THE EVALUATION OF MELATONIN AS A POSSIBLE ANTIDEPRESSIVE

A thesis presented in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

at

RHODES UNIVERSITY

by

DEBRA JEAN SKENE (B.Pharm.)

December, 1979.

"No worst, there is none. Pitched past pitch of grief
More pangs will, schooled at forepangs, wilder wring."

Gerard Manley Hopkins.

ACKNOWLEDGEMENTS

I should like to express my sincerest thanks to my supervisors, Professor B. Potgieter and Dr. B.J. Wilson, for their co-operation and guidance throughout this work.

I am also indebted to:

The Medical Research Council for a scholarship for 1979.

The Foundation for Pharmaceutical Education and Johnson and Johnson (Pty) Ltd. for a bursary for 1979.

Mr. D. Makubalo for his invaluable technical assistance.

Mrs. Theresa Naudé for typing the manuscript.

Finally, I would like to thank my parents and friends for their understanding and encouragement throughout my studies.

CONTENTS

..... - iv -/

..... - v -/

..... - vi -/

..... - vii -/

..... - viii -/

..... - ix -/

ABBREVIATIONS USED

Ach	acetylcholine
ACTH	adrenocorticotropic hormone
ADH	antidiuretic hormone
AMPT	α-methylparatyrosine
APA	American Psychiatric Association
BW	brain weight
cAMP	cyclic adenosine monophosphate
CAR	conditioned avoidance response
CEEG	computer-analyzed electroencephalogram
CNS	central nervous system
COMT	catechol-O-methyltransferase
CS	conditioned stimulus
CSF	cerebrospinal fluid
DA	dopamine
DNA	deoxyribonucleic acid
ECT	electroconvulsive therapy
EEG	electroencephalogram
ER	escape response
Fig.	figure
FSH	follicle-stimulating hormone
FSH-RF	follicle-stimulating hormone releasing factor
GABA	ɣ-aminobutyric acid
GH	growth hormone
HCl	hydrochloric acid
5-HIAA	5-hydroxyindoleacetic acid
HIOMT	hydroxyindole-O-methyltransferase
HPA	hypothalamic-pituitary-adrenal
5-HTP	5-hydroxytryptophan
HVA	homovanillic acid
I.P.	intraperitoneal
i-r	infra-red
i.v.	intravenous
L-DOPA	l-dihydroxyphenylalanine
LH	luteinizing hormone

..... - xii -/

LH-RH	luteinizing hormone releasing hormone
LSD	lysergic acid diethylamide
LVP	lysine-8-vasopressin
MAO	monoamine oxidase
5-MeODMT	5-methoxy-N,N-dimethyltryptamine
MHPG	3-methoxy-4-hydroxyphenylglycol
MIF	melanocyte-stimulating hormone release-inhibiting factor
MSH	melanocyte-stimulating hormone
NR	no response
PCPA	p-chlorphenylalanine
REM	rapid eye movement
RF	releasing factor
S.C.	subcutaneous
S.E.M.	standard error of mean
SNAT	serotonin-N-acetyltransferase
T_3	L-triiodothyronine
TAD	tricyclic antidepressive
TCP	tranylcypromine
temp.	temperature
TH	thyroid hormone
TRH	thyrotropin-releasing hormone
TSH	thyroid-stimulating hormone
UCS	unconditioned stimulus
VMA	3-methoxy-4-hydroxymandelic acid

..... - xiii -/

LIST OF FIGURES

..... - xiv -/

..... - xv -/

Page

..... - xvi -/

LIST OF TABLES

..... - xvii -/

ABSTRACT

Melatonin, a hormone of the pineal gland, was evaluated in a variety of animal models of depression.

Measurements of the frog righting reflex and rat locomotor activity showed that low doses of melatonin have a serotonin-like potentiating effect following monoamine oxidase inhibition. High doses of melatonin caused a reduction in the duration of rat immobility in the Porsolt model of depression and exerted a chlorpromazine-like effect on conditioned avoidance behaviour.

In view of the indoleamine hypothesis of depressive disorders, the possibility of melatonin being a potential antidepressive is discussed and it is concluded that melatonin might be useful in the treatment of "agitated" depressions.

C H A P T E R 1

LITERATURE REVIEW

1.1 DEPRESSION:

1.1.1 Introduction:

Depression is the most common psychiatric disorder. According to Kline (1964) more human suffering has resulted from it than from any other single disease affecting mankind.

The term depression has been used to denote a variety of conditions including (i) a normal lowering of mood in response to adversity; (ii) a symptom similar to sadness; (iii) a syndrome representing an abnormality of mood; and (iv) an illness with a recognized aetiology and prognosis. This lack of definition has led to controversy regarding its classification.

Whether depression is primarily a biological, genetic or cognitive disorder has been the subject of even more controversy since influences at all levels have been found to cause changes in each other. The various theories regarding the definition, classification and aetiology of depressive disorders are discussed.

1.1.2 Signs and Symptoms:

Although no consensus can be reached regarding the classification and aetiology of depressive illnesses, many of the symptoms are common to the entire group of

affective disorders. The signs and symptoms of depression are outlined in Table 1A.

The primary change is in mood. The depressive suffers from feelings of sadness, hopelessness, guilt, apathy and lowered self-esteem. Other disturbances in sleeping, appetite, libido and so forth are common but not essential accompaniments.

This uniformity of symptoms suggests that all depressions, regardless of the cause, are expressed in a similar neuro-physiological way.

TABLE 1:

SIGNS AND SYMPTOMS OF DEPRESSION

Manifestations	Description	Patient's expressions
Emotional		
Dysphoric mood	Crying Sad, unhappy, blue	"I can't help crying." "The pleasure has gone out of life."
Cognitive		
Negative self-concept	Lowered self-esteem Self-reproach and self-criticism Pessimism	"I am inferior." "It's all my fault." "Things will never get any better."
Poor concentration and slow thinking		
Behavioural		
Loss of interest and motivation		"I have no desire to do anything."
Neglect of personal appearance		
Psychomotor retardation		
Agitation	Restless, fearful, worried, irritable	
Physical		
Sleep disturbance	Early morning wakening Insomnia or hypersomnia	
Loss of libido	Impotence, frigidity	
Appetite disturbances	Anorexia or increase in appetite	
Constipation		
Menstrual disturbances		
Other general complaints	Headache Hot flushes Dryness of mouth Blurred vision Dizziness Dyspnoea	

..... - 4 -/

1.1.3 Concepts of Depression:

Beginning with Freud's paper on "Mourning and Melancholia" in 1917 (Freud, 1950), numerous concepts of depression have been formulated. These can be grouped into 4 general categories: psychoanalytic, cognitive, behavioural and biological.

1.1.3.1 Psychoanalytical Concepts:

1.1.3.1.1 "Aggression-turned Inward":

This concept, originally proposed by Abraham in 1911 (Abraham, 1948) and later elaborated by Freud (1950) attempted to explain depression in terms of psychoanalytic theory. Depression was seen as hostility directed inward upon the loss of a loved object.

Abraham suggested that the essential difference between grief and depression is that a mourner is consciously concerned with the lost person, whereas a depressed individual is overcome with feelings of loss, anger, guilt and low self-esteem. Freud expanded these ideas, suggesting that in mourning the loss is conscious, whereas in depression the true loss is unconscious. Depression was therefore seen as a failure of the normal mourning process.

Although this is the most widely quoted psychological theory of depression, there is little systematic evidence to support it (Weissman et al., 1971).

1.1.3.1.2 "Object Loss":

Depression was conceptualized as a reaction to the real or fantasized loss of a loved person, either by death or separation, loss of a material possession, loss of status and so forth (Spitz, 1946).

Bibring (1965) extended this by suggesting that loss of self-esteem was the essential element in depression. Bibring's hypothesis differs from the id-psychological approach of Freud and Abraham by placing more emphasis on the ego. He viewed depression as the emotional expression of a state of helplessness and powerlessness of the ego. In other words, depression appeared when one could not live up to one's ego ideals.

These theories suggest that depression is primarily a disturbance in mood.

1.1.3.2 Cognitive Hypothesis:

This concept of depression postulated by Beck (1967) suggests that the primary disturbance is in thinking which, in turn, causes the disturbed mood state.

This alteration in cognition is characterized by negative expectations. The depressed person has a negative view of himself, his experiences and of the future.

1.1.3.3 Behavioural Concepts:

Behaviourists conceptualize depression as a set of maladaptive behavioural responses elicited either by uncontrollable aversive stimuli or by loss of reinforcement.

1.1.3.3.1 "Helplessness":

The animal model of "learned helplessness" developed by Seligman (1975) has many similarities with human depression (see Section 1.4.3). He has concluded from these studies that an individual, who is unable to exercise control over his reinforcers, develops feelings of helplessness, powerlessness and passivity which constitute the depressed state.

1.1.3.3.2 Loss of Reinforcement:

Lewinsohn and his co-workers suggested that a low rate of response-contingent positive reinforcement acts as an eliciting stimulus for depression (Lewinsohn, 1974). Depression would ensue either if the environment failed to provide reinforcement or if the individual failed to emit the appropriate response to potential reinforcers.

Some depressive patients exploit the "sick role" to substitute for the loss of reinforcement, a characteristic which has been labelled "hysteroid dysphoria" by Klein and Davis (1969).

1.1.3.4 Biological Hypotheses:

(See Section 1.2 for a detailed discussion.)

1.1.3.4.1 Biogenic Amine Hypothesis:
This postulates that the primary cause of depression is a defect in biogenic amine metabolism. Depression is associated with a relative deficiency of catecholamines (Bunney and Davis, 1965; Schildkraut, 1965) or indoleamines (Glassman, 1969; Lapin and Oxenkrug, 1969) at functionally important receptor sites.

1.1.3.4.2 Cholinergic Hypothesis:
Janowsky et al. (1972b) propose that depression is a disease of central cholinergic predominance.

There are numerous other biological concepts of depression including, a disturbance in adrenocortical hormone metabolism, a disturbance in electrolyte metabolism and so forth. The question is whether these biological changes seen in depression are the cause or a consequence of the depressed state.

To conceive depression as the behavioural expression of a single input is probably a gross oversimplification. A comprehensive theory of depression requires input from all all 4 levels of analysis, rather than narrow adherence to any one approach. Akiskal and McKinney (1973; 1975) have attempted to integrate the various conceptual models of depression into a unified hypothesis. According to them, depression is seen as a "psychobiological final common pathway" of various interlocking processes at chemical, experiential and behavioural levels.

1.1.4 Classification of Depression:

Defining and classifying depressive disorders are crucial for research. Classification is of considerable importance, both in the development of new concepts of depressive illnesses and when the appropriate course of treatment is under consideration.

Confusion in the classification of affective disorders has arisen because of the multiple use of the term "depression". It has been used to describe a physiological mood state, a symptom, a syndrome and a disease entity. Depressive disorders can be classified according to aetiology, symptomatology or biochemistry.

Until recently, two schools of thought predominated. The gradualists (Lewis, 1938; Hoch, 1953; Menninger, 1963) proposed that depression is a single clinical disorder that can express itself in a variety of forms. They maintained that the difference between neurotic and psychotic depressions is one of degree and should not be classified separately.

The separatists (Kiloh and Garside, 1963; Carney, Roth and Garside, 1965; Klerman, 1971) divide depressive disorders into two distinct groups. Those which are "endogenous" and those which are "reactive". Endogenous depressions have no apparent cause, whereas reactive depressions are exaggerated reactions to adversity.

The term psychotic and neurotic depressive reactions refer to the clinical picture and a distinction is also made between them. Psychotic depressive disorders are more severe, contact with reality is lost and delusions are present. Neurotic depressive disorders are milder with no delusions. The link between these two dichotomies is that endogenous depressions often tend to be psychotic in clinical picture, while reactive depressions tend to be neurotic. In practice, this simplistic classification does little justice to the range of disorders seen and an appreciable number of cases fall within the ill-defined areas separating the two groups. The American Psychiatric Association (APA) adopted the separatist view and classified depressive disorders into two broad categories based on aetiology (APA, 1968). The classification is shown in Table 2.

The "major affective disorders" consist of "manic-depressive illnesses" and "involutional melancholias". The onset of these disorders "does not seem to be related directly to a precipitating life experience" (APA, 1968). This infers a biological origin. On the other hand, the neurotic and psychotic depressive reactions are "reactive" to life events and are therefore psychogenic. The important disadvantage of this classification system is that the affective disorders are divided on an aetiological basis even though the aetiology of depressive illnesses is still unclear.

..... - 10 -/

TABLE 2:

AMERICAN PSYCHIATRIC ASSOCIATION'S CLASSIFICATION OF THE AFFECTIVE DISORDERS

NOMENCLATURE	DESCRIPTION
I MAJOR AFFECTIVE DISORDERS	Illness not related to a precipitating life event.
A. MANIC-DEPRESSIVE ILLNESSES	
1. MANIC-DEPRESSIVE ILLNESSES, MANIC TYPE	Manic episodes only - excessive elation, irritability, talkativeness, flight of ideas, and motor activity.
2. MANIC-DEPRESSIVE ILLNESSES, DEPRESSED TYPE	Depressive episodes only. Depressed mood, mental and motor retardation.
3. MANIC-DEPRESSIVE ILLNESSES, CIRCULAR TYPE	Depressive and manic episodes.
4. OTHER MAJOR AFFECTIVE DISORDERS Includes "mixed" manic-depressive illnesses	Manic and depressive symptoms appear almost simultaneously.
B. INVOLUTIONAL MELANCHOLIAS	Occurs in the involutional period - worry, anxiety, agitation and insomnia. Delusions may also occur.
II PSYCHOTIC DEPRESSIVE REACTIONS	Depressed mood due to some experience. Delusions are present.
III DEPRESSIVE NEUROSES	Includes reactive depressions. Depression due to internal conflict or loss of a loved object.

The difficulties of classification have led to alternative ways of describing depressed patients. Pollitt (1965) classified depressive patients according to the presence or absence of the "depressive functional shift". The term describes the somatic features of depression such as loss of appetite, loss of mass and early morning wakening. He proposed that the absence of the depressive functional shift (justified or J type) was a result of psychological stress, whereas its presence (somatic or S type) represented a physiological disorder. Although the usefulness of this proposal has still to be determined, it seems unrealistic to classify the numerous forms of depressive disorders according to the presence or absence of somatic features (Mendels, 1970).

Van Praag (1962; 1976) divided depressions into "vital depression" and "personal depression". "Vital depression" describes "endogenous depression" but the term refers exclusively to a syndrome not an aetiology. Likewise "personal depression" is a descriptive concept describing "reactive depression". Mixed forms are also common.

The major problem with the previous systems of classification is that they assume depressive disorders to be either inherently psychological or inherently biological. This is seen in the mind/body, endogenous/reactive and neurotic/psychotic dichotomies. Any emotional state, including depression, however, is the behavioural expression of

..... - 12 -/

psychological, somatic and psychomotor inputs and consequently depressive disorders should be classified comprehensively taking all 3 levels of influence into consideration.

A major advance in this area is the Robins-Guze (1970) classification of depressive disorders into "primary" and "secondary depression". "Secondary depression" refers to feelings of sadness and hopelessness that occur in the presence of another illness. This illness may be either a nonaffective psychiatric disorder such as anxiety, neurosis, hysteria or it may be a medical illness such as rheumatoid arthritis, viral infections and chronic heart conditions. A primary disorder of mood with no other pre-existing psychiatric syndrome is termed "primary depression". This is further subdivided into unipolar and bipolar affective illness. Unipolar depression refers to one or more episodes of depression, whereas bipolar depression is characterized by manic attacks either in the patient or in his biological relatives. This subdivision has been supported by numerous workers, who from genetic, biochemical and pharmacological studies, have concluded that there is a quantitative difference between bipolar and unipolar depressive diseases. These differences are summarized in Table 3. The value of this classification is that depressive disorders are defined independently of aetiology and therefore it eliminates the endogenous/reactive controversy. It is also the most comprehensive to date as it incorporates genetics, biochemistry, neurophysiology and response to pharmacotherapy (Table 3).

..... - 13 -/

TABLE 3:

DIFFERENTIAL CHARACTERISTICS OF BIPOLAR, UNIPOLAR AND SECONDARY DEPRESSIONS
(Modified from Akiskal and McKinney, 1975)

VARIABLE	BIPOLAR DEPRESSION	UNIPOLAR DEPRESSION	SECONDARY DEPRESSION	REFERENCES
Clinical Features	Retarded in psychomotor activity.	Agitated in psychomotor activity.	Depressive features superimposed on non-affective psychiatric symptomatology e.g. hysteria.	Biegel and Murphy, 1971
	Postpartum episodes.			Reich and Winokur, 1970
	Manifested in affective episodes only.	Clinically heterogeneous - manifested in forms other than depression e.g. alcoholism.		Winokur et al., 1970;71
	Median age at onset 30 years.	Median age at onset 45 years.		Perris, 1966
Clinical Course	Episode lasts 3-6 months.	Episode lasts 6-9 months.	No clear-cut episodes; either transient or chronic.	Perris, 1968 Winokur, 1970
	Greater than 3 episodes per lifetime.	1-2 episodes per lifetime.		
Genetics	High genetic loading for affective illness suggesting dominant transmission.	Low genetic loading for depression. Mode of inheritance uncertain.		Perris, 1966 Winokur et al., 1969 Cadoret et al., 1970
	Either X-linked dominant,			Reich et al., 1969 Winokur et al., 1969 Mendlewicz et al., 1972
	or autosomal dominant,			Green et al., 1973
		Polygenic inheritance suggested.		Gershon et al., 1971 Baker et al., 1972
	Higher suicide rate in their families.			Murphy et al., 1971
Neurophysiology	"Augmenter" on evoked potentials.	"Reducer" on evoked potentials.		Borge et al., 1971 Buchsbaum et al., 1971
Biochemistry	Low platelet MAO[1] activity.	Normal platelet MAO activity.		Murphy and Weiss, 1972 Jacobsen et al., 1973
	Lower MHPG[2] excretion.			Maas et al., 1973 Schildkraut et al., 1973a; 1973b Beckman et al., 1975
	More marked decrease in lumber CSF 5-HIAA[3].			Mendels et al., 1972a Ashcroft et al., 1973a
	Subnormal steroid excretion.	Above normal steroid excretion.		Dunner et al., 1972
	Lower 17-hydroxycorticosteroid concentration in urine.			Jacobsen et al., 1973
Pharmacology	Lithium carbonate responsive.	Less likely to respond to lithium carbonate.		Goodwin et al., 1972 Noyes et al., 1974
	May switch to hypomania with tricyclics.	Tricyclic responsive.		Bunney et al., 1970 Murphy et al., 1971
			"Hysteroid dysphorias" and "atypical depressions" may respond to MAO inhibitor.	Robinson et al., 1973

[1]MAO = monoamine oxidase

[2]MHPG = 3-methoxy-4-hydroxyphenylglycol

[3]CSF 5HIAA = cerebrospinal fluid 5-hydroxyindoleacetic acid

1.1.4.1 Biochemical Classification:

In recent years there has been an attempt to classify depressive disorders on a biochemical rather than a clinical basis. Various biochemical classifications based on urinary MHPG levels, CSF 5-HIAA concentrations and platelet MAO activity have been proposed.

Schildkraut et al. (1973a; 1973b; 1977) have shown that bipolar manic-depressive patients excrete a significantly smaller amount of MHPG (the predominant metabolite of brain noradrenaline) than do unipolar and non-specific depressive patients. This has been supported by numerous investigators who have found low urinary MHPG levels in a substantial number of depressive patients (Maas et al., 1968; 1973; Beckmann and Goodwin, 1975; Goodwin and Post, 1975; Casper et al., 1977). These findings have led to the suggestion that there may be two biological groups of depressive disorders: one with a relatively low MHPG excretion such as bipolar manic-depressive depressions and the other with a relatively high MHPG excretion such as unipolar depressions. Patients excreting less than normal quantities of MHPG showed a temporary brightening of mood following d-amphetamine administration, whereas patients who excreted normal amounts of MHPG tended to have either no elevation of mood or depression with d-amphetamine (Fawcett and Siomopoulous, 1971; Fawcett et al., 1972). Consequently it has been suggested that d-amphetamine be used to identify various depressive subgroups (Garver and Davis, 1979).

..... - 15 -/

Further studies have demonstrated that low urinary levels of MHPG are correlated with successful desipramine and imipramine treatment (Fawcett et al., 1972; Maas et al., 1972a; Beckmann and Goodwin, 1975) and high or normal levels of MHPG with successful amitriptyline treatment (Schildkraut, 1973; Beckmann and Goodwin, 1975). Desipramine has been shown to selectively inhibit the reuptake of noradrenaline (Eckhardt and Maxwell, 1973), whereas amitriptyline selectively inhibits the reuptake of serotonin (Carlsson et al., 1969; Maas, 1975). In clinical trials, imipramine seems to be similar to desipramine with respect to correlation between therapeutic efficacy and low MHPG levels (Fawcett et al., 1972; Beckmann and Goodwin, 1975), however in the laboratory, imipramine has almost equal effects on serotonin and noradrenaline reuptake (Maas, 1975; Randrup and Braestrup, 1977). The reason for this discrepancy is not yet known.

It has been postulated from these results that at least two biochemical subtypes of depression exist. "Noradrenaline depression" characterized by (1) low urinary MHPG levels, (2) a favourable response to treatment with imipramine or desipramine and (3) an elevation in mood following d-amphetamine treatment. "Serotonin depression" characterized by (1) normal or elevated urinary MHPG levels (Maas, 1975), (2) low CSF 5-HIAA levels (Åsberg et al., 1976), (3) a favourable response to amitriptyline treatment and (4) a lack of mood change during d-amphetamine treatment.

..... - 16 -/

Additional evidence for a "serotonin depression" has been presented (van Praag and Korf, 1971a; Åsberg et al., 1973; 1976; Bertilsson and Sjoqvist, 1973).

Åsberg et al. (1973; 1976) measured the CSF concentration of 5-HIAA (a metabolite of serotonin) of a group of depressed patients. Twenty-nine % of these patients had sub-normal 5-HIAA concentrations. Within this subgroup, there was a significant negative correlation between the 5-HIAA concentration in the CSF and the severity of depression. A group of depressed patients with a subnormal 5-HIAA concentration in the CSF has also been reported by Mendels et al. (1972a) and by van Praag et al. (1970; 1973a).

The rate of accumulation of 5-HIAA in the CSF after probenecid administration is presumed to reflect the rate of synthesis of brain serotonin (van Praag et al., 1970; Korf et al., 1970). Van Praag and Korf (1971a) have shown that some depressive patients exhibit a normal rise in 5-HIAA after probenecid pretreatment whilst others show a subnormal response. These patients exhibiting a subnormal response have been reported to respond favourably to 5-hydroxytryptophan (5-HTP) administration (van Praag and Korf, 1974). Several investigators have confirmed these results (Goodwin et al., 1973; Sjöstrom, 1973; Bowers, 1974). This suggests that within the group of vital depressive patients, there is a subgroup with disturbances in serotonin

metabolism, although they are indistinguishable in psychopathological terms.

Platelet MAO activity may be useful to classify depressive disorders further. Murphy and Weiss (1972) reported low platelet MAO activity in bipolar manic-depressive depressions. These results, in conjunction with the findings of Schildkrant et al. (1973a, 1973b, 1977), suggested that a subgroup characterized by low MHPG excretion and low MAO activity exists. Recent evidence shows there to be a subgroup of depressive patients with low MHPG excretion along with relatively high platelet MAO activity. These individuals have histories of asocial, eccentric or bizarre behaviour and show psychotic disorganization when treated with tricyclic antidepressive drugs (TADs)(Schildkraut, 1977).

Growth hormone (GH) is released into the peripheral circulation following insulin-induced hypoglycaemia and it has been postulated that this is regulated in part by the central noradrenergic system (Martin, 1976). This GH response to insulin-induced hypoglycaemia has been found to be deficient in many depressed patients (Mueller et al., 1969; Sachar et al., 1971b). A subgroup of these depressives also exhibited diminished urinary MHPG levels which suggests that a defective GH response may be a characteristic of "noradrenaline depression" (Garver et al., 1975).

Evidence for a biochemical classification is summarized in Table 4. These findings suggest that various measures

..... - 18 -/

TABLE 4:

PROPOSED BIOCHEMICAL CLASSIFICATION OF DEPRESSIVE DISORDERS

NORADRENALINE DEPRESSION	SEROTONIN DEPRESSION	REFERENCES
Low MHPG excretion in association with high or low platelet MAO activity.	Normal or high MHPG excretion. Normal platelet MAO activity.	Schildkraut et al., 1973a; 1973b; 1977 Maas, 1975 Murphy and Weiss, 1972
Normal CSF 5-HIAA levels.	Low CSF 5-HIAA levels.	van Praag et al., 1970; 1973a Mendels et al., 1972a Åsberg et al., 1973; 1976
Normal rise in CSF 5-HIAA after probenecid administration.	Subnormal rise in CSF 5-HIAA after probenecid administration.	van Praag and Korf, 1971a Goodwin et al., 1973 Sjöstrom, 1973 Bowers, 1973
Low GH response to insulin-induced hypoglycaemia.	Normal GH response to insulin administration.	Garver et al., 1975
Responsive to imipramine or desipramine.	Responsive to amitriptyline.	Fawcett et al., 1972 Maas et al., 1972 Schildkraut, 1973a Beckmann and Goodwin, 1975
Includes bipolar manic-depressive depression and schizoaffective depression.	Includes unipolar chronic depressions and non-specific depression.	Schildkraut et al., 1977
Brief antidepressive response with d-amphetamine.	No antidepressive response with d-amphetamine.	Fawcett and Siomopoulous, 1971 Fawcett et al., 1972

CSF, cerebrospinal fluid; 5-HIAA, 5-hydroxyindoleacetic acid; GH, growth hormone; MAO, monoamine oxidase; MHPG, 3-methoxy-4-hydroxyphenylglycol.

related to catecholamine and indoleamine metabolism may be clinically useful in classifying depressive disorders.

1.1.5 Aetiology of Depressive Illnesses:

There is no consensus regarding the aetiology of depressive disorders. Some investigators suggest that depression is the result of a neuropharmacological dysfunction, whereas others believe it to be due to a basic fault in character development. This is discussed more fully in Section 1.1.3.

There is no single cause of depression. The specific form that a syndrome will take in a given individual results from an interaction between a number of predisposing and precipitating factors. These are summarized in Table 5.

1.1.5.1 Predisposing Factors:

In response to the same degree of loss, some people are more liable to depression than others. What constitutes the Achilles' heel of these people? A number of predisposing factors have been postulated.

1.1.5.1.1 Genetic Vulnerability:

Most workers accept that some, if not all, depressive disorders are inherited. Heredity has been shown to be an important factor in manic-depressive illnesses and involutional melancholia (Winokur and Clayton, 1967; Mendlewicz et al., 1975).

TABLE 5:

FACTORS INVOLVED IN THE AETIOLOGY

OF DEPRESSION

PREDISPOSING FACTORS	DESCRIPTION
Genetic vulnerability	
Biological and neuropharmacological abnormalities	Disturbance in catecholamine metabolism, indoleamine metabolism, sodium metabolism
Personality traits	Presence of a "depressed personality" - strong oral needs, obsessive - compulsive
Developmental events	Childhood loss - real or symbolic e.g. parental deprivation
PRECIPITATING FACTORS	
Environmental events	Loss of reinforcement Interpersonal events
Physiological stressors	Iatrogenic stressors e.g. reserpine Endocrine disturbances Somatic disorders

For example, it has been shown that if one of a set of monozygotic twins had an involutional psychiatric illness, there would be a 60% chance that the second one would also develop such an illness (Stenstedt, 1959). The percentage was much lower in dizygotic twins which suggested a hereditary factor. Combining all the twin studies, there is a concordance rate of approximately 75% for identical twins and 20% for nonidentical twins which again indicates a genetic factor (Adams and Victor, 1977).

Winokur (1969) conducted a series of family studies and found that affective illness occurred much more frequently in the first-degree relatives of a group of patients with depressive disorders than in a control group. He also found an increased incidence of alcoholism in the relatives of patients with affective disorders which suggested that alcoholism may be a manifestation of a depressive element.

A group of patients who had a history of depressive illnesses in two generations was compared with a group of patients who had no other family members with psychiatric illness (Winokur, 1969). It was demonstrated that the two-generation group contained almost all the manic patients, whereas the family-history-negative group contained very few manic patients. From these results he proposed that two different types of depressive disorders existed: manic depressive illness and depressive illness. This classification based on genetic background has been supported by Angst (1966), Perris (1966) and Leonard (1968).

..... - 22 -/

Bipolar affective illness is thought to be a dominant trait which can be transmitted as either X-linked dominant (Winokur et al., 1969; Reich et al., 1969; Mendlewicz et al., 1972) or autosomal dominant (Green et al., 1973). The mode of transmission of unipolar illness is less clear, though some studies favour polygenic inheritance (Gershon et al., 1971; Baker et al., 1972)(Table 3).

The levels of catecholamines in the brain and in the adrenal medulla are genetically determined and hence a genetic abnormality may affect the amount of functional neurotransmitter at the synaptic cleft. There are a number of possible sites of genetic dysfunction. An alteration in a protein molecule could cause enzymatic changes, resulting in altered synthesis, storage, release, reuptake or metabolism of the neurotransmitter. Other loci of dysfunction have been proposed, 4 of which deserve special mention.

(1) An alteration in the gene(s) that controls the availability of rate-limiting enzymes involved in biogenic amine synthesis.

(2) An increase in the functional capacity or the level of catechol-O-methyltransferase (COMT) or MAO which would decrease the biogenic amines in the synaptic cleft.

(3) A dysfunction in either the vesicular, presynaptic or postsynaptic membrane which would interfere with the release and reuptake of electrolytes and/or biogenic amines (Mendels

and Frazer, 1973). This may involve a deficiency in either the membrane sodium ion/potassium ion dependent adenosine triphosphatase (Singh, 1970; Bunney et al., 1972b) or the carrier protein.

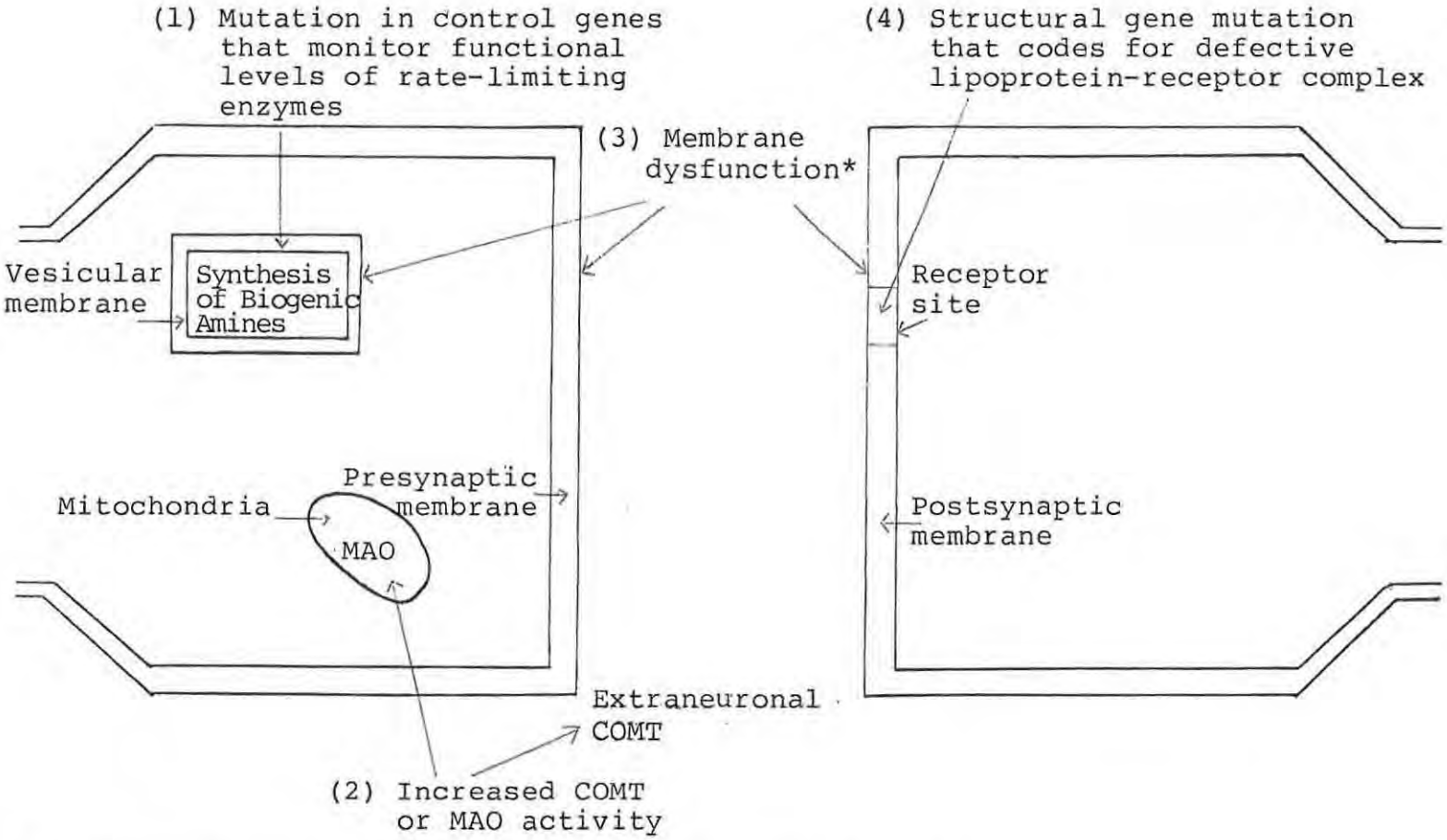
(4) An alteration in receptor site sensitivity due to a structural gene mutation that codes for an aberrant lipoprotein-receptor complex on the postsynaptic membrane. This is supported by the work of Prange et al. (1969) who postulated that the enhancement of tricyclic antidepressive action by thyroid hormone may be due to a hormone-mediated increase in receptor site sensitivity.

These 4 plausible sites of dysfunction are schematized in Fig. 1A.

Thus theoretically a defective gene could result in the deficiency of catecholamines which has been proposed as the cause of the depressive syndrome (Schildkraut, 1965; Bunney and Davis, 1965).

Although it seems likely that a genetic factor plays a part in the development of depression, it is not known whether this is necessary or whether it is a sufficient cause in itself.

1.1.5.1.2 Biological and Neuropharmacological Abnormalities: Disturbances in catecholamine metabolism (Schildkraut, 1965; Bunney and Davis, 1965), indoleamine metabolism (Coppen,



*involving Adenosine Triphosphatase or Carrier Protein

COMT, catechol-0-methyltransferase; MAO, monoamine oxidase

Fig. 1A. Schematic Presentation of 4 Possible Sites of Genetic Dysfunction in Depressive Illness. (Modified from Akiskal and McKinney, 1975).

1967; Lapin and Oxenkrug, 1969) and sodium metabolism (Coppen, 1965) have been implicated in the aetiology of depression. These and other biochemical abnormalities seen in depressed patients will be discussed in detail in Section 1.2

1.1.5.1.3 Personality Traits:

Personality traits will determine or modify ones reactivity to stress and adversity. Some studies have suggested the presence of a "depressed personality". In 1911, Abraham (1960) studied manic-depressive patients and stressed their tendency to have obsessive-compulsive personalities with unusually strong oral needs.

Laughlin (1956) described the characteristics of a person whom he believed was likely to develop a depressive disorder. These characteristics include several groups of traits: (1) overserious, overconscientious, gloomy and subdued; (2) have an unconscious need for love and a desire to be dependent, which makes them particularly vulnerable to rejection and disappointment; (3) compliance, overpoliteness and subservience, which suggests that the person denies all feelings of anger and hostility; (4) meticulousness, rigidity and perfectionism which are features of the obsessive-compulsive personality seen particularly in involutional melancholias. Although these characteristics are often seen in depressed patients, the specific relationship between them and depression in unknown.

1.1.5.1.4 Developmental Events:

Childhood loss or stress occurring at crucial periods of development are thought to sensitize the individual to depression in later life. According to Abraham (1960), the withdrawal of love from the infant during its oral phase of development results in a sense of unfulfilment and feelings of anger and despair in the child. The emotional development of the child is fixated at this oral stage and in adult life, these individuals depend on people and events to supply emotional gratification. According to this theory, repetition of the infantile loss (symbolic) later in life causes feelings of anger and despair to recur and predisposes the individual to depression.

Research studies have suggested that adolescents and adults who have experienced a parental deprivation in childhood become particularly sensitized to loss and prone to depression in the face of it (Gregory, 1966a; 1966b; Granville-Grossman, 1968; Bowlby, 1973).

There is evidence from animal studies to support this. Rhesus infants separated from their mothers or peers show a similar reaction to human infants experiencing anaclitic depression (Seay et al., 1962; McKinney et al., 1971b; 1972). Young et al. (1973) separated 3 to 4 year old monkeys who had experienced a traumatic separation during infancy, from their peers and observed a "despair-like" reaction.

Although this relationship between loss in childhood and depression in later life makes clinical sense, it has not yet been firmly established.

1.1.5.2 Precipitating Factors:

A precipitating factor alone may be sufficient to invoke a depressive illness. However, it may also trigger off a depressive syndrome in a predisposed individual.

1.1.5.2.1 Environmental Events:

The most frequent precipitating factor is loss - the real or fantasized loss of anything the patient holds dear. The concept of loss incorporates anything which leads to a loss of reinforcement, for example, loss of a personal relationship (death of a spouse, loss of children through marriage), loss of material possessions (financial troubles), loss of physical and mental capacities (debilitating, chronic illnesses), loss of self-esteem (failure to achieve a desired goal) or loss of control over ones destiny (helplessness to alter the future). The stresses of life in a wider sense, such as war, poverty, injustice, corruption, lack of communication between people and nations, and the rapid pace of our accelerating world also result in a decrease in positive reinforcement and loss of control over ones destiny. However, the relative contribution of these factors to the incidence of depression is still unclear.

The events that take place between the depressive patient

and his interpersonal environment also act as an important precipitant in depression (Feldman, 1976).

He postulated that the processes of reciprocal stimulation and reinforcement that exist between two people, for example, in marriage, are important not only in the initiation but particularly in the maintenance of the depressive illness. Interpersonal conflicts result in a decrease in positive reinforcement which precipitates depression. If the conflict is maintained in a family system, this serves to prolong the depressive disorder. The effect of interpersonal events on a depressive patient may be genetically determined. It has been reported that genetic predisposition renders 10 to 15% of the population extremely vulnerable to interpersonal stresses (Perris, 1966; Cadoret et al., 1970; Gershon et al., 1971).

Although the onset of reactive depression is usually associated with one of the above-mentioned stresses, it is not necessarily true that the stress directly caused the illness (Mendels, 1970). Often the patient has experienced similar stresses at other times of his or her life without becoming depressed. In addition, there are many other people who do not become depressed on exposure to similar experiences.

1.1.5.2.2 Physiological Stressors:

Iatrogenic stresses can induce direct physiochemical changes. Goodwin and Bunney (1971) have shown that reserpine which

depletes or displaces the biogenic amines in the CNS, precipitates depression in 15 to 20 % of hypertensive patients when given in doses of more than 0,5 mg per day. Similar depressive states have been found following α-methyldopa (Bunney and Davis, 1965). It was thus postulated that these drugs act as chemical triggers and upset a vulnerable biochemical-neurophysiological system in genetically predisposed individuals.

High doses of corticotrophin or cortisol (Clark et al., 1952) have also induced a depressive reaction in some patients. Other drugs, for example barbiturates, analgesics and phenothiazines (Simonson, 1964) may also be associated with the development of depression.

A number of endocrine disturbances have been implicated in the aetiology of depression. Depressive disorders have been observed in patients with Addison's disease (Engel and Margolin, 1942; Michael and Gibbons, 1963), Cushing's syndrome (Carpenter et al., 1972), hyperparathyroidism (Karpati and Frame, 1964) and hypothyroidism (Whybrow and Hurwitz, 1976). The exact nature of the relationship between these endocrine diseases and depressive illness is unclear.

Castelnuovo-Tedesco (1961) proposed that diseases that specifically interfere with the normal functioning of the nervous system may produce depression. These include

cerebral arteriosclerosis, senile dementia, neurosyphilis, multiple sclerosis and various vitamin deficiency syndromes. According to Castelnuovo Tedisco, other somatic disorders that are likely to be complicated by depression are: (1) certain infectious diseases, especially infectious hepatitis, influenza, infectious mononucleosis, atypical pneumonia, rheumatic fever and tuberculosis; (2) psychosomatic disorders such as ulcerative colitis, asthma, neurodermatitis and rheumatoid arthritis; (3) anaemias and (4) malignancies. The experience of somatic and psychomotor debilitation which frequently accompanies such disorders may in itself be depressionogenic.

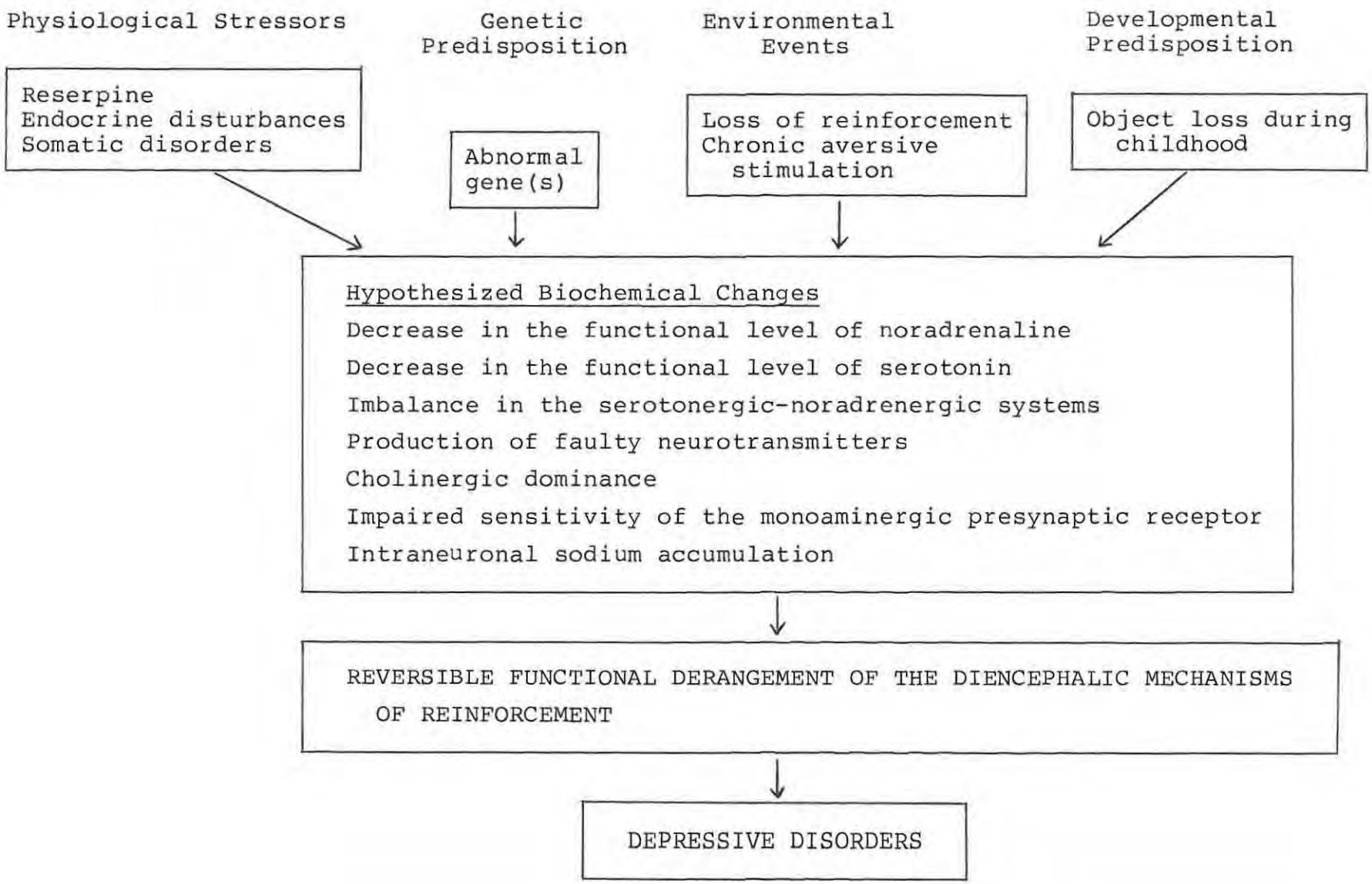
Akiskal and McKinney (1975) have attempted to integrate these diverse predisposing and precipitating factors into a unified model of depression (Fig. 1B). They proposed that genetic, physiological, environmental and developmental factors lead to biochemical alterations in the patient which, in turn, lead to a reversible functional derangement in the diencephalic mechanisms of reinforcement. The major virtue of this hypothesis is that it provides a conceptual bridge between the behavioural and biological models of depression.

1.1.6 Treatment of Depression:

There is considerable controversy regarding the treatment of depression with two schools of thought predominating. The authors who accept depression as being primarily a psychological disorder believe that psychotherapy is

Fig. 1B. Unified Model of the Aetiology of Depressive Disorders.

(Modified from Akiskal and McKinney, 1973.)



essential in its treatment. They question the use of medication to solve what they consider to be essentially a psychological or social problem. They argue that drug treatment may dilute the patient's anxiety so much that he is diverted from improving his underlying personality problem and, as a result, further episodes of depression could occur. On the other hand, those who conceive depression to be fundamentally a biochemical or genetic abnormality, suggest treatment should be aimed at correcting the disturbed state of the nervous system with anti-depressive drugs. Psychodynamics and psychotherapy are regarded as relatively unimportant except in supportive use.

However, there is evidence suggesting that depressive disorders which are biological in origin ("endogenous depression") are more responsive to psychotherapy. Greenblatt et al. (1964) demonstrated that the response rates of various depressions differed markedly when hospitalized depressives were treated with placebo. They proposed that the comparatively low placebo response rate in "manic-depressive"(1) or "involutional psychotic"(1) depressions demonstrated the need for antidepressive medication. In contrast, "neurotic" or "character disorder" depressions could be treated adequately by interpersonal techniques

---

(1) The diagnostic criteria used in this study are specified elsewhere (Greenblatt et al., 1964).

without the use of medication. This suggests that no matter what interpersonal events elicit depressive disorders, once they assume severe proportions, they become biologically autonomous and are relatively refractory to psychotherapy (Akiskal and McKinney, 1973).

These studies emphasize the need for distinguishing between the types of depressions when deciding on appropriate treatment. Therapy should be individualized according to the patient and the phase of his illness, and may involve medication, psychotherapy and/or direct guidance.

1.1.6.1 Psychotherapy:

Psychotherapy provides emotional support and reassurance to counteract the depressive patient's low self-esteem and feelings of hopelessness. Maladaptive patterns and predisposing events are investigated and restructured in order to help the patient cope with, and understand, his depression so that any future episodes can be dealt with using the individual's own resources. As recovery develops, psychotherapy is also of use when the patient is intolerant of the side effects or refractory to a given antidepressive.

There is a wide range of psychological treatments including individual and group therapy, family and marital therapy, behavioural therapies and psychoanalysis. Lazarus (1968) has discussed 3 methods of treatment effective in depression, all of which seem to involve the patient's

realization that he can control important reinforcers by his own actions. (1) Assertive training (Wolpe and Lazarus, 1969) where the patient learns to assert himself and to exercise control over his interpersonal events. (2) Affective expression in which the individual merely expresses anger. (3) Morita therapy (Kora, 1965; Burgess, 1968) which reinforces active behaviour in depressed patients. Taulbee and Wright (1971) developed the "Tuscaloosa Plan" whereby mistreatment of the depressive patient acts as a powerful reinforcer.

The effectiveness of these therapies suggests that depression may be antagonized when the patient comes to realize that his own responses are effective in alleviating his condition and producing gratifications. This approach has been supported experimentally by Seligman's helplessness model (Seligman, 1975). He demonstrated that learned helplessness can be broken down by forcing the passive dog to see that its responses produce reinforcement. Melges and Bowlby (1969) also see mitigation of helplessness as the central theme in the treatment of depression.

Some authors stress the importance of explaining to the depressed patient that depression is self-limiting (Campbell, 1953; Kraines, 1957; Ayd, 1961). They suggest that the patient be told his illness has a physical basis and that he will be cured. It is believed that this "formulation of the patient" greatly assists in alleviating

the depressive disorder.

Beck (1970) has developed a cognitive therapy for depression, the aim of which is to change the negative cognitive set of the depressive patient to a more optimistic one. This is done by reconstructing the stages in the development of the patient's depression, teaching him to recognize his cognitions and to neutralize inaccurate negative thoughts.

In short, antidepressive drugs have not replaced psychotherapy. Even in manic-depressive illness where the effectiveness of medication has been proved beyond doubt, psychotherapy is considered essential to shorten the illness and to alleviate the patient's suffering (Kraines, 1957).

1.1.6.2 Hospitalization:

Hospitalization is indicated in more severe cases of depression as it provides care and protection against suicidal risks.

In some cases a hostile, ambivalent or dependent relationship existing between the depressed patient and his or her family may contribute to the perpetuation of the illness and removal from these environmental pressures is often beneficial. Hospitalization, although being a more extreme form of environmental change, offers a temporary respite from this domestic stress. In addition, the controlled,

protective surroundings of the hospital may itself help in resolving the illness since it is well-known that many patients improve simply on being admitted to a hospital. In addition, a hospital can also provide a therapeutic regimen that is not available at home, for example ECT.

1.1.6.3 Electroconvulsive Therapy:

Cerletti and Bini introduced electroconvulsive therapy (ECT) in 1938 and up until the mid-sixties, it was the most common method of treatment for hospitalized depressive patients.

Despite the efficacy of antidepressive drugs, there is ample evidence that ECT does resolve a depressive episode in a significant number of patients. The evaluation of the efficacy of ECT, however, has been complicated by the fact that (i) some depressed patients seem to respond better to ECT than others, and (ii) the depressive disorder is often self-limiting. For example, a number of workers have found patients suffering from "endogenous depression" to be more responsive to ECT (Carney et al., 1965; Mendels, 1965; 1967; Ilaria and Prange, 1975).

Riddle (1963) and Davis (1965) reviewed the effectiveness of ECT and the two studies taken together show ECT to be superior to placebo or no treatment in 2 studies; more effective than antidepressive drugs in 4 studies; and equivalent to drug therapy in 3 studies. From the available evidence, a number of investigators have suggested

that ECT is the same as, or superior to, any drug treatment for psychotic depressives (Cole, 1964; Wechsler et al., 1965). The physiological, biochemical and psychological effects of ECT have been summarized by Holmberg (1963) and are outlined in Table 6.

ECT rapidly alleviates the depressive disorder and, as a result, is of particular use in cases where there is a risk of suicide. It is also recommended for depressed patients who are refractory or hypersensitive to antidepressive therapy or who refuse to take their medication.

However, the administration of ECT entails certain disadvantages. These include memory loss (both anterograde and retrograde), difficulty in concentration, confusion, fractures and dislocations, risk of anaesthetic, and cardiovascular complications. There is also a high relapse rate but this can be prevented by administering maintenance doses of antidepressive drugs for 6 months after ECT. Unilateral ECT (where both electrodes are placed on the nondominant cerebral hemisphere), however, has been reported to reduce the incidence and severity of these side effects to some extent.

1.1.6.3.1 Mechanism of Action:

There is a mass of widely diverse and often contradictory theories regarding the mechanism of action of ECT. Fear, hormones, muscular exertion, anoxia, amnesia and biochemical

..... - 38 -/

TABLE 6:

EFFECTS OF ELECTROCONVULSIVE THERAPY

(Holmberg, 1963)

Physiological Effects	-	An initial jerk produced by direct cortical stimulation
	-	Followed by tonic and clonic convulsions
	-	During the convulsion - spasm of glottis and respiratory muscles
		- elevation of blood CO_2 tension
		- reduction of O_2 tension
		- increase in cerebral circulation
		- increase in brain metabolism
	-	Heart rate frequently rapid and irregular
	-	Possible fluctuations in blood pressure
	-	Excitation of autonomic regulatory centres - psychomotor restlessness
	-	Cholinergic effects - salivary and bronchial secretions
		- transient arrhythmias
Biochemical Effects	-	Hyperglycaemia
	-	Elevated nitrogen compounds, potassium, calcium, phosphorus and steroids in blood
	-	Elevation of catecholamines and serotonin in blood
	-	Increased brain serotonin (especially in brain stem)
Psychological Effects	-	Memory impairment - amnesia (may be both anterograde and retrograde)

changes in the CNS have been implicated in its mechanism, but proof is lacking to substantiate these claims.

Holmberg (1963) suggested that the effectiveness of ECT is dependant upon the production of seizure activity in the brain. He reported that increasing the convulsive activity with muscle relaxants and oxygenation improved the therapeutic effects of ECT, whereas decreasing the convulsive activity with anticonvulsive premedication reduced the therapeutic effect.

Several investigators have attempted to determine whether the clinical efficacy of ECT is related to an alteration in amine metabolism (Weil-Malherbe, 1955; Havens et al., 1959; Cochran and Marbach, 1962; Holmberg, 1963). Increases in both plasma and urinary noradrenaline and adrenaline have been demonstrated in unmodified ECT, however, these alterations were diminished when barbiturates or muscle relaxants were administered prior to ECT. Rosenblatt et al. (1960) have suggested that the amine blood-CSF barrier becomes more permeable to noradrenaline after ECT. Schildkraut et al. (1967) showed that ECT lowered the brain levels of tritiated noradrenaline and increased the brain levels of normetanephrine-H^3 above those of the control animals. In addition, Schildkraut (1975) found increased urinary MHPG levels in depressed patients recovering after ECT treatment. They thus concluded that ECT might act by increasing noradrenaline synthesis and turnover, thereby increasing the

..... - 40 -/

neuronal discharge of noradrenaline onto the receptors. This has been confirmed by other investigators (Ebert et al., 1973; Modigh, 1976a). Noradrenaline-stimulated synthesis of cyclic AMP has also been shown to be reduced in rat brain tissues after chronic treatment with ECT (Vetulani et al., 1976). In a recent report, Pandey et al. (1979) observed a significant decrease in β-adrenergic sensitivity in the rat brain following chronic ECT treatment. This effect was shown to be similar to the effects of other antidepressives, for example, desipramine (Pandey et al., 1979), doxepin and iprindole (Banerjee et al., 1977). However, the question of whether this action is related to the antidepressive effect of ECT is still unknown.

The effect of ECT on the serotoninergic nervous system has also been investigated in an attempt to verify the indole-amine hypothesis. Ebert et al. (1973) reported that the cerebral concentrations of serotonin, 5-HIAA and tryptophan are increased in test animals for a few days following a series of electroshocks which might indicate increased serotonin turnover. However, the baseline concentration of 5-HIAA in human CSF is unaffected after ECT. Some depressive patients show a low CSF 5-HIAA response after probenecid pretreatment which is said to indicate reduced turnover in the CNS (Roos and Sjöstrom, 1969; Sjöström and Roos, 1972; van Praag et al., 1970; 1973a). Van Praag (1977a) has shown that ECT increases the post-probenecid 5-HIAA accumulation in the CSF of these patients, suggesting that

ECT has some influence on serotonin turnover.

1.1.6.4 Pharmacotherapy:

Antidepressive drugs have proved to be of considerable value in the treatment of depressive disorders. They have probably resulted in a reduced use of ECT, brought about improvement in patients who were chronically ill and resistant to other treatment, reduced the need for and duration of hospitalization, and made patients more responsive to psychotherapy.

However a number of factors complicate the evaluation of the success rates for the various treatments.

(1) Depression is often a self-limiting illness and thus the statistics on the efficacy of the different drugs are often unreliable. There is a high spontaneous remission rate which is said to vary from a median of 3 to 4 months among outpatients to a median of 6 to 18 months among in-patients (Beck, 1973). Therefore many apparently drug-induced remissions are probably spontaneous.

(2) Depressed patients often show a nonspecific placebo response. It is well known that the response to any drug (including a placebo) is dependent upon the patient's attitude and expectations. The placebo response has been found to vary with the type of drug, the social background and educational level of the patient, and whether or not the patient was seen in a clinic or by a private psychiatrist (del Giudice, 1970).

(3) The population of depressed patients is heterogeneous with different subgroups of depressives responding selectively to drugs. It has been suggested, for example, that patients suffering from "endogenous" depression are particularly responsive to TADs, whereas patients with "neurotic" and "atypical" depressions respond better to MAO inhibitors (Sargant, 1961; Sargant and Dally, 1962; Crane, 1970).

Caffey et al. (1970) compared the efficacy of imipramine with thioridazine and found that imipramine was superior in "retarded" depression, thioridazine superior in "anxious" depression, while both were equally effective in "hostile" depression. This selective responsivity to treatment could account for the discrepancies in findings among the various studies, since the clinical population of most studies has included a heterogeneous group of depressed patients.

Another major problem with pharmacotherapy arises from the delay in clinical activity of the antidepressive drugs.

The clinical effects of reserpine, the tricyclic drugs and lithium carbonate, take several weeks to become apparent, whereas their effects on biogenic amines are immediately evident in in vivo animal studies. The reasons for this latency in the onset of improvement are not understood.

Thus, it is evident that the choice of a treatment programme

must be individualized according to the particular clinical features present, instead of having a standard approach that is applied to every depression.

1.1.6.4.1 Tricyclic Antidepressives:

Based on controlled trials, it would seem that the tricyclic antidepressives (TADs) are currently more effective and safer than non-tricyclic drugs in the treatment of depression. Beck (1973, review) has summarized the controlled studies comparing the tricyclics to a placebo and concluded that out of 67 inpatient trials, 42 (63%) showed statistically significant improvement. The trend appears to be that 50 to 70% of patients are moderately improved while on TAD therapy, whereas 30 to 50% improve in placebo groups (Klerman and Paykel, 1970). Patients with retardation and other clinical features resembling "endogenous" depression (hyposomnia, early morning wakening, decreased appetite, mass loss and decreased libido) have been shown to be particularly responsive to TAD treatment which remains the drug of choice for this condition. Moreover, in comparison with other antidepressive treatments, the side effects of the tricyclics are far less frequent and serious.

The effectiveness of imipramine was first described by Kuhn (1958) and since then a number of other tricyclic compounds have been developed. These include (i) the iminodibenzyls (desipramine, trimipramine and clomipramine)

and (ii) the dibenzocycloheptenes (amitriptyline, nortriptyline, protriptyline and doxepin). In clinical studies, the "sedative" tricyclics (amitriptyline, nortriptyline, doxepin) have been reported to be particularly useful in the treatment of "agitated" depressions (associated with anxiety and insomnia), whereas the "stimulant" type of antidepressives (imipramine, desipramine, protriptyline) are more suitable in "retarded" depressions (Cole and Davis, 1967; Shepherd et al., 1968). It has also been observed that some groups of depressed patients fail to respond to amitriptyline (a relatively specific serotonin uptake inhibitor) but do respond to imipramine or desipramine, while other groups of depressed patients fail to respond to imipramine or desipramine (noradrenaline uptake inhibitors) but do respond to amitriptyline (Beckman and Goodwin, 1975). However, it is not yet known why imipramine, which has almost equal effects on both serotonin and noradrenaline reuptake, exerts a similar action to desipramine and not to amitriptyline in clinical trials. These findings support the hypothesis of two distinct biological subtypes of depression (See Section 1.1.4.1) and emphasize the need to identify those individuals among the heterogeneous depressed population who may be specifically responsive to the different TADs.

The most common side effects associated with TAD administration are related to the anticholinergic action of the drug. These include dry mouth, constipation, increased perspiration, visual distortions, urinary retention and

postural hypotension. In addition, it has been reported that imipramine administration may intensify an episode of mania in manic-depressive patients and could lead to an exacerbation of the psychosis in schizophrenic patients (Kuhn, 1958; Shepherd et al., 1968). Mild, transient, self-limited, hypomania symptomatology has also been observed in patients with "endogenous" depressions during imipramine treatment (Schildkraut et al., 1966a).

While the TADs represent an important advance compared to previous therapies, their efficacy is limited since they require prolonged administration (usually 1 to 4 weeks) before their antidepressive effects can be observed. This is a disadvantage, particularly in the treatment of a suicidal patient. It was proposed that the delay in therapeutic effect be explained by the fact that imipramine must first be converted into its active desmethyl metabolite before it can be clinically effective.

The desmethyl derivatives of imipramine and amitriptyline, desipramine and nortriptyline respectively, were then synthesized in the hope that they would act more rapidly than their parent compounds but there is little clinical evidence to support this (Shepherd et al., 1968). Further research must discover the reasons for this delay in therapeutic effect and develop a new antidepressive with a more rapid onset of action.

1.1.6.4.1.1 Mechanism of Action:

The precise mechanism of action of the TADs in alleviating depression is unknown but it has been suggested that its pharmacological property of blocking the cellular reuptake of the biogenic amines (noradrenaline, dopamine and/or serotonin) may be important in this respect (Schildkraut, 1965; Lapin and Oxenkrug, 1969; Mendels et al., 1976; Randrup and Braestrup, 1977).

On nerve stimulation, newly synthesized transmitter is released by the process of exocytosis from the storage vesicles in the presynaptic neuron (Axelrod, 1972). This diffuses across the synaptic cleft and reacts briefly with the postsynaptic receptors. The action of the neurotransmitter is terminated primarily by reuptake of the amine from the synaptic cleft by the presynaptic neurons (Cooper et al., 1974). Most of the resorbed transmitter is restored in the storage vesicles but some is metabolized by the enzyme, monoamine oxidase (MAO), which is located in the mitochondria of the nerve cell. Any noradrenaline remaining in the synaptic cleft is metabolized to normetanephrine by catechol-0-methyltransferase (COMT). The normal process of release and reuptake is illustrated in Figure 2A. Figures 3 and 4 illustrate the synthesis and metabolism of noradrenaline and serotonin respectively. The tricyclics block the reuptake of biogenic amines from the synapse and are therefore presumed to increase the quantity of functional neurotransmitter at the postsynaptic

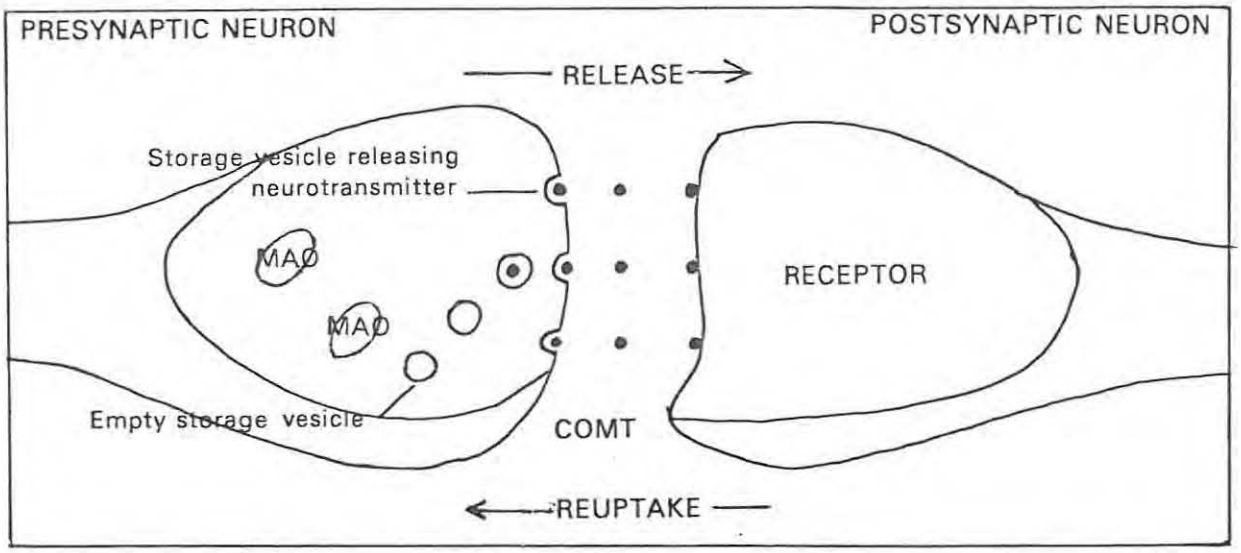


Fig. 2A. Schematic Representation of the Normal Process of Release, Reuptake and Metabolism of Neurotransmitters.

COMT, catechol-O-methyltransferase; MAO, monoamine oxidase.

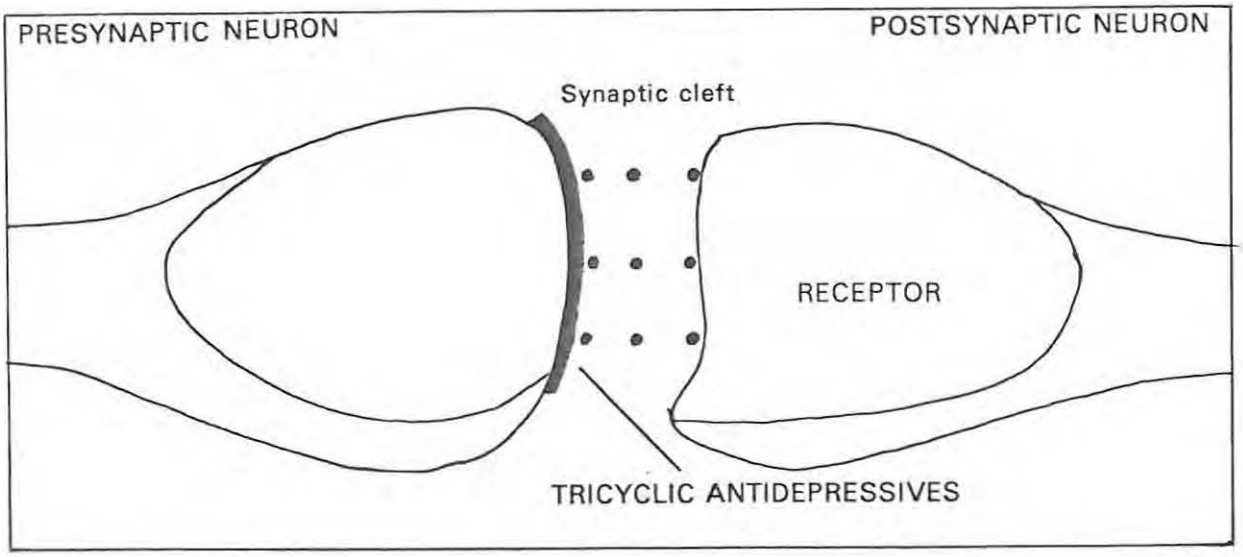


Fig. 2B. Schematic Representation of the Proposed Mechanism of Action of the Tricyclic Antidepressives.

The tricyclic antidepressives block the presynaptic nerve terminal, increasing the concentration of neurotransmitter (noradrenaline, dopamine or serotonin) at the receptor site and thus potentiating its effect.

..... - 48 -/

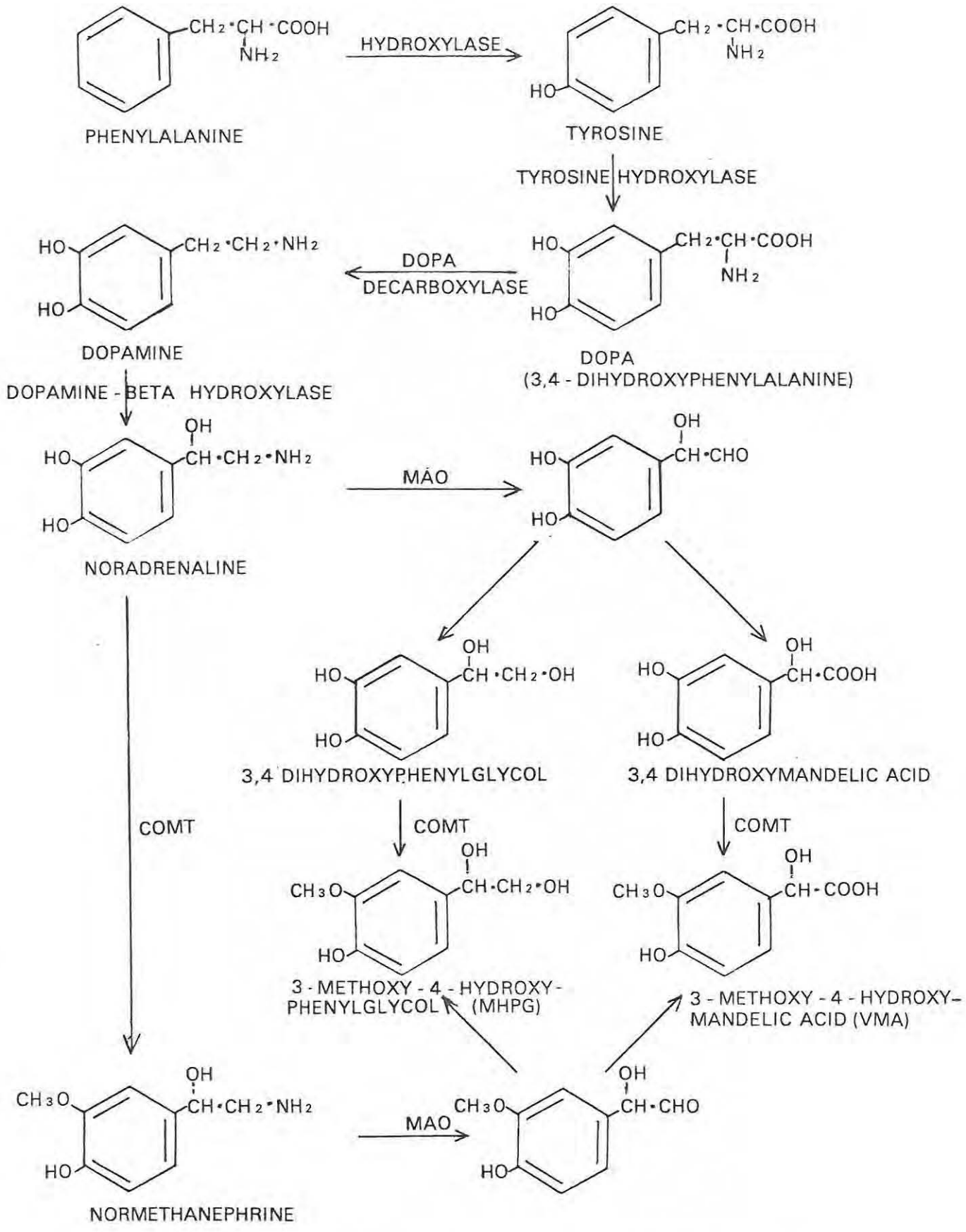


FIG. 3: THE SYNTHESIS AND METABOLISM OF NORADRENALINE

..... - 49 -/

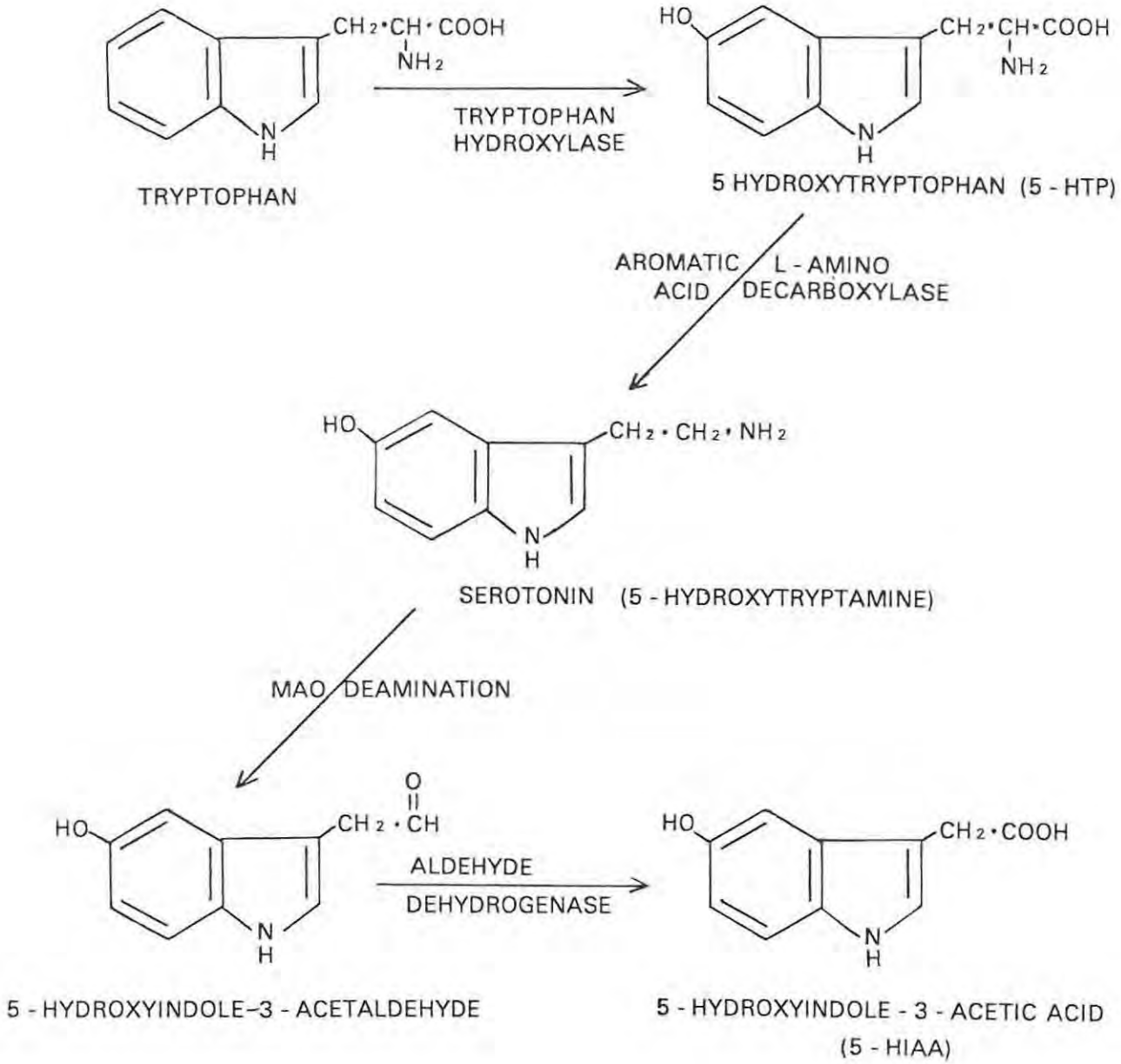


FIG. 4: THE SYNTHESIS AND METABOLISM OF SEROTONIN

..... - 50 -/

receptor sites. This is illustrated in Figure 2B.

In 1959, Sigg suggested that imipramine exerts a "sensitizing" influence on the adrenergic receptors (Sigg, 1959). This was supported by numerous investigators who found imipramine potentiated both the response to sympathetic nerve stimulation in several experimental situations as well as many of the peripheral effects of exogenously administered noradrenaline in animals and in man (Ryall, 1961; Gershon et al., 1962; Sigg et al., 1963; Thoenen et al., 1964).

The effects of the tricyclics upon the brain metabolism of radioactive noradrenaline were then investigated. Glowinski and Axelrod (1964) were the first to demonstrate that the tricyclic derivatives (imipramine, desipramine and amitriptyline) inhibit the uptake of tritiated noradrenaline in the rat brain. Previously, desipramine and imipramine had been found to block the uptake of noradrenaline-H^3 into peripheral tissues (Axelrod et al., 1961; Hertting et al., 1961) and brain slices (Dengler et al., 1961). Rat brain normetanephrine-H^3 levels were also shown to be increased by imipramine administration (Glowinski and Axelrod, 1965; Schildkraut et al., 1967). These results were supported by clinical studies which demonstrated increased normetanephrine levels and decreased VMA levels in the urine of patients treated with imipramine (Schildkraut et al., 1964; 1965). The decreased VMA excretion was thought to be due to the reduced intracellular degradation of

noradrenaline by MAO brought about by the ability of the tricyclics to prevent the reuptake of extracellular noradrenaline. This, in turn, led to an increased normetanephrine excretion.

From studies conducted by Haefely et al. (1964) it was concluded that the tricyclics exert at least 3 types of activity at the peripheral adrenergic synapses: (1) a sympathomimetic effect (thought to be due to the release of active noradrenaline from its binding sites); (2) inhibition of reuptake of noradrenaline from the synaptic cleft; and (3) a sympatholytic effect in higher doses.

The tricyclics' ability to increase monoamine availability by blocking reuptake has been confirmed by numerous investigators (Ross and Renyi, 1967; Schildkraut et al., 1969a; Lidbrink et al., 1971; Hamberger and Tuck, 1973) and it has been hypothesized that this might account for its therapeutic effect (Schildkraut, 1965; Bunney and Davis, 1965; Mendels et al., 1976).

The effect of TAD administration on the serotoninergic processes was also investigated. Tricyclics were found to potentiate the peripheral (Sigg et al., 1963; Gyermek, 1966) and central effects (Himwich et al., 1961; Gyermek, 1966) of serotonin. Although tricyclic derivatives showed an antiserotonin action on some isolated organs, the potentiation of serotonin effects has been proposed to be

..... - 52 -/

more relevant to its mechanism of action in depression (Gyermek, 1966). Tissue uptake of injected serotonin was shown to be inhibited by imipramine administration thus causing an increase in the concentration of free serotonin acting on the receptors (Axelrod and Inscoe, 1963). Carlsson et al. (1968) demonstrated that imipramine inhibits the cellular reuptake of serotonin by serotoninergic neurons, while desipramine and protriptyline are inactive in this test. This finding has been supported by other workers (Lidbrink et al., 1971; Hamberger and Tuck, 1973) and some authors suggest that the efficacy of the tricyclics is based on this serotonin potentiating property (Coppen, 1967; Lapin and Oxenkrug, 1969). In addition, the tricyclics have been reported to reduce central serotonin turnover (Schuberth, 1973). This phenomenon, however, may be secondary to receptor stimulation and may result from feedback inhibition of serotonin synthesis. Inhibition of the uptake of tryptophan into the neurons by the tricyclic compounds (Bruinvels, 1972) is another possible explanation. In humans, the tricyclics (imipramine, amitriptyline and clomipramine) have been shown to reduce the post-probenecid accumulation of 5-HIAA which indicates a decreased serotonin turnover (Bowers, 1972b; Post and Goodwin, 1974).

Not all the tricyclic compounds have the same inhibitory effect on the reuptake of noradrenaline and serotonin. Laboratory studies have demonstrated that desipramine

..... - 53 -/

selectively inhibits the reuptake of noradrenaline (Carlsson et al., 1969a; Eckhardt and Maxwell, 1973; Ross and Renyi, 1975; Uzan and Le Fur, 1975; Koe, 1976) with little effect on serotonin (Carlsson et al., 1969b; Maas, 1975; Ross and Renyi, 1975; Uzan and Le Fur, 1975; Koe, 1976). Amitriptyline, on the other hand, has been shown to selectively inhibit the reuptake of serotonin with little effect on noradrenaline reuptake (Carlsson et al., 1969b; Maas, 1975; Ross and Renyi, 1975). Imipramine inhibits serotonin and noradrenaline reuptake almost equally, although at half the activity of either amitriptyline or desipramine (Maas, 1975). This is summarized in Table 7.

The hypothesis that the tricyclics' therapeutic effect be attributed to their ability to inhibit the uptake of noradrenaline and/or serotonin has been questioned with the discovery of the newer tricyclics, butriptyline and iprindole. These drugs have been shown to exhibit a weak inhibitory effect on noradrenaline and serotonin reuptake (butriptyline: Jaramillo and Greenberg, 1975; iprindole: Ross et al., 1971; Fann et al., 1972; Sanghvi and Gershon, 1975; Horn, 1976) even though they are clinically effective antidepressives (butriptyline: Levinson, 1974; iprindole: Fann et al., 1972, review; Rickels et al., 1973; Briant and George, 1975; Tait and Todrick, 1975).

More recently the neurotransmitter, dopamine (DA) has been implicated in the aetiology of depression and the TADs'

TABLE 7:

SYNAPTOSOMAL UPTAKE INHIBITION OF NORADRENALINE AND SEROTONIN AT CLINICALLY ACHIEVABLE LEVELS OF TRICYCLICS (Maas, 1975)

Tricyclic Antidepressive	Noradrenaline	Serotonin
Amitriptyline	0	++++
Nortriptyline	++	++
Imipramine	++	+++
Desipramine	++++	0

0 = probable lack of activity

++++ = most active

mechanism of action (Randrup et al., 1975, review). Amitriptyline and doxepin have been shown to be potent inhibitors of the DA sensitive adenyl cyclase (like the neuroleptics) while the other tricyclic derivatives were moderate or weak inhibitors (Karobath, 1975). Modigh (1975) reported that protriptyline administration resulted in DA supersensitivity, the duration of which paralleled the duration of the antidepressive effect (Modigh, 1976b). Initially it was thought that the neuronal uptake of DA was not inhibited by the TADs, but more recently, Randrup and Braestrup (1977) have shown that the classical tricyclic drugs (together with the more recently developed drugs, butriptyline and iprindole) all inhibit the uptake of DA in crude striatal synaptosomes at concentrations between 10^{-6} to 10^{-5} M (Table 8).

All the clinically effective tricyclics display considerable anticholinergic properties both centrally (Sulser et al., 1964; Vallant, 1967) and peripherally (Sigg, 1959; Vernier et al., 1962; Sigg et al., 1963). It has been proposed that the blockade of central cholinergic responses may account for the drugs' antidepressive effect (Biel et al., 1962; Biel, 1966; Janowsky et al., 1972b).

In conclusion, the various tricyclic drugs exert differing inhibitory effects on noradrenaline, serotonin and dopamine reuptake (see Table 8) and at present, it is not known which transmitter is of prime importance for its antidepressive

TABLE 8:

SYNAPTOSOMAL UPTAKE INHIBITION OF BIOGENIC AMINES BY ANTIDEPRESSIVE DRUGS. (Randrup and Braestrup, 1977)

Drug	Concentration causing 50% inhibition of uptake (μM)		
	Dopamine	Noradrenaline	Serotonin
Butriptyline	5,2	1,7	10,0
Trimipramine	6,8	1,0	8,2
Maprotiline	8,6	0,02	12,0
Desipramine	8,7	0,0015	2,0
Clomipramine	3,8	0,044	0,015
Nortriptyline	4,4	0,011	0,42
Protriptyline	4,9	0,003	1,62
Amitriptyline	5,6	0,02	-
Imipramine	12,5	0,02	0,24
Dibenzepin	113,0	-	-
Iprindole	7,9	2,6	10,0
Mianserin	19,0	0,084	11,0
Nomifensine	0,10	0,007	6,6

- Comparative values not available.

effect. Some authors believe that the mood-elevating action is due to the blockade of serotonin reuptake and that activation of motor activity is related to the effect on noradrenaline (Lapin and Oxenkrug, 1969; Carlsson, 1976). However, it is possible that all 3 of these amines are involved in antidepressive effects and their interactions are important.

1.1.6.4.2 Monoamine Oxidase Inhibitors:

The monoamine oxidase (MAO) inhibitors constitute the second major group of antidepressive drugs. They are subdivided according to structure into hydrazine derivatives (phenelzine, isocarboxazid and nialamide) and nonhydrazine derivatives (tranylcypromine and pargyline).

Iproniazid was the first compound shown to be effective in reversing depressive symptoms (Crane, 1957; Loomer et al., 1957; Kline, 1958) and since then, the clinical effectiveness of the MAO inhibitors has been studied fairly extensively (Hordern, 1965; Cole and Davis, 1967; Davis et al., 1967; Shepherd et al., 1968). Discrepancies between the various studies have been reported which supports the suggestion that only a specific subgroup of depressed patients may be responsive to MAO inhibitor treatment (West and Dally, 1959; Pare et al., 1962). It has been proposed that while "endogenous" depressions respond particularly well to tricyclic antidepressive administration, patients with "neurotic" and "atypical" depressions improve more

with the MAO inhibitors (Sargant, 1961; Sargant and Dally, 1961; Crane, 1970). In addition, it has been found that the family members of the responders of MAO inhibitors have a tendency to react favourably to such compounds, and negatively to TADs which suggests a genetic factor.

In general, MAO inhibitors seem less effective than the TADs. The tricyclics were found to be significantly superior in 9 studies, whereas no study has shown the reverse (Beck, 1973, review). In addition, the side effects associated with the MAO inhibitors (dizziness, dry mouth, constipation, impotence, orthostatic hypotension and paroxysmal hypertension) were reported to be more numerous and more severe than with the tricyclic drugs (Beck, 1973). A potentially more serious side effect of MAO inhibition is the hypertensive crises ("the cheese effect") that can occur in patients who have ingested food rich in either sympathomimetic amines or their precursors, such as, tyramine (Blackwell, 1963; Blackwell and Mabbitt, 1965; Shepherd et al., 1968; Youdim, 1976). This adverse reaction has limited their clinical usefulness.

Because of the variability of clinical response and the possibility of toxic side effects, these agents are not the first drugs to be prescribed in the treatment of depression. MAO inhibitors are usually only used if the depressed patient has shown a previous favourable reaction to them or if treatment with a TAD has failed.

1.1.6.4.2.1 Mechanism of Action:

MAO isoenzymes which are associated with the mitochondria of nerve terminals, exist in at least 2 different forms - type A and type B. The classification is based on substrate specificity and sensitivity to inhibition by selected inhibitors (Christmas et al., 1972; Sandler and Youdim, 1972). The A type enzyme has a substrate preference for noradrenaline and serotonin and is selectively inhibited by clorgyline. The B type enzyme has a substrate preference for β-phenylethylamine and benzylamine and is selectively inhibited by deprenyl. Dopamine, tyramine and tryptamine are equally good substrates for both forms of the enzyme. However, the relative importance of these 2 types of MAO in depressive disorders is currently unknown.

The MAO inhibitor antidepressives inhibit the activity of these enzymes (Zeller et al., 1952; Pletscher, 1968) which, in turn, causes an increase in the biogenic amines and a decrease in their deaminated metabolites within the nerve terminal (Kopin, 1964; Pschneidt, 1964). These higher concentrations of transmitters are then available for release from the presynaptic terminals, following stimulation. The degree of inhibition of MAO activity has been reported to be related to the clinical anti-depressive effects (Feldstein et al., 1965; Ravaris et al., 1976). The mechanism of action of the MAO inhibitors is illustrated in Figure 5A.

..... - 60 -/

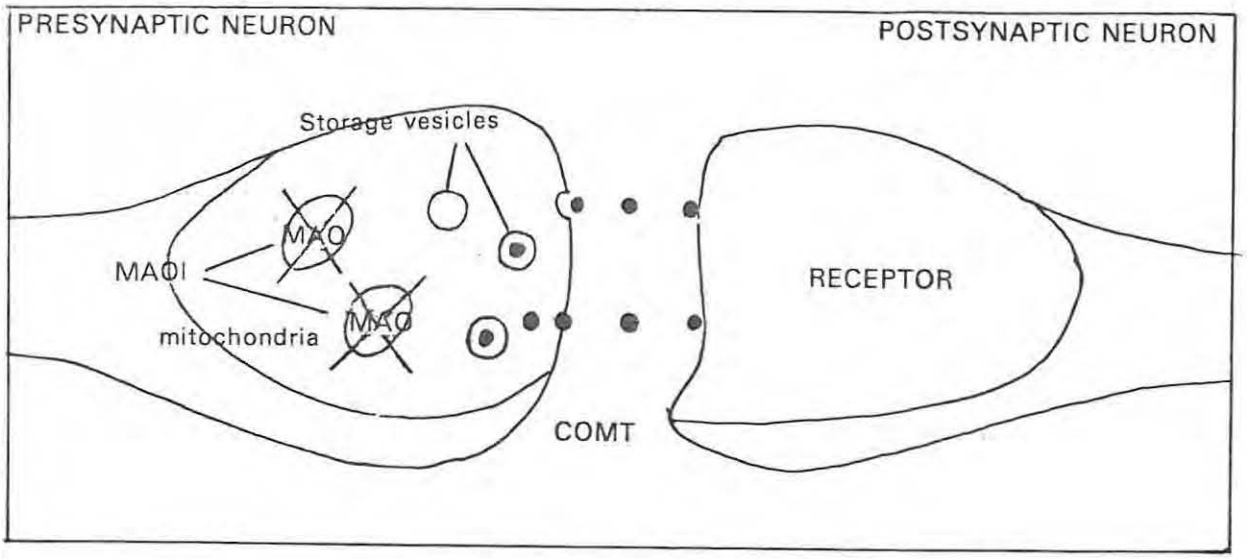


Fig. 5A. Schematic Representation of the Mechanism of Action of the Monoamine Oxidase Inhibitors.

The MAO inhibitors block the degradation of the neurotransmitter (noradrenaline and serotonin) by MAO.

COMT, catechol-O-methyltransferase, MAOI, monoamine oxidase inhibitor

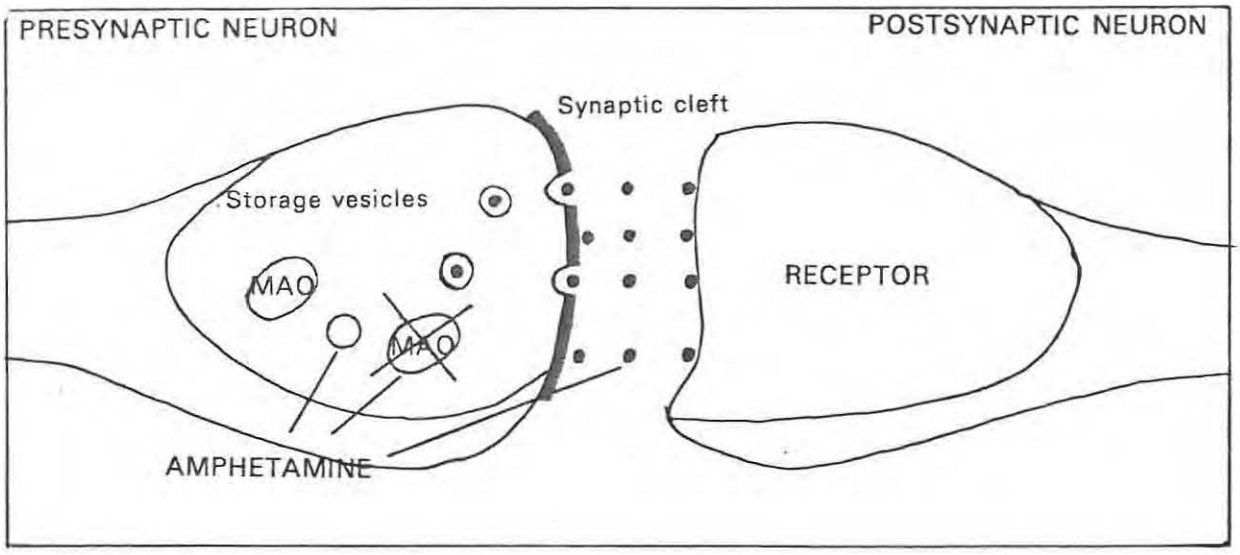


Fig. 5B. Schematic Representation of the Proposed Mechanism of Action of the Psychostimulants.

Psychostimulants (e.g. amphetamine) stimulate the release of stored noradrenaline and dopamine, inhibit the reuptake of the neurotransmitter from the synaptic cleft and inhibit MAO.

It has also been proposed that the MAO inhibitors block the reuptake of released noradrenaline into the storage vesicles, thereby producing an increased concentration of noradrenaline around the adrenergic synapses (Pletsher, 1966). In addition, the MAO inhibitors have been reported to reduce central serotonin turnover (Glowinski et al., 1972; Schuberth, 1973). This, however, may be a result of receptor stimulation and subsequent feedback inhibition of serotonin synthesis.

Inhibitors of MAO elevate the brain levels of both noradrenaline and serotonin; which of these amines plays the major role in these drugs' mechanism of action is a point of contention. Some authors regard the increase of noradrenaline as being of primary importance (Pletscher, 1964), whereas others suggest the increased serotonin level is more crucial (Dahlström and Fuxe, 1964; Bartoniček, 1966). Moreover, the particular effect may vary with the MAO inhibitor used (Gorkin, 1966) - for example, phenelzine and nialamide inhibit the deamination of serotonin more strongly than the deamination of tyramine, whereas other MAO inhibitors inhibit inactivation of both amines equally. Chronic treatment with MAO inhibitor drugs has been shown to cause a greater increase of brain serotonin than of noradrenaline (Spector et al., 1960; Green et al., 1962). In addition, the absolute and relative increase of amines after administration of MAO inhibitors, varies from region to region of the brain - for instance, in the limbic structures of intact rats the ratio

..... - 62 -/

of serotonin to noradrenaline is normally 1 : 1, but 4 hours after tranylcypromine administration this ratio was found to be 5 : 1 (Valzelli and Garattini, 1968).

Inhibition of MAO has been linked with the "False Neurochemical Transmitter" theory promulgated by Kopin et al. (1965). This proposes that MAO inhibition will produce an accumulation of sympathomimetic metabolites which normally are not present in the body, but which have sufficient affinity for the adrenergic receptor sites to displace the regularly present neurotransmitters from sympathetic nerve endings. Moreover, sympathetic nerve stimulation will release these agents in the same way as it does noradrenaline. However, the resultant effect at the postsynaptic receptor sites will be greatly diminished, since the false neurotransmitters have distinctly weaker sympathomimetic properties (Kopin, 1968; 1971). Normally tyramine is rapidly metabolized by MAO so that little octopamine is formed. However, following MAO inhibition, this acts as a false neurotransmitter having only one-hundredth the efficacy of noradrenaline at postsynaptic receptor sites (Molinoff and Axelrod, 1969; Kopin, 1971). This has been supported by clinical studies which have shown octopamine to accumulate in platelets from depressed patients treated with MAO inhibitors (Murphy, 1972). Octopamine has also been found in platelets from patients with endogenously reduced MAO activity.

1.1.6.4.3 Lithium:

Well-controlled studies have indicated that lithium is highly effective in the treatment of manic and hypomanic states (Schou, 1959; 1967; Gershon and Yuwiler, 1960; Maggs, 1963). More recently it has been suggested that lithium maintenance therapy might exert a prophylactic effect in the treatment of recurrent manic-depressive disorders (bipolar) and in recurrent depression in which no manic episode occurs (unipolar)(Hartigan, 1963; Baastrup and Schou, 1967). These authors have reported that lithium maintenance treatment may prevent, delay or alleviate these recurrent affective episodes. The efficacy of lithium salts in the prophylactic treatment of manic-depressive disorders has, however, been questioned (Blackwell and Shepherd, 1968). Numerous other workers have studied the efficacy of lithium as a preventative agent in depression and the results are conflicting. In 9 controlled studies lithium was reported to be significantly superior to placebo (Angst et al., 1970; Coppen et al., 1971a; Schou, 1971) but not significantly superior in another 3 studies (Fieve et al., 1968; Melia, 1970; Polackova et al., 1971).

The use of lithium salts in the treatment of depression is controversial. Controlled studies investigating the effectiveness of lithium in the treatment of depression have been published and are outlined in Table 9. Differences in the response pattern to lithium between

..... - 64 -/

TABLE 9:

TREATMENT OF DEPRESSION WITH LITHIUM

Number of Patients	Diagnosis	Drugs Used	Results	Reference
12	Acute depression	Lithium or placebo	No significant difference between lithium and placebo.	Hansen et al., 1959
30	Depression	Lithium or placebo	Bipolar depressions responded more favourably than unipolar depressions.	Goodwin et al., 1969
29	Acutely depressed manic-depressives	Lithium or imipramine	Imipramine significantly superior to lithium although both groups showed improvement.	Fieve et al., 1968
70	Manic-depressives	Lithium or imipramine	Imipramine significantly superior to lithium.	Platman, 1970
24	Acute depression	Lithium or desipramine	Both exhibited similar antidepressive activities.	Mendels et al., 1972b

bipolar and unipolar depressive patients have been revealed. It has been reported that both the manic and depressive phases of bipolar patients respond more favourably to lithium than the depressive phase of unipolar patients (Hartigan, 1963; Baastrup, 1964; Goodwin et al., 1972; Klerman, 1974; Noyes et al., 1974; Prien, 1974). This responsiveness to lithium has been used in the classification of affective disorders to identify bipolar depressive patients (Table 3).

Although no definite conclusion can be drawn concerning lithium's antidepressive effect, there is some evidence that lithium may be of value in the treatment of patients with recurrent depressions that are resistant to other therapies (Dyson and Mendels, 1968; Greenspan et al., 1968; Zall et al., 1968). These lithium responsive patients are characterized by a capacity to develop higher red blood cell lithium concentrations during treatment (Mallinger et al., 1975; Frazer and Mendels, 1977).

1.1.6.4.3.1 Mechanism of Action:

The mechanism by which lithium exerts its psychiatric effects is not yet known and it is difficult to determine which of the many known lithium effects are relevant to its clinical action.

A number of hypotheses regarding its mode of action have been put forward. Coppen et al. (1962) suggested that

..... - 66 -/

lithium exerts its therapeutic effect by replacing sodium intracellularly and thereby restoring the resting electrolyte balance across the cell membrane. Coppen (1967) and Bunney et al. (1972b) hypothesized that the sodium pump (which is responsible for removing sodium from the cell) is defective in affective illness and lithium administration corrects this imbalance. In support of this, lithium carbonate treatment of manic patients has been shown to be accompanied by decreased residual sodium levels in 2 of the 3 studies (Coppen et al., 1967a; Baer et al., 1970; Mendels, 1971).

It is likely that the intracellular displacement of sodium by lithium also has an effect on the electrophysiology of the cell (Whybrow and Mendels, 1969). The studies of Garside et al. (1966) and Barratt et al. (1968) have suggested that lithium administration reduces CNS excitability. The literature was reviewed in detail by Schou (1957).

An alternative hypothesis suggests that lithium's therapeutic effect is mediated via the biogenic amines. After a transient increase in catecholamine turnover rate (Schildkraut, 1973b), lithium has been shown to inhibit the release (Baldessarini, 1975) and increase the reuptake of catecholamines (noradrenaline and dopamine) at presynaptic neurons (Colburn, et al., 1967; Baldessarini and Yorke, 1970; Kuriyama and Speken, 1970). Synaptosomes, isolated from the brain of rats and pretreated with lithium,

..... - 67 -/

have been shown to take up noradrenaline to a significantly greater extent than their controls (Colburn et al., 1967). Blood platelets (which have similar membrane functions to synaptosomes) from patients treated with lithium carbonate also show an increased uptake of noradrenaline (Murphy et al., 1969). Lithium has also been shown to interfere with some cyclic adenosine monophosphate (c-AMP) mediated hormones to stimulate specific adenylate cyclases (Forrest, 1975) which are important components of the postsynaptic receptor mechanisms in the mediation of responses to catecholamines (Forn and Valdecasas, 1971).

The hypotheses which implicate either biogenic amines or electrolytes in the mechanism of lithium are not mutually exclusive. Extracellular sodium is required for the neuronal uptake of noradrenaline and serotonin, thus in the case of a defective sodium pump, the lack of extracellular sodium may prevent the uptake mechanism and maintain the manic syndrome (Bunney et al., 1972b; Ban, 1977). In addition, intracellular sodium has been found to cause a steady release of noradrenaline from the synaptic vesicles which, in the case of a defective sodium pump, would maintain the manic state unless counteracted by lithium administration (Bunney et al., 1972b; Mendels et al., 1974).

An alternative hypothesis on the action mechanism of lithium suggests that lithium exerts its therapeutic effect by producing a shift in the metabolism of monoamines from

..... - 68 -/

extracellular O-methylation to intracellular deamination. In favour of this hypothesis are the findings that there is a decrease in the urinary excretion of normetanephrine, and an increase in the urinary excretion of VMA and 5-HIAA during successful lithium treatment (Schildkraut et al., 1967; Mendels et al., 1973).

Lithium effects on biogenic amines are compatible with the catecholamine hypothesis as long as lithium is considered only as an antimanic drug. Current hypotheses, however, fail to explain the effectiveness of lithium in the prevention of recurrent depression.

Lithium administration also exerts an effect on indoleamines. Knapp and Mandell (1973) have reported that chronic lithium treatment increases tryptophan uptake but decreases the activity of tryptophan hydroxylase in rat synaptosomes.

In addition to its effect on the catecholamines and indoleamines, lithium has been found to decrease both the synthesis and release of acetylcholine (Dawes and Vizi, 1973; Vizi, 1975) as well as increase the synthesis of glutamate and γ-aminobutyrate (GABA)(Gottesfeld et al., 1971; 1973).

In short, the mechanism of action of lithium, according to Baldessarini and Lipinski (1975), "has not yet been explained by a coherent and convincing body of data or theory, although it seems likely that important effects at neuronal and

hormonally sensitive membranes are involved.

1.1.6.4.4 Psychostimulants:

The psychostimulants, amphetamine, methamphetamine and methylphenidate, cause hyperactivity in animals and mood elevation, increased alertness and enhanced performance in humans (Weiss and Laties, 1962; Innes and Nickerson, 1965). Amphetamine has been used for many years with variable results in the treatment of depression. Although the drug has been reported to possess some clinical anti-depressive activity (Prange, 1975a), it is generally agreed that it has little to offer in the treatment of the major depressive disorders (Hordern, 1965; Cole and Davis, 1967; Davis et al., 1968; Shepherd et al., 1968). Amphetamine administration may alleviate the symptoms of mild or "reactive" depressions but such beneficial effects are often transient and may be accompanied by a number of unwanted side effects, such as insomnia and nervousness. There is also the possibility of tolerance and addiction developing and as a result, the drug has limited usefulness as an antidepressive (General Practitioner Research Group, 1964; Davis et al., 1968). In addition, it has been observed clinically that after the chronic administration of large doses of amphetamine, a "rebound period" of mental depression and fatigue develops which has been attributed to a temporary depletion of noradrenaline stores (McLean and McCartney, 1961; Moore and Lariviere, 1963). This interpretation is supported by the finding that large doses

..... - 70 -/

of amphetamine significantly lower the concentration of brain noradrenaline in animals (Smith, 1965).

The mechanism of action of amphetamine is quite complex as it has been reported to have several actions on the nerve terminal. It increases the concentration of catecholamines (noradrenaline and dopamine) in the synaptic cleft by releasing them from the intraneuronal storage vesicles (Iversen, 1964; Hanson, 1967). There is also evidence that amphetamine blocks the cellular reuptake of the monoamines in a similar manner to the TADs (Carlsson et al., 1966; Rutledge, 1970; Fawcett et al., 1972; Maas, 1975). Amphetamine may also inhibit the enzyme, MAO (Costa and Garattini, 1970). The proposed mechanisms of action of amphetamine are illustrated in Figure 5B.

Amphetamine may, however, have some value in separating the various types of depressive disorders. Roberts (1959a; 1959b) found that patients suffering from "neurotic" depression showed a euphoric response after an acute dose of methamphetamine, whereas patients with "psychotic" depressions experienced dysphoria. Fawcett et al. (1968) used a three-day trial of amphetamine to predict which depressed patients would respond to the TADs. Those patients who exhibited elation and a temporary improvement in depressive symptoms after amphetamine administration, have been found to be responsive to subsequent tricyclic therapy, whereas those who were unaffected by the amphetamine

treatment failed to respond to the tricyclics (Fawcett and Siomopoulous, 1971).

1.1.6.4.5 Atypical Antidepressives:
Although these drugs are clinically effective, they differ in both chemical structure and pharmacological activity from the classical TADs or MAO inhibitors.

1.1.6.4.5.1 Iprindole:
Iprindole, a tricyclic indole, has been shown in several double-blind studies to be an effective antidepressive (Johnson and Maden, 1967; Imlah et al., 1968; Sutherland et al., 1970) with its clinical efficacy comparable with imipramine (Ayd, 1969; Fann et al., 1972, review; Rickels et al., 1973; Briant and George, 1975; Tait and Todrick, 1975).

Pharmacologically the drug is neither a strong inhibitor of noradrenaline and serotonin reuptake (Ross et al., 1971; Fann et al., 1972; Sanghvi and Gershon, 1975; Horn, 1976; Koe, 1976), nor an inhibitor of MAO (Rosloff and Davis, 1974). This indicates that its antidepressive activity is mediated by a mechanism different from that suggested for the classical antidepressives. Iprindole has been reported to possess about the same potency in inhibiting striatal dopamine uptake as the classical tricyclics (Halaris et al., 1975; Randrup and Braestrup, 1977) which may explain its antidepressive effect (Table 8).

..... - 72 -/

Noradrenaline-sensitive limbic cyclase activity in rats is also reduced after chronic administration of iprindole (Vetulani and Sulser, 1975). If the hypothesis that some depressed patients may exhibit an increased receptor sensitivity to noradrenaline is correct, then iprindole (as well as the TADs, MAO inhibitors and ECT) may exert its therapeutic effect by this mechanism (Vetulani and Sulser, 1975; Vetulani et al., 1975).

1.1.6.4.5.2 Mianserin:

The tetracyclic compound, mianserin, although pharmacologically and biochemically different from the classical TADs, has been shown to be an effective antidepressive in man (Wheatley, 1975; Murphy, 1975; Ghose et al., 1976; Pichot et al., 1978; Smith et al., 1978). Although the drug inhibits the brain uptake of noradrenaline in vitro (Koe, 1976; Raiteri et al., 1976), neither biogenic amine uptake (Leonard, 1974; Burgess et al., 1978) nor MAO activity (Coppen et al., 1978a) is inhibited in vivo. In fact, mianserin has been found to be a potent serotonin antagonist both centrally and peripherally (Van Riezen, 1972; Jalfre et al., 1974; Maj et al., 1976). Moreover, an increase in the synthesis and turnover of noradrenaline has been demonstrated in animals who were pretreated with mianserin both acutely (Kafoe and Leonard, 1973) and chronically (Kafoe et al., 1976). This acceleration of noradrenaline synthesis has been suggested as a possible mechanism of mianserin's antidepressive action (Garver and Davis, 1978).

..... - 73 -/

1.1.6.4.5.3 Nomifensine:

The antidepressive activity of nomifensine has been confirmed by numerous investigators (Franchin, 1973; Madalena et al., 1973; Pecknold et al., 1975; Acebal et al., 1976). In addition to blocking noradrenaline uptake (Samanin et al., 1975; Koe, 1976), it is also a potent blocker of dopamine uptake in the rat brain (Hunt et al., 1974; Randrup and Braestrup, 1977; Tuomisto, 1977). This is illustrated in Table 8. Serotonin uptake, however is not inhibited to any significant extent (Schacht and Heptner, 1974; Samanin et al., 1975).

1.1.6.4.5.4 Other Atypical Antidepressives:

The drugs fenfluramine, maprotiline, toloxatone and viloxazine have been shown to possess antidepressive activity despite their widely differing mechanisms of action (fenfluramine: Murphy et al., 1976; maprotiline: Ludiomil Symposium, 1975; Kielholz, 1972; 1973; 1974; Briant and George, 1975; toloxatone: Martin, 1973; Suttel and Duplan, 1973; viloxazine: Vivalan Symposium, 1975; Moizeszowicz and Subira, 1977).

Fenfluramine inhibits the uptake of serotonin into blood platelets (Wielosz et al., 1976) and causes the release of serotonin from central storage vesicles (Trulson and Jacobs, 1976).

Maprotiline is a stong inhibitor of noradrenaline uptake

..... - 75 -/

(Waldmeier et al., 1976) but only has a weak inhibitory effect on serotonin uptake (Maitre et al., 1975; Benesova and Benes, 1974). Striatal dopamine uptake is also inhibited by this drug (Halaris et al., 1975; Randrup and Braestrup, 1977).

Toloxatone, an oxazolidinone derivative, appears to be a relatively specific type A MAO inhibitor (Kan et al., 1977).

Viloxazine, a β-blocker derivative, neither inhibits MAO nor blocks the uptake of monoamines in the brain (Mallion et al., 1972; Koe, 1976; Lippman and Pugsley, 1976).

1.2 BIOCHEMICAL THEORIES OF DEPRESSION:

1.2.1 Catecholamine Hypothesis:

In 1965, Schildkraut (1965) put forward the "catecholamine hypothesis". In essence, this hypothesis postulates that depression is associated with an absolute or relative deficiency of catecholamines, particularly noradrenaline, at functionally important adrenergic receptor sites in the brain. Elation, or mania, on the other hand, is presumed to be associated with an excess of the neurotransmitters.

The proposal was based on two fundamental observations. Reserpine, a drug which is capable of precipitating severe depression in some humans (Muller et al., 1955; Harris, 1957), lowers both the catecholamine and indoleamine concentrations in the brain (Shore, 1962). Iproniazid, a therapeutically effective antidepressive (Crane, 1957; Cole et al., 1961), raises the concentration of biogenic amines in the brain by blocking the action of MAO (Spector et al., 1960; 1963). Additional pharmacological evidence which supported this hypothesis is summarized in Table 10. Largely on the basis of this indirect evidence, other workers supported the proposal that an alteration in brain catecholamines may be involved in the pathophysiology of affective disorders (Bunney and Davis, 1965; Coppen, 1967).

There are, however, numerous problems with the pharmacological evidence that supports the catecholamine hypothesis. For

TABLE 10:

PHARMACOLOGICAL OBSERVATIONS CONSISTENT WITH THE CATECHOLAMINE HYPOTHESIS

(Modified from Schildkraut (1965) and Davis (1970))

DRUG	ACTION	EFFECTS ON BEHAVIOUR IN ANIMALS	EFFECTS ON MOOD IN HUMANS
RESERPINE	DEPLETES BRAIN OF NA AND 5HT	SEDATION	SEDATION CAUSES DEPRESSION (in some patients)
TETRABENAZINE	DEPLETES BRAIN OF NA AND 5HT	SEDATION	SEDATION CAUSES DEPRESSION (in some patients)
MAO INHIBITORS	ELEVATES BRAIN LEVELS OF NA AND 5HT BY BLOCKING MAO	EXCITEMENT PREVENTS AND REVERSES RESERPINE-INDUCED SEDATION	RELIEVES DEPRESSION
TRICYCLIC ANTI-DEPRESSIVES	BLOCKS UPTAKE OF NA AND 5HT	PREVENTS RESERPINE-INDUCED SEDATION POTENTIATES THE EFFECTS OF AMPHETAMINE	RELIEVES DEPRESSION
AMPHETAMINE	INHIBITS CELLULAR UPTAKE AND INACTIVATION OF NA RELEASES NA	EXCITEMENT	ELATION
ECT	INCREASES NA TURNOVER		RELIEVES DEPRESSION
LITHIUM	INCREASES NET UPTAKE OF NA AND 5HT REDUCES RELEASE OF NA AND 5HT	PREVENTS THE HYPER-ACTIVITY INDUCED BY DMI AND RESERPINE	RELIEVES MANIA
L-DOPA	INCREASES NA	EXCITEMENT REVERSES RESERPINE EFFECTS	RELIEVES DEPRESSION?
PROPRANOLOL	β-ADRENERGIC BLOCKER		CAUSES DEPRESSION

DMI, desipramine; NA, noradrenaline; 5HT, serotonin; MAO, monoamine oxidase.

example, it is not clear whether the therapeutic effects of these agents (reserpine, TADs, MAO inhibitors, lithium, ECT) result from their effects on the catecholamines or on serotonin. In addition, the clinical effects of reserpine, TADs and lithium take from several days to weeks to become apparent, whereas their effects on biogenic amines are immediately evident in _in vivo_ studies. Thus the chronic effects of these drugs are probably more complicated.

Two major research strategies have been employed to test the catecholamine hypothesis. One approach, the precursor loading technique, consists of administration of the catecholamine precursor amino acids and observation of the therapeutic response.

L-DOPA has been used in the treatment of depression with mixed results. Pare and Sandler (1959) were the first to administer L-DOPA to depressed individuals and they reported no therapeutic effect. This has been confirmed by several investigators (Klerman _et al._, 1963; Schildkraut _et al._, 1963; Turner and Merlis, 1964; Davis, 1970). In contrast Ingvarsson (1965) reported dramatic remissions of long standing drug resistant depressions following the administration of L-DOPA every other day. Marked improvement was seen within a few hours and relapses occurred on discontinuance of the L-DOPA therapy. More recent reports by Goodwin _et al._ (1970) and Matussek _et al._ (1970) have shown that if large doses of L-DOPA are used, with or without

..... - 78 -/

a peripheral decarboxylase inhibitor, there is a therapeutic response in about one third of the patients, although others may deteriorate. Bunney et al. (1971) have reported L-DOPA to be particularly useful in the treatment of "retarded" depressions. This has led to the suggestion that within a heterogeneous depressive population, there is a subgroup of patients who will respond to L-DOPA therapy.

There are a number of possible explanations for L-DOPA's lack of effect. L-DOPA does not pass through the blood brain barrier easily, thus it may be difficult to reach therapeutic levels in the brain (Berlter et al., 1966). In addition, although L-DOPA treatment leads to increased levels of dopamine in the brain, only a small fraction is converted to noradrenaline (Glowinski and Iversen, 1966). In most studies noradrenaline brain levels are either unchanged or are lower following L-DOPA administration (Butcher and Engel, 1967; Iversen, 1967; Everett and Borcherding, 1970), although the turnover of noradrenaline may be increased (Dairman and Udenfriend, 1971). Another complication of L-DOPA treatment is that the metabolic response to the drug is not confined to the catecholamine system. L-DOPA has been reported to decrease rat brain serotonin in a dose-dependent manner (Everett and Borcherding, 1970). The behavioural responses of animals to L-DOPA can be prevented by PCPA which suggests that serotonin is necessary for the drug's behavioural response (Carroll, 1971). Decreased serotonin synthesis has also been observed

..... - 79 -/

in depressed patients following L-DOPA administration (Goodwin et al., 1971; Dunner and Goodwin, 1972). Platelet serotonin content has also been found to decrease significantly during L-DOPA treatment (Murphy, 1972). A number of mechanisms purporting to explain the above findings have been put forward and are discussed in detail by Carroll (1971) and Murphy (1972).

The negative results following L-DOPA administration challenge the adequacy of the catecholamine hypothesis of depression. The results suggest functional interactions between catecholamines and indoleamines at the cellular level which, in turn, underlies the need for combined study of the different neurotransmitter systems instead of narrow adherence to one system.

Another more direct approach for confirming the catecholamine hypothesis involves measuring the postmortem catecholamine metabolites in the brain and the body fluids of patients with affective illnesses.

Raised urinary noradrenaline levels have been reported in depressed patients (Curtis et al., 1960). However, this has also been seen in mania (Strom-Olsen and Weil-Malherbe, 1958), schizophrenia (Mattock et al., 1967) and in a psychotically depressed population (Bunney et al., 1967). In addition, Bliss and co-workers (1966) found a reduction in cerebral noradrenaline concentration when animals were

subjected to a variety of stresses. The above results led Whybrow and Mendels (1969) to conclude that disturbances in peripheral noradrenaline levels represent a general response to stress rather than aetiologic disruption of amine metabolism. In addition, there is evidence to suggest that turmoil, psychosis as well as muscular activity may be associated with alterations in the excretion of noradrenaline and its metabolites (Sachar et al., 1963; Schildkraut et al., 1965; Nelson et al., 1966; Bunney et al., 1967).

The most direct test of the catecholamine hypothesis would be the measurement of noradrenaline levels in the human brain. However, these studies are obviously limited. Shaw et al. (1967) and Bourne et al. (1968) examined the brains of depressed patients who had committed suicide and observed no marked difference in noradrenaline levels when compared with a control group who died from other causes. Dencker et al. (1966a) and Moses and Robins (1975) also found no difference in the brain noradrenaline levels of depressed patients.

MHPG has been shown to be the major metabolite of noradrenaline in the brains of many different species including man (Glowinski et al., 1965; Rutledge and Jonason, 1967; Schanberg et al., 1968a; 1968b; Wilk and Watson, 1973). It has, therefore, been suggested that the amount of MHPG excreted in the urine may reflect central noradrenaline

..... - 81 -/

metabolism (Maas and Landis, 1967; Schanberg et al., 1968a).

In a pilot study, it was reported that a heterogeneous group of depressed individuals excreted significantly less MHPG into urine than did a healthy control group, whereas the quantities of urinary normetanephrine and metanephrine excreted by these two groups were similar (Maas et al., 1968). Since then numerous investigators have shown the levels of urinary MHPG to be relatively lower during depression and higher during mania or hypomania than after clinical remissions (Greenspan et al., 1970; Schildkraut et al., 1971; 1972; Bond et al., 1972; Watson et al., 1972; Jones et al., 1973). However, this was not confirmed by Bunney et al. (1972a) and Shopsin et al. (1973b). In addition, one should interpret urinary MHPG levels with caution, since these levels may be a function of activity (Ebert et al., 1972) or stress (Rubin et al., 1970; Maas et al., 1971).

Maas et al. (1968; 1972) observed that not all depressed patients excrete low levels of MHPG and proposed the existence of various subgroups of depressives, those excreting normal, less than normal or greater than normal quantities of MHPG. In support of this, Schildkraut et al. (1977) reported significantly lower MHPG excretion in "schizoaffective" and "bipolar manic-depressive" depressions than in "unipolar chronic characterological" and "non-specific" depressions. (The diagnostic criteria for this classification of depressive disorders were based on clinical picture and are described in

..... - 82 -/

a previous paper (Schildkraut and Klein, 1975)). On the basis of these findings, they have proposed that examination of MHPG levels may provide a biochemical basis for classifying the different types of depressions (Section 1.1.4.1).

Recent studies of MHPG levels in the CSF of depressed patients confirm the existence of subgroups of depressed patients, since Gordon and Oliver (1971) and Post et al. (1973) reported a significant decrease in MHPG in the depressed group while Shaw et al. (1973) and Shopsin et al. (1973a) did not. In addition, Goodwin (1976) found that the concentration of MHPG in the CSF of bipolar depressed patients was lower than in unipolar depressive patients.

Therefore, although the pharmacological evidence supporting the catecholamine hypothesis is indirect and indefinite, the more recent clinical findings provide evidence that alteration in central noradrenaline metabolism may be of importance in the underlying pathophysiology of at least some types of depressive disorders.

1.2.2 Indoleamine Hypothesis:

The indoleamine hypothesis of depressive disorders is a variation of the catecholamine hypothesis, which states that depression is associated with a deficiency of brain serotonin (Lapin and Oxenkrug, 1969).

Since reserpine decreases brain serotonin concentrations as well as brain noradrenaline levels, and since most antidepressive drugs (tricyclics, MAO inhibitors) that increase noradrenaline concentrations usually also increase serotonin concentrations, the question of whether mood states are related to changes in the concentration of serotonin or noradrenaline has remained unanswered to date. There are, however, a number of findings which suggest that serotonin may be of major importance in the aetiology of depressive disorders.

Treatment with TADs has been shown to reduce brain serotonin turnover (Goodwin and Post, 1974). These findings are consistent with those of Bowers (1972a) as well as those from animal studies demonstrating that imipramine decreases brain 5-HIAA when administered with probenecid (Bruinvels, 1972), decreases serotonin depletion after synthesis inhibition (Corrodi and Fuxe, 1968; 1969; Bruinvels, 1972) and retards the disappearance of labelled serotonin from the brain (Schildkraut et al., 1969b). In addition, there is evidence that treatment with imipramine in usual therapeutic doses significantly decreases the concentration of 5-HIAA in human spinal fluid (Papeschi and McClure, 1971). Imipramine therefore apparently decreases CNS turnover of serotonin. Another point in favour of the above hypothesis is the observation that elevation of serotonin occurs several weeks after the onset of treatment with MAO inhibitors coinciding with the time at which the antidepressive effect

..... - 84 -/

occurs (McClean et al., 1965). Modification of serotonin levels may thus be relevant to the treatment of depression.

Van Praag (1977a) reported an increased 5-HIAA accumulation in CSF following probenecid treatment on the day ECT was administered. Moreover, patients who had shown a low 5-HIAA response prior to therapy, showed a normal response 1 week after a successful ECT course. Therefore ECT also seems to influence serotonin turnover in a direction to be expected on the basis of the indoleamine hypothesis.

There have been two major strategies employed to test more directly the relationship between serotonin and affective disorders. One strategy is to manipulate the level of serotonin pharmacologically using serotonin precursors.

There is no consensus on the efficacy of the serotonin precursor, l-tryptophan, in depression. Some authors have reported l-tryptophan to be as effective as imipramine (Broadhurst, 1970; Coppen and Noguera, 1970; Coppen et al., 1972a) and ECT (Coppen et al., 1967b) in the treatment of depression. These studies, however, are open to serious questions since no placebo group was included and the study was not double-blind. L-tryptophan has also been found useful in depression by Cocheme (1970) and van Praag and Korf (1970). The apparent prophylaxis by l-tryptophan of an annual episode of depression has also been reported (Hertz and Sulman, 1968). Other investigators, however,

..... - 85 -/

have not confirmed these findings (Bowers, 1970; Carroll et al., 1970; Bunney et al., 1971; Carroll, 1971; Murphy et al., 1973). Use of tryptophan pyrrolase inhibitors, such as allopurinol and nicotinamide, has been suggested as adjuncts in the treatment of depression (Curzon, 1969). These drugs would protect tryptophan from excessive degradation along the irreversible pyrrolase-catalysed kynurenine pathway and thus favour its passage to the brain where it can form serotonin. In a preliminary study (Chouinard et al., 1977), nicotinamide has been used successfully in combination with l-tryptophan, all the depressed patients showing significant improvement over a 4-week period.

A study indicating l-tryptophan's ability to potentiate the antidepressive effect of a MAO inhibitor more positively connects indoleamines with depression. This potentiation was initially demonstrated by Coppen et al. (1963) and has been replicated by other workers (Pare, 1963; Glassman and Platman, 1969; Van Praag, 1970). Van Praag (1962), however, had previously been unable to demonstrate this potentiation, either in terms of intensity or of rapidity of the antidepressive effect. Van Praag and Korf (1971a) in order to explain the variability of the reported results, proposed that there is a serotonin deficient subgroup of depressed patients who respond to l-tryptophan. The combination of l-tryptophan with clomipramine has also been reported to produce a more intensive antidepressive

..... - 86 -/

effect than clomipramine alone (Wålinder et al., 1975). The immediate metabolic precursor of serotonin, 5-hydroxy-tryptophan (5-HTP), has also been tried alone and in combination with MAO inhibitors in the treatment of depression but with inconclusive results. 5-HTP alone has been reported by 2 groups of investigators (Sano, 1972; Takahashi et al., 1973b) to be an effective treatment for some depressed patients. In other studies, this anti-depressive activity was demonstrated in only a subgroup of the depressed population (Persson and Roos, 1967; Brodie et al., 1973). In addition, it has been demonstrated that patients who exhibit decreased accumulation of 5-HIAA following probenecid administration respond to 5-HTP treatment while those exhibiting higher accumulations do not (Van Praag et al., 1972). Where other investigators have failed to demonstrate a significant antidepressive effect with 5-HTP (Pare and Sandler, 1959; Kline et al., 1964; Glassman and Jaffe, 1969), the discrepancies might be explained by the proposal that only those patients with decreased serotonin turnover respond to 5-HTP (Van Praag et al., 1973b).

A single report (Kline and Sacks, 1963) indicates that the effects of MAO inhibitors are potentiated by 5-HTP, while other reports show no beneficial effects from this combination (Pare and Sandler, 1959; Glassman, 1969). In normal test subjects, however, 5-HTP has been observed to exert a positive influence on mood level (Trimble et al., 1975).

..... - 87 -/

There are, unfortunately, limitations to the strategy of testing the indoleamine hypothesis by the use of precursors, since these agents also have effects on other systems. Tryptophan raises central serotonin levels but may also alter the levels of other transmitters. 5-HTP is taken up not only into the serotoninergic neurons but also into the catecholaminergic neurons (Van Praag, 1977a).

Murphy et al. (1976) tested fenfluramine, a serotonin releaser in depressed patients. They reported that there was improvement in a majority of the bipolar depressives after a few weeks of treatment but not in the unipolar cases. Moreover, there are indications that 4-chloramphetamines, compounds which potentiate central serotonin activity, behave like true antidepressives (Van Praag and Korf, 1973a; 1976).

If the indoleamine hypothesis is correct, then drugs which decrease serotonin brain levels should cause depression. There is evidence that PCPA, which inhibits tryptophan hydroxylase and lowers the brain concentration of serotonin, can produce depressive symptoms in subjects without a previous history of depression (Bunney et al., 1969). More recently, Shopsin and co-workers reported that PCPA abolished the therapeutic effect of imipramine (1974) and tranylcypromine (1976) within a few days. PCPA administration caused the patients to revert to the depressed state, whereas its withdrawal permitted the re-establishment of mental well-being. This finding points to an important

..... - 88 -/

role of serotonin, although it should be borne in mind that this drug may also have effects other than reduction of serotonin formation.

Methysergide, a specific antagonist of serotonin, has been reported to be effective in the treatment of mania (Dewhurst, 1968b), however, this has been disputed by Coppen et al. (1969).

Thus the results obtained from pharmacological studies using drugs which potentiate or diminish serotoninergic activity, lend support to the hypothesis that a deficiency in indoleamines in the CNS plays an important aetiological role in the pathogenesis of at least some depressive disorders.

A more direct way of testing the indoleamine hypothesis is by direct measurement of serotonin and its metabolites in the biological fluids of depressed patients. The concentration of 5-HIAA in the CNS is related to the amount of serotonin metabolized in the CNS and consequently, the 5-HIAA concentration is considered to be an indication of the amount of serotonin degraded in the CNS. An argument in favour of this is that procedures that stimulate (administration of serotonin precursors) or inhibit (PCPA administration) central serotonin synthesis elicit an increase or decrease, respectively, in the 5-HIAA concentration in the CSF (Chase, 1972; Dunner and Goodwin, 1972; Van Praag et al., 1973b).

..... - 89 -/

Numerous investigators have reported lowered levels of 5-HIAA in the lumbar CSF of depressive and manic patients, while others have been unable to confirm these findings. A summary of the work down in this field is presented in Table 11. The low 5-HIAA levels can be interpreted as arising from a defect in the synthesis of brain serotonin or a slower rate of its release and metabolism. This abnormality has been shown to persist even after apparent full recovery (Coppen et al., 1972b; Mendels et al., 1972a). It is therefore suggested that serotonin deficiency determines the patients' predisposition to depression and mania, while other factors may be involved in triggering the affective illness (Prange et al., 1974).

The variable results in many reports of spinal fluid 5-HIAA levels in depression might be explained by several factors. Post et al. (1973) demonstrated that 5-HIAA levels are correlated with motor activity. This relationship had previously been noted by Fotherby et al. (1963) and Bowers et al. (1969). Variability might also result from different amounts being removed during the spinal tap (Siever et al., 1975) and age (Bowers and Gerbode, 1968; Åsberg et al., 1973). These discrepancies might also be based on differences in the nature of the control group, differences in the procedure for collection of the CSF or other factors such as drugs and diet (Goodwin and Post, 1973). A recent study was controlled for age of the subject, amount of fluid removed and the time of day of the test and again

..... - 90 -/

TABLE 11:

RESULTS OF STUDIES OF CSF 5-HIAA CONCENTRATIONS IN AFFECTIVE ILLNESS

AFFECTIVE ILLNESS	MEAN 5-HIAA LEVEL WITHOUT PROBENECID		MEAN 5-HIAA LEVEL WITH PROBENECID	
	LOW	NORMAL	LOW	NORMAL
DEPRESSION	Ashcroft et al., 1966 Dencker et al., 1966b Van Praag et al., 1970 Van Praag and Korf, 1971a Coppen et al., 1972 b McLeod and McLeod, 1972 Mendels et al., 1972a Coppen, 1973	Fotherby et al., 1963 Bowers et al., 1969 Roos and Sjöstrom, 1969 Papeschi and McClure, 1971 Sjöstrom and Roos, 1972 Goodwin and Post, 1973	Roos and Sjöstrom, 1969 Sjöstrom and Roos, 1972 Van Praag and Korf, 1973b Van Praag et al., 1970; 1973a	Bowers, 1972b. Goodwin and Post, 1973
MANIA	Dencker et al., 1966b Coppen et al., 1972b Coppen, 1973	Ashcroft et al., 1966 Bowers et al., 1969 Roos and Sjöstrom, 1969 Goodwin and Post, 1973		

significantly lower CSF 5-HIAA concentrations were seen in depressed patients when compared to controls (Coppen, 1973).

The probenecid technique (Van Praag et al., 1973a) has refined spinal fluid studies and has increased the reliability of these determinations (Van Praag, 1977a). Probenecid inhibits the active transport of serotonin from the CNS to the bloodstream (Neff et al., 1967) and thus it has been assumed that the rate of 5-HIAA accumulation following probenecid administration is proportional to the turnover rate of serotonin in the brain. The accumulation of 5-HIAA with time following probenecid administration has been reported to be lower in depressed patients than in control subjects, although this has not been confirmed by all investigators (Table 11).

There are, however, also complications with the probenecid test. It is possible that the level of probenecid achieved differs from patient to patient or that a given level of probenecid produces different levels of transport inhibition (Zarcone et al., 1977). Moreover, it is also possible that probenecid may affect serotonin metabolism directly (Korf et al., 1972).

Åsberg et al. (1976) measured the 5-HIAA concentration in the CSF of 68 depressed patients and demonstrated that this measurement is distributed bimodally. In these patients,

..... - 92 -/

29% had less than 15 ng/ml of 5-HIAA and a significant correlation between the severity of the depression and 5-HIAA concentration in this low level subgroup was observed. These results support the hypothesis of a subgroup of depressed patients with low CSF 5-HIAA levels (Section 1.1.4.1). Åsberg's bimodal distribution may explain the difficulties investigators have in establishing significant differences in 5-HIAA levels in the CSF, with or without probenecid. In other words, if a patient group included 2 kinds of different modal values and these were pooled, there would be little hope of detecting differences. The proposed bimodal distribution of 5-HIAA values has been supported by Goodwin (1976) and Van Praag (1977b). Kripke (1976), however, suggested that bimodal disturbances should not be postulated until the possibility that a diurnal rhythm has shifted out of place, has been ruled out.

Several investigators have measured the urinary excretion of 5-HIAA in depressed patients. Pare and Sandler (1959) and Van Praag and Leijnse (1963) reported that those depressed patients who responded to iproniazid had a lower initial urinary excretion of 5-HIAA than those who did not respond. Burgermeister et al. (1963), however, could not replicate these findings.

Studies on tryptamine excretion in depressed patients have shown that urinary tryptamine levels can be either increased (McNamee et al., 1972) or decreased (Rodnight, 1961; Coppen et al.,

..... - 93 -/

1965a) in depressive disorders. However, since diet was not standardized and classification of depressive disorders received little attention; these findings cannot be regarded as very significant (Van Praag, 1977a).

Another method for biochemical investigation of patients with depression involves the assay of levels of serotonin and 5-HIAA in the brains of suicide victims. Early studies suggested that either serotonin (Shaw et al., 1967; Pare et al., 1969) or 5-HIAA (Bourne et al., 1968) was decreased in the brainstem of suicide victims as compared to a control group whose deaths were from other causes. In a more recent study, Lloyd et al. (1974) found that serotonin, but not 5-HIAA, was lower in certain raphe nuclei (the dorsal nucleus and the inferior central nucleus) of suicide victims than in controls.

Limitations of this strategy, however, are numerous. For example, it is difficult to obtain reliable diagnostic data on these subejcts, the victims' nutritional state as well as their drug history is often uncertain, the time of day when they committed suicide is often difficult to determine and so forth. Moreover, the duration of the interval between death and postmortem examination is a factor that is difficult to standardize.

The above data on serotonin metabolism (postmortem and CSF studies) in depressed patients, although not unequivocal,

provide strong evidence suggesting a disturbance of either serotonin synthesis or turnover in depressive disorders.

There are also natural and iatrogenic states that seem to involve serotonin deficiency and these cause mental depression. Patients with Parkinson's disease who receive L-DOPA sometimes become depressed and this has been attributed to interference with the intestinal absorption of tryptophan by competition between the two amino acids (Lehmann, 1973) or to the excessive formation of DA in the brain which, in turn, displaces the serotonin from its storage sites (Birkmayer and Neumayer, 1972). "Natural" diseases such as carcinoid syndrome and regional enteritis cause serotonin depletion and the depression occuring with these has been reported to be relieved by l-tryptophan administration (Lehmann, 1972; Johansson et al., 1973).

Since both the blood platelets and CNS serotonin synaptosomes share common biochemical as well as many morphological characteristics, the blood platelets have been used as an easily obtainable model of serotoninergic neurons. The uptake of serotonin into platelets of depressed patients has been shown to be decreased (Tuomisto and Tukiainen, 1976; Ehsanullah and Mulgirigama, 1979) and the capacity of serotonin transport through the platelet membrane has been reported to be impaired in some depressed patients, the impairment being independent of the psychiatric status

of the patient (Coppen et al., 1978b).

The above results are far from unequivocal but overall, are more in favour of than against the indoleamine hypothesis. The evidence points to an abnormality in serotonin metabolism in both mania and depression, but it seems that clinical recovery is not accompanied by any change in this abnormality. This suggests that these changes in serotonin and possibly other amines, are only part of the chemical pathology and may be a necessary predisposition that requires another factor - perhaps endocrinological - to produce the clinical picture of depression and mania (Coppen, 1972).

1.2.3 Dopamine Hypothesis:

Recently DA has also been implicated in depression (Randrup et al., 1975, review). Support for this hypothesis has come from the findings that most of the TADs as well as some newer antidepressives (iprindole, maprotiline, mianserin, nomifensine) are fairly potent inhibitors of DA uptake in rat brain synaptosomes (Randrup and Braestrup, 1977). A comparison of the inhibitory effects of the various antidepressives on DA, noradrenaline and serotonin reuptake is shown in Table 8. In addition, piribedil, a DA receptor agonist, has been reported to be effective in the reversal of depressive symptoms in some patients (Post et al., 1978). Nomifensine, an antidepressive drug which is a dopamine agonist (Costall et al., 1975; Schacht et al., 1977) as

well as an inhibitor of noradrenaline reuptake (Schacht et al., 1977) has been found to be particularly useful in depressed patients with psychomotor retardation (van Scheyen et al., 1977). The efficacy of L-DOPA, the immediate precursor of DA, in depression is controversial and is reviewed in Section 1.2.1. At best, L-DOPA seems to be effective in the treatment of only a few retarded, depressed patients (Bunney et al., 1971).

An indirect piece of evidence from neuroendocrinology pointing to deficient dopaminergic mechanisms in the hypothalamus of depressed patients is the subnormal GH response to insulin-induced hypoglycaemia seen in some depressives (Section 1.2.9.2.2)(Casper et al., 1977).

Post et al. (1973) reviewed the studies measuring the concentration of homovanillic acid (HVA), the main degradation product of DA, in the CSF of depressed patients. Some investigators have reported slightly lower (Nordin et al., 1971) or definitely lower (Sjöstrom, 1973) HVA levels in the CSF of depressed patients than of controls, while others have found no significant difference from the control group (van Praag and Korf, 1971b; Goodwin et al., 1973; van Praag et al., 1973a). Probenecid inhibits the active transport of HVA from the CSF and the amount of HVA accumulating after probenecid administration is believed to reflect the rate of DA turnover. Post and Goodwin (1975) using the probenecid method concluded that, while the baseline concentrations of

HVA were not abnormal among manic-depressive patients, the rate of increase of HVA during probenecid administration was definitely below normal. This finding has been confirmed by numerous investigators (Roos and Sjöstrom, 1960; Nordin et al., 1971; van Praag and Korf, 1971b; Sjöstrom and Roos, 1972; Sjöstrom, 1973; Goodwin et al., 1973; van Praag et al., 1973a; Post and Goodwin, 1975). These observations suggest that there may be a deficiency of DA synthesis or turnover in the state of depression. A point against this hypothesis, however, is the observation that ECT (Nordin et al., 1971), as well as various anti-depressive drugs, seem to have little or no effect upon the accumulation of HVA in the probenecid test (Sourkes, 1977). In addition, motor activity has been shown to affect HVA levels in the CSF (Post et al., 1973).

As with noradrenaline, no significant changes in the concentration of DA in various parts of the brain of depressive suicides has been reported (Moses and Robins, 1975).

The above findings thus suggest that DA may also play a role in depressive disorders, particularly in the motor component of depression.

1.2.4 Permissive Amine Hypothesis:

This hypothesis, originally proposed by Kety (1971) and developed by Prange and his co-workers (1974), states that

..... - 98 -/

a central serotonin deficiency indicates a vulnerability to affective illness, but in itself is not sufficient for its cause. In addition, a change in catecholamine levels is needed to produce the clinical syndrome, lowered catecholamines correlating with depression and increased catecholamines with mania. A comparison between this proposal and the other biogenic amine hypotheses is outlined in Table 12.

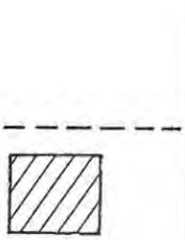
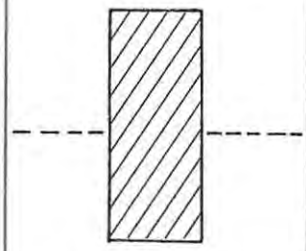
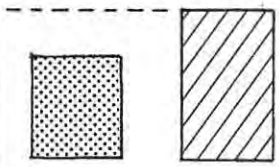
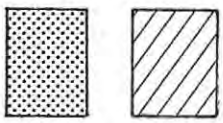
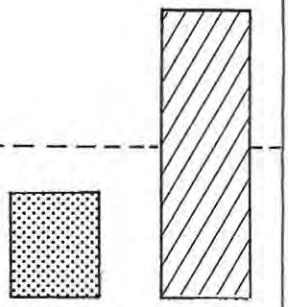
The permissive amine hypothesis supports the view that depression and mania are on a continuum rather than being polar opposites. This model, originally proposed by Court (1968), suggests that mania and depression share a similar biological dysfunction, with mania representing a more severe deviation from normal mood.

Support for this continuum model comes from the observation that both depression and mania may show some response to the same antidepressive therapies. ECT is widely used in both states. Imipramine, a clinically effective antidepressive, has been reported by one investigator to be useful in the treatment of mania (Akimoto et al., 1960). Moreover, lithium, an effective treatment for mania, has been shown to have some therapeutic efficacy in depression (Goodwin et al., 1969). In addition, some drugs, such as cortisone, can precipitate episodes of either depression or mania - sometimes in the same individual (Fawcett and Bunney, 1967).

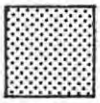
..... - 99 -/

TABLE 12:

BIOGENIC AMINE HYOPTHESES OF DEPRESSIVE ILLNESSES: COMPARISON

HYPOTHESIS	PREDISPOSITION	DEPRESSION	MANIA
CATECHOLAMINE	Unspecified		
INDOLEAMINE	Unspecified		Unspecified
PERMISSIVE AMINE HYPOTHESIS			

 CATHECHOLAMINES

 INDOLEAMINES

----- NORMAL

Electrolyte abnormalities have been reported to deviate from normal in the same direction in mania and depression (Coppen, 1965). In a review of the evidence, Whybrow and Mendels (1969) proposed that mania and depression are neurophysiologically very similar, both representing "an unstable state of CNS hyperexcitability". In addition, the clinical observation of depression during mania (Kotin and Goodwin, 1972) can only be explained by a continuum model.

Since there is data implicating a deficiency of catecholamines and indoleamines in depression as well as increased catecholamine levels in mania (Section 1.2.1, 1.2.2 and 1.2.3), only evidence suggesting decreased indoleamine activity in mania is necessary to verify the permissive amine hypothesis.

L-tryptophan has been reported to be useful in mania (Prange et al., 1974) and depression (Coppen et al., 1967b; 1972a; van Praag and Korf, 1970). Prange et al. (1974) proposed that l-tryptophan exerts its antimanic effect by correcting the postulated indoleamine deficit while only slightly reducing the catecholamine level. Lithium not only decreases catecholamine levels (Schildkraut et al., 1966b) but also increases serotoninergic activity (Sheard and Aghajanian, 1970; Tagliamonte et al., 1971; Knapp and Mandell, 1973), which, according to this hypothesis, would account for its effective antimanic activity. This

..... - 101 -/

hypothesis would also explain why L-DOPA precipitates mania in some individuals (Murphy et al., 1971), while not being a particularly effective antidepressive (Bunney et al., 1970). L-DOPA has been shown to decrease 5-HIAA levels in the CSF of depressed patients (Goodwin et al., 1971), a decrease which would aggravate the already deficient indoleamine system.

Prange et al. (1974) believe that if this hypothesis were true in the strictest sense, a carefully adjusted dose of l-tryptophan would not only cure depression and mania but also prevent their recurrence. This, however, has not been reported. Thus, even though this hypothesis probably represents only half the story, it, unlike the previous biogenic amine hypotheses, deals with the interrelations of amines and is of heuristic value in providing further stimulus for research.

1.2.5 Kynurenine Hypothesis:

There is ample evidence for defective serotonin synthesis and metabolism as well as abnormally high adrenocortical secretion in some groups of depressed patients (Section 1.2.2 and 1.2.9.2.1). A possible link between these two conditions has been proposed (Lapin and Oxenkrug, 1969; Curzon, 1969; Curzon and Bridges, 1970).

Tryptophan is known to be metabolised by several pathways. Less than 3% of dietary tryptophan is converted to serotonin,

..... - 102 -/

its formation being regulated by the enzyme, tryptophan hydroxylase. A quantitatively more important route involves the formation of nicotinic acid which is regulated by the liver enzyme, tryptophan pyrrolase, via the kynurenine intermediate. The metabolic pathway is presented in Fig. 6.

The activity of liver pyrrolase has been reported to be increased by hydroxycorticosteroids (Knox, 1951; Knox and Auerbach, 1955; Shan et al., 1968; Curzon, 1969; Young and Sourkes, 1977). This observation led to postulation of the hypothesis that the raised plasma cortisol levels seen in depressed patients, may induce tryptophan pyrrolase which, in turn, may directly divert tryptophan away from serotonin formation (thereby lowering brain serotonin levels) and instead, increase metabolism along the tryptophan nicotinic acid pathway (Curzon, 1969; Curzon and Bridges, 1970). Thus depression is proposed to be associated with a decreased utilization of tryptophan in the formation of serotonin with a consequent increased utilization of tryptophan as a substrate for kynurenine formation.

Direct investigation of this relationship between pyrrolase activity and brain serotonin metabolism requires determination of brain serotonin. Rat brain serotonin levels were shown to decrease (maximally by 30%) after a single intraperitoneal injection of 5 mg/kg hydrocortisone (Curzon and Green, 1968). This reduction was shown to be associated with a comparable fall of 5-HIAA levels which indicated decreased serotonin

..... - 103 -/

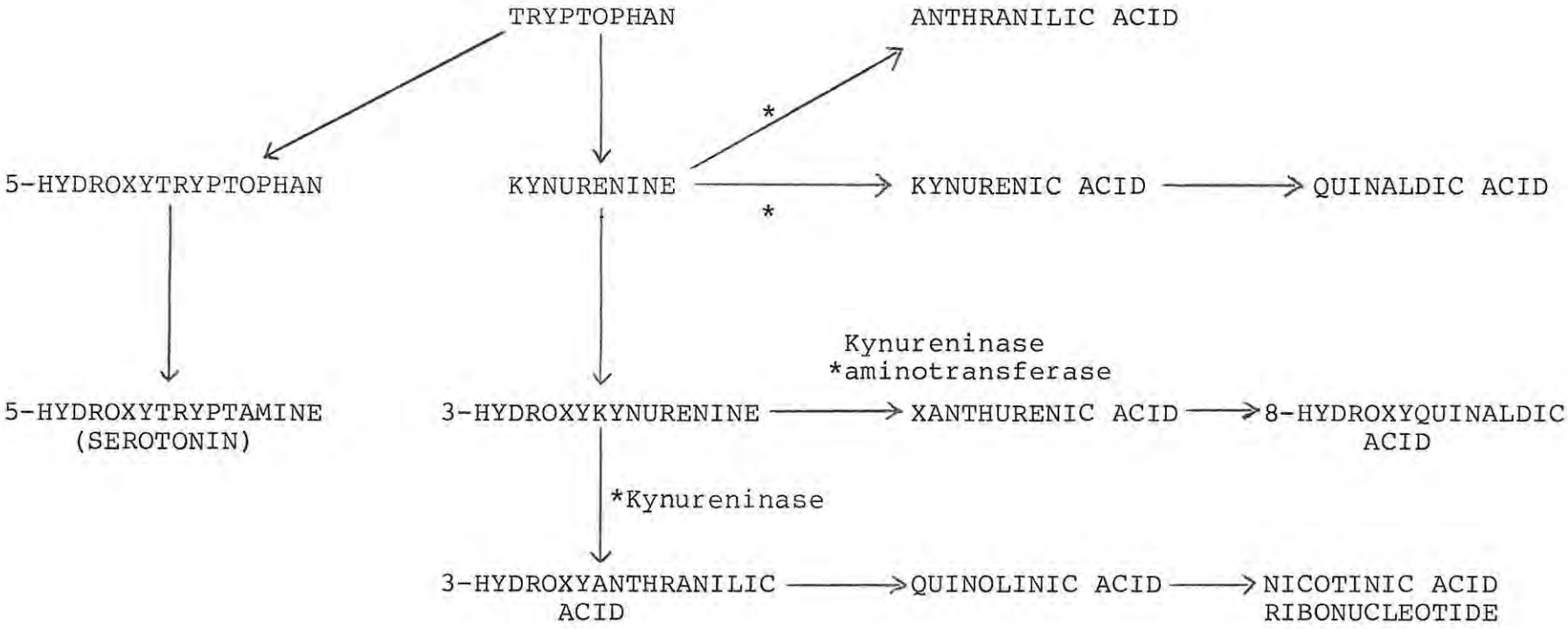


Fig. 6. The Metabolic Pathway for the Biosynthesis of Nicotinic Acid Ribonucleotide and Serotonin from Tryptophan and its Important Side Reactions.

*known pyridoxal 5-phosphate-dependent reactions.

synthesis. In this study, it was also noted that the injection of pyrrolase inhibitors (allopurinol and yohimbe) nullified hydrocortisone's effect in reducing serotonin. Furthermore, α-methyltryptophan, which causes pyrrolase activity to increase, also led to a fall of brain serotonin. Studies using human brains are obviously impossible. However, indirect evidence comes from the observation that a group of female patients suffering from "endogenous" depression excreted significantly greater amounts of kynurenine and 3-hydroxykynurenine than the female controls (Curzon and Bridges, 1970). As already mentioned, this fall in brain serotonin could be explained by the diversion of tryptophan. However, another explanation is possible. Injection of kynurenine or other tryptophan metabolites formed subsequent to pyrrolase action causes brain serotonin levels to fall (Curzon and Green, 1968). Therefore, increased formation of these substances due to increased pyrrolase activity may be involved in the mechanism by which brain serotonin levels diminish.

Lapin and Oxenkrug (1969) proposed that the depression of brain serotonin levels may result in weakening of the inhibitory processes on the amygdaloid complex. In man, activation of this area has been reported to increase plasma cortisol levels, presumably by stimulating the anterior pituitary to produce more ACTH. Thus a vicious circle would be set up - low serotonin brain levels would lead to activation of the amygdaloid complex, increased formation

..... - 105 -/

of hydroxycorticosteroids and increased activity of tryptophan pyrrolase, which, in turn, would "shunt" the degradation of tryptophan away from serotonin to kynurenine. This is illustrated in Fig. 7.

If this hypothesis were true, treatment of depressive disorders would involve attacking the links in the chain. Serotonin precursors would perhaps be effective in restoring the serotonin brain levels. Chronic administration of imipramine or desipramine has been found to inhibit liver tryptophan pyrrolase activity in mice (Paracchi, 1967) which suggests that this action could be a component of their antidepressive effect. According to this hypothesis, pyrrolase inhibitors (nicotinamide and allopurinol) would be of use in the treatment of depression (Curzon, 1969). These drugs would inhibit the degradation of tryptophan along the pyrrolase-catalysed kynurenine pathway thereby increasing the formation of brain serotonin. In support of the theory Chouinard et al. (1977) reported that the combination of l-tryptophan and nicotinamide has an enhanced antidepressive effect in some depressed patients.

A number of questions involving the kynurenine hypothesis, however, are still unresolved. Firstly there is the question of whether the activation of tryptophan pyrrolase is sufficiently marked to draw a substantial amount of tryptophan away from serotonin synthesis. It may bein acute experiments involving rats (Curzon and Green, 1968), but it

..... - 106 -/

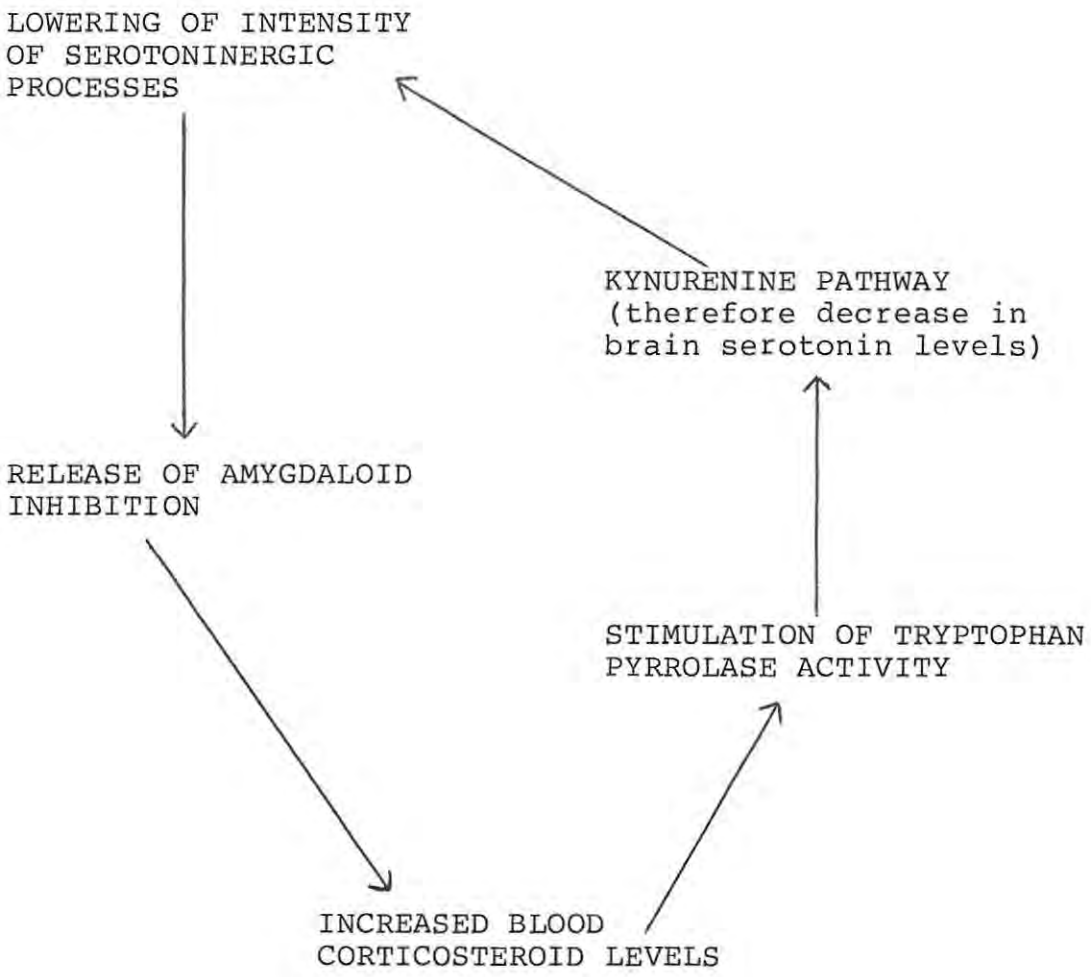


Fig. 7. The Kynurenine Hypothesis and Sequence of Events Proposed by Lapin and Oxenkrug (1969).

is not known whether this mechanism is also involved in depressed patients. A drawing off of available tryptophan, however, seems unlikely since the pyrrolase pathway is only one of the important metabolic pathways of tryptophan. Moreover, as noted by van Praag (1977a), the correlation between cortisol and plasma/CSF tryptophan has not been studied in depressed patients. Furthermore, normal CSF tryptophan levels have been reported in depressions (Ashcroft et al., 1973b). Thus at this stage, the kynurenine hypothesis is mere speculation.

1.2.6 Tryptamine Hypothesis:

As an alternative to the monoamine theory, Dewhurst (1968a) proposed a hypothesis to account for the role of noradrenaline and serotonin in terms of central receptors. He classified central monoamines into two distinct groups: excitant amines (type A) and depressant amines (type C).

Type A amines have a general formula $R.CH_2CH_2NH_2$ where R is a fat-soluble planar hydrocarbon, such as indolyl or phenyl. These amines, of which tryptamine is the most potent, produce behavioural and physiological excitation in immature chicks, probably by activation of specific central receptors. Depressant amines (type C) have a general structure $R'CH(OH)CH_2.NH.CH_3$ where R' is polar, for example, catechol. Such amines, of which adrenaline is the most potent, produce drowsiness and sleep in the immature chick.

..... - 108 -/

Dewhurst (1968a) proposed that some forms of depressive illness are caused by a deficiency of type A amines or insensitivity of the type A receptor, and some forms of mania are caused by an excess of type A amines or increased sensitivity of the type A receptor. He explained the efficacy of some antidepressive treatments in terms of this theory. Inhibitors of MAO principally increase tryptamine (type A amine). Amphetamine acts directly on the brain type A receptors. Tryptophan is the precursor of tryptamine, which could explain its antidepressive effect. In some studies methysergide, a specific competitive antagonist of the type A receptor (Dewhurst and Marley, 1965), has been claimed to be able to control manic disorders (Dewhurst, 1968b). Although these findings provide support for the above hypothesis, objections to it remain. For example, adrenaline does not produce sedation in man, but excitation. Moreover, this theory suggests that noradrenaline and serotonin have opposing actions in man, however, this is probably an oversimplification. It is more likely that they have both excitatory and inhibitory effects in different areas of the brain and that satisfactory treatment of affective disorders depends on restoring the normal balance of neurotransmitters. In addition, this theory does not take other transmitter substances, such as dopamine, into consideration.

1.2.7 False Neurotransmitter Theory:

Murphy (1972) proposed that the state of decreased central

..... - 109 -/

adrenergic activity during depression could be due to the effects of endogenously produced false transmitters, instead of resulting from a reserpine-like depletion of central biogenic amines as has been previously hypothesized (Schildkraut, 1965).

Although no definite role for false central neurotransmitters has been established, there is evidence to suggest that they may be involved in depressive disorders. Administration of the precursor of the false transmitter, α-methyldopa, to hypertensive patients has been found to precipitate depressive reactions and suicidal behaviour, particularly in those individuals with a history of depressive episodes (Fullerton and Morton-Jenkins, 1963; Smirk, 1963; McKinney and Kane, 1967). Octopamine, a false transmitter with one-hundredth the efficacy of noradrenaline, has been reported to accumulate in platelets from depressives treated with MAO inhibitors (Murphy, 1972). In the same study octopamine was found in the platelets of patients with endogenously reduced MAO activity.

Thus preliminary reports indicate that false neurotransmitters may be important in affective disorders, the exact function of which needs further evaluation.

1.2.8 Cholinergic-Adrenergic Hypothesis:

It is possible that brain amine systems other than those previously discussed, are involved in the genesis of affective

..... - 110 -/

disorders. Janowsky et al. (1972a; 1972b; 1973a; 1973b) have postulated that the cholinergic nervous system is also involved in the regulation of affect. They have suggested that a balance between adrenergic and cholinergic factors exists in the brain areas which regulate affect and have hypothesized that depression is a disease of central cholinergic predominance and mania a disease of central adrenergic (or serotoninergic) predominance. In other words, high biogenic amine levels and low acetylcholine (Ach) levels are associated with mania, while low catecholamine levels and high Ach levels are associated with depression.

There is both direct and indirect evidence suggesting the involvement of cholinergic activity in the aetiology of depression.

Reserpine, which can produce depression in hypertensive patients, has central cholinomimetic properties in both animals (Bogdanski et al., 1961) and man (Goodman and Gilman, 1975). According to the above hypothesis, reserpine would be expected to shift the adrenergic-cholinergic balance to a cholinergic predominance. It is interesting to note that α-methylparatyrosine (AMPT) which specifically blocks catecholamine synthesis without altering central Ach levels, has not been reported to produce depression in hypertensive patients (Charalampous and Brown, 1967).

The TAD drugs have central anticholinergic effects (Janowsky

..... - 111 -/

et al., 1972b). In man, these drugs cause a toxic, confusional state characterized by agitation, disorientation, loss of short-term memory and hallucinations which can be reversed by physostigmine. In addition, imipramine has been shown to block the increase in central cholinergic activity occuring with reserpine administration (Sulser et al., 1964) and to antagonize physostigmine-induced suppression of operant behaviour in pigeons (Vallant, 1967). Thus the tricyclics block central cholinergic activity, as well as increasing functionally available neurotransmitters, shifting the adrenergic-cholinergic balance towards an adrenergic predominance.

Numerous workers have reported data from animal studies which indicate that central cholinergic activation causes depressant inhibitory effects, while anticholinergic agents or adrenergic stimulation causes behavioural activation and arousal (Carlton, 1963; Vallant, 1967; Fibiger et al., 1970). For example, brain self-stimulation (Jung and Boyd, 1966) and locomotor activity (Fibiger et al., 1970) are inhibited by centrally active cholinomimetic agents. Centrally acting anticholinergic drugs block these cholinomimetic effects (Jung and Boyd, 1966; Vallant, 1967) and have themselves been shown to increase locomotor activity and self-stimulation (Stein, 1964).

The most convincing direct evidence that central cholinergic activity plays a significant role in the regulation of affect,

..... - 112 -/

however, comes from studies of the effects of centrally active cholinomimetic agents in man. Patients, who have a pre-existing depression, have been reported to experience worsening of their symptoms after physostigmine (Janowsky, 1972b; 1973a; Modestin et al., 1973) and choline administration (Tamminga et al., 1976). Moreover, several studies have reported that depression is a complication of poisoning with cholinesterase-inhibitor insecticides (Rowntree et al., 1959; Gershon and Shaw, 1961; Bowers et al., 1964). Severe depression has also been seen in patients treated with physostigmine while on a marihuana-induced "high" (El-Yousef et al., 1973). Physostigmine administered to methylphenidate-stimulated humans has been shown to cause a dramatic decrease in methylphenidate activation (Janowsky et al., 1972b). These workers have proposed that physostigmine acts by increasing central Ach levels so as to balance the increase in central adrenergic activity caused by methylphenidate.

Physostigmine has also been reported to exert an antimanic effect (Janowsky et al., 1972a; 1972b; Carroll et al., 1973). Janowsky et al. (1972a; 1972b) demonstrated that an acute dose of physostigmine caused a rapid decrease in the manic state which was converted to a state of psychomotor retarded depression.

The above findings support the hypothesis that the major affective disorders result from an imbalance between

..... - 113 -/

cholinergic and aminergic neurotransmission in the CNS.

1.2.9 Biochemical Abnormalities in Depression:

1.2.9.1 Electrolyte Abnormalities:

Electrolytes play a vital role in the normal functioning of the nervous system. The resting potential is dependent on the ratio of the concentration of potassium inside and outside the cell, while the action potential is dependent on the ratio of the concentration of intracellular to extracellular sodium. Electrolytes also play an important role in the synthesis, storage, release and inactivation of the neurotransmitters. Thus it is conceivable that abnormalities in electrolyte distribution and metabolism per se or through interaction with the biogenic amine systems, could lead to altered neuronal functioning that could underlie depressive disorders.

1.2.9.1.1 Sodium:

Schottstaedt and co-workers (1956) reported a reduction in the urinary excretion of sodium during depressive episodes. Other early studies also found a retention of sodium in depressed patients (Russell, 1960; Anderson and Dawson, 1963). More recently radioisotope techniques have been used to study electrolyte metabolism in affective disorders (Coppen, 1965; Shaw, 1966). Gibbons (1960) observed a decrease in exchangeable sodium after recovery from a depressive illness, although exchangeable potassium showed no significant alteration. These results led to the

hypothesis that during depression sodium is retained, with total body sodium increasing until recovery occurs, by which time the excess sodium has been excreted.

Subsequent studies by Coppen and Shaw (Coppen and Shaw, 1963; Coppen, 1965; Coppen et al., 1965b; 1966; Shaw, 1966) however, strongly indicate that there is no change in total body sodium, but that during depression there is a redistribution of sodium within the body. For example, a significant increase in residual sodium (which represents intracellular sodium together with a small amount of exchangeable bone sodium) was demonstrated in both depressed (Coppen and Shaw, 1963) and manic patients (Coppen et al., 1966). The magnitude of the increase was 50% in depression and 200% in mania, levels returning to normal after recovery. In addition, total body potassium and intracellular potassium were found to be low in depression but this did not change with clinical recovery.

The rate of entry of radioactive sodium from the blood to the CSF was investigated by Coppen (1960). He reported a reduced entry rate in severly depressed patients when compared with normal subjects, schizophrenic patients and patients who had recovered from a depressive disorder. Shaw et al. (1969) found that the brains of depressed patients who had committed suicide had an increased water content and reduced sodium concentration as compared to brains from subjects who died of natural causes.

Changes in sodium could arise in several ways. The increased sodium retention observed in the above studies may be secondary to the elevated cortisol seen in depression (Gibbons, 1963; Durele and Schildkraut, 1966; Baer et al., 1969). Sodium distribution is also affected by progesterone, aldosterone and ADH. Therefore it is important to know whether these abnormalities are primary to affective disorders or secondary to changes in glucocorticoids and mineralocorticoids, or in adrenergic functioning (Shaw, 1966).

Based largely on the data from Coppen's laboratory (Coppen and Shaw, 1963; Coppen et al., 1966), Whybrow and Mendels (1969, review) hypothesized that there is an unstable hyperexcitability of the CNS in depression. They suggested that states of central arousal could result from intraneuronal sodium accumulation with a consequent lowering of the resting potential and hyperexcitability.

Clinical evidence to support the proposal that electrolytes do play a role in the pathogenesis of affective disorders comes from the finding that lithium appears to have a prophylactic value in reducing the incidence and severity of depressive disorders (Section 1.1.6.4.3). Lithium is believed to exert its therapeutic effect by replacing sodium intracellularly, thereby re-establishing the "resting" electrolyte balance across the cell membrane (Coppen et al., 1962; 1965b; Bunney et al., 1972b).

..... - 116 -/

It has been proposed that the disturbance in biogenic amines seen in depressed patients (Sections 1.2.1 and 1.2.2) could secondarily result from intraneuronal sodium accumulation, since the transport and release of monoamine neurotransmitters across the presynaptic membrane is partially determined by the distribution of electrolytes intra- and extraneuronally (Bogdanski et al., 1968; Maas, 1972; White and Paton, 1972). Transport of electrolytes across the presynaptic membrane involves Na^+/K^+ dependent adenosine triphosphatase (ATPase) and the carrier protein. A deficiency (could be a genetic deficiency) in either the enzyme or the carrier would interfere with the transport of electrolytes and thus the transport of biogenic amines. Bunney et al. (1972b) hypothesized that increased intracellular sodium could decrease the intracellular release of noradrenaline from its carrier transport protein, thereby decreasing net noradrenaline transport. Additional support for a defective transport system comes from investigators who have demonstrated reduced erythrocyte Na^+/K^+ ATPase activity in patients suffering from depressive illness (Naylor et al., 1973; Hokin-Nyverson et al., 1974; Hesketh et al., 1977). Previous studies by Naylor and co-workers (1970a; 1970b; 1971) showed that the mean erythrocyte sodium concentration as well as active sodium transport was lower in depressive females in comparison to that of a control group.

Pandey et al. (1976) and Mendels et al. (1977) found that

..... - 117 -/

the ability to extrude lithium ions from red blood cells is impaired in many manic patients and some first degree relatives. Reduced platelet serotonin uptake and accumulation has also been observed in some depressives (Tuomisto and Tukiainen, 1976; Coppen et al., 1978b).

Thus current evidence suggests that there is a subgroup of depressed patients who have an abnormality, possibly genetic, in some property of the cell membrane that regulates electrolyte distribution. This hypothesis is not incompatible with the biogenic amine hypotheses of affective disorders since it is possible that an abnormality in one system may be responsible for changes in other systems. Thus an impairment of the ionic fluxes responsible for depolarization and repolarization of the neurons, could disrupt critical neurotransmitter levels which, in turn, may lead to depressive symptomatology.

1.2.9.1.2 Magnesium:

Cade (1964) reported considerably elevated total plasma magnesium levels in depressed patients both before and after recovery. Frizel et al. (1969), however, found total plasma magnesium to be significantly lowered in depressed patients which increased significantly with recovery. Ionized magnesium, however, did not differ between the control and depressed group, and since it is presumably ionized magnesium that is physiologically active, the authors concluded that it was unlikely that magnesium was involved in

the pathophysiology of depression.

1.2.9.1.3 Calcium:

Calcium plays a role in regulating sodium and potassium flux across the cell membrane. There is evidence to suggest that this ion may be involved in affective disorders.

Severe depression or catatonic stupor has often been observed in hypercalcaemic disorders, for example, hyperparathyroidism (Mandel, 1960; Hockaday et al., 1966; Petersen, 1968) and vitamin D intoxication (Andersen et al., 1968).

In an early study, Flach (1966) found a reduced calcium excretion in the urine of depressed patients who had improved as compared with a small rise in urinary calcium excretion in those patients who did not respond to the treatment. He thus suggested that clinical improvement is associated with calcium retention.

Carman and Wyatt (1977) in a review of the evidence reported that a decrease in extracellular calcium levels reduces the activity of tyrosine hydroxylase and tryptophan hydroxylase, and increases the activity of acetylcholinesterase, COMT and adenylate cyclase. Reduced extracellular calcium thus diminishes neurotransmitter release, precursor uptake and transmitter uptake.

Lowering calcium levels in the CSF produces behavioural

excitation in several animal species (Pappenheimer et al., 1962; Veale and Myers, 1971), whereas increasing the calcium concentration in the CSF produces sedation or catatonic states (Marquardt and Riemschneider, 1951; Feldberg and Sherwood, 1957). In addition, a decrease in calcium levels in the CSF has been associated with the antidepressive response to ECT (Carman et al., 1974) and sleep deprivation (Jimerson et al., 1976), while CSF calcium levels remained unaltered when no antidepressive response was evident.

Thus, even though no definite role for calcium has been shown to date, there is evidence to suggest it may be a factor in affective disorders.

1.2.9.2 Neuroendocrine Abnormalities:

1.2.9.2.1 Hypothalamic-pituitary-adrenal cortical (HPA) system:

Associations between affective disorders and diseases of the HPA axis have been noted by many clinicians (Michael and Gibbons, 1963; Rubin and Mandell, 1966; Whybrow and Hurwitz, 1976). Addison in his original description of hypoadrenalism, included mental depression among the clinical symptoms. Since then numerous workers have confirmed that symptoms such as depression, apathy, insomnia, apprehension and sleep disturbances are associated with Addison's disease (Engel and Margolin, 1941; Cleghorn, 1951). A significant proportion of patients with hyperadrenalism, Cushing's syndrome, or under long term treatment with ACTH or cortisol

..... - 120 -/

have also been found to exhibit profound mental changes such as euphoria, depression and overt psychosis (Borman and Schmallenberg, 1951; Glaser, 1953; Cobb, 1960). Although euphoria has been reported, depression is a more common finding in chronic cases (Fawcett and Bunney, 1967). These findings seem contradictory but might be explained by the fact that in both Addison's disease and some forms of Cushing's disease, pituitary ACTH levels can be raised. This clinical association of mood changes with abnormalities of adrenal function raised the question of the role of glucocorticoids in affective disorders.

Many workers have shown that the production (or secretion) rate of cortisol is elevated in most patients suffering from depression (Gibbons, 1964; 1966; Carpenter and Bunney, 1971; Sachar et al., 1971a; 1973b). Carroll (1972), in a review of the evidence, reported that the plasma cortisol levels of depressed patients are usually elevated over that of normal patients. These plasma cortisol levels provide an indirect indication of the rate of release of ACTH from the anterior pituitary. Similar findings have been observed by many other investigators (for example, Gibbons and McHugh, 1962; Michael and Gibbons, 1963; Rubin and Mandell, 1966). However, it should be noted that in most studies there are usually a number of patients who do not show any significant alteration in cortisol levels. In a review, Carroll and Mendels (1976) concluded that the adrenal cortex of depressed patients responds normally to ACTH which thus suggests that

..... - 121 -/

the high cortisol secretion rate observed, reflects increased ACTH release rather than exaggerated adrenal cortical responsiveness to ACTH.

Researchers studying CSF cortisol levels in depressed patients have reported conflicting results. Some authors have demonstrated a deficiency in brain cortisol (McClure and Cleghorn, 1968; 1970; Carpenter and Bunney, 1971; Coppen et al., 1971), while others have found elevated CSF cortisol levels in depressed subjects (Carroll, 1972; Carroll and Mendels, 1976). The latter finding would be in keeping with the elevation of plasma cortisol levels described above. Urinary free cortisol excretion has also been reported to be elevated in most of the depressed patients studied (Ferguson et al., 1964; Carroll, 1976).

All these findings (elevated plasma free and total cortisol, normal plasma corticosteroid-binding globulin capacity (King, 1973), elevated CSF cortisol and elevated urinary free cortisol excretion) suggest that there is increased exposure of peripheral tissues and the CNS to free cortisol in depressed patients. It is therefore surprising that cortisol levels have been found to be lower than normal in the suicide brains of depressed patients (Brooksbank et al., 1972; 1973). These findings, however, cannot be evaluated properly since not only are suicide brain studies fraught with difficulties of interpretation, but also because little is known about the rate of postmortem decay of cortisol in

..... - 122 -/

nervous tissue (Carroll and Mendels, 1976).

Initially attempts were made to suggest an aetiological role for the steroids in depression. This interest diminished however, when it was known that cortisol levels could be elevated in a number of conditions that involve stress such as combat, examinations and hospitalization. Thus it was proposed that the elevated cortisol plasma levels found in some depressives, probably reflect a non-specific stress response which is secondary to the psychic distress of the disorder (Brooksbank and Coppen, 1967; Sachar, 1967). Even though the hypersecretion of cortisol may only be a secondary effect of certain depressive disorders, Curzon (1969) suggested that the elevated cortisol secretion may not simply be a stress response, but may reflect "apparent limbic dysfunction, along with disturbances in mood, affect, appetite, sleep, aggressive and sexual drives and autonomic nervous system activity".

The HPA axis of man exhibits a definite circadian variation in spontaneous activity (Doe et al., 1956; Kobberling and von zur Muhlen, 1974). Instead of operating continuously with a minute-to-minute feedback control, the HPA axis is activated in an episodic manner which is interspersed by periods of "total adrenocortical quiescence" (Hellman et al., 1970; Krieger et al., 1971). During most of the day the brain exerts an inhibitory influence on the HPA axis, with a relaxation in this inhibition being observed mainly during the morning peak of cortisol secretory episodes (Carroll, 1972).

..... - 123 -/

The diurnal rhythm of plasma cortisol levels persists in most depressed patients (where rhythm is defined as morning values being higher than night values). However, the elevation of evening plasma cortisol levels over control values is usually much greater than the elevation of morning values i.e. night values are more abnormal than are morning values (Doig et al., 1966; McClure, 1966; Knapp et al., 1967; Fullerton et al., 1968). In other words, morning plasma cortisol levels of depressed patients are generally within the normal range, whereas the levels measured during the night lie in the pathological range. Sachar et al. (1973b) studied the diurnal rhythm of cortisol secretion in depressives using the venous catheter technique (Hellman et al., 1970), and found a circadian HPA disturbance. These patients were shown to have an increased number of secretory episodes, increased total cortisol secretion and increased time per day during which cortisol secretion was occuring. Of particular interest was the fact that active cortisol secretion was observed during the late evening and early morning hours when cortisol secretion is minimal in normal subjects. They proposed that these findings indicated a failure of the normal CNS circadian inhibitory influences on ACTH-cortisol release. These results have been replicated by Carroll and his co-workers (Carroll and Mendels, 1976) and this has led to the hypothesis that "the essential neuroendocrine lesion in depression appears to be a disinhibition of the HPA axis".

..... - 124 -/

A number of standard clinical tests of HPA function has been used to provide additional evidence of central neuro-endocrine dysfunction in depressed patients.

Lysine-8-vasopressin (LVP), by a yet undefined mechanism, causes a rapid increase in plasma cortisol levels in normal subjects. Jakobson et al. (1969) and Carroll (1972) have observed that some depressed patients fail to respond normally to LVP, which suggests an impairment of the central mechanisms mediating the HPA response to LVP.

An intravenous injection of insulin produces a rapid fall in blood glucose levels followed by a large HPA activation. Some depressed patients have been found to have impaired responses to insulin-induced hypoglycaemia (Carroll, 1969; 1972; Perez-Reyes, 1972), while other depressives have been shown to respond in a similar manner to normal subjects (Sachar et al., 1971b; Endo et al., 1974).

In normal subjects a midnight dose of dexamethasone (2 mg) suppresses plasma cortisol levels maximally for at least 24 hours (McHardy-Young et al., 1967). Numerous investigators have reported that depressed patients fail to show normal suppression of plasma cortisol levels after dexamethasone administration (Butler and Besser, 1968; Carroll et al., 1968; Platman and Fieve, 1968; Carpenter and Bunney, 1971; Stokes, 1972; Endo et al., 1974; Carroll and Mendels, 1976) but this has not been confirmed

..... - 125 -/

by all workers (Shopsin and Gershon, 1971; Verghese et al., 1973). The abnormality of dexamethasone suppression observed by Carroll et al. (1968) in a group of depressed patients, was shown to be correlated with the severity of the depressive illness as well as with the physiological symptoms (Carroll, 1972).

Studies by Carroll (1972) and Carroll and Mendels (1976) have indicated that although some depressed patients had initial suppression of HPA function following dexamethasone administration, this was followed by escape from suppression abnormally early. In addition, they have found that with increasing severity of depression, escape from suppression occurs earlier on the post dexamethasone day. On the basis of these results they have proposed that the abnormality previously called "nonsuppression" is really an early escape from suppression and that it is a graded phenomenon rather than an all-or-none event. Carroll and Mendels (1976) proposed that the above abnormalities seen in depressed patients, reflect the same basic disturbance of HPA function as described by Sachar et al. (1973b).

To summarize, Addison's disease, diencephalic Cushing's disease and primary depressive illness have been shown to share all the common features of mood disorder and disinhibition of the central HPA mechanisms. In addition, the limbic areas involved in HPA regulation (hippocampus, amygdala, septal region, midbrain) are closely associated

..... - 126 -/

with the limbic sites which regulate mood and emotions. Thus experimental evidence suggests that depressive states may be a result of suprahypophyseal brain dysfunction which amongst other things causes stimulation of the anterior pituitary.

1.2.9.2.2 Hypothalamic-pituitary-growth hormone system: Another important neuroendocrine system to be studied in affective disorders is the hypothalamic-pituitary-growth-hormone system. Various stimuli are able to increase the secretion of growth hormone (GH), including slow-wave sleep, L-DOPA, apomorphine, ACTH fragment 1-24 and amphetamine, but the most widely used clinical test procedure is insulin-induced hypoglycaemia.

Release of GH is thought to be regulated at least in part by the central noradrenergic system, since adrenergic blockers, such as phentolamine, reduce such insulin-induced release (Martin, 1976). Basal GH levels are normal in depressed patients and the GH response to slow-wave sleep, L-DOPA, apomorphine and ACTH fragment 1-24 has been shown to be within the normal range (Carroll and Mendels, 1976). However, in many depressed patients, the GH response to insulin-induced hypoglycaemia (Mueller et al., 1969; Sachar et al., 1971b; Carroll, 1972) and to amphetamine administration (Langer et al., 1976) is deficient. Garver et al. (1975; 1976) reported a correlation between diminished GH response and diminished urinary MHPG levels in some depressed patients.

This suggests that the depressives who show evidence of reduced noradrenaline turnover, also exhibit a functional deficit in noradrenaline neurotransmission. Amphetamine causes release of both noradrenaline and dopamine. However, since the GH responses to L-DOPA and apomorphine were shown to be unimpaired (Frazer, 1975), the low GH responses to amphetamine support the hypothesis of deficient central noradrenaline systems in a group of depressed patients (Garver, 1979).

The above findings are consistent with the catecholamine hypothesis of depression. However, serotonin (Bivens et al., 1973) as well as somatostatin (Besser, 1974) may also play a role in mediating the GH response.

1.2.9.2.3 Hypothalamic-pituitary-thyroid system:
Thyroid dysfunction is associated with changes in mood. Numerous investigators have reported a marked depression of mood in patients suffering from hypothyroidism (Whybrow et al., 1969, review; Whybrow and Ferrell, 1974; Whybrow and Hurwitz, 1976).

L-triiodothyronine (T_3) has been reported to potentiate the action of the TADs in women with primary depression (Prange et al., 1969; 1976). They have hypothesized that this may be due to a hormone-mediated increase in receptor site sensitivity (Section 1.2.11).

Thyrotropin-releasing hormone (TRH) is a tripeptide that controls the secretion of thyroid-stimulating hormone (TSH). In some studies TRH administration has been found effective in depressed patients (Kastin et al., 1972a; Prange et al., 1972), although other investigators have reported no anti-depressive effect (Takahashi et al., 1973a; Ehrensing et al., 1974; Gorden, 1975; Hollister et al., 1975a).

Itil (1976) has shown TRH to have CEEG (computer-analyzed electroencephalogram) profiles similar to the psychostimulant, d-amphetamine, which could account for its occasional anti-depressive effect.

Thus although there is no consensus regarding the efficacy of TRH in depression, it has been suggested that the TSH rise induced by TRH may be a diagnostic aid, since every study measuring the TSH response to TRH has found a lowered response in a subgroup of depressed patients (Kastin et al., 1972a; Prange et al., 1972; Takahashi et al., 1973a; Ehrensing et al., 1974; Itil et al., 1975). This has led to the suggestion that they may be a subgroup of depressed patients in whom TRH acts as a mood elevator. Prange (1975b) reviewed the patterns of pituitary responses to TRH in 12 studies involving 153 depressed patients and concluded that a diminished TSH response occurs in about 50% of the depressives. Moreover, this response deficit seems to be unrelated to the type and severity of the depressive disorder.

..... - 129 -/

At present, it is not clear why there is a diminished TSH response in depressed patients, although high cortisol levels have been implicated. In addition, it is not yet known whether the deficit in TSH response is related to the state of being depressed or to predisposition to a depressive disorder.

1.2.10 Sex Steroids:

Research has not yet succeeded in relating depression to any specific hormone. There is, however, evidence suggesting that the sex steroids may play a role.

Clinical depressions occur more frequently in women than in men. Published surveys show the sex ratios to be between 2:1 and 3:1 (Weissman and Paykel, 1974). The reason for the predominance of female depressives, however, need not be hormonal. Social factors could be important, for example, "the tendency in our culture for tearfulness and helplessness to be regarded as feminine manifestations that cannot be acknowledged by males" (Weissman and Paykel, 1974). Furthermore, it has been suggested that alcohol may be used by males in an attempt to alleviate depression, and as an alternative to psychiatric treatment (Winokur et al., 1970; 1971). The higher rate of alcoholism seen among men supports this suggestion.

Weissman and Paykel (1974) have cited additional evidence which suggests a link between sex hormones and depressive

..... - 130 -/

disorders. Minor feelings of depression are a common part of the premenstrual syndrome. Depression is also common in the first few days after childbirth (postpartum period) when major hormonal changes occur. Furthermore, depression also occurs in both men and women during the involutional period (menopause) which is also characterized by significant hormonal changes. Research by Rosenthal (1968) and Winokur (1973), however, has questioned whether there is, in fact, an increase in the occurrence of depressions around menopause. Daykin et al. (1966) have suggested that these depressions may be more related to psychological causes such as changes in social status, departure of children from home etc. Another piece of suggestive evidence is that oral contraceptives both cause and relieve depressive symptomatology (Section 1.2.12.2).

Sex steroids have variable effects on neurotransmitters. The administration of steroids of the oestrogen and nor-testosterone type utilized as contraceptives has been reported to modify the uptake and turnover of catecholamines in the hypothalamic median eminence (Fuxe et al., 1973). In addition, these drugs have a marked effect on central DA metabolism (Greengrass and Tonge, 1973; 1974; Jori and Dolfini, 1976). Endogenous ovarian steroids also influence the metabolism of monoamines in the brain (Stefano et al., 1965; Fuxe et al., 1973; Greengrass and Tonge, 1974; Wirz-Justice et al., 1974).

..... - 131 -/

Testosterone has been reported to exert catecholamine-like effects on brain function (Brovermann et al., 1968; Stenn et al., 1972). It has also been shown that testosterone directly inhibits MAO activity in plasma (Klaiber et al., 1967). Thus it has been speculated that testosterone might exert its catecholaminergic effect through an elevation of brain catecholamine levels as a result of MAO inhibition. Testosterone has been found to be effective in the treatment of depression, particularly in climateric depression in males (Bleuler, 1954; Klaiber et al., 1975). This anti-depressive effect could be explained by its MAO inhibitory effects. Sachar et al. (1973a) however, have reported that the mean plasma testosterone level of depressed male patients is not significantly changed after recovery from illness.

Itil et al. (1974) examined the CEEG profiles of patients receiving 2 sex steroids. Mesterolone, an androgen, was shown to resemble the psychostimulant, d-amphetamine in low doses, while in high and very high doses it had an effect similar to the "stimulant" TADs. The CEEG profile of patients receiving low doses of cyproterone acetate, an antiandrogen, was similar to the "sedative" TADs, whereas in high doses the profile was similar to that of an anxiolytic. On the basis of these results, mesterolone and cyproterone acetate were evaluated in a clinical trial. Mesterolone was shown to be dramatically effective in some male depressives while having no effect in others. The reason for this discrepancy in unknown. Itil et al. (1974)

..... - 132 -/

proposed that mesterolone's antidepressive effect could be related to the general MAO inhibitory effects of androgens. Low doses of cyproterone acetate were reported to be effective in male anxiety and high doses were found useful in the relief of the female premenstrual syndrome.

The above study is the most positive to date in which a role for sex steroids in affective illness has been implied. The study, however, was small and uncontrolled and therefore, double-blind controlled trials are needed to verify these results.

1.2.11 Adrenergic Receptor Abnormalities:

Altered neuronal transmission in depression may result not only from alteration in transmitter availability but also from alteration in postsynaptic receptor sensitivity. Recent research has offered some support for this concept of an altered receptor sensitivity in depressive disorders.

The chronic administration of TADs and MAO inhibitors or ECT has been shown to cause a reduction of noradrenaline-sensitive limbic cyclase activity (Vetulani and Sulser, 1975; Vetulani et al., 1975). Since this effect closely parallels the time course of the clinical antidepressive response, these authors proposed it to be the most relevant mechanism of action of the antidepressives. Moreover, iprindole, which neither inhibits the uptake of noradrenaline or serotonin nor inhibits MAO (Fann et al., 1972;

..... - 133 -/

Rosloff and Davis, 1974), also causes a similar reduction of limbic cyclase activity (Vetulani and Sulser, 1975). This pharmacological evidence suggests that depression might be accompanied by an increased receptor sensitivity to noradrenaline which could be corrected by the reduction of noradrenaline-sensitive limbic cyclase activity produced by antidepressive therapy.

Supersensitive noradrenergic receptors could be expected to cause feedback inhibition of noradrenaline synthesis, which might explain the diminished urinary MHPG levels observed in some depressed patients (Maas, 1968; Jones et al., 1973; Schildkraut et al., 1977). This hypothesis is thus not inconsistent with the reports which suggest a noradrenaline depression subtype (Maas, 1975; Garver, 1979).

In contrast, Ashcroft (1972) and Prange et al. (1972) suggest impaired sensitivity of the postsynaptic receptor in depressive disorders. This is supported by the finding that thyroid hormone (TH), which affects receptor sensitivity, enhances the antidepressive action of the tricyclics (Prange et al., 1969; 1970; Coppen et al., 1972a; Wheatley, 1972). In addition, a deficient GH response to clonidine (Matussek et al., 1977) is observed in some depressed patients, suggesting diminished α-adrenergic receptor sensitivity.

These conflicting reports emphasize the need for further study of receptor function in affective states.

..... - 134 -/

1.2.12 Vitamin Abnormalities:

1.2.12.1 Folate Deficiency:

Hunter et al. (1967) found a high incidence of low serum folate concentrations in patients suffering from various psychiatric syndromes. A large number of patients in this study, however, took alcohol and/or barbiturates to excess or were on anticonvulsant therapy, which could partly account for this result. Carney (1967) and Reynolds et al. (1970) also reported that a substantial proportion of patients suffering from affective disorders had low serum folic acid levels.

Although the relationship of folic acid deficiency to depression is not yet clear, a possible link with the biogenic amine theories of depression has been postulated (Reynolds et al., 1970). Since pteridine cofactor is necessary in the hydroxylation of tyrosine (Udenfriend, 1966) and tryptophan (Lovenberg et al., 1968), the proposed folic acid deficiency could interfere with this rate-limiting hydroxylation step in the synthesis of the catecholamines or serotonin.

1.2.12.2 Pyridoxine Deficiency:

The enzymes kynureninase and kynureninase aminotransferase, which convert 3-hydroxykynurenine to 3 hydroxyanthranilic acid and xanthurenic acid respectively, are pyridoxal-phosphate-dependent (Fig. 6). Pyridoxine deficiency results in an elevated urinary excretion of kynurenine,

3-hydroxykynurenine and xanthurenic acid, apparently because kynureninase is more sensitive to a lack of available co-enzymes (Ogasawara et al., 1962). Pyridoxine deficiency has been implicated in depression.

Clinically it has been known for a considerable time that oral contraceptive intake can lead to depression. A survey of the literature (British Medical Journal, 1969) showed the frequency of this side effect to vary between 2 and 30%. Nilsson and Sölvell (1967) conducted a controlled trial and reported that 12% of the test patients complained of tiredness and/or depression. Oral contraceptive administration has been shown to induce a state of pyridoxine deficiency (Salkeld et al., 1973) with a consequent increase in the urinary excretion of these abnormal tryptophan metabolites (kynurenine, 3-hydroxykynurenine and xanthurenic acid)(Brown et al., 1969; Rose, 1969; Briggs, 1976). It has been reported that the oestrogenic component of the oral contraceptive induces tryptophan pyrrolase which results in altered tryptophan metabolism (Rose, 1969; Salkeld et al., 1973; Sauberlick, 1975). According to the kynurenine hypothesis (Section 1.2.5) induction of tryptophan pyrrolase would decrease brain serotonin levels which, in turn, could cause depression. Another proposed mechanism is that pyridoxine deficiency in the brain would lead to low 5-HTP decarboxylase activity and thus to low brain serotonin and depression. In support of this, Nistico and Preziosi (1970) found that rats injected with

..... - 136 -/

an oral contraceptive for 10 days showed a significant fall of brain serotonin with high pyrrolase activity.

Pyridoxine supplements have been reported to restore the tryptophan metabolite levels to normal (Brown et al., 1969; Rose, 1969; Salkeld et al., 1973; Briggs, 1976). In addition, supplementary pyridoxine has been shown to relieve the depression in women taking oral contraceptives (Winston, 1973; Leeton, 1974).

The above findings thus indicate that pyridoxine deficiency may well contribute to the state of mental depression. However, the exact nature of the relationship is currently unknown.

1.2.13 Abnormal Glucose Utilization:

One of the earliest biochemical changes noted in depressive disorders was a decrease of glucose tolerance indicating an inhibition of glucose utilization (van Praag and Leijnse, 1965). Moreover, this change was reported to persist for some time after clinical recovery (Pryce, 1958).

Treatment of depressed patients with MAO inhibitors has been found to improve their glucose utilization. The fact that MAO inhibitors potentiate insulin's effects could account for this observation (Weil-Malherbe, 1970). How abnormal glucose utilization is linked to depression remains unknown however.

1.3 MELATONIN:

1.3.1 Introduction:

In 1958, Lerner and his co-workers isolated the melanophore-lightening principle from bovine pineals and identified its structure as N-acetyl-5-methoxytryptamine (melatonin). Melatonin is found in the pineal, brain and eye of amphibians (Quay, 1965a; Cardinali and Rosner, 1971). In mammals, this hormone is synthesized primarily in the pineal gland (Wurtman et al., 1968b), its synthesis undergoing diurnal variations in response to the photoperiod (Section 1.3.4).

1.3.2 Physiology and Pharmacology:

Melatonin is a highly lipophilic compound that is uncharged at physiological pH and which enters the brain with little difficulty.

Melatonin has a unique tissue distribution: most of the indole present at any time is located within its pineal organ. The amounts of melatonin present in the pineal are very small (0,5 to 7 ng in the rat pineal) and they fluctuate according to the photoperiod (Lynch, 1971).

There is indirect evidence that melatonin is actually secreted from the pineal since it is found in human plasma and urine and is also present in tissues that lack the enzymes necessary to synthesize it (Lerner et al., 1959). However, it is not yet known whether melatonin is secreted into the bloodstream or into the CSF. The electron

..... - 138 -/

microscopic and histological evidence is in favour of secretion into a rich capillary network that surrounds the pineal cells (Kappers, 1971). On the other hand, there is as good evidence suggesting secretion into the CSF. Anton-Tay and Wurtman (1969) showed that the indole concentrates in the brain a 100 times better if it is placed in the CSF instead of in the circulation. Thus, in determining the effects of exogenously-administered melatonin, the dose and route of administration are of particular importance since 100 μg of intravenously-administered melatonin would be equivalent to about 1 μg of the hormone introduced into the CSF.

If isotopically labelled melatonin is injected into the venous blood, the hormone enters all tissues, including the brain (Kopin et al., 1961; Wurtman et al., 1964b). If labelled melatonin is placed into the CSF, it is concentrated within several brain areas, especially by the hypothalamus and midbrain (Anton-Tay and Wurtman, 1969; Cardinali et al., 1973). Both brain regions are considered possible sites of action of melatonin. In a recent paper, Cohen et al. (1978), using specific binding techniques, presented evidence for the existence of a specific cytoplasmic melatonin receptor. Radioactive melatonin binding was detected in animal and human ovarian tissue as well as in the testis, uterus, skin, liver and eye of the rodent species. These binding areas correlated well with those already mentioned in the study by Wurtman et al. (1964b).

..... - 139 -/

Melatonin is detectable in rat and human plasma during the dark period of the day (Lynch and Wurtman, 1972). Most of the hormone in plasma (60-80%) is bound to serum albumin (Cardinali et al., 1972). Circulating radioactive melatonin disappears rapidly from the blood and is mostly inactivated by 6-hydroxylation within the liver, followed by conjugation and excretion as the sulphate or glucuronide (Kopin et al., 1960; 1961). Cardinali et al. (1973) showed that ^{3}H-melatonin taken up from the CSF also disappears very rapidly from the brain. They reported that its delay curve exhibits 2 major components, an early phase lasting 20 minutes (half-life about 5 minutes) and a slower phase lasting 20 to 180 minutes (half-life about 40 minutes). A major fraction of brain melatonin was shown to be metabolized to undefined water-soluble metabolites. In addition, Hirata et al. (1974) have shown that melatonin in the brain is also metabolized by an enzyme that cleaves the indole nucleus (Fig. 8).

1.3.3 Synthesis of Melatonin:

1.3.3.1 Biosynthetic Pathway:

The synthesis of melatonin from tryptophan is shown in Fig. 8.

The biosynthesis of melatonin is initiated by the uptake of the amino acid tryptophan from the circulating blood. Once within the pineal, some of the tryptophan is utilized for the synthesis of pineal proteins, while a larger fraction

..... - 140 -/

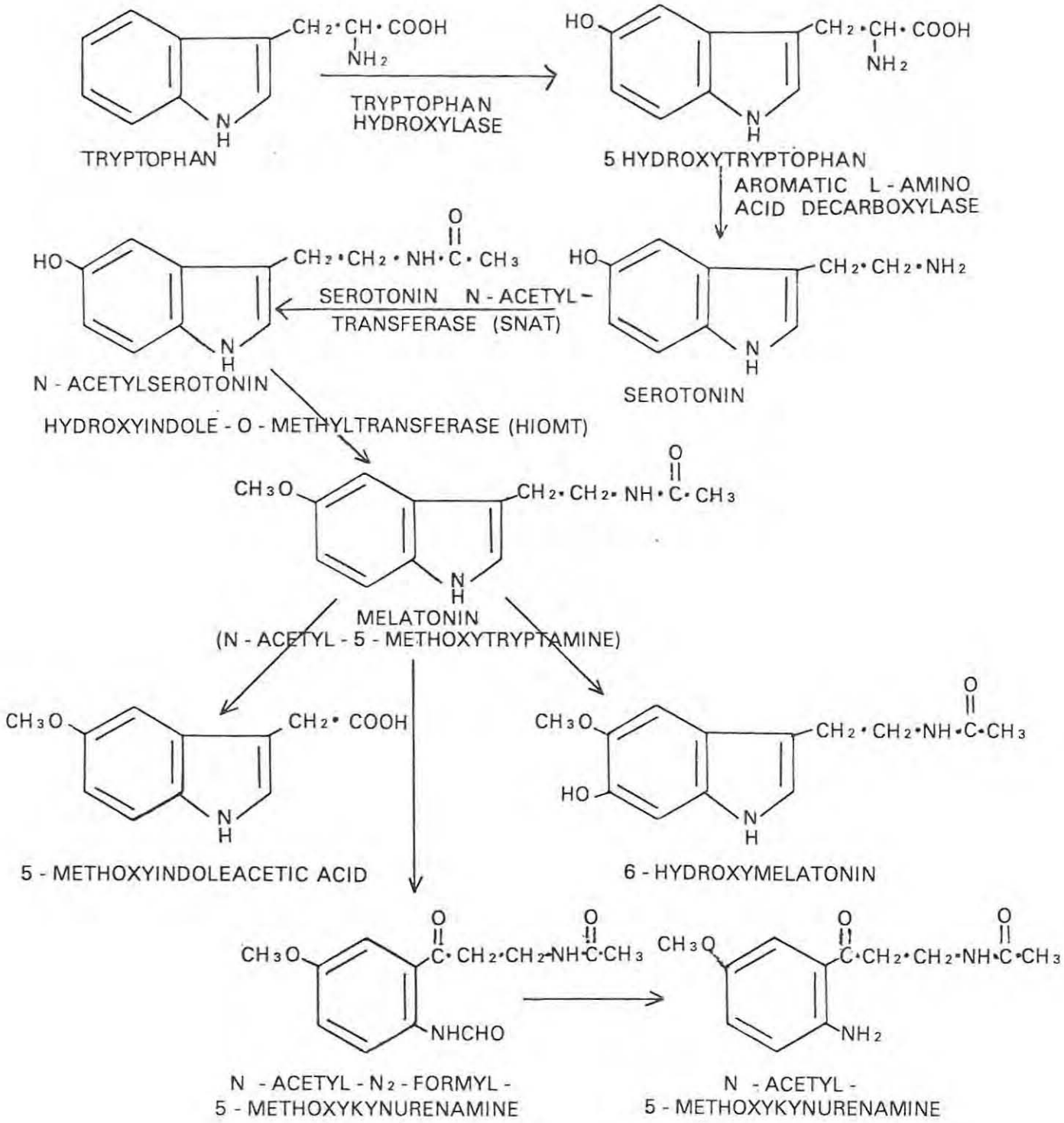


FIG. 8: THE SYNTHESIS AND METABOLISM OF MELATONIN

is converted to serotonin (Wurtman et al., 1968c).

The first step in melatonin's biosynthesis is hydroxylation which is catalysed by the enzyme, tryptophan hydroxylase (Lovenberg et al., 1967), and requires the presence of oxygen, ferrous iron and a reduced pteridine cofactor (Lovenberg et al., 1968). Next, 5-HTP is decarboxylated to form serotonin, which is found in very high concentrations in the pineal (Giarman and Day, 1959). This step is pyridoxal-phosphate-dependent. Part of the serotonin is oxidized to 5-HIAA by MAO and part is converted into melatonin (Fig. 8).

In mammals serotonin-N-acetyltransferase (SNAT) activity is found in many organs besides the pineal, whereas hydroxyindole-0-methyltransferase (HIOMT) activity has been reported to be confined to the pineal gland (Axelrod and Weissbach, 1961; Axelrod et al., 1961a). the retina (Cardinali and Rosner, 1971) and the harderian gland (Vlahakes and Wurtman, 1972). However, it remains to be demonstrated that the mammalian retina or harderian gland actually synthesizes melatonin in vivo or that such melatonin is released. In a recent paper, however, Raikhlin and Kvetnoy (1976) presented evidence suggesting that melatonin is synthesized in the enterochromaffine cells of the gastro-intestinal tract.

..... - 142 -/

1.3.3.2 Neural Control:

Exposure of rats to constant illumination has been shown to reduce the activity of pineal HIOMT (Wurtman et al., 1963), SNAT (Klein and Weller, 1970) and melatonin content in the pineal (Ralph et al., 1971). Making a number of discrete neural transections in various brain areas of the rat, Wurtman et al. (1968b) established the pathway by which light reaches the pineal:retina → inferior accessory optic tract → medial forebrain bundle → preganglionic sympathetic nerves → superior cervical ganglion → postganglionic sympathetic nerves → pineal gland (Fig. 9).

Noradrenaline (Axelrod et al., 1969) or cAMP (Shein and Wurtman, 1970) added to rat pineal gland cultures stimulated melatonin synthesis from ^{14}C-labelled tryptophan and enhanced the cellular uptake of labelled tryptophan. This stimulation of melatonin synthesis was shown to be blocked by propranolol but not by phenoxybenzamine (Wurtman et al., 1971). In addition, adenyl cyclase activity (Weiss and Costa, 1968) and cAMP content (Strada et al., 1972) of the rat pineal were increased by noradrenaline.

Based on these results, it has been proposed that in response to information received from the retina, the sympathetic nerves release noradrenaline, which then acts at β-adrenergic receptor sites in the pineal to activate the adenyl cyclase system which, in turn, triggers HIOMT activity and melatonin synthesis.

..... - 143 -/

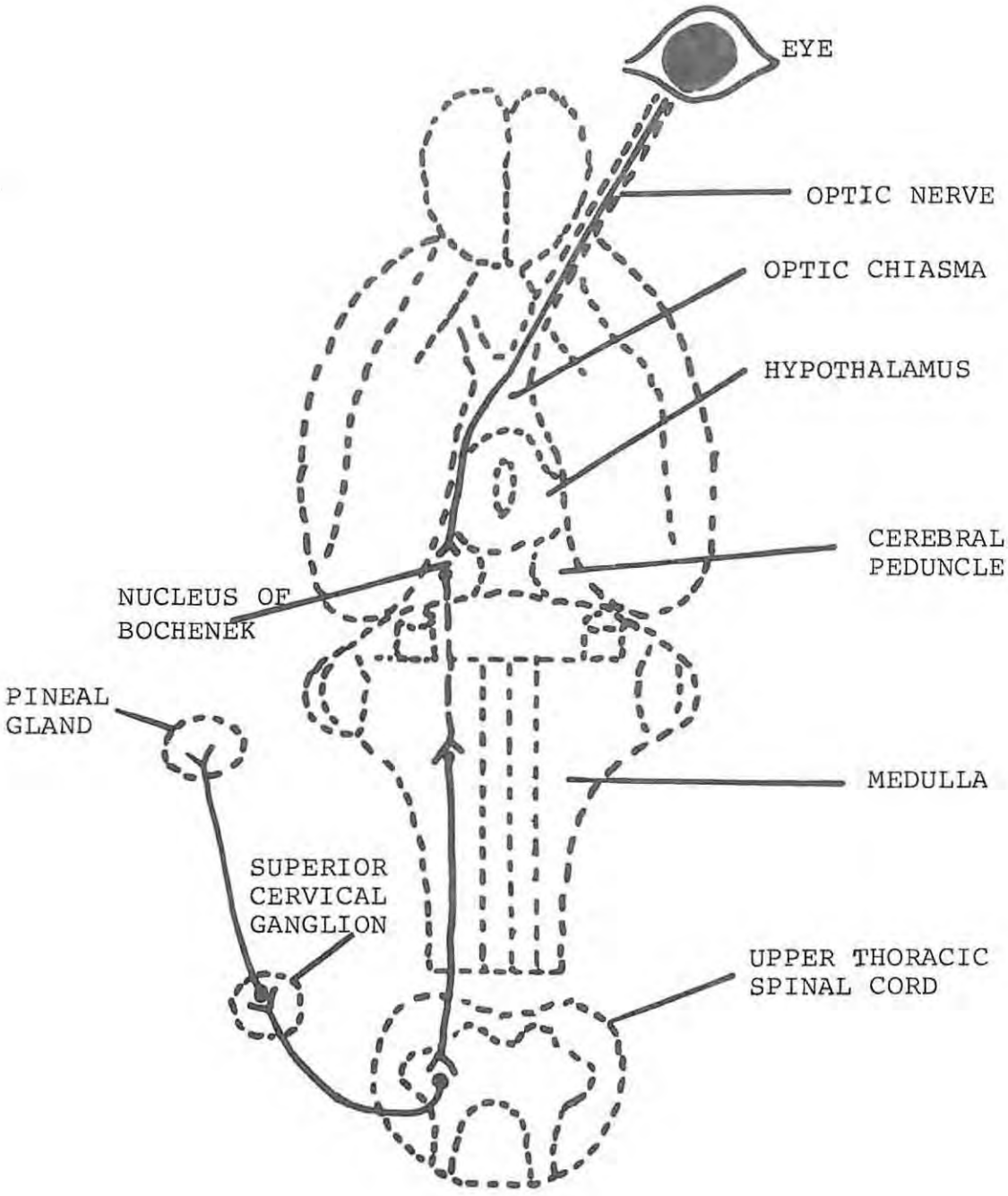


Fig. 9. Neuro-optic Pathway Connecting the Pineal Gland to the Eye in the Rat (Wurtman et al., 1968b).

..... - 144 -/

However, there is the possibility that the neural signal is generated by factors other than light, since the daily rhythms in pineal HIOMT and SNAT activity as well as melatonin content persist in continuous darkness (Section 1.3.4).

1.3.3.3 Factors Affecting Melatonin Synthesis:
Not only environmental lighting affects the pineal. Melatonin synthesis has been shown to be activated by stress and hypoglycaemia (Lynch et al., 1973a; Sampson, 1975). Lynch et al. (1973a) showed that animals immobilized for several hours or made hypoglycaemic by the administration of insulin, exhibit a marked elevation of pineal melatonin as well as increased SNAT activity even while maintained in a environment of continuous illumination.

1.3.3.3.1 Drug Effects:
Since melatonin formation is stimulated by the release of noradrenaline from the sympathetic nerves innervating the pineal, it is conceivable that catecholaminergic drugs and hormones that influence the release of noradrenaline will affect melatonin synthesis.

Several investigators have shown that the rate at which cultured rat pineals synthesize ^{14}C-melatonin from labelled ^{14}C-tryptophan is increased by catecholaminergic drugs, such as d-noradrenaline, l-epinephrine, dopamine, tyramine, octopamine, tryptamine (Axelrod et al., 1969). The MAO

inhibitor, pheniprazine (Axelrod et al., 1969) and harmine (Klein and Rowe, 1970) have also been reported to increase melatonin synthesis.

A variety of psychotomimetic agents have also been shown to increase the production of melatonin. These include dimethyltryptamine, bufotenine, mescaline, lysergic acid diethylamide (LSD) and dimethoxyphenylethylamine (Hartley and Smith, 1973) as well as amphetamine (Backstrom and Wetterberg, 1973) and cocaine (Holtz et al., 1974). On the other hand, neuroleptics have been found to decrease this synthesis (Hartley et al., 1972). A possible relationship between these drug effects, melatonin and schizophrenia is discussed in Section 1.3.8.3.1.

In rats, L-DOPA administration increased the activity of pineal SNAT and HIOMT and caused a rapid acceleration of the synthesis and secretion of melatonin (Lynch et al., 1973b). The mechanism by which L-DOPA acts probably involves either the release of noradrenaline from the pineal sympathetic nerve terminals or its conversion to DA which acts directly on the pineal parenchymal cells to stimulate melatonin synthesis (Minneman and Wurtman, 1976).

Numerous compounds have been shown to affect pineal HIOMT and SNAT activity, thereby indirectly influencing melatonin production. These are reviewed by Minneman and Wurtman, (1976).

..... - 146 -/

1.3.3.3.2 Effects of Sex Hormones:

Although there is much literature on the effect of melatonin on gonadal function, little is known about the mechanism that relates gonadal function to changes in pineal melatonin levels.

Wurtman et al. (1965) showed that pineal HIOMT activity can be correlated with stages of the rat oestrous cycle. Enzyme activity was found to be twice as great in meta-oestrous and di-oestrous than during pro-oestrous or oestrous. Weiss and Crayton (1970a; 1970b) demonstrated that sex hormones influence the response of pineal adenyl cyclase to catecholamines. Noradrenaline stimulated pineal adenyl cyclase activity to a greater extent in male than in female rats. Ovariectomy increased the noradrenaline-induced activation of adenyl cyclase whereas oestrogen treatment had the opposite effect. Testosterone and progesterone were without effect in this system. The authors therefore suggested that the reciprocal relationship that exists between the pineal and ovary involves the adenyl cyclase-cAMP system.

Pineal HIOMT activity has also been shown to be affected by gonadal steroids. Oestrogen has been reported to both increase (Cardinali et al., 1974a) and decrease (Nagle et al., 1972a) this activity in rats. Moreover, progesterone decreased HIOMT activity (Houssay and Barcelo, 1972), whereas testosterone administration produced a dose-dependent

..... - 147 -/

variation in enzyme activity (Nagle et al., 1974). Cardinali et al. (1975a) reported that superior cervical ganglionectomy decreases the pineal uptake of both oestradiol and testosterone. It was thus suggested that sympathetic transmission plays a role in the effects of these hormones on the pineal.

Further evidence suggesting that the pineal gland is a target organ for sex hormones, is the observation that labelled ^{3}H-oestradiol concentrates within the rat pineal following intraperitoneal injection (Neuspiller et al., 1972). In a study conducted by Rosner et al. (1971), pineal ^{3}H-oestradiol concentrations were shown to be twice those found in the uterus. The above observations suggest that an oestradiol "receptor" may be present in the pinealocytes.

1.3.4 Diurnal Rhythms in the Pineal:

The activity of rat pineal SNAT undergoes a 24-hour rhythm which is in phase with changes in HIOMT activity, noradrenaline and melatonin secretion but is 180° out of phase with the pineal serotonin rhythm. Its activity is more than 15 times higher at night than during the day (Klein and Weller, 1970). The rhythm persists in complete darkness or in blinded animals (Klein and Weller, 1970) but can be suppressed by maintaining the animals in constant light (Klein and Weller, 1970), removing the superior cervical ganglion which sends nerve fibres to the pineal, or deafferentation of this ganglion (Klein et al., 1971). Pineal SNAT rhythmicity thus

appears to be endogenous, although dependent on the sympathetic neural input and subject to modification by external lighting conditions.

Pineal serotonin content also shows diurnal changes with highest levels during the daylight (peak at midday) and falling rapidly with the onset of darkness (Quay, 1963; Snyder et al., 1965). Like the SNAT rhythm, the serotonin rhythm is endogenous although cued by environmental lighting conditions (Synder et al., 1965; 1967).

HIOMT activity shows a diurnal rhythm similar to SNAT activity (Wurtman et al., 1964a; Axelrod et al., 1965) but its variability is much less pronounced and is, therefore, not thought to have a major rate-limiting role in melatonin synthesis. This rhythm also persists when animals are placed in constant darkness (Nagle et al., 1972b).

Pineal noradrenaline also has a diurnal rhythm corresponding to the photoperiod (Wurtman et al., 1967). This rhythm is 180° out of phase with serotonin and in contrast to serotonin, is not endogenous, since it is readily abolished by continuous light or darkness.

A circadian rhythm in rat pineal melatonin has also been observed. During the day melatonin levels are low but at night, values 7 to 10 times the day levels are reached (Quay, 1964; Lynch, 1971).

..... - 149 -/

Persistence of this rhythm in constant darkness (Ralph et al., 1971) suggests that pineal melatonin production is influenced by extrapineal circadian oscillators which, in turn, appear to influence pineal responsiveness to alterations in environmental light. Melatonin concentration in the blood has been shown to follow a similar rhythmic pattern in chickens (Pang et al., 1974; Pelham, 1975), sheep (Rollang and Niswender, 1976) calves (Hedlund et al., 1977) and humans (Vaughan et al., 1976). In addition, Vaughan et al. (1976) showed that 5-HIAA excretion followed a cycle similar to that of blood melatonin. Comparable diurnal changes in urinary melatonin have been demonstrated in man (Lynch et al., 1975). Jimerson et al. (1977) measured the urinary melatonin rhythms in depressed and normal patients and found similar diurnal rhythms. Moreover, following 1 night's sleep deprivation, the diurnal rhythm in melatonin excretion remained unchanged. Similarly, Vaughan et al. (1976) observed a continuation of the melatonin rhythm for $2\frac{1}{2}$ days following the onset of constant light. This suggests that neither sleep nor darkness governs the rhythm of melatonin excretion. These findings are inconsistent with previous studies in rats showing rapid light-induced suppression of the nocturnal rise in pineal melatonin synthesis (Wurtman et al., 1963; Axelrod et al., 1965). However, in a recent study using rats, Lynch (1977) reported that 1 night's exposure to continuous light failed to decrease the nocturnal rise in urinary melatonin excretion, in agreement with the human data.

..... - 150 -/

Based on experimental evidence, Klein and Weller (1970) put forward the following hypothesis to explain the diurnal rhythm of the pineal compounds. The initial event is a dark-induced release of noradrenaline from the sympathetic nerve endings. Noradrenaline stimulates adenyl cyclase in the pinealocytes, resulting in an increase in cAMP production which, in turn, stimulates SNAT (Section 1.3.3.2). A rapid rise in SNAT at night produces a decrease in serotonin concentration and results in elevated levels of N-acetyl-serotonin and melatonin. It was suggested that pineal serotonin probably decreases because serotonin production cannot keep pace with the increased rate of acetylation. Thus, apparently it is the cycles in the activity of SNAT which are responsible for the cycles observed in serotonin, N-acetylserotonin and melatonin.

There has been a suggestion that melatonin itself may contribute to the maintenance of rhythmic changes in the pineal (Fiske and Huppert, 1968). These authors found that when melatonin was administered in the middle of the light period, the characteristic diurnal changes in serotonin were suppressed, whereas melatonin injected at the beginning of the dark period had no significant effect.

Circadian rhythms have been observed in such functions as body temperature, adrenocortical secretion, sleep, motor activity and eating behaviour. It has been proposed that the mammalian pineal may function as a "biological clock" which

..... - 151 -/

delivers time signals generated by environmental lighting conditions to centres in the brain that mediate other biological rhythms (Wurtman and Axelrod, 1965). Research, at present, is directed towards discovering which organs "watch the pineal clock".

1.3.5 Endocrine Effects:

1.3.5.1 Pituitary Gland and Gonadotropin Secretion:

The reports of investigations on the effects of melatonin administration on blood and pituitary levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are conflicting (Table 13).

In an early study, Adams et al. (1965) reported an increase in the pituitary LH content following the administration of melatonin to immature rats which suggested LH suppression. Other investigators, however, have failed to detect any change in pituitary LH levels (Debeljuk, 1969; Debeljuk et al., 1970; Roche et al., 1970). Subsequent research demonstrated that the implantation of melatonin into selective brain areas, such as the median eminence and the reticular formation of the brain stem, suppressed the post-castration rise in pituitary LH content (Fraschini et al., 1968; 1971). Likewise the castration-induced rise in serum LH was suppressed by melatonin in some studies (Roche et al., 1970; Fraschini et al., 1971) but not in others (Talbot and Reiter, 1973).

..... - 152 -/

TABLE 13:

THE EFFECT OF MELATONIN ON LH AND FSH MEASUREMENTS

(Modified from Reiter et al., 1975a)

SPECIES	SEX	AGE	DOSE	ROUTE	MODEL	LH		FSH		REFERENCES
						PITUITARY	BLOOD	PITUITARY	BLOOD	
Rat	F	21-35 day old	100µg/ day	S.C.	Prepubertal Daily injection	↓ (blocked pubertal drop)	-	-	-	Adams et al., 1965
Rat	M	25-44 day old	100-500µg/ day	S.C.	Immature Constant light Daily injection	No effect	-	No effect	-	Debeljuk, 1969
Rat	M	21-25 day old	300µg/ day	Systemic	Immature Daily injection	No effect	-	↓	-	Debeljuk et al., 1970
Sheep	F	Adult	2000µg/ day	I.V.	Ovariectomy then daily injection day 15-30	No effect	↓	-	-	Roche et al., 1970
Rat	M	Adult	1mg/week beeswax implants	S.C.	Castrated	No effect	No effect	No effect	No effect	Talbot and Reiter, 1973
Rat	F	30-58 day old	10µg/ day	I.P.	Immature Daily injection	-	-	-	↓	Sorrentino, 1968
Rat	F	Adult	30µg/day	I.P.	Adult, UO Daily injection day 0-18	-	-	-	↓	
Rat	F	Immature	1500µg/ day	I.P.	Immature, Pro-oestrous PMS induced ovulation	-	↓ovulatory surge	-	-	Reiter and Sorrentino, 1971
Rat	F	Adult	1250-5000 µg	Intra-cardiac	Pro-oestrous cyclic ovulation	-	↓ovulatory surge	-	-	Ying and Fiske, 1972
Rat	M	Adult	Small amount in cocoa butter	Pituitary	Castrated	No effect	-	-	-	Fraschini et al., 1968; 1971
Rat	M	Adult	Small amount in cocoa butter	Median eminence	Castrated	↓	↓	No effect	-	
Rat	M	Adult	Small amount in cocoa butter	Reticular formation		↓	↓	-	-	
Rat	M	Adult	1-50µg	III Ventricle	Acute injection	-	↓	↓	↓	Kamberi et al., 1971

F, female; FSH, follicle-stimulating hormone; I.P., intraperitoneal; I.V., intravenous; LH, luteinizing hormone; M, male; PMS, pregnant mare's serum; UO, unilateral ovariectomy.

- comparative figures not available.

Melatonin administration has also been reported to decrease pituitary mass among untreated (Narang et al., 1967; Vaughan et al., 1971), light-exposed (Debeljuk, 1969) and castrated rats (Debeljuk et al., 1970). DeProspo and Hurley (1971a), however, did not observe any effect on pituitary mass.

In addition, melatonin administration has been shown to block the pre-ovulatory surge in serum LH (Reiter and Sorrentino, 1971; Ying and Fiske, 1972) as well as decrease serum FSH (Sorrentino, 1968; Kamberi et al., 1971).

A possible mechanism explaining the above effects has been proposed by Moszkowska et al. (1973). These investigators found that microinjections of melatonin into the rat lateral ventricle resulted in a significant decrease in hypothalamic releasing factor (RF) levels, particularly FSH-RF content. It was, therefore, suggested that melatonin may act by decreasing the synthesis or release of the gonadotropin RFs in the hypothalamus. This hypothesis was supported by the studies of Kamberi et al. (1970) who showed that melatonin injected directly into the pituitary gland had no effect on serum LH levels. In contrast, the more recent reports by Martin and co-workers (Martin and Klein, 1976; Martin et al., 1977) have demonstrated that melatonin can act directly on the neonatal pituitary gland and suppress the response to LH releasing hormone (LH-RH) which, in turn, suppresses pituitary LH release. It is possible, however, that

..... - 154 -/

melatonin acts both on the hypothalamus to inhibit LH-RH secretion and on the pituitary to suppress the stimulatory effect of the hypothalamic hormone on LH release.

1.3.5.2 Gonads:

Melatonin's antigonadal effects have been well-reported but are not the subject of this study. For excellent reviews, the reader is referred to Reiter et al. (1975a) and Minneman and Wurtman (1976).

1.3.5.3 Adrenal Glands:

1.3.5.3.1 Glucocorticoids:

Studies investigating the effect of melatonin administration on ACTH secretion and corticosterone levels are conflicting. Following reports on the inhibitory effect of pineal material on adrenal function (Kinson and Singer, 1967a; 1967b), Gromova et al. (1967a; 1967b) found that corticosterone production increased significantly after melatonin treatment (200 µg/100 g BW). This effect disappeared following hypophysectomy which suggested that melatonin has a stimulatory effect on pituitary ACTH. Fraschini et al. (1968), however, observed decreased adrenal mass following intrahypothalamic implantation of melatonin, whilst Barchas et al. (1969) reported no alteration in corticosterone or ACTH blood levels after either acute or chronic administration of melatonin.

In order to increase the amount of melatonin gaining access

to the neuroendocrine axis, De Prospo and Hurley (1971a) injected melatonin (100 µg/day) into the lateral cerebral ventricles of female rats for 10 days. Judging from the normal adrenal mass observed in the melatonin-treated animals, this treatment also failed to depress the pituitary-adrenal axis.

Despite reports to the contrary, Motta et al. (1971) demonstrated that an acute intraventricular injection of melatonin caused a marked reduction of plasma cortisol levels in normal male rats. Further experiments indicated that melatonin also suppresses stress-induced corticosterone production. However, following reserpine pretreatment, the corticosterone response was unaffected by melatonin treatment. These results support the proposal that ACTH is under inhibitory control by a central adrenergic mechanism (Giuliani et al., 1966) and suggest that melatonin might act via this inhibitory mechanism. A central action of melatonin is supported by the in vitro evidence that, when the hormone is added to pituitary incubates, it does not change ACTH secretion (Jouan and Samperez, 1965). Melatonin has also been shown to alter the synthesis of corticoids in incubated adrenal slices (Giordano et al., 1967; 1970).

In a recent paper, Ogle and Kitay (1977) demonstrated that daily intraperitoneal administration of melatonin (2 x 50 µg/day) for 1 week stimulated adrenal 5 α-reductase activity

..... - 156 -/

in ovariectomized and hypophysectomized rats. This finding suggests that melatonin may play a role in the physiological regulation of adrenal steroidogenesis.

1.3.5.3.2 Aldosterone:

Although numerous investigators have studied the influence of the pineal and pinealectomy on aldosterone production (Reiter and Fraschini, 1969, review; Reiter et al., 1975a; review), only 1 report concerning melatonin's effect on this variable is presently available. Gromova et al. (1967a; 1967b) reported that acute administration of melatonin significantly reduced aldosterone production, while hypophysectomy abolished this effect.

1.3.5.4 Thyroid Gland:

Melatonin administration has been reported to inhibit thyroid function though the results are far from uniform. Baschieri et al. (1963) noted a highly significant depression of ^{131}I uptake by the thyroid after daily subcutaneous injections of melatonin. This finding was confirmed by Reiter et al. (1965). Baschieri et al. (1963) also demonstrated that melatonin blocked the thyroid hyperplasia caused by methylthiouracil. These authors concluded that melatonin might interfere with either the release or production of TSH. De Prospo et al. (1968; 1969) also reported depression of ^{131}I uptake following melatonin administration which they found unrelated to the inhibitory effect of melatonin on gonadal secretion. In contrast, Thiéblot et al. (1966) showed that administration of melatonin (100 µg/day) led to gland changes normally associated with increased activity, whilst Naber et al. (1969) found melatonin to have neither stimulatory nor depressant effects on ^{131}I uptake into the thyroid.

..... - 157 -/

The TH secretion rate in rats was also shown to be inhibited by chronic melatonin administration (Narang et al., 1967). The animals, however, became progressively less sensitive to melatonin with advancing age.

Several investigators have attempted to identify the site of action of melatonin on the hypothalamic-pituitary-thyroid axis (De Prospo and Hurley, 1971b; Panda and Turner, 1968). Panda and Turner (1968) observed that daily administration of melatonin (100 µg/day) to rats for 10 days, increased their TSH levels which was associated with an increase in the DNA content of the thyroid. As a result of their findings, the authors concluded that melatonin acts directly on the thyroid to inhibit either the synthesis or release of TH.

1.3.5.5 Growth Hormone:

Smythe and Lazarus (1973) showed that melatonin administration blocked the GH response to serotonin while 5-HTP administration enhanced it. This action could explain melatonin's well-known inhibitory effect on growth. In a subsequent study (1974a), these authors demonstrated a reduced GH response to insulin-induced hypoglycaemia in 10 subjects following oral administration of melatonin. Initially, however, a significant elevation of serum GH levels was observed.

This increase in serum GH levels was again demonstrated in a later experiment (Smythe and Lazarus, 1974b). On the basis of these results, the authors suggested that the

..... - 158 -/

ability of melatonin to increase serum GH in man is due to interaction with serotonin receptor sites in the hypothalamus which, in turn, mediates the release of GH from the pituitary. They proposed that once melatonin had occupied the serotonin receptors and had displaced any serotonin present, it acted as a competitive inhibitor of serotonin and consequently blocked GH stimulation via serotoninergic pathways.

The ability of melatonin to block serotonin effects has also been demonstrated *in vitro* in several types of smooth muscle (Rahamimoff *et al.*, 1965; Quastel and Rahamimoff, 1965).

1.3.5.6 Melanocyte-Stimulating Hormone and Melanocyte-Stimulating Hormone Release-Inhibiting Factor:

The significance of melanocyte-stimulating hormone (MSH) in mammals has been reviewed in detail by Thody (1977). The possible relationship between MSH, MSH release-inhibiting factor (MIF) and melatonin is briefly discussed here.

In an early study, Kastin and Schally (1967) reported that melatonin administration decreased the MSH content of the rat pituitary gland. They suggested that the release of MSH from rat pituitaries is stimulated by an increased concentration of circulating melatonin and inhibited by raised levels of circulating MSH. The exact mechanism by which melatonin stimulates MSH secretion was not known, although they proposed that the hormone might act by inhibiting MIF or by direct action on the pituitary. A

..... - 159 -/

possible interaction between the pineal gland, hypothalamus and pituitary involving melatonin, MSH and MIF is suggested in a later paper by Kastin et al. (1972b).

Tilders and Smelik (1975), however, found no changes in MSH content of the rat pituitary gland following melatonin administration. Furthermore, melatonin had no effect on the diurnal variation of pituitary MSH levels (Tilders, 1973) and it was therefore concluded that this rhythm as well as resting MSH levels was not controlled by melatonin (Tilders and Smelik, 1975).

1.3.5.6.1 Effect on Mood:

In animals MSH administration produces an exaggerated response to noxious stimuli (Kastin et al., 1973; Stratton et al., 1973) and in man, produces feelings of nervousness, anxiety and motor restlessness (Kastin et al., 1968). Since melatonin and MSH have been shown to exert opposing actions on frog skin colour (Kastin and Ross, 1965; Kastin and Schally, 1966), rat pituitary MSH release (Kastin and Schally, 1967) rat thyroid (Baschieri et al., 1963; Bowers et al., 1964) and melanocyte-lightening activity in the rat (Kastin et al., 1972), it is possible that melatonin may have an antianxiety or antidepressive effect on mood (Section 1.3.10).

Further support for this suggestion comes from studies investigating the antidepressive effect of MIF; MIF is a peptide, isolated from hypothalamic tissue, which antagonizes

..... - 160 -/

the action of MSH by inhibiting the latter's release from the pituitary (Celis et al., 1971; Kastin et al., 1971). This peptide has been reported to be effective in the DOPA response potentiation test (Plotnikoff et al., 1971), a test for screening possible antidepressives (Everett, 1966). In another animal model of depression, oral administration of the peptide reversed the sedative effects of deserpidine in mice and monkeys (Plotnikoff et al., 1973). In a double-blind study, Ehrensing and Kastin (1974) investigated the efficacy of MIF in female depressives and reported MIF to be more effective than placebo in 4 of the 5 patients. Itil (1976) using CEEG profiles reported that MIF exhibits bimodal effects, in low dosages CNS depression and in high doses CNS stimulation. This response, however, was subject to biological variability, subjects with α-background activity showed a marked response to MIF, whereas those with a β-pattern showed no significant response.

1.3.5.7 Prolactin:

Melatonin administration has been shown to increase the concentration of prolactin in the blood. Following an injection of melatonin into the rat cerebral ventricles, Kamberi et al. (1971) reported increased prolactin release into the circulation. Since this effect was not obtained when melatonin was injected directly into the anterior pituitary, these investigators suggested that melatonin might block the release of prolactin inhibiting factor (dopamine) from the hypothalamus, which, in turn, would

explain the observed elevation in plasma prolactin levels. This finding has been confirmed by Lu and Meites (1973).

1.3.5.8 Glucose Metabolism:

Pinealectomy creates a paradiabetic state with low glucose tolerance, hyperglycaemia and a rise in insulin levels, whilst administration of pineal extracts leads to increased glucose tolerance and hypoglycaemia (Milcu et al., 1971, review). It has been suggested that these effects can be explained either by direct action on the pancreas, possibly mediated via serotoninergic mechanisms, or by indirect action involving altered adrenocorticosteroid production. Thus, although the pineal gland clearly plays a role in glucose metabolism, the function of melatonin is less clear.

1.3.6 Central Metabolic Effects:

Melatonin administration has been shown to influence the metabolism of various central neurotransmitters.

1.3.6.1 Serotonin:

Anton-Tay et al. (1968) reported that melatonin (500 µg) administered intraperitoneally to rats caused an increase in brain serotonin content, especially in the midbrain and hypothalamus, 1 hour after administration. These were the sites in the brain at which circulating ^{3}H-melatonin was shown to be most concentrated (Section 1.3.2). Piezzi and Wurtman (1970) showed that melatonin administration (1 mg/kg

I.P. per day) for 5 days resulted in a significant increase in the serotonin concentration of the pars intermedia without influencing the concentration of serotonin in the other areas of the pituitary. Clinical studies have indicated increased 5-HIAA levels in the urine of Parkinsonian patients (Anton-Tay, 1974) as also in the CSF of depressed patients (Carmen et al., 1976) following the administration of melatonin, which again suggests increased serotoninergic activity. Additional effects of melatonin administration on serotonin and catecholamine release and uptake are discussed in Section 1.3.6.4.

1.3.6.2 GABA:

Intraperitoneal injection of melatonin (50 µg/kg) increased the hypothalamic concentration of GABA twofold, as well as significantly increasing its concentration in the rat cerebral cortex 2 hours after administration (Anton-Tay, 1971).

1.3.6.3 Pyridoxal Phosphokinase:

Pyridoxal phosphokinase catalyzes the formation of pyridoxal phosphate in the brain (McCormick and Snell, 1959). Pyridoxal phosphate is the prosthetic group of the enzyme, aromatic L-amino acid decarboxylase, and this is essential for the synthesis of DA, serotonin and GABA (Roberts et al., 1958; Lovenberg et al., 1962).

Anton-Tay et al. (1970) reported that intraperitoneal injections of melatonin (0,25 to 4 mg/kg) gave rise to a rapid increase

..... - 163 -/

in the activity of rat brain pyridoxal phosphokinase, 90 minutes after administration. This effect was dose-related. Following a single dose of melatonin (200 µg/kg) a good correlation between the changes in pyridoxal kinase activity and serotonin levels was noted. The above data led Anton-Tay et al. (1970) to propose that the biochemical effects of melatonin administration (i.e. increased serotonin and GABA brain levels) might be related to its effect on pyridoxal phosphokinase. Since melatonin had no effect on pyridoxal phosphokinase activity in vitro, the authors suggested that melatonin enhanced the enzyme's activity in vivo by increasing its de novo synthesis. This is supported by the finding that 2 mg/kg of actinomycin was able to block the effects of melatonin (Anton-Tay, 1971).

1.3.6.4 Catecholamines:

Cotzias et al. (1971a) found no significant alteration in brain DA content of mice following an intraperitoneal injection of melatonin (400 mg/kg). Previously Anton-Tay et al. (1968) had reported no changes in noradrenaline levels in brain tissue after melatonin administration. Wendel et al. (1974), however, dosed rats with either an intraarterial or intracisternal injection of melatonin and found significant increases in DA and noradrenaline levels in the rat brain. An intraarterial injection of melatonin (250 µg/g) nearly doubled the DA content and significantly increased the noradrenaline concentration 1 hour following administration. Melatonin injected intracisternally to circumvent the blood brain

..... - 164 -/

barrier, also caused significant increases in both DA and noradrenaline levels. The elevated levels were noted within 15 minutes after hormone administration, rose to a maximum between 30 minutes and 1 hour and returned to control levels approximately 2 hours later.

These authors suggested that the failure of the previous workers to demonstrate an effect of melatonin on catecholamine levels may have been due to its rapid conversion to 6-hydroxy-melatonin following intraperitoneal administration.

In addition, melatonin has been shown to non-competitively inhibit the uptake of noradrenaline, serotonin, DA and glutamate into synaptosome-rich homogenates of the rat hypothalamus (Cardinali et al., 1975b). This effect was dose-dependent. Noradrenaline, serotonin, DA and glutamate accumulation fell by 43, 69, 48 and 32% respectively following administration of 0,5 mM melatonin and by 60, 82, 66 and 55% respectively following 1,0 mM melatonin. Transmitter release evoked by increasing $[K^+]$ to 30 mM was also augmented by melatonin in a dose-dependent manner. Noradrenaline, serotonin, DA and glutamate release was increased by 9, 25, 12 and 4% (0,5 mM) and by 22, 34, 19 and 11% (1,0 mM), respectively. The results suggested that melatonin interferes more easily with transmitter uptake and release in serotonin- than in catecholamine-containing neurons. This proposal was supported in a subsequent report by Cardinali (1975) who showed that melatonin-treated rats exhibited a depressed hypothalamic

..... - 165 -/

serotonin uptake whilst the uptake and accumulation of noradrenaline, DA and glutamate was unaffected.

1.3.6.5 Protein Synthesis:

Melatonin administration has been reported to decrease protein synthesis (Orsi et al., 1973; Cardinali et al., 1974b) and microtubule protein content (Cardinali and Freire, 1975). Brain microtubule protein (Thoa et al., 1972) or actin-like protein (Nicklas et al., 1973; 1974) has been implicated in the process of neurotransmitter uptake and release by the nerve endings. Cardinali et al. (1975b), therefore, speculated that melatonin may affect transmitter uptake and release by interacting with brain microtubule or actin-like protein.

1.3.7 Behavioural Effects:

1.3.7.1 Sleep and Electrophysiological Changes:

Several investigators have evaluated the effect of melatonin administration on sleep and EEG patterns in a variety of animal species. Marczynski et al. (1964) implanted melatonin into the hypothalamus of cats and reported the appearance of behavioural and EEG signs of sleep. Systemic administration of the hormone into young chicks was also found to induce sleep (Barchas et al., 1967; Hishikawa et al., 1969) and EEG desynchronization (Hishikawa et al., 1969).

Likewise, melatonin administration has been shown to induce and prolong sleep in healthy human subjects. In an early study, Lerner and Case (1960) gave a patient 200 mg of

..... - 166 -/

melatonin intravenously and observed mild sedation. Anton-Tay et al. (1971) administered an acute dose of melatonin (1,25 mg/kg) to 11 healthy volunteers and reported cortical deactivation, sleep and an increase of EEG alpha activity. In a more extensive study, these authors showed Phase II enhancement, Phase IV shortening and an increase in rapid eye movement (REM) sleep (Anton-Tay, 1974). Cramer et al. (1974), however, did not observe any significant changes in total sleeping time or sleep stage percentages although sleep onset time was reduced by 50%. These findings led them to conclude that "melatonin-induced sleep strikingly resembles natural sleep". This has been confirmed by another group using EEG studies (Hollister et al., 1975a).

On the other hand, pinealectomy led to increased cortical EEG activity (Nir et al., 1969).

The above data suggest that melatonin among other factors may play a neurohumoral role in modulating the state of wakefulness and sleep. A proposed mechanism of action is discussed in Section 1.3.9.

1.3.7.2 Barbiturate Potentiation:

Additional support for the sedative role of melatonin has been presented by Barchas et al. (1967) who demonstrated a potentiation of hexobarbital sleeping time in mice following a systemic injection of melatonin (25 mg/kg I.P.). This inhibitory effect on the CNS had been previously shown by Arutyunyan et al. (1964),

..... - 167 -/

who found that melatonin potentiated the effect of the somnifacients, sodium hexenal and chloral hydrate. In addition, Martini and Fioretti (1971) reported that intraventricular injections of melatonin prolonged the effect of pentobarbitone and that this effect was dose-related.

Since melatonin is metabolized by the same liver enzymes which metabolize barbiturates (Kopin et al., 1961), it has been suggested by Axelrod (1971) that prolongation of the sleeping time by melatonin might be due to the hormone blocking the metabolism of the barbiturate.

Furthermore, Nir (1971) has demonstrated that the electrophysiological reaction of the rat brain to lethal doses of pentobarbitone is different after pinealectomy. In the sham-operated and intact controls death occurred within 7 to 10 minutes, while in the pinealectomized rats it was delayed until at least the fifteenth minute. These results, therefore, suggest that pinealectomy decreases the lethality of barbiturates which is consistent with Axelrod's hypothesis (1971).

1.3.7.3 Avoidance Behaviour:

A study evaluating the effect of melatonin administration on avoidance behaviour in rats as well as a review of previous reports is presented in Chapter 2.

1.3.7.4 Effect on Locomotor Activity:

Numerous investigators have studied the influence of the pineal gland and melatonin on the locomotor activity of many animal species under a variety of experimental conditions.

Gaston and Menaker (1968) studied the effects of pinealectomy on the locomotor activity rhythm of the house sparrow (Passer domesticus) in different environmental lighting conditions. They found that when pinealectomized birds were exposed to light-dark cycles, synchronization of rhythmic aspects of their locomotor activity with changes in the photoperiod did not differ from those of the sham-operated control birds. However, when the birds from which the pineal had been removed, were kept in constant darkness, their rhythm of locomotor activity was abolished. The control birds, on the other hand, continued to exhibit rhythmic activity patterns. From these observations the authors concluded that the pineal of the sparrow is essential for the persistence of the circadian locomotor rhythm in constant conditions and consequently they suggested that the pineal may be an important component of the endogenous time measuring system ("biological clock") which serves to correlate activity rhythms with periodic changes in the external environment. These studies have been partially replicated in the iguanid lizard (Sceloporus olivaceus) by Underwood and Menaker (1970).

The laboratory rat has been used extensively to study pineal involvement in locomotor activity levels and rhythm with mixed

..... - 169 -/

results. The effect of pinealectomy and melatonin administration on rat locomotor activity is summarized in Tables 14 and 15. In an early study, Reiss et al. (1963) reported that the neonatal pinealectomy of both male and female rats resulted, 12 weeks later, in increased treadwheel activity during a 2-hour test period. This experiment, however, suffered serious methodological problems including the lack of a sham-operated control group. In a later experiment Reiss et al. (1967) studied the pineals of normal male rats microscopically and found that the "slow runners" exhibited a greater pineal cell density, suggesting greater hormone production, than the "fast runners". Wong and Whiteside (1968) found that daily intraperitoneal injections of melatonin (10 µg/rat) significantly decreased the wheel-running activity of food-deprived male rats during a 2-hour test period for the first 5 days of testing. However, these differences were small and the curves of increasing activity over time appeared very similar in the 2 groups.

All these early studies, therefore, suggested that pineal functioning retards the total level of motor activity in the rat. This conclusion, however, is inconsistent with the more recent finding that pineal activity, specifically melatonin production, as well as rat locomotor activity is higher at night.

Recent, better-controlled experiments relating pineal function and rat motor activity have contradicted these

..... - 170 -/

TABLE 14:

EFFECT OF PINEALECTOMY ON RAT LOCOMOTOR ACTIVITY

Pinealectomy	Species	Sex	Age	Lighting conditions	Test period and apparatus	Other conditions	Results	Reference
Neonatal (2-4 days after birth)	Sprague-Dawley	Male & female	12 weeks	-	2 hours in a treadwheel	Lack of sham-operated control group	PE rats were more active	Reiss et al., 1963
Postpubertal	Long-Evans	Male	28 weeks	Continuous light then continuous dark	Tested in an activity wheel for 5 months		No effect on activity rhythms	Quay, 1968
Postpubertal	Sprague-Dawley	Male	25 weeks	12L:12D (14 days) then constant illumination	Activity recorded twice daily in activity wheels		No effect on wheel running activity	Remley et al., 1969
Postpubertal	Local S_1 strain	Female	28 weeks	Varied lighting conditions	Tested in an activity wheel for a year		No effect on activity levels or rhythms	Quay, 1970a
Neonatal (2-4 days after birth) or postpubertal	Wistar strain	Male	7 weeks	8L:16D	1 hour in a treadwheel	Food-deprived	No effect on activity levels	Relkin, 1970a
Neonatal (2-4 days after birth) or postpubeptal	Wistar strain	Male	6 weeks	8L:16D	1 hour in a maze	Food-deprived	No effect on maze performance	Relkin, 1970b
Neonatal (24 hrs after birth)	Holtzman and Charles River strain	Female	13 weeks	14L:10D then 10L:14D	1 month in activity cages		PE rats showed reduced motor activity. Also synchronize to new light-dark cycles more readily	Kincl et al., 1970
Neonatal (2-3 days after birth)	Charles River strain	Female	12 weeks	Constant darkness	3-minute test period in an open-field box		PE rats were more active in the open field	Sampson and Bigelow, 1971
Postpubertal	Sprague-Dawley	Male	-	15L:9D	1 day in activity cages		PE rats showed increased activity in the darkness	Karppanen et al., 1973
Postpubertal	R-Amsterdam albino	Female	-	12L:12D	3-minute test period in an open-field box		No effect on open-field activity	Kovács et al., 1973
Prepubertal (22 days after birth)	Sprague-Dawley	Female	-	14L:10D	2-minute test period in an open-field box	Rats were rendered blind and anosmic	Pinealectomy reversed hyperactivity	Sackman and Reiter, 1977

PE, pinealectomized

- not reported

TABLE 15:

EFFECT OF MELATONIN ADMINISTRATION ON RAT LOCOMOTOR ACTIVITY

Melatonin Dose and Duration	Species	Sex	Age	Lighting conditions	Test period and apparatus	Other conditions	Results	Reference
10 µg/rat daily for 2 weeks	Wistar albino	Male	9 weeks	-	2 hours per day in an activity wheel	Deprived of food	Melatonin decreased activity for the first 5 days of testing	Wong and Whiteside, 1967
100 µg/rat daily for 2 weeks	Charles River strain	Female	12 weeks	Constant darkness	3-minute test period on day following the final injection in an open-field box	Administered to PE and control rats	No effect on exploratory activity in the open-field	Sampson and Bigelow, 1971
200 µg/rat daily for 13 days	Sprague-Dawley	Male	-	Constant illumination	Daily 3-minute test period in an activity cage	Administered to hypophysectomized and control rats	No effect on activity	Kastin et al., 1973
50 µg/rat and 100 µg/rat for 3 days	R-Amsterdam albino	Male	-	12L:12D	3-minute test period in an open-field box		No effect on open-field activity	Kovács et al., 1973
Weekly S.C. implants (1 mg melatonin) for 4 weeks	Sprague-Dawley	Female	3 weeks	14L:10D	2-minute test period in an open-field box	Administered to blind-anosmic rats	Reversed hyperactivity	Sackman and Reiter, 1977

PE, pinealectomized; S.C., subcutaneous.

- not reported

earlier reports. For example, Remley et al. (1969) demonstrated that the total activity of male rats which had been pinealectomized as adults, did not differ significantly from sham-operated controls, either during light-dark cycles or during constant illumination. These findings were confirmed by Relkin (1970a; 1970b) who showed that neither prepubertal nor postpubertal pinealectomy affected the treadwheel-running activity (1970a) or maze performance (1970b) of adult male rats that had been deprived of food.

Quay in a series of well-controlled studies demonstrated that pinealectomy did not influence the wheel-running activity rhythms of either male rats kept in constant conditions (1965b; 1968) or of female rats under varied environmental lighting conditions (1970a). The total level of wheel-running activity of adult female rats was also shown to be unaffected by pinealectomy (1970a). Quay, however, found that following reversal of the photoperiod, pinealectomy affected the shifting of the daily start of the rat's activity phase (1970a; 1970b). The direction of this effect of pinealectomy on shifting the activity phase was reported to change with age - initially pinealectomized rats phase-shifted more rapidly than their controls, however, at early adulthood, this difference reversed and the pinealectomized animals phase-shifted more slowly (1972). In addition, he showed that the activity levels of pinealectomized rats synchronized to the new light-dark cycles more rapidly than those of the control animals.

Kincl et al. (1970) replicated these findings using neonatally pinealectomized female rats. Neonatal pinealectomy was also reported to reduce the spontaneous motor activity of female rats and this was said to be independent of the oestrous cycle. These authors thus suggested that the pineal gland acts as a "brake" which adjusts the activity of nocturnal animals in response to changing photoperiod, and, in the absence of such a "brake", rapid adjustment of motor activity to light changes occurs.

Sampson and Bigelow (1971) supported the work of Kincl et al. (1970) by showing that female rats which had been pinealectomized 2 to 3 days after birth, exhibited more exploratory activity in the open field than either sham-operated or unoperated littermate controls. Daily injections of aqueous pineal extract for 2 weeks reversed this effect, while similar treatment with melatonin (100 µg/rat) had no significant effect on this behavioural variable.

In a study performed by Kastin et al. (1973) a single injection of melatonin (200 µg/rat) had no significant effect on the motor activity of intact or hypophysectomized male rats under conditions of constant illumination. Kovács et al. (1973) reported that neither melatonin (50 µg/rat and 100 µg/rat) nor pinealectomy had any effect on exploratory activity in the open field. Karppanen et al. (1973) demonstrated that during a 15-hour-light:9-hour-dark schedule pinealectomized male rats showed increased motor activity in the dark. In addition,

..... - 174 -/

oxypertine (a methoxyindole resembling melatonin) was shown to reduce locomotor activity in pinealectomized rats less than in unoperated controls.

In a recent report, Sackman and Reiter (1977) found that pinealectomy or weekly implants of melatonin (1 mg/rat) reduced the hyperactivity produced in blind-anosmic female rats. In this case pinealectomy and melatonin implants acted similarly in reducing hyperactivity which supports the suggestion that chronic treatment of rats with melatonin leads to functional pinealectomy (Reiter et al., 1975b).

The influence of the pineal and melatonin on rat locomotor activity is thus not clear cut. In order to demonstrate the complex relationship between pineal activity and motor activity, it is essential that experiments be well-controlled in terms of photoperiod, sex, age and species of the animal. The above findings show that the pineal gland per se does not exert a direct control over motor activity. Were this the case, pinealectomy would render the rodents either continuously active or continuously inactive. The pineal has, however, been implicated indirectly in the circadian control of spontaneous locomotor activity (Kincl et al., 1970; Quay, 1970b). Melatonin also does not exert a simple effect on locomotor activity and conflicting reports have been published. Since serotonin is concerned with the control of sleep, the inhibitory effect of melatonin on motor activity reported by some workers, might be due to the drug's effect on the serotoninergic mechanisms of the midbrain and hypothalamus.

..... - 175 -/

1.3.7.5 Psychotomimetic Effect?:

Abnormal methylation has long been implicated in the aetiology of schizophrenia (Osmond and Smythies, 1952; Kety, 1965). It has been hypothesized that enzyme systems involved in transmethylation reactions are defective in schizophrenic patients, which, in turn, allows for the build-up of toxic levels of pyschotomimetics in the brain. HIOMT is a methylating enzyme and it has been suggested that this enzyme may be congenitally defective in schizophrenics (Greiner, 1970). Since the function of HIOMT is to catalyse the formation of melatonin, this indole has been implicated in the disease (McIsaac et al., 1961; Jones et al., 1969). It was suggested that melatonin might undergo a cyclic dehydration to form 10-methoxyharmalan, a psychotomimetic compound. There is indirect evidence supporting the concept that melatonin may be associated with schizophrenia. In animal studies, haloperidol (Naylor and Olley, 1969), a drug used in the treatment of the disease, and LSD (Drab et al., 1971), a psychotomimetic, have been shown to be concentrated in the rat pineal gland. In addition, Hartley et al. (1972) reported the inhibition of pineal HIOMT by haloperidol and fluphenazine in vitro. Moreover, in a subsequent paper, it was shown that psychotomimetic drugs activate HIOMT activity in vivo (Hartley and Smith, 1973). On the basis of these results, the authors proposed that the ability of haloperidol and fluphenazine to inhibit pineal HIOMT activity and melatonin synthesis may account for the beneficial action of these drugs in the treatment of schizophrenia. Unfortunately, however, there have been few

..... - 176 -/

clinical studies to substantiate this hypothesis (Section 1.3.8.3.1).

1.3.8 Clinical Studies:

1.3.8.1 Normal Subjects:

Numerous investigators have administered melatonin to normal healthy volunteers. A summary of these studies and the psychological effects of melatonin administration is outlined in Table 16. Common effects of melatonin treatment seem to be sedation (Section 1.3.7.1), tranquilization and a general feeling of well-being. Cutaneous flush, abdominal cramps, diarrhoea, scotoma lucidum and migraine headaches have also been noted in occasional patients. These effects suggest serotoninergic activity.

1.3.8.2 Neurological Disorders:

1.3.8.2.1 Epilepsy:

Anton-Tay et al. (1971) administered melatonin to 3 epileptic patients and reported the same well-being and elation seen in normal subjects. Recordings of the EEG showed a reduction in the electrical activity over the temporal lobe, depression of paroxysmal activity and increase in REM sleep. The observed rise in the convulsive threshold suggested a deactivation of the subcortical structures that integrate the general seizure (Penfield and Jasper, 1964). Melatonin, therefore, may be of use in the treatment of epilepsy, however, further studies are needed to confirm this suggestion.

TABLE 16:

PSYCHOLOGICAL EFFECTS OF MELATONIN ADMINISTERED TO NORMAL SUBJECTS
(Modified from Carman et al. (1976))

Study	Number of Subjects	Length of Study (days)	Peak Daily Dose (mg)	Route of Administration	Psychological Effects
Lerner and Case, 1960	2	1	200	Intravenous	Mild sedation
Anton-Tay et al., 1971	15	1	125	Intravenous	Sleep induction, elation, visual imagery
Anton-Tay et al., 1974	6	6	1000	Oral	Sleep EEG effects, increased REM sleep, increased phase 2 and decreased phase 4
Cramer et al., 1974	15	1	50	Intravenous	Immediate sedation, decreased psychomotor activity, shortened sleep latency, increased emotional stability noted on the day following bedtime infusion
Smythe and Lazarus, 1974	10	1	1000	Oral	Behaviour not reported
Hollister et al., 1975a	-	-	1,25	-	Sleep EEG effects
Norlund and Lerner, 1975	6	28	1000	Oral	Sedation

..... - 178 -/

1.3.8.2.2 Parkinsonism:

Studies investigating the efficacy of melatonin in the treatment of Parkinsonism are conflicting. Anton-Tay et al. (1971) administered 1,2 g of melatonin daily to 2 Parkinsonians for 4 weeks and reported a striking improvement in their clinical picture. Rigidity and tremor improved and in addition, both reported a general feeling of well-being associated with an improvement in the performance of daily tasks. In both cases, placebo substitution led to overall deterioration. These beneficial effects of melatonin administration have been replicated in a study by Anton-Tay and Fernandez-Guardiola (1976) but have not been confirmed by other investigators. Papavasiliou et al. (1972) reported that various oral doses of melatonin had no effect on the signs of Parkinsonism in 11 patients suffering from the disease. Shaw et al. (1973) administered 1 g of melatonin daily for 4 weeks to 4 Parkinsonian patients and likewise reported no change in the disabilities. Cotzias et al. (1971b), however, showed that melatonin administration controlled the tremor of 1 Parkinsonian patient.

1.3.8.2.3 Huntington's Chorea:

In a single study, Carmen et al. (1976) reported that 2 patients suffering from Huntington's chorea showed depression and psychomotor retardation following melatonin administration.

1.3.8.3 Psychiatric Disorders:

1.3.8.3.1 Schizophrenia:

In a small study, Jones et al. (1969) investigated the metabolism of melatonin in 3 schizophrenic and 2 non-schizophrenic patients. Although they reported a somewhat higher excretion of an unidentified acidic metabolite by the schizophrenic patients, no mention was made of the behavioural or possible therapeutic effects of melatonin on the subjects.

In contrast to melatonin, aqueous extracts of pineal glands have been shown to produce clinical improvement in some patients with chronic schizophrenia (Altschule, 1957; Eldred et al., 1961; Bigelow, 1974).

1.3.8.3.2 Depression:

To date, only 1 study by Carman et al. (1976) has investigated the effects of melatonin administration in depressed patients. Melatonin was administered in varying amounts either orally or by intravenous infusion. During therapy, depression ratings increased and this was accompanied by a loss of sleep and mass and a drop in oral temperature. Melatonin, however, was shown to increase the 5-HIAA and calcium levels in the CSF in 3 of the 4 patients studied.

1.3.9 Proposed Mechanism of Action on the CNS:

The exact mechanism of action of melatonin on the CNS is unknown, although various theories have been proposed. Melatonin might elevate brain neurotransmitter levels by

..... - 180 -/

stimulating synthesis, inhibiting release, enhancing reuptake or by inhibiting the intraneuronal or extraneuronal metabolism of the transmitter. Anton-Tay et al. (1970) proposed that the increased concentration of serotonin and GABA observed in the midbrain and hypothalamus following melatonin administration could be explained by the hormone's effect on pyridoxal phosphokinase activity (Section 1.3.6.3).

Another explanation involving cAMP has been put forward. This is schematically outlined in Fig. 10. Anton-Tay (1974) hypothesized that the changes in brain cAMP observed after melatonin administration (Ortega et al., 1974) suggest that the hormone acts on at least 2 different kinds of receptors, one located in the midbrain, the other in the cerebellum. He proposed that the midbrain receptor responds to melatonin by a decrease in cAMP level while the cerebellum receptor produces an increase of nucleotide in response to the hormone. Following melatonin administration, maximal changes in cAMP levels are seen after 10 minutes, in pyridoxal phosphokinase activity after 45 minutes and in midbrain serotonin concentration after 60 minutes. Thus, although the physiological role of cAMP in the CNS is not clear, the above sequence of metabolic events induced by melatonin suggests a causal relationship between them.

Anton-Tay (1974) also postulated other mechanisms. He suggested that melatonin might compete with serotonin at receptor sites in the telencephalon, causing an increased

..... - 181 -/

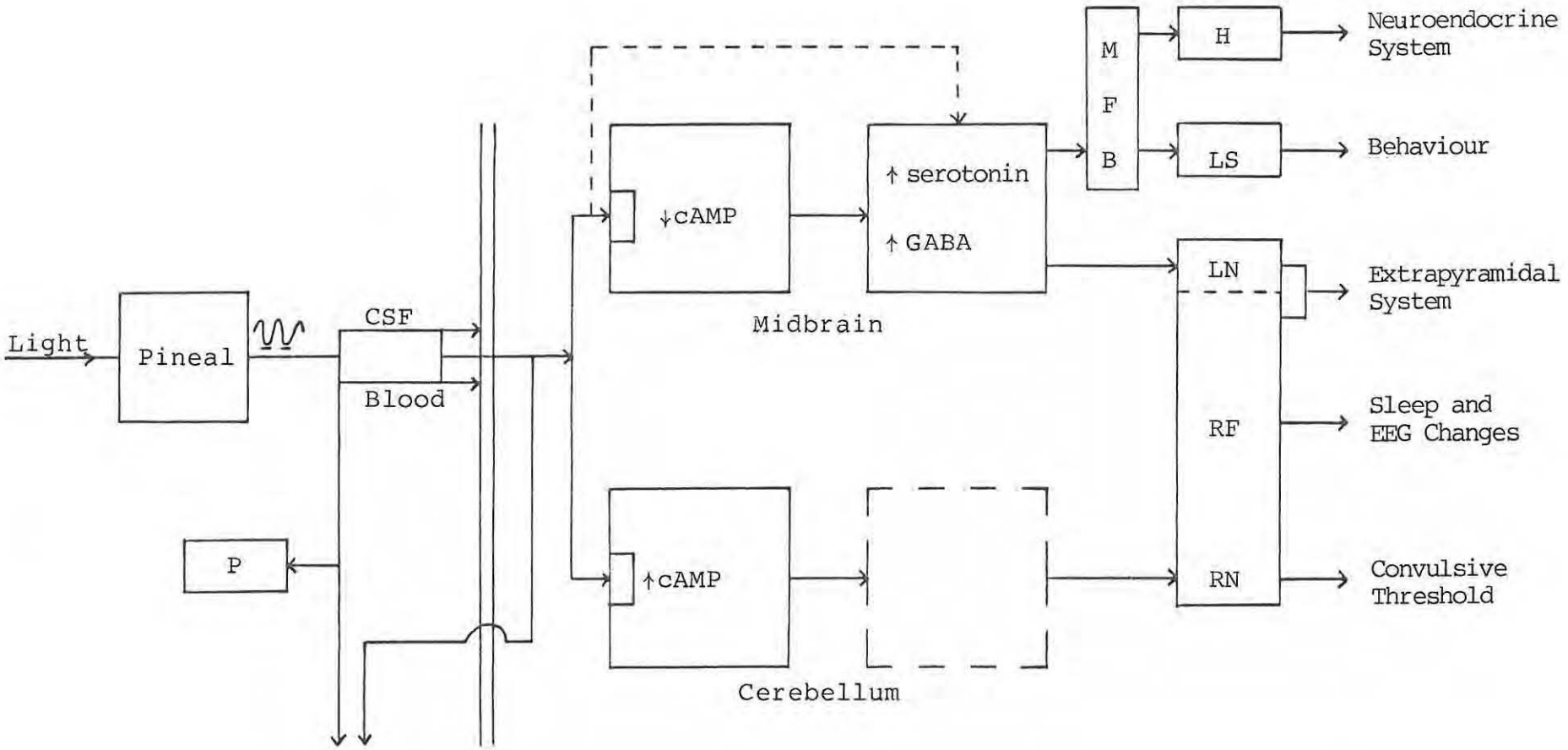


Fig. 10. Schematic Diagram of the Proposed Mechanism of Melatonin Action on the CNS (Anton-Tay, 1974).

CSF, cerebrospinal fluid; H, hypothalamus; MFB, medial forebrain bundle; LN, locus niger; LS, limbic system; P, peripheral tissues; RF, reticulum formation; RN, red nucleus.

synthesis of the monoamine in the midbrain. Melatonin may also act on other brain systems, for example, the hypothalamus. The fact that the hypothalamus shows a rich uptake of melatonin (Anton-Tay and Wurtman, 1969) and that hypothalamic levels of melatonin remain unaltered after pinealectomy (Green et al., 1972) support this assumption.

The above actions of melatonin on the midbrain and cerebellum would explain the variety of effects observed after its administration. Since the serotoninergic neurons of the midbrain have axons projecting to the hypothalamus and limbic system through the medial forebrain bundle, it is possible that the neuroendocrine and behavioural effects of melatonin could be mediated by the activation of these serotoninergic pathways. Similarly, activation of the neural pathways that leave the cerebellum and enter the red nucleus and reticular formation may explain the observed changes in sleep pattern and convulsive threshold.

Electrical stimulation of the cerebellum has been reported to depress convulsive activity (Dow et al., 1962) and it is possible that melatonin triggers the same mechanism. In addition, increased neuronal activity of the red nucleus is followed by depression of the spinal monosynaptic reflex through the rubrospinal tract (Fernández-Guardiola et al., 1964), a mechanism that could account for the effects of melatonin on the tremor of Parkinson's disease. These postulations, however, remain to be verified by other researchers.

..... - 183 -/

1.3.10 Melatonin as an Antidepressive?:

There is a lot of circumstantial evidence suggesting that melatonin may be therapeutically effective in depressive disorders.

The hypothalamus is involved in the regulation of a variety of functions known to be disturbed in depression, such as appetite, sleep, libido and hormone secretion and this brain area has been proposed as the site of action of melatonin. Labelled melatonin whether injected intravenously or intraventricularly has been shown to concentrate largely in the brain limbic structures (involved in mood regulation) and the hypothalamus (Wurtman et al., 1964b; Anton-Tay and Wurtman, 1969; Cardinali et al., 1973). In addition, Kappers et al. (1974) have provided evidence suggesting that the antigonadotropic effects of melatonin are mediated by the hypothalamus.

In depression sleep is frequently reduced and melatonin has been reported to induce and prolong sleep in animals and man (Section 1.3.7.1).

A deficiency of brain catecholamines and/or serotonin has long been implicated in the aetiology of depressive disorders (Sections 1.2.1, 1.2.2 and 1.2.3). Melatonin, on the other hand, has been shown to increase the content of these monoamines, especially serotonin, in the brain (Anton-Tay et al., 1968; Anton-Tay, 1971; Wendel et al., 1974; Cardinali, 1975;

..... - 184 -/

Cardinali et al., 1975). In addition, 5-HIAA levels in the urine (Anton-Tay, 1974) and CSF (Carman et al., 1976) are increased in depressives and Parkinsonian patients following melatonin administration.

Reiter and his co-workers (Reiter and Morgan, 1972; Reiter et al., 1972) found that when the pineal was removed from immature rats which had previously been either thyroparathyroidectomized or parathyroidectomized, most of the animals experienced severe convulsive seizures. Miline (1971) showed that pinealectomized rats were more sensitive to lowered environmental temperatures than control animals. On the basis of these results and similar experiments, Sampson (1975) proposed that "pineal functioning protects the organism against stress". However, the exact role of melatonin in these experimental situations is unclear.

MSH, an antagonist of melatonin (Cotzias et al., 1971b) causes an exaggerated response to noxious stimuli in animals and feelings of nervousness and anxiety in humans (Section 1.3.5.6.1). Conversely, MIF has been shown to be more effective than placebo in depressed patients (Ehrensing and Kastin, 1974).

The above findings suggest that melatonin could be an antidepressive. Currently, only 1 study has tested this hypothesis. Carman et al. (1976) administered melatonin to 6 depressed patients and reported an "exacerbation of the symptoms of dysphoria" in all the depressives (Section 1.3.8.3.2).

..... - 185 -/

In the light of the findings of Anton-Tay (1971) and Cardinali (1975), these results seem paradoxical. As a possible explanation, Watson and Madden (1977) proposed that melatonin's effect on the GABA neurons may outweigh the monoamine increases. In addition, 4 of the 6 patients used in Carman's study had been diagnosed as suffering from "retarded" depressions and in view of melatonin's known depressant and sedative effect on the CNS (Sections 1.3.7.1 and 1.3.7.2), the results are not surprising. Moreover, since this was a small study, this author suggests that melatonin should be evaluated in a larger group of depressives, preferably using patients suffering from "agitated" depressions, before a definite conclusion regarding melatonin's antidepressive effect is drawn.

The above findings and discrepancies have prompted the investigation of the effects of melatonin in current animal models of depression. This study was undertaken not only to evaluate melatonin as a possible antidepressive but also to determine whether the newer pharmacological models of depression are, in fact, viable models for the screening of "antidepressives" of unknown merit and mechanism of action.

..... - 186 -/

1.4 ANIMAL MODELS OF DEPRESSION:

1.4.1 Need for an Animal Model of Depression:

No satisfactory experimental animal model of depression exists at present. Depression in man is a poorly defined entity and an animal model would help in clarifying the clinical phenomenon. Moreover, the theories relating the monoamines and other biological variables to depression are based on limited and indirect evidence and a model would allow this relationship to be studied directly. In an animal model, the social and environmental variables thought to be important in depression could be systematically manipulated and their relationship to depression clarified, since there is only a limited opportunity to control these variables in humans. An experimental model could also provide a system in which potential antidepressive treatments could be evaluated.

Any animal model of a human affective disorder, however, is limited by the relative inability of animals to communicate with us as well as by our tendency to anthropomorphise. McKinney and Bunney (1969) proposed that a model meet the following minimum requirements: the symptoms of the induced animal depression should be reasonably similar to those seen in human depression; these should be observable behavioural changes; the treatment techniques effective in human depression should reverse the "depressive state" seen in the animals; and the model should be reproducible by other investigators.

..... - 187 -/

1.4.2 Separation-Loss Model:

This model is a concrete version of the psychoanalytic object-loss concept. The separation of an infant from either its mother or peers has been shown to be a powerful inducer of depression in humans and non-human primates.

In 1945, Spitz reported a deprivational reaction in human infants separated from their mothers in the second half of the first year. The syndrome, termed "anaclitic depression", was characterized by: (i) apprehension and crying; (ii) withdrawal; (iii) psychomotor retardation and rejection; and (iv) insomnia, mass loss and anorexia (Spitz, 1945; 1946). A similar reaction was demonstrated in older children (Robertson and Bowlby, 1952; Bowlby, 1960). The syndrome had 3 phases; a "protest" stage in which the behaviour of the child was characterized by restlessness and tearfulness; a "despair" phase with apathetic withdrawal; and a "detachment" phase seen only in some children and characterized by rejection of the mother on reunion.

The nature and variety of reactions to separation have also been studied in non-human primates by numerous investigators and are summarized in Table 17. In general, the monkey infants' reaction to separation whether from mother or peers, was similar to that described by Spitz (1945) and Robertson and Bowlby (1952). Initially they went through a "protest" phase with loud screaming, crying and random locomotion, followed by a "depressive" phase characterized

TABLE 17:

SEPARATION EXPERIMENTS IN NON-HUMAN PRIMATES
(Modified from McKinney and Bunney, 1969)

Investigator	Animals	Behavioural Changes	Precipitating Incident	Course	Duration of Change
Harlow (1961)	Rhesus monkeys (Macaca mulatta)	(1) ↑crying (2) ↓play (3) ↓social interaction (4) ↓appetite (5) sleep disturbance	Experimental mother-infant separation	Immediate re-attachment in most instances following reunion	Until reunion
Jensen and Tolman (1962)	Pigtail monkeys (Macaca nemestrina)	Infants constant screaming	Experimental mother-infant separation	Increased intensity of mother-infant relationship following reunion	Less than 1 hour
Hinde et al. (1966)	Rhesus monkeys	(1) ↓activity (2) Sitting huddled in corner	Experimental mother-infant separation	Preseparation activity level regained within 1 week after reunion	Separation period of 6 days
Kaufman and Rosenblum (1967)	Pigtail monkeys	(1) Agitation (2) Sitting hunched over with head down between legs (3) ↓Activity (4) ↓Social interaction (5) ↓Play behaviour	Removal of mother from group living situation	(1) Reintroduction of mother led to greatly increased closeness of infants and mothers (2) Some spontaneous recovery in stages	6-8 days
Suomi et al. (1970)	Rhesus monkeys	(1) ↑Vocalization (2) ↑Self-clasping and huddling (3) ↓Activity	Experimental peer-peer separation	Increased ventral clinging and social contact following reunion	Multiple separations of 3 days each
Bowden and McKinney (1972)	Rhesus monkeys	(1) ↑Locomotion (2) ↑Self-directed behaviour	Experimental peer-peer separation	One day of intense social activity followed by a return to preseparation activity levels	Until reunion 2 weeks later
McKinney et al. (1972)	Rhesus monkeys	(1) ↑Locomotion (2) ↑Environmental exploration (3) ↓Passivity	Experimental peer-peer separation	Return to base-line levels upon reunion	Until reunion 2 weeks later

..... - 189 -/

by withdrawal behaviour, an increase in huddling and self-clasping and a marked decrease in locomotor activity. In addition, the age at which separation takes place (McKinney et al., 1972), prior experience with separation (Young et al., 1973) and the preseparation mother-infant relationship (Hinde and Spencer-Booth, 1970) have been shown to influence the nature of the separation response. Thus the monkey model of object loss may provide important information with regards to the aetiology and treatment of depression.

The attachment of one human being to another, be that infant-mother or peer-peer, is a powerful reinforcer. These experiments have demonstrated that a disruption of the attachment bond by separation represents a loss in reinforcement which, in turn, leads to a breakdown of motivated behaviour and depression.

1.4.3 Learned Helplessness:

Seligman and his associates have proposed the phenomenon of "learned helplessness" in animals as a model of depression in man (Overmier and Seligman, 1967; Seligman and Maier, 1967; Seligman et al., 1968; Seligman and Groves, 1970).

Dogs while strapped in a Pavlovian harness were subjected to repeated, inescapable electric shock. When these dogs subsequently received electric shock in a shuttle-box they failed to cross the barrier and instead seemed to "give up"

and passively accept the shock. This behavioural state was termed "learned helplessness". In contrast, experimentally naive dogs who had not been previously exposed to inescapable shock, during escape avoidance training soon learnt that they could terminate the shock by jumping over the barrier.

Learned helplessness is not restricted to dogs. Deficits in escaping and avoiding shock after experience with uncontrollable shock, have been seen in rats (Dinsmoor, 1958; Mullin and Mogenson, 1963; Weiss et al., 1968), cats (Seward and Humphrey, 1967), fish (Behrend and Bitterman, 1963; Padilla et al., 1970) and humans (Thornton and Jacobs, 1971; Hiroto, 1974).

According to Seligman (1974; 1975) this behavioural condition has many similarities with human depressive symptomatology. It may also offer important clues to the unresolved questions of aetiology, treatment and prevention of depression. Seligman (1974; 1975) reported 6 characteristics associated with learned helplessness which are relevant to human depressions: (i) animals become passive in the face of trauma; (ii) animals are retarded at learning that their responses produce relief; (iii) learned helplessness dissipates with time; (iv) lowered aggression with a possible loss of dominant status; (v) anorexia and mass loss; (vi) physiological changes - helpless rats show noradrenaline depletion (Weiss et al., 1970) and helpless cats may be cholinergically over-active

..... - 191 -/

(Thomas and Balter, 1974). This similarity of symptoms indicates that learned helplessness may serve as a useful animal model of depression. According to Seligman, the cause of learned helplessness is not the trauma as such, but rather not having control over the trauma. He proposes that the depression-prone individual has had a life-long history characterized by failure in exercising control over his reinforcers in his environment. When the person perceives himself losing all control over such reinforcers, he is paralyzed by helplessness and passivity and depression results.

Learned helplessness was reversed by forcibly dragging the animal to the non-electrified section of the shuttle-box i.e. teaching the dog that its own responses could bring relief and reinforcement. Hence therapy, according to this model, involves having the patient find out and come to believe that his responses can produce the gratifications he desires. Beck's cognitive therapy of depression (1970) as well as the "Tuscaloosa Plan" (Taulbee and Wright, 1971), assertive training (Wolpe and Lazarus, 1969) and Morita therapy (Kora, 1965; Burgess, 1968) have similar goals, namely, to show the patient that he can control important reinforcers by his own actions (Section 1.1.6.1).

In addition, it was demonstrated that animals that had previously experienced controllable trauma, were immunized against the helplessness caused by inescapable trauma

..... - 192 -/

(Richter, 1957; Seligman and Maier, 1967). The extrapolation of this information to human depression suggests that people who have had extensive experience in controlling and manipulating the sources of reinforcement in their lives, might be more resistant to depression, whereas those who have had little control over their reinforcements, may be particularly susceptible to depressive disorders. Seligman (1974) speculates that "a childhood of experiences in which ones own actions are instrumental in bringing about gratification and removing annoyances" may prevent depression in later life.

1.4.4 Porsolt Model:

This is described in detail in Chapter 5.

1.4.5 Pharmacological "Depression":

Biochemical abnormalities which have been reported in human depressive patients, have been simulated in animals to serve as models of depression. High doses of reserpine have been observed to induce depression in approximately 15 % of hypertensive patients (Bunney and Davis, 1965). Similar depressive states have been reported following α-methyldopa (Fullerton and Morton-Jenkins, 1963; McKinney and Kane, 1967) and tetrabenazine administration (Lingjaerde, 1963). Research has shown that reserpine depletes the intraneuronal vesicles of noradrenaline (Holzbauer and Vogt, 1956) and serotonin (Pletscher et al., 1955) by disrupting the capacity of the intracellular storage vesicles to accumulate and thereby protect the

..... - 193 -/

biogenic amines from oxidative deamination by mitochondrial MAO (Kopin and Gordon, 1962; 1963; Stjarne, 1964). Tetrabenazine is thought to reduce brain noradrenaline and serotonin in a similar manner. Alpha-methyldopa depletes central catecholamines and serotonin by synthesis inhibition (Henning, 1969) and, in addition, the metabolites of α-methyldopa, α-methyldopamine and α-methylnoradrenaline, act as false transmitters and displace the catecholamines from nerve endings (Henning, 1969; Cooper et al, 1974).

Drug-induced depressions have been considered possible pharmacological models of the naturally occuring depressive disorders. Rhesus monkeys have been reported to exhibit depressive-like symptoms (decrease in locomotion and visual exploration, increase in huddling behaviour) after chronic reserpine administration (McKinney et al., 1971). Redmond et al. (1971) found that adult stump tail monkeys showed decreased social interaction, withdrawal behaviour and retarded motor activity following prolonged treatment with α-methyl-p-tyrosine, a specific blocker of the synthesis of dopamine and noradrenaline (Spector et al., 1965). However, no appreciable behavioural effects were seen in monkeys treated with PCPA, the selective depletor of serotonin (Redmond et al., 1971).

Reserpine produces sedation in experimental animals and this has been proposed as a possible animal analogue of depression in man (Sulser et al., 1964; 1966; 1967;

..... - 194 -/

Brodie, 1965). Other reserpine effects include ptosis, miosis, hypothermia, gastric ulcers, diarrhoea, salivation and bradycardia. At present, the reserpine model is the most prevalent experimental model of depression in use.

Studies by numerous investigators have suggested that reserpine-induced sedation is related to the depletion of central monoamines (Brodie and Shore, 1957; Carlsson, 1961; Häggendal and Lindqvist, 1964), but it is not known which monoamine plays the major role in its action. Administration of the catecholamine precursor, L-DOPA, to animals reverses the sedation induced by reserpine, whereas the serotonin precursor, 5-HTP, does not (Carlsson et al., 1957; McGeer et al., 1963; Wada et al., 1963). Degkwitz et al. (1960) demonstrated that L-DOPA administration counteracted the psychological effects of reserpine in human subjects. These findings suggest that the catecholamines are important in the reserpine syndrome. However, it has been proposed that dopamine rather than noradrenaline may produce the behavioural effects of reserpine (Everett and Wiegand, 1962; Creveling et al., 1968). Other workers have implicated serotonin in reserpine-induced sedation. Some have suggested that serotonin produces central excitation and the depletion of serotonin results in sedation (Woolley, 1962), while others have proposed that serotonin and noradrenaline antagonize each other in the brain and the free serotonin causes sedation (Brodie et al., 1966). Miller and Maickel (1969)

..... - 195 -/

suggest that it is the relative ratios of free noradrenaline and serotonin that are important in producing the behavioural depression.

Pretreatment with tricyclic drugs prevents or reverses reserpine-induced sedation in animals (Carlsson, 1961; Scheckel and Boff, 1964; Sulser et al., 1964). Sulser et al. (1964) have proposed that this phenomenon depends on the availability and release rate of the catecholamines. It has been suggested that the imipramine-like drugs may act either by limiting the access of noradrenaline to the mitochondrial MAO, thereby allowing free active noradrenaline to leave the cell (Schildkraut et al., 1964), or by inhibiting the inactivation of noradrenaline by blocking the cellular reuptake (Glowinski and Axelrod, 1964). Another possible explanation, however, has been put forward. The neuronal uptake and subsequent action of guanethidine has been reported to be inhibited by the TADs, thus it has been proposed that the tricyclics may act similarly in blocking reserpine uptake which would, in turn, abolish reserpine's effect (Goodman and Gilman, 1975).

The reserpine syndrome is also antagonized by prior treatment with MAO inhibitors (Chessin et al., 1957; Carlsson, 1961; Spector et al., 1963). These drugs are believed to inhibit the intracellular degradation of the reserpine-released noradrenaline which may then leak out of the cell onto the postsynaptic receptor sites (Kopin,

..... - 196 -/

1964). Thus, although differing in their mechanism of action, both the MAO inhibitors and the TADs may reverse the effects of reserpine by increasing noradrenaline at central adrenergic receptor sites (Scheckel and Boff, 1964; Schildkraut et al., 1964; Sulser et al., 1964).

It has been suggested that this reversal of reserpine-induced depression may be a useful test for evaluating new antidepressive drugs for use in man (Brodie et al., 1961; Garattini et al., 1962; Sulser et al., 1962).

The reserpine model, however, has some limitations as a model of depression. Reserpine releases both catecholamines and serotonin and thus the phenomenon of reserpine-induced sedation cannot be related to an effect of one or another specific amine. Hence the model does not provide evidence which could relate the aetiology of depression to a deficit of any single amine. The effectiveness of this model in screening potential antidepressives has also been questioned. Zbinden (1962) has shown that the effects of reserpine can be antagonized by drugs which do not have any antidepressive effects. Conversely, some clinically effective anti-depressives, such as iprindole (Gluckman and Baum, 1969) and mianserin (Van Riezen, 1972; Gouret et al., 1977), have only showed slight, transient antireserpine activity. Neither of these 2 drugs is a potent blocker of uptake and thus one is led further to believe that TADs reverse reserpine sedation by preventing its entry into nerves.

..... - 197 -/

In addition, it seems unlikely that this empirical model will lead to the discovery of antidepressives with mechanisms of action different from those already in use.

In conclusion, any model of depression should be based on behavioural as well as biochemical concepts. The reserpine model may be merely drug-induced sedation rather than anything resembling human depression.

1.4.6 Other Behavioural Models Used in Drug Evaluation:

1.4.6.1 Locomotor Activity:

1.4.6.1.1 Serotonin and Locomotor Activity:

Brodie and Reid (1968) postulated that serotonin mediates the effects of a trophotrophic system, a system which when activated causes drowsiness, sleep, increased central parasympathetic output and decreased motor activity. Noradrenaline, on the other hand, is postulated to mediate the activity of neuronal pathways underlying an ergotrophic system producing arousal, excitation, increased central sympathetic output and hyperactivity. The work of several investigators, however, strongly suggests that in some circumstances serotonin produces excitation (Dewhurst, 1968a; Grahame-Smith, 197la; Modigh, 1972).

1.4.6.1.1.1 5-Hydroxytryptophan Administration:

When given alone, 5-HTP has been shown to induce sedation in mice and rats, whereas after inhibition of MAO it produces excitation with increased motor activity (Garattini and

..... - 198 -/

Valzelli, 1965, review).

In studies quantitatively evaluating the effects of 5-HTP on motor activity in mice, Brown (1960) reported reduced spontaneous activity following the administration of 5-HTP (0,1 to 100 mg/kg), whilst Smith and Dews (1962) found no changes in activity following 5-HTP administration (10 to 1000 mg/kg). The findings of Modigh (1972), however, indicated that the decreased motor activity following the administration of large doses of 5-HTP is a peripheral effect since this decrease could be abolished after the inhibition of peripheral decarboxylase. Moreover, he showed that the central effects of 5-HTP are excitatory producing increased motor activity in mice. The central stimulatory effects resulting from 5-HTP administration could be a result of both stimulation of the serotonin receptors and displacement of catecholamines from the catecholaminergic neurons. However, since the depletion of brain catecholamine stores did not counter this increased motor activity, it was concluded that excessive stimulation of the serotonin receptors is of major importance for the observed central excitation. Horita and Hamilton (1970) found corroborative evidence in a similar experiment using rabbits.

1.4.6.1.1.2 L-Tryptophan Administration Following the Inhibition of MAO:

Hyperactivity has been reported following the administration of l-tryptophan to rats pretreated with a MAO inhibitor

(Hess and Doepfner, 1961; Grahame-Smith, 1971a). In a series of experiments, Grahame-Smith and Green (Grahame-Smith 1971a; 1971b; Grahame-Smith and Green, 1974a; 1974b; Green and Grahame-Smith, 1974; 1976) have tried to evaluate the role of brain neurotransmitters, particularly serotonin, in the production of this hyperactivity syndrome.

Although no hyperactivity was observed following l-tryptophan administration (in doses up to 1g/kg) to rats, after pre-treatment with a MAO inhibitor (tranylcypromine 20 mg/kg) marked hyperactivity and hyperpyrexia were observed (Grahame-Smith, 1971a). This hyperactivity syndrome was shown to be dependent not only on inhibition of MAO but also on intact tryptophan hydroxylase and intact aromatic l-amino acid decarboxylase activity. Tranylcypromine (TCP) either alone or in combination with l-tryptophan however, was found to have no effect on the brain concentrations of DA and noradrenaline. Although these results suggested that serotonin is the monoamine responsible for the observed hyperactivity, the development and severity of the syndrome was found to be related not to the absolute concentration of brain serotonin but to the rate of accumulation of serotonin in the brain. In addition, both tetrabenazine and reserpine pretreatment increased the speed of onset and development of hyperactivity.

As a result of these findings, Grahame-Smith (1971a) proposed the following hypothesis. Under normal conditions the

..... - 200 -/

amount of serotonin synthesized is in excess of that required to fulfil the functional needs of the brain and this excess is metabolized by intraneuronal MAO to become functionally inactive. Following MAO inhibition, the increased rate of brain serotonin synthesis produced by tryptophan loading exceeds the capacity of intraneuronal serotonin stores to cope with and inactivate serotonin so that serotonin "spills over" into functional activity resulting in hyperactivity. On the other hand, when MAO activity is intact, tryptophan administration still increases the rate of serotonin synthesis but no hyperactivity results because the increased serotonin is metabolized by intraneuronal MAO and thereby prevented from becoming functionally active. The demonstrated excitatory effect of serotonin is inconsistent with the findings of Brodie and Reid (1968) who reported a correlation between the lowering of brain serotonin and the development of reserpine sedation. Although these authors deduced that the observed sedation was caused by the release of serotonin onto its receptor sites, another interpretation based on Grahame-Smith's model is possible. If reserpine releases serotonin from its intraneuronal granular binding sites not onto its receptor sites but onto MAO by which it is metabolized without becoming functionally active, then the amount of serotonin available within the neurons for functional "excitatory" activity would be lowered and sedation would ensue.

This same syndrome of hyperactivity is also produced in rats

..... - 201 -/

by 5-methoxy-N,N-dimethyltryptamine (5-MeODMT)(Ahlborg et al., 1968) and pretreatment with TCP (20 mg/kg) potentiated the hyperactive response (Grahame-Smith, 1971b). Pretreatment with PCPA, however, did not diminish this hyperactivity, thereby indirectly suggesting that this action of 5-MeODMT is not mediated by the release of endogenous brain serotonin. Chlorpromazine pretreatment, on the other hand, inhibited the hyperactivity caused by either tryptophan or 5-MeODMT after MAO inhibition (Grahame-Smith, 1971b). On the basis of these results, Grahame-Smith postulated that chlorpromazine acts either by competition with serotonin or 5-MeODMT at the receptor sites or by physiological antagonism.

In a subsequent paper, Grahame-Smith and Green (1974a) reported that administration of a tyrosine hydroxylase inhibitor (AMPT) abolished the hyperactivity syndrome while L-DOPA administration restored the response. These findings suggested a role for brain DA in the hyperactivity syndrome produced in rats after the administration of TCP and l-tryptophan. These authors therefore proposed that there may be a group of dopaminergic neurons situated between the serotoninergic neurons initiating the response and those mechanisms responsible for the expression of the syndrome.

A more recent report by Foldes and Costa (1975) supported this proposal that a catecholamine, perhaps DA, could be involved in the hyperactivity response since these authors found that destruction of the central sympathetic nerves

..... - 202 -/

with 6-hydroxydopamine prior to the administration of TCP and l-tryptophan abolished the hyperactivity due to l-tryptophan. The doses of 6-hydroxydopamine used in this study had been shown previously to result in a 70 to 80% decrease in brain catecholamine levels (Uretsky and Iversen, 1970), however, this treatment produced no significant change in the rate of accumulation of brain serotonin (Foldes and Costa, 1975). On the basis of this finding and the fact that the hyperactivity had been shown not to correlate with serotonin synthesis or rate of accumulation; Foldes and Costa suggested that serotonin was not involved in the hyperactivity response. In addition, they found that exogenous tryptamine in doses as low as 0,5 mg/kg I.P. also exhibited hyperactivity and this was antagonized by PCPA, reserpine and AMPT pretreatments. In order to explain their findings, they suggested that l-tryptophan loading of MAO-inhibited rats may result in the accumulation of other indole metabolites, perhaps tryptamine, which affects some catecholaminergic, probably dopaminergic, pathways leading to the observed hyperactivity.

1.4.6.1.1.3 Melatonin and Locomotor Activity:
The effect of melatonin on animal locomotor activity is reviewed in Section 1.3.7.4.

1.4.6.2 Avoidance Behaviour:
Spontaneous motor activity and avoidance behaviour are commonly used for the routine evaluation of new drugs.

..... - 203 -/

The advantage of studying animal behaviour using conditioning techniques, however, is that 1 specific type of behaviour can be brought under the experimenter's control, this behaviour can be defined with precision and the effects which drugs have on it can be measured objectively. Locomotor activity determinations do not discriminate between the different types of depressant drugs, whereas avoidance behaviour responds differentially to depressant drugs.

There are 2 types of shock avoidance schedules in use: (1) discrete avoidance where the shock is preceded by a warning signal, such as a light or buzzer; and (2) continuous avoidance where no warning signal is provided and each response (lever pressing) postpones the occurrence of shock for a specified period of time. Discrete avoidance conditioning is the most useful for studying chlorpromazine-like drugs but it has the disadvantage of not providing a good measure of stimulant drug activity. On the other hand, continuous avoidance conditioning is sensitive to both stimulant and depressant drug effects.

Neuroleptics and hypnotic drugs exert different effects on continuous avoidance behaviour. The phenothiazines (such as chlorpromazine) block conditioned avoidance responses (CAR) in doses which do not interfere with escape responses (ER)(Cook and Weidley, 1957). In other words, the animals are sufficiently sedated so as not to avoid the shock, but

..... - 204 -/

their ability to respond to and escape the shock is unimpaired. Hypnotic drugs (such as barbiturates) however, only block avoidance behaviour at the same doses that suppress escape behaviour and cause ataxia (Cook and Weidley, 1957). Thus chlorpromazine has a selective effect in that only 1 type of behaviour is blocked, whereas the barbiturates produce a non-specific depression of all behaviour. In contrast, antianxiety drugs (such as chlordiazepoxide) have no characteristic effect on avoidance behaviour. High doses suppress CAR but this is most likely due to muscle relaxation and ataxia and not to a behavioural effect per se. However, these drugs attenuate conflict behaviour and increase food reinforced behaviour, while antipsychotics have non-specific depressant effects on these variables (Scheckel, 1970). If only schedules that involve shock behaviour are considered, it is seen that antipsychotics and antianxiety drugs affect different types of avoidance behaviour; antipsychotics selectively reduce active avoidance (lever pressing to avoid shock is blocked) whereas antianxiety drugs reduce passive avoidance (lever withholding to avoid shock is blocked).

1.4.6.2.1 Avoidance Conditioning:

(1) High intensities of electric shock interfere with the acquisition of the avoidance response. Moyer and Korn (1964) found that the percentage of avoidance responses of a rat in a shuttle-box decreased as shock intensity increased and reported the optimum shock intensity to be 0,5 mA and 1,0 mA. Apparently high-intensity shock elicits motor

..... - 205 -/

responses (for example freezing) that are incompatible with instrumental avoidance conditioning. However, if an extremely low shock intensity is used, the mean response latency is long (Kimble, 1955) and few animals learn the avoidance response (Brush, 1957).

(2) Pseudoconditioning has been found to occur more readily if a buzzer instead of a light is used as the conditioned stimulus (CS). Pseudoconditioning is a situation in which the CS becomes a noxious stimulus (which elicits a fear response) and many examples of this have been reported (for example, Smith et al., 1961).

(3) An animal will learn an avoidance response more rapidly if (a) it has had prior conditioning experience (Church and Solomon, 1956), (b) the same response is used for avoidance as for escape (Mowrer and Lamoreaux, 1946), (c) the noxious stimulus terminates at the time of the escape response, and (d) the warning signal terminates at the time of the escape response (Kamin, 1959; Kamin et al., 1959).

In the present study, the conditions specified above were therefore complied with in order to optimize the avoidance conditioning.

C H A P T E R 2

THE EFFECT OF MELATONIN ON CONDITIONED AVOIDANCE BEHAVIOUR

2.1 INTRODUCTION:

Melatonin has been shown by many investigators to induce sedation in both humans and experimental animals (Section 1.3.7.1). Serotonin has been implicated in sleep (Jouvet, 1969, review) and since melatonin has been reported to increase serotonin levels in selected brain areas (Anton-Tay et al., 1968), it is suggested that this could explain melatonin's sedative effect. In addition both serotonin (Cook and Weidley, 1957) and high doses of melatonin (Section 3.3.2) have been shown to produce a general depression of spontaneous motor activity in rats. Consequently it has been postulated that melatonin exerts a sedating effect on CNS activity and thus may be important in the homeostatic system of the organism (Sampson, 1975).

If this is the case, then perhaps this sedating or calming action of melatonin could be demonstrated on behavioural measures, particularly those that impose some kind of stress upon the animals. This effect has been demonstrated in a study by Martini and Fioretti (1971). Subcutaneous injections of 250 µg of melatonin were administered daily to male rats and, although this treatment was reported not to influence the rate of acquisition of an avoidance response, significant differences in the rate of extinction were demonstrated in

the melatonin-treated animals. These rats showed a more rapid extinction of the CAR than the control animals. Apart from this perhaps being due to a central effect of melatonin, another interpretation is possible. Melatonin has been shown to suppress the secretion of ACTH (Motta et al., 1971) and ACTH has been reported to be able to retard the extinction of avoidance responses (De Wied, 1969), consequently melatonin's effect may be mediated indirectly by the suppression of the secretion of ACTH. This finding was confirmed by Kovács et al. (1974) who demonstrated that melatonin in a daily dose of 50 µg/rat facilitated the extinction of an active avoidance response as well as decreasing the intertrial activity during acquisition. In addition, melatonin (100 µg/rat) given on two consecutive days facilitated the passive avoidance behaviour in water deprived animals in two different experimental situations. These results obtained in passive avoidance learning indicated that melatonin might affect the memory fixation processes under certain circumstances.

The aim of this study was to investigate qualitatively the behavioural suppression induced by melatonin. For this purpose conditioned avoidance behaviour, which is affected differently by neuroleptic and hypnotic drugs, was used. This is discussed in more detail in Section 1.4.6.2.

..... - 208 -/

2.2 MATERIALS AND METHODS:

2.2.1 Animals:

Female rats (BD IX Agonti - S.A.B.S.) with a mass between 190 and 220 g were used. They were housed individually in a controlled temperature room with free access to food and water.

2.2.2 Apparatus:

A shuttle-box (60 x 30 x 35 cm) was divided into two compartments by a thin aluminium barrier (7,5 cm high). The floor was a grid composed of 32 stainless steel rods, 5 mm in diameter and placed 15 mm apart. Intermittent shock (24 V and 1 mA a.c. current as provided by a Ralph Gerbrande shock generator) was delivered to the rats' feet via a shock scrambler circuit connected to the grid floor. This shock served as the unconditioned stimulus (UCS). The conditioned stimulus (CS) was a light (275 V, 15 W) which illuminated the entire apparatus. The continual sound of the shock scrambler counteracted any disturbing noise which occurred during training.

2.2.3 Training:

The rats were trained every day between 09h00 and 13h00 for 6 months (May - October, 1979), each rat being trained at the same time every day. The training session for each rat lasted 30 minutes and consisted of an acclimatization period (5 minutes) followed by 50 30-second trials. In

each trial the CS (light) was presented for 10 seconds, followed by the CS plus UCS (shock) for another 10 seconds and the following variables were recorded. Conditioned avoidance response (CAR): the rat jumped the barrier within 10 seconds after the CS had been presented. Escape response (ER): a jump within 10 seconds after the shock had been delivered. No response (NR): the rat remained in the same compartment for the entire trial (>20 seconds). The rats were trained until at least 3 consecutive sessions with 85% CAR had been achieved.

2.2.4 Drugs and Drug Treatment:

Melatonin was dissolved in polyethylene glycol 400. Chlorpromazine and pentobarbitone sodium were used as the prepared injection (Largactil: May and Baker, Ltd.; Sagatal: May and Baker Ltd.). All the rats were dosed at 09h00, the drugs being injected intraperitoneally on a mg/kg basis. The test session consisted of a 3-minute acclimatization period followed by 20 30-second trials. Each rat was tested before drug administration and thereafter at 30 minute intervals until the drug effect had subsided. A minimum period of 2 weeks was allowed between the different drug treatments in order to avoid any carry-over drug effects.

2.3 RESULTS:

2.3.1 Effect on Untreated Rats:

On the first trial the rats exhibited random escape move-

..... - 210 -/

ments and manifested considerable emotional upset (vocalization, urination, defaecation). Eventually, by chance, the animal jumped across the barrier to the other compartment, a response that resulted in escape from the shock and termination of the CS. Within a few trials the rat became motionless at the onset of the light signal with signs of emotionality and when the shock was delivered, the animal moved rapidly into the other compartment (ER). A few training sessions later, when the light was switched on, the rat moved into the other compartment (CAR), terminating the light signal and the shock. During subsequent trials the proportion of avoidance responses increased and the latency of the avoidance responses decreased. A typical example of a rat's progress, as a function of trials, is presented in Fig. 11.

2.3.2 Effect of Pentobarbitone Sodium:

Rats (n = 3) were treated with various concentrations of pentobarbitone sodium (10 mg/kg, 25 mg/kg and 50 mg/kg). Pentobarbitone (10 mg/kg) exerted no significant effect on avoidance conditioning, apart from a transient block of the CAR (maximum 20%) during the first hour after drug administration. Initially the rats showed slight signs of ataxia (loss of balance and difficulty in climbing over the barrier), however, 1,5 hours after the injection their appearance had returned to normal.

Pentobarbitone in doses of 25 mg/kg and 50 mg/kg, however,

..... - 211 -/

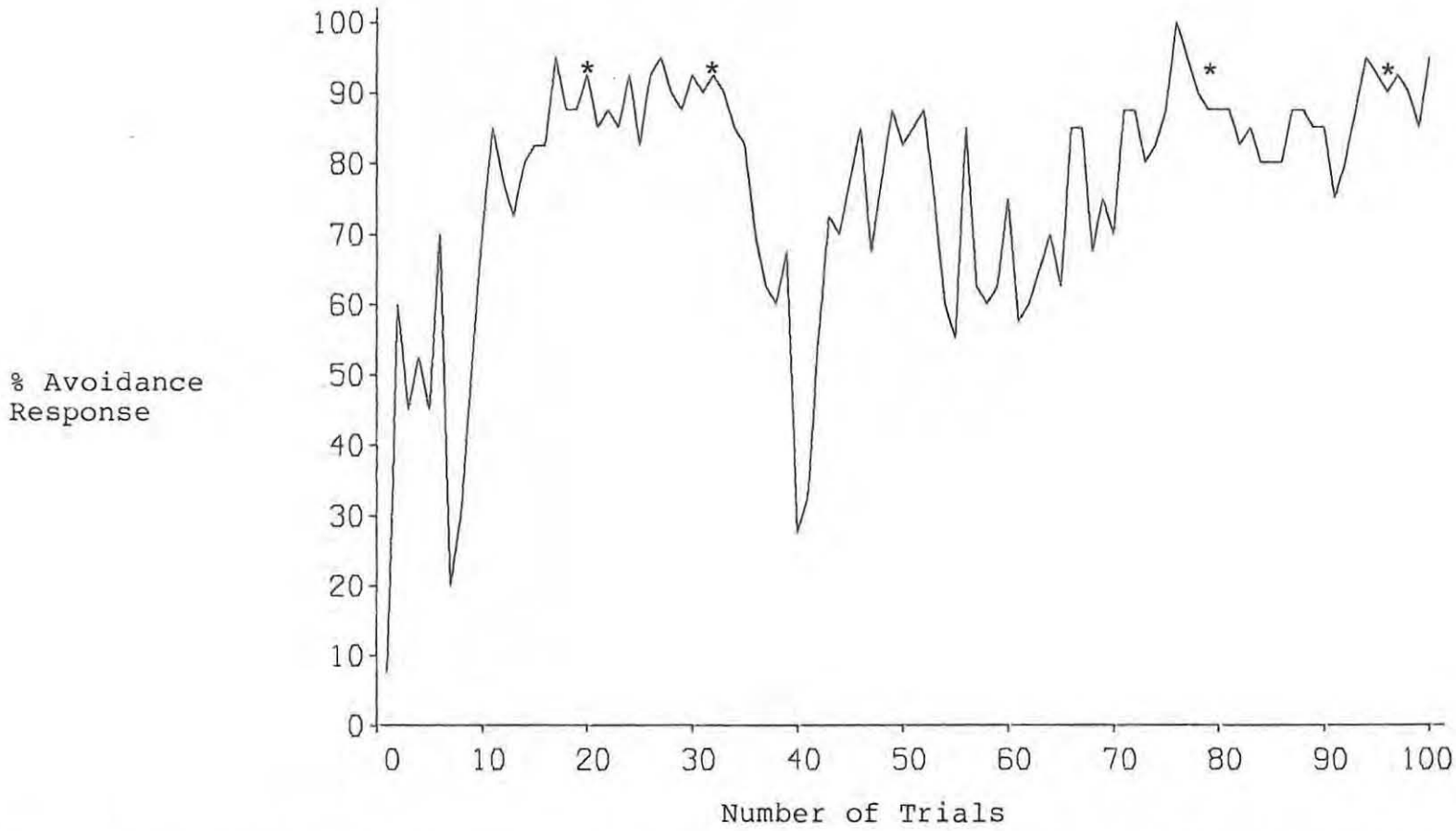


Fig. 11. Acquisition of an Avoidance Response as a Function of Trials for a Single Female Rat.

Each rat was trained at the same time every day and when at least 3 consecutive sessions with 85% CAR had been achieved, drugs were administered. This is represented by *. A minimum period of 2 weeks was allowed between the different drug treatments.

..... - 212 -/

caused a complete block of the CAR and ER (Fig. 12). Marked ataxia was observed, the rats being unable to move when the shock was delivered. The avoidance performance of the rats treated with pentobarbitone, 25 mg/kg, returned to predrug levels 5 hours after drug administration, whereas the animals injected with pentobarbitone, 50 mg/kg, recovered 8 hours after drug treatment (Table 18). This return to normal was characterized by a rapid changeover from high NR levels to high CAR levels with a relatively short ER phase.

This non-specific depression of both the CAR and ER is characteristic of the barbiturate-like drugs.

2.3.3 Effect of Chlorpromazine:

Chlorpromazine (10 mg/kg) caused a marked suppression of the CAR up to 10 hours after drug administration with a corresponding increase in both the ER and NR. This effect is illustrated in Figure 13. Some residual effects were seen 24 hours after drug treatment, however, all the rats were responsive to the CS 2 days later.

Initially the ER was high (maximum level occurring 30 minutes after treatment) but this later decreased with maximal suppression occurring 2 to 3 hours after drug administration. Suppression of the ER was caused by ataxia, the rat being unable to move and cross the barrier, which in turn led to an increase in the NR. Approximately 6 to 7 hours after

..... - 213 -/

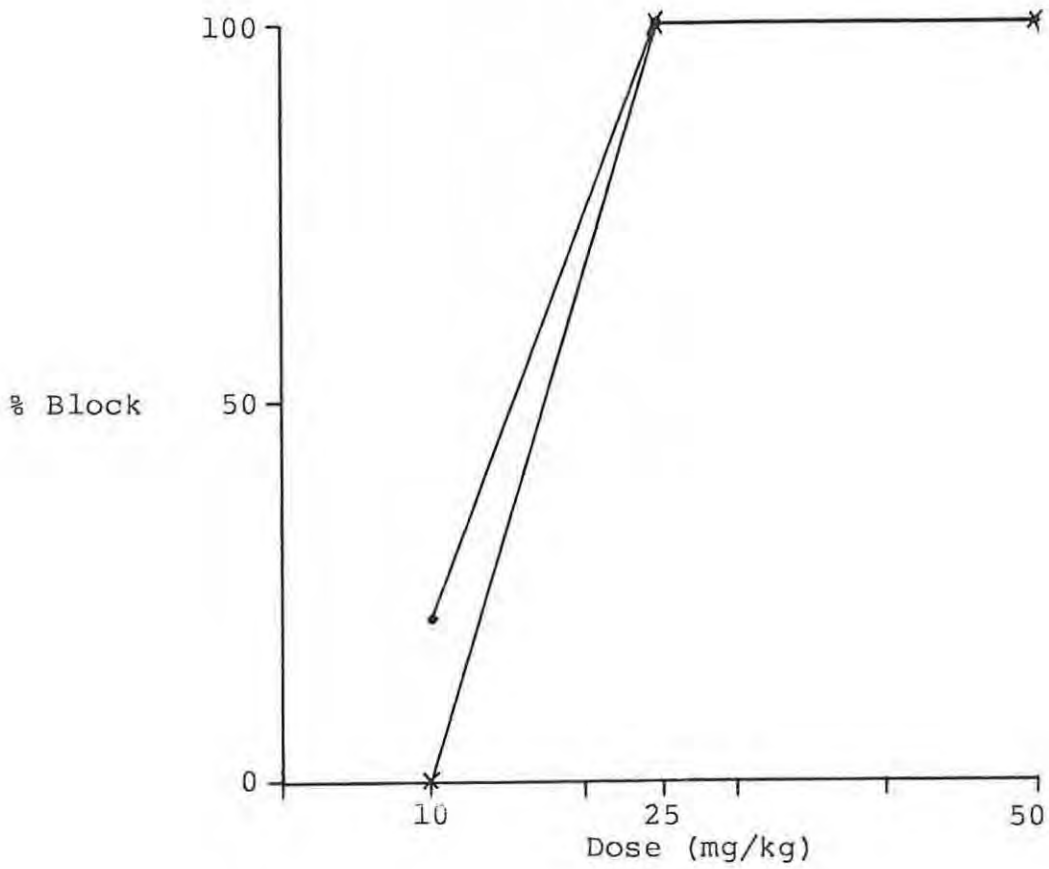


Fig. 12. Block of Conditioned (CAR) and Escape Responses (ER) by Various Doses of Pentobarbitone Sodium.

•——• Block of CAR
×——× Block of ER

Each point on the curve represents the maximum effect produced by that dose, regardless of the time it occurred. Each drug group consisted of 3 rats.

..... - 214 -/

TABLE 18:

THE EFFECT OF VARIOUS DOSES OF PENTOBARBITONE SODIUM ON THE DURATION OF BEHAVIOURAL SUPPRESSION.

Pentobarbitone Sodium Dose (mg/kg)	Hours After Drug Administration	CAR	ER	NR
25	3	0	0	20
	4	5	13	2
	5	18	2	0
50	6	0	2	18
	7	2	8	10
	8	18	2	0

The values represent the mean responses of 3 rats during 20 trials.

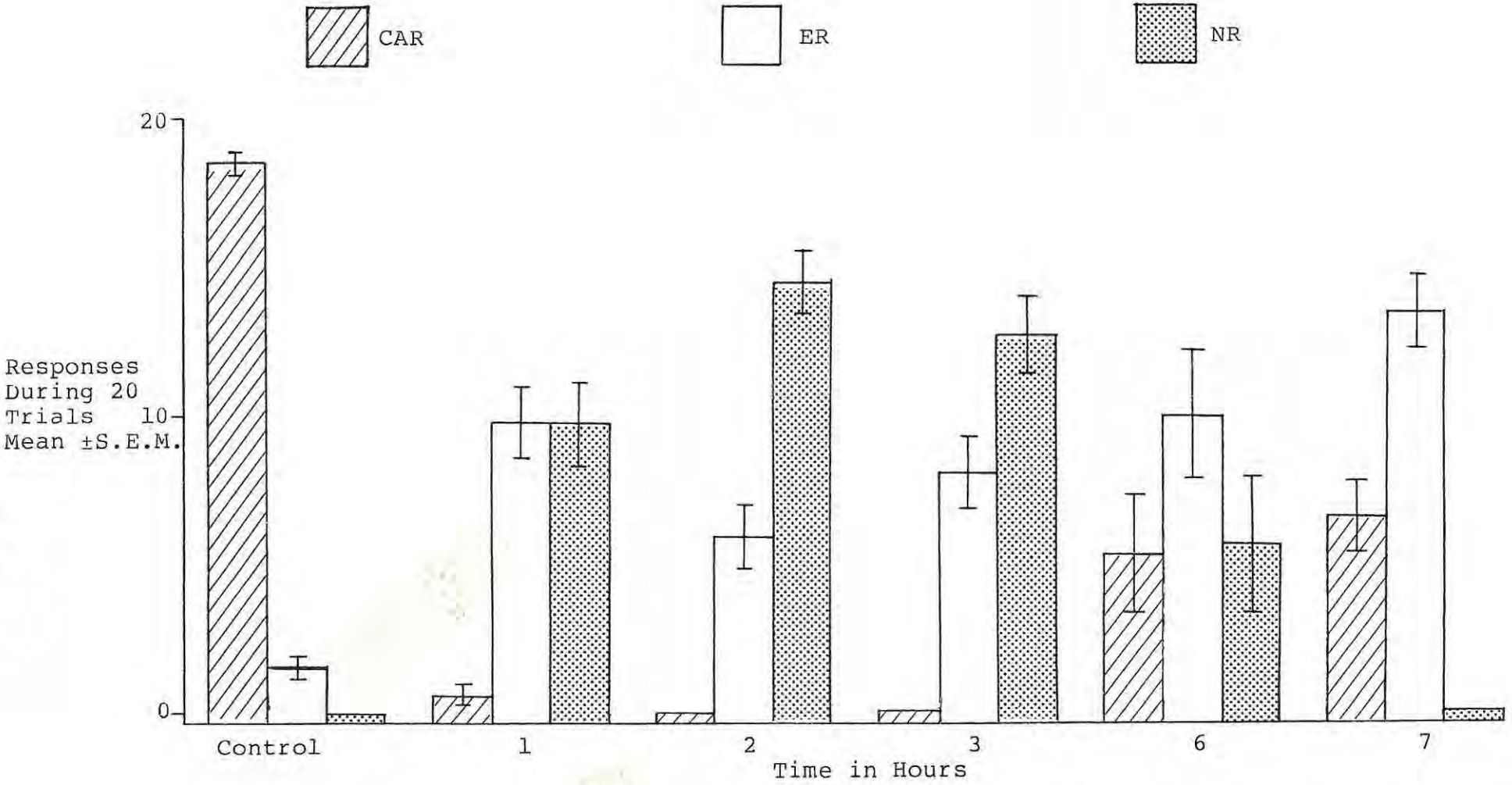


Fig. 13. The Effect of Chlorpromazine (10 mg/kg) on Conditioned Avoidance Responses (CAR), Escape Responses (ER) and No Responses (NR).

The control represents the test session immediately before the administration of chlorpromazine. Four rats were used.

treatment, the rats appeared to walk normally and the NR levels decreased to zero. However, the CAR remained suppressed and high ER levels were observed. This was in contrast to the action of phenobarbitone which caused a rapid changeover from high NR to high CAR levels (Table 18).

These results confirm previous findings that chlorpromazine has a specific action on the CAR, being able to suppress the CAR without effecting the ER at low doses. However, at relatively high doses of chlorpromazine (10 mg/kg) moderate ataxia was observed which caused suppression of the ER with a resultant increase in the NR.

2.3.4 Effect of Melatonin:

Melatonin was shown to have a dose-dependent effect on CAR. At doses of 50 mg/kg and 100 mg/kg, the drug had no significant effect on CAR, however, at 200 mg/kg a significant reduction of CAR was observed ($p < 0,01$; Wilcoxon matched-pairs signed-ranks test). These results are shown in Figure 14. The effect on the CAR was evident 30 minutes after melatonin administration and continued for 5 hours. At the 6-hour interval the avoidance performance of the rats had returned to the pre-drug levels.

The effect of melatonin (200 mg/kg) on CAR, ER and NR as a function of time is illustrated in Figure 15. Initially melatonin caused an increase in ER but 1,5 hours after drug administration this was also blocked and NR predominated.

..... - 217 -/

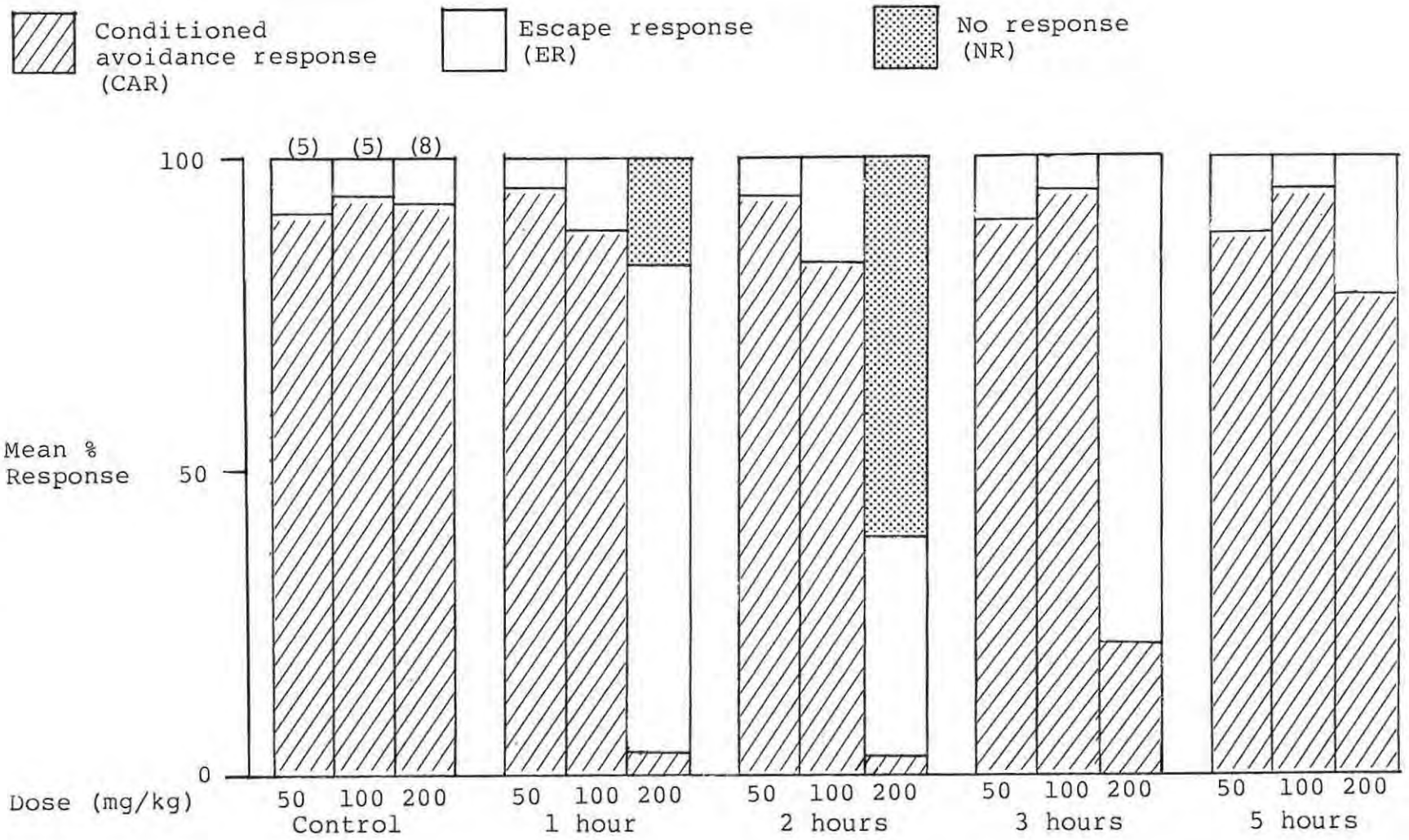


Fig. 14. The Effect of Different Concentrations of Melatonin on Avoidance Conditioning.

The control represents the test session immediately before the administration of melatonin. The number of rats used is indicated in brackets at the top of the control bars.

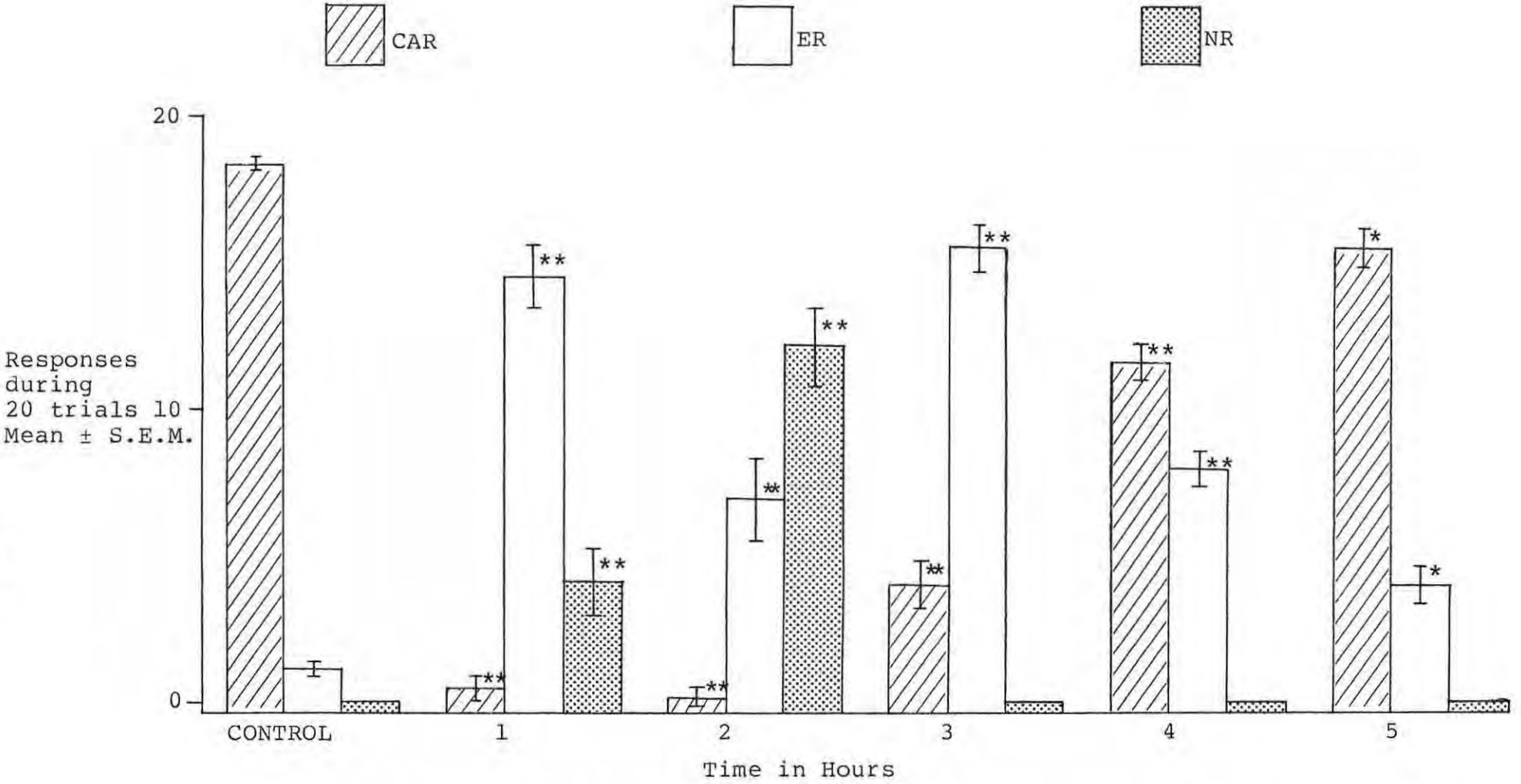


Fig. 15. The Effect of Melatonin (200 mg/kg) on Conditioned Avoidance Responses (CAR), Escape Responses (ER) and No Responses (NR).

The control represents the test session immediately before the administration of melatonin. The results were assessed statistically using the Wilcoxon matched-pairs signed-ranks test (n=8).

* $P < 0,05$ difference from control

** $P < 0,01$ difference from control

This effect was short-lived however, since an hour later the NR returned to zero and an increase in ER was again observed. This ER then gradually decreased in favour of CAR.

As assessed by gross observation the rats were sedated, relaxed and exhibited no movement except when shocked. During the first hour, some rats, showing slight signs of ataxia, appeared uncoordinated and had difficulty in balancing. However, after 3 hours, all the rats were able to walk properly (no NR was observed) and appeared normal. The animals' eyes remained open throughout the test period.

In order to qualitatively evaluate the effect of melatonin on avoidance conditioning, the drug was compared with chlorpromazine (10 mg/kg) and pentobarbitone (25 mg/kg). The comparison between the effect of these drugs on the CAR, ER and NR is illustrated in Figs. 16, 17 and 18 respectively. The results indicate that high doses of melatonin (200 mg/kg) have a similar effect to chlorpromazine (10 mg/kg) on conditioned avoidance behaviour. Both drugs suppressed the CAR, although chlorpromazine had a longer duration of action. Thirty minutes after treatment, both drugs caused an increase in the ER but this was later decreased in favour of NR. The NR levels eventually returned to zero (3 hours after melatonin administration; 7 hours after chlorpromazine administration) which resulted in a corresponding increase in the ER. This high ER

..... - 220 -/

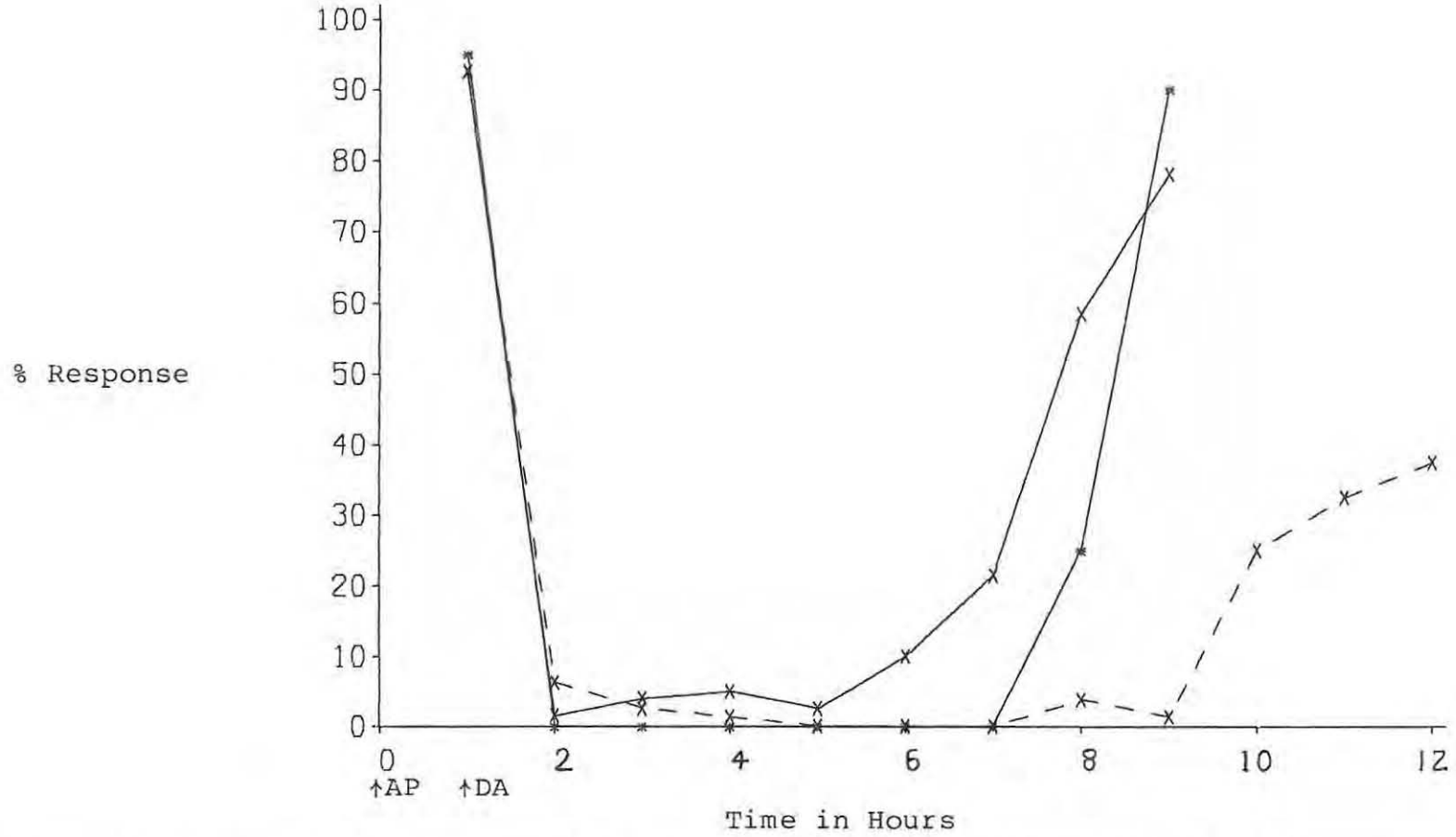


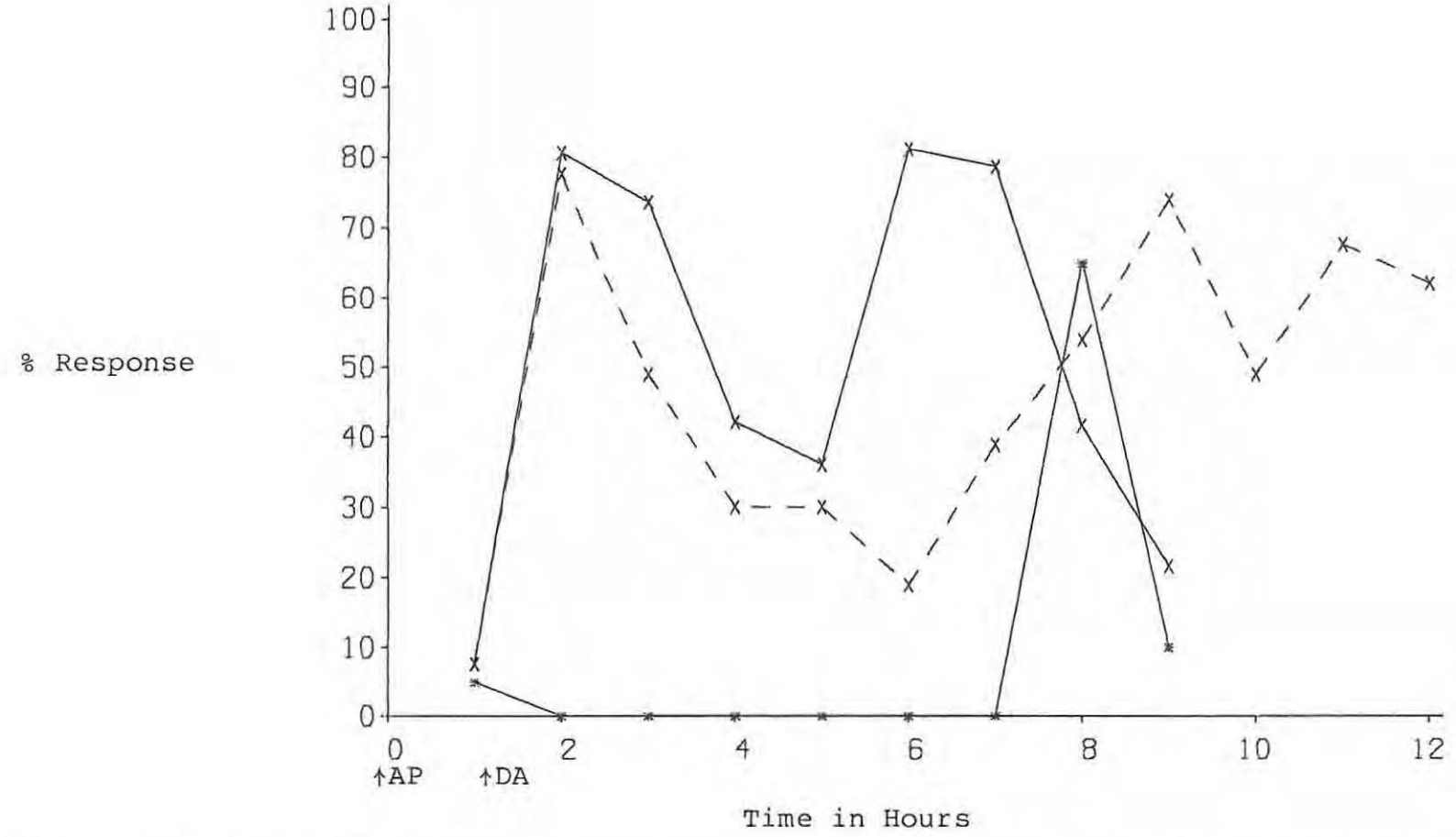
Fig. 16. The Effect of Melatonin, Chlorpromazine and Pentobarbitone on the Conditioned Avoidance Response of Trained Female Rats.

Melatonin 200 mg/kg (8)
Chlorpromazine 10 mg/kg (4)
Pentobarbitone 25 mg/kg (3)

Percent responses are represented by the mean value obtained during 20 test trials.

The figures in brackets refer to the number of rats used.

AP, acclimatization period; DA, drug administration.



..... - 222 -/

Fig. 17. The Effect of Melatonin, Chlorpromazine and Pentobarbitone on the Escape Response of Trained Female Rats.

×——× Melatonin 200 mg/kg (8)
×---× Chlorpromazine 10 mg/kg (4)
—— Pentobarbitone 25 mg/kg (3)

Percent responses are represented by the mean value obtained during 20 test trials.

The figures in brackets refer to the number of rats used.

AP, acclimatization period; DA, drug administration.

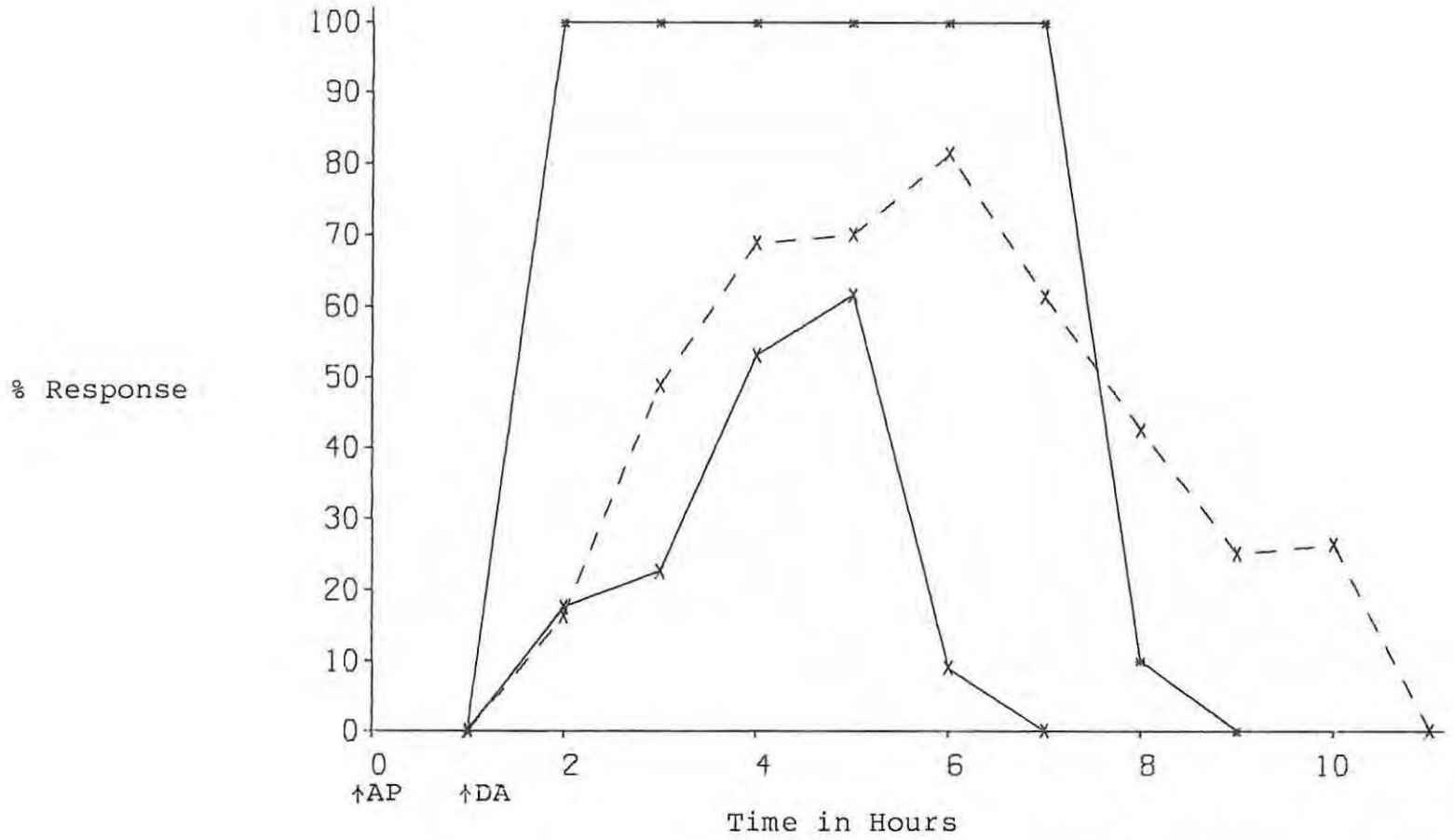


Fig. 18. The Effect of Melatonin, Chlorpromazine and Pentobarbitone on the No Response of Trained Female Rats.

×——× Melatonin 200 mg/kg (8)
×---× Chlorpromazine 10 mg/kg (4)
×——× Pentobarbitone 25 mg/kg (3)

Percent responses are represented by the mean value obtained during 20 test trials.

The figures in brackets refer to the number of rats used.

AP, acclimatization period; DA, drug administration.

..... - 223 -/

gradually returned to pre-drug levels, the transition being complete 48 hours after drug treatment.

Pentobarbitone differed from melatonin and chlorpromazine in that it caused a complete block of the CAR and ER and, upon recovery, a rapid transition from high NR to high CAR levels was observed.

2.4 DISCUSSION:

The above findings suggest that high doses of melatonin may have a chlorpromazine-like effect on the CAR in rats.

A similarity between the effect of chlorpromazine and melatonin on avoidance behaviour is not inconsistent with previous findings. Chlorpromazine and melatonin have both been shown to alter EEG patterns, to interfere with the ovulatory or oestrous cycle of rats, to block some of the effects of serotonin on smooth muscle and to alter mammalian pigmentation (Wurtman and Axelrod, 1966, review). On the basis of these findings, the authors suggested that chlorpromazine might produce some of its effects by elevating the levels of melatonin in the tissues. This hypothesis was supported by a subsequent study in which chlorpromazine was shown to inhibit the *in vivo* metabolism of melatonin (Wurtman *et al.*, 1968a). In contrast, however, melatonin and chlorpromazine have opposing effects on MSH release from the pituitary (Domino, 1971).

..... - 224 -/

However, melatonin's effect on CAR could be explained by another mechanism. Cook and Weidley (1957) have demonstrated that serotonin (10 mg/kg, 20 mg/kg and 40 mg/kg) produces a specific block of the CAR.

Serotonin has also been shown to facilitate the inhibitory processes involved in the habituation of activity and spontaneous alternation in a Y-maze (Swŏnger and Rech, 1972), suppress self-stimulation (Wise et al., 1973), strengthen behavioural reactions based on punishment mechanisms (Stein et al., 1973; Wise et al., 1973) and inhibit the stress-induced activation of the hypothalamic-pituitary-adrenal system (Vermes and Telegdy, 1972; Telegdy and Vermes, 1973). Therefore it may be possible that melatonin's effect on CAR is mediated via serotonin. In addition, high doses of the precursor, tryptophan (600 mg/kg and 800 mg/kg), have been shown to produce a specific block of the CAR, although this effect was said to be unrelated to its effect on serotonin brain levels (Engel and Modigh, 1974). The maximum effect produced by serotonin (40 mg/kg) was a 50% block of the CAR (Cook and Weidley, 1957) and tryptophan (800 mg/kg) produced a maximum block of 60% (Engel and Modigh). In the present study, however, melatonin (200 mg/kg) caused a 100% block of the CAR. Anton-Tay et al. (1968) showed that low doses of melatonin (1 mg/kg) increase brain serotonin levels, whereas in this study, only high doses of melatonin blocked the CAR. These findings suggest that mechanisms other than the increased concentration of brain serotonin are involved in the behavioural effect of melatonin.

..... - 225 -/

C H A P T E R 3

LOCOMOTOR ACTIVITY STUDIES

3.1 INTRODUCTION:

Grahame-Smith (1971a) postulated that the hyperactivity observed in rats following combined treatment with a MAO inhibitor and l-tryptophan is a result of excess serotonin which "spills over" into the synaptic cleft producing stimulation of the postsynaptic receptors (Section 1.4.6.1.1.2).

This hyperactivity syndrome produced in rats may be a useful model of depression since the combination of l-tryptophan plus a MAO inhibitor has antidepressive properties (Section 1.2.2). In addition, the administration of lithium, which has been reported to exert antidepressive effects in some depressed patients (Section 1.1.6.4.3), followed by the administration of a MAO inhibitor (TCP 20 mg/kg or pargyline 75 mg/kg) has been shown to produce a hyperactivity response identical to that produced by TCP and l-tryptophan (Grahame-Smith and Green, 1974b). The administration of PCPA abolished this hyperactivity suggesting a role for serotonin. Moreover, TRH which may have some antidepressive properties in man (Prange et al., 1972), potentiated the hyperactivity produced by the MAO inhibitor and l-tryptophan combination (Green and Grahame-Smith, 1974). On the other hand, the administration of chlorpromazine (Grahame-Smith, 1971b) and propranolol (Green and Grahame-Smith, 1976), which are both effective in the treatment of schizophrenia, inhibited the

..... - 226 -/

characteristic syndrome produced by the administration of a MAO inhibitor and l-tryptophan.

Thus, it can be inferred from the studies of Grahame-Smith and Green that a drug which produces a hyperactivity syndrome in rats following MAO inhibition, is acting in some way to increase the stimulation of serotonin receptors in the brain although this has been disputed by Foldes and Costa (1975). These authors have postulated that the observed hyperactivity is catecholamine- and not indoleamine-mediated (Section 1.4.6.1.1.2).

In view of the biogenic amine hypothesis of depression (Sections 1.2.1 and 1.2.2), any drug which produces hyperactivity in MAO-inhibited rats, may have potential antidepressive effects. As a preliminary screening test in the evaluation of melatonin as a possible antidepressive, melatonin was investigated in this behavioural model.

3.2 MATERIALS AND METHODS:

3.2.1 Animals:

Naive male albino Wistar rats with a mass between 120 and 180 g were used. Female rats were not used because of their known tendency for increased activity during oestrous and, in addition, the possibility of an interaction between hormonal changes and melatonin could be avoided. Between weaning and testing the rats were housed 3 to a cage in a room with a regular day/night lighting cycle (12L:12D) with

..... - 227 -/

free access to food and water.

3.2.2 Apparatus:

Locomotor activity was recorded by an improved version of the photocell activity cage (Physics Department, Rhodes University). The main part of the system is a black box measuring 50 cm by 50 cm by 20 cm high which is incorporated onto a base 80 cm high. The light source consists of 8 infra-red (i-r) light emitting diodes situated equidistantly (10 cm apart) in 2 of the cage walls, opposite to which are placed 8 corresponding phototransistors. The i-r source eliminates the use of a bright visible light source usually employed in photocell activity cages and which is said to provide a mildly stressful situation (Sampson, 1975).

When a rat interrupts an i-r beam, the circuit is broken and a switching pulse is fed into an operational amplifier where it is amplified to logic levels and fed into a microprocessor. The microprocessor scans the circuit every $\frac{1}{100}$ of a second and any change from the one frame to the next is registered as a count. By means of a timer, sum counts for either 1,5 or 10 minute intervals can be obtained. At the end of the required time interval these counts are stored in a memory and the microprocessor is reset. This resetting process is in the order of micro-seconds, thus, no noticeable time lag in counting occurs. The data is then read out of the memory into a digital printer.

Another advantage is that the cage is designed to measure

..... - 228 -/

not only horizontal locomotor activity but also exploratory behaviour (a component of a rat's overall activity). This is made possible by the existence of 9 holes (6 cm diameter) each placed 11 cm apart in the floor of the cage with 6 i-r transmitter-receiver pairs placed beneath the floor. A rat peering through the hole will, therefore, break the circuit and this is counted and printed out separately as a measure of exploratory behaviour.

In addition, the transmitter-receiver pairs and holes are placed in such a way that if a rat blocks a beam, a second rat moving past will break another circuit and thus be counted. Moreover, since the microprocessor measures changes in the circuits, a rat sitting in front of a beam will only be counted once when it enters and once when it leaves the beam. These modifications, therefore, make the registrations very accurate measurements of the rats' activity.

3.2.3 Drugs and Drug Treatment:

Pargyline HCl and TCP were dissolved in saline (0,9% w/v NaCl), while dl-tryptophan was dissolved in a small amount of dilute HCl (8% v/v) before being made up to volume with saline. Melatonin, depending on the amount, was dissolved in various concentrations of ethanol. All the drugs were administered by intraperitoneal injection on a mg/kg basis.

..... - 229 -/

3.2.4 Procedure:

Since environmental lighting could affect the phototransistors, the cages were fitted with black tops and the experiments were carried out in subdued lighting. All the experiments were performed at the same time each day starting at 16h00, each animal being allowed a 30-minute period of adaptation before drug administration.

After a preliminary study it was decided to use groups of 3 rats per cage and combine at least 6 groups i.e. the mean activity of 18 rats per drug treatment.

In order to eliminate the biological variables which may affect locomotor activity such as mass, age, reaction to drug etc., each group of rats was used as its own control. On Day 1 the control drug plus test drug solvent was administered and on Day 2, the control plus test drug and, therefore, any reduction or potentiation of motor activity could be observed. Furthermore, occasionally the drug treatments were reversed in order to check that the observed effect on locomotor activity was, in fact, a true drug effect and not due to the rats' acclimatizing to the experimental situation. Moreover, preliminary experiments showed that neither ethanol nor dilute HCl in the concentrations used had any effect on rat locomotor activity.

The rats' activity was recorded every 5 minutes on a digital printout. However, for graphic representation and statistical

..... - 230 -/

treatment of the results, the average activity over each 30-minute period was used. Computer programmes were written to facilitate these calculations, as well as to test for statistical significance (Wilcoxon matched-pairs signed-ranks test; Student's t-test) and to draw graphs.

3.3 RESULTS:

3.3.1 Effect of MAO Inhibition and DL-Tryptophan on Rat Locomotor Activity:

On Day 1 rats were dosed with TCP (20 mg/kg) and 24 hours later were pretreated with TCP (20 mg/kg) 30 minutes before the administration of various concentrations of dl-tryptophan. Following TCP pretreatment, the effect of 1 mg/kg, 10 mg/kg and 100 mg/kg dl-tryptophan on rat locomotor activity is presented in Figs. 19, 20 and 21 respectively. Although a significant increase in activity was recorded in all cases, the time and number of deaths was dependent on the amount of dl-tryptophan administered (Table 19). An hour after dl-tryptophan (100 mg/kg) and TCP (20 mg/kg) administration, death of some rats occurred which accounts for the decrease in locomotor activity observed in Fig.21.

The administration of TCP (20 mg/kg) alone on Day 2 also caused a significant increase in activity as compared to TCP administration on Day 1 (Fig.22), however, no deaths were recorded. Furthermore, in all the treatment groups the symptoms of drug treatment (defaecation, increased squeaking during handling, forepaw padding, piloerection,

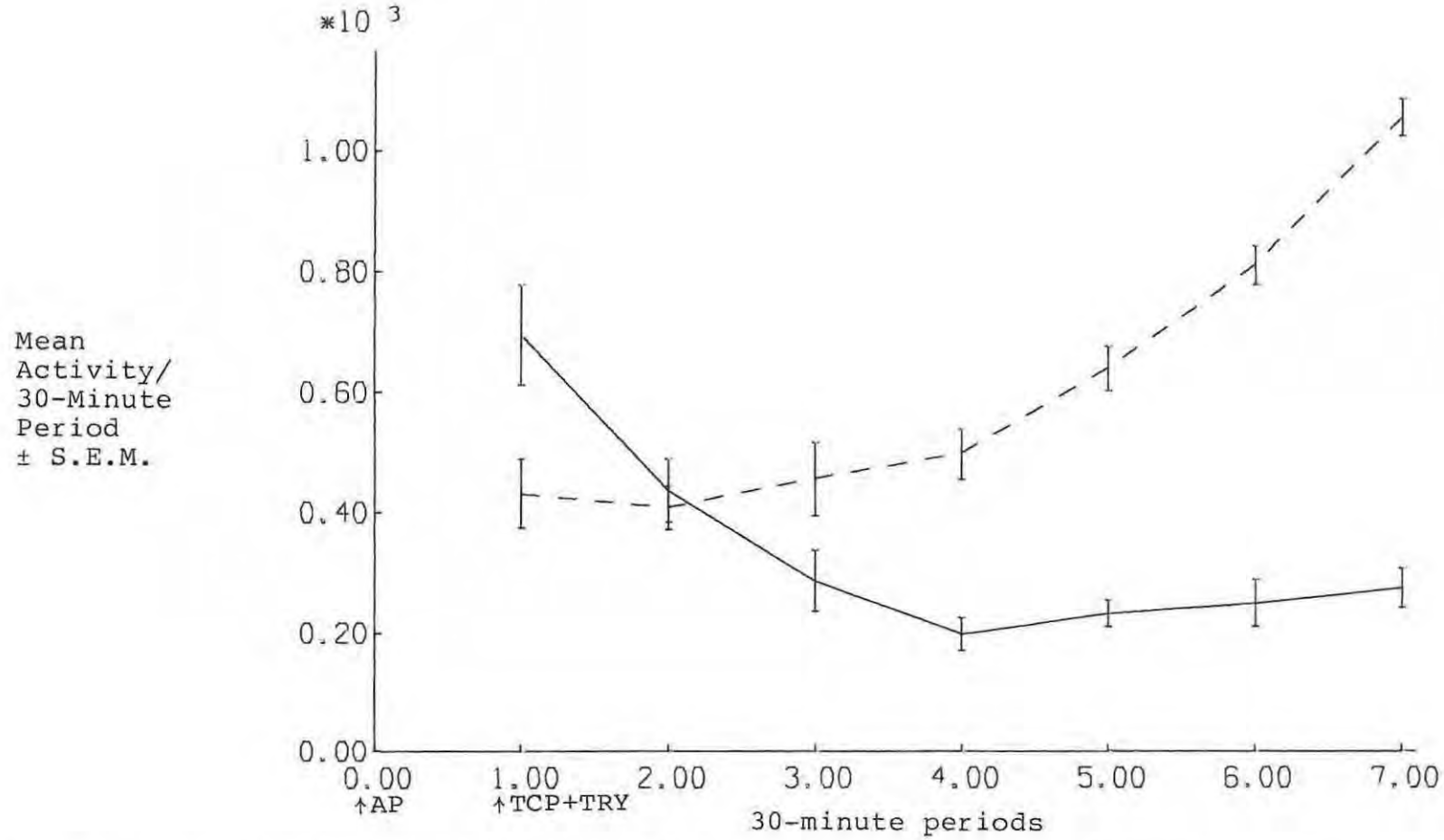


Fig. 19. The Effect of 1 mg/kg DL-Tryptophan on Rat Locomotor Activity over a Period of 3 Hours Following Tranylcypromine Administration.

⊢—⊣ Control (TCP, 20 mg/kg)
⊢- -⊣ TCP, 20 mg/kg, followed by TRY, 1 mg/kg, 30 minutes later.

Six groups of rats (3 per group) were used.

AP, acclimatization period; TCP, tranylcypromine; TRY, dl-tryptophan.

P < 0,05 as compared to control (Wilcoxon matched-pairs signed rank test).

..... - 232 -/

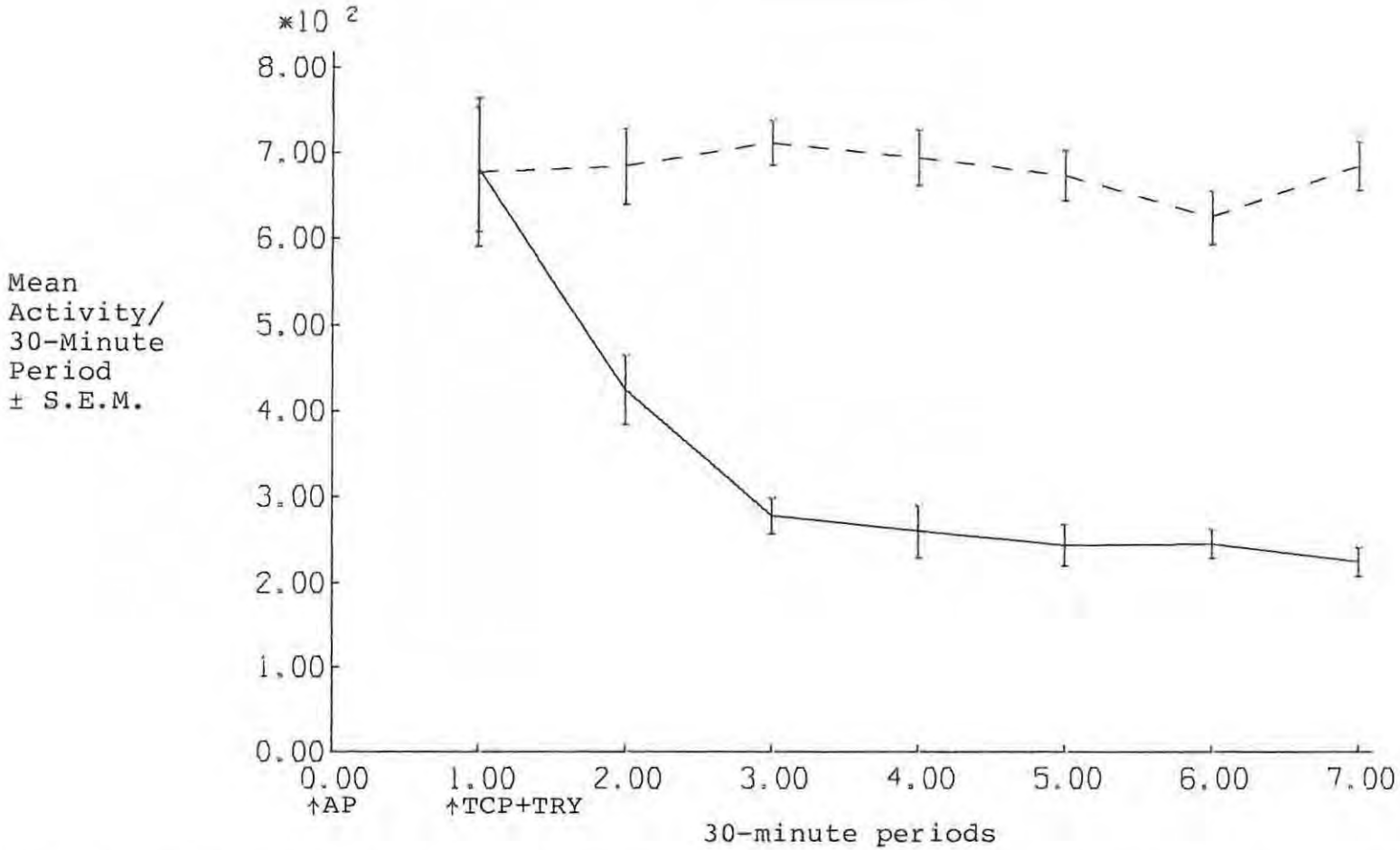


Fig. 20. The Effect of 10 mg/kg DL-Tryptophan on Rat Locomotor Activity over a Period of 3 Hours Following Tranylcypromine Administration.

⊢—⊣ Control (TCP, 20 mg/kg)
⊢---⊣ TCP, 20 mg/kg, followed by TRY, 10 mg/kg, 30 minutes later.

Six groups of rats (3 per group) were used.

AP, acclimatization period; TCP, tranylcypromine; TRY, dl-tryptophan.

P < 0,05 as compared to control (Wilcoxon matched-pairs signed-rank test).

..... - 233 -/

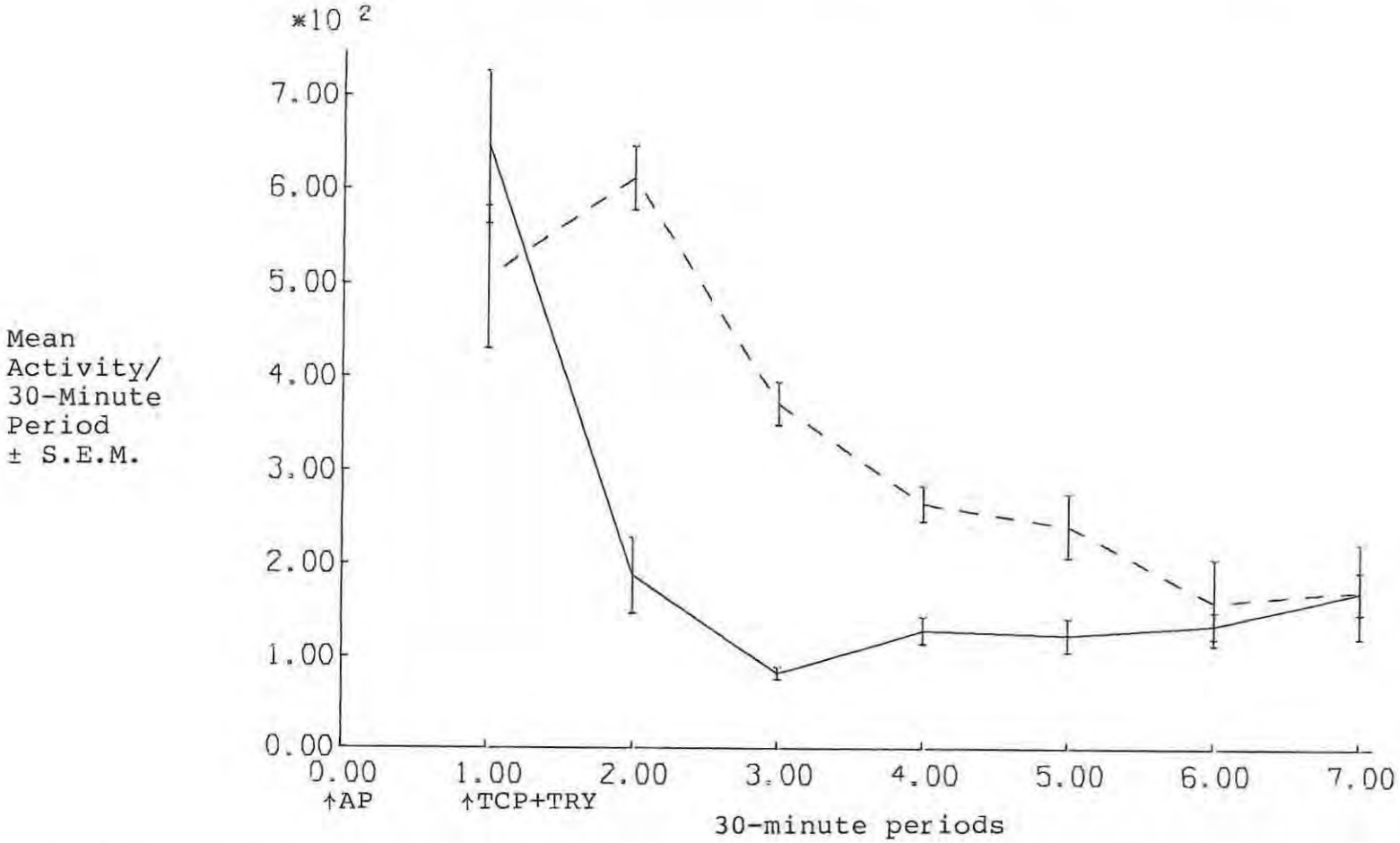


Fig. 21. The Effect of 100 mg/kg DL-Tryptophan on Rat Locomotor Activity over a Period of 3 Hours Following Tranylcypromine Administration.

⊢—⊣ Control (TCP, 20 mg/kg)
⊢---⊣ TCP, 20 mg/kg, followed by TRY, 100 mg/kg, 30 minutes later.

Six groups of rats (3 per group) were used.

AP, acclimatization period; TCP, tranylcypromine; TRY, dl-tryptophan.

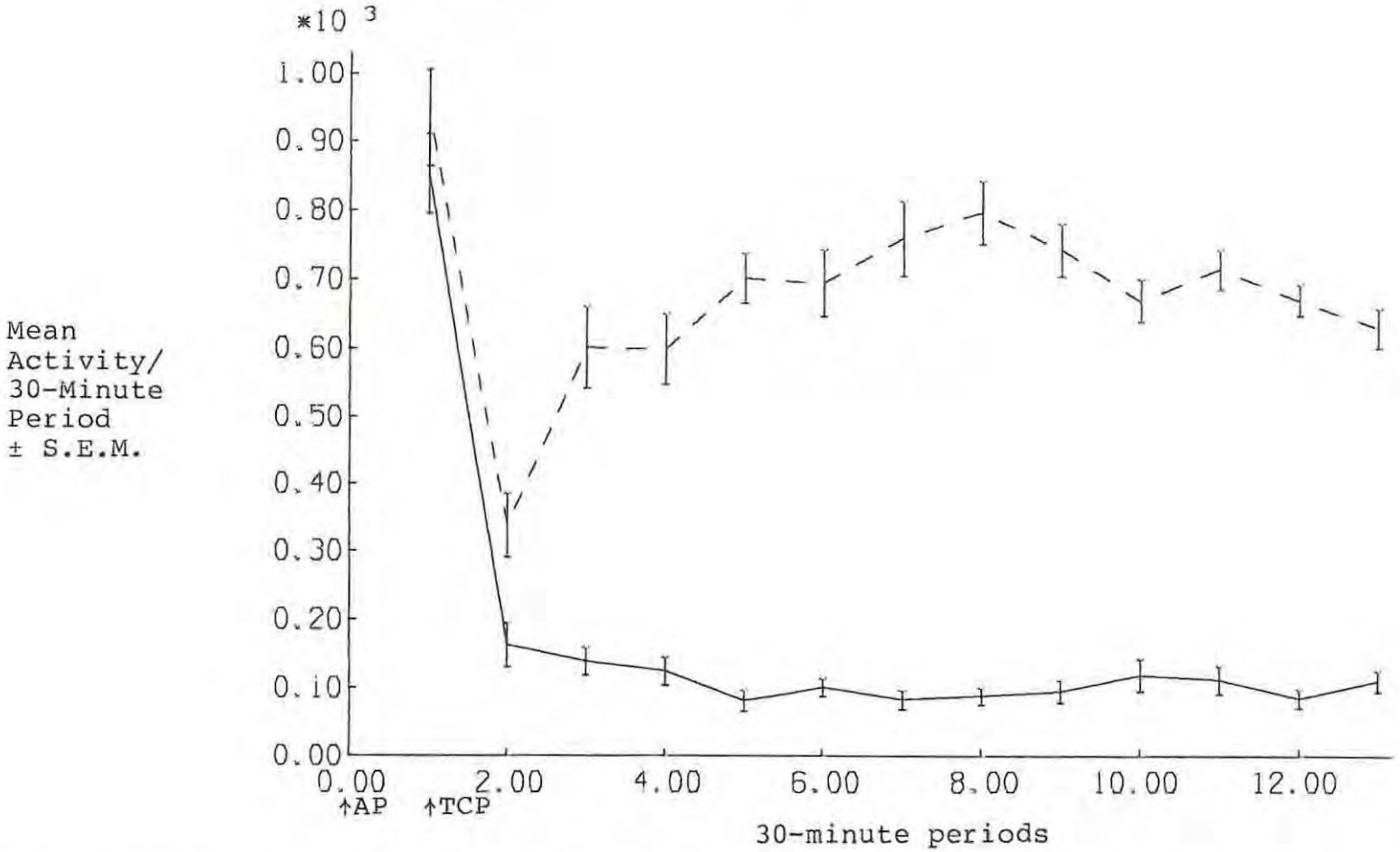
P < 0,05 as compared to control (Wilcoxon matched-pairs signed-rank test).

TABLE 19:

EFFECT OF VARIOUS DOSES OF DL-TRYPTOPHAN AND MELATONIN ON THE RAT DEATH RATE FOLLOWING THE ADMINISTRATION OF TRANYLCYPROMINE

Concentration of drug (mg/kg I.P.)		Number of rats used	Death Rate %
dl-tryptophan	1	18	0
	10	18	44
	100	9	100
melatonin	1	18	0
	10	24	17
	100	18	67

..... - 235 -/



..... - 236 -/

Fig. 22. The Effect of Tranylcypromine Administration on Rat Locomotor Activity over a Period of 6 Hours.

⊢—⊣ Control (TCP, 20 mg/kg, on Day 1)
⊢- -⊣ TCP, 20 mg/kg, on Day 2

Six groups of rats (3 per group) were used.

AP, acclimatization period; TCP, tranylcypromine.

$P < 0,05$ as compared to control (Wilcoxon matched-pairs signed-rank test).

penile erections, salivation) were more marked on Day 2.

The results show that TCP alone or in combination with dl-tryptophan increased rat locomotor activity. However, following TCP pretreatment, dl-tryptophan administration was found to further increase the absolute counts (Fig.23), this potentiation being statistically significant.

In order to confirm that it was dl-tryptophan that was potentiating the locomotor activity and not just the effect of TCP administration, another MAO inhibitor, pargyline, was used. Pargyline (75 mg/kg) alone does not cause any significant increase in locomotor activity when administered alone either on Day 1 or on Day 2 (Fig.24). The administration of dl-tryptophan (100 mg/kg) 30 minutes after pargyline pretreatment, however, resulted in a significant increase in motor activity (Fig.25). Thus the observed hyperactivity is a result of dl-tryptophan following MAO inhibition.

3.3.2 Effect of MAO Inhibition and Melatonin on Rat Locomotor Activity:

Thirty minutes after TCP (20 mg/kg) pretreatment, rats were dosed with various concentrations of melatonin and the locomotor activity was recorded. They had been injected on the previous day with TCP, 20 mg/kg. The effect of 1 mg/kg, 10 mg/kg and 100 mg/kg melatonin in this experimental situation is illustrated in Figs. 26, 27 and 28 respectively. Hyperactivity was observed following

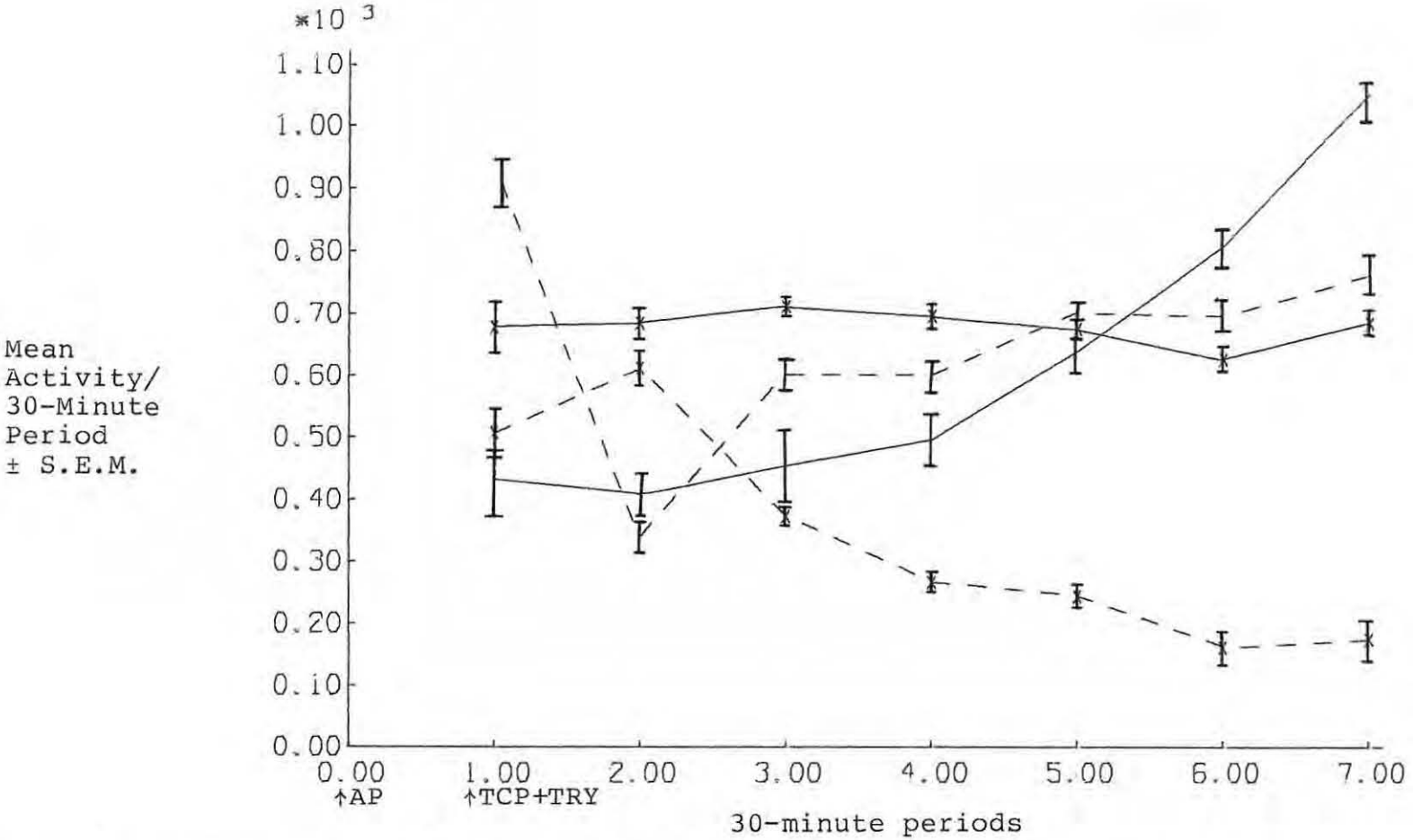


Fig. 23. Comparison Between the Effects of Various Doses of DL-Tryptophan and Tranylcypromine on Rat Locomotor Activity over a Period of 3 Hours.

⊢—⊣ Tryptophan 1 mg/kg (18)
✕—✕ Tryptophan 10 mg/kg (18)*
✕--✕ Tryptophan 100 mg/kg (9)
⊢---⊣ TCP 20 mg/kg (18)

All the rats were pretreated 24 hours previously with TCP (20 mg/kg). The numbers in brackets refer to the number of rats used.

AP, acclimatization period; TCP, tranylcypromine; TRY, dl-tryptophan.

*P < 0,05 as compared to the TCP-treated rats (Student's t-test).

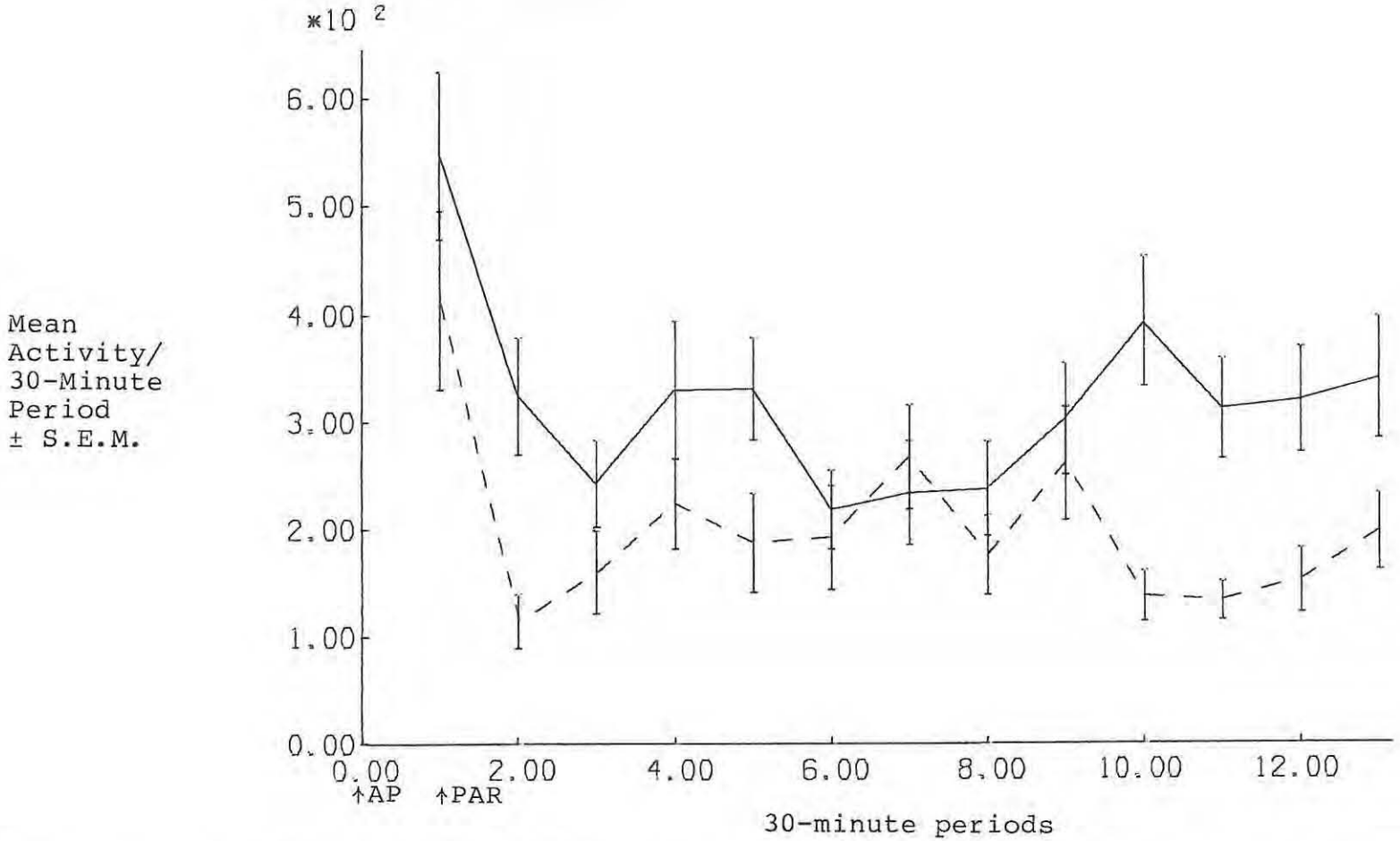


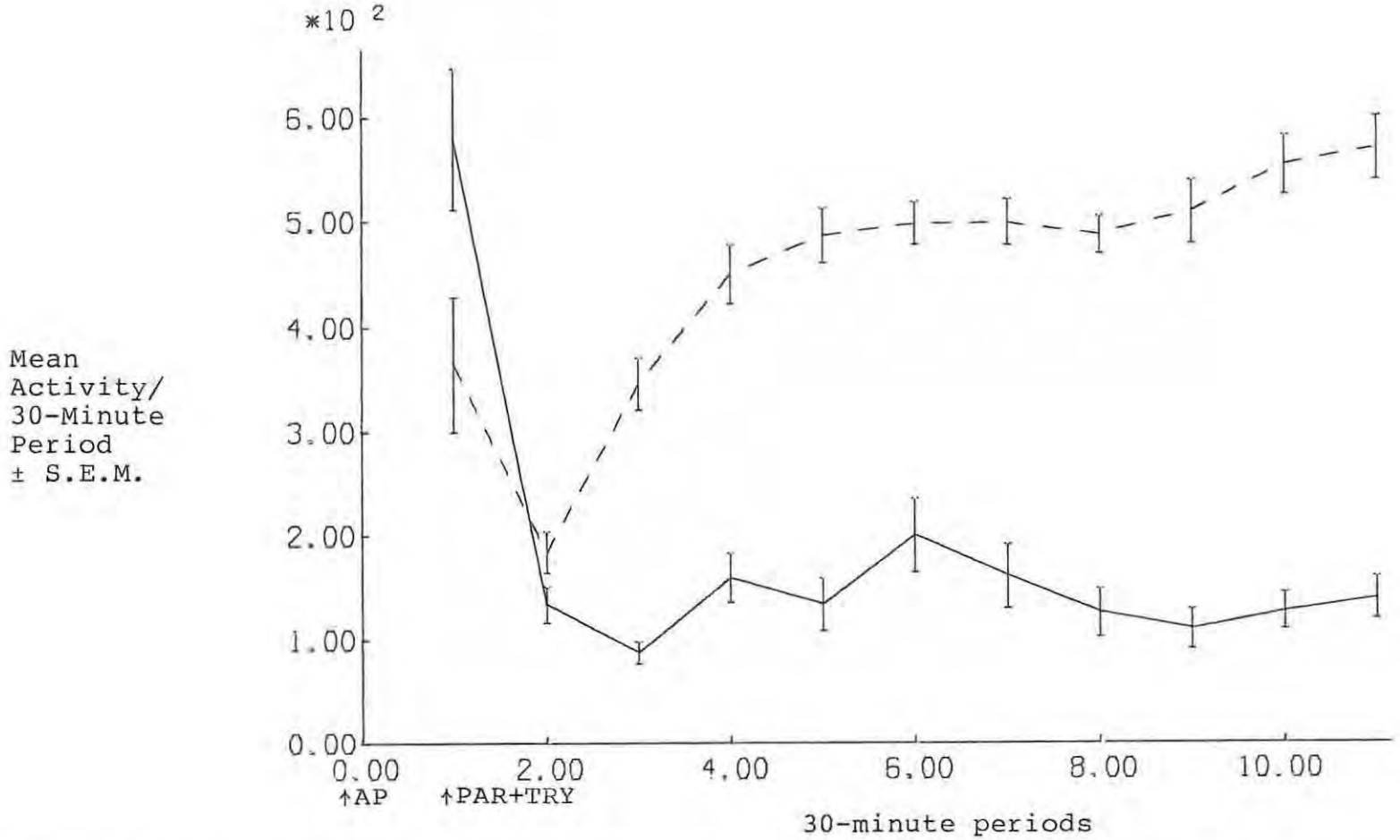
Fig. 24. The Effect of Pargyline Administration on Rat Locomotor Activity over a Period of 6 Hours.

⊢—⊣ Control (Pargyline, 75 mg/kg, on Day 1)
⊢- -⊣ Pargyline, 75 mg/kg, on Day 2

Six groups of rats (3 per group) were used.

AP, acclimatization period; PAR, pargyline.

$P < 0,05$ as compared to control (Wilcoxon matched-pairs signed-rank test).



..... - 240 -/

Fig. 25. The Effect of 100 mg/kg DL-Tryptophan on Rat Locomotor Activity over a Period of 5 Hours Following Pargyline Administration.

⊢—⊣ Control (pargyline, 75 mg/kg)
⊢---⊣ Pargyline, 75 mg/kg, followed by tryptophan, 100 mg/kg, 30 minutes later.

Six groups of rats (3 per group) were used.

AP, acclimatization period; PAR, pargyline, TRY, dl-tryptophan.

$P < 0,05$ as compared to control (Wilcoxon matched-pairs signed-rank test).

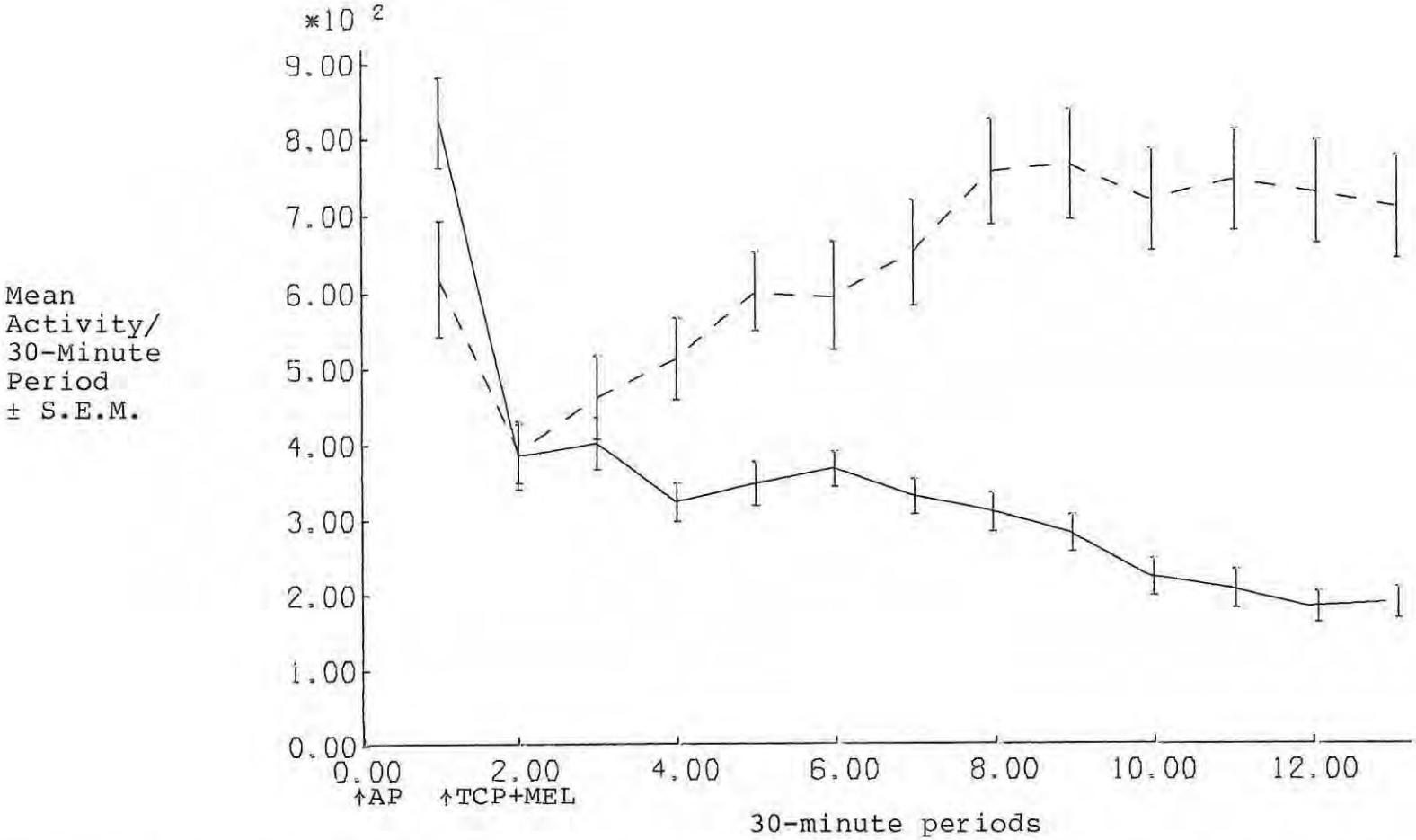


Fig. 26. The Effect of 1 mg/kg Melatonin on Rat Locomotor Activity over a Period of 6 Hours Following Tranylcypromine Administration

⊢——⊣ Control (TCP, 20 mg/kg)
⊢- - -⊣ TCP, 20 mg/kg, followed by melatonin, 1 mg/kg, 30 minutes later.

Six groups of rats (3 per group) were used.

AP, acclimatization period; MEL, melatonin; TCP, tranylcypromine.

$P < 0,05$ as compared to control (Wilcoxon matched-pairs signed-rank test).

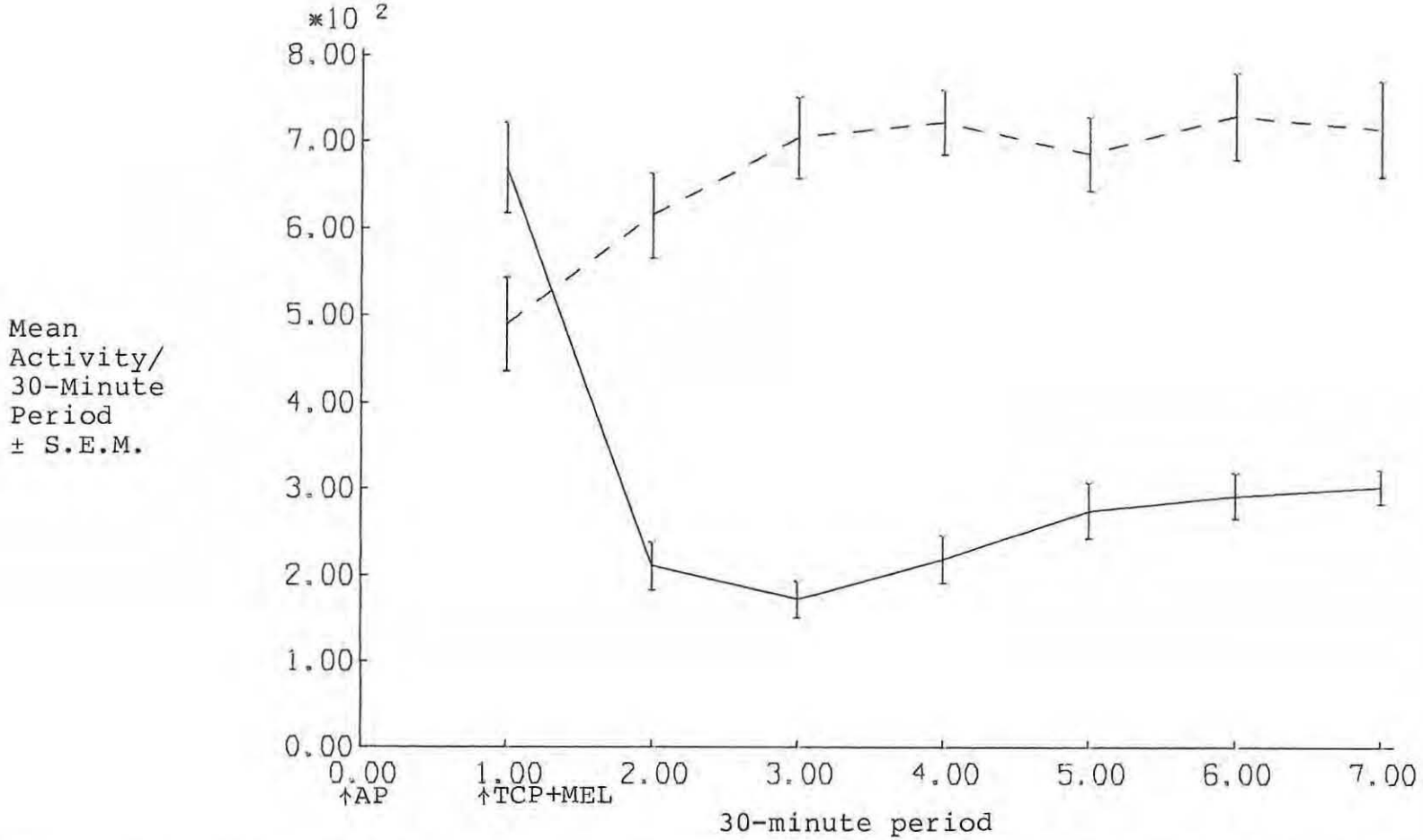


Fig. 27. The Effect of 10 mg/kg Melatonin on Rat Locomotor Activity over a Period of 3 Hours Following Tranylcypromine Administration.

⊢——⊣ Control (TCP, 20 mg/kg)
⊢---⊣ TCP, 20 mg/kg, followed by melatonin, 10 mg/kg, 30 minutes later.

Eight groups of rats (3 per group) were used.

AP, acclimatization period; MEL, melatonin; TCP, tranylcypromine.

P < 0,01 as compared to control (Wilcoxon matched-pairs signed-rank test).

..... - 242 -/

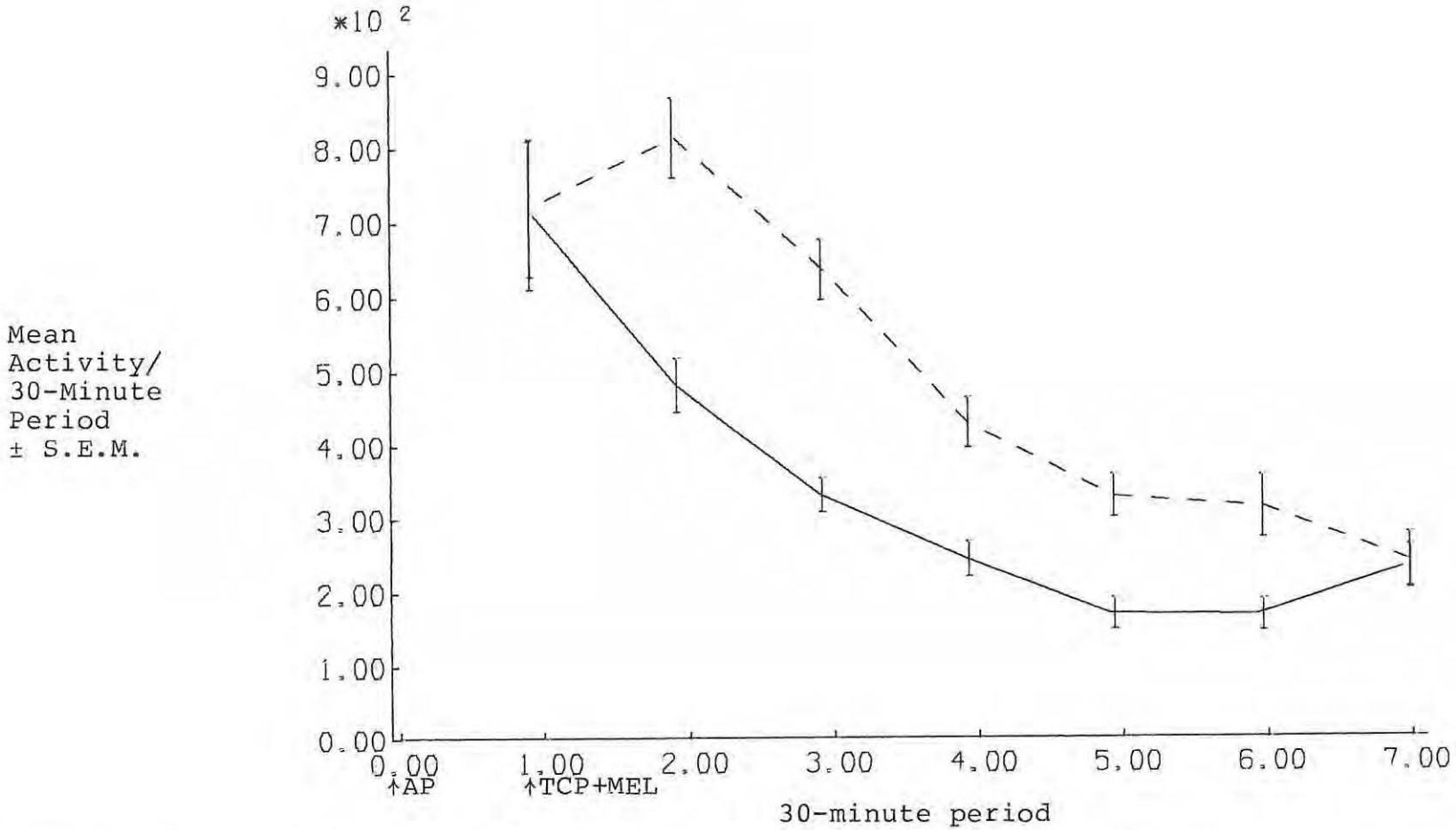


Fig. 28. The Effect of 100 mg/kg Melatonin on Rat Locomotor Activity over a Period of 3 Hours Following Tranylcypromine Administration.

⊢—⊣ Control (TCP, 20 mg/kg)
⊢- - -⊣ TCP, 20 mg/kg, followed by melatonin, 100 mg/kg, 30 minutes later.

Six groups of rats (3 per group) were used.

AP, acclimatization period; MEL, melatonin; TCP, tranylcypromine.

P < 0,05 as compared to control (Wilcoxon matched-pairs signed-rank test).

the administration of melatonin and TCP, while melatonin, 30 mg/kg and 100 mg/kg, administered alone on Day 1 and Day 2 showed no potentiation of rat locomotor activity (Figs. 29 and 30, respectively). Furthermore the absolute activity values observed show that the administration of melatonin alone decreases locomotor activity just as serotonin (Brodie and Reid, 1968; Goodman and Gilman, 1975) and tryptophan does (Modigh, 1973).

Following TCP administration, melatonin in the concentrations used behaved in a manner similar to dl-tryptophan (Section 3.3.1). At a dose of 1 mg/kg both melatonin and dl-tryptophan had no significant effect on rat locomotor activity as compared to those rats which received TCP alone. However, at 10 mg/kg both drugs produced a significant increase in motor activity. Melatonin and dl-tryptophan at a dose of 100 mg/kg also resulted in elevated motor activity but this effect was lost due to the death of some of the rats. A comparison between the various doses of dl-tryptophan or melatonin and TCP alone is presented in Figs. 23 and 31 respectively.

Melatonin administration (100 mg/kg) following pargyline (75 mg/kg) pretreatment also resulted in a significant increase in locomotor activity (Fig.32).

3.4 DISCUSSION:

Melatonin and tryptophan appear to affect the locomotor

..... - 244 -/

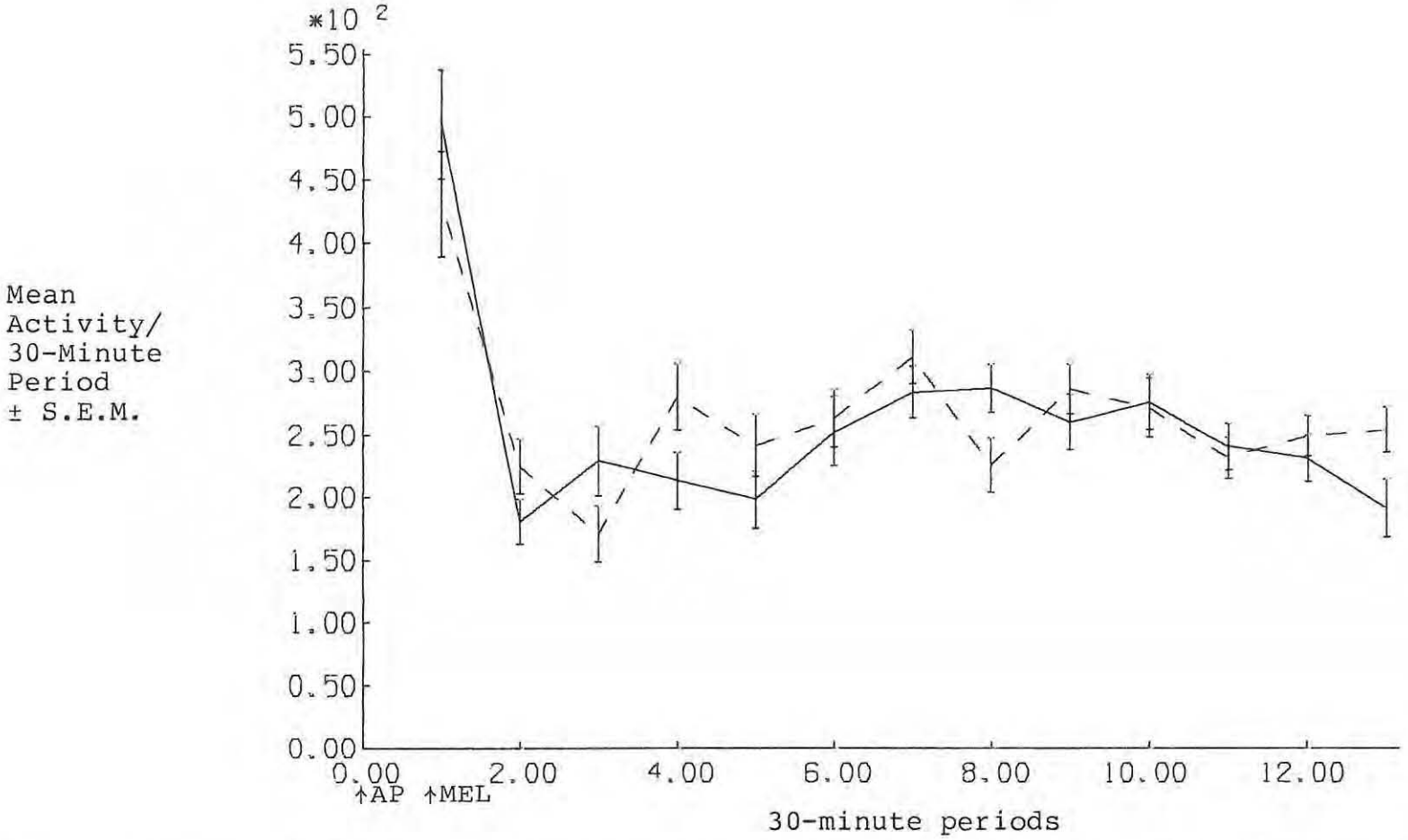


Fig. 29. The Effect of 30 mg/kg Melatonin on Rat Locomotor Activity over a Period of 6 Hours.

⊢——⊣ Control (melatonin, 30 mg/kg, on Day 1)
⊢- - -⊣ Melatonin, 30 mg/kg, on Day 2

Ten groups of rats (3 per group) were used.

AP, acclimatization period; MEL, melatonin.

..... - 245 -/

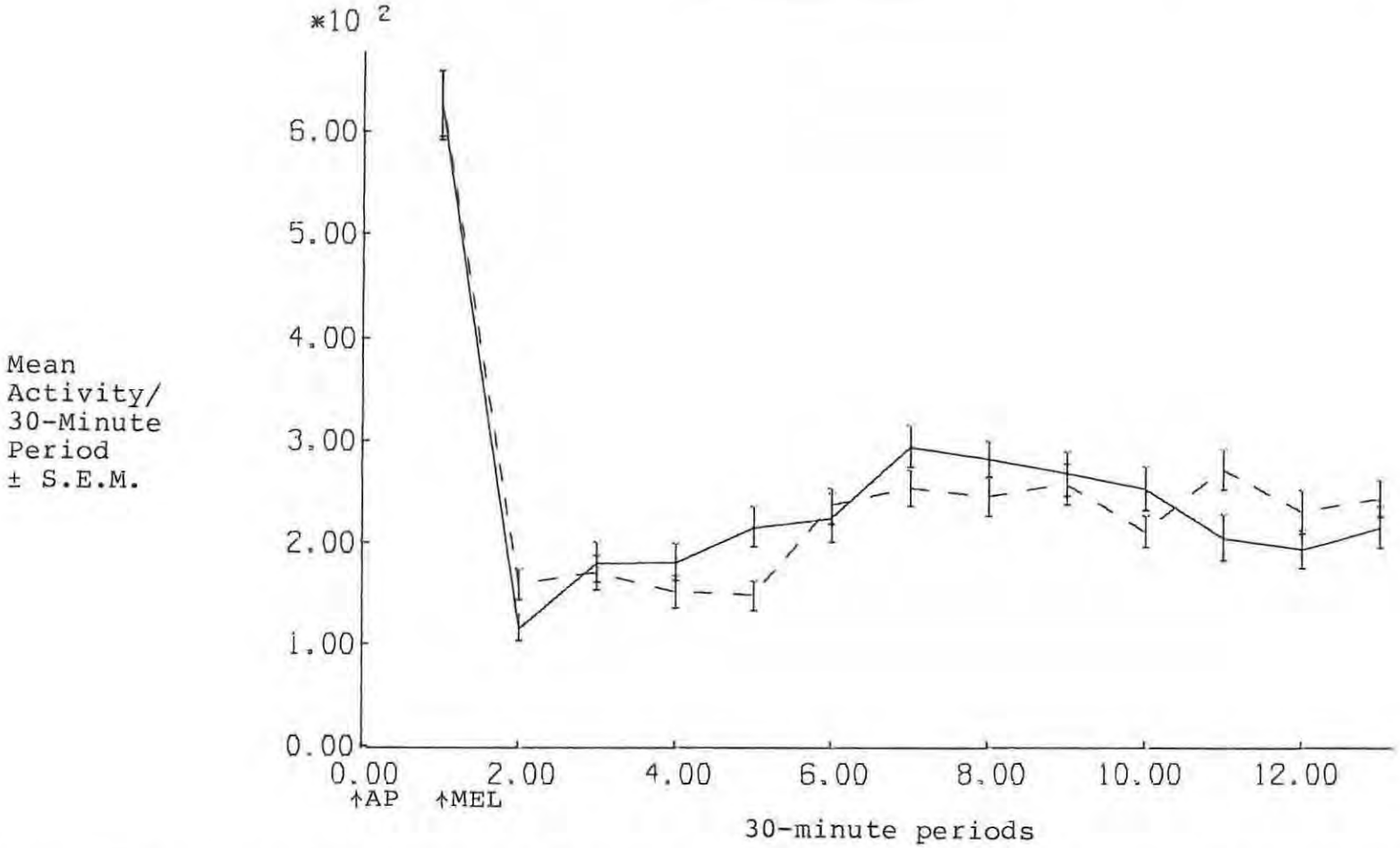


Fig. 30. The Effect of 100 mg/kg Melatonin on Rat Locomotor Activity over a Period of 6 Hours.

⊢—⊣ Control (melatonin, 100 mg/kg, on Day 1)
⊢---⊣ Melatonin, 100 mg/kg, on Day 2

Thirteen groups of rats (3 per group) were used.

AP, acclimatization period; MEL, melatonin.

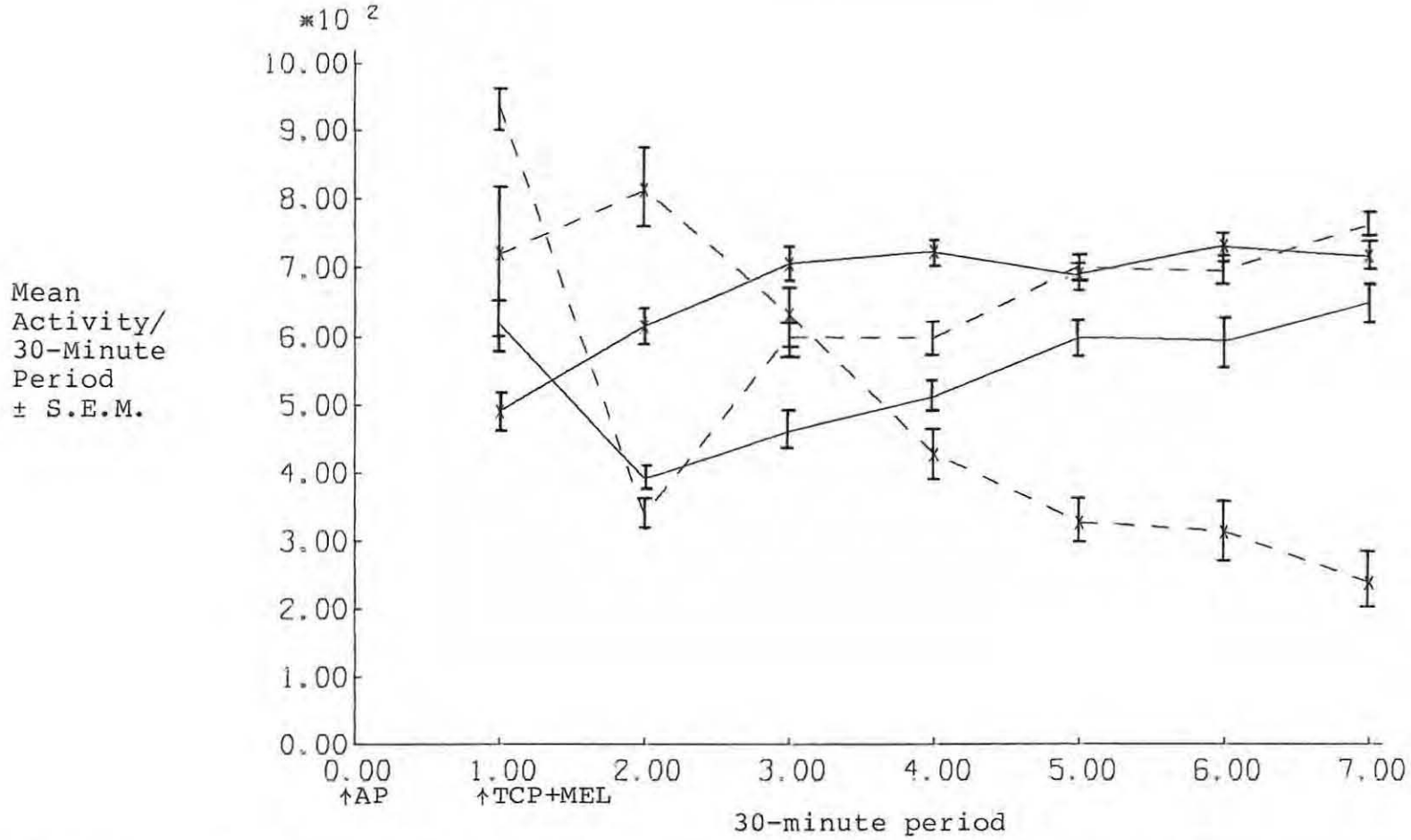


Fig. 31. Comparison Between the Effects of Various Doses of Melatonin and Tranylcypromine on Rat Locomotor Activity over a Period of 3 hours.

⊢—⊣ Melatonin 1 mg/kg (18)
×—× Melatonin 10 mg/kg (24)*
×--× Melatonin 100 mg/kg (18)
⊢--⊣ TCP 20 mg/kg (18)

All the rats were pretreated 24 hours previously with TCP (20 mg/kg). The numbers in brackets refer to the number of rats used.

AP, acclimatization period; MEL, melatonin; TCP, tranylcypromine.

*P < 0,05 as compared to the TCP-treated rats (Student's t-test).

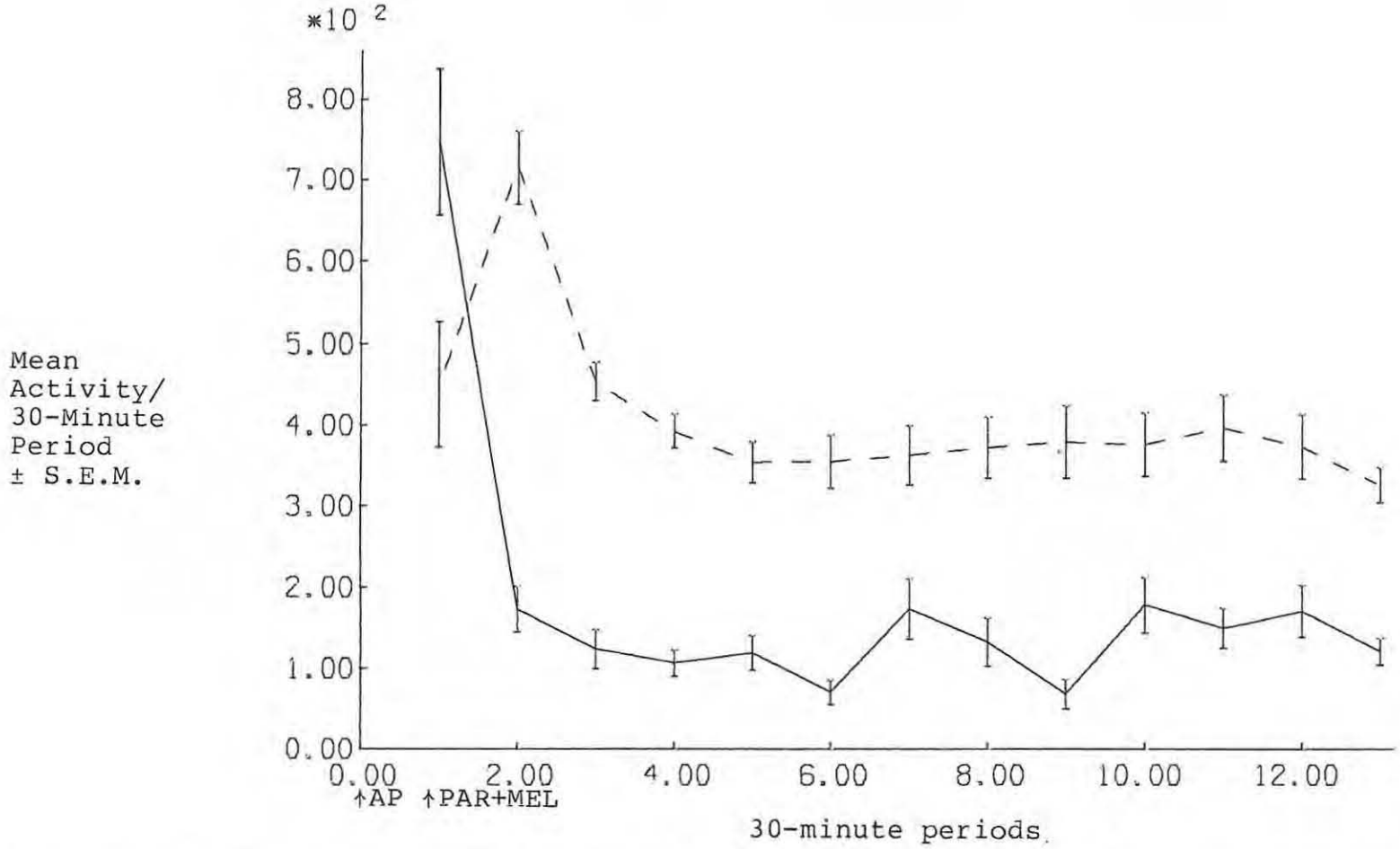


Fig. 32. The Effect of 100 mg/kg Melatonin on Rat Locomotor Activity over a Period of 6 Hours Following Pargyline Administration.

⊢—⊣ Control (Pargyline, 75 mg/kg)
⊢- -⊣ Pargyline, 75 mg/kg, followed by melatonin, 100 mg/kg, 30 minutes later.

Six groups of rats (3 per group) were used.

AP, acclimatization; MEL, melatonin; PAR, pargyline

$P < 0,05$ as compared to control (Wilcoxon matched-pairs signed-rank test).

..... - 248 -/

activity of rats pretreated with a MAO inhibitor in a similar dose-dependent manner.

Brain serotonin is presumed to be implicated in the observed hyperactivity although there is evidence to suggest that other central neurotransmitters may be involved (Section 1.4.6.1.1.2). Based on the proposal put forward by Grahame-Smith (1971a), these findings suggest that melatonin acts by increasing brain serotonin which results in the production of hyperactivity. This may either be by a direct (excess serotonin "spills over" and stimulates its receptors) or indirect (increased serotonin influences the dopaminergic neurons) mechanism.

Since tryptophan has been shown to have some antidepressive properties particularly in combination with a MAO inhibitor (Section 1.2.2) and in view of the fact that tryptophan (Grahame-Smith, 1971a) and melatonin (Anton-Tay et al., 1968) both increase serotonin brain levels and produce hyperactivity in rats following MAO inhibition, it is suggested that melatonin may have antidepressive properties.

C H A P T E R 4

MELATONIN AND A FROG MODEL OF DEPRESSION

4.1 INTRODUCTION:

Reserpine-induced sedation has been proposed as an animal analogue of depression in man. This sedation has been associated with decreased levels of noradrenaline, dopamine and serotonin in the brain (Brodie and Shore, 1957; Carlsson, 1961) but there is no equivocal opinion about which of these amines plays the most important role in the action of reserpine (Section 1.4.5). Since the mammalian brain contains more or less equal amounts of serotonin and noradrenaline (Bogdanski et al., 1963) and since reserpine and the tricyclic antidepressives affect both the catecholamine and serotonin brain levels, it is difficult to evaluate the effect of the drug on one specific amine. The amphibian brain, however, has been found to differ from other vertebrate brains in that it contains adrenaline instead of noradrenaline (Bogdanski et al., 1963; 1964; Brodie and Bogdanski, 1964) (Table 20). The reason for this is not understood. Although there is no data for the Xenopus species, it was assumed that they would not differ significantly from the Rana species, as it seems this pecularity occurs throughout the amphibian class. In addition, these authors found that the concentration of serotonin in the amphibian brain is high relative to that in mammals. This makes the frog an ideal species for the evaluation of the influence of antidepressives

TABLE 20:

DISTRIBUTION OF SEROTONIN AND CATECHOLAMINES IN VARIOUS SPECIES OF VERTEBRATES

(Adapted from Bogdanski et al., 1963 and Brodie and Bogdanski, 1964)

Species	Level of amine in brain (µg/g of tissue)		
	Serotonin	Adrenaline	Noradrenaline
MAMMAL			
Rabbit	0,67 (6)		0,60 (6)
Rat	0,48 (6)		0,49 (6)
BIRD			
Pigeon	0,70 (7)		0,38 (7)
Chicken	1,0 (3)		0,60 (3)
REPTILE			
Lizard - S. cyanogenys	3,1 (2)		1,6 (2)
AMPHIBIAN			
Frogs - R. pipiens	3,8 (18)	2,3 (21)	
- R. cinerea	2,1 (2)	0,94 (2)	
- R. temporaria*	—	0,84 (11)	<0,01 (9)
Toads - B. americanus	9,1 (4)	0,83 (2)	
B. marinus	1,5 (2)	2,4 (2)	
Salamander - Desmognathus	2,8 (1)	1,24 (1)	
A. tigrinum	2,9 (2)	0,75 (3)	
N. maculosus	1,1 (1)	0,39 (1)	
FISH			
C. auratus	0,15 (3)		0,49 (2)
A. calva	0,48 (1)		0,33 (1)

*Lapin et al., 1968.

Figures in brackets refer to the number of determinations.

— Comparative figures not available.

..... - 251 -/

on the serotoninergic system alone and the effects of this influence on the action of reserpine.

Brodie and Bogdanski (1964) have demonstrated that reserpine has a selective action on the serotonin stores of the frog brain (Rana pipiens). At 23° C, reserpine (100 mg/kg) depleted serotonin levels by 30%, whereas the content of brain adrenaline decreased by 15%. This was more pronounced at 37° C; serotonin stores were depleted by 70% while adrenaline brain levels diminished by 40%. In addition, pargyline was shown to cause a 38% rise in the level of brain serotonin with little or no rise in the adrenaline level.

Reserpine, even in high doses (50 mg/kg) was shown to have no visible effect in the frog (Brodie and Bogdanski, 1964; Brodie et al., 1964). However, after pretreatment with the MAO inhibitor, pargyline, a loss of the righting reflex was observed which demonstrated an enhancement of the sedative effect of reserpine. This sedative effect was thought to be due to the accumulation of free serotonin in the brain and any enhancement of this would indicate serotonin potentiation.

It has been proposed that the thymoleptic effect is mediated through the activation of the central serotoninergic processes (Carlsson et al., 1969; Lapin and Oxenkrug, 1969; Section 1.2.2). Consequently a drug which potentiates the sedative effect of reserpine after MAO inhibition may have a

..... - 252 -/

thymoleptic effect. The tricyclic antidepressives were shown to enhance the sedative action of reserpine in the frog model (Lapin et al., 1968; Lapin and Oxenkrug, 1970) which further supports this hypothesis.

Melatonin was, therefore, investigated in the frog model to evaluate its effect on the serotoninergic system and to determine any possible thymoleptic effect.

4.2 MATERIALS AND METHODS:

Male and female frogs (Xenopus laevis) weighing 20 to 45 g were used. These were housed in a glass tank (90 x 40 x 35 cm) containing water maintained at 23 - 25° C. Room temperature was 20 - 22° C. During the experiment the frogs were placed individually in plastic tanks (30 x 12 x 12 cm) containing 100 ml of water.

The water-soluble salts of imipramine, amitriptyline and pargyline were freshly dissolved in distilled water. Melatonin was dissolved in a solution containing 25% w/v polyethylene glycol 400. Reserpine was first dissolved in a small amount of glacial acetic acid (8% w/v) and then made up to volume with distilled water. All the drugs were injected into the supra femoral lymph sac. All the experiments were carried out at the same time of day starting at 09h00. Pargyline was administered two hours before, and imipramine, amitriptyline or melatonin thirty minutes before, reserpine administration. The righting

..... - 253 -/

reflex was tested 10 times in every frog. This was recorded before and for up to 6 hours after reserpine administration. Vocalizations and any twitches of the extremities were also registered.

4.3. RESULTS:

4.3.1 Effect of MAO Inhibition Followed by Reserpine Administration upon the Frog Righting Reflex:

In preliminary studies, low doses of pargyline had no apparent effect on the righting reflex of the frog. However, a transient sedative effect, which returned to normal after approximately 3 hours, was produced by higher doses (200 mg/kg and 300 mg/kg) of the drug. This effect was shown to be significantly altered by temperature ($p < 0,005$; Student's t-test). At 10° C, the frogs showed profound sedation, loss of the righting reflex and subsequent death, whereas at 23° C, the depression was less pronounced and of shorter duration (Table 21A).

Reserpine, in doses up to 50 mg/kg, did not elicit any sedative effect in the frog. Reserpine, 100 mg/kg, caused a slight loss of the righting reflex but this was not significant (Table 21B).

Sedation and a loss of the righting reflex were observed, however, when the frogs were dosed with reserpine 2 hours after pargyline pretreatment (Fig. 33). This

..... - 254 -/

TABLE 21A:

EFFECT OF PARGYLINE ADMINISTRATION ON THE FROG RIGHTING REFLEX AT 23°C AND 10°C

Temp. (° C)	Pargyline (mg/kg)	Number of Frogs	Number of rightings from 10 trials Mean±S.E.M.			
			1 hour	2 hours	3 hours	4 hours
23° C	200	22	8,0±0,6	9,8±0,1	10±0	10±0
	300	12	1,1±0,6*	2±1,1*	9,75±0,25	10±0
10° C	200	15	0±0*	0±0*	0±0*	DEAD
	300	5	0±0	0±0	0±0*	DEAD

*p < 0,005 when compared with pargyline, 200 mg/kg, at 23° C (Student's t-test).

TABLE 21B:

EFFECT OF VARIOUS DOSES OF RESERPINE ON THE FROG RIGHTING REFLEX

Reserpine (mg/kg)	Number of Frogs	Number of rightings from 10 trials Mean ± S.E.M.			
		2 hours	4 hours	6 hours	24 hours
12,5	5	10 ± 0	10 ± 0	10 ± 0	10 ± 0
25	5	10 ± 0	10 ± 0	10 ± 0	10 ± 0
50	5	9,8±0,2	9,2±0,6	10 ± 0	9,6±0,4
100	9	9 ± 0,5	8,8±1,2	8,8±1,2	9,2±0,8

..... - 255 -/

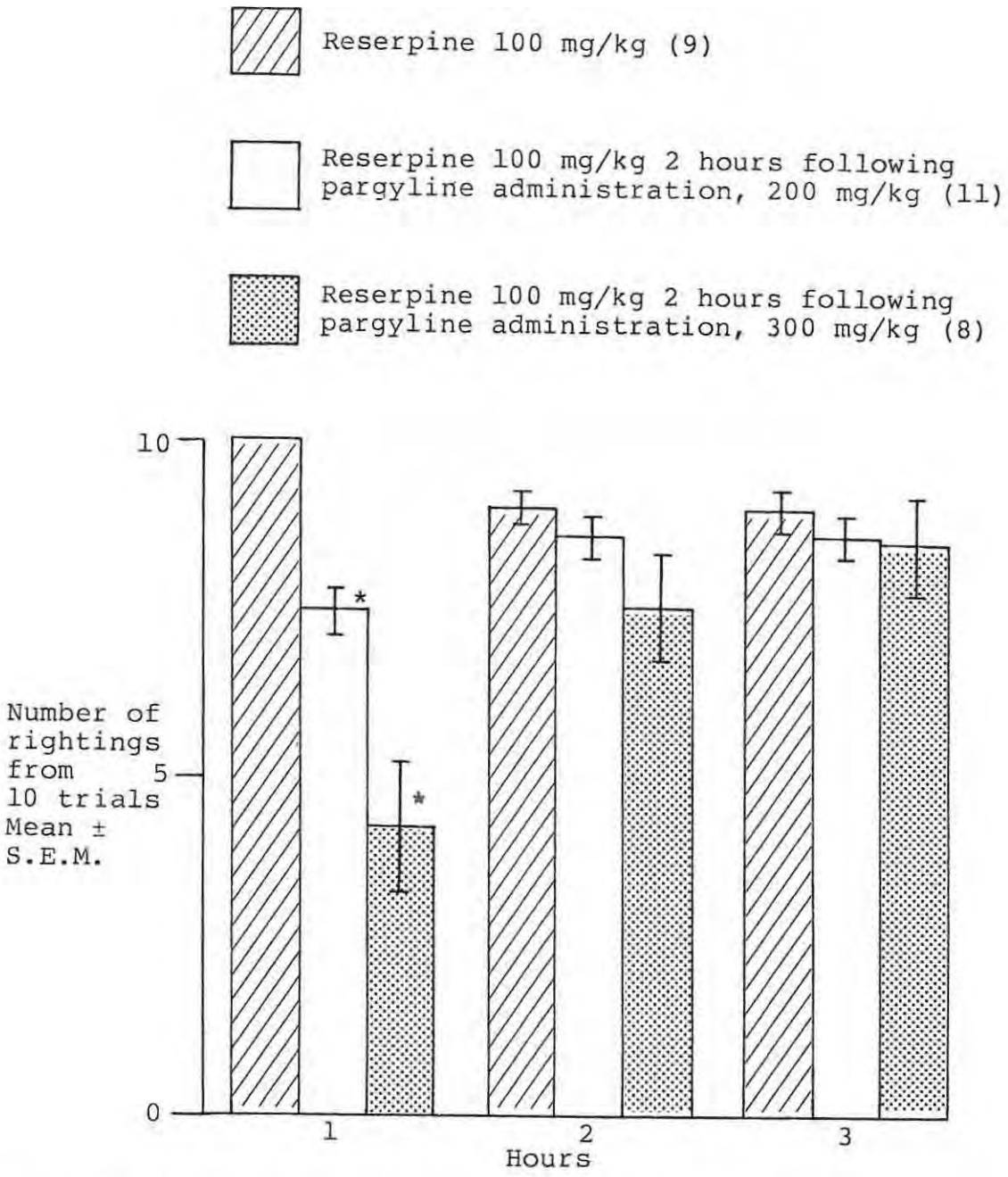


Fig. 33. The Effect of Reserpine on the Frog Righting Reflex Following Pargyline Pretreatment.

The figures in parentheses represent the number of frogs used in the trial.

*P < 0,005 when compared with reserpine-treated frogs (Student's t-test).

..... - 256 -/

potentiation was dose-dependent and most evident within the first hour after reserpine administration ($p < 0,005$; Student's t-test). The doses of reserpine and pargyline were then manipulated so as to give a very slight sedative effect and thus allow any synergism between the test drug and the pargyline-reserpine combination to be evaluated. Table 22 shows that concentrations of reserpine lower than 100 mg/kg had no appreciable effect upon the righting reflex after pargyline pretreatment. On the other hand, pargyline (300 mg/kg) and reserpine (100 mg/kg) exhibited a marked sedative effect within the first hour after drug administration ($p < 0,05$; Student's t-test) and therefore, pargyline (200 mg/kg) administered 2 hours before reserpine (100 mg/kg) appeared to be the most suitable dose regimen.

4.3.2 Enhancement of the Sedative Action of Reserpine by Imipramine, Amitriptyline and Melatonin:

Following pargyline pretreatment (200 mg/kg), imipramine (10 mg/kg) and amitriptyline (10 mg/kg) significantly enhanced the action of reserpine (100 mg/kg) (Fig. 34). The potentiation of the effect of reserpine was accompanied by the appearance of twitches of the extremities.

As presented in Table 23, various doses of melatonin (5 mg/kg, 10 mg/kg, 25 mg/kg and 50 mg/kg) alone, or in combination with reserpine (100 mg/kg), had no visible effect on the frog righting reflex, except in high doses (100 mg/kg). When melatonin was administered with reserpine

..... - 257 -/

TABLE 22:

THE EFFECT OF VARIOUS DOSES OF RESERPINE AND PARGYLINE ON THE FROG RIGHTING REFLEX

Pargyline (mg/kg)	Reserpine (mg/kg)	Number of Frogs	Number of rightings from 10 trials Mean ± S.E.M.				
			1 hour	2 hours	3 hours	4 hours	5 hours
200	10	4	10 ± 0	9,75 ± 0,3	0,5 ± 0,5	9,5 ± 0,5	9,75 ± 0,3
200	25	5	9 ± 0,8	10 ± 0	10 ± 0	9,2 ± 0,6	9,8 ± 0,2
200	50	5	10 ± 0	10 ± 0	10 ± 0	10 ± 0	10 ± 0
200	100	11	7,5 ± 0,8	8,6 ± 0,6	8,6 ± 0,6	8,5 ± 0,6	8,9 ± 0,6
300	50	4	10 ± 0	10 ± 0	10 ± 0	10 ± 0	10 ± 0
300	100	8	4,25 ± 2*	7,5 ± 1,6	8,5 ± 1,5	8,75 ± 1	9 ± 1

Reserpine was injected 2 hours after pargyline administration.

*$p < 0,05$ when compared with pargyline (200 mg/kg) followed by reserpine (100 mg/kg)

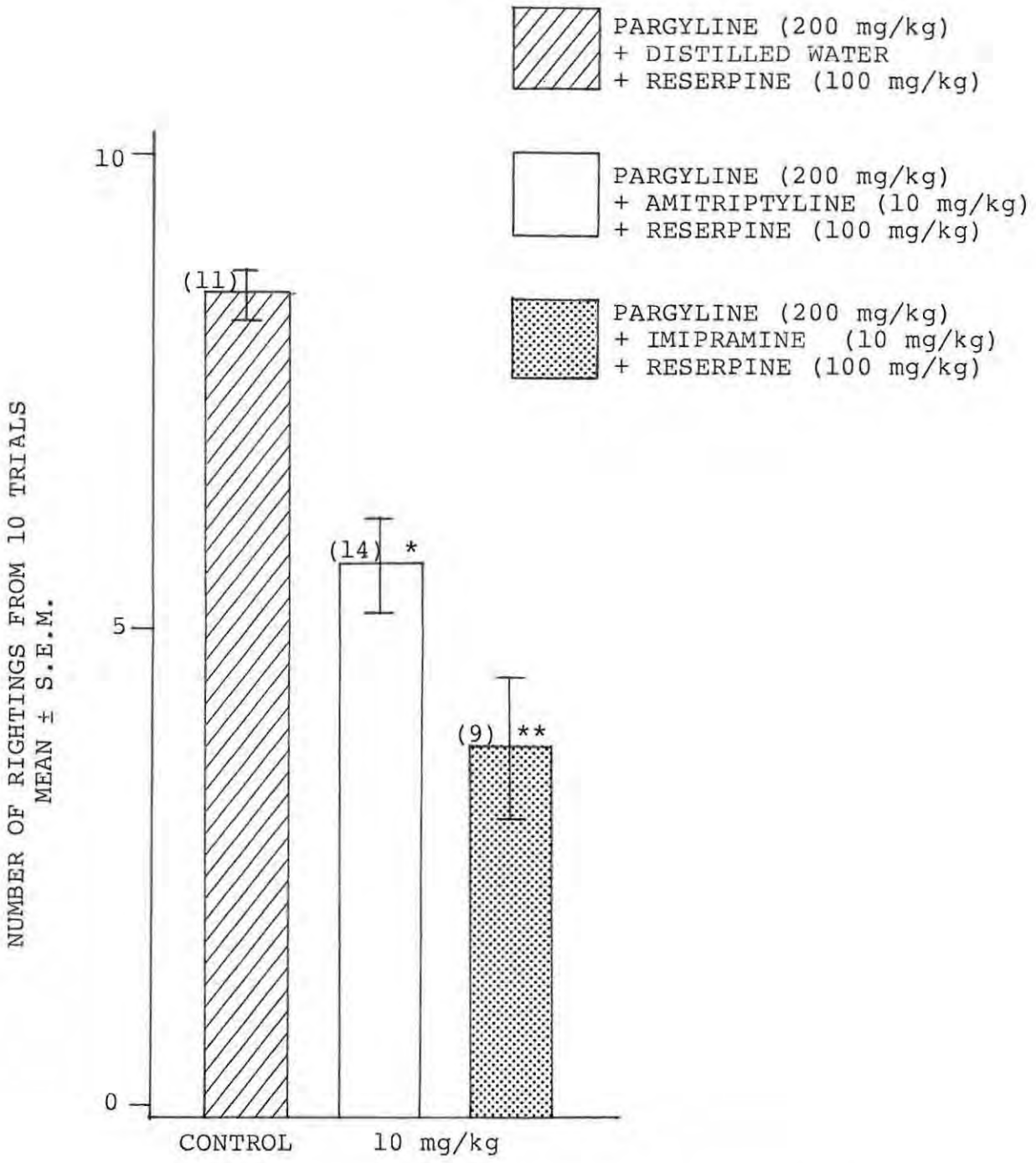


Fig 34. POTENTIATION OF THE SEDATIVE EFFECT OF RESERPINE BY IMIPRAMINE AND AMITRIPTYLINE.

The frogs were pretreated with pargyline 1,5 hours before the administration of the tricyclic drug. Reserpine was injected 30 minutes following tricyclic administration and the righting reflex was recorded two hours later.

The figures in brackets refer to the number of frogs used.

* $P < 0,05$ when compared with control (Student's t-test).

** $P < 0,001$ when compared with control (Student's t-test).

..... - 259 -/

TABLE 23:

ENHANCEMENT OF THE SEDATIVE ACTION OF RESERPINE BY VARIOUS DOSES OF MELATONIN

Drugs (mg/kg)	Number of frogs	1 hour Number of		5 hours Number of	
		rightings from 10 trials Mean ± S.E.M.	frogs lost righting reflex with twitches	rightings from 10 trials Mean ± S.E.M.	frogs lost righting reflex with twitches
Control PAR 200 + DW + Res 100	11	7,5 ± 0,8	0	8,9 ± 0,6	0
DW + mel 5 + Res 100	5	10 ± 0	0	10 ± 0	0
PAR 200 + mel 5 + Res 100	5	10 ± 0	0	10 ± 0	0
DW + mel 10 + DW	8	10 ± 0	0	10 ± 0	0
DW + mel 10 + Res 100	5	10 ± 0	0	10 ± 0	0
PAR 200 + mel 10 + Res 100	13	4,3 ± 1,2*	5	4,8 ± 1,4*	6
DW + mel 25 + Res 100	5	10 ± 0	0	10 ± 0	0
PAR 200 + mel 25 + Res 100	9	3,9 ± 1,5*	4	2,2 ± 1,5**	7
DW + mel 50 + Res 100	4	10 ± 0	0	10 ± 0	0
PAR 200 + mel 50 + Res 100	9	2,6 ± 1,0**	4	2,8 ± 1,2**	4
DW + mel 100 + DW	4	7,25 ± 1,4	0	9,3 ± 0,5	0
DW + mel 100 + Res 100	4	5,0 ± 1,1	0	7,0 ± 1,5	0
PAR 200 + mel 100 + Res 100	4	0 ± 0**	4	4,0 ± 1,8**	1

Differences from the control were determined statistically using the Student's t-test.

DW, distilled water; mel, melatonin; PAR, pargyline; Res, reserpine.

* $p < 0,05$ as compared to control.

** $p < 0,001$ as compared to control.

(100 mg/kg), following pargyline pretreatment (200 mg/kg) however, marked sedation and loss of the righting reflex were observed. This effect was significantly different from the control group (Table 23) and was shown to be dose-dependent (Fig. 35). A loss of the righting reflex was always accompanied by twitching of the extremities, similar to that observed with tricyclic antidepressive treatment.

4.4 DISCUSSION:

The effect of pargyline administration on the frog righting reflex was shown to be temperature dependent. This could be explained by the fact that MAO activity changes markedly at different temperatures. Brodie and Bogdanski (1964) demonstrated that at 2° C, the activity of MAO in the frog brain was 110±18 µg/g/hr, whereas at 37° C, the activity was 1218±150 µg/g/hr. Temperature is also an important variable in the action of reserpine, brain amines being released more readily at 37° C than at 23° C (Brodie et al., 1964).

The low turnover of serotonin in the frog brain (Brodie et al., 1964) could account for the failure of reserpine to elicit sedation by itself. However, when pretreated with pargyline, sedation and loss of the righting reflex occurred. This effect is associated with the selective release of brain serotonin and its protection from destruction by MAO inhibition.

..... - 261 -/

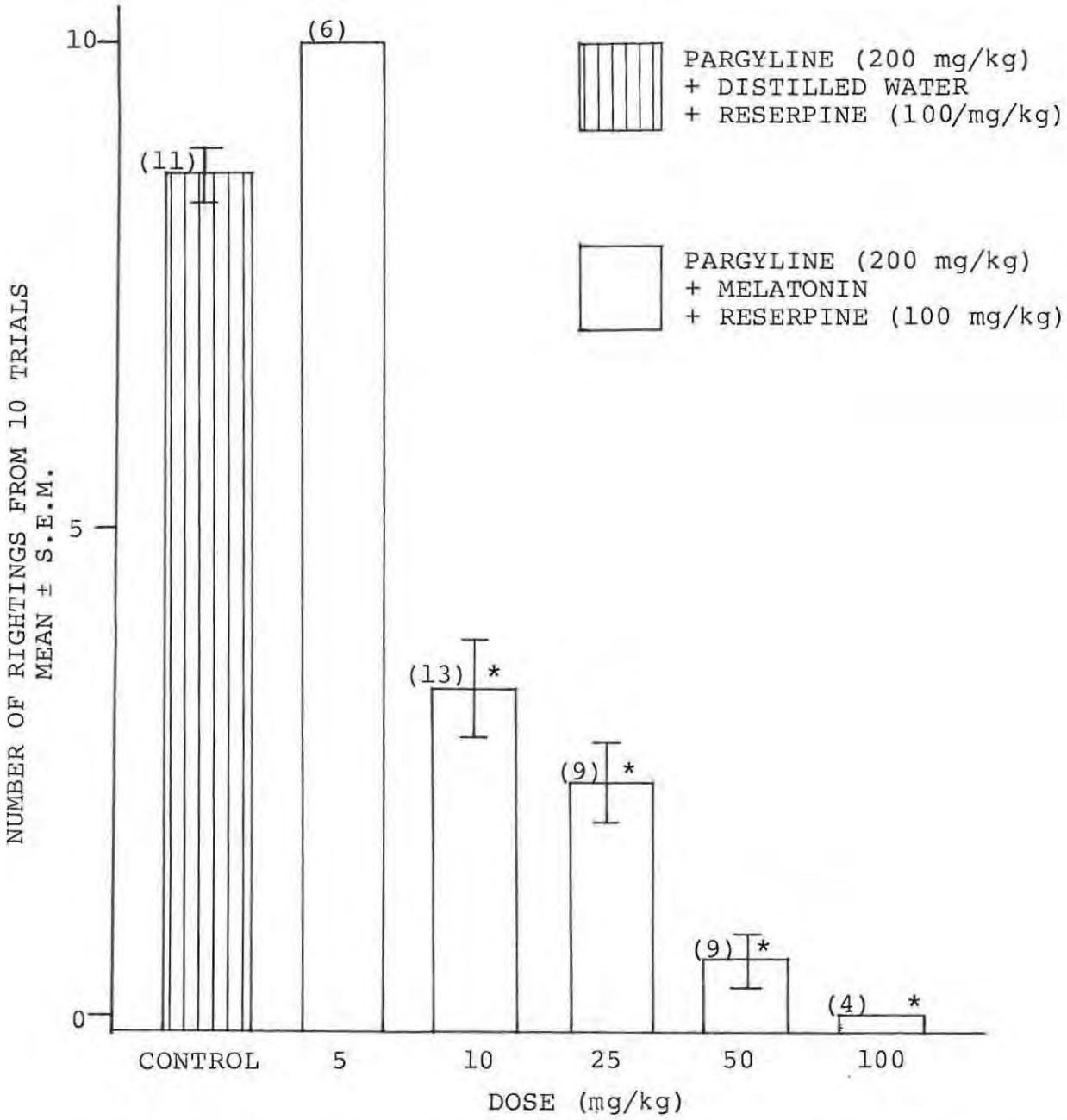


Fig. 35. POTENTIATION OF THE SEDATIVE EFFECT OF RESERPINE BY VARIOUS DOSES OF MELATONIN.

The frogs were pretreated with pargyline 1,5 hours before the administration of melatonin. Reserpine was injected 30 minutes following melatonin administration and the righting reflex was recorded two hours later.

The figures in brackets refer to the number of frogs used.

* $P < 0,001$ when compared with control (Student's t-test).

..... - 262 -/

Imipramine and amitriptyline enhanced the sedative action of reserpine after pargyline pretreatment. Both drugs are potent serotonin reuptake blockers (Table 7). Desipramine, however, being a relatively ineffective blocker of serotonin uptake, has previously been shown to be less effective in this model (Lapin et al., 1968). The potentiation of reserpine's action therefore is probably related to the inhibition of serotonin reuptake, which in turn, increases the level of free serotonin and causes sedation. The fact that melatonin administration also caused profound sedation and loss of the righting reflex in the test system could be explained by its reported effect on serotonin brain levels (Section 1.3.6.1).

Enhancement of the action of reserpine by melatonin and imipramine and amitriptyline was accompanied by twitches of the extremities. Head twitches have also been observed in mice after serotonin treatment (Corne et al., 1963). This suggests that both these syndromes may be due to the activation of the serotoninergic processes in the CNS. In addition, twitching has been shown to be blocked by the administration of the serotonin antagonist, BOL-148 (Lapin et al., 1968) which further supports this assumption. Twitching has thus been used to differentiate between drugs which have a non-specific effect on the frog righting reflex, such as chlorpromazine and benactyzine (Lapin and Oxenkrug, 1970) and drugs which potentiate serotoninergic effects.

..... - 263 -/

In conclusion, therefore, the results indicate that melatonin has a central serotoninergic potentiating effect in the frog which, in turn, could suggest a possible thymoleptic effect.

..... - 264 -/

C H A P T E R 5

THE PORSOLT MODEL

5.1 INTRODUCTION:

There is a need for an animal model of depression which is (1) similar to depression in man; (2) sensitive to the clinically effective antidepressives; and (3) based on behavioural as well as biochemical concepts (Section 1.4.1).

The helplessness and despair seen in animals that have been subjected to uncontrollable trauma ("learned helplessness" model), as well as the depressive-like behaviour induced in non-human primates by separation from either mothers or peers ("separation-loss" model) serve as convincing behavioural models of depression. These models are discussed in more detail in Sections 1.4.2 and 1.4.3. However, because of cost and practicability they cannot be used to screen for potential antidepressive drugs.

The Porsolt model (Porsolt et al., 1977) has attempted to overcome these limitations. In this test, rats are forced to swim in a situation from which they cannot escape and it has been proposed that the characteristic immobility which develops, may represent a state of depression and despair. In addition, drugs which are effective in the treatment of depression, have been reported to reduce the duration of this immobility (Porsolt et al., 1977; 1978). Consequently

the authors have suggested that the model could serve as a simple and inexpensive animal model of depression which would be useful in the evaluation of possible antidepressive drugs.

The tricyclic drugs, which are known to be effective in the treatment of depressive illness, were tested in the model in order to confirm the findings of Porsolt and his co-workers. Melatonin, a potential antidepressive, was also evaluated in this system.

5.2 MATERIALS AND METHODS:

5.2.1 Animals:

Male albino Wistar rats weighing between 200 and 300 g were used. They were housed in a constant temperature room, in single cages with free access to food and water.

5.2.2 Modifications of the Porsolt Model:

Two modifications to the Porsolt model were made.

(1) The depth of the water level was increased form 15 to 25 cm. In this study large rats (200 - 300 g), which were able to stand in 15 cm of water, were used and thus less expenditure of energy would have been required and a less stressful situation produced. Consequently the development and duration of the despair or immobility phase might have been affected.

..... - 266 -/

(2) When the rats were dosed with amphetamine, it was observed that some rats were active for the entire test period. Thus the test period was increased in order to obtain a quantitative measure of the duration of immobility. In addition, there is a brief burst of hyperactivity which frequently occurs after 7 to 8 minutes and this would have been undetected using a 5-minute test period. The 5-minute test period on Day 2 was extended to 10 minutes.

5.2.3 Induction and Measurement of Immobility:

All experiments were performed between 14h00 and 17h00. Naive rats were plunged individually into a glass tank (30 x 17 x 35 cm) containing water (depth: 25 cm) and maintained at a constant temperature of 25 - 27° C. After swimming for 15 minutes, they were removed, dried in an oven (30 - 32° C) for another 15 minutes and then returned to their original cages. This procedure was repeated 24 hours later (Day 2).

A rat was judged to be immobile in the water when all escape movements had ceased and it was floating passively, only making slight movements to keep its nose above the water. The duration of this immobility was measured during the first 10 minutes on both Day 1 and Day 2, each rat, therefore acting as his own control.

5.2.4 Drugs and Drug Treatment:

The water soluble salts of imipramine, desipramine,

amitriptyline and amphetamine were freshly dissolved in normal saline (0,9% w/v NaCl solution). Melatonin, depending on its concentration, was dissolved in various amounts of polyethylene glycol 400. The doses, expressed in terms of the salt, were given on a mg/kg basis. Control rats were given the vechile only.

The first dose of a given drug was administered intraperitoneally after the rat had been removed from the oven on Day 1, and then the dose was repeated 5 hours and 1 hour before the test period on Day 2.

5.3 RESULTS:

5.3.1 Swimming Behaviour without Drugs:

Many rats (n = 206) were tested in this experimental situation and a characteristic behavioural pattern emerged.

Rats which were placed in the water for the first time, showed an initial period of hyperactivity consisting of vigorous swimming, splashing at the sides, diving and frequent defaecation. This activity subsided after 2 to 3 minutes and a phase of headshaking and face wiping ensued. During this phase the rats' movements slowed down and periods of immoblity of increasing duration followed. This immobility was characterized by the animal floating in a vertical but hunched position, resting its tail on the bottom of the tank and making occasional movements to keep its nose above the water (Fig. 36). Frequently,

..... - 268 -/

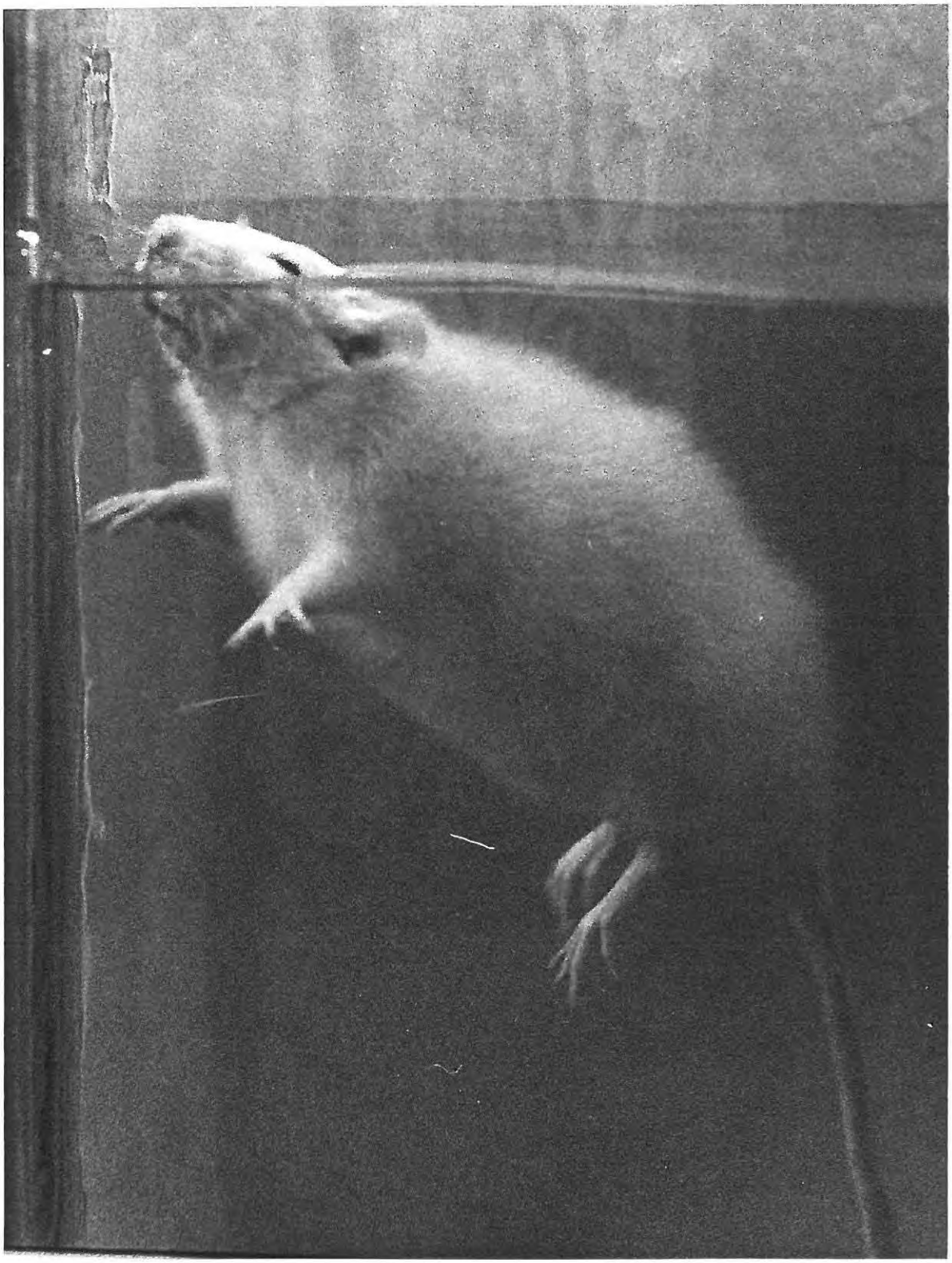


Fig. 36. Rat Showing the Characteristic Posture of Immobility.

..... - 269 -/

after 7 to 8 minutes, another brief period of hyperactivity was noted, perhaps as part of a final bid to escape. Upon removal from the water, the rats were hypothermic (-2° C) and hypoactive.

On Day 2, when the rats were retested in the water, a similar behavioural pattern was observed. The duration of immobility, however, was shown to increase significantly ($p < 0,001$; Wilcoxon matched-pairs signed-rank test). This is shown in Fig. 37. In addition, the amount of diving, apparently in search of an exit, was significantly reduced ($p < 0,001$; χ^2 test). A decrease in the amount of headshaking and defaecating was also noted, although this was not significant. This suggests that the rat, having been rescued on Day 1, may have adapted to the situation and be less fearful on Day 2.

In addition, different rats showed large variations in the duration of immobility and consequently, each rat was used as his own control to eliminate the high standard deviation which would have resulted.

5.3.2 Effects of Different Drugs on the Duration of Immobility:

5.3.2.1 Amphetamine:

Fig. 38 shows that amphetamine administration caused a dose-dependent reduction in the duration of immobility ($p < 0,001$; Dunnett test). The rats were generally hyperactive, splashing and diving for the major part of

..... - 270 -/

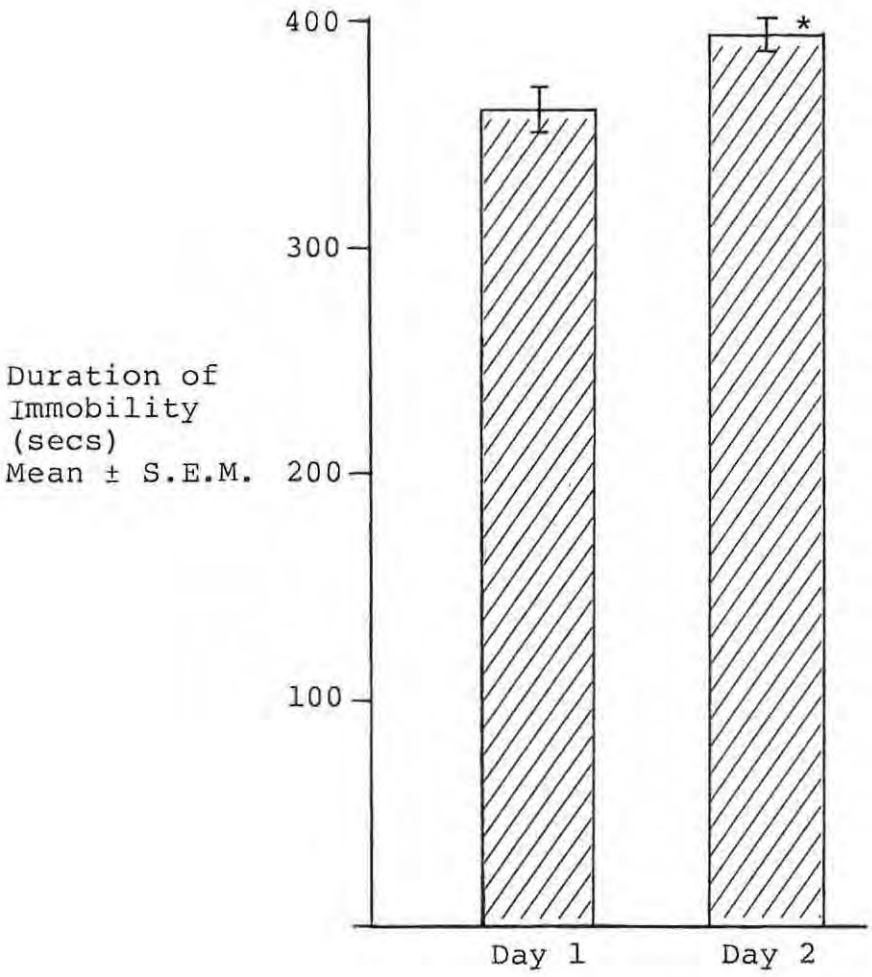


Fig. 37. Mean Duration of Rat Immobility on Day 1 and Day 2. On Day 1, rats (n=26) were placed in the water for the first time and the duration of immobility was measured during a 10-minute test period. This was repeated 24 hours later (Day 2).

*P < 0,001 (Wilcoxon matched-pairs signed-rank test).

..... - 271 -/

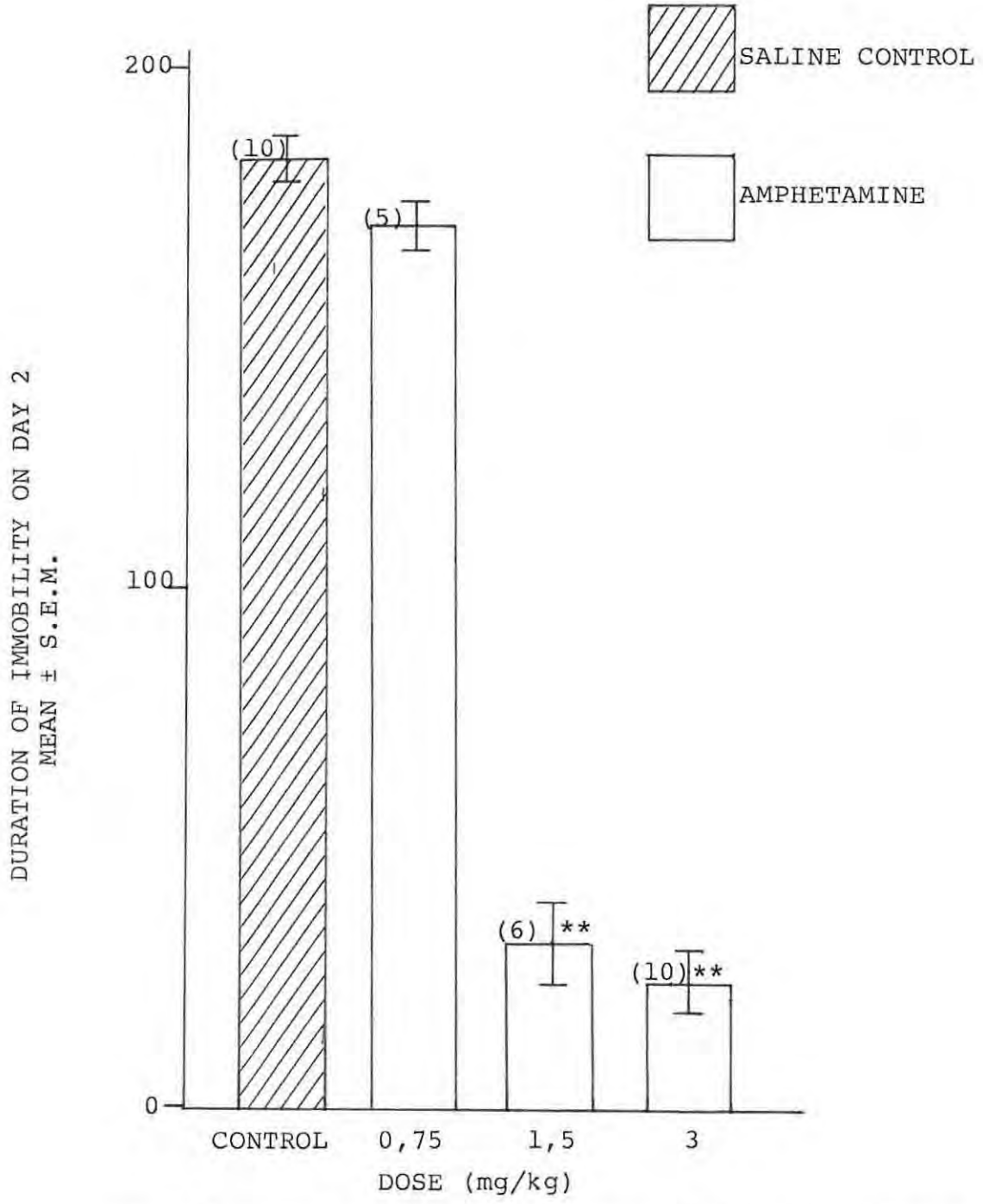


FIG. 38. THE EFFECT OF AMPHETAMINE ADMINISTRATION ON THE DURATION OF IMMOBILITY DURING A 5-MINUTE TEST PERIOD.

The figures in brackets refer to the number of rats used.

** $P < 0,001$ difference from control (Dunnett test).

..... - 272 -/

the test and stereotype head movements were observed at higher doses (1,5 mg/kg and 3,0 mg/kg). However, the stereotype behaviour is thought to be due to the drug's motor stimulating effect rather than to any possible anti-depressive effect.

5.3.2.2 Tricyclic Antidepressives:

Rats dosed with a tricyclic antidepressive showed a marked reduction in diving, headshaking and defaecation ($p < 0,001$ χ^2 test).

As presented in Fig. 39, desipramine (10 mg/kg and 20 mg/kg) and imipramine (15 mg/kg and 30 mg/kg) caused a significant reduction in the duration of immobility measured on Day 2. Amitriptyline was found to be less effective in this test, only reducing the immobility period at relatively high doses (30 mg/kg). In contrast to the effect of amphetamine administration, slow but continual movements of the limbs were observed with tricyclic antidepressive treatment.

5.3.2.3 Melatonin:

Although a wide range of concentrations were used (5 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg), melatonin was only found to significantly decrease the duration of immobility at a very high dose (200 mg/kg). This is shown in Fig. 40. The amount of diving, headshaking and defaecating, however, did not differ markedly from the control group.

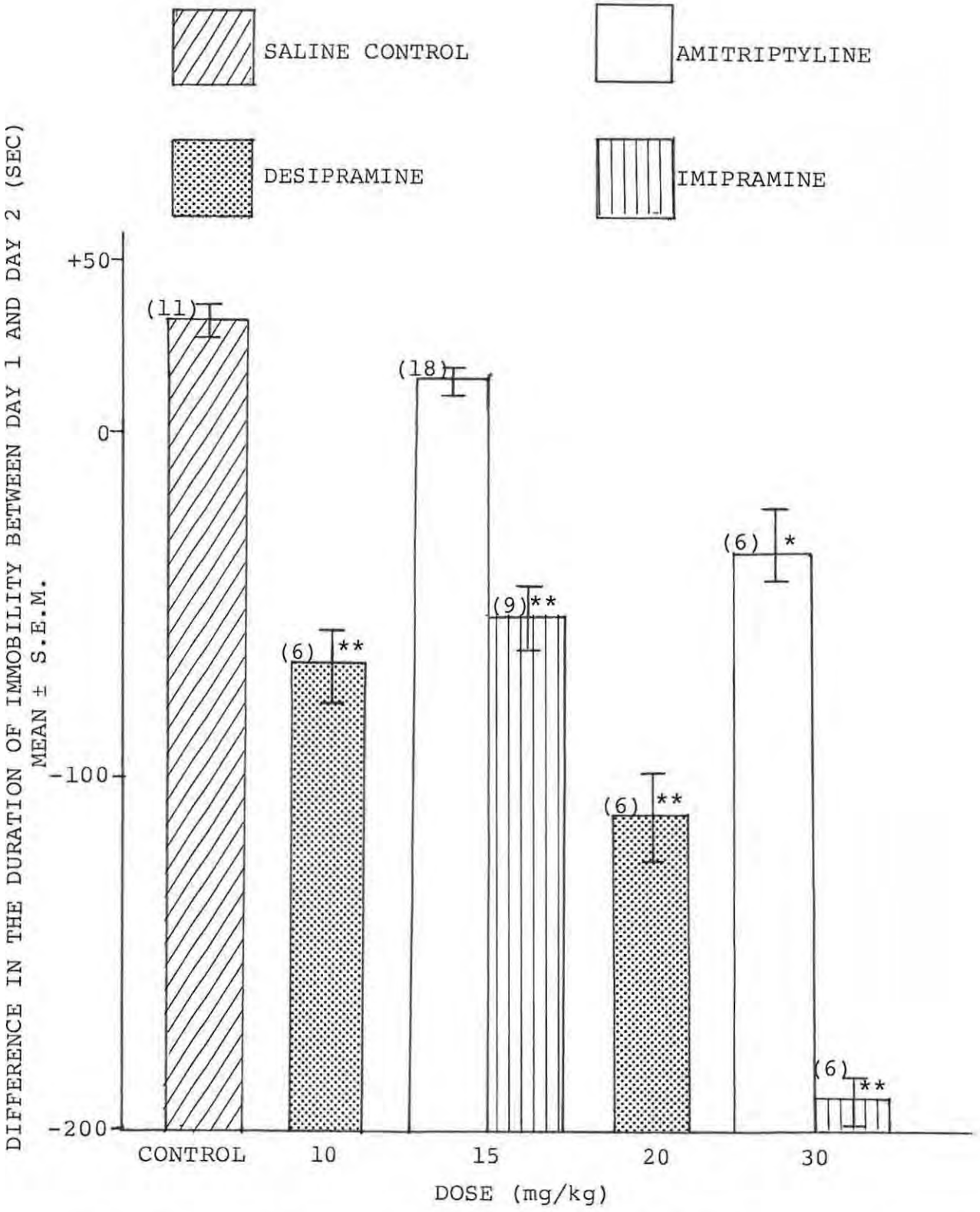


Fig. 39. THE EFFECT OF VARIOUS TRICYCLIC ANTIDEPRESSIVES ON THE DURATION OF IMMOBILITY DURING A 10-MINUTE TEST PERIOD.

The figures in brackets refer to the number of rats used.

* $P < 0,01$ difference from control (Student's t-test).

** $P < 0,001$ difference from control (Student's t-test).

..... - 274 -/

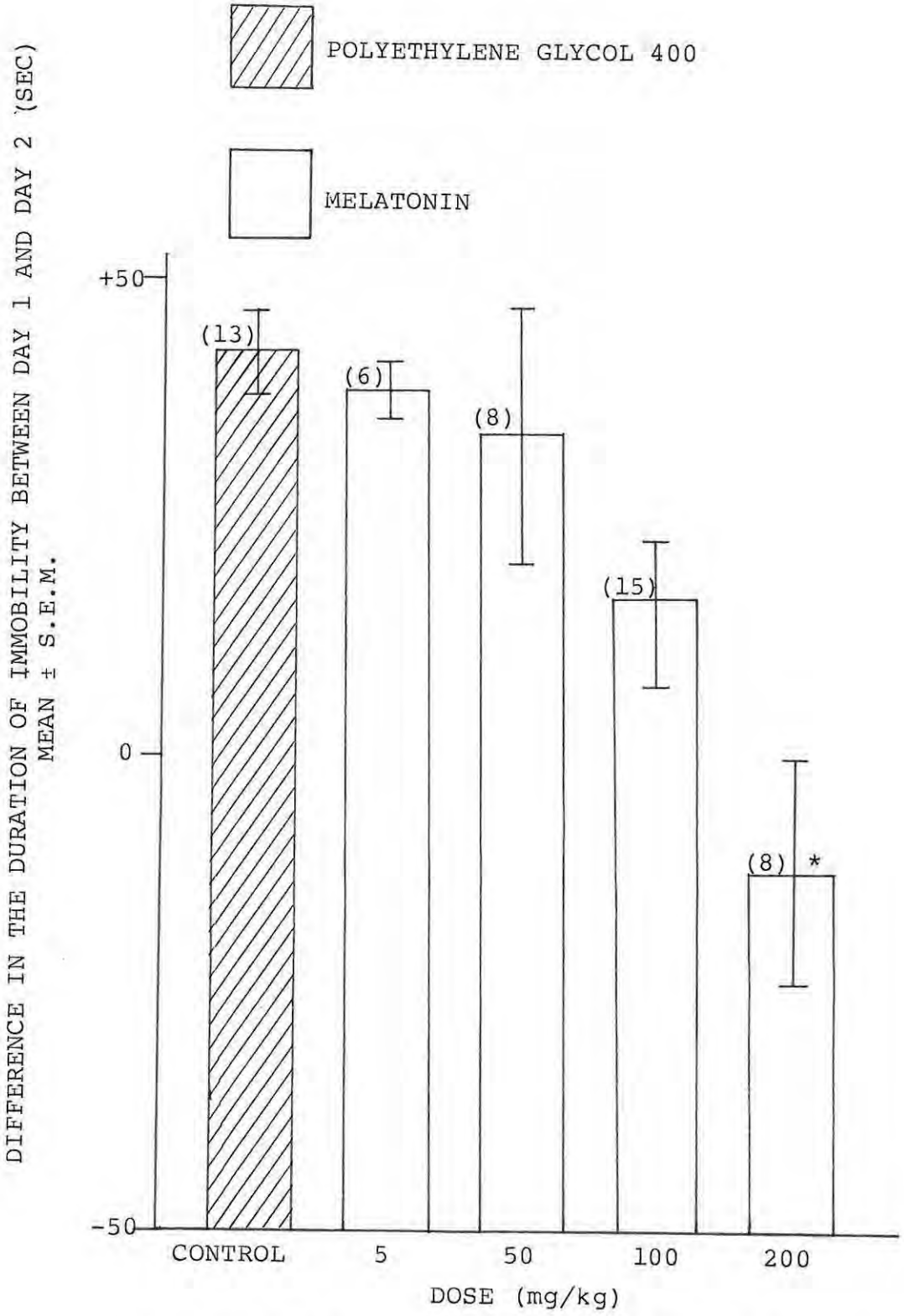


Fig. 40. THE EFFECT OF MELATONIN ON THE DURATION OF IMMOBILITY DURING A 10-MINUTE TEST PERIOD.

The figures in brackets refer to the number of rats used.

* $P < 0,05$ difference from control (Student's t-test).

..... - 275 -/

5.4 DISCUSSION:

The immobility induced in this experiment is thought to reflect a "state of lowered mood" in the rat (Porsolt et al., 1977; 1978) and thus it has been suggested that this model may be used to discover new antidepressive drugs.

The tricyclic antidepressives (desipramine, imipramine and amitriptyline) were shown to differ in their effects on the duration of immobility. Desipramine, a relatively selective noradrenaline reuptake blocker, was the most effective in reducing the duration of immobility. Imipramine which has approximately equal effects on noradrenaline and serotonin reuptake, was also active in the test at slightly higher concentrations. Amitriptyline, a relatively selective blocker of serotonin reuptake, only increased the swimming time at a relatively high concentration (30 mg/kg). Thus the stimulant tricyclic drugs appear to be more effective than the sedative tricyclics in this model. Likewise, melatonin which has been shown to exert a sedative effect in animals and humans, only had a significant effect on the duration of immobility at very high doses.

The above results suggest that a reduction in the duration of immobility may be due to the motor stimulating properties of a drug and not necessarily reflect an antidepressive effect. The fact that amphetamine, which has little antidepressive action, was active in this test further supports this assumption.

..... - 276 -/

Thus the Porsolt model may be useful in discovering "stimulant" antidepressives acting primarily on the catecholaminergic system but its effectiveness in detecting "sedative" antidepressives is debatable.

..... - 277 -/

C H A P T E R 6

SUMMARY

Chapter 1:

Numerous theories involving the aetiology of depressive disorders have been proposed. A deficiency in the concentration of serotonin at functionally important receptor sites has been implicated, although whether this is a primary defect or secondary to developmental and interpersonal events is not known. Melatonin, a possible antidepressive, was investigated in a number of behavioural models, since the indoleamine had previously been reported to increase serotonin levels in selective brain areas.

Chapter 2:

In a preliminary screening test using avoidance conditioning, high doses of melatonin were found to exert a similar effect to chlorpromazine administration on rat avoidance behaviour, causing suppression of the conditioned avoidance response.

Chapter 3:

Spontaneous rat locomotor activity was suppressed following melatonin administration. However, in rats pretreated with a MAO inhibitor, locomotor activity was significantly increased following the administration of melatonin 30 minutes later. This effect indicated a potentiation of the serotoninergic processes and was similar to that observed following the

..... - 278 -/

administration of an equivalent amount of dl-tryptophan.

Chapter 4:

In the frog model, following MAO inhibition, melatonin administration produced a loss of the righting reflex which was dose-dependent. Amitriptyline and imipramine, in similar doses, produced the same effect. The action of melatonin in this experimental situation again indicated a serotonin-like potentiating effect which was confirmed by the appearance of characteristic twitching of the extremities.

Chapter 5:

Melatonin, although only in high doses, also reduced the duration of immobility in the Porsolt model of depression, indicating a possible antidepressive effect.

The TADs were shown to differ in their effects when investigated in the 2 newer animal models of depression. The relatively selective serotonin reuptake blockers were more effective in the frog model whereas the noradrenaline reuptake inhibitors were more effective in the Porsolt model. These findings suggest that the Porsolt model may be useful in discovering stimulant antidepressives acting primarily on the catecholaminergic system, whereas the frog model may be used in detecting potential sedative antidepressives which act on the serotoninergic system.

..... - 279 -/

CONCLUSION:

The results show that melatonin has a serotonin-like potentiating effect in the behavioural models used and consequently may be a useful antidepressive. In view of its known sedative effect in humans, it is suggested that melatonin be investigated in a well-controlled clinical trial of patients suffering from "agitated" depressions in order to confirm these predictions from animal pharmacology.

..... - 280 -/

REFERENCES

ABRAHAM, K. Selected Papers of Karl Abraham. London, Hogarth Press, p. 137, 1948.

ABRAHAM, K., in Selected Papers on Psychoanalysis, New York, Basic Books, p. 137, 1960.

ACÉBAL, E., SUBIRÁ, S., SPATZ, J., FALENI, R., MERZBACHER, B., GALES, A. and MOIZESZOWICZ, J., Eur. J. Clin. Pharmacol. 10, 109 (1976).

ADAMS, R.D. and VICTOR, M. Principles of Neurology. New York, McGraw-Hill, 1977.

ADAMS, W.C., WAN, L. and SOHLER, A., J. Endocr. 31, 295 (1965).

AHLBORG, V., HOLMSTEDT, B. and LINDGREN, J.-E., Adv. Pharmacol. 6B, 213 (1968).

AKIMOTO, H., NAKAKUKI, M. and MACHIYAMA, Y., Dis. Nerv. Syst. 21, 645 (1960).

AKISKAL, H.S. and McKINNEY, W.T., Jr., Science 182, 20 (1973).

AKISKAL, H.S. and McKINNEY, W.T., Jr., Arch. Gen. Psychiat. 32, 285 (1975).

ALTSCHULE, M.D., New Engl. J. Med. 257, 919 (1957).

AMERICAN PSYCHIATRIC ASSOCIATION. Diagnostic and Statistical Manual of Mental Disorders. Second edition. Washington, D.C., The Association, 1968.

ANDERSEN, D.C., COOPER, A.F. and NAYLOR, G.J., Br. Med. J. 4, 744 (1968).

ANDERSON, W.McC. and DAWSON, J., Br. J. Psychiat. 109, 225 (1963).

ANGST, J., Monogr. Neurol. Psychiat. 112, 1 (1966).

ANGST, J., WEIS, P., GROF, P., BAASTRUP, P.C. and SCHOU, M., Br. J. Psychiat. 116, 604 (1970).

ANTON-TAY, F., in The Pineal Gland, eds. G.E.W. Wolstenholme and J. Knight, London, Churchill Livingstone, p. 213, 1971.

ANTON-TAY, F., in Advances in Biochemical Psychopharmacology, eds. E. Costa, G.L. Gessa and M. Sandler, New York, Raven Press, vol. 11, p. 315, 1974.

ANTON-TAY, F. and FERNÁNDEZ-GUARDIOLA, A.,(1976), cited in Watson, S.J. and Madden, J., in Neuroregulators and Psychiatric Disorders, eds. E. Usdin, D.A. Hamburg and J.D. Barchas, New York, Oxford University Press, p. 196, 1977.

ANTON-TAY, F. and WURTMAN, R.J., Nature 221, 474 (1969).

ANTON-TAY, F., CHOU, C., ANTON, S. and WURTMAN, R.J., Science 162, 277 (1968).

ANTON-TAY, F., SEPULVEDA, J. and GONZÁLES, S., Life Sci. 9, 1283 (1970).

ANTON-TAY, F., DÍAZ, J.L. and FERNÁNDEZ-GUARDIOLA, A., Life Sci. 10, 841 (1971).

ARUTYUNYAN, G.S., MASHKOVSKII, M.D. and ROSHCHINA, L.F., Fedn. Proc. 23, T1330 (1964).

ÅSBERG, M., BERTILSSON, L., TUCK, D., CRONHOLM, B. and SJÖQVIST, F., Clin. Pharmacol. Ther. 14, 277 (1973).

ÅSBERG, M., THOREN, P. TRÄSKMAN, L., BERTILSSON, L. and RINGBERGER, V., Science 191, 478 (1976).

ASHCROFT, G.W., Lancet 2, 573 (1972).

ASHCROFT, G.W., CRAWFORD, T.B.B., ECCLESTON, D. SHARMAN, D.F., McDOUGALL, E.J., STANTON, J.B. and BINNS, J.K., Lancet 2, 1049 (1966).

ASHCROFT, G.W., CRAWFORD, T.B.B., CUNDALL, R.L., DAVIDSON, D.L., DOBSON, J., DOW, R.C., ECCLESTON, D., LOOSE, R.W. and PULLAR, I.A., Psychol. Med. 3, 326 (1973a).

ASHCROFT, G.W., BLACKBURN, I.M., ECCLESTON, D., GLEN, A.I.M., HARTLEY, W., KINLOCH, N.E., LONERGAN, M., MURRAY, L.G. and PULLAR, I.A., Psychol. Med. 3, 319 (1973b).

AXELROD, J., in The Pineal Gland, eds. G.E.W. Wolstenholme and J. Knight, London, Churchill Livingstone, p.371, 1971.

AXELROD, J., Semin. Psychiat. 4, 199 (1972).

AXELROD, J. and INSCOE, J.K., J. Pharm. exp. Ther. 141, 161 (1963).

AXELROD, J. and INSCOE, J.K., J. Pharmacol. 141, 101 (1965).

AXELROD, J. and WEISSBACH, H., J. Biol. Chem. 236, 211 (1961).

AXELROD, J., MacLEAN, P.D., ALBERS, R.W. and WEISSBACH, H., in Regional Neurochemistry, eds. S.S. Kety and J. Elkes, Oxford, Pergamon Press, p.307, 1961a.

..... - 282 -/

AXELROD, J., WHITBY, L.G. and HERTTING, G., Science 133, 383, (1961b).

AXELROD, J., WURTMAN, R.J. and SNYDER, S.H., J. Biol. Chem. 240, 949 (1965).

AXELROD, J., SHEIN, H. and WURTMAN, R.J., Proc natl. Acad. Sci. U.S.A. 62, 544 (1969).

AYD, F.J., Jr. Recognizing the Depressed Patient. New York, Grune and Stratton, 1961.

AYD, F.J., Jr., Dis. Nerv. Syst. 30, 818 (1969).

BAASTRUP, P.C., Compreh. Psychiat. 5, 396 (1964).

BAASTRUP, P.C. and SCHOU, M., Arch. Gen. Psychiat. 16, 162, (1967).

BACKSTROM, M. and WETTERBERG, L., Acta Physiol. Scand. 87, 113 (1973).

BAER, L., DURELL, J., BUNNEY, W.E., Jr., LEVY, R.B. and CARDON, P.V., J. Psychiat. Res. 6, 289 (1969).

BAER, L., DURELL, J., BUNNEY, W.E., Jr., LEVY, B.S., MURPHY, D.L., GREENSPAN, G.R. and CARDON, P.V., Arch. Gen. Psychiat. 22, 40 (1970).

BAKER, M., DORZAB, J., WINOKUR, G. and CADORET, R., Arch. Gen. Psychiat. 27, 320 (1972).

BALDESSARINI, R.J., in Handbook of Neuropsychopharmacology, eds. L.L. Iversen, S. Iversen and S.H. Snyder, New York, Plenum Press, 1975.

BALDESSARINI, R.J. and LIPINSKI, J.F., Ann. Intern. Med. 83, 527 (1975).

BALDESSARINI, R.J. and YORKE, C., Nature 228, 1301 (1970).

BAN, T.A., Psychosomatics 18, 59 (1977).

BANERJEE, S.P., KUNG, L.S., RIGGI, S.J. and CHANDA, S.K., Nature 271, 380 (1977).

BARCHAS, J.D., DA COSTA, F. and SPECTOR, S., Nature 214, 919 (1967).

BARCHAS, J.D., CONNER, R., LEVINE, S. and VERNIKOS-DANELLIS, J., Experientia 25, 413 (1969).

BARRATT, E.S., CRESON, D.L. and RUSSELL, G., Am. J. Psychiat. 125, 530 (1968).

BARTONIČEK, V., Medna. Pharmacol. exp. 14, 365 (1966).

BASCHIERI, L., DE LUCA, F., CRAMAROSSA, L., DE MARTINO, C., OLIVERIO, A. and NEGRI, M., Experientia 19, 15 (1963).

BECK, A.T. Depression: Clinical, Experimental and Theoretical Aspects. New York, Harper and Row, 1967.

BECK, A.T., Behavior Therapy 1, 184 (1970).

BECK, A.T. The Diagnosis and Management of Depression. Second edition. Philadelphia, University of Pennsylvania Press, 1973.

BECKMANN, H. and GOODWIN, F.K., Arch. Gen. Psychiat. 32, 17 (1975).

BEHREND, E.R. and BITTERMAN, M.E., J. exp. Anal. Beh. 13, 229 (1963).

BENESOVÁ, O. and BENES, V., J. Pharmacol. 5 (suppl. 2), 7 (1974).

BERTILSSON, L. and SJÖQVIST, F., Pharmacol. Bull. 9, 35 (1973).

BERTLER, A., FALCK, B., OWMAN, C. and ROSENGRENN, E., Pharmacol. Rev. 18, 369 (1966).

BESSER, G.M., Br. Med. J. 3, 613 (1974).

BIBRING, E., in Affective Disorders, ed. P. Greenacre, New York, International Universities Press, p.13, 1965.

BIEGEL, A. and MURPHY, D., Arch. Gen. Psychiat. 24, 215 (1971).

BIEL, J.H., NUHFER, P.A., HOYA, W.H. and LEISER, H.A., Ann. N. Y. Acad. Sci. 96, 251 (1962).

BIGELOW, L.B., Biol. Psychiat. 8, 5 (1974).

BIRKMAYER, W. and NEUMAYER, E., Nervenarzt 43, 76 (1972).

BIVENS, C.H., LEBOVITZ, H.E. and FELDMAN, J.M., New Engl. J. Med. 289, 236 (1973).

BLACKWELL, B., Lancet 2, 849 (1963).

BLACKWELL, B. and MABBITT, L.A., Lancet 1, 938 (1965).

BLACKWELL, B. and SHEPHERD, M., Lancet 1, 968 (1968).

BLEULER, M., (1954), cited in Itil, T.M., in Hormones, Behaviour and Psychopathology, ed. E.J. Sachar, New York, Raven Press, p.31, 1976.

BLISS, E.L., WILSON, V.B. and ZWANZIGER, J., J. Psychiat. Res. 4, 59 (1966).

..... - 284 -/

BOGDANSKI, D.F., SULSER, F. and BRODIE, B.B., J. Pharm. exp. Ther. 132, 176 (1961).

BOGDANSKI, D.F., BONOMI, L. and BRODIE, B.B., Life Sci. 1, 80 (1963).

BOGDANSKI, D.F., TISSAN, A. and BRODIE, B.B., in Psychopharmacology: A Review of Progress 1957-1967, ed. D.Efron, Washington, D.C., U.S. Government Printing Office, p. 17, 1968.

BOND, P.A., JENNER, F.A. and SAMPSON, G.A., Psychol. Med. 2, 81 (1972).

BORGE, F., BUCHSBAUM, M., GOODWIN, F., MURPHY, D. and SILVERMAN, J., Arch. Gen. Psychiat. 24, 501 (1971).

BORMAN, M.C. and SCHMALLENBERG, H.C., J. Am. med. Assoc. 146, 337 (1951).

BOURNE, H.R., BUNNEY, W.E., Jr., COLBURN, R.W., DAVIS, J.M., DAVIS, J.N., SHAW, D.M. and COPPEN, A.J., Lancet 2, 805 (1968).

BOWDEN, D. and McKINNEY, W.T., Jr., Develop. Psychobiol. 5, 353 (1972).

BOWERS, C.Y., REDDING, T.W. and SCHALLY, A.V., Endocrinology 74, 559 (1964).

BOWERS, M.B. Jr., Neuropharmacology 9, 599 (1970).

BOWERS, M.B., Jr., Neuropharmacology 11, 101 (1972a).

BOWERS, M.B., Jr., Psychopharmacology (Berl.) 23, 26 (1972b).

BOWERS, M.B., Jr. and GERBODE, F.A., Nature 219, 1256 (1968).

BOWERS, M.B.,Jr., GOODMAN, E. and SIM, V.M., J. Nerv. Ment. Dis. 138, 383 (1964).

BOWERS, M.B.,Jr., HENINGER, G.R. and GERBODE, F.A., Int. J. Neuropharmacol. 8, 255 (1969).

BOWLBY, J., Psychoanal. Study Child 15, 9 (1960).

BOWLBY, J. Separation: Anxiety and Anger. New York, Basic Books, 1973.

BRIANT, R.H. and GEORGE, C.F., Br. J. Clin. Pharmacol. 2, 94 (1975),

BRIGGS, M., Ad. Steroid Biochem. Pharmacol. 5, 65 (1976).

British Medical Journal, 1969.

BROADHURST, A.D., Lancet 1, 1392 (1970).

..... - 285 -/

BRODIE, B.B., in The Scientific Basis of Drug Therapy in Psychiatry, Oxford, Pergamon Press, 1965.

BRODIE, B.B. and BOGDANSKI, D.F., Prog. Brain Res. 9 234 (1964).

BRODIE, B.B. and REID, W.D., Adv. Pharmacol. 6B, 97 (1968).

BRODIE, B.B. and SHORE, P.A., Ann. N.Y. Acad. Sci. 66, 631 (1957).

BRODIE, B.B., DICK, P., KIELHOLZ, P., PÖLDINGER, W. and THEOBALD, W., Psychopharmacologia (Berl.) 2, 467 (1961).

BRODIE, B.B., BOGDANSKI, D.F. and BONOMI, L., in Comparative Neurochemistry, ed. D. Richter, London, Pergamon Press, p.367, 1964.

BRODIE, B.B., COMER, M.S., COSTA, E. and DLABAC, A., J. Pharm. exp. Ther. 152, 340 (1966).

BRODIE, H.K.H., SACK, R. and SIEVER, L., in Serotonin and Behaviour, eds. J.D. Barchas and E. Usdin, New York, Academic Press, p.549, 1973.

BROOKSBANK, B.W.L., BRAMMALL, M.A., CUNNINGHAM, A.E., SHAW, D.M. and CAMPS, F.E., Psychol. Med. 2, 56 (1972).

BROOKSBANK, B.W.L., BRAMMALL, M.A. and SHAW, D.M., Steroids Lipids Res. 4, 162 (1973).

BROOKSBANK, B.W.L. and COPPEN, A., Br. J. Psychiat. 113, 395 (1967).

BROVERMANN, D.M., KLAIBER, E.L., KOBAYASHI, Y., Psychol. Rev. 75, 23 (1968).

BROWN, B.B., Arch. int. Pharmacodyn. Ther. 128, 391 (1960).

BROWN, R.R., ROSE, D.P., PRICE, J.M. and WOLF, H., Ann. N.Y. Acad. Sci. 166, 44 (1969).

BRUINVELS, J., Eur. J. Pharmacol. 20, 231 (1972).

BRUSH, F.R., J. Comp. Physiol. Psychol. 50, 547 (1957).

BUCHSBAUM, M., GOODWIN, F., MURPHY, D. and BORGE, G., Am. J. Psychiat. 128, 19 (1971).

BUNNEY, W.E., Jr. and DAVIS, J.M., Arch. Gen. Psychiat. 13, 483 (1965).

BUNNEY, W.E., Jr., DAVIS, J.M., WEIL-MALHERBE, H. and SMITH, E.R.B., Arch. Gen. Psychiat. 16, 448 (1967).

BUNNEY, W.E., Jr., DAVIS, J.M., CARPENTER, W.T. and ENGLEMAN, K., (1969), cited in Coppen, A., J. Psychiat. Res. 9, 163 (1972).

..... - 286 -/

BUNNEY, W.E., Jr., MURPHY, D.L. and GOODWIN, F.K., Lancet 2, 1022 (1970).

BUNNEY, W.E., Jr., BRODIE, H.K.H., MURPHY, D.L. and GOODWIN, F.K., Am. J. Psychiat. 127, 872 (1971).

BUNNEY, W.E., Jr., GOODWIN, F.K., MURPHY, D.L., HOUSE, K.M. and GORDON, E.K., Arch. Gen. Psychiat. 27, 304 (1972a).

BUNNEY, W.E., Jr., GOODWIN, F.K. and MURPHY, D.L., Arch. Gen. Psychiat. 27, 312 (1972b).

BURGERMEISTER, J.J., DICK, P., GARRONE, G., GUGGISBERG, M. and TISSOT, R., Pr. Med. 71, 1116 (1963).

BURGESS, C.D., TURNER, P. and WADSWORTH, J., Br. J. Clin. Pharmacol. 5 , 215 (1978).

BURGESS, E., in Advances in Behaviour Therapy, eds, R. Rubin and C. Frank, New York, Academic Press, 1968.

BUTCHER, L.L. and ENGEL, J., Brain Res. 15, 233 (1967).

BUTLER, P.W.P. and BESSER, G.M., Lancet 2, 1234 (1968).

CADE, J.F., Med. J. Australia 1, 195 (1964).

CADORET, R., WINOKUR, G. and CLAYTON, P., Br. J. Psychiat. 116, 625 (1970).

CAFFEY, E.M., Jr., HOLLISTER, L.E., KLETT, C.J. and KAIRN, S.C., in Principles of Psychopharmacology, eds. W.G. Clark and del Giudice, J., New York, Academic Press, p. 429, 1970.

CAMPBELL, J.D. Manic-Depressive Disease. Philadelphia, Lippincott, 1953.

CARDINALI, D.P., Neuroendocrinology 19, 91 (1975).

CARDINALI, D.P. and FREIRE, F., (1975), cited in Cardinali, D.P., Nagle, C.A., Friere, F. and Rosner, J.M., Neuroendocrinology 18, 72 (1975).

CARDINALI, D.P. and ROSNER, J.M., Endocrinology 89, 301 (1971).

CARDINALI, D.P., LYNCH, H.J. and WURTMAN, R.J., Endocrinology 91, 1213 (1972).

CARDINALI, D.P., HYYPPA, M.T. and WURTMAN, R.J., Neuroendocrinology 12, 30 (1973).

CARDINALI, D.P., NAGLE, C.A. and ROSNER, J.M., Hormone Res. 5, 304 (1974a).

..... - 287 -/

CARDINALI, D.P., NAGLE, C.A. and ROSNER, J.M., Neuroendocrinology 16, 74 (1974b).

CARDINALI, D.P., NAGLE, C.A. and ROSNER, J.M., Life Sci. 16, 93 (1975a).

CARDINALI, D.P., NAGLE, C.A., FREIRE, F. and ROSNER, J.M., Neuroendocrinology 18, 72 (1975b).

CARLSSON, A., Neuropsychopharmacology 2, 417 (1961).

CARLSSON, A., Pharmacol. Rev. 18, 541 (1966).

CARLSSON, A., Pharmakopsychiat. 9, 2 (1976).

CARLSSON, A., LINDQVIST, M. and MAGNUSSON, T., Nature 180, 1200 (1957).

CARLSSON, A., FUXE, K., HAMBERGER, B. and LINDQVIST, M., Acta Physiol. Scand. 67, 481 (1966).

CARLSSON, A., FUXE, K. and UNGERSTEDT, U., J. Pharm. Pharmac. 20, 150 (1968).

CARLSSON, A., CORRODI, H., FUXE, K. and HÖKFELT, T., Eur. J. Pharmacol. 5, 367 (1969a).

CARLSSON, A., CORRODI, H., FUXE, K. and HÖKFELT, T., Eur. J. Pharmacol. 5, 357 (1969b).

CARLTON, P.L., Psychol. Rev. 70, 19 (1963).

CARMAN, J.S. and WYATT, R.J., in Neuroregulators and Psychiatric Disorders, eds. E. Usdin, Hamburg, D.A. and Barchas, J.D., New York, Oxford University Press, p.488, 1977.

CARMAN, J.S., POST, R.M., BUSWELL, R. and GOODWIN, F.K., Am. J. Psychiat. 133, 1181 (1976).

CARMAN, J.S., POST, R.M., TEPLITZ, T.A., GOODWIN, F.K. and BUNNEY, W.E., Jr., cited in Neuroregulators and Psychiatric Disorders, eds. E. Usdin, D.A. Hamburg and J.D. Barchas, New York, Oxford University Press, p.491, 1977.

CARNEY, M.W.P., Br. Med. J. 4, 512 (1967).

CARNEY, M.W.P., ROTH, M. and GARSIDE, R.F., Br. J. Psychiat. 3, 659 (1965).

CARPENTER, W.T., Jr. and BUNNEY, W.E., Jr., Am. J. Psychiat. 128, 31 (1971).

CARPENTER, W.T., Jr., STRAUSS, J.S. and BUNNEY, W.E. Jr., in Psychiatric Complications of Medical Drugs, ed. R.I. Shader New York, Raven Press, 1972.

..... - 288 -/

CARROLL, B.J., Br. Med. J. 3, 27 (1969).

CARROLL, B.J., Clin. Pharmacol. Ther. 12, 743 (1971).

CARROLL, B.J., Depressive Illness: Some Research Studies, eds. B. Davies, B.J. Carroll and R.M. Mowbray, Springfield, Ill., Charles C. Thomas, p. 23, 1972.

CARROLL, B.J., Psychosom. Med. 38, 106 (1976).

CARROLL, B.J. and MENDELS, J., in Hormones, Behaviour and Psychopathology, New York, Raven Press, p.193, 1976.

CARROLL, B.J., MARTIN, F.I.R. and DAVIES, B.M., Br. Med. J. 3, 285 (1968).

CARROLL, B.J., MOWBRAY, R.M. and DAVIS, B.M., Lancet 1, 967 (1970).

CARROLL, B.J., FRAZER, A., SCHLESS, A. and MENDELS, J., Lancet 1, 427 (1973).

CASPER, R.C., DAVIS, J.M., PANDEY, G.N., GARVER, D.L. and DEKIRMENJIAN, H., Psychoneuroendocrinology 2, 105 (1977).

CELIS, M.E., TALEISNIK, S. and WALTER, R., Proc. natl. Acad. Sci. U.S.A. 68, 1428 (1971).

CHARALAMPOUS, K.D. and BROWN, S., Psychopharmacologia (Berl.) 11, 422 (1967).

CHASE, T.N., Arch. Neurol. 27, 354 (1972).

CHESSIN, M., KRAMER, E.R. and SCOTT, C.T., J. Pharm. exp. Ther. 119, 453 (1957).

CHOUINARD, G., YOUNG, S.N., ANNABLE, L. and SOURKES, T.L., Lancet 1, 249 (1977).

CHRISTMAS, A.J., COULSON, C.J., MAXWELL, D.R. and RIDDELL, D., Br. J. Pharmacol. 45, 490 (1972).

CHURCH, R.M. and SOLOMON, R.L. Psychological Reports 2, 357, (1956).

CLARK, L.D., BAUER, W. and COBB, S., New Engl. J. Med. 246, 206 (1952).

CLEGHORN, R.A., Can. Med. Assoc. J. 65, 449 (1951).

COBB, S., J. Nerv. Ment. Dis. 130, 97 (1960).

COCHEME, M.A.X., Lancet 1, 1392 (1970).

COCHRAN, B., Jr. and MARBACH, E.P., Recent Advances Biol. Psychiat. 4, 154 (1962).

..... - 289 -/

COHEN, M., ROSELLE, D., CHABNER, B., SCHMIDT, T.J. and LIPPMAN, M., Nature 274, 894 (1978).

COLBURN, R.W., GOODWIN, F.K., BUNNEY, W.E., Jr. and DAVIS, J.M., Nature, 215, 1395 (1967).

COLE, J.O., J. Am. Med. Assoc. 190, 445 (1964).

COLE, J.O. and DAVIS, J.M., in Comprehensive Textbook of Psychiatry, eds. A.M. Freedman and H.I. Kaplan, Baltimore, Williams and Wilkins, p.1263, 1967.

COLE, J.O., JONES, R.T. and KLERMAN, G.L., Prog. Neurol. Psychiat. 16, 539 (1961).

COOK, L. and WEIDLEY, E., Ann. N.Y. Acad. Sci. 66, 740 (1957).

COOPER, J.R., BLOOM, F.E. and ROTH, R.H. The Biochemical Basis of Neuropharmacology. New York, Oxford Press, 1974.

COPPEN, A., J. Neurol. Neurosurg. Psychiat. 23, 156 (1960).

COPPEN, A., Br. J. Psychiat, 111, 1133 (1965).

COPPEN, A., Br. J. Psychiat. 113, 1237 (1967).

COPPEN, A., J. Psychiat. Res. 9, 163 (1972).

COPPEN, A., in Serotonin and Behaviour, eds. J.D. Barchas and E. Usdin, New York, Academic Press, p.523, 1973.

COPPEN, A. and NOGUERA, R., Lancet 1, 111 (1970).

COPPEN, A. and SHAW, D.M., Br. Med. J. 2, 1439 (1963).

COPPEN, A., SHAW, D.M. and MANGONI, A., Br. Med. J. 2, 295 (1962).

COPPEN, A., SHAW, D.M. and FARRELL, J.P., Lancet 1, 79 (1963).

COPPEN, A., SHAW, D.M., MALLESON, A., ECCLESTON, E. and GUNDY, G., Br. J. Psychiat. 111, 996 (1965a).

COPPEN, A., MALLESON, A. and SHAW, D.M., Lancet 1, 682 (1965b).

COPPEN, A., SHAW, D.M., MALLESON, A. and COSTAIN, R., Br. Med. J. 1, 71 (1966).

COPPEN, A., SHAW, D.M. and MURRAY, D., Lancet 2, 805 (1967a).

COPPEN, A., SHAW, D.M., HERZBERG, B. and MAGGS, R., Lancet 2, 1178 (1967b).

COPPEN, A., PRANGE, A.J., WHYBROW, P.C., NOGUERA, R. and PAEZ, J.M., Lancet 2, 388 (1969).

..... - 290 -/

COPPEN, A., NOGUERA, R., BAILEY, J., BURNS, B.H., SWANI, M.S., HARE, E.H., GARDNER, R. and MAGGS, R., Lancet 2, 275 (1971a).

COPPEN, A., BROOKSBANK, R., NOGUERA, R. and WILSON, D., J. Neurol. Neurosurg. Psychiat. 34, 432 (1971b).

COPPEN, A., WHYBROW, P.C., NOGUERA, M.B.R., MAGGS, R. and PRANGE, A.J.,Jr., Arch. Gen. Psychiat. 26, 234 (1972a).

COPPEN, A., PRANGE, A.J., Jr., WHYBROW, P.C. and NOGUERA, R., Arch. Gen. Psychiat. 26, 474 (1972b).

COPPEN, A., GHOSE, K., SWADE, C. and WOOD, K., Br. J. Clin. Pharmacol. 5, 135 (1978a).

COPPEN, A., SWADE, C. and WOOD, K., Clinica Chimica Acta 87, 165 (1978b).

CORNE, S.J., PICKERING, R.W. and WARNER, B.T., Br. J. Pharmacol. 20, 106 (1963).

CORRODI, H. and FUXE, K., J. Pharm. Pharmac. 20, 230 (1968).

CORRODI, H. and FUXE, K., Eur. J. Pharmacol. 7, 56 (1969).

COSTA, E. and GARATTINI, S. Amphetamines and Related Compounds. New York, Raven Press, 1970.

COSTALL, B., KELLEY, D.M. and NAYLOR, R.J., Psychopharmacologia (Berl.) 41, 153 (1975).

COTZIAS, G.C., TANG, L.C., MILLER, S.I. and GINOS, J.Z., Science 173, 450 (1971a).

COTZIAS, G.C., PAPAVASILIOU, P.S. and GINOS, J., Ann. Rev. Med. 22, 305 (1971b).

COURT, J.H., Br. J. Psychiat. 114, 1523 (1968).

CRAMER, H., RUDOLPH, H., CONSBRUCH, U. and KENDEL, K., in Advances in Biochemical Psychopharmacology, eds, E. Costa, G.L. Gessa and M. Sandler, New York, Raven Press, vol. 11, p.187, 1974.

CRANE, G.E., Psychiat. Res. Rep. Am. Psychiat. Ass. 8, 142 (1957).

CRANE, G.E., in Principles of Psychopharmacology, eds. W.G. Clark and del Giudice, J., New York, Academic Press, p.643, 1970.

CREVELING, C.R., DALY, J., TOKUYAMA, T. and WITKOP, B., Biochem. Pharmacol. 17, 65 (1968).

CURTIS, G.C., CLEGHORN, R.A. and SOURKES, T.L., J. Psychosom. Res. 4, 176 (1960).

..... - 291 -/

CURZON, G., Br. J. Psychiat. 115, 1367 (1969).

CURZON, G. and BRIDGES, P.K., J. Neurol. Neurosurg. Psychiat. 33, 698 (1970).

CURZON, G. and GREEN, A.R., Life Sci. 7, 657 (1968).

DAHLSTRÖM, A. and FUXE, K., Acta Physiol. Scand. 62 (suppl. 232), 1 (1964).

DAIRMAN, W. and UDENFRIEND, S., Science 171, 1022 (1971).

DAVIS, J.M., Arch. Gen. Psychiat. 13, 552 (1965).

DAVIS, J.M., Int. Rev. Neurobiol. 12, 145 (1970).

DAVIS, J.M., KLERMAN, G.L. and SCHILDKRAUT, J.J., in Psychopharmacology: A Review of Progress, ed. D.H. Efron Washington, D.C., U.S. Govt. Printing Office, p.719, 1968.

DAWES, P.M. and VIZI, E.S., Br. J. Pharmacol. 48, 225 (1973).

DEBELJUK, L., Endocrinology 84, 937 (1969).

DEBELJUK, L., FEDER, V.M. and PAULUCCI, O.A., J. Reprod. Fertil. 21, 363 (1970).

DEGKWITZ, R., FROWEIN, R., KULENKAMPFF, C. and MOHS, U., Klin. Wschr. 38, 120 (1960).

DEL GIUDICE, J., in Principles of Psychopharmacology, eds. W.G. Clark and del Giudice, J., New York, Academic Press, p.425, 1970.

DENCKER, S.J., HÄGGENDAL, J. and MALM, U., Lancet 2, 754 (1966a).

DENCKER, S.J., MALM, U., ROOS, B.-E. and WERDINIUS, B., J. Neurochem. 13, 1545 (1966b).

DENGLER, H.G., SPIEGEL, H.E. and TITUS, E.O., Nature 191, 816 (1961).

DE PROSPO, N.D. and HURLEY, J., J. Endocr. 49, 545 (1971a).

DE PROSPO, N.D. and HURLEY, J., Agents Actions 2, 14 (1971b).

DE PROSPO, N.D., DE MARTINO, L.J. and McGUINESS, E.T., Life Sci. 7, 183 (1968).

DE POSPO, N.D., SAFINSKI, K.J., DE MARTINO, L.J. and McGUINNESS, E.T., Life Sci. 8, 837 (1969).

DEWHURST, W.G., Nature 218, 1130 (1968a).

..... _ 292 -/

DEWHURST, W.G., Nature 219, 506 (1968b).

DEWHURST, W.G. and MARLEY, E., Br. J. Pharmacol. 25, 705 (1965).

DE WIED, D., in Frontiers in Neuroendocrinology, ed. W.G. Ganong and Martini, L., New York and London, Oxford University Press, p.97, 1969.

DEYKIN, E.Y., JACOBSON, S., KLERMAN, G.L. and SOLOMON, M., Am. J. Psychiat. 122, 1422 (1966).

DIAB, I.M., FREEDMAN, D.X. and ROTH, L.J., Science 173, 1022 (1971).

DINSMOOR, J., Psychological Reports 4, 531 (1958).

DOE, R.P., FLINK, E.B. and GOODSELL, M.G. J. clin.Endocr. Metab. 16, 196 (1956).

DOIG, R.J., MUMMERY, R.V., WILLS, M.R. and ELKES, A., Br. J. Psychiat. 112, 1263 (1966).

DOMINO, E.F., in Drill's Pharmacology in Medicine, fourth edition, ed. J.R. Di Palma, New York, McGraw-Hill, p.463 1971.

DOW, R.S., FERNÁNDEZ-GUARDIOLA, A. and MANNI, E., Electro-encephalography and Clinical Neurophysiology 14, 383 (1962).

DUNNER, D. and GOODWIN, F.K., Arch. Gen. Psychiat. 26, 364 (1972).

DUNNER, D., GOODWIN, F.K., GERSHON, E., MURPHY, D. and BUNNEY, W.E., Jr., Arch. Gen. Psychiat. 26, 360 (1972).

DURELL, J. and SCHILDKRAUT, J.J., in American Handbook of Psychiatry, ed. S. Arieti, New York, Basic Books, vol. 3, p.423, 1966.

DYSON, W.L. and MENDELS, J., Curr. Ther. Res. 10, 601 (1968).

EBERT, M.H., POST, R.M. and GOODWIN, F.K., Lancet 2, 766 (1972).

EBERT, M.H., BALDESSARINI, R.J., LIPINSKY, J.F. and BERV, K., Arch. Gen. Psychiat. 29, 397 (1973).

ECKHARDT, S.B. and MAXWELL, R.A., Pahiavi Med. J. 4, 461 (1973).

EHRENSING, R.H. and KASTIN, A.J., Arch. Gen. Psychiat. 30, 63 (1974).

EHRENSING, R.H., KASTIN, A.J., SCHALCH, D.S., TRIESEN, H.G., VARGAS, J.R. and SCHALLY, A.V., Am. J. Psychol. 131, 714 (1974).

EHSANULLAH, R.S. and MULGIRIGAMA, D.L., Br. J. Clin. Pharmacol. 7, 434P, (1979).

..... - 293 -/

ELDRED, S.H., BELL, N.W. and SHERMAN, L.J., New Engl. J. Med. 263, 1330 (1961).

EL-YOUSEF, M.K., JANOWSKY, D.S., DAVIS, J.M. and ROSENBLATT, J.R., Br. J. Addict. 68, 321 (1973).

ENDO, M., ENDO, J., NISHIKUBO, M., YAMAGUCHI, T. and HATOTANI, N., in Psychoneuroendocrinology, ed. N. Hatotani, Basel, Karger, p.22, 1974.

ENGEL, G.L. and MARGOLIN, S.G., Arch. Neurol. Psychiat. 45, 881 (1941).

ENGEL, G.L. and MARGOLIN, S.G., Arch. Intern. Med. 70, 236 (1942).

ENGEL, J. and MODIGH, K., in Advances in Biochemical Psychopharmacology, eds. Costa, E., Gessa, G.L. and Sandler, M., New York, Raven Press, vol. 11, p.405, 1974.

EVERETT, G.M., in Proceedings of the First International Symposium on Antidepressant Drugs, Excerpta Medica, International Congress Series No. 122, p.164 (1966).

EVERETT, G.M. and BORCHERDING, J.W., Science 168, 849 (1970).

EVERETT, G.M. and WIEGAND, R.G., in Proc. 1st Intern. Pharmacol. Meeting, Stockholm, 1961, Oxford, Pergamon Press, vol. 8, p.85, 1962.

FANN, W.E., DAVIS, J.M., JANOWSKY, D.S., KAUFMANN, J.S., GRIFFITH, J.D. and OATES, J.A., Arch. Gen. Psychiat. 26, 158 (1972).

FAWCETT, J.A. and BUNNEY, W.E., Jr., Arch. Gen. Psychiat. 16, 517 (1967).

FAWCETT, J.A. and SIOMOPOULOUS, V., Arch. Gen. Psychiat. 25, 247 (1971).

FAWCETT, J.A., MAAS, J.W., SIOMOPOULOS, V. and DEKIRMENJIAN, H., Psychosom. Med. 30, 550 (1968).

FAWCETT, J.A., MAAS, J.W. and DEKIRMENJIAN, H., Arch. Gen. Psychiat. 26, 246 (1972).

FELDBERG, W. and SHERWOOD, S.L., J. Physiol.(Lond.) 139, 408 (1957).

FELDMAN, L.B., Family Process 15, 389 (1976).

FELDSTEIN, A., HOAGLAND, H., OKTEM, M.R. and FREEMAN, H., Int. J. Neuropsychiat. 1, 384 (1965).

FERGUSON, H.C., BARTRAM, A.C.G., FOWLIE, H.C., CATHRO, D.M., BIRCHALL, K. and MITCHELL, F.L., Acta Endocr. (Kbh.) 47, 58 (1964).

..... - 294 -/

FERNÁNDEZ-GUARDIOLA, A., MUÑÓZ-MARTIŃEZ, E. and VERLAZCO, M., Boletin del Instituto de Estudios Médicos y Biológicos 22, 205 (1964).

FIBIGER, H.C., LYTLE, L.D. and CAMPBELL, B.A., J. Comp. Physiol. Psychol. 72, 384 (1970).

FIEVE, R.R., PLATMAN, S.R. and PLUTCHIK, R.R., Am. J. Psychiat. 125, 492 (1968).

FISKE, V.M. and HUPPERT, L.C., Science 162, 279 (1968).

FLACH, F.F., Proceedings of the IVth World Congress of Psychiatry, Madrid, Spain, Excerpta Medica International Congress Series No. 150, Amsterdam, Excerpta Medica Foundation, 1966.

FOLDES, A. and COSTA, E., Biochem. Pharmacol. 24, 1617 (1975).

FORN, J. and VALDECASAS, F.G., Biochem. Pharmacol. 20, 2773 (1971).

FORREST, J.N., Jr., New Engl. J. Med. 292, 423 (1975).

FOTHERBY, K., ASHCROFT, G.W., AFFLECK, J.W. and FORREST, A.D., J. Neurol. Neurosurg. Psychiat. 26, 71 (1963).

FRANCHIN, E.C., Rev. bras. Clin. Therap. 2, 317 (1973).

FRASCHINI, F., MESS, B. and MARTINI, L., Endocrinology 82, 919 (1968).

FRASCHINI, F., COLLU, R. and MARTINI, L., in The Pineal Gland, eds. G.E.W. Wolstenholme and J. Knight, London, Churchill Livingstone, p.259, 1971.

FRAZER, A., in The Psychobiology of Depression, ed. J. Mendels, New York, Spectrum, p. 7, 1975.

FRAZER, A. and MENDELS, J. in Neuroregulators and Psychiatric Disorders, eds. E. Usdin, D.A. Hamburg and J.D. Barchas, New York, Oxford University Press, p.478, 1977.

FREUD, S., in Collected Papers, ed. J. Strachey, London, Hogarth Press, vol. 4, p.152, 1950.

FRIZEL, D., COPPEN, A. and MARKS, V., Br. J. Psychiat. 115, 1375 (1969).

FULLERTON, A.G. and MORTON-JENKINS, D., Br. Med. J. 1, 538 (1963).

FULLERTON, D.T., WENZEL, F.J., LOHRENZ, F.N. and FAHS, H., Arch. Gen. Psychiat. 19, 674 (1968).

..... - 295 -/

FUXE, K., HÖKFELT, T., JONSSON, G. and LOFSTROM, A., in Fronteirs in Catecholamine Research, eds. E. Usdin and S. Snyder, New York, Pergamon Press, p.787, 1973.

GARATTINI, S. and VALZELLI, L. Serotonin. Amsterdam, Elsevier, p.206, 1965.

GARATTINI, S., GIACHETTI, A., JORI, A., PIERI, L. and VALZELLI, L., J. Pharm. Pharmac. 14, 509 (1962).

GARSIDE, I.B., LIPPOLD, C.J. and MELDRUM, B.S., Clin. Neurophysiol. 20, 382 (1966).

GARVER, D.L. and DAVIS, J.M., Life Sci. 24, 383 (1979).

GARVER, D.L., PANDEY, G.N., DEKIRMENJIAN, H. and JONES, F.D., Am. J. Psychiat. 132, 1149 (1975).

GARVER, D.L., PANDEY, G.N., DEKIRMENJIAN, H. and DAVIS, J.M., in Hormones, Behaviour and Psychopathology, ed. E.J. Sachar, New York, Raven Press, p.233, 1976.

GASTON, S. and MENAKER, M., Science 160, 1125 (1968).

General Practitioner Research Group Practitioner 192, 151 (1964).

GERSHON, S. and SHAW, F.H., Lancet 1, 1371 (1961).

GERSHON, S. and YUWILER, A., J. Neuropsychiat. 1, 229 (1960).

GERSHON, S., HOLMBERG, G., MATTSSON, E., MATTSSON, N. and MARSHALL, A., Arch. Gen. Psychiat. 6, 96 (1962).

GERSHON, E., DUNNER, D. and GOODWIN, F., Arch. Gen. Psychiat. 25, 1 (1971).

GHOSE, K., COPPEN, A. and TURNER, P., Psychopharmacology 49, 201 (1976).

GIARMAN, N.J. and DAY, M., Biochem. Pharmacol. 1, 235 (1959).

GIBBONS, J.L., Clin. Sci. 19, 133 (1960).

GIBBONS, J.L., Postgrad. Med. J. 39, 19 (1963).

GIBBONS, J.L., Arch. Gen. Psychiat. 10, 572 (1964).

GIBBONS, J.L., J. Psychosom. Res. 10, 263 (1966).

GIBBONS, J.L. and McHUGH, P.R., J. Psychiat. Res. 1, 162 (1962).

..... - 296 -/

GIORDANO, G., BALESTRERI, R., JACOPINO, G.E., FOPPIANI, E. and BERTOLINI, S., (1967), cited in Reiter, R.J., Vaughan, G.K., Vaughan, G.M., Sorrentino, S., Jr. and Donofrio, R.J., in Fronteirs of Pineal Physiology, ed. M.D. Altschule, Cambridge, Mass., MIT Press, p.54, 1975.

GIULIANI, G., MOTTA, M. and MARTINI, L., Acta Endocr. 51, 203 (1966).

GLASSER, G.H., Psychosom. Med. 15, 280 (1953).

GLASSMAN, A., Psychosom. Med. 2, 107 (1969).

GLASSMAN, A. and JAFFE, F., cited in Glassman, A., Psychosom. Med. 31, 107 (1969).

GLASSMAN, A. and PLATMAN, S.R., J. Psychiat. Res. 7, 83 (1969).

GLOWINSKI, J. and AXELROD, J., Nature 204, 1318 (1964).

GLOWINSKI, J. and AXELROD, J., J. Pharm. exp. Ther. 149, 43 (1965).

GLOWINSKI, J. and IVERSEN, L.L., J. Neurochem. 13, 655 (1966).

GLOWINSKI, J., KOPIN, I.J. and AXELROD, J., J. Neurochem. 12, 25 (1965).

GLOWINSKI, J., HAMON, M., JAVOY, O. and MOROT-GAUDRY, Y., Adv. Biochem. Psychopharmacol. 5, 423 (1972).

GLUCKMAN, M.I. and BAUM, T., Psychopharmacologia (Berl.) 15, 169 (1969).

GOODMAN, L.S. and GILMAN, A., The Pharmacological Basis of Therapeutics. Fifth edition. Toronto, MacMillan, 1975.

GOODWIN, F.K., 10th Cong. Collegium Internationale Neuorpsychopharmacologicum, Quebec, p.23, 1976.

GOODWIN, F.K., and BUNNEY, W.E., Jr., Semin. Psychiat. 3, 435 (1971).

GOODWIN, F.K. and POST, R.M., in Serotonin and Behaviour, eds. J.D. Barchas and E. Usdin, New York, Academic Press, p. 469, 1973.

GOODWIN, F.K. and POST, R.M., in Advances in Biochemical Psychopharmacology, eds. E. Costa, G.L. Gessa and M. Sandler, New York, Raven Press, vol. 11, p.341, 1974.

GOODWIN, F.K., MURPHY, D.L. and BUNNEY, W.E., Jr., Arch. Gen. Psychiat. 21, 486 (1969).

GOODWIN, F.K., BRODIE, H.K.H., MURPHY, D.L. and BUNNEY, W.E., Jr., Lancet 1, 908 (1970).

..... - 297 -/

GOODWIN, F.K., DUNNER, D.L. and GERSHON, E.S., Life Sci. 10, 751 (1971).

GOODWIN, F.K., MURPHY, D., DUNNER, D. and BUNNEY, W.E., Jr., Am. J. Psychiat. 129, 44 (1972).

GOODWIN, F.K., POST, R.M., DUNNER, D.L. and GORDON, E.K., Am. J. Psychiat. 130, 73 (1973).

GORDEN, A.E., Psychopharmacol. Bull. 11, 20 (1975).

GORDON, E.K. and OLIVER, J., Clinica Chimica Acta 35, 145 (1971).

GORKIN, V.Z., Vop. med. Khim. 10, 115 (1964).

GORKIN, V.Z., in Molecular Basis of Pathology, ed. V. Orekhovic, Moscow, p.179, 1966.

GOTTESFELD, Z., EBSTEIN, B.S. and SAMUEL, D., Nature 234, 124 (1971).

GOTTESFELD, Z., SAMUEL, D. and ICEKSON, I., Experientia 29, 68 (1973).

GOURET, C., MOCQUET, G., COSTON, A. and RAYNAUD, G., J. Pharmacol. 8, 333 (1977).

GRAHAME-SMITH, D.G., J. Neurochem. 18, 1053 (1971a).

GRAHAME-SMITH, D.G., Br. J. Pharmacol. 43, 856 (1971b).

GRAHAME-SMITH, D.G. and GREEN, A.R., Br. J. Pharmacol. 50, 442P (1974a).

GRAHAME-SMITH, D.G. and GREEN, A.R., Br. J. Pharmacol. 50, 443P (1974b).

GRANVILLE-GROSSMAN, K., in Recent Developments in Affective Disorders, eds. A. Coppen and A. Walk, London, Headley Brothers, p.65, 1968.

GREEN, A.R. and GRAHAME-SMITH, D.G., Nature 251, 524 (1974).

GREEN, A.R. and GRAHAME-SMITH, D.G., Nature 262, 594 (1976).

GREEN, A.R., KOSLOW, S.H., SPANO, P.F. and COSTA, E., in Proc. Vth Int. Congr. Pharmacol., p. 87, 1972.

GREEN, H., SAWYER, J.L., ERICKSON, R.W. and COOK, L., Proc. Soc. exp. Biol. Med. 109, 347 (1962).

GREEN, R., GOETZE, V., WHYBROW, P. and JACKSON, R., J. Am. med. Assoc. 223, 1289 (1973).

GREENBLATT, M., GROSSER, G.H. and WECHSLER, H., Am. J. Psychiat. 120, 935 (1964).

..... - 298 -/

GREENGRASS, P.M. and TONGE, S.R., Br. J. Pharmacol. 47, 660P (1973).

GREENGRASS, P.M. and TONGE, S.R., Arch. int. Pharmacodyn. Ther. 211, 291 (1974).

GREENSPAN, K., GREEN, R. and DURELL, J., Am. J. Psychiat. 125, 512 (1968).

GREENSPAN, K., SCHILDKRAUT, J.J., GORDON, E.K., BAER, L., ARANOFF, M.S. and DURELL, J., J. Psychiat. Res. 7, 171 (1970).

GREGORY, I., Arch. Gen. Psychiat. 15, 354 (1966a).

GREGORY, I., Arch. Gen. Psychiat. 15, 362 (1966b).

GREINER, A.C., Can. Psychiat. Assoc. 15, 433 (1970).

GROMOVA, E.A., KRAUS, M. and KRECEK, J., J. Endocr. 39, 345 (1967a).

GROMOVA, E.A., KRAUS, M. and KRECEK, J., Gen. Comp. Endocr. 9, 455 (1967b).

GYERMEK, L., Int. Rev. Neurobiol. 9, 95 (1966).

HAEFELY, W., HUERLIMANN, A. and THOENEN, H., Helv. Physiol. Acta 22, 315 (1964).

HÄGGENDAL, J. and LINDQVIST, M., Acta Physiol. Scand. 60, 315 (1964)

HALARIS, A., BELENDUIK, K. and FREEDMAN, D.X., Biochem. Pharmacol. 24, 1896 (1975).

HAMBERGER, B. and TUCK, J.R., Eur. J. Clin. Pharmacol. 5, 229 (1973).

HANSEN, C.J., RETBOLL, K. and SCHOU, M., cited in SCHOU, M., Psychopharmacologia (Berl.) 1, 65 (1959).

HANSON, L.C.F., Psychopharmacologia (Berl.) 10, 289 (1967).

HARLOW, H.F., in Determinants of Infant Behaviour, ed. B.M. Foss, New York, Wiley and Sons, p.75, 1961.

HARRIS, T.H., Am. J. Psychiat. 113, 950 (1957).

HARTIGAN, G.P., Br. J. Psychiat. 109, 810 (1963).

HARTLEY, R. and SMITH, J.A., J. Pharm. Pharmac. 25, 751 (1973).

HARTLEY, R., PADWICK, D. and SMITH, J.A., J. Pharm. Pharmac. 24, 100P (1972).

HAVENS, L.L., ZILELI, M.S., DiMASCIO, A., BOLING, L. and GOLDFIEN, A., in Biological Psychiatry, ed. J.H. Masserman, New York, Grune and Stratton, vol. 1, p.120, 1959.

HEDLUND, L., LISCHKO, M.M., ROLLAG, M.D. and NISWENDER, G.D., Science 195, 686 (1977).

HELLMAN, L., NAKADA, F., CURTI, J., WEITZMAN, E.D., KREAM, J., ROFFWARG, H., ELLMAN, S., FUKUSHIMA, D.K. and GALLAGHER, T.F., J. clin. Endocr. Metab. 30, 411 (1970).

HENNING, M., Acta Physiol. Scand. suppl. 322, 1 (1969).

HERTTING, G., AXELROD, J. and WHITBY, L.G., J. Pharm. exp. Ther. 134, 146 (1961).

HERTZ, T. and SULMAN, F.G., Lancet 1, 531 (1968).

HESKETH, J.E., GLEN, A.I.M. and READING, H.W., J. Neurochem. 28, 1401 (1977).

HESS, S.M. and DOEPFNER, W., Arch. int. Pharmacodyn. Ther. 134, 89 (1961).

HIMWICH, W.A., COSTA, E. and HIMWICH, H.E., Neuropsychopharmacology 2, 485 (1961).

HINDE, R.A. and SPENCER-BOOTH, Y., J. Child Psychol. Psychiat. 11, 159 (1970).

HINDE, R.A., SPENCER-BOOTH, Y. and BRUCE, M., Nature 210, 1021 (1966).

HIRATA, F., HAYAISHI, O., TOKUYAMA, T. and SENOH, S., J. Biol. Chem. 249, 1311 (1974).

HIROTO, D.S., J. exp. Psychol. 102, 187 (1974).

HISHIKAWA, Y., CRAMER, H. and KUHLO, W., Exp. Brain Res. 7, 84 (1969).

HOCH, P.H., in Current Problems in Psychiatric Diagnosis, eds. P.H. Hoch and J. Zubin, New York, Grune and Stratton, p.46, 1953.

HOCKADAY, T.D.R., KEYNES, W. and McKENZIE, J., Br. Med. J. 1, 85 (1966).

HOKIN-NYVERSON, M., SPIEGEL, D.A. and LEWIS, W.C., Life Sci. 15, 1739 (1974).

HOLLISTER, L.E., DAVIS, K.L. and DAVIS, B.M., Hosp. Pract. 10, 103 (1975a).

HOLLISTER, L.E., BERGER, P., OGLE, F.L., ARNOLD, R.C. and JOHNSON, A., Psychopharmacol. Bull. 11, 27 (1975b).

HOLMBERG, G., Int. Rev. Neurobiol. 5, 389 (1963).

HOLTZ, R.W., DEGUCHI, T. and AXELROD, J., J. Neurochem. 22, 205 (1974).

..... - 300 -/

HOLZBAUER, M. and VOGT, M., J. Neurochem. 1, 8 (1956).

HORDERN, A., New Engl. J. Med. 272, 1159 (1965).

HORITA, A. and HAMILTON, A.E., J. Pharm. Pharmac. 22, 389 (1970).

HORN, A.S., Postgrad. Med. J. 52 (suppl. 3), 25 (1976).

HOUSSAY, A. and BARCELO, A., Experientia 28, 478 (1972).

HUNT, P., KANNENGIESSER, M.-H. and RAYNAUD, J.-P., J. Pharm. Pharmac. 26, 370 (1974).

HUNTER, R., JONES, M., JONES, T.G. and MATTHEWS, D.M., Br. J. Psychiat. 113, 1291 (1967).

ILARIA, R. and PRANGE, A.J., in The Nature and Treatment of Depression, eds. F.F. Flach and S.C. Draghi, New York, Wiley and Sons, p. 271, 1975.

IMLAH, N.W., MURPHY, K.P. and MELLOW, C.S., Clin. Trails J. 5, 927 (1968).

INGVARSSON, C.G., Arzneimittel Forsch. 15, 849 (1965).

INNES, I.R. and NICKERSON, M., in The Pharmacological Basis of Therapeutics, third edition, eds. L.S. Goodman and A. Gilman, New York, Macmillan, p.477, 1965.

IVERSEN, L.L., J. Pharm. Pharmac. 16, 435 (1964).

IVERSEN, L.L., Nature 214, 8 (1967).

ITIL, T.M., in Hormones, Behaviour and Psychopathology, eds. E.J. Sachar, New York, Raven Press, p.31, 1976.

ITIL, T.M., CORA, R., AKPINAR, S., HERRMANN, W. and PATTERSON, C.J., Curr. Ther. Res. 16, 1147 (1974).

ITIL, T.M., PATTERSON, C.J., POLVAN, N., MEHTA, S. and BERGEY, B., Dis. Nerv. Syst. 36, 529 (1975).

JAKOBSON, T., BLUMENTHAL, M., HAGMAN, H. and HEIKKINEN, E., J. Psychosom. Res. 13, 263 (1969).

JALFRE, M., RUCH-MONACHON, M.A. and HAEFELY, W., Adv. Biochem. Psychopharmacol. 10, 121 (1974).

JANOWSKY, D.S., EL-YOUSEF, M.K., DAVIS, J.M., HUBBARD, B. and SEKERKE, H.J., Lancet 1, 1236 (1972a).

JANOWSKY, D.S., EL-YOUSEF, M.K., DAVIS, J.M. and SEKERKE, H.J., Lancet 2, 632 (1972b).

JANOWSKY, D.S., EL-YOUSEF, M.K., DAVIS, J.M. and SEKERKE, H.J., Arch. Gen. Psychiat. 28, 542 (1973a).

..... - 301 -/

JANOWSKY, D.S., EL-YOUSEF, M.K., DAVIS, J.M. and SEKERKE, H.J., Am. J. Psychiat. 130, 1370 (1973b).

JARAMILLO, J. and GREENBERG, R., Can. J. Physiol. Pharmacol. 53, 104 (1975).

JENSEN, G.D. and TOLMAN, C.W., J. Comp. Physiol. Psychol. 55, 131 (1962).

JIMERSON, D.C., POST, R.M., CARMAN, J.S., VAN KAMMEN, D.P., WOOD, J.H., GOODWIN, F.K. and BUNNEY, W.E., Jr., (1976), cited in Neuroregulators and Psychiatric Disorders, eds. E. Usdin, D.A. Hamburg and J.D. Barchas, New York, Oxford University Press, p.491, 1977.

JIMERSON, D.C., LYNCH, H.J., POST, R.M., WURTMAN, R.J. and BUNNEY, W.E., Jr., Life Sci. 20, 1505 (1977).

JOHANSSON, C., ALLGÉN, L.G. and HELLSTROM, L., Acta Med. Scand. 194, 551 (1973).

JOHNSON, H. and MADEN, J.G., Clin. Trials J. 4, 787 (1967).

JONES, F.D., MAAS, J.W., DEKIRMENJIAN, H. and FAWCETT, J.A., Science 179, 300 (1973).

JONES, R.L., McGEER, P.L. and GREINER, A.C., Clinica Chimica Acta 26, 281 (1969).

JORI, A. and DOLFINI, E., Neuroendocrinology 21, 74 (1976).

JOUAN, P. and SAMPEREZ, S., C.R. Soc. Biol. (Paris) 159, 316 (1965).

JOUVET, M., Science 163, 32 (1969).

JUNG, O.H. and BOYD, E.S., Am. J. Physiol. 210, 432 (1966).

KAFOE, W.F. and LEONARD, B.E., Arch. int. Pharmacodyn. Ther. 206, 389 (1973).

KAFOE, W.F., DeRIDDER, J.J. and LEONARD, B.E., Biochem. Pharmacol. 25, 2455 (1976).

KAMBERI, I.A., MICAL, R.S. and PORTER, J.C., Endocrinology 87, 1 (1970).

KAMBERI, I.A., MICAL, R.S. and PORTER, J.C., Endocrinology 88, 1288 (1971).

KAMIN, L.J., Psychological Reports 5, 455 (1959).

KAMIN, L.J., CAMPBELL, D., JUDD, R., RYAN, T. and WALKER, J., J. Comp. Physiol. Psychol. 52, 202 (1959).

..... - 302 -/

KAN, J.P., MALNOE, A. and STROLIN BENEDETTI, M., Proc. 8th Ann. Meeting Amer. Soc. Neurochem., p.149, 1977.

KAPPERS, J.A., in The Pineal Gland, eds. G.E.W. Wolstenholme and J. Knight, London, Churchill Livingstone, p.3, 1971.

KAPPERS, J.A., SMITH, A.R. and DeVRIES, R.A.C., in Progress in Brain Research, eds. D.F. Swabb and J.P. Schade, Amsterdam, Elsevier, vol. 44, 1974.

KAROBATH, M.E., Eur. J. Pharmacol. 30, 159 (1975).

KARPATI, G. and FRAME, B., Arch. Neurol. 10, 387 (1964).

KARPPANEN, H., AIRAKSINEN, M.M. and SÄRKIMÄKI, J., Annales Medicinae Experimentalis et Biologiae Fenniae 51, 93 (1973).

KASTIN, A.J. and ROSS, G.T., Endocrinology 77, 45 (1965).

KASTIN, A.J. and SCHALLY, A.V., Experientia 22, 389 (1966).

KASTIN, A.J. and SCHALLY, A.V., Nature 213, 1238 (1967).

KASTIN, A.J., KULLANDER, S., BORGLIN, N.E., DAHLBERG, B., DYSTER-AAS, K., KRAKAU, C.E.T., INGVAR, D.H., MILLER, M.C., BOWERS, C.Y. and SCHALLY, A.V., Lancet 1, 1007, (1968).

KASTIN, A.J., SCHALLY, A.V. and VIOSCA, S., Proc. Soc. exp. Biol. Med. 137, 1437 (1971).

KASTIN, A.J., EHRENSING, R.H., SCHALCH, D.S. and ANDERSON, M.S., Lancet 2, 740 (1972a).

KASTIN, A.J., VIOSCA, S., NAIR, R.M.G., SCHALLY, A.V. and MILLER, M.C., Endocrinology 91, 1323 (1972b).

KASTIN, A.J., MILLER, M.C., FERRELL, L. and SCHALLY, A.V., Physiol. Beh. 10, 399 (1973).

KAUFMAN, I.C. and ROSENBLUM, L.A., Science 155, 1030 (1967).

KETY, S.S., Int. J. Psychiat. 1, 409 (1965).

KETY, S.S., in Brain Chemistry and Mental Disease, eds. B.T. Ho and W.M. McIsaac, New York, Plenum Press, p.237, 1971 .

KIELHOLZ, P. Depressive Illness: Diagnosis, Assessment, Treatment, An International Symposium, Bern, Stuttgart, Vienna, H. Huber, 1972.

KIELHOLZ, P. Masked Depression. Bern, Stuttgart, Vienna, H. Huber, 1973.

..... - 303 -/

KIELHOLZ, P. Depression in Everyday Practice. Bern, Stuttgart, Vienna, H. Huber, 1974.

KILOH, L.G. and GARSIDE, R.F., Br. J. Psychiat. 109, 451 (1963).

KIMBLE, G., J. Comp. Physiol. Psychol. 48, 281 (1955).

KINCL, F.A., CHANG, C.C. and ZBUZKOVA, V., Endocrinology 87, 38 (1970).

KING, D.J., Psychol. Med. 3, 53 (1973).

KINSON, G.A. and SINGER, B., J. Endocr. 2, 257 (1967a).

KINSON, G.A. and SINGER, B., Neuroendocrinology 2, 283 (1967b).

KLAIBER, E.L., BROVERMANN, D.M. and KOBAYASHI, Y., Psychopharmacologia (Berl.) 11, 320 (1967).

KLAIBER, E.L., BROVERMANN, D.M., VOGEL, W. and KOBAYASHI, Y., (1975), cited in Itil, T.M., in Hormones, Behaviour and Psychopathology, ed. E.J. Sachar, New York, Raven Press, p.31, 1976.

KLEIN, D.C. and DAVIS, J.M. Diagnosis and Drug Treatment of Psychiatric Disorders. Baltimore, Williams and Wilkins, p.180, 1969.

KLEIN, D.C. and ROWE, J., Mol. Pharmacol. 6, 164 (1970).

KLEIN, D.C. and WELLER, J.L., Science 169, 1093 (1970).

KLEIN, D.C., WELLER, J.L. and MOORE, R.J., Proc. natl. Acad. Sci. U.S.A. 68, 3107 (1971).

KLERMAN, G.L., in Classification and Prediction of Outcome of Depression, ed. J. Angst, New York, Schattauer, 1974.

KLERMAN, G.L. and PAYKEL, E.S., in Principles of Psychopharmacology, eds. W.G. Clark and del Giudice, J., New York, Academic Press, p.627, 1970.

KLERMAN, G.L., SCHILDKRAUT, J.J., HASENBUSH, L.L., GREENBLATT, M. and FRIEND, D.G., J. Psychiat. Res. 1, 289 (1963).

KLINE, N.S., J. Clin. exp. Psychopath. 19 (suppl. 1), 72 (1958).

KLINE, N.S., J. Am. med. Assoc. 190, 732 (1964).

KLINE, N.S. and SACKS, W., Am. J. Psychiat. 120, 274 (1963).

KLINE, N.S., SACKS, W. and SIMPSON, G.M., Am. J. Psychol. 121, 379 (1964).

KNAPP, S. and MANDELL, A.J., Science 180, 645 (1973).

..... - 304 -/

KNAPP, M.S., KEANE, P.M. and WRIGHT, J.G., Br. Med. J. 2, 27 (1967).

KNOX, W.E., Br. J. exp. Path. 32, 462 (1951).

KNOX, W.E. and AUERBACH, V.H., J. Biol. Chem. 214, 307 (1955).

KOBBERLING, J. and VON ZUR MUHLEN, A., J. clin. Endocr. Metab. 38, 313 (1974).

KOE, B.K., J. Pharm. exp. Ther. 199, 649 (1976).

KOPIN, I.J., Pharmacol. Rev. 16, 179 (1964).

KOPIN, I.J., Ann. Rev. Pharmacol. 10, 377 (1968).

KOPIN, I.J., Fedn. Proc. 30, 904 (1971).

KOPIN, I.J. and GORDON, E.K., J. Pharm. exp. Ther. 138, 351 (1962).

KOPIN, I.J. and GORDON, E.K., J. Pharm. exp. Ther. 140, 207 (1963).

KOPIN, I.J., PARE, C.M.B., AXELROD, J. and WEISSBACH, H., Biochim. Biophys. Acta 40, 377 (1960).

KOPIN, I.J., PARE, C.M.B., AXELROD, J. and WEISSBACH, H., J. Biol. Chem. 236, 3072 (1961).

KOPIN, I.J., FISCHER, J.E., MUSACCHIO, J.M., HORST, W.D. and WEISE, V.K., J. Pharm. exp. Ther. 147, 186 (1965).

KORA, T., Int. J. Psychiat. 1, 611 (1965).

KORF, J., VAN PRAAG, H.M. and SEBENS, J.B., Brain Res. 42, 239 (1972).

KOTIN, J. and GOODWIN, F.K., Am. J. Psychiat. 129, 679 (1972).

KOVÁCS, G.L., GAJÁRI, I., TELEGDY, G. and LISSÁK, K., Physiol. Beh. 13, 349 (1974).

KRAEPELIN, E., in Textbook of Psychiatry, trans. by Barclay, R.M., Edinburgh, Livingstone, 1913.

KRAINES, S.H. Mental Depressions and Their Treatment. New York, Macmillan, 1957.

KRIEGER, D., ALLEN, W., RIZZO, F. and KRIEGER, H., J. clin. Endocr. Metab. 32, 266 (1971).

KRIPKE, D.F., Science 194, 214 (1976).

KUHN, R., Am. J. Psychiat. 115, 459 (1958).

..... - 305 -/

KURIYAMA, K. and SPEKEN, R., Life Sci. 9, 1213 (1970).

LANGER, G., HEINZE, G., REIM, B. and MATUSSEK, N., Arch. Gen. Psychiat. 33, 1471 (1976).

LAPIN, I.P. and OXENKRUG, G.F., Lancet 1, 132 (1969).

LAPIN, I.P. and OXENKRUG, G.F., J. Pharm. Pharmac. 22, 781 (1970).

LAPIN, I.P., OSIPOVA, S.V., USKOVA, N.V. and STABROVSKI, E.M., Arch. int. Pharmacodyn. Ther. 174, 37 (1968).

LAUGHLIN, H.P. The Neuroses in Clinical Practice. Philadelphia, W.B. Saunders Co., 1956.

LAZARUS, A., Behaviour Research and Therapy 6, 83 (1968).

LEETON, J., Aust. N.Z. J. Psychiat. 8, 85 (1974).

LEHMANN, J., Acta Psychiat. Scand. Suppl. 237, 5 (1972).

LEHMANN, J., Acta Med. Scand. 194, 181 (1973).

LEONARD, B.E., Psychopharmacologia (Berl.) 36, 221 (1974).

LEONARD, K., Der Nervenarzt 39, 104 (1968).

LERNER, A.B. and CASE, J.D., Fedn. Proc. 19, 590 (1960).

LERNER, A.B., CASE, J.D., MORI, W. and WRIGHT, M.R., Nature 27, 1821 (1959).

LEVINSON, B., S. Afr. Med. J. 48, 873 (1974).

LEWINSOHN, P., in The Psychology of Depression: Contemporary Theory and Research, eds. R.J. Friedman and M.M. Katz, Washington, D.C., Winston and Sons, 1974.

LEWIS, A., Br. Med. J. 2, 875 (1938).

LIDBRINK, P., JONSSON, G. and FUXE, K., Neuropharmacology 10, 521 (1971).

LINDJAERDE, O., Acta Psychiat. Scand. 39 (suppl. 170), 1 (1963).

LIPPMANN, W. and PUGSLEY, T.A., Can. J. Physiol. Pharmacol. 54, 491 (1976).

LLOYD, K.G., FARLEY, I.J., DECK, J.H.N. and HORNYKIEWICZ, O., in Advances in Biochemical Psychopharmacology, eds. E. Costa, G.L. Gessa and M. Sandler, New York, Raven Press, vol. 11, p. 387 (1974).

LOOMER, H.P., SAUNDERS, J.C. and KLINE, N.S., Psychiat. Res. Rep. 8, 129 (1957).

..... - 306 -/

LOVENBERG, W., WEISSBACH, H. and UDENFRIEND, S., J. Biol. Chem. 237, 89 (1962).

LOVENBERG, W., JEQUIER, E. and SJOERDSMA, A., Science 155, 217 (1967).

LOVENBERG, W., JEQUIER, E. and SJOERDSMA, A., Adv. Pharmacol. 6A, 21 (1968).

LU, K.H. and MEITES, J., Endocrinology 93, 152 (1973).

Ludiomil Symposium, ed. J.E. Murphy, J. Intern. Med. Res. 3 (Suppl. 2), 1 (1975).

LYNCH, H.J., Life Sci. 10, 791 (1971).

LYNCH, H.J., cited in Jimerson, D.C., Lynch, H.J., Post, R.M., Wurtman, R.J. and Bunney, W.E., Jr., Life Sci. 20, 1501 (1977).

LYNCH, H.J. and WURTMAN, R.J.,(1972), cited in Cardinali, D.P. and Wurtman, R.J., in Frontiers of Pineal Physiology, ed. M.D. Altschule, Cambridge, Mass., MIT Press, p. 12, 1975.

LYNCH, H.J., ENG, J.P. and WURTMAN, R.J., Proc. natl. Acad. Sci. U.S.A. 70, 1704 (1973a).

LYNCH, H.J., PANG, P. and WURTMAN, R.J., Life Sci. 12, 145 (1973b).

LYNCH, H.J., WURTMAN, R.J., MOSKOWITZ, M.A., ARCHER, M.C. and HO, M.H., Science 187, 169 (1975).

MAAS, J.W., J. Psychiat. Res. 9, 227 (1972).

MAAS, J.W., Arch. Gen. Psychiat. 32, 1357 (1975).

MAAS, J.W. and LANDIS, D.H., Fedn. Proc. 26, 463 (1967).

MAAS, J.W., FAWCETT, J. and DEKIRMENJIAN, H., Arch. Gen. Psychiat. 19, 129 (1968).

MAAS, J.W., DEKIRMENJIAN, H. and FAWCETT, J.A., Nature 230, 330 (1971).

MAAS, J.W., FAWCETT, J.A. and DEKIRMENJIAN, H., Arch. Gen. Psychiat. 26, 252 (1972).

MAAS, J.W., DEKIRMENJIAN, H., GARVER, D., REDMOND, D.E. and LANDIS, D.H., Eur. J. Pharmacol. 23, 121 (1973).

MACLEAN, R., NICHOLSON, W.J., PARE, C.M.B. and STACEY, R.S., Lancet 2, 205 (1965).

..... - 307 -/

MADALENA, J.C., AZEVEDO, O.F., MORAIS, M.L., SANTAN, E.L., RZEZINSKY, P.C., ALMEIDA, M.J. and LOVENKROU, T.S., Rev. bras. Therap. 2, 311 (1973).

MAGGS, R., Br. J. Psychiat. 109, 56 (1963).

MAITRE, L., WALDMEIER, P.C., GREENGRASS, P.M., JAEKEL, J., SEDLACEK, S. and DELINI-STULA, A., Int. J. Med. Res. 3 (Suppl. 2), 2 (1975).

MAJ, J., BARAN, L., RAWLOW, A. and SOWINSKA, H., Proc. 10th Congr. C.I.N.P., ed. D. Baronet-Lacroix, p. 163, 1976.

MALLINGER, A.G., KUPFER, D.J., POUST, R.I. and HANEN, I., Clin. Pharmacol. Ther. 18, 467 (1975).

MALLION, K.B., TODD, A.H., TURNER, R.W., BAINBRIDGE, J.G., GREENWOOD, D.T., MADINAVEITA, J., SOMERVILLE, A.R. and WHITTLE, B.A., Nature 238, 157 (1972).

MANDEL, M.M., Am. J. Psychiat. 117, 234 (1960).

MARCYZNSKI, T.J., YAMAGUCHI, N., LING, G.M. and GRODZINSKA, L., Experientia 20, 435 (1964).

MARQUARDT, P. and RIEMSCHNEIDER, H.H.T., Arch. int. Pharmacodyn. 85, 273 (1951).

MARTIN, J.B., in Frontiers of Neuroendocrinology, eds, L. Martini and W.F. Ganong, New York, Raven Press, vol. 4, p. 129, 1976.

MARTIN, J.E. and KLEIN, D.C., Science 191, 301 (1976).

MARTIN, J.E., ENGEL, J.N. and KLEIN, D.C., Endocrinology 100, 675 (1977).

MARTIN, M., Inform. Psychiat. 49, 1023 (1973).

MARTINI, L. and FIORETTI, M.C., in The Pineal Gland, eds. G.E.W. Wolstenholme and J. Knight, London, Churchill Livingstone, p. 368, 1971.

MATTOCK, G.L., WILSON, D.L. and HOPPER, A., Nature 213, 1189 (1967).

MATUSSEK, N., BENKERT, O., SCHNEIDER, K., OTTEN, H. and POHLMEIER, H., Lancet 2, 660 (1970).

MATUSSEK, N., ACKENHEIL, M., HIPPIUS, H., SCHRODER, H-Th., SCHULTES, H. and WASILEWSKI, B., Abst. VI World Congress of Psychiat. 96, (1977).

McCLURE, D.J., J. Psychosom. Res. 10, 189 (1966).

McCLURE, D.J. and CLEGHORN, R.A., Can. Psychiat. Assoc. J. 13, 477 (1968).

McCLURE, D.J. and CLEGHORN, R.A., in Science and Psychoanalysis, ed. J.H. Masserman, New York, Grune and Stratton, vol. 17, p.12, 1970.

McCORMICK, D.B. and SNELL, E.E., Proc. natl. Acad. Sci. U.S.A. 45, 1371 (1959).

McGEER, P.L., McGEER, E.G. and WADA, J.A., Arch. Neurol. 9, 81 (1963).

McHARDY-YOUNG, S., HARRIS, P.W.R., LESSOFF, M.H. and LYNE, C., Br. Med. J. 1, 740 (1967).

McISAAC, W.M., KHAIRALLAH, P.A. and PAGE, I.H., Science 134, 674 (1961).

McKINNEY, W.T., Jr., and BUNNEY, W.E., Jr., Arch. Gen. Psychiat. 21, 240 (1969).

McKINNEY, W.T., Jr., and KANE, F.S., Am. J. Psychiat. 124, 80 (1967).

McKINNEY, W.T., Jr., EISING, R., MORAN, E., SUOMI, S. and HARLOW, H., Dis. Nerv. Syst. 32, 735 (1971a).

McKINNEY, W.T., Jr., SUOMI, S. and HARLOW, H., Am. J. Psychiat. 127, 1313 (1971b).

McKINNEY, W.T., Jr., SUOMI, S. and HARLOW, H., Arch. Gen. Psychiat. 27, 200 (1972).

McLEAN, J.R. and McCARTNEY, M., Proc. Soc. exp. Biol. Med. 107, 77 (1961).

McLEOD, W.R. and McLEOD, M., in Depressive Illness: Some Research Studies, eds. B.M. Davies, B.J. Carroll and R.M. Mowbray, Springfield, Ill., Charles C. Thomas, p.209, 1972

McNAMEE, H.B., MOODY, J.P. and NAYLOR, G.J., J. Psychosom. Res. 16, 63 (1972).

MELGES, F.T. and BOWLBY, J., Arch. Gen. Psychiat. 20, 690 (1969).

MELIA, P.I., Br. J. Psychiat. 116, 621 (1970).

MENDELS, J., Br. J. Psychiat. 3, 675 (1965).

MENDELS, J., Am. J. Psychiat. 124, 153 (1967).

MENDELS, J. Concepts of Depression. New York, Wiley and Sons, 1970.

MENDELS, J., Lancet 1, 342 (1971).

..... - 309 -/

MENDELS, J. Biological Psychiatry. New York, Wiley and Sons, 1973.

MENDELS, J. and FRAZER, A., J. Psychiat. Res. 10, 9 (1973).

MENDELS, J., FRAZER, A., FITZGERALD, R.G., RAMSEY, T.A. and STOKES, J.W., Science 175, 1380 (1972a).

MENDELS, J., SECUNDA, S.K. and DYSON, L., Arch. Gen. Psychiat. 26, 154 (1972b).

MENDELS, J., FRAZER, A. and SECUNDA, S.K., Int. Pharmacopsychiat. 9, 206 (1974).

MENDELS, J., STERN, S. and FRAZER, A., Dis. Nerv. Syst. 37, 3 (1976).

MENDELS, J., RAMSEY, T.A. and FRAZER, A., Scientific Proceed. 130th Annual Meeting Am. Psychiat. Assoc. 150 (1977).

MENDLEWICZ, J., FLEISS, J.L. and FIEVE, R.R., J. Am. med. Assoc. 222, 1624 (1972).

MENDLEWICZ, J., FLEISS, J.L. and FIEVE, R.R., in Genetic Research in Psychiatry, eds, R.R. Fieve, D. Rosenthal and H. Brill, Baltimore, John Hopkins University Press, p. 219, 1975.

MENNINGER, K., MAYMAN, M. and PRUYSER, P. The Vital Balance. New York, Viking Press, 1963.

MICHAEL, R.P. and GIBBONS, J.L., in International Review of Neurobiology, eds. C. Pfeifer and J. Smythies, New York, Academic Press, vol. 5, p. 243, 1963.

MILCU, S.M., NANU-IONESCU, L. and MILCU, I., in The Pineal Gland, eds. G.E.W. Wolstenholme and J. Knight, London, Churchill Livingstone, p. 345, 1971.

MILINE, R., The Pineal Gland, eds. G.E.W. Wolstenholme and J. Knight, London, Churchill Livingstone, p. 372, 1971.

MILLER, F.P. and MAICKEL, R.P., Life Sci. 8, 487 (1969).

MINNEMAN, K.P. and WURTMAN, R.J., Ann. Rev. Pharmacol. Toxicol. 16, 33 (1976).

MODESTIN, J.J., HUNGERM, R.B. and SCHWARTZ, R.B., Arch. Psychiat. Nerwenkr. 218, 67 (1973).

MODIGH, K., Psychopharmacologia (Berl.) 23, 48 (1972).

MODIGH, K., Psychopharmacologia (Berl.) 30, 123 (1973).

MODIGH, K., J. Neural Transm. 36, 19 (1975).

..... - 310 -/

MODIGH, K., Psychopharmacology 49, 179 (1976a).

MODIGH, K., Acta Physiol. Scand. Suppl. 440, 37 (1976b).

MOIZESZOWICZ, J. and SUBIRA, S., J. Clin. Pharmacol. 17, 81 (1977).

MOLINOFF, P.B. and AXELROD, J., Science 164, 428 (1969).

MOORE, K.E. and LARIVIERE, E.W., Biochem. Pharmacol. 12, 1283 (1963).

MOSES, S.G. and ROBINS, E., Psychopharmacol. Commun. 1, 327 (1975).

MOSZKOWSKA, A., SCEMAMA, A., LOMBARD, M.N. and HÉRY, M., J. Neural Transm. 34, 11 (1973).

MOTTA, M., SCHIAFFINI, O., PIVA, E. and MARTINI, L. in The Pineal Gland, eds, G.E.W. Wolstenholme and J. Knight, London, Churchill Livingstone, p. 279, 1971.

MOWRER, O.H. and LAMOREAUX, R.R., J. Comp. Psychol. 39, 29 (1946).

MOYER, K.E. and KORN, J.H., J. exp. Psychol. 67, 352 (1964).

MUELLER, P.S., HENINGER, G.R. and McDONALD, R.K., Arch. Gen. Psychiat. 21, 587 (1969).

MULLER, J.C., PRYER, W.W., GIBBONS, J.E. and ORGAIN, E.S., J. Am. med. Assoc. 159, 836 (1955).

MULLIN, A.D. and MOGENSON, G.J., Psychological Reports 13, 707 (1963).

MURPHY, D.L., Am. J. Psychiat. 129, 141 (1972).

MURPHY, D.L. and WEISS, R., Am. J. Psychiat. 128, 1351 (1972).

MURPHY, D.L., COLBURN, R.W., DAVIS, J.M. and BUNNEY, W.E., Jr., Life Sci. 8, 1187 (1969).

MURPHY, D.L., BRODIE, H.K.H. and GOODWIN, F.K., Nature 229, 135 (1971).

MURPHY, D.L., CAHAN, D. and MOLINOFF, P.B., cited in Murphy, D.L., Am. J. Psychiat. 129, 55 (1972).

MURPHY, D.L., BAKER, M., KOTIN, J. and BUNNEY, W.E.,Jr., in Serotinin and Behaviour, eds. J.D.Barchas and E. Usdin, New York, Academic Press, p. 529, 1973.

MURPHY, D.L., SLATER, S., and de la VEGA, E., Proc. 10th Congr. C.I.N.P., ed. D. Baronet-Lacroix, p. 125, 1976.

MURPHY, J., Int. J. Med. Res. 3, 251 (1975).

NABER, S.P., GOLDMAN, M. and PEASLEE, M.H., Proc. Soc. Dak. Acad. Sci. 48, 44 (1969).

NAGLE, C.A., NEUSPILLER, N.R., CARDINALI, D.P. and ROSNER, J.M., Life Sci. 11, 1109 (1972a).

NAGLE, C.A., CARDINALI, D.P. and ROSNER, J.M., Endocrinology 91, 423 (1972b).

NAGLE, C.A., CARDINALI, D.P. and ROSNER, J.M., Neuroendocrinology 14, 14 (1974).

NARANG, G.D., SINGH, D.V. and TURNER, C.W., Proc. Soc. exp. Biol. Med. 125, 184 (1967).

NAYLOR, G.J. and OLLEY, J.E., Br. J. Pharmacol. 36, 208 (1969).

NAYLOR, G.J., McNAMEE, H.B. and MOODY, J.P., J. Psychosom. Res. 14, 173 (1970a).

NAYLOR, G.J., McNAMEE, H.B. and MOODY, J.P., J. Psychosom. Res. 14, 179 (1970b).

NAYLOR, G.J., McNAMEE, H.B. and MOODY, J.P., Br. J. Psychiat. 118, 219 (1971).

NAYLOR, G.J., DICK, D., DICK, E., LE POIDEVIN, D. and WHYTE, S., Psychol. Med. 3, 502 (1973).

NEFF, N.H., TOZER, T.N. and BRODIE, B.B., J. Pharmac. exp. Ther. 158, 213 (1967).

NELSON, G.N., MASUDA, M. and HOLMES, T.H., Psychosom. Med. 28, 216 (1966).

NEUSPILLER, N.R., NAGLE, C.A., ORSI, L. and ROSNER, J.M., (1971), cited in Cardinali, D.P. and Wurtman, R.J., in Frontiers of Pineal Physiology, ed. M.D. Altschule, Cambridge, Mass., MIT Press, p. 12, 1975.

NICKLAS, W.J., PUSZKIN, S. and BERL, S., J. Neurochem. 20, 109 (1973).

NICKLAS, W.J., PUSZKIN, S. and BERL, S., Nature 247, 471 (1974).

NILSSON, L. and SÖLVELL, L., Acta Obstretricia et Gynecologica Scand. 46, Suppl. 8 (1967).

NIR, I., in The Pineal Gland, eds. G.E.W. Wolstenholme and J. Knight, London, Churchill Livingstone, p. 222, 1971.

NIR, I., BEHROOZI, M., ASSAEL, I., IVRIANI, I. and SULMAN, F.G., Neuroendocrinology 4, 122 (1969).

..... - 312 -/

NISTICO, G. and PREZIOSI, P., Lancet 2, 213 (1970).

NORDIN, G., OTTOSSON, J.O. and ROOS, B.-E., Psychopharmacologia (Berl.) 20, 315 (1971).

NORLAND, J.J. and LERNER, A.B., cited in Frontiers of Pineal Physiology, ed. M.D. Altschule, Cambridge, Mass., MIT Press, p. 42, 1975.

NOYES, R., DEMPSEY, M. and BLUM, A., Compreh. Psychiat. 15, 187 (1974).

OGASAWARA, N., HAGINO, Y. and KOTAKE, Y., J. Biochem. (Tokyo) 52, 162 (1962).

ORSI, L., DENARI, J.H., NAGLE, C.A., CARDINALI, D.P. and ROSNER, J.M., J. Endocr. 58, 131 (1973).

ORTEGA, B.G., ANTON-TAY, F., ESPARZA, N. and CANCINO, F.M., (1974), cited in Anton-Tay, F., in Advances in Biochemical Psychopharmacology, New York, Raven Press, vol. 11, p. 315, 1974.

OSMOND, H. and SMYTHIES, J.R., J. Ment. Sci. 98, 309 (1952).

OVERMIER, J.B. and SELIGMAN, M.E.P., J. Comp. Physiol. Psychol. 63, 28 (1967).

PADILLA, A.M., PADILLA, C., KETTERER, T. and GIACOLONE, D., Psychonomic Sci. 20, 295 (1970).

PANDA, J.N. and Turner, C.W., Acta Endocr.(Kbh.) 57, 363 (1968).

PANDEY, G.N., JAVAID, J.I., DAVIS, J.M. and TOSTESON, D.C., Physiologist 19, 321 (1976).

PANDEY, G.N., HEINZE, W.J., BROWN, B.D. and DAVIS, J.M., Nature 280, 234 (1979).

PANG, S.F., RALPH, C.L. and REILLY, D.P., Gen. Comp. Endocr. 22, 499 (1974).

PAPAVASILIOU, P.S., COTZIAS, G.C., DUBY, S.E., STECK, A.J., BELL, M. and LAWRENCE, W.H., J. Am. med. Assoc. 222, 88 (1972).

PAPPENHEIMER, J.R., HEISEY, S.R., JORDAN, E.F. and DOWNER, R.J.D., Am. J. Physiol. 203, 763 (1962).

PARACCHI, G., Boll. Soc. ital. Biol. sper. 43, 960 (1967).

PARE, C.M.B., Lancet 1, 529 (1963).

PARE, C.M.B. and SANDLER, M., J. Neurol. Neurosurg. Psychiat. 22, 247 (1959).

..... - 313 -/

PARE, C.M.B., REES, L. and SAINSBURY, M.J., Lancet 2, 1340 (1962).

PARE, C.M.B., YEUNG, D.P.H., PRICE, K. and STACEY, R.S., Lancet 2, 133 (1969).

PAPESCHI, R. and McCLURE, D.J., Arch. Gen. Psychiat. 25, 354 (1971).

PECKNOLD, J.C., BAN, T.A., LEHMANN, H.E. and KLINGNER, A., Int. J. Clin. Pharmacol. 11, 304 (1975).

PELHAM, R.W., Endocrinology 96, 543 (1975).

PENFIELD, W. and JASPER, H. Epilepsy and the Functional Anatomy of the Human Brain. London, Churchill, p. 164, 1964.

PEREZ-REYES, M., in Recent Advances in the Psychobiology of the Depressive Illnesses, eds. T. Williams, M. Katz and Shield, J., Washington, D.C., U.S. Government Printing Office, p. 131, 1972.

PERRIS, C., Acta Psychiat. Scand. 42 (Suppl. 194), 7 (1966).

PERRIS, C., Acta Psychiat. Scand. 44, 238 (1968).

PERSSON, T. and ROOS, B.-E., Lancet 2, 987 (1967).

PETERSEN, P., J. Clin. Endocr. 28, 1491 (1968).

PICHOT, P., DREYFUS, J.F. and PULL, C., Br. J. Clin. Pharmacol. 5, 875 (1978).

PIEZZI, R.S. and WURTMAN, R.J., Science 169, 285 (1970).

PLATMAN, S.R., Dis. Nerv. Syst. 31, 132 (1970).

PLATMAN, S.R. and FIEVE, R.R., Arch. Gen. Psychiat. 18, 591 (1968).

PLETSCHER, A., Arzneimittel Forsch. 14 (Suppl.), 479 (1964).

PLETSCHER, A., Pharmacol. Rev. 18, 121 (1966).

PLETSCHER, A., in Psychopharmacology: A Review of Progress, ed. D.H. Efron, Washington, D.C., U.S. Government Printing Office, p. 649, 1968.

PLETSCHER, A., SHORE, P.A. and BRODIE, B.B., Science 122, 374 (1955).

PLOTNIKOFF, N.P., KASTIN, A.J., ANDERSON, M.S. and SCHALLY, A.V., Life Sci. 10, 1279 (1971).

PLOTNIKOFF, N.P., KASTIN, A.J., ANDERSON, M.S. and SCHALLY, A.V., Neuroendocrinology 11, 67 (1973).

..... - 314 -/

POLACKOVA, J., BILY, J. and HANUS, H., Acta Nerv. Sup. 13, 171 (1971).

POLLITT, J. Depression and its Treatment. Springfield, Ill., Charles C. Thomas, 1965.

PORSOLT, R.D., LE PICHON, M. and JALFRE, M., Nature 266, 730 (1977).

PORSOLT, R.D., ANTON, G., BLAVET, N. and JALFRE, M., Eur. J. Pharmacol. 47, 379 (1978).

POST, R.M. and GOODWIN, F.K., Arch. Gen. Psychiat. 30, 234 (1974).

POST, R.M. and GOODWIN, F.K., in The Psychobiology of Depression ed. J. Mendels, Toronto, Spectrum Publications, chap. 4, 1975.

POST, R.M., GORDON, E.K., GOODWIN, F.K. and BUNNEY, W.E., Jr., Science 179, 1002 (1973).

POST, R.M., GERNER, R.H., CARMAN, J.S., JIMERSON, D.C., GILLIN, J.C., GOODWIN, F.K. and BUNNEY, W.E., Jr., Arch. Gen. Psychiat. 35 (1978).

PRANGE, A.J., Jr., in The Nature and Treatment of Depression, eds. F.F. Flach and S.C. Draghi, New York, Wiley and Sons, p. 255, 1975a.

PRANGE, A.J., Jr., in Phenomenology and Treatment of Depression, ed. W.E. Fann, New York, Spectrum Press, 1975b.

PRANGE, A.J., Jr., WILSON, I.C., RABON, A.M. and LIPTON, M.A., Am. J. Psychiat. 126, 457 (1969).

PRANGE, A.J., Jr., WILSON, I.C., KNOX, A., McCLARE, T. and LIPTON, M., Am. J. Psychiat. 127, 191 (1970).

PRANGE, A.J., Jr., WILSON, I.C., LARA, P.P., ALLTOP, L.B. and BREESE, G.R., Lancet 2, 999 (1972).

PRANGE, A.J., Jr., WILSON, I.C., LYNN, C., ALLTOP, L.B. and STIKELEATHER, R., Arch. Gen. Psychiat. 30, 56 (1974).

PRANGE, A.J., Jr., WILSON, I.C., BREESE, G.R. and LIPTON, M.A., in Hormones, Behaviour and Psychopathology, ed. E.J. Sachar, New York, Raven Press, p. 41, 1976.

PRIEN, R.F., in Classification and Prediction of Outcome of Depression, ed. J. Angst, New York, Schattauer, 1974.

PRYCE, I.G., J. ment. Sci. 104, 421 (1958).

..... - 315 -/

PSCHNEIDT, G.R., Int. Rev. Neurobiol. 7, 191 (1964).

QUASTEL, M.R. and RAHAMIMOFF, R., Br. J. Pharmacol. 24, 455 (1965).

QUAY, W.B., Gen. Comp. Endocr. 3, 473 (1963).

QUAY, W.B., Proc. Soc. exp. Biol. Med. 115, 710 (1964).

QUAY, W.B., Life Sci. 4, 983 (1965a).

QUAY, W.B., Photochem. Photobiol. 4, 425 (1965b).

QUAY, W.B., Physiol. Behav. 3, 109 (1968).

QUAY, W.B., Physiol. Behav. 5, 353 (1970a).

QUAY, W.B., Physiol. Behav. 5, 1281 (1970b).

QUAY, W.B., Trans. N.Y. Acad. Sci. 34, 239 (1972).

RAHAMIMOFF, R., BRUDERMAN, I. and GOLSHANI, G., Life Sci. 4, 228 (1965).

RAIKHLIN, N.T. and KVETNOY, I.M., Acta histochem. Bd. 55, 519 (1976).

RAITERI, M., ANGELINI, F. and BERTOLLINI, A., J. Pharm. Pharmac. 28, 483 (1976).

RALPH, C.L., MULL, D., LYNCH, H.J. and HEDLUND, L., Endocrinology 89, 1361 (1971).

RANDRUP, A. and BRAESTRUP, C., Psychopharmacology 53, 309 (1977).

RANDRUP, A., MUNKVAD, I., FOG, R., GERLACH, J., MOLANDER, L., KJELLBERG, B. and SCHEEL-KRÜGER, J., in Current Developments in Psychopharmacology 2, eds. W.B. Essman and L. Valzelli, Spectrum Publications, p. 206, 1975.

RAVARIS, C.L., NIES, A., ROBINSON, D.S., IVES, J.O., LAMBORN, K.R. and KORSON, L., Arch. Gen. Psychiat. 33, 347 (1976).

REDMOND, D., MAAS, J., KLING, A. and DEKIRMENJIAN, H., Psychosom. Med. 33, 97 (1971).

REICH, T. and WINOKUR, G., J. Nerv. Ment. Dis. 151, 60 (1970).

REICH, T., CLAYTON, P. and WINOKUR, G., Am. J. Psychiat. 125, 1358 (1969).

REISS, M., DAVIS, R.H., SIDEMAN, M.B. and PLICHTA, E.S., J. Endocr. 28, 127 (1963).

..... - 316 -/

REISS, M., SIDEMAN, M.B. and PLICHTA, E.S., J. Endocr. 37, 475 (1967).

REITER, R.J. and FRASCHINI, F., Neuroendocrinology 5, 219 (1969).

REITER, R.J. and MORGAN, W.W., Physiol. Behav. 9, 203 (1972).

REITER, R.J. and SORRENTINO, S., Jr., Contraception 4, 385 (1971).

REITER, R.J., HOFFMAN, R.A. and HESTER, R.J., Am. Zool. 5, 727 (1965).

REITER, R.J., SORRENTINO, S., Jr. and HOFFMAN, R.A., Life Sci. 11, 123 (1972).

REITER, R.J., VAUGHAN, M.K., VAUGHAN, G.M., SORRENTINO, S., Jr. and DONOFRIO, R.J., in Frontiers of Pineal Physiology, ed. M.D. Altschule, Cambridge, Mass., MIT Press, p. 54, 1975a.

REITER, R.J., BLASK, D.E. and VAUGHAN, M.K., Neuroendocrinology 19, 72 (1975b).

RELKIN, R., Physiol. Behav. 5, 341 (1970a).

RELKIN, R., Am. J. Physiol. 218, 328 (1970b).

REMLEY, N.R., BRYON, D.M. and WILSON, F.R., Psychonomic Sci. 15, 175 (1969).

REYNOLDS, E.H., PREECE, J.M., BAILEY, J. and COPPEN, A., Br. J. Psychiat. 117, 287 (1970).

RICHTER, C., Psychosom. Med. 19, 191 (1957).

RICKELS, K., CHUNG, H., CSANALOSI, I., SABLOSKY, L. and SIMON, J., Br. J. Psychiat. 123, 329 (1973).

RIDDLE, S.A., Arch. Gen. Psychiat. 8, 546 (1963).

ROBERTS, E., ROTHSTEIN, M. and BAXTER, C.F., Proc. Soc. exp. Biol. med. 97, 796 (1958).

ROBERTS, J.M., J. ment. Sci. 105, 693 (1959a).

ROBERTS, J.M., J. ment. Sci. 105, 703 (1959b).

ROBERTSON, J. and BOWLBY, J., Cour. cent. Int. Enfant 2, 131 (1952).

ROBINS, E. and GUZE, S., Am. J. Psychiat. 126, 983 (1970).

ROBINSON, D.S., NIES, A., RAVARIS, L. and LAMBORM, K.R., Arch. Gen. Psychiat. 29, 407 (1973).

..... - 317 -/

ROCHE, J.F., FOSTER, D.L., KARSCH, F.J. and DZUIK, P.J., Endocrinology 87, 1205 (1970).

RODNIGHT, R., Int. Rev. Neurobiol. 3, 251 (1961).

ROLLAG, M. and NISWENDER, G., Endocrinology 98, 482 (1976).

ROOS, B.-E. and SJÖSTROM, R.S., Pharmacologia Clinica 1, 153 (1960).

ROOS, B.-E. and SJÖSTROM, R.S., J. Clin. Pharmacol. 1, 153 (1969).

ROSE, D.P., in Metabolic Effects of Gonadal Hormones and Contraceptive Steroids, eds. H.A. Salhanick, D.M. Kipnis and R.L. van de Wiele, New York, Plenum Press, p. 352, 1969.

ROSENBLATT, S., CHANLEY, J.D., SOBOTKA, H. and KAUFMAN, M.R., J. Neurochem. 5, 172 (1960).

ROSENTHAL, S.H., Am. J. Psychiat. 124, 21 (1968).

ROSLOFF, B.N. and DAVIS, J.M., Psychopharmacologia (Berl.) 40, 53 (1974).

ROSNER, J.M., MACOME, J.C., CASTRO, A., BRUNENGO, A., DE CARLI, N., IMAS, B., DENARI, J.H., MARTINEZ, I., PEDROZA, E. and CARDINALI, D.P., in Recent Advances in Endocrinology, Amsterdam, Excerpta Medica, p. 168, 1971.

ROSS, S.B. and RENYI, A.L., Eur. J. Pharmacol. 2, 181 (1967).

ROSS, S.B. and RENYI, A.L., Acta Pharmacol. (Kbh.)Toxicol. 36, 382 (1975).

ROSS, S.B., RENYI, A.L. and ÖGREN, S.-O., Life Sci. 10, 1267 (1971).

ROWNTREE, D.W., NEVIN, S. and WILSON, A., J. Neurol. Neurosurg. Psychiat. 13, 47 (1959).

RUBIN, R.T. and MANDELL, A., Am. J. Psychiat. 123, 387 (1966).

RUBIN, R.T., MILLER, R.G., CLARK, B.R., POLAND, R.E. and ARTHUR, R.J., Psychsom. Med. 32, 589 (1970).

RUSSELL, G.F.M., Clin. Sci. 19, 327 (1960).

RUTLEDGE, C.O., J. Pharmac. exp. Ther. 171, 188 (1970).

RUTLEDGE, C.O. and JONASON, J., J. Pharmac. exp. Ther. 157, 493 (1967).

RYALL, R.W., Br. J. Pharmacol. 17, 339 (1961).

..... - 318 -/

SACHAR, E.J., Arch. Gen. Psychiat. 16, 461 (1967).

SACHAR, E.J., MASON, J.W., KOLMER, H.S. and ARTISS, K.L., Psychosom. Med. 25, 510 (1963).

SACHAR, E.J., HELLMAN, L., FUKUSHIMA, D.K. and GALLAGHER, T.F., Arch. Gen. Psychiat. 23, 289 (1971a).

SACHAR, E.J., FINKELSTEIN, J. and HELLMAN, L., Arch. Gen. Psychiat. 25, 263 (1971b).

SACHAR, E.J., HALPERN, F., ROSENFELD, R.S., GALLAGHER, T.F. and HELLMAN, L., Arch. Gen. Psychiat. 28, 15 (1973a).

SACHAR, E.J., HELLMAN, L., ROFFWARG, H., HALPERN, F., FUKUSHIMA, D.K. and GALLAGHER, T.F., Arch. Gen. Psychiat. 28, 19 (1973b).

SACKMAN, J.W. and REITER, R.J., Physiol. Beh. 18, 321 (1977).

SALKELD, R.M., KNORR, K. and KORNER, W.F., Clinica Chimica Acta 49, 195 (1973).

SAMANIN, R., BERNASCONI, S. and GARATTINI, S., Eur. J. Pharmacol. 34, 377 (1975).

SAMPSON, P.H., in Frontiers of Pineal Physiology, ed. M.D. Altschule, Cambridge, Mass., MIT Press, p. 204, 1975.

SAMPSON, P.H. and BIGELOW, L., Physiol. Beh. 7, 713 (1971).

SANDLER, M. and YOUDIM, M.B.H., Pharmacol. Rev. 24, 331 (1972).

SANGHVI, I. and GERSHON, S., Biochem. Pharmacol. 24, 2103 (1975).

SANO, I., Folia Psychiat. Neurol. Jap. 26, 7 (1972).

SARGANT, W., Br. Med. J. 1, 225 (1961).

SARGANT, W. and DALLY, P., Br. Med. J. 1, 6 (1962).

SAUBERLICK, H.E., S. Afr. Med. J. 49, 2235 (1975).

SCHACHT, U. and HEPTNER, W., Biochem. Pharmacol. 23, 3413 (1974).

SCHACHT, U., LEVEN, M. and BACKER, G., Br. J. Clin. Pharmacol. 4, 775 (1977).

SCHANBERG, S.M., SCHILDKRAUT, J.J., BREESE, G.R. and KOPIN, I.J., Biochem. Pharmacol. 17, 247 (1968a).

SCHANBERG, S.M., BREESE, G.R., SCHILDKRAUT, J.J., GORDON, E.K. and KOPIN, I.J., Biochem. Pharmacol. 17, 2006 (1968b).

SCHECKEL, C.L., in Principles of Psychopharmacology, eds. W.G. Clark, and J. del Giudice, New York, Academic Press, p. 235, 1970.

SCHEKEL, C.L. and BOFF, E., Psychopharmacologia (Berl.) 5, 198 (1964).

SCHILDKRAUT, J.J., Am. J. Psychiat. 122, 509 (1965).

SCHILDKRAUT, J.J., Am. J. Psychiat. 130, 695 (1973a).

SCHILDKRAUT, J.J., J. Nerv. Ment. Dis. 158, 348 (1973b).

SCHILDKRAUT, J.J., Adv. Biochem. Pharmacol. 13, 137 (1975).

SCHILDKRAUT, J.J. and KLEIN, D.C., in Manual of Psychiatric Therapeutics, ed. R.I. Shader, Boston, Little and Brown, p. 39, 1975.

SCHILDKRAUT, J.J., KLERMAN, G.L., FRIEND, D.G. and GREENBLATT, M., Ann. N.Y. Acad. Sci. 107, 1005 (1963).

SCHILDKRAUT, J.J., KLERMAN, G.L., HAMMOND, R. and FRIEND, D.G., J. Psychiat. Res. 2, 257 (1964).

SCHILDKRAUT, J.J., GORDON, E.K. and DURELL, J., J. Psychiat. Res. 3, 213 (1965).

SCHILDKRAUT, J.J., GREEN, R., GORDON, E.K. and DURELL, J., Am. J. Psychiat. 123, 690 (1966a).

SCHILDKRAUT, J.J., SCHANBERG, S.M. and KOPIN, I.J., Life Sci. 5, 1479 (1966b).

SCHILDKRAUT, J.J., SCHANBERG, S.M., BREESE, G.R. and KOPIN, I.J., Am. J. Psychiat. 124, 54 (1967).

SCHILDKRAUT, J.J., DODGE, G.A. and LOGUE, M.A., J. Psychiat. Res. 7, 29 (1969a).

SCHILDKRAUT, J.J., SCHANBERG, S.M., BREESE, G.R., GORDON, E.K. and KOPIN, I.J., Biochem. Pharmacol. 18, 1971 (1969b).

SCHILDKRAUT, J.J., WATSON, R., DRASKOCZY, P.R. and HARTMANN, E., Lancet 2, 485 (1971).

SCHILDKRAUT, J.J., KEELER, B.A., ROGERS, M.P. and DRASKOCZY, P.R., Psychosom. Med. 34, 470 (1972).

SCHILDKRAUT, J.J., KEELER, B.A., GRAB, E.L., KANTROWICH, J. and HARTMANN, E., Lancet 1, 1251 (1973a).

SCHILDKRAUT, J.J., KEELER, B.A., PAPOUSEK, M. and HARTMANN, E., Science 181, 762 (1973b).

..... - 320 -/

SCHILDKRAUT, J.J., ORSULAK, P.J., GUDEMAN, J.E., SCHATZBERG, A.F., ROHDE, W.A., LABNE, R.A., CAHILL, J.F., COLE, J.O. and FRAZIER, S.H., in Neuroregulators and Psychiatric Disorders, eds. E. Usdin, D.A. Hamburg and J.D. Barchas, New York, Oxford University Press, p. 122, 1977.

SCHOTTSTAEDT, W.W., GRACE, W.J. and WOLFF, H.G., J. Psychosom. Res. 1, 287 (1956).

SCHOU, M., Pharmacol. Rev. 9, 17 (1957).

SCHOU, M., Psychopharmacologia (Berl.) 1, 65 (1959).

SCHOU, M., in Antidepressant Drugs, eds. S. Garattini and M.N.G. Dukes, Amsterdam, Excerpta Medica, p. 80, 1967.

SCHOU, M., Nerv. Psychiat. 10, 217 (1971).

SCHUBERTH, J., Ph.D. Thesis, Stockholm, 1973.

SEAY, B., HANSEN, E. and HARLOW, H., J. Child Psychol. Psychiat. 3, 123 (1962).

SELIGMAN, M.E.P., in The Psychology of Depression: Contemporary Theory and Research, eds. R.J. Friedman and M.M. Katz, Washington, D.C., Winston and Sons, p. 83, 1974.

SELIGMAN, M.E.P. Helplessness: On Depression, Development and Death, San Francisco, Freeman, 1975.

SELIGMAN, M.E.P. and GROVES, D., Psychonomic Sci. 19, 191 (1970).

SELIGMAN, M.E.P. and MAIER, S.F., J. exp. Psychol. 74, 1 (1967).

SELIGMAN, M.E.P., MAIER, S.F. and GEER, J., J. Abnorm. Psychol. 73, 256 (1968).

SEWARD, J. and HUMPHREY, G.L., J. Comp. Physiol. Psychol. 63, 338 (1967).

SHAN, N.A., STEVENS, S. and HIMWICH, H.E., Arch. int. Pharmacodyn. Ther. 171, 285 (1968).

SHAW, D.M., Br. Med. J. 2, 262 (1966).

SHAW, D.M., CAMPS, F.E. and ECCLESTON, E.G., Br. J. Psychiat. 113, 1407 (1967).

SHAW, D.M., FRIGEL, D., CAMPS, F.E. and WHITE, S., Br. J. Psychiat. 115, 69 (1969).

SHAW, D.M., O'KEEFFE, R., MACSWEENEY, D.A., BROOKSBANK, B.W.L., NOGUERA, R. and COPPEN, A., Psychol. Med. 3, 333 (1973).

..... - 321 -/

SHAW, K.M., STERN, G.M. and SANDLER, M., Lancet 1, 271 (1973).

SHEARD, M.H. and AGHAJANIAN, G.K., Life Sci. 9, 285 (1970).

SHEIN, H.M. and WURTMAN, R.J., Science 166, 519 (1970).

SHEPHERD, M., LADER, M. and RODNIGHT, R. Clinical Psychopharmacology. London, English Universities Press, 1968.

SHOPSIN, B. and GERSHON, S., Arch. Gen. Psychiat. 24, 320 (1971).

SHOPSIN, B., WILK, S., GERSHON, S., DAVIS, K. and SUBL, M., Arch. Gen. Psychiat. 28, 230 (1973a).

SHOPSIN, B., WILK, S., GERSHON, S., ROFFMAN, M. and GOLDSTEIN, M., in Frontiers in Catecholamine Research, eds. E. Usdin and S. Snyder, New York, Pergamon Press, p. 173, 1973b.

SHOPSIN, B., GERSHON, S., GOLDSTEIN, M., FRIEDMAN, E. and WILK, S., Psychopharmacol. Bull. 10, 52 (1974).

SHOPSIN, B., FRIEDMAN, E. and GERSHON, S., Arch. Gen. Psychiat. 33, 811 (1976).

SHORE, P.A., Pharmacol. Rev. 14, 531 (1962).

SHORE, P.A., PLETSCHER, A., CARLSSON, A., KUNTZMAN, R. and BRODIE, B.B., Ann. N.Y. Acad. Sci. 66, 609 (1959).

SIEVER, L., KRAEMER, H., SACK, R., ANGWIN, P., BERGER, P.A., ZARCONE, V., BARCHAS,J.D. and BRODIE, H.K.H., Dis. Nerv. Syst. 36, 13 (1975).

SIGG, E.B., Can. Psychiat. Assoc. J. 4 (Suppl. 1), 75 (1959).

SIGG, E.B., SOFFER, L. and GYERMEK, L., J. Pharmac. exp. Ther. 142, 13 (1963).

SIMONSON, M., J. Neuropsychiat. 5, 259 (1964).

SINGH, M., Psychiat. Q 44, 706 (1970).

SJÖSTROM, R., Eur. J. Clin. Pharmacol. 6, 75 (1973).

SJÖSTROM, R. and ROOS, B.-E., Eur. J. Clin. Pharmacol. 4, 170 (1972).

SMIRK, H., Br. Med. J. 1, 146 (1963).

SMITH, A.H.W., NAYLOR, G.S. and MOODY, J.P., Br. J. Clin. Pharmacol. 5, 675 (1978).

SMITH, C.B., J. Pharmac. exp. Ther. 147, 96 (1965).

SMITH, C.B. and DEWS, P.B., Psychopharmacologia (Berl.) 3, 55 (1962).

SMITH, O.A., Jr., McFARLAND, W.L. and TAYLOR, E., J. Comp. Physiol. Psychol. 54, 154 (1961).

SMYTHE, G.A. and LAZARUS, L., Nature 224, 230 (1973).

SMYTHE, G.A. and LAZARUS, L., J. Clin. Invest. 54, 116 (1974a).

SMYTHE, G.A. and LAZARUS, L., Science 184, 1373 (1974b).

SNYDER, S.H., AXELROD, J., ZWEIG., M. and FISCHER, J.E., Proc. natl. Acad. Sci. U.S.A. 53, 301 (1965).

SNYDER, S.H., AXELROD, J. and ZWEIG, M., J. Pharmac. exp. Ther. 158, 206 (1967).

SORRENTINO, S., Jr., Anat. Rec. 160, 432 (1968).

SOURKES, T.L., Can. Psychiat. Assoc. J. 22, 467 (1977).

SPECTOR, S., SHORE, P.A. and BRODIE, B.B., J. Pharmac. exp. Ther. 128, 15 (1960).

SPECTOR, S., HIRSCH, C.W. and BRODIE, B.B., Int. J. Neuropharmacol. 2, 81 (1963).

SPECTOR, S., SJOERDSMA, A. and UDENFRIEND, S., J. Pharmac. exp. Ther. 147, 86 (1965).

SPITZ, R.A., Psychoanal. Study Child 1, 53 (1945).

SPITZ, R.A., Psychoanal. Study Child 2,213 (1946).

STEFANO, F.J.E., DONOSO, A.O. and CUKIER, J., Acta Physiol. Lat. Am. 15, 425 (1965).

STEIN, L., Fedn. Proc. 28, 836 (1964).

STEIN, L., WISE, C.D. and BERGER, B.D., in The Benzodiazepines, ed. S. Garattini, New York, Raven Press, p. 299, 1973.

STENN, P.G., KLAIBER, E.L., VOGEL, W. and BROVERMAN, D.M., Percept. Mot. Skills 34, 371 (1972).

STENSTEDT, A., Acta Psychiat. Scand. Suppl. 127 (1959).

STJARNE,L., Acta Physiol. Scand. Suppl. 228, 49 (1964).

STOKES, P., in Psychobiology of Depressive Illness, eds. T. Williams, M. Katz and J.A. Shield, Washington, D.C.,U.S. Government Printing Office, p. 199, 1972.

STRADA, S.J., KLEIN, D.C., WELLER, J.L. and WEISS, B., Endocrinology 90, 1470 (1972).

STRATTON, L.O., KASTIN, A.J. and COLEMAN, W.P., Physiol. Behav. 11, 907 (1973).

STROM-OLSEN, R. and WEIL-MALHERBE, H., J. Ment. Sci. 104, 696 (1958).

SULSER, F. and BRODIE, B.B., Biochem. J. 8, 16 (1961).

SULSER, F. and SOROKO, F., Psychopharmacologia (Berl.) 8, 191 (1965).

SULSER, F., WATTS, J. and BRODIE, B.B., Ann. N.Y. Acad. Sci. 96, 279 (1962).

SULSER, F., BICHEL, M.H. and BRODIE, B.B., J. Pharmac. exp. Ther. 144, 321 (1964).

SULSER, F., OWENS, M.L. and DINGELL, J.V., Life Sci. 5, 2005 (1966).

SULSER, F., OWENS, M.L. and DINGELL, J.V., Pharmacologist 9, 213 (1967).

SUOMI, S., DOMEK, C. and HARLOW, H., J. Abnorm. Psychol. 76, 161 (1970).

SUTHERLAND, M.S., SUTHERLAND, S.S. and PHILLIP, A.E., Clin.Trials J. 7, 857 (1970).

SUTTEL, R. and DUPLAN, J., Actual. Psychiat. 2 (Suppl. 3), (1975).

SWÖNGER, A.K. and RECH, R.H., J. Comp. Physiol. Psychol. 81, 509 (1972).

TAGLIAMONTE, A., TAGLIAMONTE, P., PEREZ-CRUET, J., STERN, S. and GIAN, L., J. Pharmac. exp. Ther. 177, 475 (1971).

TAIT, A.C. and TODRICK, A., Br. J. Clin. Pharmacol. 2, 93 (1975).

TAKAHASHI, S., KONDO, H., YOSHIMURA, M. and OCHI, Y., Folia Psychiat. Neurol. Jap. 27, 305 (1973a).

TAKAHASHI, S., KONDO, H. and KATO, N., in Abstracts of the Fourth International Congress of the International Society of Psychoneuroendocrinology, Berkeley, California, 1973b.

TALBOT, J. and REITER, R., Neuroendocrinology 13, 164 (1973-1974).

TAMMINGA, C., SMITH, R.C., CHANG, S., HARASZTI, J.S. and DAVIS, J.M., Lancet 2, 905 (1976).

..... - 324 -/

TAULBEE, E.S. and WRIGHT, H.W., in Current Topics in Clinical and Community Psychology, ed. C.D. Spielberger, New York, Academic Press, vol. 3, 1971.

TELEGDY, G. and VERMES, I., in Brain-Pituitary-Adrenal Interrelationship, eds. A. Brodish and E.S. Redgate, Basel, Karger, p. 332, 1973.

THIÉBLOT, L., BERTHELAY, J. and BLAISE, S., Ann. Endocr. (Paris) 27, 69 (1966).

THOA, N.B., WOOTEN, G.F., AXELROD, J. and KOPIN, I.J., Proc. natl. Acad. Sci. U.S.A. 69, 520 (1972).

THODY, A.J., in Advances in Drug Research, eds. N.J. Harper and A.B. Simmonds, London, Academic Press, vol. 11, p. 23, 1977.

THOENEN, H., HUERLIMANN, A. and HAEFELY, W., J. Pharmac. exp. Ther. 144, 405 (1964).

THOMAS, E. and BALTER, A., (1974), cited in Seligman, M.E.P., in Helplessness: On Depression, Development and Death, San Francisco, Freeman, p. 70, 1975.

THOMAS, D.R., FREEMAN, F., SVINICKI, J.G., BURR, D.E. and LYONS, J., J. exp. Psychol. 83, 1 (1970).

THORNTON, J.W. and JACOBS, P.D., J. exp. Psychol. 87, 369 (1971).

TILDERS, F.J.H., J. Endocr. 57, XXXV (1973).

TILDERS, F.J.H. and SMELIK, P.G., Neuroendocrinology 17, 296, (1975).

TRIMBLE, M., CHADWICK, D., REYNOLDS, E.H. and MARSDEN, C.D., Lancet 1, 583 (1975).

TRULSON, M.E. and JACOBS, B.L., Eur.J. Pharmacol. 36, 49 (1976).

TURNER, W.J. and MERLIS, S., Dis. Nerv. Syst. 25, 538 (1964).

TUOMISTO, J., Eur. J. Pharmacol. 42, 101 (1977).

TUOMISTO, J. and TUKIAINEN, E., Nature 262, 596 (1976).

UDENFRIEND, S., The Harvey Lectures Series 60, 57 (1966).

UNDERWOOD, H. and MENAKER, M., Science 170, 190 (1970).

URETSKY, N.J. and IVERSEN, L.L., J. Neurochem. 17, 269 (1970).

UZAN, A. and LE FUR, G., Ann. Pharm. Franc. 33, 345 (1975).

VALLANT, G.E., J. Pharmac. exp. Ther. 157, 636 (1967).

..... - 325 -/

VALZELLI, L. and GARATTINI, S., J. Neurochem. 15, 259 (1968).

VAN PRAAG, H.M., Ph.D. Thesis, Utrecht, 1962.

VAN PRAAG, H.M., Psychiat. Neurol. Neurochir. 73, 9 (1970).

VAN PRAAG, H.M. Depression and Schizophrenia: A Contribution on Their Chemical Pathology, New York, Spectrum Publications 1976.

VAN PRAAG, H.M., in Neuroregulators and Psychiatric Disorders, eds. E. Usdin, D.A. Hamburg and J.D. Barchas, New York, Oxford University Press, p. 163, 1977a.

VAN PRAAG, H.M., Biol. Psychiat. 12, 101 (1977b).

VAN PRAAG, H.M. and KORF, J., Lancet 2, 612 (1970).

VAN PRAAG, H.M. and KORF, J., Psychopharmacologia (Berl.) 19, 148 (1971a).

VAN PRAAG, H.M. and KORF, J., Psychopharmacologia (Berl.) 19, 199 (1971b).

VAN PRAAG, H.M. and KORF, J., Biol. Psychiat. 3, 105 (1971c).

VAN PRAAG, H.M. and KORF, J., J. Clin. Pharmacol. 13, 3 (1973a).

VAN PRAAG, H.M. and KORF, J., in Serotonin and Behaviour, eds. J.D. Barchas and E. Usdin, New York, Academic Press, p. 457, 1973b.

VAN PRAAG, H.M. and KORF, J., Int. Pharmacopsychiat. 9, 35 (1974).

VAN PRAAG, H.M. and KORF, J., in Psychotherapeutic Drugs, eds. E. Usdin and I.S. Forrest, New York, Dekker, 1976.

VAN PRAAG, H.M. and LEIJNSE, B., Psychopharmacologia (Berl.) 4, 1 (1963).

VAN PRAAG, H.M. and LEIJNSE, B., Psychopharmacologia (Berl.) 8, 67 (1965).

VAN PRAAG, H.M., KORF, J. and PUITE, J., Nature 225, 1259 (1970).

VAN PRAAG, H.M., KORF, J., DOLS, L.C.W. and SCHUT, T., Psychopharmacologia (Berl.) 25, 14 (1972).

VAN PRAAG, H.M., KORF, J. and SCHUT, T., Arch. Gen. Psychiat. 28, 827 (1973a).

VAN PRAAG, H.M., FLENTGE, F., KORF, J., DOLS, L.C.W. and SCHUT, T., Psychopharmacologia (Berl.) 33, 141 (1973b).

VAN SCHEYEN, J.D., VAN PRAAG, H.M. and KORF, J., Br. J. Clin. Pharmacol. 4, 179S (1977).

..... - 326 -/

VAN RIEZEN, H., Arch. int. Pharmacodyn. Ther. 198, 256 (1972).

VAUGHAN, G.M., PELHAM, R.W., PANG, S.F., LOUGHLIN, L.L., WILSON, K.M., SANDOCK, K.L., VAUGHAN, M.K., KOSLOW, S.H. and REITER, R.J., J. clin. Endocr. Metab. 42, 752 (1976).

VAUGHAN, M.K., VAUGHAN, G.M. and O'STEEN, W., J. Endocr. 51, 211 (1971).

VEALE, W.L. and MYERS, R.D., Physiol. Beh. 7, 601 (1971).

VERGHESE, A., MATTHEW, J., MATHAI, G., KOTHOOR, A., SAXENA, B. and KOSHY, T., Indian J. Psychiat. 15, 72 (1973).

VERMES, I. and TELEGDY, G., Acta Physiol. Acad. Sci. Hung. 42, 49 (1972).

VERNIER, V.G., HANSEN, H.M. and STORE, C.A., in Psychosomatic Medicine, eds. J.H. Nodine and J.H. Moyer, Philadelphia, Lea and Febiger, p. 683, 1962.

VETULANI, J. and SULSER, F., Nature 257, 495 (1975).

VETULANI, J., STAWARY, R.J., BLUMBERG, J.B. and SULSER, F., Fedn. Proc. 34, 269 (1975).

VETULANI, J., STAWARY, R.J., DINGELL, J.V. and SULSER, F., Naunyn-Schmiedebergs Archs. Pharmak. 293, 109 (1976).

VIVALAN SYMPOSIUM, J. Intern. Med. Res. 3 (Suppl. 3), (1975).

VIZI, E.S., in Lithium Research and Therapy, ed. F.N. Johnson, London, Academic Press, p. 391, 1975.

VLAHAKES, G. and WURTMAN, R.J., Biochim. Biophys. Acta 261, 194 (1972).

WADA, J.A., WRINCH, J., HILL, D., McGEER, P.L. and McGEER, E.G., Arch. Neurol. 9, 69 (1963).

WALDMEIER, P.C., GREENGRASS, P.M., BAUMANN, P. and MAITRE, L., Postgrad. Med. J. 52 (Suppl. 3), 33 (1976).

WÅLINDER, J., SKOTT, A., NAGY, A., CARLSSON, A. and ROOS, B.-E., Lancet 1, 984 (1975).

WATSON, R.E., HARTMANN, E. and SCHILDKRAUT, J.J., Am. J. Psychiat. 129, 263 (1972).

WATSON, S.J. and MADDEN, J., in Neuroregulators and Psychiatric Disorders, eds. E. Usdin, D.A. Hamburg and J.D. Barchas, New York, Oxford University Press, p. 193, 1977.

WECHSLER, H., GROSSER, G. and GREENBLATT, M., J. Nerv. Ment. Dis. 141, 231 (1965).

..... - 327 -/

WEIL-MALHERBE, H., J. Ment. Sci. 101, 733 (1955).

WEIL-MALHERBE, H., in Principles of Psychopharmacology, eds. W.G. Clark and J. del Giudice, New York, Academic Press, p. 127, 1970.

WEISS, B. and COSTA, E., J. Pharmac. exp. Ther. 161, 310 (1968).

WEISS, B. and CRAYTON, J.W., Endocrinology 87, 527 (1970a).

WEISS, B. and CRAYTON, J.W., in Advances in Biochemical Psychopharmacology, eds. P. Greengard and E. Costa, New York, Raven Press, vol. 3, p. 217, 1970b.

WEISS, B. and LATIES, V.G., Pharmacol. Rev. 14 (1962).

WEISS, J.M., KRIECKHAUS, E.E. and CONTE, R., J. Comp. Physiol. Psychol. 65, 413 (1968).

WEISS, J.M., STONE, E.A. and HARRELL, N., J. Comp. Physiol. Psychol. 72, 153 (1970).

WEISSMAN, M.M. and PAYKEL, E.S. The Depressed Woman. Chicago, University of Chicago Press, 1974.

WEISSMAN, M.M., PAYKEL, E.S. and KLERMAN, G.L., Am. J. Psychiat. 128, 261 (1971).

WENDEL, O.T., WATERBURY, L.D. and PEARCE, L.A., Experientia 30, 1167 (1974).

WEST, E.D. and DALLY, P.J., Br. Med. J. 1, 1491 (1959).

WHEATLEY, D., Arch. Gen. Psychiat. 26, 229 (1972).

WHEATLEY, D., Curr. Ther. Res. 18, 849 (1975).

WHITE, T. and PATON, D., Biochim. Biophys. Acta 266, 116 (1972).

WHYBROW, P.C. and FERRELL, R., in The Thyroid Axis, Drugs and Behaviour, ed. A.J. Prange, Jr., New York, Raven Press, p. 5, 1974.

WHYBROW, P.C. and HURWITZ, T., in Hormones, Behaviour and Psychopathology, ed. E.J. Sachar, New York, Raven Press, p. 125, 1976.

WHYBROW, P.C. and MENDELS, J., Am. J. Psychiat. 125, 45 (1969).

WHYBROW, P.C., PRANGE, A.J., Jr. and TREADWAY, C.R., Arch. Gen. Psychiat. 20, 48 (1969).

WIELOSZ, M., SALMONA, M., DE GAETANO, G. and GARATTINI, S., Naunyn-Schmiedeb. Arch. Pharmacol. 296, 59 (1976).

WILK, S. and WATSON, E., in Frontiers in Catecholamine Research, eds. E. Usdin and S. Snyder, New York, Pergamon Press, p. 1067, 1973.

WINOKUR, G., in Psychochemical Research in Man, eds. A.J. Mandell and M.P. Mandell, New York, Academic Press, 1969.

WINOKUR, G., Semin. Psychiat. 2, 451 (1970).

WINOKUR, G., Am. J. Psychiat. 130, 92 (1973).

WINOKUR, G. and CLAYTON, P.J., in Recent Advances in Biological Psychiatry, ed. J. Wortes, New York, Plenum Press, vol. 9, p. 35, 1967.

WINOKUR, G., CLAYTON, P. and REICH, T. Manic-Depressive Illness. St. Louis, C.V. Mosby, 1969.

WINOKUR, G., REICH, T., RIMMER, J. and PITTS, F., Arch. Gen. Psychiat. 23, 104 (1970).

WINOKUR, G., CADORET, R., DORZAB, J. and BAKER, M., Arch. Gen. Psychiat. 24, 135 (1971).

WINSTON, F., Am. J. Psychiat. 130, 1217 (1973).

WIRZ-JUSTICE, A., HACKMANN, E. and LICHTSTEINER, M., J. Neurochem. 22, 187 (1974).

WISE, C.D., BERGER, B.D. and STEIN, L., Biol. Psychiat. 6, 3 (1973).

WOLPE, J. and LAZARUS, A.A. Behaviour Therapy Techniques. Oxford, Pergamon Press, 1969.

WONG, R. and WHITESIDE, C.B.C., J. Endocr. 40, 383 (1968).

WOOLLEY, D.W. The Biochemical Basis of Psychoses or the Serotonin Hypothesis about Mental Diseases, New York, Wiley and Sons, 1962.

WURTMAN, R.J. and AXELROD, J., Scient. Am. 213, 50 (1965).

WURTMAN, R.J. and AXELROD, J., Nature 212, 312 (1966).

WURTMAN, R.J., AXELROD, J. and PHILLIPS, L.S., Science 142, 1071 (1963).

WURTMAN, R.J., AXELROD, J. and FISHER, J.E., Science 143, 1328 (1964a).

WURTMAN, R.J., AXELROD, J. and POTTER, L.T., J. Pharmac. exp. Ther. 143, 314 (1964b).

WURTMAN, R.J., AXELROD, J., SNYDER, S.H. and CHU, W.E., Endocrinology 76, 798 (1965).

WURTMAN, R.J., AXELROD, J., SEDVALL, G. and MOORE, R.Y., J. Pharmac. exp. Ther. 157, 487 (1967).

WURTMAN, R.J., AXELROD, J. and ANTON-TAY, F., J. Pharmac. exp. Ther. 161, 367 (1968a).

WURTMAN, R.J., AXELROD, J. and KELLY, D.E. The Pineal. New York, Academic Press, 1968b.

WURTMAN, R.J., SHEIN, H.M., AXELROD, J. and LARIN, F., Proc. natl. Acad. Sci. U.S.A. 62, 749 (1968c).

WURTMAN, R.J., SHEIN, H.M. and LARIN, F., J. Neurochem. 18, 1683 (1971).

YING, S. and FISKE, V.M., Fedn. Proc. 31, 277 (1972).

YOUDIM, M.B.H., in Neuroregulators and Psychiatric Disorders, eds. E. Usdin, D.A. Hamburg and J.D. Barchas, New York, Oxford University Press, p. 57, 1976.

YOUNG, L., SUOMI, S., HARLOW, H. and McKINNEY, W.T., Jr., Am. J. Psychiat. 130, 400 (1973).

YOUNG, S.N. and SOURKES, T.L., Adv. Neurochem. 2, 133 (1977).

ZALL, H., THERMAN, P.O.G. and MYERS, J.M., Am. J. Psychiat. 125, 549 (1968).

ZARCONE, V.P., Jr., BERGER, P.A., BRODIE, H.K.H., SACK, R. and BARCHAS, J.D., Dis. Nerv. Syst. 38, 646 (1977).

ZBINDEN, G., Int. J. Neuropharmacol. 1, 435 (1962).

ZELLER, E.A., BARSKY, J., FOUTS, J.R., KIRCHHEIMER, W.F. and VAN ORDEN, L.S., Experientia 8, 349 (1952).