sensitive adenylate cyclase in homogenates of rat brain striatum, with amitriptyline and doxepin being particularly potent (Karobath, 1975). A discrepancy exists between the rather potent
effects of some TCAs on DA-sensitive adenylate cyclase in vitro and the low or no antipsychotic activity of these drugs in vivo. TCAs also inhibit the activity of cyclic nucleotide phosphodiesterase in brain (Roberts and Simonsen, 1970; Abdulla and Hamadak, 1970). The physiological significance of this biochemical effect is difficult to assess as it is shared by many other drugs, e.g., benzodiazepines and promethazine (Muschek and McNeill, 1971; Beer et al, 1972; Berndt and Schwabe, 1973), and because no class of drugs has yet been shown to act physiologically via their effect on cyclic nucleotide phosphodiesterases in either peripheral organs or in the CNS.

In 1978, U'Prichard and coworkers the TCAs to have a high affinity for -noradrenergic receptor sites in brain as determined by the competition for the binding of the -antagonist ($^{3}$H)-WB-4101 (2-2',6'-dimethoxy phenoxylethylomine methylbenzodiazoxan) to membrane fractions of rat brain. The relative affinities of tertiary and secondary amines of TCAs as determined in such binding assays may be correlated with some of the side effects of these drugs.

Recent evidence suggests the existence of post synaptic regulatory mechanism involving the NE-receptor-coupled adenylate cyclase system in the CNS. Following the administration of drugs which can precipitate despair in primates or severe depressive reactions in man (e.g. 6-hydroxydopamine, reserpine), an increased responsiveness of the cyclic AMP generating system to NE occurs in cortical slices (Palmer, 1972; Huang et al, 1973; Kalisker, 1973), in slices from the hypothalamus and brain stem (Palmer et al, 1973), and from the limbic forebrain area (Vetulani et al,
Administration of TCAs have been reported by Vetulani et al, (1976) and some other workers to decrease the responsiveness of the limbic cyclic AMP generating system to NE. While MAO inhibitors have been reported to cause an enhanced cyclic AMP response to NE when given acutely and treatment with these agents for 3 weeks resulted in a significant desensitization of NE-receptor; coupled adenylate cyclase system. Tricyclic antidepressive agents such as desipramine (a potent blocker of NE) also reduced the reactivity of the system to (R)-NF following their chronic but not acute administration. The reduction in the neurohormonal response is not related to the concentration of the antidepressant drugs in brain tissue but depend on time. TCAs, regardless of their action on presynaptic sites, thus share this delayed action on the limbic NE-receptor-coupled adenylate cyclase system with MAO inhibitor type antidepressants.

The reduced sensitivity of the NE-receptor coupled adenylate cyclase system resulting from prolonged administration of different prototypes of effective antidepressant drugs suggest that the therapeutic action may be related to this common delayed postsynaptic change in noradrenergic receptor function in the limbic forebrain and other brain areas rather than to acute and inconsistent action at presynaptic site. Data obtained from a number of studies aimed at the elucidation of molecular basis of reduced noradrenergic receptor function suggest that at least with regard to β-adrenergic receptors, the resistant state of adenylate cyclase towards NE after chronic administration of TCAs is linked to a decrease in the actual number of β-adrenergic receptors (Sulser, 1978).
The majority of drugs used to treat depression has been in clinical use for more than twenty years. Until about ten years ago there were a clear, although unproven, hypothesis of their mode of action. Recently, the advent of newer antidepressants, the so called atypical or second generation antidepressants has led to a sharp increase in research as they do not markedly affect the monoamines (Green and Nutt, 1983). It also led to the realization that the existing monoamine hypothesis of depression is not supported by much of the current experimental evidence. There is no explanation for the time a between the time of onset of antidepressant action and the speed with which the drugs inhibit monoamine uptake. These observations led to a re-evaluation of the monoamine hypothesis of depression (Luchins (1976), Murphy et al, (1978) which is now considered as unsatisfactory. However, there are so many observations and experimental results in favour of this hypothesis that it cannot be rejected abruptly. The study of the effects of reserpine, the mode of action of antidepressants and the metabolite of antidepressants are the main arguments in favour of monoamine hypothesis.

Reserpine is known to reduce drastically the brain content of both NA and 5HT may in some people provoke depressions that are quite similar to "typical" endogenous ones. The depressant effect of reserpine does not speak in favour of either the noradrenaline or the serotonin hypothesis, since reserpine has about the same effect on both monoamines. Anyhow, the reserpine depression is one of the strongest arguments in favour of a primary monoaminergic hypoactivity in endogenous depression. The mechanism of action of antidepressant drugs seems to fit very
nicely into the monoamine hypothesis. In fact, this is not surprising, since the hypothesis was partly based on the supposed mode of action of such drugs: The "classical", or "first generation", tricyclic antidepressants (TCA) have an inhibitory effect on the reuptake of NA and 5-HT at the synapses. This means an inhibition of inactivation, and thus a stimulation of synaptic activity. Monoamine oxidase inhibitors (MAOI), on the other hand, inhibit the enzymatic degradation of the monoamines, and thereby stimulate their activity by a different mechanism. However, it probably is not that simple. As for MAOI, they are not very effective in typical endogenous depression, but probably more in "atypical" ones, for whatever reason.

Among the TCA, some have a rather weak inhibitory effect on the 5-HT or the NA pump, or even on both, but still seem to be about as effective in endogenous depression as the stronger uptake inhibitors. Among the "second generation" TCA, some are specific 5HT uptake inhibitors whereas others, like mianserin, have weak effects on both pumps. Thus, it seems that TCAs also work by other mechanisms than by reuptake inhibition, and this means that the mode of action of these drugs no longer can be considered a strong argument for the monoamine hypothesis.

More direct proof of reduced turnover and function of NA and 5-HT might be obtained by demonstrating reduced levels of their metabolites in the brain itself, in the CSF, in plasma, or in urine. The urinary excretion of MHPG shows good correlation with MHPG in CSF (Agren, 1981; Maas et al, 1982). Several investigators have found reduced amount of urinary MHPG in endogenous depression Maas et al, 1968), and there even seems to be a corre-
lation between low MHPG excretion and response to those TCA that are supposed to stimulate noradrenergic function, like imipramine (Beckman & Goodwin, 1975). However, others have found no definite abnormality in MHPG excretion, and it has also been claimed that some depressed patients (mainly unipolar) have a higher than normal excretion of MHPG, whereas other (mainly bipolar depressions) have reduced excretion (Schatzberg et al, 1982). Low levels of MHPG in CSF has also been demonstrated by some (Post et al, 1973). Thus, there are some indications of reduced NA turnover in endogenous depression, but the picture is far from clear.

As to the 5-HT metabolite 5HIAA, urinary excretion mainly reflects peripheral metabolism. 5HIAA in CSF is reduced in endogenous depression according to several investigators, but the findings are by no means unanimous (Murphy et al, 1978). Asberg and coworkers (1976) have found a bimodal distribution of CSF 5HIAA in depression. The smaller or lower mode probably represents a subgroup of "serotonin depression", and is associated with more typical "endogenous" clinical features than the upper mode, as well as with the serious suicidal behaviour (Asberg et al, 1976; Banki et al, 1981). It may also be associated with clinical symptoms like anxiety (Asberg et al, 1981; Banki et al, 1981), although this was not confirmed by Agren (1981).

There is no association between CSF 5HIAA and the unipolar-bipolar dichotomy. If there exists a subgroup of "serotonin depression", it should, according to current theory, react favorably to drugs having a special tendency to stimulate serotonergic function, like the potent 5HT uptake inhibitor clomipramine. However, clomipramine does not seem to be particularly
effective in low 5HIAA depression (Traskman et al, 1979). On the other hand, van Praag (1981b) has reported therapeutic effect of the serotonin precursor 5-hydroxytryptophan especially in patients with low CSF 5HIAA.

A very interesting recent study supporting the assumption of a connection between low CSF 5HIAA and affective disorder, is published by Sedvall and coworkers (1980): they found a significant correlation between low CSF 5HIAA in healthy volunteers, and a family history of depression. If there is a presynaptic deficiency of NA or 5HT in endogenous depression, monoamine precursors may have an antidepressant effect. (There is no use giving the monoamines themselves, since they do not readily cross the blood-brain barrier; they also have too strong peripheral effects). Such an effect in depression would be analogous with the L-DOPA effect in parkinsonism.

Both tryptophan and 5-hydroxytryptophan (5-HTP) have been tried in several controlled clinical studies in endogenous depression, and although some trials have failed to reveal any effect, several studies have given positive results (van Praag, 1981b; Gelenberg et al, 1982). The effect of 5HT precursors is probably most favorable when they are given together with MAOI or TCA. When given alone, tryptophan and 5-HTP do not seem to have the same efficacy as TCA alone - at least according to most investigators. Therefore, the antidepressant effect of these compounds is of more theoretical than practical importance. The results with the N.A. precursor tyrosine and L-DOPA are less clear. However, promising results with tyrosine have recently been reported (Glebberg et al, 1982).
As a whole, the clinical results obtained with monoamine precursors, although not always very convincing, support the hypothesis that there is a functional deficiency of 5-HT and/or NA in at least a subgroup of endogenous depression, and is certainly not in accordance with the theory of a primary hyperactivity in the function of these amines.

These therapeutic actions became an indirect support for the belief that blockade of monoamine uptake or inhibition of monoamine oxidase (MAO) activity were the molecular mechanism whereby TCAs and MAOIs relieve the symptoms of depression. Yet the mechanisms of action proposed by the biogenic amine hypothesis of depression are not consistent with the delayed onset of therapeutic effects of antidepressant treatments nor with acute effects of more recently developed second generation antidepressant drugs. These observations were further corroborated by the finding that cocaine, an inhibitor of monoamine uptake failed to relieve the symptoms of depression (Post, Kotin and Goodwin, 1974). And it became necessary to look for an explanation to the mechanism of action of antidepressants. It has recent been speculated that a reduction in \(-\)adrenoceptor and serotonergic receptor density and a desensitization of noradrenergic cAMP generating systems in the brain induced by chronic administration of TCAs in rats (clements-Jewery, 1978; Wolfe et al, 1978; Bergstrom and Keller, 1979; Peroutka & Snyder, 1980; Hertz et al, 1981; Miyauchi et al, 1984) may represent the basic neurochemical mechanism of therapeutic action of these drugs (Stone, 1978; Mobley and Sulser, 1981).

It has been further supported by the observation that repeated administration of both desipramine and nialamide, a MAO inhibitor,
decreases the maximum elevation of cAMP to norepinephrine stimulation in pineal glands. Lesioning of the norepinephrine input to this organ prevented down regulation of adenylate cyclase, although the lesion alone reduced cAMP production, making interpretation more difficult (Moyer et al, 1980).

The acute effects of antidepressant drugs on amine reuptake can not completely account for their therapeutic activity. Hence, research into the mechanism of action of antidepressants has recently been switched from short-term observations of drug effects to the adaptive changes in norepinephrine and serotonin receptor functions induced by long-term administration of antidepressant agents.

Behavioral (Sypraki and Fibiger, 1980) and biochemical (Surgue, 1980) studies in laboratory animals have demonstrated that administration of desipramine decreases -adrenergic receptor sensitivity. These studies were further supported by clinical observations that chronic but not acute administration of desipramine attenuates the clonidine induced decrease in plasma 3-methoxy-4-hydroxy phenethyleneglycol MHPG (Charney et al, 1981). The hypotensive drug clonidine, reduces norepinephrine turnover by acting as a selective agonist at -adrenoceptors. The mechanism involved in the decrease in clonidine sensitivity during long-term desipramine treatment may be a consequence of the persistent increase of synaptic norepinephrine at -receptor. Thus, the receptor may become subsensitive with long-term desipramine treatment, resulting in an increased amount of norepinephrine release per nerve impulse and desensitization of -adrenergic
receptors for agonists such as clonidine. This subsensitivity of 2-adrenoceptors induced by desipramine is shared by imipramine and the atypical antidepressant mianserin (a well known 2-adrenergic antagonist) (Surgue, 1983, Maggi et al, 1980) but is not possessed by most other antidepressant agents. Therefore, such effects on the presynaptic adrenergic receptors do not account for the clinical efficacy of all antidepressants.

Recently, most of the research interest has been directed toward studying the adaptive changes in norepinephrine and serotonin at postsynaptic receptors induced by chronic administration of antidepressants.

Electrophysiological and behavioral studies indicate that sensitivity of central $\alpha_1$-adrenoceptor is enhanced by chronic administration of antidepressants. Both electroconvulsive therapy (ECT) and long-term treatment with desipramine, imipramine, amitriptyline, and the atypical antidepressant drugs such as iprindole are associated with an augmented response to iontophoretic norepinephrine in the rat facial motor nucleus (Menkes et al, 1980). Furthermore clonidine-induced increase in spontaneous motor activity has been shown in rats treated chronically, but not acutely with imipramine, amitriptyline and mianserin (Maj et al, 1979). Interesting indirect evidence indicates that the adrenergic receptor function may be disturbed in populations of depressed patients (Menkes et al, 1980). This supersensitivity of $\alpha_1$-adrenergic receptor induced by chronic administration of some antidepressant drugs is not shared by all the types of antidepressants.
Recently, most of the work has centered around the effect of chronic treatments with antidepressants on the beta-adrenoreceptors. The majority of antidepressants have been found to affect the action function (receptor-coupled adenylate cyclase system) and/or recognition function (receptor number). Thus, radioligand binding studies have revealed that the chronic administration of tricyclic antidepressants such as desipramine, imipramine, nortriptyline and, amitriptyline, MAO inhibitors (Pargyline, clorgyline) and repeated electroconvulsive shock therapy (ECT) is always associated with a reduction in the number of -adrenoceptor recognition sites present in the rat cortex (Keller et al, 1981). Interestingly, the combination of -adrenoceptor blocker (e.g., Yohimbine) with either NE uptake inhibitor or MAO inhibitor accelerates and intensifies the reduction in the density of -adrenoceptors (Crews et al, 1981).

The break through in the mechanism of action of antidepressant therapy was the observation by Vetulani and Sulser (1975) that the activity of the norepinephrine stimulated adenylate cyclase system present in the rat limbic forebrain slices was diminished by long-term administration of both desipramine and iprindole. This observation was further supported by the finding that all forms of antidepressants studied so far shared this effect (Sulser, 1982).

The down-regulation of norepinephrine receptor coupled adenylate cyclase system induced by antidepressant is indirect and requires the availability of the agonist norepinephrine for the regulation of both the receptor number and the sensitivity of the receptor system to exogenous norepinephrine and/or isoproterenol (Sulser et al, 1983). Thus, the destruction of nerve endings by 6-hydroxydopamine, or locus
coeruleus lesion as well as \(-\text{adrenoceptors blockade (e.g. Proprano-}
lo)\), all resulted in a failure of desipramine and iprindole to down-
regulate the NE-sensitive adenylate cyclase system (Surgue, 1983;
Sulser et al, 1983). It is worth noting that the atypical antidepres-
sant (iprindole) which does not affect the availability of NE has
also failed to elicit subsensitivity to NE on the lesioned side of
locus coeruleus. This may indicate that iprindole does not have a
direct effect at the postsynaptic receptors and it is more likely
that presynaptic mechanisms or neuronal signal is a concurrent require-
ment for eliciting desensitization of \(\beta\)-adrenoceptors.

As a consequence of the finding that virtually all forms of
chronic antidepressant therapy down-regulate central NE-stimulated
adenylate cyclase, it has been speculated that depression may, in
part, be related to functional hyperactivity of certain NE neuronal
systems. These hypersensitive receptors may serve to amplify incoming
stimuli and thus cause central hyperexcitability (Segal et al, 1974).
The pharmacotherapy and ECT treatments, however, induced a delayed
deamplification of the system and hence may represent a means of
facilitated adaptation which has a therapeutically relevant biochemical
action. The specificity of the down-regulation of central NE-stimulated
adenylate cyclase induced by antidepressants has been challenged by
the observation that the antipsychotic drug chlorpromazine when given
chronically down-regulated this system (Schultz, 1976).

The involvement of central 5-HT synapses in the mechanism of
action of antidepressant action has been studied extensively by
Fuxe and his associates (1983). They found that chronic antidepres-
sant treatment with desipramine, imipramine, zimelidine and alapro-
clate led to adaptive change in both the pre- and postsynaptic 5-
hydroxy tryptamine (5-HT) receptor mechanisms which seem to result in sub- or supersensitivity development depending upon the 5-HT nerve terminal system analyzed.

By and large the terminals of serotonergic projections in the cortex coincide with those of noradrenergic projections. Complex interconnections are present among central putative neurotransmitters and modulatory systems. Thus the activity of one monoaminergic system can be modified by changes in another system. Hertz et al, (1981) have demonstrated that in a preparation of cultured mouse astrocytes incubated in the presence of 1 mol amitriptyline there was a marked decrease in cyclic AMP accumulation following adenylate cyclase stimulation with isoproterenol. Chronic exposure of astrocytes, therefore, leads to a down-regulation of -adrenoceptor function. This raises the question as to the relative contribution of neuronal and non-neuronal tissue to the changes reported in adenylate cyclase activity in slices or homogenates, and indeed suggests that altered synaptic function may not be necessary for the changes produced by at least some tricyclics.

In contrast to the reported changes in norepinephrine turnover which occur following chronic treatment with 5-HT antagonist (Przegalinski et al., 1981 alterations in the availability of 5-HT did not change norepinephrine sensitive adenylate cyclase systems in the brain. While chronic administration of clomipramine or amitriptyline (drugs which have 5-HT uptake-inhibitory activity) decreased the sensitivity of the norepinephrine-sensitive cyclic AMP-generating system, neither increasing 5-HT availability with the specific 5-HT uptake inhibitor fluoxetine nor selectively reducing 5-HT concentrations with raphe nucleus lesions altered the sensitivity of nore-
pinephrine-coupled adenylate cyclase. Mishra et al, 1981 suggest, therefore, that the action of both clomipramine and amitriptyline on the norepinephrine-sensitive adenylate cyclase system is probably due to their respective metabolism to demethylclomipramine and nor-triptiline, both affect inhibition of norepinephrine reuptake.

Following the data of Sulser (1978), showing that chronic antidepressant treatment decreased norepinephrine-sensitive adenylate cyclase activity, several groups have investigated whether such treatment altered the binding characteristics of \( \beta \)-adrenoceptor. A significant reduction in \( \beta \)-adrenoceptor binding has been demonstrated by several investigators (Banerji et al, 1977; Wolf et al, 1978; Sellinger-Barnett, et al, 1980; Bergstrom and Keller, 1979). However, the change has not been seen with every antidepressant examined since mianserin and zimelidine do not produce such effects.

Subtyping of the \( \beta \)-adrenoceptor change has been performed by Minneman et al, 1979, who showed that the desipramine-induced decrease was specific to the \( \beta_1 \)-adrenoceptor subtype. The rate of onset of these changes varied in different brain regions, being slower in the hippocampus than in the cortex (Bergstrom and Keller, 1979), which may explain why Kinnier et al, (1980) found that ten days of treatment with imipramine down-regulated the central cerebellar but not the hippocamal \( \beta \)-adrenoceptors.

If the \( \beta \)-adrenoceptor down-regulation is secondary to raised concentrations of norepinephrine in the synaptic cleft, then this down-regulation should be accelerated by inhibition of the presynaptic \( \alpha_2 \)-adrenoceptors, which are thought to have an inhibitory action on norepinephrine release (Langer, 1977). On the basis of this hypothesis several groups have attempted to accelerate the adrenoceptor
desensitization by combining \( \alpha \)-adrenoceptor antagonists with the antidepressant. Crews et al, (1981) for example, added the non-specific \( \beta \)-adrenoceptor antagonist, phenoxybenzamine, to desipramine treatment (7.5 mg/kg/day and showed that this combination produced a significantly faster decrease in \(^3\text{H}\)-dihydroalprenolol binding. This decrease in the maximum number of binding sites occurred after a single dose, whereas desipramine administration alone took six days to produce a similar reduction in receptor number, a change which in itself is faster than in most other studies. Phenoxybenzamine itself had no effect. Similarly, Ursillo et al, (1980) and Wiech and Ursillo (1980), accelerated \( \alpha \)-receptor down-regulation using the selective \( \alpha_2 \)-adrenoceptor antagonist, yohimbine, in conjunction with desipramine, iprindole or amitriptyline. Using a dose of 5 mg/kg/day of desipramine they obtained a significant fall in \( \alpha \)-adrenoceptor binding after three days of combined treatment with yohimbine, whereas this dose of desipramine alone took more than three weeks to produce a significant effect. Yohimbine alone did not affect \( \alpha \)-adrenoceptor number.

The same group of investigators have reported that the combination of amphetamine (a drug which increases the concentration of norepinephrine in the synaptic cleft) and iprindole, at doses with which either drug alone has no effect, will produce down-regulation of \( \alpha \)-adrenoceptor binding in the cortex and cerebellum after only three days of treatment (Reisine, 1980). Unfortunately it appears that this phenomenon is not due to a potentiation of the effects of iprindole by amphetamine, for it seems that there is a pharmacokinetic explanation (Manier, et al, 1980). Despite the fact that amphetamine
increases the availability of norepinephrine at the receptor site, chronic administration of the drug, even at high doses, only produced a slight subsensitivity of the norepinephrine-sensitive cyclic AMP-generating system in the rat brain (Mobley et al, 1979; Stone, 1978). It appears that following S-amphetamine administration, there is an accumulation of parahydroxy-norepinephrine (PHN) in adrenergic neurons and this is an antagonist of norepinephrine on the adenylate cyclase system. This compound, therefore, hinders the development of postsynaptic subsensitivity, since sustained action seems to be necessary for the down-regulation of \(-\)-adrenoceptor responses by norepinephrine (Mobley et al, 1979). Iprindole inhibits the aromatic hydroxylation of amphetamine, thereby increasing the half-life of amphetamine four- to five-fold and preventing the accumulation of PHN (Freeman and Sulser, 1972).

It should be noted that Hu et al, 1980 demonstrated no change in \(-\)-adrenoceptor density in the guinea pig cerebral cortex following chronic antidepressant treatment. This lack of change might reflect a difference in the \(-\)-adrenoceptor function in the two species.

The inhibition of monoamine oxidase in gastrointestinal tract and liver, however, exposes patients to the potentially toxic effects of dietary amines such as tyramine and \(-\)-phenylethylamine, which are normally degraded by monoamine oxidase and prevented from entering the general circulation. This undesirable side-effect (the 'wine and cheese syndrome') together with the authoritative Medical Research Council trial (1965) which showed the monoamine oxidase inhibitor phenelzine to be inferior to placebo in the treatment of a group of severely depressed patients, have understandably led to a sharp decline in the popularity of this group of drugs. Their place in the
treatment of depressive illness seems to be confined to those cases in whom anxiety is a major presenting feature of the illness.

There are, however, some reasons for retaining interest in the monoamine-oxidase inhibitors. Alternative pathways for the metabolism of toxic amines such as tyramine, exist in man, and a better understanding of individual differences in such metabolic pathways might make it possible to distinguish the patients likely to be at risk if treated with monoamineoxidase inhibitors. Furthermore, it is known that multiple forms (A and B) of the enzyme monoamine-oxidase exist in most tissues (Tipton et al, 1976). Monoamine oxidase-A is distinguished by its sensitivity to the inhibitor clorgyline (Johnston 1968) and it oxidizes noradrenaline and 5-HT, but not -phenylethylamine. Monoamine oxidase-B is preferentially inhibited by deprenyl (Knoll and Magyar, 1972) and oxidizes phenylethylamine but not no-repinephrine or 5-HT. Tyramine is a substrate for both forms. There is, thus, potential for the use of selective inhibitors of the two forms of monoamine oxidase as less hazardous antidepressant drugs (Knoll 1976).

The recent clinical studies of Rothe et al, (1976) and others also suggest that monoamine-oxidase inhibitors may have a valid clinical use in alleviating certain features of anxiety and phobic states.

Tricyclic Antidepressants And Other Monoamine Uptake Inhibitors

The high affinity, energy dependent uptake of NA, 5-HT and DA by nerve endings are recognized as the most important mechanisms by which the synaptic action of these neurotransmitters is terminated
(Iversen, 1971, 1975). Thus, drugs which interfere with these uptake processes will prolong the CNS actions of the monoamines.

Precise measurements of drug effects on the various amine uptake processes can be made in in vitro experiments, usually with synaptosome preparations from norepinephrine, 5-HT, or dopamine-rich areas of animal brain (Synder and Coyle, 1969). The in vivo potencies of drugs can also be assessed by pre-treating animals with various doses of test drugs before removal of the brain for in vitro uptake experiments. Alternatively the ability of drugs to prevent the depletion of brain norepinephrine by the compound 4-a-dimethyl-meta-tyramine offers an in vivo model for assessing inhibition of norepinephrine uptake (Carlsson et al, 1969) and interference with 5-HT depletion induced by 4-dimethyl-meta-tyramine (Carlsson et al, 1969) or by p-chloroamphetamine (Meek et al, 1971) are in vivo models for 5-HT uptake inhibition (Carlsson et al, 1969).

Sigg (1959) first reported that the dibenzazepine-tricyclic compound imipramine potentiated the action of NA on the cat nictitating membrane and it has since been clearly established that imipramine is a potent inhibitor of NA uptake into norepinephrine nerve endings. Imipramine is structurally related to another family of tricyclic compounds, the phenothiazines, and differs from promazine only by the replacement of the sulphur atom in the phenothiazine nucleus by an ethylene linkage. Four other tricyclic antidepressants have a central ring structure isosteric with imipramine and its monodesmethylated derivative desipramine (DMI) amitriptyline, nortriptyline, doxepin, and protriptyline.

The relative potencies of this series of tricyclic drugs in inhibiting NA, 5-HT, and DA uptake have been thoroughly investigated
(Horn et al, 1971; Horn 1976). Although effects on NA uptake occur at drug concentrations lower than those affecting 5-HT or DA uptake, there is a spectrum of activity spanning all three monoamine systems. Non-planar tricyclic molecules appear to be most potent as NA-uptake inhibitors (Horn 1976; Maxwell et al, 1969), and the presence of methyl or other substituents on the side chain nitrogen decreases potency against NA uptake.

The molecular characteristics favouring inhibition of 5-HT uptake, on the other hand, are (1) N-substitution or even a quaternary methyl structure, (2) a 3-carbon side chain, and (3) chlorine in the 3-position of the tricyclic nucleus (Horn 1976; Fuller and Wong, 1977).

Clomipramine is the most potent 5-HT uptake inhibitor of the tricyclic series. Although clomipramine is more selective than imipramine against 5-HT uptake, it remains a potent inhibitor of both 5-HT and norepinephrine uptakes.

A convenient clinical model for investigations of drug effects on 5-HT uptake is the blood platelet, which possesses 5-HT uptake sites with kinetic characteristics very similar to those in nerve endings (Sneddon 1973). The potency hierarchy for tricyclic antidepressants as inhibitors of 5-HT $^3$H uptake into human platelets is as follows: clomipramine > imipramine > amitriptyline > nortriptyline > desipramine (Waldmeier et al, 1976). Significantly decreased 5-HT-$^3$H uptake by platelets can also be demonstrated after in vivo drug treatment. Inhibition of 5-HT uptake by platelets taken from normal subjects after 4 days of clomipramine treatment, for example, was described by Waldmeier et al, (1976). Further support for the effects of clomipramine on central 5-HT and NA uptake in man
comes from the studies of Asberg et al (1977) who reported reduced 5-HIAA and MHPG in cerebrospinal fluid of patients who had received the drug of 3 weeks.

Horn et al (1971) demonstrated that most conventional tricyclic-antidepressants do not potently inhibit DA uptake by dopaminergic nerve terminals in brain. Horn et al (1971) investigated the structure-activity relationships of a large series of drugs with regard to inhibition of DA and NA uptake into rat brain synaptosomes. Replacement of an alkylamino side chain by a tropine ring system enhanced affinity for the DA-uptake mechanism, as did a relative lack of constraint of the aromatic ring.

Although there is little evidence that conventional tricyclic antidepressants interact directly with CNS dopaminergic systems, it is possible that they may influence such systems indirectly in vivo. Molander and Randrup (1976), for example found that imipramine, desipramine, clomipramine, and amitriptyline potentiated the apomorphine at postsynaptic DA receptors. Fuxe et al (1978) also reported that low doses of amitriptyline and desipramine reduced DA turnover in vivo in the rat nucleus accumbens, olfactory tubercle, and anterior caudate. It is still, however, doubtful whether effects on DA systems contribute importantly to the clinical actions of the antidepressants. Meltzer et al (1977), for example, failed to find any change in serum prolactin concentrations in ten depressed patients who were treated with tricyclic antidepressants, although prolactin secretion is known to be under inhibitory control by CNS dopaminergic neurones.
In addition to the tricyclic antidepressants, other classes of psychoactive drugs are capable of acting as inhibitors of monoamine uptake. Hendley and Synder (1968), for example claimed that the rank order of potency of six monoamine oxidase inhibitors as inhibitors of norepinephrine-^3H uptake into brain slices correlated better with their clinical potencies than did their ability to inhibit monoamine oxidase. Horn et al (1971) showed weak inhibitory properties in a series of phenothiazines tested on DA-^3H and NA-^3H uptake into rat brain synaptosomes, the most active drug, chlorpromazine, was only weakly active (approximate IC_{50}=5 μM) in blocking NA-^3H uptake.

The ability of the tricyclic antidepressants at nanomolar concentrations to inhibit NA and 5-HT uptake by monoaminergic neurones in CNS remains the most outstanding biological activity shared by these drugs. Monoamine uptake inhibition has become a key property in screening for new antidepressant drugs, and a number of compounds have recently been developed which have highly selective actions on NA or 5-HT uptake in CNS.

Maprotiline is a tetracyclic compound chemically related to benzoctamine. The side-chain attached to the tetracyclic nucleus is identical to that of desipramine and although it is similar to imipramine in its potency as an inhibitor of NA uptake it has only a very weak effect on 5-HT uptake (Maitre et al, 1975). Greengrass et al (1976) found maprotiline to be without effect on 5-HT-^3H uptake into platelets of human volunteers who had taken the drug, and it has been shown to have only weak effects on 5-HT-^3H uptake in rat brain synaptosomes (Maitre et al, 1974; Waldmeier et al, 1976).
The behavioural effects of maprotiline in animals are unlike those of imipramine - it is sedative and antiaggressive in rats. These differences may be due not only to a more selective effect of the drug on the NA uptake mechanism, but also to the lack of anticholinergic and post-synaptic -adrenoceptor antagonist-activity (Maitre et al., 1975). The assessment of clinical effectiveness is not within the scope of this discussion, but doubts have been expressed as to the efficacy of maprotiline in the treatment of primary depressive illness (Tait and Todrick, 1975).

Viloxazine is structurally different from imipramine in that it possesses a bicyclic nucleus. It is reported to be an inhibitor of NA uptake on the basis of its ability to protect against the NA-depleting effects of 6-hydroxydopamine in the mouse heart \textit{in vivo}, and appears to be of similar potency to amitriptyline, although weaker than imipramine, in this test (Fuller and Wong, 1977). Lippman and Pugsley (1976) reported that viloxazine inhibited the uptake of intravenously administered NA-\textsuperscript{3}H into mouse and rat hypothalamus or medulla. Desipramine, imipramine, and amitriptyline all inhibited drenal uptake of NA-\textsuperscript{3}H when administered in similar doses. A recent report by Waldmeier and Baumann (1978) however, showed viloxazine to be comparable in potency to clomipramine in inhibiting NA uptake in rat brain \textit{after in vivo} administration. In one study the drug failed to protect against brain 5-HT depletion by H 75/12 at an \textit{in vivo} dose of 32 mg/kg (Fuller and Wong, 1977), whereas in another a weak protective effect was observed at a dose of 25 mg/kg (Lippman and Pugsely, 1976). In conclusion, the evidence suggests a moderate selectivity of inhibition of NA uptake \textit{in vivo}. 
Viloxazine is able to cause a reversal of the behavioural depressant effects of reserpine and tetrabenazine in mice, and potentiates the effects of applied NA in the isolated rat vas deferens (Mallion et al., 1972). It appears to be less potent in these respects than imipramine. Observations in the intact animal suggest some similarities to amphetamine (EEG arousal pattern) and to benzodiazepines (reduced aggression in septal rat preparation) which distinguish it from tricyclic antidepressants (Greenwood, 1975) but its clinical efficacy as an antidepressant remains to be established.

Zimelidine is a bicyclic derivative of pheniramine and is less potent than clomipramine as an inhibitor of 5-HT $^3$H uptake into mouse-brain slices but has greater selectivity than clomipramine for 5-HT uptake relative to NA and DA uptake (Ross et al., 1976). Its potency in vivo, however, appears roughly equal to clomipramine and the possibility of an active metabolite has been investigated. Ross and Renyi (1977) investigated the effects of the drug and some of its metabolites on 5-HT $^3$H uptake into rat-brain homogenates and slices and concluded that most of the biological activity in vivo may derive from the monodesmethyl metabolite. The drug is isomeric and only the cis isomer is biologically active against 5-HT uptake (Abrahamson et al., 1976).

Trazodone is a new psychoactive drug which possesses an unusual neurochemical profile with regard to its action on brain monoamines and particular attention has been devoted to its effect on brain 5-HT mechanisms. High doses of the drug (50 mg/kg) given acutely to rats have no effect on the whole brain 5-HT of 5-hydroxyindoleacetic acid (5-HIAA) concentrations (Garattini et al., 1976).
Relatively low doses of trazodone are capable of protecting the rat brain against the 5-HT-depleting action of fenfluramine. In this respect trazodone is equipotent with clomipramine but more potent than desipramine. This effect of the parent drug is apparently shared by one of its metabolites (m-chlorophenylpiperazine, CPP). Trazodone and CPP inhibit the uptake of 5-HT-\textsuperscript{14}C into rat platelets with IC\textsubscript{50} values of approximately 10 uM and 7 uM respectively; values roughly ten times higher than clomipramine when tested in the same system (Garatini et al, 1976). These observations indicate that trazodone, and its congener CPP, share with the tricyclic antidepressants the property of blocking the membrane uptake for 5-HT into brain and platelets. The theoretical metabolite CPP has never been detected after administration but two other metabolites which have been identified, oxatrazolepyridinepropionic acid and trazodone N-oxide, are without biological activity. The potentiation of the central effects of 5-HTP observed after trazodone administration may be related to its 5-HT uptake blocking action.

**Toxicology of Antidepressants**

Generally the adverse and toxic effects of potential drugs are predictable from animal studies. However, some important toxic effects are not predictable from animal studies (WHO, 1966) and this applies particularly to drugs acting on the central nervous system, such as antidepressants. Recognition of species differences and similarities in responses is considered as an important means of predicting toxic effects in man. Moreover, tricyclic antidepressants are not innocuous drugs and toxicity due to these drugs is characterized by
central nervous system and cardiovascular involvements. Jefferson;
1975; Elonen, 1974; Thomann and Hess, 1980; Blackwell, 1983). In
addition to TCAs, other forms of therapies used to treat depression
have been found to have equally or to say more severe toxic effects.
For example, MAO inhibitors are reported to cause multifocal cardiac
necrosis with inflammatory reactions, mononuclear cell infiltration
and even myocardial fibrosis. Cases of adenoma and adenocarcinoma
of lungs and angiomia and angiosarcoma of blood vessels in female
mice have also been reported (Toth, 1976). Nialamide, at oral
doses of 60-80 mg/kg, markedly diminished fertility in rats and
cased fetal resorptions and marked changes in the estrus cycle
(Tuchmann-Duplessis and Mercier-Parot, 1961, 1962, 1963). In the
case of phenelzine, the antifertility effect was suggested to be
due to partial depression of pituitary gonadotropin activity
(Poulson and Robson, 1964).

In drug interaction studies of MAOIs, Milner (1968) found combi-
nation of ethanol and phenelzine to increase the duration of coma
and loss of righting reflex due to alcohol. Tranylcypromine caused
fetal hyperpyrexia when given to rats pretreated with lithium chlo-
ride for four consecutive days (Shimomura et al, 1977). Pretreat-
ment with iproniazid or tranylcypromine increased the acute toxi-
city of morphine, pethidine and phenazocine in mice. All MAO inhib-
itors, regardless of chemical structure or biological potency
increased the toxicity of amphetamine in mice (O'Dea et al, 1969).

Fatalities in man after tranylcypromine over dosage have
occurred at doses as low as 170 mg though most cases involved a dose
range above 300 mg. With phenelzine lethality may be observed in
the range of 375-1500 mg. Doses up to 1500 mg of nialamide have been fatal (Davis et al, 1968). Though MAO inhibitors alone are toxic enough to cause death, a number of fatalities have been reported to occur due to interaction with other drugs or certain types of food. MAO inhibitors not only block the deamination of biogenic amines but also interfere with various other enzymes and are able to prolong and intensify the effects of other drugs. Interference occurs with central depressant agents such as barbiturates, alcohol, potent analgesics, meperidine and drugs like tricyclic antidepressants, anticholinergics and precursors of biogenic amines.

MAO inhibitors possess a considerable potential for chronic toxic effects. Though the incidence of hepatotoxicity with currently used MAO inhibitors is low, hepatic effects are among the most dangerous. Excessive central stimulation is observed covering the range from tremors, insomnia, agitation and, rarely, hallucinations or confusion to convulsions. Orthostatic hypotension has been reported to occur with all currently used MAO inhibitors (Goodman and Gillman, 1980; Hamilton and Mahapatra, 1972).

Electroconvulsive therapy (ECT) is yet another form of therapy used to treat depression but is not without risk and 62 deaths were reported in England between 1947 and 1952 due to ECT. The major toxic effects due to ECT are loss of memory and induction of epileptic seizures (Prafitt, 1942). The impairment of memory associated with ECT is well-documented (Harper and Wiens, 1975, Holliday, Davison, Brown and Kreeger, 1968). It appears that the impairment of memory is probably related to the number of shocks given (Squire and Miller, 1974; Squire, 1975; Crow and Johnstone, 1979).
Typical tricyclic antidepressant drugs have been used for more than two decades, they are most often accompanied by many unwanted side effects. Most of the typical antidepressant drugs have moderate to strong anticholinergic effects at therapeutic doses (Baldessarini, 1980; Blackwell et al, 1980). These anticholinergic side effects are manifested in blurred vision, dry mouth, tardive dyskinesia, constipation, the most serious one being urinary retention. The tertiary amines possess the highest incidence of these effects, while the secondary amines like desipramine have relatively less anticholinergic effects (Baldessarini, 1981; 1982; Murphy et al, 1979).

Acute poisoning by TCAs usually leads to symptoms of central excitation followed at the higher and lethal dose levels of central inhibition. The symptomatology includes muscular weakness, twitching, stupor, respiratory disorders, ataxia and tonic-clonic convulsions.

The toxicity due to TCAs is more marked in the cardiovascular system. Through a combination of anticholinergic activity, diet myocardial depressant activity and an effect on the adrenergic neurons, they can cause a combination of arrhythmias, blood pressure abnormalities and congestive heart failure. These change, although most common with over dose, can occur at therapeutic levels. In addition a number of drug-drug interactions can occur between the tricyclics and agents used to treat other disease conditions especially the cardiovascular diseases.

In a recent study Glassman and Bigger (1981) have concluded that the most serious adverse effect of TCAs is the orthostatic hypotension but that these compounds are essentially free of adverse
cardiac actions in usual therapeutic concentrations obtained in prospective plasma-level controlled studies. In patients with pre-existing bundle branch disease there is a heightened risk of heart block but patients with ventricular arrhythmias are likely to benefit from the quinidine-like action of these drugs (Burgess, 1981). In a randomized clinical trial of 34 patients on nomifensine or maprotiline (75 mg daily each), Bethge et al, (1982) found no cardiac irregularities or conduction changes in the electrocardiographic analyses before treatment, after three weeks and one week after withdrawal. One novel cardiotoxic effect is the report of a case of arrhythmia associated with discontinuation of imipramine in a 54 year old woman treated for 13 years with 125 mg daily (Boisvert and Chouinard, 1981).

Tachycardia is another undesired common side effect of the typical antidepressants. The tachycardia is mainly attributed to the blockade of norepinephrine uptake in the heart and partially to the anticholinergic effect. Other serious side effects on the cardiovascular system reported include lowering of the blood pressure, increased tendency for arrhythmias, myocardial infarction and the precipitation of congestive heart (Carney, 1979, Hollister, 1981). Reports of sudden unexpected death (Moire et al, 1972) have been confirmed in tricyclic-treated in patients with myocardial disease. Therefore, tricyclic antidepressants are contra-indicated in patients having a history of heart disease. Unfortunately, many depressed people fall in the category of having both depressive illness as well as cardiovascular disease (Baldessarini, 1980).

In animal experiments TCAs have been found to have a dual effect on the cardiovascular system; small doses tend to cause an
increase in blood pressure, heart rate, cardiac contractility and coronary artery blood flow; larger dose cause a decrease in these measurements (Sigg et al, 1963; Langslet et al, 1971). It has been shown that the increased myocardial contraction occurring at low doses is mediated through activation of cardiac glycogen phosphorylase (Satchell et al, 1964). TCAs, in general, depress the pressor response to bilateral carotid artery occlusion or stellate ganglion stimulation (Kaumann et al, 1965; Sigg et al, 1963). Nortriptyline has been shown to cause right axis deviation, repolarization disturbances, intraventricular conduction disturbances and arrhythmias such as bradycardia and variable degrees of atrioventricular block. Higher doses led to cardiac arrest in asystole (Gaultier et al, 1965).

The ECG changes produced by tricyclics are probably caused by a combination of actions related to a direct effect on the myocardium anticholinergic activity and an effect on the adrenergic neuron. In rabbits amitriptyline-induced ECG changes were slightly normalized by quinidine and procainamide (antiarrhythmic agents), normalized by pyridostigmine (an anticholinesterase) and prevented by propranolol (beta adrenergic blocker) (Nymark and Rasmussen, 1966).

The direct arrhythmogenic effects of TCA may be due to interference with sodium-potassium transport across cell membranes. In the perfused isolated rat heart, imipramine caused a dose-dependent decrease in potassium ion, and in isolated rabbit atria it has been shown to cause changes in transmembrane potential (Matsuo, 1967). It was suggested that TCA-induced ECG abnormalities are increased by an intra to extracellular potassium shift in cardiac
tissue (Slovis, 1971). ATP phosphohydrolase is involved in the active transport of these ions, and imipramine has been shown to be an inhibitor of this enzyme (Tarve and Brochtlova, 1967).

When administered in therapeutic amounts, TCA can cause electrocardiographic changes in man. Results are somewhat variable from study to study, but for the most part involve reversible T-wave alterations.

Iprindole, an indole-based tricyclic with an antidepressive effectiveness similar to imipramine, was reported to have caused no ECG changes in man and to have little or no effect on ECG configuration in animals. Doxepin, in therapeutic doses, was associated with no change in serial ECGs in 140 patients after 2, 3, 4 and 6 weeks of therapy. In another doxepin study, 37 patients had one or more electrocardiograms over 18th and 41st months with no adverse changes except for a mild transient tachycardia in five patients taking over 200 mg/day. (Ayd, 1971).

Tricyclic antidepressants are known to interfere with the uptake of hydrophilic amines (e.g. guanethidine, metarominol etc.) by blocking the rather unspecific transport system in neuronal membranes. Such an effect can explain the prevention by TCAs of the adrenergic neurone blockade and depletion of catecholamines caused by guanethidine. It also explains the reduction by TCAs of the 6-hydroxydopamine-induced depletion of NE and DA as well as the reduction in 5-HT depletion caused by p-chloroamphetamine because the biochemical effects of 6-hydroxydopamine and p-chloroamphetamine depend on their neuronal uptake (Evetts and Iversen, 1970; Meek, 1971; Fuller et al, 1975). Amine depleting drugs
requiring active transport into monoaminergic neurons are used as a tool for the evaluation of uptake inhibition by potential antidepressants. This blockade of the transport system in neuronal membranes by TCAs has clinical consequences like the nullification of the antihypertensive effects of guanethidine, bethanidine, and debrisoquin (Mitchel et al, 1970).

Many a behavioural effects of amphetamine are enhanced or prolonged by TCAs due to the para-hydroxylation of amphetamine (Groppetti and Costa, 1969; Lewander, 1969; Freeman and Sulser, 1972). The peripheral effects of amphetamine, the indirectly acting sympathomimetic amine, are reduced or blockade by TCAs. Tricyclics are also known to inhibit the metabolism of a number of other drugs such as guanethidine, tremorine, oxotremorine, pento-barbital and hexobarbital.

The central stimulating properties of TCAs may come to light in combination with other centrally acting drugs. TCAs antagonize the various symptoms of reserpine syndrome. Imipramine in a dose of 50 mg/kg was found to counteract the potentiating effect of 2 mg/kg reserpine on anaesthesia produced by 2-M-4A. Similarly the spontaneous motor activity reduced by 2 mg/kg reserpine was antagonized by 50 mg/kg imipramine. Later on TCAs were found to be active at more reasonable dose levels and imipramine and amitriptyline were active at dose levels of 3.5 mg/kg and 13 mg/kg respectively against reserpine-induced ptosis. Secondary amine compounds were the most active compounds. Instead of reserpine, synthetic benzo-quinolizines, e.g., R04-1284 or tetrabenazine, have also been used.
Reserpine-induced hypothermia is antagonized by thymoleptics. Imipramine, desipramine, amitriptyline and nortriptyline given before reserpine enhances the hyperthermic phase of reserpine and prevents the subsequent hypothermic phase (Jori and Garattini, 1965; Garattini and Jori, 1967). These drugs given after reserpine elicit a marked rise in body temperature above the reserpine-induced hypothermic level (Morpurgo and Theobald, 1965; Votava et al, 1965; Garattini and Jori, 1967). Reserpine-induced gastric ulcers are prevented by rather large doses of imipramine, desipramine, amitriptyline or nortriptyline (Votava et al, 1965).

These effects of thymoleptics are most likely a result of potentiation of central norepinephrine mechanisms. The same mechanism may be responsible for the potentiating effect of TCAs on the central action of amphetamine. They enhance and prolong the hyperthermic effects of amphetamine in rats. Scheckel and Boff (1964) found that TCAs in rather low doses potentiated the effects of amphetamine and cocaine in a Sidman continuous avoidance test. Anticholinergics were also active but, in contrast to these drugs, TCAs also produced stimulant effect when combined with a small nondepressant dose of tetrahydrozine. While imipramine had no effect per se on self stimulation in the medial forebrain bundle it greatly augmented the effect of methamphetamine (Stein, 1967). Since imipramine and desipramine inhibit the parahydroxylation of amphetamine the potentiation and prolongation
of amphetamine effect may be, at least partly, due to increased brain levels of amphetamine. (Valzelli et al, 1967).

TCAs also potentiate the effect of catecholamine precursor dopa (dihydroxyphenylalanine) e.g., desipramine enhances the hyperthemic effect of dopa. In mice pretreated with an MAO inhibitor, TCAs enhance the behavioural effects of dopa producing a syndrome of piloerection, salivation, increased excitability, jumping, squeaking and aggressive fighting (Everett, 1967).

In mice, TCAs modify and intensify the behavioural effects of apomorphine, causing an intense gnaw compulsion syndrome (Pedersen, 1967). TCAs also potentiate the gnawing in mice induced by dopa following the decarboxylase inhibitor R04-4602 (Molander and Randrup, 1976). Amitriptyline and imipramine have been reported to be the most potent in provoking the apomorphine-induced gnawing. Pretreatment with -MT, a tyrosine hydroxylase inhibitor but not diethyldithiocarbomzte (dopamine -hydroxylase inhibitor) abolished the gnaw compulsion indicating that catecholamine probably dopamine plays an important role for the gnaw compulsion syndrome (Pedersen, 1968).

The effects of 5-HT precursors tryptophan and 5-hydroxytryptophan (5-HTP) are potentiated by some tricyclics. In mice, 5-HTP given after a tricyclic compound which blocks the reuptake of 5-HT in central serotonergic neurones results in an intense syndrome consisting of excitatio, lordosis, and abduction
of hind limbs (Hyttel and Fjalland, 1972). This syndrome is not seen after 5-HTP alone. Among the TCAs, chlomipramine was the most potent in this respect. The tertiary amines, imipramine and amitriptyline were also active although much weaker than clomipramine. Secondary amines were inactive. Among the newer nontricyclics which were tested, femoxetine was found to be slightly less potent than clomipramine, fluoxetine three times more potent and citalopram six times more potent than clomipramine (Christensen et al, 1977).

Given after an MAO inhibitor to rabbits, TCAs are often fatal, and the syndrome is characterized by hyperthermia and excitation (Carlsson et al, 1969, Gong and Rogers, 1971). This happens because following MAOI administration, brain levels of NA, DA and 5-HT are elevated and even pretreatment with p-MT did not prevent the hyperthermic reaction. While pretreatment with p-chlorophenylalanine (PCPA), a 5-HT synthesis inhibitor, prevented the increase in brain 5-HT and the hyperthermic reaction does not occur (Goerg and Rogers, 1971). Also, citalopram which is bicyclic specific 5-HT uptake inhibitor (Hyttel, 1977) causes marked hyperthermia after an MAOI, which is completely inhibited by pretreatment with PCPA (Christensen et al, 1977).
ANIMAL MODELS

Although no satisfactory experimental animal model of depression exists, there have been persistent attempts to create behavioral models of these syndromes in the laboratory. The creation of such a model is important for the understanding of the neural mechanism of depression and also they might be valuable test systems for the evaluation of new and effective antidepressant drugs.

The first experiments of this kind were performed in direct response to the original formulation of the amine hypothesis of depression that low levels of cerebral amines are associated with this state (SchildKrant, 1965). It was also found that some of the hypotensive patients receiving about 0.5 mg/kg reserpine developed depressive symptoms (Goodwin and Buneev, 1971). In an experiment on rhesus monkeys McKinney and coworkers (1971) administered reserpine to the animals for 81 days and found them to show a significantly decreased locomotion and visual exploration and increased huddling. In addition to these symptoms, reserpine also depleted the brain (and periphery) of both catecholamines and indoleamines. In order to assess the relative importance of these amines, Redmond et al (1971) carried out a number of experiments with \(-methyl-p-tyrosine (AMPT) which depletes catecholamines and p-chlorophenylalanine (PCPA) which depletes 5-HT mainly. From these experiments Redmond et al (1971) concluded that since the symptoms with AMPT resembled those of retarded depressives and since PCPA, despite the use of larger doses, produced no comparable symptoms of depression, the results are supportive of the catecholamine hypothesis of depression, despite the fact that L-dopa did not reverse the effect.
of AMPT in one animal to which it was administered. In another experiments, Redmond et al (1973) administered massive doses of the neurotoxin 6-hydroxydopamine (6-OHDA) intravenously for 3 days. Their findings were again supportive of the catecholamine hypothesis.

Though the results from these and other experiments on amine depletion and primate social behaviour are interesting, it cannot be readily accepted as a useful model of depression in man for the reason that the drug AMPT is nonspecific in that catecholamines are depleted everywhere in brain and periphery and has devastating physiological consequences. To assert that the behavioral changes are a specific result of cerebral catecholamine depletion, it must primarily require that they are reversed by the administration of precursors which restore the amines to their pretreatment levels. Further doubt is shed on the specificity of behavioral changes since surgical ablation of amygdala has apparently similar effects (Redmond et al, 1973; Dicks, 1969). It is obvious that the most valuable experimental model should involve primates. But it is apparent that models utilizing drug-induced alterations in the levels of amines in socially grouped monkey, even though they produce predictable neurochemical changes (which are not consistently seen in depressive subjects) are unlikely to be of real value in clarifying the neural basis of depression or aiding the development of new therapies.

Two particularly interesting approaches to correlate the depression in man and the classical psychological theories of depression are those based on reinforcement (reward) mechanisms in the brain (Olds, 1977), and the key behavioural sign in depression - helplessness or despair (Grinker, 1961).

Helplessness may be taken to indicate loss of control by the
patient over reinforcing stimuli in the environment, which results in the negative expectations about the effectiveness of one's efforts in bringing the environment under one's control (Akiskal and McKinney, 1973). Thus hopeless and helplessness about life become dominant sign quite early on, and may progress to withdraw from social contacts (since they are no longer reinforcing) because of an inability to both initiate and respond to them. In an ingeneous series of experiments Seligman et al (1967, 1968, 1970) proposed an animal model for this symptom of depression. Harnessed dogs were subjected to repeated, inescapable electric shocks and subsequently placed in a conventional shuttle-box situation. Exposure to shocks in this situation would normally lead to rapid avoidance by jumping to the safe side. But Seligman's dogs failed to learn this simple response, instead remained in their place and accepted the noxious stimulus. This behaviour was defined as "Learned helplessness".

The idea of learned helplessness has been challenged and criticized and by a number of workers (Weiss and Glazer, 1975; Weiss, Glazer and Pohorecky, 1976; Glazer and Weiss, 1976, a,b.) on the grounds that the phenomenon disappears within 48 hours. Thus they argued that such rapid disappearance of 'learned helplessness' is unlikely to occur if the helplessness following inescapable shock is really learned. And in a series of behavioural, pharmacological and neurochemical experiments, Weiss et al (1976) demonstrated that the temporary (24 hours) interference effect was mediated by a stress-induced disturbance in central neurotransmitter activity following the intense inescapable shocks and was viewed by them as performance deficit. This led them to propose the 'motot activation hypothesis' to replace the learned helplessness hypothesis.'
Exposure of animals to inescapable shocks of long duration (five seconds or more) produced long term interference effects (i.e. one week). And Seligman's subsequent work on this long-term phenomenon reconfirmed his original views and led to the restatement of the 'learned helplessness' hypothesis (Seligman et al 1975). However, further experiments by Glazer and Weiss (1976b) have provided the important observation, that with shocks of longer duration, the animal's response are biphasic: at onset there is much movement but, as the shock is continued, the animals become quiescent - a state which coincides with termination of shock. Glazer and Weiss (1976) suggested that this association of inactivity with shock termination explains the long term interference effects of such experiments. They therefore, contended that animals exposed to inescapable shocks do not learn to be 'helpless' but to be 'inactive'.

The basic assumption in the mechanism of the reward system in the brain is that animals will self-stimulate through electrodes implanted in the brain because the sensation so produced is pleasure-able or rewarding. Sites which support such behaviour are, essentially, within the medial forebrain bundle (which runs in the lateral hypothalamus and most important in the context of self-stimulation and reward, carries ascending aminergic projections from the brain stem to the diencephalon and telencephalon, as well as descending projections from the limbic forebrain (Olds, 1977).

Stein (1966) and others (Olds, 1977 etc.) in their pharmacological analysis of self-stimulation (reward) demonstrated that depletion of catecholamines (using AMPT) or blockade of dopamine (DA) or norepinephrine (NE) receptors using chlorpromazine or destruction of catecholamine neurons (using 6 OHDA) all disrupt...
self-stimulation, implying that they decrease its reinforcing value. Likewise, enhancement of catecholamine transmission, especially of NA, increases the rate of self-stimulation, i.e. increases the reinforcement value of the operant response. TCAs also possess this property particularly if used in combination with a drug which releases catecholamine, e.g., amphetamine. The views of Stein and other workers on depression as a failure of brain's reinforcement system are consistent with these findings. Regarding the controversial role of 5-HT it has been reported that depletion of 5-HT (as with PCPA) or blockade of 5-HT receptors both enhance self-stimulation and elevation of central 5-HT activity suppresses self-stimulation (Shaw et al, 1977). Hence it is proposed that drugs which decrease 5-HT levels in the brain should have some antidepressant property (Fuxe et al, 1978; Shaw et al, 1977).

The behavioural effects of clinically used compounds with known antidepressant effects have also been employed for the creation of models by comparing the behavioural effects of the known antidepressant with the new, untried compounds. A similarity of effects may be taken to indicate a therapeutic value for the compound. The main problem with such models is that the behavioural effects seen here are the depression itself and no attempt has been made to correlate them. A good example is the Horovitz's (1966) model of muricide based on the argument that TCAs block muricide and so do the central amygdaloid lesions - thus the two effects may be related to one another. In support of this, it was found that imipramine and some other TCAs block the muricide when injected into the centromedial amygdala but not elsewhere in the amygdala or the hypothalamus or the septal nuclei. Thus Horovitz (1981)
contended that there is a fairly clear evidence or relationship between the amygdala and the actions of antidepressants and that the disease entity of depression may be a reflection of abnormal activity of this portion of brain and that amygdala would have an inhibitory role and its increased activity (possibly acting on the hypothalamus) brings on depression.

Despite the elegance of this one experiment out of the many widely used, the data derived from such experiments say little more about the drug's potential therapeutic effect than the initial observations, for example, the inhibition of monoamine uptake.

Since abnormal behavioural pattern can be induced in non-human primates, KcKinney and Bunney (1969) suggested four essential conditions to be met before such animal models could be considered as representative of the human syndrome. The four conditions laid down are:

1) The external behaviour and expressions of the animal should be easily identifiable with those of man.
2) The biochemical background of the disturbed animal should mirror that of the disturbed human.
3) The aetiological conditions predisposing or causing human depression should be similar for animals.
4) The kinds of therapy effective for man should also be effective for the animal species.

Unfortunately none of the animal models developed so far has come up to these expectations.

The most widely used parameters for the evaluation of anti-
depressant activity are hypothermia and ptosis produced by amine-depleting agents (Garattini et al, 1962; Askew, 1963; Halliwell et al, 1964; Lapin, 1976; Tedeschi, 1974). It is generally agreed that the reversal of hypothermia is not specific for antidepressants. The fall in body temperature that occurs in amine-depleted animals can be reversed by many other drugs with no proven antidepressant activity such as antihistamines, analgesics, sympathomimetic and anticholinergics (Lapin, 1967 a,b). But the hypothermia produced by the blockade of D.A. receptors by pimozide is not countered by despramine (Doggett et al, 1975a), this seemingly implies that the antagonizing effects of PCAs are NA-selective. Although apomorphine is a direct DA-receptor stimulant drug, the hypothermia induced by it can be counteracted by a number of TCAs (Maj et al, 1974; Schelkunov, 1977).

TCAs also prevent and reverse the hypothermia induced by reserpine and like drugs. But mianserine and some other atypical antidepressants do not counteract the effects produced by either reserpine or tetrabenazine in mice (Foll et at, 1973; Fleischhauer et al, 1973). It appears that the mechanism involved in the control of body temperature are complex and that besides NE-activating (Cox, 1975; Svensson, 1971) other concomittant actions of TCAs (e.g. postsynaptic - receptor blockade etc.) can interfere with magnitude of antihypothermic effect.

The limitations experienced in the case of hypothermia are also valid for the case of tosis induced by reserpine or related drugs (Lapin, 1967 b; Sigg et al, 1965; Nimegeers 1975). However, effects exerted by other types of drugs can be discriminated from those of antidepressants. Directly acting sympathomimetics can
be cited as an example to produce exophtalmos in untreated animals and in terms of doses this effect coincides with their antagonistic action against ptosis. It is also true for amphetamine like drugs. Antidepressants are known to prevent ptosis by means of their ability to increase central adrenergic output (Halliwell et al, 1964; Tedeschi, 1974) through inhibition of NE-uptake. Fielden and Green (1965) has also suggested a peripheral mode of action.

Antidepressants also reverse sedation and produce hyperactive behaviour in animals treated with amine depleters. But they, the classical TCAs, do not produce a true reversal of symptoms already established because their activity depends on the presence of functionally active amines. In many later studies such effects of various imipramine related TCAs were not fully confirmed. And it was more so if attempts were made to quantity locomotion. Schmitt and Schmitt (1966) reported various imipramine-like drugs not to reduce signs of motor depression to an appreciable extent. According to Delini-Stula (1972) TCAs produce in general rather weak, inconsistent and mostly short-lived activating effect in amine-depleted animals.

Although animal models in which behavioural excitation induced by drugs believed to activate directly or indirectly central adrenergic functions, have also been introduced simultaneously as the amine-depleted animal models. They are less valid and less apt to discriminate antidepressants from other types of drugs than the amine-depleted models. Among the most widely used drug-induced behavioural models, are those of amphetamine and like drugs, apomorphine and L-dopa. Hyperthermia, stereotyped behaviour and self stimulation are the symptoms of the
amphetamine group of drugs (Morpurezo & Theobald, 1967; Halliwell et al, 1964; Stein, 1962). Apomorphine produces hypothermia and stereotyped behaviour (Maj et al, 1974; SchelKunor, 1977; Ther and Schram, 1962), whereas L-dopa causes a behavioural excitation (Everett, 1967; Molander and Randrup, 1976a). These drugs are not alone in producing such behavioural disturbances in animals, a number of other drugs, structurally or otherwise related to imipramine, produce similar effects. At present a parallel between the biochemical events and functional manifestation of the interaction between amphetamines and TCAs cannot (possibly) be drawn. Imipramine, desipramine which prolong the effects of amphetamine, have been shown to inhibit its metabolic degradation and decrease the rate of its disappearance from the brain (Sulser et al, 1966; Valzelli et al, 1967; Lemberger et al, 1970; Freeman & Sulser, 1972).

The catecholamine procurssor, L-dopa produces in animals autonomic and behavioural changes (piloerection, sweating and salivation, acceleration of respiratory rate, hyperactivity and irritability) and these effects have been attributed to the newly formed products of catabolic degradation of L-dopa, the NA, DA and adrenaline, similar effects have been observed by antihistaminic and anticholinergic drugs. Certain hormones, such as thyrotropin-releasing hormone (TRH), or luteinizing hormone releasing hormone (LH-RH) and growth hormone release inhibiting (Somatostatin, GHRH) hormones markedly increase behavioural effects produced by L-dopa (Plotnikoff et al, 1971, 1972, 1975). Similar action was also described by Plotnikoff and coworkers (1976) for some peptides and morphine. Though an association between the bio-
Chemical effects of antidepressants (TCAs) on dopamine metabolism in brain (Pycock et al, 1977) and the behavioural effects of L-dopa have been suggested (Molander and Randrup, 1976a; Randrup and Braestrup, 1977), at present it is not possible to show/draw a clear parallel between either of the biochemical effects of TCAs and potentiation of L-dopa response.

Potentiation of the effects of apomorphine (a direct DA-receptor stimulant), like stereotyped grooming, licking and biting, are not specific for antidepressants and has been reported for drugs of various pharmacological classes, e.g. analgesics, anticholinergics, antihistamines and biochemical changes underlying the functional effects of apomorphine still remain to be elucidated.