THE EFFECT OF TRICYCLIC ANTIDEPRESSANT DRUGS ON THE UPTAKE AND METABOLISM OF SEROTONIN BY THE PINEAL GLAND, IN ORGAN CULTURE

THESIS

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MANORANJENNI PILLAY

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic Hormone
AMI	Amitriptyline
ATP	Adenosine Triphosphate
CSF	Cerebro-spinal Fluid
CLOMI	Clomipramine
p-CPA	p-Chlorophenylalanine
cpm	Counts Per Minute
c AMP	Cyclic-adenosine Monophosphate
DNA	Deoxyribose Nucleic Acid
DESI	Desipramine
dpm	Disintegrations Per Minute
DA	Dopamine
ECG	Electrocardiogram
ECT	Electroconvulsive Therapy
EEG	Electroencephalogram
FSH	Follicular Stimulating Hormone
FSH-RF	Follicular Stimulating Hormone Releasing Factor
GABA	Gama-aminobutyric Acid
GH	Growth Hormone
HIAA	Hydroxyindoleacetic Acid
HIOMT	Hydroxyindole-o-methyl Transferase
HTP	Hydroxytryptophan
HTOH	Hydroxytryptophol
HVA	Homovanillic Acid
IMI	Imipramine
iv	Intravenous
l/kg	Litres per Kilogram
LH	Luteinizing Hormone
LH-RH	Luteinizing Hormone Releasing Factor
MEL	Melatonin
MSH	Melanocyte-stimulating Hormone
MIF	Melanocyte-stimulating Hormone Inhibiting Factor
MIAN	Mianserin
MHPG	3-Methoxy-4-Hydroxy-Phenylglycol
MIAA	Methoxyindole Acetic Acid
MT	Methoxytryptamine
MTOH	Methoxytryptophol
µl	Microlitre
mg	Milligram
mmol	Millimole

M	Molar
NAS	N-Acetyl Serotonin
ng	Nanogram
N/A	Noradrenaline
PEA	Phenylethylamine
PLM	Pinealectomy
PGs	Prostaglandins
RNA	Ribonucleic Acid
SAH	S-Adenosylhomocysteine
5-HT	Serotonin
SNAT	Serotonin-N-Acetyl Transferase
TLC	Thin - Layer Chromatography
TAD	Tricyclic Antidepressant

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ABSTRACT

The effect of tricyclic antidepressants (TADs) on a variety of pineal functions was assessed.

TADs affected the uptake of ^{3}H-5HT into bovine pineal slices within a particular concentration range of these drugs. DESI, CLOMI and IMI appeared to inhibit uptake slightly, within a limited concentration range. Surprisingly, DESI appeared to be a relatively potent 5HT uptake inhibitor. The 5-HT re-uptake system in the pineal probably differs from that in brain tissue.

TADs had a marked effect on the metabolism of ^{3}H-5HT in the rat pineal, in an organ culture system. MEL and N-acetylserotonin synthesis increased for the first 11 days and thereafter a slight decrease was observed. HTOH and HIAA also showed an initial increase followed by a slight decrease in synthesis. The synthesis of MTOH and MIAA was decreased. The possibility that TADs could affect HIOMT and SNAT synthesis and thereby change the metabolic pattern of 5-HT was investigated.

TADs appeared to stimulate SNAT initially and thereafter a slight decrease from peak activity was observed. This is probably due to stimulation followed by development of subsensitivity of β-receptors. HIOMT activity also appeared to be affected by TADs. The existence of two types of HIOMT is suggested.

There is a possibility that these changes in the metabolism of 5-HT could be implicated in the mechanism of action of TADs.

CHAPTER ONE

1.1 THE TRICYCLIC ANTIDEPRESSANT DRUGS

1.11 INTRODUCTION

The nucleus of the tricyclic antidepressant drugs (TAD's) was synthesized in 1889 by Thiele and Holzinger (1). In 1948 Häfliger and Schindler (2) synthesized a series of iminodibenzyl derivatives for possible uses as antihistamines, sedatives, analgesics and anti-parkinsonism drugs. In 1954, during a clinical investigation of these compounds, Kuhn (3) found quite fortuitously that while imipramine (IMI) showed little antipsychotic action, elevation of the mood of depressed patients was evident. Thereafter, Kuhn (3) found that IMI was most useful in "endogenous" depressions characterized by regression and inactivity. Further evidence for the effectiveness of this compound has accumulated since then (4). These observations were followed by a search for compounds chemically related to IMI.

Today, the TAD's play a major role in the treatment of depression. Other drugs which have been reported to possess antidepressant activity include the following:-

(a) Monoamine oxidase inhibitors, e.g. iproniazid, isocarboxazid, nialamide, phenelzine sulphate, tranylcypromine sulphate (5 - 12)

(b) Lithium carbonate (13 - 18)

(c) Amphetamine (19)

(d) Tetracyclics, e.g. maprotiline (20 - 22) and mianserin (23 - 28)

(e) Bicyclics, e.g. viloxazine (29 - 31) and zimelidine (32 - 33)

(f) Trazodone (34)

(g) Nomifensine (35 - 39)

(h) Amoxapine (40 - 42)

(i) Benzodiazepines, e.g. alprazolam (43)

(j) β -adrenergic stimulants, e.g. salbutamol (44 - 46)

The newer drugs offer no great therapeutic advantage over the TAD's but they may prove to have a more acceptable profile of side effects (47).

Electroconvulsive therapy is also effective in the treatment of certain types of depression (48 - 53).

In this chapter, the pharmacological properties of the TAD's are discussed with special reference to depression.

1.12 CHEMISTRY AND STRUCTURE-ACTIVITY RELATIONSHIP

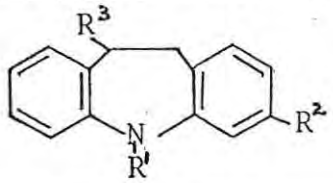
The 3-ring nucleus characteristic of these drugs has given them the epithet "tricyclics". IMI and amitriptyline (AMI) are regarded as the prototypes. The structure of some of the TAD's is shown in Figure I.

When the nitrogen atom on their aminopropyl side chain has three substituents, they are termed tertiary, e.g. IMI, AMI and clomipramine (CLOMI). The monodemethylated side chain metabolites, e.g. desipramine (DESI) and nortriptyline are the secondary amines.

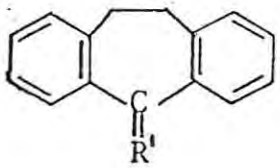
DESI the demethylated congener of IMI has a similar antidepressant activity to that of IMI (54 - 56). Brodie and his associates (55, 57 - 58) have suggested that the pharmacologically active form of IMI may be DESI. However, it is now certain that DESI is no more effective or rapidly acting than IMI. The same generalizations can be made from the comparison between AMI and it's desmethyl derivative, nortriptyline.

FIGURE 1

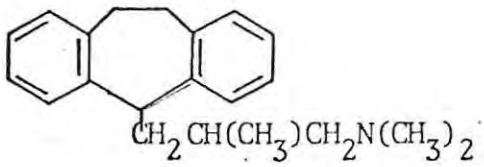
STRUCTURES OF SOME OF THE TRICYCLIC ANTIDEPRESSANTS



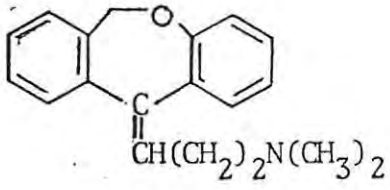
	R'	$R^{2'}$	R^3
IMIPRAMINE	$(CH_2)_3N(CH_3)_2$		
DESIPRAMINE	$(CH_2)_3NHCH_3$		
CLOMIPRAMINE	$(CH_2)_3N(CH_3)_2$	Cl	
TRIMIPRAMINE	$CH_2CH(CH_3)CH_2N(CH_3)_2$		
KETIPRAMINE	$(CH_2)_3N(CH_3)_2$		=O



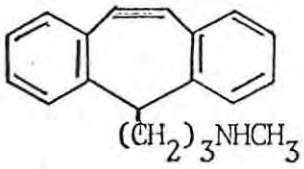
	R'
AMITRIPTYLINE	$CH(CH_2)N(CH_3)_2$
NORTRIPTYLINE	$CH(CH_2)_2NHCH_3$



BUTRIPTYLINE



DOXEPIN



PROTRIPTYLINE

CLOMI is the 3-chloro derivative of IMI which has a mood elevating effect (59 - 60). Imipramine-N-Oxide has been reported to combine the actions of IMI and DESI (61 - 62) while ketipramine (60, 63) resembles IMI and CLOMI.

Doxepin and protriptyline differ from the other TAD's largely in the minor modifications of the tricyclic ring structure. Doxepin has geometric isomers, and both are active (64, 65). The pharmacological profile of protriptyline resembles those of other members of the dibenzocycloheptene series (66 - 69) but in contrast to AMI, which possesses hypnotic actions, protriptyline stimulates psychomotor activity and produces restlessness and insomnia.

1.13 PHARMACOKINETIC PROPERTIES

In recent years, many researchers have focussed their attention on the pharmacokinetic properties of the TAD's.

1.131 ABSORPTION AND DISTRIBUTION

TAD's are well absorbed after oral administration. The transfer of IMI and its metabolites (except for glucuronides) across membranes is believed to be accomplished by non-ionic passive diffusion, a process governed by physicochemical rather than biochemical parameters (70). The polarity of the molecule is of great importance in this respect. IMI has a low polarity (and is therefore lipophilic) while the polarity of DESI is slightly higher than that of IMI. The hydroxylated metabolites and IMI-N-Oxide have a higher polarity when compared to that of IMI and DESI. The glucuronides have a very high polarity (71).

Once absorbed, the lipophilic drugs are widely distributed. IMI and DESI reach considerable concentrations in all tissues, including brain (71 - 74). Douglas et al (75) have reported that IMI crosses the placental barrier. All the polar metabolites are restricted to extra cellular compartments such as plasma, urine, bile and the lower intestinal lumen (72).

The lipophilic drugs easily penetrate cells and reach high tissue concentrations, which contrast with low concentrations in plasma. This accounts for their large volumes of apparent distribution which are typically 15 - 40 ℓ/kg body mass (76). Steady-state plasma levels determined in patients under IMI therapy were 2 - 120 ng/ml for unchanged drug and 3 - 567 ng/ml for metabolically formed desipramine (77 - 80).

TAD's and their active metabolites are highly but variably bound to plasma proteins (81). Sixty to ninety five per cent of the therapeutic concentration of IMI is reported to be bound (82 - 84). The human serum albumin molecule (which has 6 binding sites), well as α-globulins and β-globulins participate in the binding of these drugs (83 - 85). The nature of the binding was thought to be hydrophobic (86) but Sharples (87) has reported that the major factor is electronic with only a minor contribution from the hydrophobic parameters.

It has been reported that the uptake of IMI and DESI corresponds to a near-quantitative extraction in the liver (88 - 90) and also in the lung (91 - 93). Subcellular distribution studies (89 - 90) have shown that when IMI enters hepatocytes, it is rapidly concentrated in the membranes of the endoplasmic reticulum, which are also sites of metabolism. DESI is also bound

to membranes whereas the polar metabolites such as glucuronides are released and appear in the plasma. Bickel et al (94) have reported the presence of high-affinity low-capacity and low-affinity high-capacity binding sites for these lipophilic drugs in microsomes and mitochondria, from liver and other tissues but not nuclei and cytosol. Since the major part of the drug fraction is localized in tissue, minor changes in tissue binding may lead to major fluctuations in plasma levels (95).

Recently, several studies have been made to define the therapeutic plasma concentration of TAD's (96 - 102). In patients with endogenous depression, nortriptyline shows an inverted U-shaped relationship between plasma concentration and clinical response (99). This pattern of response does not apply to other TAD's. However, it has been emphasized that the TAD's are maximally effective only within a rather narrow range.

As much as a 36-fold difference in steady state plasma concentrations of tricyclics have been reported in patients given the same oral dosing regimen (76). The following factors might account for this difference.

(a) Individual variation in liver metabolism (76, 104).

(b) Variation in plasma protein binding (105 - 106).

(c) First pass metabolism (107).

(d) Differences in metabolism in males and females (108).

The individual variation in gastrointestinal absorption has been reported to be unimportant (109).

1.132 METABOLISM

The metabolism of IMI is discussed in detail in this section because IMI has become a model drug for the study of a complex drug metabolic situation that has important pharmacokinetic and pharmacodynamic consequences.

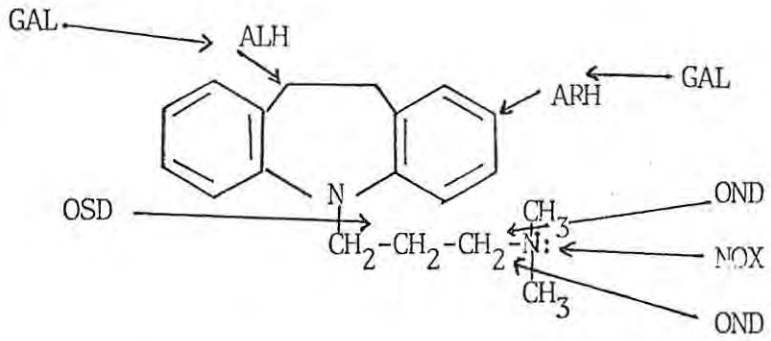
IMI undergoes metabolism by multiple pathways leading to about 30 metabolites. The metabolically vulnerable groups or bonds and the known metabolites are shown in Figure 2. The order of the metabolites follows the chronology of this discovery.

All biotransformations of IMI result in peripheral molecular changes only, leaving the tricyclic nucleus. The number of metabolites far surpasses the number of metabolically vulnerable positions because two or more positions can be attacked simultaneously or in sequence, leading to the formation of a combination of metabolites e.g. 2-hydroxydesmethylimipramine is both demethylated and hydroxylated. There are vast differences in the amounts of individual metabolites produced. The major IMI metabolites in most species are DESI, 2-hydroxyimipramine, 2-hydroxydesipramine and the o-glucuronides of the latter two hydroxylated metabolites. It is important to note, however, that a so-called minor metabolite may well be qualitatively important i.e. in terms of potential pharmacological or toxic action.

The liver is a very important site of metabolism of TAD's (120 - 126). Oxidative N-demethylation may occur in 2 steps; transforming IMI into its secondary amine analogue (DESI) and then it's primary amine analogue (desdimethylimipramine).

FIGURE 2

METABOLICALLY VULNERABLE PARTS OF THE IMIPRAMINE MOLECULE AND METABOLITES OF IMIPRAMINE



Metabolite	Reference
2-Hydroxyimipramine	110
Desmethylimipramine	111
2-Hydroxyimipramine glucuronide	111
2-Hydroxydesmethylimipramine	112
2-Hydroxydesmethylimipramine glucuronide	112
Desdimethylimipramine	112
Imipramine N-oxide	113
Iminodibenzyl	114
2-Hydroxyiminodibenzyl	114
10-Hydroxyimipramine	115
10-Hydroxydesmethylimipramine	115
10-Hydroxydidesmethylimipramine	115
10-Hydroxyiminodibenzyl	115
10-Hydroxyimipramine glucuronide	115
10-Hydroxydesmethylimipramine glucuronide	115
10-Hydroxydidesmethylimipramine glucuronide	115
10-Hydroxyiminodibenzyl glucuronide	115
Unidentified non conjugated metabolite	116 72
Unidentified glucuronides	116 72
2-Hydroxydesdimethylimipramine	72
2-Hydroxydesdimethylimipramine glucuronide	72
2-Hydroxyiminodibenzyl glucuronide	72
Unidentified non glucuronide conjugate	117
Desmethylimipramine-N-glucuronide	118
N-Hydroxydesmethylimipramine	119
N-Hydroxydesdimethylimipramine	120

Figure 2 .....continued....

ALH	:	aliphatic hydroxylation
ARH	:	aromatic hydroxylation
GAC	:	glucuronide acid conjugation
NOX	:	N-oxidation
OND	:	oxidative N-demethylation
OSD	:	oxidative side chain dealkylation

Iminodibenzyl formation may occur non-enzymatically or by oxidative side-chain dealkylation. Aromatic hydroxylation in position 2 is a major pathway, while aliphatic hydroxylation in position 10 is of minor importance. The phenolic and alcoholic metabolites resulting from the latter pathways of O-glucuronides can be hydrolysed to unconjugated hydroxylated metabolites (72, 117). The pathway of N-glucuronidation can apply to secondary and primary amines only.

IMI can undergo N-oxidation and the resulting N-oxide can be subjected to N-oxide reduction or N-oxide demethylation. IMI-N-oxide is a clinically active antidepressant (61, 62) which does not cross the blood-brain barrier but is rapidly metabolised into IMI and DESI (121, 126).

Desdimethylimipramine and iminodibenzyl (non polar, minor metabolites) have some antidepressant activity. Theobald et al (127) screened seven major metabolites of IMI for pharmacological activity. All of them had peripheral activities, such as catecholamine potentiation, but the central actions were not elicited by the polar metabolites, particularly glucuronides. AMI (while mainly demethylated to nortriptyline) and nortriptyline undergo preferential oxidation at position 10, followed by glucuronidation. The 10-hydroxymetabolite thus formed, has some biological activity (128).

The major routes of metabolism of doxepin appear to be similar to those of AMI and IMI i.e. demethylation, N-oxidation, hydroxylation and glucuronide formation (129 - 130). N- demethylation results in the formation of desmethyldoxepin which is pharmacologically active (131 - 132).

Desmethylclomipramine is a major metabolite of CLOMI in man, where it is detected in high concentrations in plasma of depressed patients receiving treatment with the drug (133).

Factors such as species differences (122, 125, 134), sex (135) and strain differences (58, 136) contribute to the differences in metabolism and hence plasma level of TAD's. Nagy et al (137) observed a marked variation in the ratio of methylated to demethylated molecules of IMI and AMI in human subjects. This variation, as well as that of the concentration of drug in blood appears to be characteristic of the individual and is presumably under genetic control (138). However, in general the proportion of AMI to nortriptyline favours the parent drug while the converse is true for IMI and its metabolite DESI (139).

Marked differences in metabolism, absorption and bioavailability can occur as a result of varying routes of administration (73, 140). After oral or intraperitoneal administration, bioavailability is low and variable due to extensive first-pass metabolism in the liver (107).

The species variability in metabolism of TAD's may limit the extrapolation of results obtained in one species to another. The metabolism of IMI illustrates this point. Man converts IMI to DESI slowly but the metabolism of DESI is much slower. The rat converts IMI to DESI rapidly. In both the above species desipramine accumulates. Mice and rabbits metabolise IMI and DESI at roughly the same rate. Therefore DESI does not accumulate in the tissues of mice and rabbits (134).

1.133 EXCRETION

Renal excretion is the major route of elimination of this group of drugs. Thirty to fifty per cent of a single dose of IMI is excreted within the first 24 hours but a single dose can take several days before it is excreted completely (116, 141). The bulk of the urinary drug fraction consists of polar metabolites, particularly glucuronides, while 10% or less occurs as unchanged drug (72, 76, 116, 117). The excretion of the glucuronides is pH-dependent and obeys the pH partition law (71, 76).

Unconjugated hydroxylated metabolites, which amounts to only about one quarter of the bulk excreted via the urine, are excreted via the faeces (76, 117, 141). The source of the material excreted in the faeces is the bile (72, 89).

A summary of the pharmacokinetic parameters of various TAD's is presented in Table I .

1.14 PHARMACOLOGICAL PROPERTIES

The pharmacological profile of TAD's is characterized by a multitude of actions, some of which apparently do not correlate with the clinical antidepressant actions.

1.141 EFFECTS ON THE CENTRAL NERVOUS SYSTEM

TAD's produce an elevation of mood in some depressed patients when given over a period of time. The therapeutic effects of these drugs become evident after about one to two weeks of treatment. The explanation of the slow onset of effects remains a matter of conjecture (see section 1.147).

TABLE I : SOME PHARMACOKINETIC PARAMETERS OF TAD's

DRUG	BIOAVAILABILITY (%)	PROTEIN BINDING (%)	PLASMA $t_{1/2}$ (h)	VOLUME OF DISTRIBUTION (L/kg)
IMIPRAMINE	30 - 55 29 - 77	76 - 95 96 88 - 93	9 - 20 19 - 24	20 - 40
AMITRIPTYLINE	31 - 61	82 - 96	31 - 46 32 - 40 17	
NORTRIPTYLINE	56 - 79 46 - 59 32 - 63	93 95	18 - 93 18 - 28 24 - 86	21 - 31 21 - 57 17 - 26
DESIPRAMINE		90 73 - 92	14 - 62 34 - 76	22 - 59
PROTRIPTYLINE			54 - 198 55 - 124	19 - 26 20 - 57
CLOMIPRAMINE			22 - 84 12 - 40	7 - 20
DOXEPIN	13 - 45		8 - 24	9 - 33

h - hour

L/kg - litre per Kilogram

Modified from Hollister (139)

Hallucinations, excitement, confusion, hyperactivity, disorientation and seizures (142 - 148), which may occur in a small percentage of patients, may represent anticholinergic effects (149). These reactions can be aggravated by concomitant administration of other drugs with anticholinergic action e.g. antipsychotics or anti-parkinsonism drugs (150). Convulsions (143,151 - 154) are more likely to occur in patients with a history of epilepsy or with previous EEG abnormalities (155). The above symptoms usually subside within forty eight hours after withdrawal of the drug, but have been reported to persist up to eight weeks, even with physostigmine administration (147 - 149, 156 - 158).

TAD's may cause sedation and decreased psychomotor activity. Complaints of lassitude, fatigue and loss of energy are common, especially early in treatment. Sedative effects can be mitigated by providing a single daily dose to be taken at night. Alternatively, a less sedative drug can be used for daytime treatment while a more sedative drug can be used at night. AMI, nortriptyline, trimipramine and doxepin are regarded as "sedative" antidepressives while DESI, IMI and protriptyline are regarded as the "stimulant" antidepressives. CLOMI has a position between IMI and AMI.

There have been reports of EEG changes produced by intravenous doses of IMI in rabbits and cats (159 - 160) and also in man (161 - 162).

Extrapyramidal reactions are rare. Paresthesias may be indicative of the rare development of peripheral neuropathy (163). There have been reports of gross disturbance of motor functions occurring in elderly patients (164, 165).

1.142 EFFECTS ON THE AUTONOMIC NERVOUS SYSTEM

The anticholinergic features caused by TAD's are well documented (166 - 171). The TAD can be placed in the following approximate rank order of antimuscarinic potency: AMI > IMI > doxepin = nortriptyline > DESI = protriptyline (172 - 174). The common antimuscarinic symptoms are dry mouth, dizziness, blurred vision, loss of visual accommodation, epigastric distress, constipation, urinary retention and paralytic ileus. Postural hypotension and paradoxically excessive sweating may also occur. The incidence of dizziness, postural hypotension, constipation edema, muscle tremors and urinary retention is higher in older patients (166). Treatment with a peripherally acting cholinomimetic (e.g. bethanecol) may counter the peripheral anticholinergic action of TAD's.

Tremor, which is a sympathomimetic effect of TAD's, is common. A β-blocking drug such as propranolol may be useful in the treatment of this effect.

1.143 EFFECTS ON THE CARDIOVASCULAR SYSTEM

Cardiovascular difficulties have been reported with both therapeutic doses (175 - 181) and toxic doses (3, 153, 182 - 195) of TAD's.

Moir et al (187) have reported that sudden deaths among cardiac patients receiving TADs were four times more frequent than in those who were not so treated. No single factor could be isolated as responsible for the high incidence of sudden death although most of the deaths occured in patients who were acute medical emergencies when admitted (182, 196).

Tachycardia and postural hypotension have also been reported (180, 197, 198, 199). Some authors (197, 199) have reported that significant postural hypotension is a frequent occurrence However, this cardiovascular effect was not found to be common in other studies (200, 201).

TAD administration has also been associated with coronary thrombosis (153, 184, 203), pulmonary emboli (3, 184), the precipitation of congestive heart failure (176, 179, 184, 185, 203, 204) and myocardial infarction (179, 180, 204).

The current impression is that AMI, protriptyline and IMI may be more dangerous than doxepin, maprotiline, mianserin or nomifensine in the treatment of depressed patients with cardiac disease (22, 28, 29, 65, 205 - 207).

1.144 ALLERGIC AND TOXIC EFFECTS

When allergic skin reactions occur with TAD's, they are usually noted early in therapy and often subside with reduced dosage (208).

Jaundice has been reported in less than 0,5% of cases treated with IMI (208).

Agranulocytosis is a rare but serious complication of TAD treatment. A number of cases, including three fatalities, have been reported (151, 209 - 215). Leukocytosis, leukopenia

and low grade eosinophilia have also been reported (3, 146, 216). The incidence of agranulocytosis seems to be higher in elderly women (66).

1.145 MISCELLANEOUS EFFECTS

Weight gain is frequent with these drugs (133,217). Increased appetite and caloric intake are usually implicated (217).

Lactation during IMI treatment has been reported (218).

The safety of TAD treatment during pregnancy or during lactation is not well established. Evidence concerning the possibility of teratogenic effects of TADs (219 - 222) is mixed but unconvincing.

Inappropriate secretion of antidiuretic hormone has been reported with the use of various TAD's (223).

1.146 EFFECTS OF TAD's ON BIOGENIC AMINES

The effect of TADs on biogenic amines (serotonin, noradrenaline and dopamine) at the nerve terminal has been associated with their mechanism of action. Many researchers have examined this aspect of TAD's.

On nervous stimulation, the neurotransmitter (biogenic amine) is released from the storage vesicle in the presynaptic neuron, by a process of exocytosis (224). It diffuses across the synaptic cleft and reacts briefly with postsynaptic receptors. The action

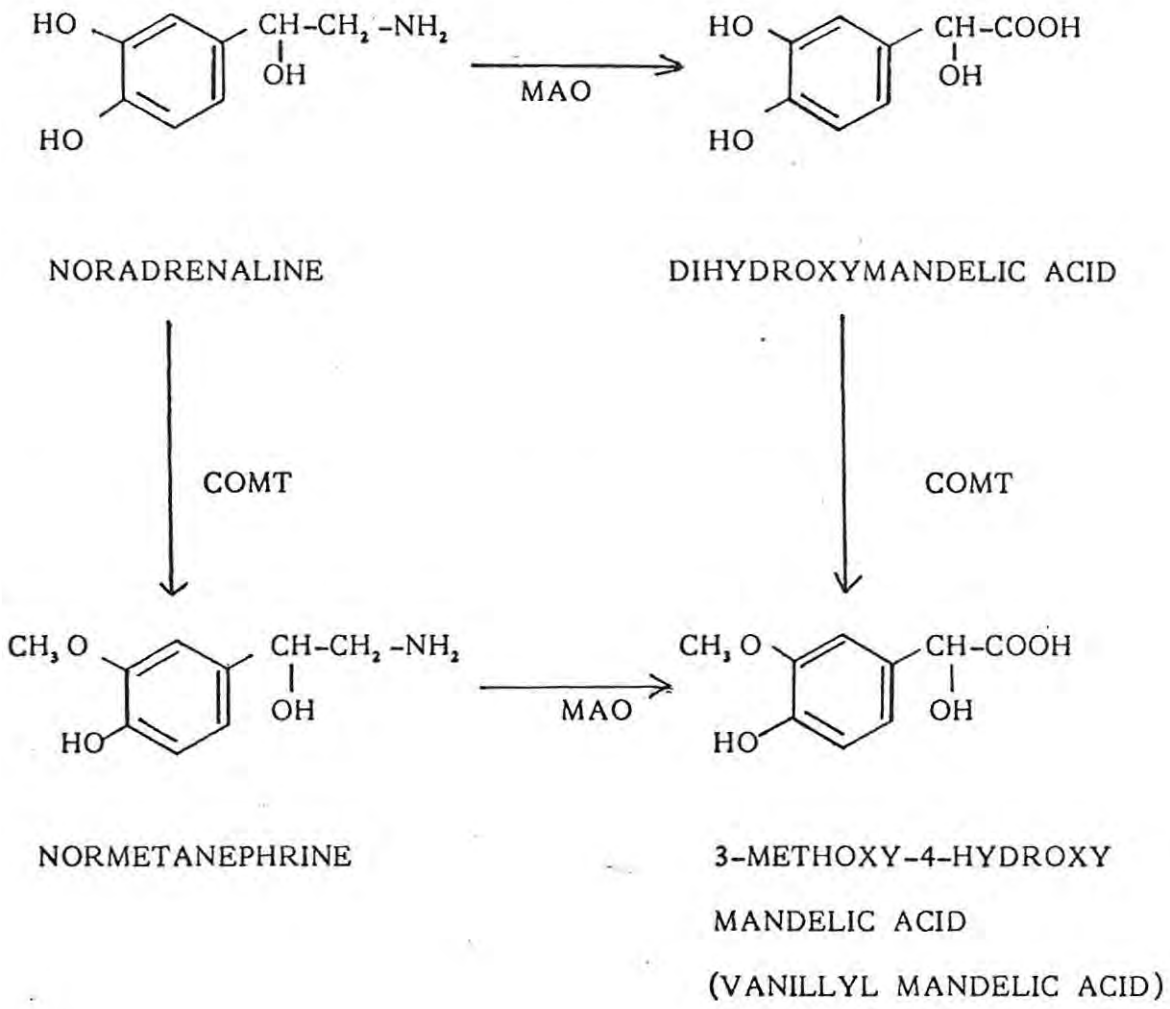
of the transmitter is terminated primarily by reuptake of the amine from the synaptic cleft by the presynaptic neurons. Most of the resorbed transmitter is restored in the storage vesicles but some is metabolised by the enzyme monoamine oxidase (MAO) to 3, 4 - dihydroxymandelic acid. MAO is located in the mitochondria of the nerve cell. Any noradrenaline remaining in the synaptic cleft is metabolised to normetanephrine by catechol-o-methyltransferase (COMT). Figure 3 illustrates the action of MAO and COMT on noradrenaline (N/A). The normal process of release and reuptake of neurotransmitters is illustrated in figure 4.

The TAD's block the reuptake of biogenic amines from the synapse and are therefore presumed to increase the quantity of functional neurotransmitter at the postsynaptic receptor sites.

The suggestion that IMI exerts a "sensitizing" influence on the adrenergic receptor (225) is supported by numerous investigators (226 - 229), who found that IMI potentiated both the response to sympathetic nerve stimulation and the peripheral effects of exogenously administrated N/A, in animals and man.

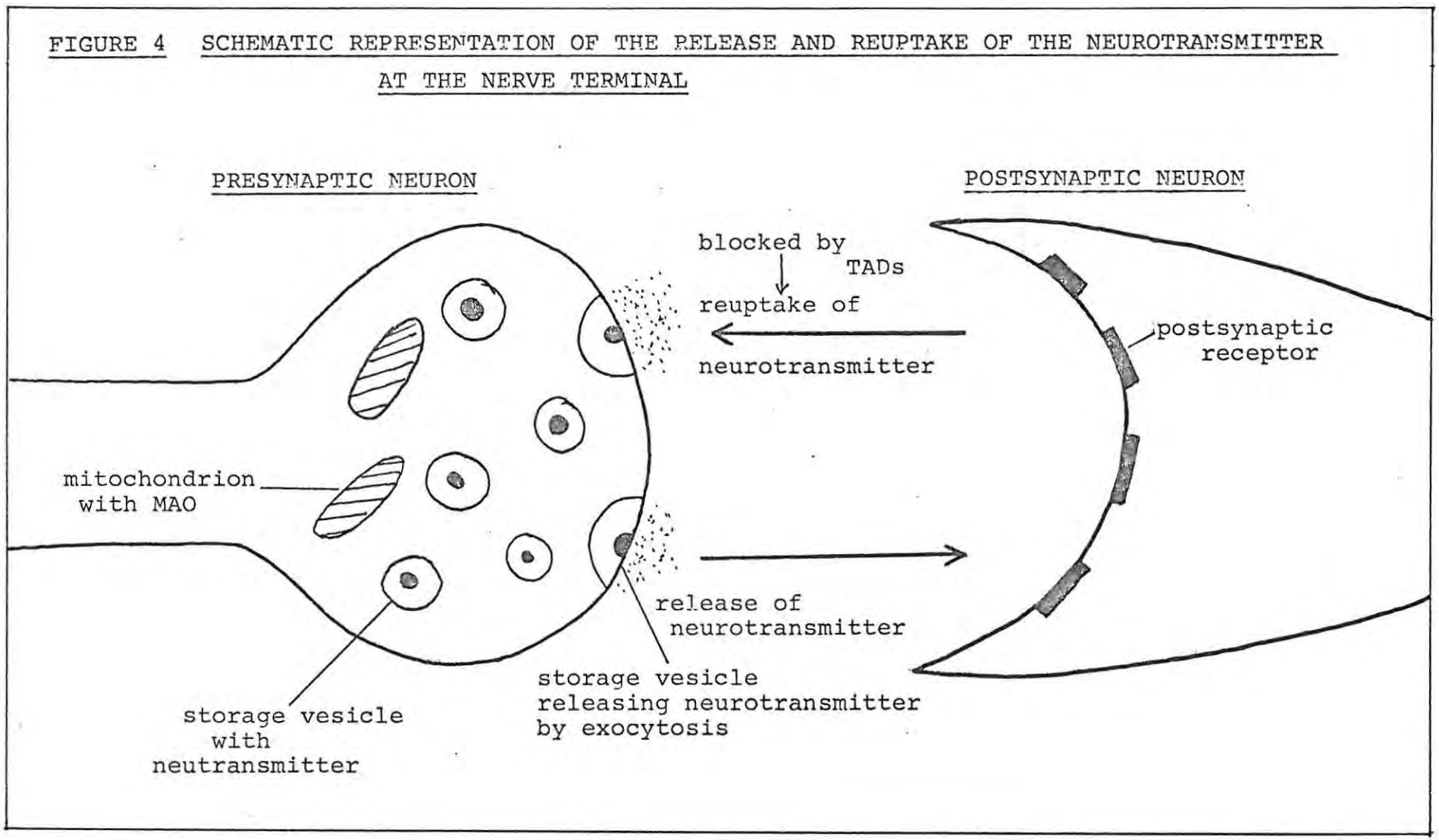
The blocking action of IMI and DESI on the reuptake of N/A was first shown in peripheral tissues (230, 231) and brain (232). Glowinski and Axelrod (233) then demonstrated the inhibition of uptake of ^{3}H-N/A in intact rat brain by IMI, DESI and AMI <u>in vitro</u>. Rat brain ^{3}H-N/A levels were also shown to be increased by IMI administration (234 - 235). These results were supported

FIGURE 3



AN ILLUSTRATION OF THE ACTION OF THE ENZYMES MAO AND COMT ON NORADRENALINE

FIGURE 4 SCHEMATIC REPRESENTATION OF THE RELEASE AND REUPTAKE OF THE NEUROTRANSMITTER AT THE NERVE TERMINAL



by clinical studies which demonstrated increased normethanephrine levels and decreased vanillylmandelic acid in urine of patients treated with IMI (236, 237). In 1964, Haefely et al (238) proposed that the TAD exert at least three types of activity at the peripheral adrenergic synapse:-

(a) A sympathomimetic effect, which was thought to be due to the release of active N/A from its binding sites;

(b) inhibition of reuptake of N/A from the synaptic cleft, and

(c) a sympatholytic effect in higher doses.

Other researchers have shown that TAD administration potentiates the peripheral (229, 239) and central effects (239, 240) of serotonin (5-HT). Tissue uptake of injected 5-HT was shown to be inhibited by IMI administration, thus causing an increase in concentration of free 5-HT acting on the receptor (241). IMI was also shown to cause a slight increase in 5-HT levels in rat brain (242 - 243).

Many workers have confirmed that TAD's are potent inhibitors of neuronal uptake of N/A (244 - 249) and 5-HT (250 - 254) in brain tissue. The uptake of dopamine was also inhibited by some of these drugs (244, 249, 255, 256).

The sensitivities of the N/A and 5-HT uptake mechanisms to the TAD's are not the same. It has been shown that the secondary amines such as DESI, nortriptyline and protriptyline selectively inhibit the reuptake of N/A (257 - 265) with little effect on 5-HT reuptake (246, 253, 259, 260, 261, 266, 269).

CLOMI and AMI selectively inhibit the reuptake of 5-HT with little effect on N/A uptake (253, 259, 262, 267, 268). IMI inhibits 5-HT and N/A reuptake almost equally (268). Doxepin is only a moderate inhibitor of N/A reuptake and a weak inhibitor of 5-HT uptake by rat brain synaptosomes (65). The order of potency of TAD, determined by comparing the molar concentration of the antidepressant which inhibited N/A uptake into synaptosomes of rat brain by 50%, was found to be as follows:- DESI $>$ nortriptyline $\geq$ chlordesipramine $\geq$ IMI $>$ AMI $\geq$ CLOMI (253, 259, 262). These researchers also demonstrated that the order of activity of TAD in inhibiting 5-HT uptake into synaptosomes was:- CLOMI $>$ IMI $\geq$ AMI $\geq$ CHLORDESIPRAMINE $>$ Nortriptyline = DESI. The ratio of activity against 5-HT and N/A uptake was found to be 4,5 : 1 for CLOMI and 1 : 9 for DESI (262).

TAD's have been reported to inhibit uptake of dopamine in crude striatal synaptosomes at concentrations between 10^{-6} M and 10^{-5} M (270). Koe (261) reported that TAD's exert only weak effects on dopamine uptake in synaptosomal preparations of rat brain. AMI and doxepin have been shown to be potent inhibitors of dopamine-sensitive adenyl cyclase while the other tricyclic derivatives were moderate or weak inhibitors (271). Dopamine supersensitivity was shown to result from protriptyline administration (272). Modigh (273) later reported that the duration of dopamine supersensitivity parallelled the duration of antidepressant effect. Since most of the TAD's have only a weak effect on dopamine-associated processes, not many researchers have been particularly interested in this effect on TAD's.

In 1973 Scubeth (274) reported that TAD's reduce central 5-HT turnover. It was suggested that this effect could be secondary to receptor stimulation and could result from feedback inhibition of 5-HT synthesis. Another possible explanation for the decrease in 5-HT turnover could be inhibition of uptake of tryptophan into neurons by TAD's (275). The reduction in post-probenecid accumulation of 5-hydroxyindole acetic acid (5-HIAA) after administration of IMI, AMI and CLOMI in humans could also be an indication of decreased 5-HT turnover (276 - 277).

TAD's have been shown to inhibit the uptake of 5-HT by blood platelets (278 - 284). The order of potency of the TAD to inhibit the uptake of 5-HT by platelets in vitro is as follows:-

CLOMI > IMI > Desmethylclomipramine > AMI > nortriptyline = desi.

It has been reported that IMI, AMI, doxepin and desmethylclomipramine have a pure competitive inhibitory effect on uptake of 5-HT by platelets while CLOMI has both a competitive and non-competitive inhibitory component (287). The non-competitive inhibitory component has been attributed to interference by CLOMI with the important role of chloride in the transport process (287). Riemers et al (282) reported a slow release of endogenous 5-HT from platelets exposed to TAD's in vitro. In vivo experiments have shown that patients receiving therapy with IMI and DESI gradually lose up to 80% of the original 5-HT content of their platelets (285, 286). The fate of the 5-HT from the platelets is not known.

TAD's also block the action of histmine at the H_1-receptor (225, 287 - 290) and also the H_2-receptor (290 - 291). The action of histamine in stimulating adenylate cyclase is blocked. The pharmacological significance of this effect is currently being investigated.

Table 2 presents a summary of the effect of TAD's on biogenic amines.

1.147 MODE OF ACTION OF ANTIDEPRESSANT DRUGS

Despite the widespread use of TAD's, their mechanism of antidepressant action in man remains to be elucidated. This is probably due, at least in part, to the inability to define the precise mechanism underlying depression. Various postulations regarding the cause of depression have been made. Until recently, catecholamine hypothesis was the most viable theory of depression available.

The catecholamine hypothesis states that depressions may be associated with an absolute or relative deficiency of N/A and other catecholamines at functionally important receptor sites in the brain (294 - 295). This postulation was based on two fundamental observations. Firstly, reserpine, a drug which is capable of precipitating severe depression in some humans (296 - 297) lowers both catecholamine and indoleamine concentrations in the brain (298). Secondly, iproniazid, a therapeutically effective antidepressant (5, 299), raises the concentration of biogenic amines in the brain by blocking the action of MAO (300 - 301).

TABLE 2

SYNAPTOSOMAL UPTAKE INHIBITION OF BIOGENIC AMINES BY TRYCYCLIC ANTIDEPRESSANTS

Modified from Randrup et al (270)

DRUG	CONCENTRATION CAUSING 50% INHIBITION OF UPTAKE (UM)		
	DOPAMINE	NORADRENALINE	SEROTONIN
Desipramine	8,7	0,0015	2,0
Clomi pramine	3,8	0,044	0,015
Imipramine	12,5	0,02	0,24
Amitriptyline	5,6	0,02	-
Nortriptyline	4,4	0,011	0,42
Trimipramine	6,8	1,0	8,2
Butriptyline	5,2	1,7	10,0
Protriptyline	4,9	0,003	1,62

Additional evidence in support of this hypothesis is present in Table 3

Lapin and Oxenkrus (302) proposed the indoleamine hypothesis of depressive disorders which is a variation of the catecholamine hypothesis. It states that depression is associated with a deficiency of brain 5-HT. The mechanism by which the postulated decreased aminergic function is mediated in depressed patients is not clear. The relative importance of 5-HT versus N/A has also been debated, although present theories accommodate both neurotransmitters (303 - 305).

It has been generally accepted that at least part of the therapeutic action of TAD's may be associated with an increased availability of N/A and/or 5-HT at the post-synaptic receptor sites. It is difficult to determine whether the clinical antidepressant effect is more closely related to the ability of the drugs to increase 5-HT or N/A since the TAD's affect transport mechanisms of both amines. The parent drugs, which may be selective to one amine system, are converted to their metabolites in vivo and these metabolites may affect the other amine system. If differences in the relative degree of blocking the uptake of N/A and 5-HT are of clinical significance, controlled clinical trials with more selective uptake inhibitors should provide a more definite answer to this problem (306). In this respect, fluoxetine is of particular interest as it is a selective inhibitor of 5-HT uptake into specific brain regions in vivo and N- demethylation to its primary amine does not alter its potency or selectivity towards inhibiting uptake of 5-HT (307).

TABLE 3

PHARMACOLOGICAL OBSERVATIONS CONSISTENT WITH THE CATECHOLAMINE HYPOTHESIS OF DEPRESSION

DRUG	ACTION	EFFECTS ON BEHAVIOUR IN ANIMALS	EFFECTS ON MOOD IN HUMANS
Tricyclic anti-depressants	Blocks uptake of N/A and 5-HT	Prevents reserpine-induced sedation Potentiates effects of amphetamine	Relieves Depression
MAO INHIBITORS	Elevates brain levels of NA and 5-HT by blocking MAO	Excitement. Prevents and reverses reserpine induced sedation	Relieves Depression
Reserpine	Depletes brain of N/A and 5-HT	Sedation	Sedation Causes depression (in some patients)
Amphetamine	Inhibits cellular uptake and in-activation of N/A Releases N/A	Excitement	Elation
Lithium	Increases Net up-take of N/A and 5-HT Reduces release of N/A and 5-HT	Prevents the hyper-activity induced by desipramine and reserpine	Relieves Depression
ECT	Increases N/A turnover		Relieves Depression
Propranolol	β-adrenergic blocker		Causes Depression

Although the arguments in favour of the biogenic amine hypothesis are quite impressive, various counter arguments have been presented. The validity of the biogenic amine hypothesis is now being questioned because of the following observations:-

(a) Not all the effective antidepressants have been shown to effect the amine pump. No clearcut interference with noradrenergic mechanisms could be demonstrated in animal experiments with trimipramine (270) and bupropion (309). Butriptyline has only a weak inhibitory effect on N/A and 5-HT uptake (310) yet it shows a clinical antidepressant effect (311). Iprindole is reported to be a clinically effective antidepressant (312 - 317) but it exhibits only a weak inhibitory effect on neuronal reuptake of N/A and 5-HT (318 - 322) and it does not alter the metabolism of N/A (321) or the turnover of N/A (322) or 5-HT (323). However, Vetulani et al (324) and Banergee et al (325) have recently reported that iprindole induces subsensitivity of postsynaptic β -receptors after chronic treatment and this is thought to be brought about by an increased concentration of N/A in the synaptic cleft.

(b) Femoxetine and cocaine are potent re-uptake blockers but are not effective antidepressants (326 - 328).

(c) L-Dopa is a natural precursor of catecholamines. If depression was due to a reduced amount of these amines, L-dopa should alleviate depression. However, L-dopa lacks antidepressant activity (329 - 330).

(d) Tyrosine hydroxylase is the rate-limiting enzyme of catecholamine biosynthesis. ∝- methyl-p-tyrosine inhibits the action of tyrosine hydroxylase but does not attenuate the antidepressant effect of IMI (331).

(e) Tyramine can cause an increase in blood pressure by releasing N/A after it is taken up into the sympathetic neuron via the N/A uptake mechanism. This uptake mechanism is also blocked by TAD's and therefore TAD's reduce the effects of tyramine on blood pressure. The plasma levels of nortriptyline and AMI correlate with the reduction in the blood-pressure response to tyramine but not with the antidepressant effects of the above TAD's (332).

(f) The slow onset of the therapeutic effects of TAD, in contrast to the almost instantly occuring pharmacological effects in in vitro animal experiments (306, 333) is probably the most important argument against the amine hypothesis of depression.

The need for the re-evaluation of the biogenic amine hypothesis of depression is therefore clear.

Recently, various adaptational changes to receptor function have been reported after chronic antidepressant treatment. Attempts have been made to correlate the adaptational changes with the latency of onset of the antidepressant effects of TAD's.

In acute animal studies, TAD's were found to reduce the synthesis and turnover (334 - 336) of N/A. This reduction in turnover could be mediated by postsynaptic receptors via a feedback mechanism or by presynaptic release-regulating α^2-receptors (337). After chronic treatment with N/A uptake inhibitors (2 - 3 weeks), normalization or a slight increase was observed, indicating that an adaptational mechanism had come into play (338 - 340). Warnack et al (341) have suggested that presynaptic α-receptors may become subsensitive after repeated administration of drugs that tend to increase the concentration of N/A in the synaptic cleft.

In animal experiments, repeated treatment with antidepressant drugs leads to a reduction in the number of post synaptic β-receptors and their responsiveness to stimulation (324, 325, 342 - 350). This effect is probably due to increased noradrenergic transmission as a consequence of the treatment (342, 344). There is some discrepancy with respect to time-course of development of this effect. Some authors have observed this effect after only a few days (342 - 344), while others have found that it occurs after four to eight weeks of treatment (324, 325). It is possible that these discrepancies could be due to differences in experimental techniques and methodology. Further studies in this field should prove to be useful.

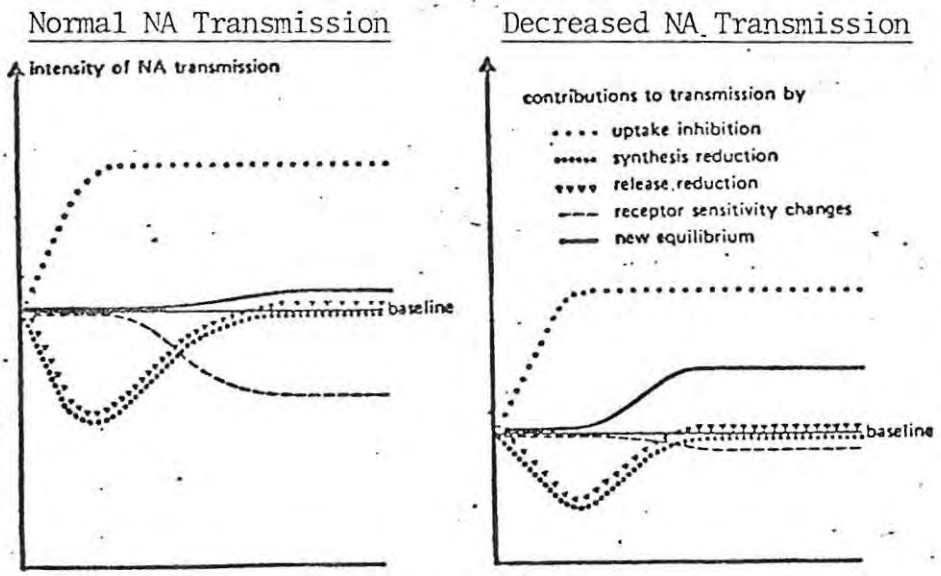
Modifications in the circadian rhythms in rat brain and adrenergic receptors have also been associated with chronic administration of TAD [351 - 352].

Waldmeier (353) has reported on the possible consequences of these adaptational phenomena on the antidepressant effect of drugs. According to his hypothesis, the above adaptive processes continue to occur once steady-state levels of the drug is reached, until a new equilibrium of the noradrenergic transmission is attained. This equilibrium,may or may not differ from the pretreatment situation, depending on how much the adaptive processes have been able to neutralize the effect of the drug. This concept is presented in the schematic diagram in Figure 5.

The above considerations may explain the latency in onset of antidepressant effects and also present a possible reason for the lack of response in some people taking antidepressant drugs. If it is assumed that a disturbance of N/A transmission is causally related to depression, it is possible that adaptational phenomena reduce the effect of antidepressant treatment on synaptic transmission to varying degrees, depending on the nature and site of the underlying defective transmission. The position of the new equilibrium state of noradrenergic transmission during treatment relative to the pretreatment situation may differ in each patient. Waldmeier (353) believes that the best improvement should be expected in a case where this difference is the greatest.

FIGURE 5

Effects of the uptake inhibition and its consequences on NA release, synthesis, and receptor sensitivity on the intensity of noradrenergic synaptic transmission.



Waldmeier (353)

This hypothesis is an amplification of the catecholamine hypothesis of depression. The author has emphasized that reduced noradrenergic transmission contributes towards depressive symtomology but does not necessarily cause depression per se. In this hypothesis, the effect of TADs on 5-HT reuptake has not been considered. Since there is no conclusive evidence that 5-HT or dopamine is not implicated in the therapeutic effects of TAD; the above hypothesis would have been more impressive had it accommodated the other neurotransmitters as well, since he was not discussing a "subtype" of depression.

It has been reported that when long-term administration of antidepressants is studied in animals and the consequences on the postsynaptic receptor are measured by the generation of cyclic AMP, a subsensitivity of the postsynaptic receptor is observed (354 - 357). This effect closely parallels the time course of the clinical antidepressant response. These observations form the basis of the views presented by Sulser et al (306). They have suggested that noradrenergic transmission in the depressed is enhanced, due to oversensitive receptors. Antidepressant treatment desensitize these receptors and thereby restore a more or less normal intensity of transmission. These views are directly opposite to those presented in the amine hypothesis of depression.

In contrast, Ashcroft (358) and Prange et al (359) suggest impaired sensitivity of the postsynaptic receptor in depressive disorders. The observation that thyroid hormone, which affects

receptor sensitivity, enhances the antidepressant action of the TADs supports the above suggestion (360 - 363).

These conflicting reports emphasize the need for further studies of noradrenergic receptor function in the depressed.

After the observation that TAD's reduce specific high affinity ^{3}H-d-LSD binding in the rat brain and also have a high affinity for ^{3}H-d-LSD binding sites in the brain (364), the possible implication of the postsynaptic receptor in depression was investigated. Aprison and coworkers (365 - 366) have recently proposed a theory named the "hypersensitive serotonergic receptor theory of depression." This theory suggests that in some types of depression a hypersensitive postsynaptic receptor develops due to decreased release of 5-HT. Other researchers (364 - 367) have lent support to this theory. Ogren et al (368) believe that the therapeutic action of antidepressant drugs may in part be due to producing a reduced functional activity of some central 5-HT systems. These conclusions were drawn from binding studies in the dorsal neocortex of the rat and behavioural studies in mice, using drugs such as AMI, nortriptyline, mianserin , doxepin and nomifensine.

Using an animal model of depression, Nagayama et al (369) demonstrated that TAD's can act as antagonists of 5-HT at the postsynaptic 5-HT receptor and they suggest that the therapeutic effects of some antidepressants may be explained by their postsynaptic rather than presynaptic effects at central 5-HT receptors. However, these studies were based on acute admin-

istration of the TAD's. Studies with chronic administration of the drugs are essential before comparisons are made with the clinical situation.

It is evident from the above reports that the biogenic amine hypothesis of depression is no longer as popular as it used to be. Attention has been diverted from the neurotransmitter itself to the neurotransmitter receptor.

Other possible mechanisms of action have also been explored. Experiments on rabbit lung and brain and human platelet MAO suggest that many TAD's inhibit type B MAO (370 - 373). Type B MAO seems to show greater activity in depressed patients. The urinary excretion of phenylethylamine (PEA), an endogenous substrate of type B MAO, was found to be reduced in depressed subjects (374). These observations aroused interest in the possible role of PEA in depression. Fischer (375) reported that IMI increased PEA levels in human urine. In vitro experiments on the rat brain showed that IMI, pargyline and iproniazid increased levels of PEA (375 - 376). However, this effect was not seen in vivo with either acute or chronic treatment with IMI, AMI or iprindole (377). It has been suggested that TADs may prevent loss of 5-HT and N/A by inhibiting MAO but evidence concerning the effect of TAD's on type A MAO is conflicting (378 - 380). Thus, the pharmacological significance of the in vitro effect of TADs on MAO remains uncertain.

Some authors have associated dopamine with the therapeutic effects of TAD's (381). Many of the newer antidepressives are potent inhibitors of dopamine (DA) uptake (197, 270). There are several reports claiming that DA uptake is blocked by TAD's (197, 382 - 383). However, the drug concentrations required for such activity is much greater than those needed for inhibition of NA and 5-HT uptake. The generally accepted view is that TAD's at realistic doses have little or no effect on DA uptake (364, 384). Pugsley and Lippmann (385) have reported that chronic DESI administration had no effect on DA uptake in vivo. Although L-dopa is the immediate precursor of dopamine, its use in the treatment of depression is controversial (386 - 390). Some researchers (39, 392) have reported a reduced concentration of homovanillic acid (HVA), the main degradation product of dopamine, in the CSF of depressed patients, but other workers (393 - 395) found no significant difference from the control. Acute administration of CLOMI or AMI but not DESI or protriptyline elevates rat striatal levels of HVA (396 - 397). Keller et al (397) have attributed the increased DA turnover to blockade of DA receptors. Chronic administration of DESI (398 - 400), protriptyline (398) or CLOMI (400) has no effect on DA turnover in rat brain. Chronic administration of IMI (401) or AMI (398) produced slight reductions in central DA turnover. It would therefore appear that there is no conclusive evidence for the involvement of DA in the mechanism of action of TAD's.

It has been postulated that the cholinergic nervous system is also involved in depression (402 - 405). Depression is believed to be a disease of central cholinergic predominance while mania is associated with central adrenergic (or serotoninergic) predominance. Many pharmacological studies lend credence to this theory. Reserpine has central cholinomimetic properties in both animals (406) and man (128) and it can produce depression. The centrally active cholinomimetic agents, physostigmine and choline, were associated with the worsening of depressive symptoms in patients with pre-existing depression (403, 404, 407, 408). Since the TAD's have anticholinergic properties, their ability to interact with rat brain muscarinic cholinergic receptors was investigated (174, 409). They were found to differ considerably in their affinity for central muscarinic receptors. This observation, together with the weak action of MAO inhibitors, suggests that central anticholinergic activities are not involved in the antidepressant action of TAD's (410).

The above studies suggest that various neurotransmitters could be implicated in the antidepressant action of TAD's but the precise mechanism(s) of action of TAD's remains elusive. It is possible that antidepressants may act by a common, as yet unknown, mechanism of action. Elucidation of the mechanism of action of TAD's would result in a better insight into the pharmacology of these drugs and it may also assist in unravelling the etiology of depression.

Various indoles are present in the pineal gland. This organ contains a very high concentration of serotonin. Noradrenalin also plays a significant role in the pineal as it is responsible for the stimulation of the enzyme serotonin N-acetyl transfrase, which is responsible for the synthesis of melatonin. Melatonin, a metabolite of serotonin, has been associated with certain behavioural effects (see Section 1.246) and it is regarded as the most important secretion of the pineal. It is therefore possible that this organ could have some influence on the mechanism of action of TAD.

1.148 CLINICAL USE

TAD's are used mainly in the treatment of depression. There have also been reports of their potential use in the following conditions: -

(a) Enuresis nocturna (411)
(b) School phobia (412, 413)
(c) Blatophobia(414)
(d) Chronic pain (415 - 417)
(e) Allergy (418, 419)
(f) Parkinsonism (414, 420 - 423)

1.149 CHOICE OF THE DRUG IN DEPRESSION

Depressed patients with clear endogenous characteristics, including psychomotor retardation, poor appetite and weight loss, are believed to respond favourably to TAD's (424).

Some researchers have based the selection of a particular TAD on the urinary excretion of 3-methoxy-4-hydroxy-phenylglycol (MHPG), a metabolite of noradrenaline (425 - 426). Patients excreting "low" amounts of this metabolite are assumed to have a "noradrenergic-deficient" depression. It is believed that these patients are best treated with drugs that tend to selectively block the uptake of noradrenaline. Conversely, patients excreting "high" amounts of MHPG are assumed to have a "serotonergic-deficient" depression and respond well to drugs like AMI, which more selectively block the reuptake of 5-HT. Some authors (427) disagree with this approach because of the difficulty in defining a "low" or a "high" rate of MHPG excretion.

Maas (268) has suggested that the patient's response to dextroamphetamine administered for 1 to 2 days initially will predict response to TAD's. Patients whose moods brighten are said to respond to IMI or DESI while those patients who do not improve should respond better to AMI.

Although the reasoning in the above approaches is justifiable, experimental proof is lacking. Thus, the choice of the drug seems to depend entirely on the individual being treated.

1.2 THE PINEAL INDOLES

1.21 INTRODUCTION

Anatomically, the pineal of the rat is a small organ (1 mg wet mass) and is situated in the rat brain, just rostral to the cerebellum and between the occipal poles of the cerebral hemispheres (428). A very slender pineal stalk provides connection between the pineal and the commisural region. A recent report has suggested that the bulk of the electrical inputs reaches the pineal via the pineal stalk (429). The physiological significance of this is not known.

The major part of the pineal parenchyma is composed of pinealocytes, which contain granular vesicles. A second category of cells that do not contain granular vesicles can be found close to the perivascular space (430).

The pineal has an independent arterial supply consisting of up to four branches of the posterior cerebral artery. The capillary network is as dense as in the cerebral cortex. Venous drainage consists of twelve to sixteen short veins which drain into the great cerebral vein and immediately into the systemic venous circulation via the confluens sinuum (431).

The mammalian pineal is innervated by pinealopetal orthosympathetic fibres and pinealopetal parasympathetic fibres (432 - 434). So far, the function of the parasympathetic

innervation is unknown. The sympathetic fibres originate in the superior cervical ganglia and reach the organ by way of the nervii conarii and along pineal vessels (435). The sympathetic nerve endings contain N/A (436 - 438) and this innervation is of primary importance for pineal secretory function in mammals.

Although the sympathetic fibres to the pineal are adrenergic, the intra-pineal portions contain large amounts of serotonin which are synthesized in the parenchymal cells and taken up by the intrapineal adrenergic terminals (439 - 441). Both N/A and 5-HT are localized within granulated vesicles present in nerves (442). This phenomenon probably reflects a competion between N/A and 5-HT for the storage sites.

In vitro experiments have shown that rat pineal "gliocyte" cells can concentrate another presumed neurotransmitter, aminobutyric acid (GABA) (443). Stimuli originating in parts of the limbic system of the brain run to the pineal via the medullary striae, the habenular nuclei and the pineal stalk (444 - 446). It has been suggested that extra hypothalamic neurosecretory fibres exist in the pineal gland of the rat (448 - 449), cattle and sheep (447) and the monkey (450). The function of such neurosecretory fibres remains to be established.

The biosynthesis of various indoles and peptides, many of which have been shown to be physiologically active, occurs in the pineal gland. In recent years the pineal has become known as a neuroendocrine transducer.

In this section the effect of neurotransmitters, hormones and drugs on pineal function as well as the effect of pineal constituents on mammals, will be discussed.

1.22 REGULATION OF PINEAL INDOLE BIOSYNTHESIS

Most substances that affect pineal function do so by acting either at the noradrenergic synapses or at one or more of the many steps involved in the conversion of tryptophan to melatonin. The influence of the sympathetic nervous system, hormones and drugs on pineal indole biosynthesis is discussed.

1.221 NEURAL CONTROL OF PINEAL INDOLE BIOSYNTHESIS

In vivo, tryptophan is taken up readily from the bloodstream by the pineal (451, 452) and some of it is converted to 5-hydroxy and 5-methoxyindoles (453) or protein (454). The major route of tryptophan metabolism in the pineal appears to be oxidative cleavage of the pyrrole ring to kynurenine by the enzyme indoleamine-2,3,- dioxygenase (455). The effect of N/A on pineal indole metabolism has been studied in organ culture systems with isotopically labelled tryptophan (456, 457). The synthesis of 5-HT and melatonin (MEL) from tryptophan is accelerated by N/A in cultured pineals, by a process involving β -adrenergic receptors (458 - 461). The β -receptor activates adenylcyclase activity (462 - 464), the enzyme responsible for the conversion of ATP to cyclic AMP (cAMP). Indirect evidence that implicates cAMP in the control of melatonin synthesis is as follows:-

(1) Adenylcyclase in pineal homogenates is activated by N/A (462, 463, 465 - 467).

(2) Dibutyryl cAMP, but not cAMP itself accelerates MEL synthesis from C-tryptophan within cultured pineals (457, 458, 468, 469).

(3) Some studies in cultured pineals have shown that dibutyryl c AMP enhances activity of serotonin-N-acetyl transferase (SNAT) (459, 470).

Using cultured pineals, Pelayo et al (471) have shown that α_2-receptors affect N/A release. Besides the β-receptor-mediated effects, N/A is also believed to act at postsynaptic α-receptors (472).

The first step in pineal indole metabolism is the hydroxylation of tryptophan by tryptophan hydroxylase to form 5-hydroxytryptophan (473, 474). 5-Hydroxytryptophan decarboxylase then converts 5-hydroxytryptophan to 5-hydroxytryptamine, i.e. Serotonin (475, 476). 5-HT is a precursor for biologically active compounds like melatonin and 5-methoxytryptophol but may also have effects of its own since its abrupt decline in rats after the onset of darkness is quite in excess of that required for methoxyindole synthesis (477).

Three pathways (478) account for the decrease of pineal 5-HT levels in darkness:-

(a) Oxidative deamination by monoamine oxidase (MAO);

(b) N-acetylation;

(c) release to the extracellular space.

Cardinali (478) has suggested that the availability of the 5-HT to any of these metabolic routes probably depends upon the control of the equilibrium
"granular 5-HT $\rightleftharpoons$ free 5-HT"; only in the latter compartment is 5-HT subjected to further metabolism.

Both type A and type B MAO are reported to be present in the pineal (479). The action of MAO on 5-HT results in the formation of 5-hydroxyindole acetaldehyde. The latter compound is an unstable intermediate which is either oxidized to 5-hydroxyindole acetic acid (480) or reduced to 5-hydroxytryptophol by an alcohol dehydrogenase (481).

N-acetylation of 5-HT by a relatively specific N-acetyltransferase (serotonin N-acetyl transferase; SNAT) results in the formation of N-acetyl serotonin (482). The acetyl group is provided by the cosubstrate acetyl coenzyme A, whose concentration is controlled by an active acetyl coenzyme A hydrolase (483).

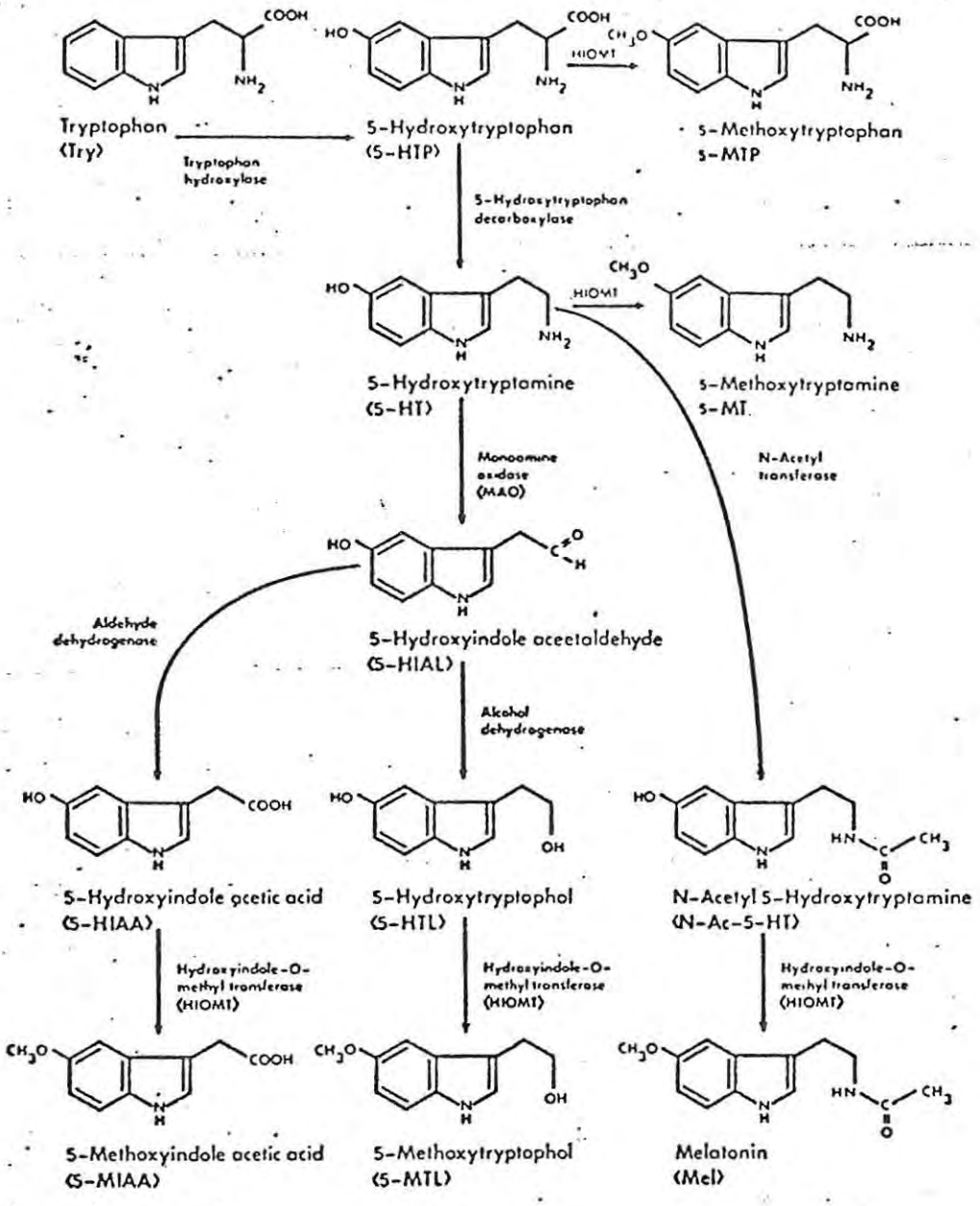
The conversion of N-acetylserotonin to melatonin is catalysed by the enzyme hydroxyindole-o-methyl transferase (HIOMT). This conversion involves the transferance of a methyl group from 5-adenosylmethionine to the 5-hydroxy group of N-acetyl serotonin (484). The pineal 5-adenosylmethionine levels are high and this is probably due to the high concentration of enzymes ATP-L-methionine-5-adenosyltransferase which catalyses the formation of 5-adenosylmethionine from ATP to L-methionine (485).

5-Hydroxyindoleacetic and, 5-hydroxytryptophol and 5-hydroxytryptaphan can also be methylated by HIOMT (484, 486 - 488). 5-methoxytryptophol, the methylated product from 5-hydroxytryptophol, is considered to be a circulating secretory product of the pineal gland (489). Figure 6 represents the synthesis and metabolism of pineal indoles.

Recently, Smith et al (490) have demonstrated the presence of O-acetyl-5-methoxy tryptophol in the pineal, using gas chromatography-mass spectrometry. According to these authors, o-acetyl-5-methoxytryptophol is rapidly hydrolyzed in plasma to 5-methoxy-tryptophol with a half-life of about 2 minutes.

No active mechanism of melatonin secretion has yet been described and it seems that melatonin is secreted by the pineal gland by simple diffusion. Earlier studies (491, 492) seemed to suggest that the primary compartment into which mammalian pineal melatonin was secreted was the cerebrospinal fluid (CSF). Subsequent studies have shown that plasma melatonin concentrations were higher than CSF melatonin concentrations in adult humans (493, 494); rhesus monkey (495), rats (496) and sheep (497). Suggesting that melatonin is secreted into the bloodstream. The observation that darkness induces increases in melatonin levels in plasma of rat without concomitant increases in CSF (497), lent credence to this view. The differences between the earlier studies and the more recent studies could be due to the method of sampling CSF or of drug treatment.

FIGURE 6



THE INDOLE METABOLISM IN THE PINEAL GLAND

Balemans , M.G.M (870)

It has been reported that melatonin rapidly crosses into the CSF and equilibrates to about 40% of plasma levels (495). About 60% - 70% of melatonin in plasma is bound to albumin and none is bound to protein in CSF (498). A recent study suggests that albumin-bound melatonin can be transported across the blood-brain barrier (499).

An intravenous dose of radioactive melatonin disappears rapidly from the blood (495, 500). 92 - 97% is converted to 6-hydroxy-melatonin after a single pass through the liver (499). The 6-hydroxy melatonin is conjugated with sulfate (70 - 80%) or glucuronide (5%) and excreted into the urine (500). The remaining melatonin appears either unchanged (1%); as 5-methoxyindoleacetic acid (0,5%) by deacetylation in the liver (501) or as N-acetyl-5-methoxykynurenamine (15%) (502). The biosynthesis and metabolism of melatonin is illustrated in Figure 7.

1.2211 EFFECT OF LIGHT ON NEURAL CONTROL

Changes in the light/dark periods have a marked influence on pineal indole synthesis and metabolism.

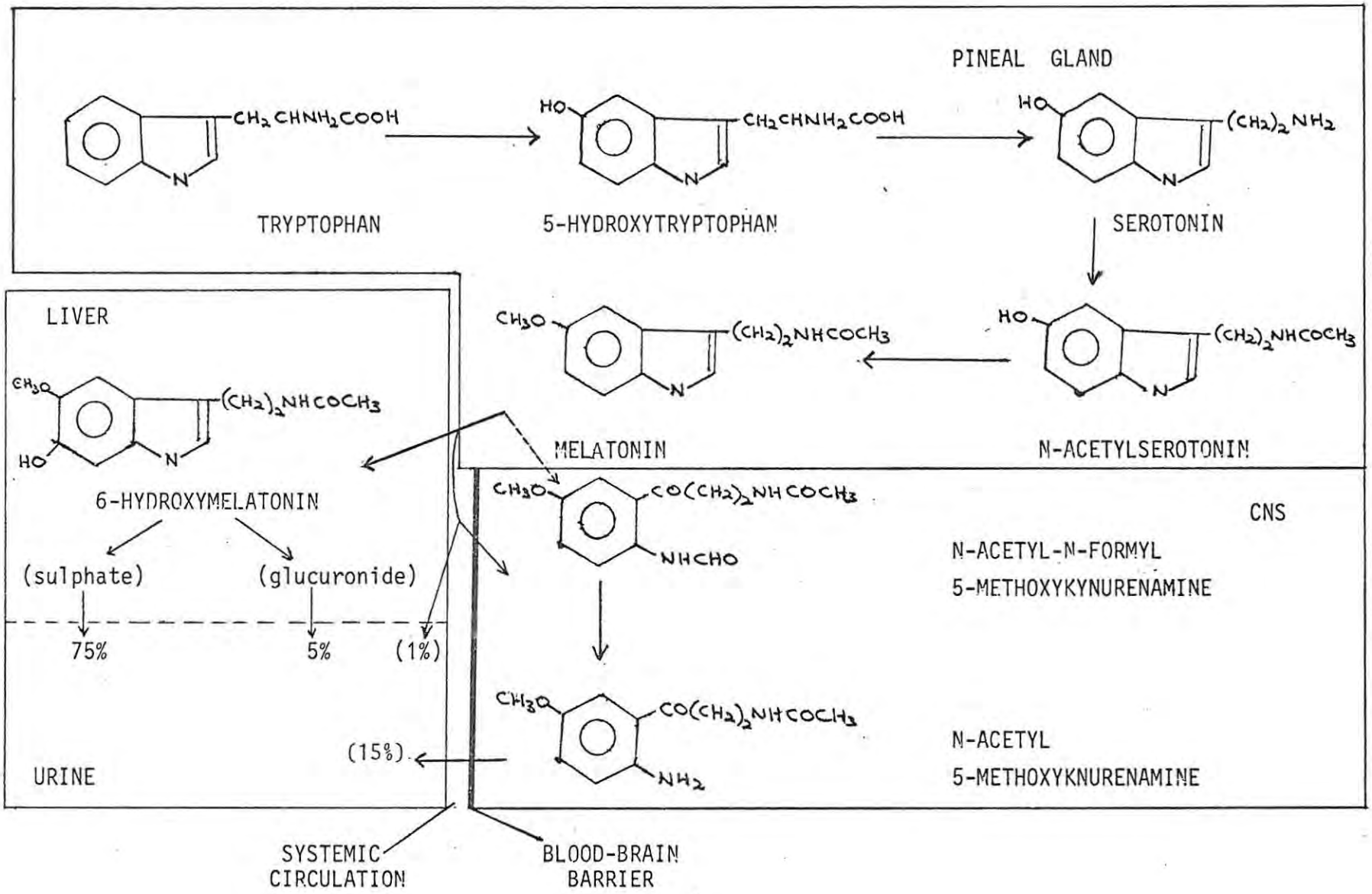
Wurtman et al (428) outlined the pathway along which light reaches the pineal as follows:-

light ⟶ retina ⟶ inferior accessory optic tract ⟶ medial forebrain bundle ⟶ preganglionic sympathetic nerves ⟶ superior cervical ganglia ⟶ post ganglionic sympathetic nerves ⟶ pineal gland.

FIGURE 7

MELATONIN BIOSYNTHESIS AND METABOLISM

CARDINALI D.P. (630)



Darkness activates the sympathetic nerves to the pineal (503). Dark adapted rats show an increased concentration and turnover of N/A in the pineal (504). The amount of N/A in the pineal usually increases during the night and decreases after the onset of light so that minimal amounts are found during day time (505 - 507). The level of N/A influences the level of 5HT, melatonin (MEL) and the enzymes involved in their synthesis and metabolism.

1.22111 ENZYMES

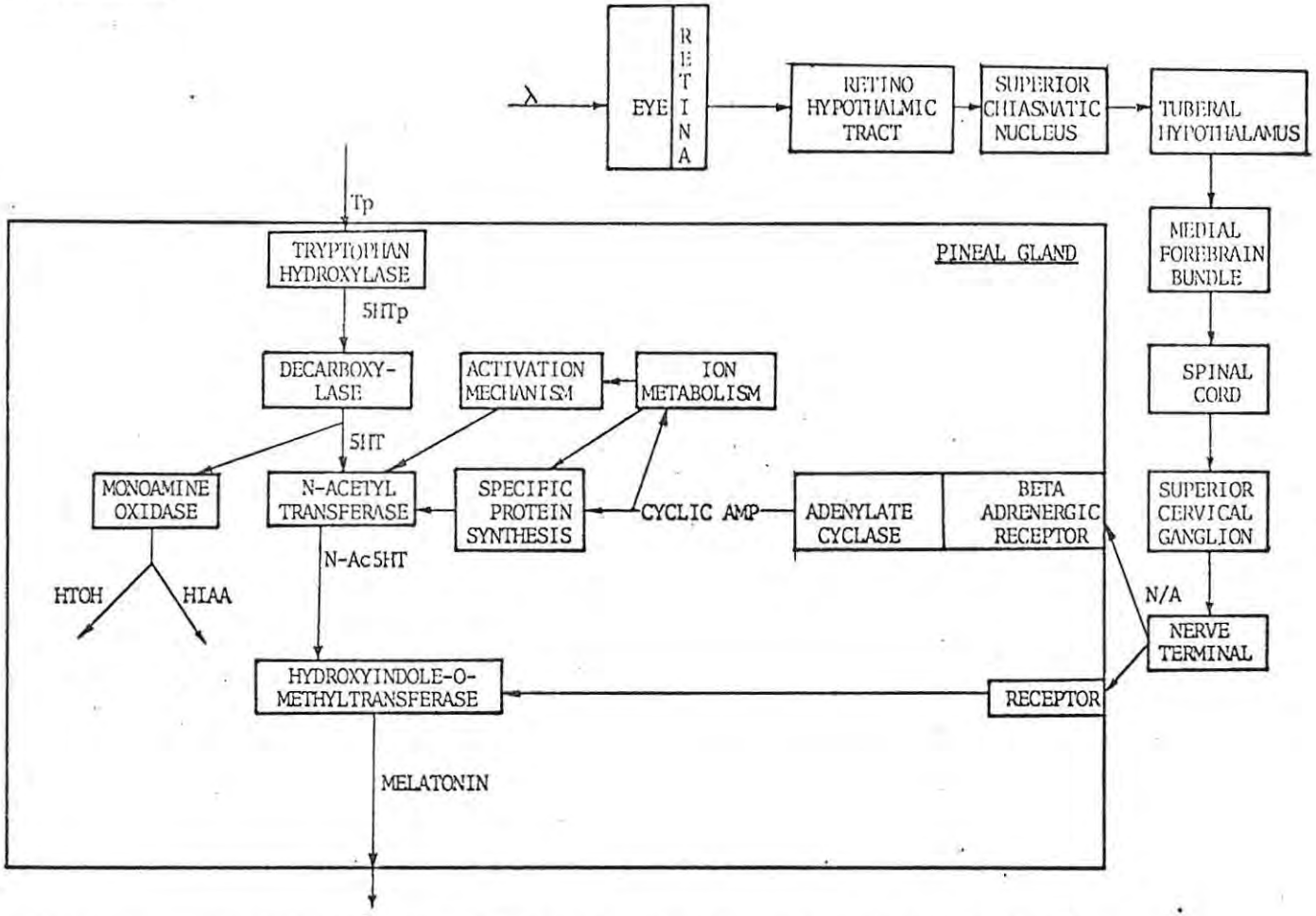
1.221111 SNAT

During the night, N/A stimulates tryptophan hydroxylase (508, 509) and N-acetyltransferase (469, 470, 510). Consequently, SNAT activity increases 20 to 50 fold in darkness (503). The release of N/A from sympathetic nerve endings results in general stimulation of the pineal gland. Zatz (511) has described the "cascade of sequential events" that ensues. N/A interacts with the β -adrenergic receptors and thereby activates adenyl cyclase (462 - 464, 512 - 513), which is responsible for the conversion of ATP to cyclic AMP (cAMP). Some of the cyclic AMP binds to and activates protein kinase (514, 515) while the rest is destroyed by phosphodiesterase (512, 516). The activation of protein kinase leads to phosphorylation of a specific nuclear protein (517), and the synthesis of a specific messenger RNA (518, 519). The translation of this messenger RNA into new protein is also induced by cyclic AMP and this accounts for the marked increase in SNAT activity. A third role for cyclic AMP

is the maintenance of SNAT in an active form (460, 520). The increased SNAT activity provides the substrate for HIOMT, which synthesizes melatonin. Thus, the signal provided to the β-adrenergic receptors is transduced by a series of steps, each activated by the previous step, which results in the large changes in pineal indoleamine metabolism at night (511). Figure 8A presents a schematic representation of the above events.

In contrast to the maximal amount of N/A present at the end of night, the activity of both enzymes (i.e. SNAT and tryptophan hydroxylase) decrease as the end of night approaches. Romero and Axelrod (521, 522) have attempted to explain this phenomenon on the basis of the increased sensitivity (super-sensitivity) of the β-receptor during the daytime. cAMP is not produced in a sufficient quantity during daytime because N/A decreases rapidly. However, the increasing amount of N/A produces a rapid increase in the quantity of cAMP after the onset of darkness, although the sensitivity of the β-receptor is declining during the night (due to continuous stimulation) (514, 524 - 526). The increasing amount of cAMP is sufficient for the stimulation of both enzymes. A further decrease in receptor sensitivity results in a decreased synthesis of cAMP at the end of the night (514). This probably accounts for the decrease in tryptophan hydroxylase and N-acetyltransferase at the end of night. The phenomenon of supersensitivity and subsensitivity of the β-adrenergic receptors has been attributed to an increase or decrease, respectively, in the number of β-adrenergic

FIGURE 8A



A SCHEMATIC REPRESENTATION OF THE NEURAL CONTROL OF THE PINEAL GLAND

KLEIN D.C. AND MOORE R.Y. (570)

receptors available (522 , 527). Wilkonson (528) has reported that the sensitivity of pineal β-receptors appears to be dependent upon calcium ions, possibly by controlling enzyme induction at an intracellular site beyond the β-receptor.

The wavelength of light also seems to affect SNAT activity. As previously mentioned, in the presence of the normal light/dark photoperiod, peak SNAT activity is seen at mid-night. Red light produces a shift in enzyme activity towards the preceding light period. With green light, enzyme activity is shifted to later periods (529).

5-Hydroxytryptophan decarboxylase, which is also under sympathetic nervous control, is stimulated by light (530, 531).

1.221112 HIOMT

Some authors (532) have suggested that sympathetic stimulation results in the inhibition of HIOMT activity. Wurtman (533 believes that acetyl choline causes an increase in HIOMT activity. The influence of acetylcholine is observed only during darkness. It is possible that acetylcholine cancels the inhibition of NA, which shows a maximal concentration in darkness. HIOMT activity is maximal during the night and is inhibited by the presence of light (534). Green light decreases HIOMT activi ty while red light was found to have no effect on HIOMT activity.

1.22112 SEROTONIN

In some mammals the pineal contains a higher concentration of 5-HT than reported in any other part of the brain (535 - 537). Quay (538) demonstrated that pineal 5-HT content undergoes a diurnal circadian rhythm, with highest levels during the day and lowest levels at night. This occurs in both nocturnal and diurnal animals. Experiments (539, 540) that have shown that this rhythm persists in continuous darkness and in blinded animals suggest that the rhythm is endogeous. Wurtman et al (541) have reported that neither hypophysectomy, ovariectomy, adrenalectomy or thyroidectomy affects the 24-hour serotonin rhythm. This indicates that the rhythm is not generated by circulating hormones.

Fiske (542) and Snyder (540, 543) have shown that the serotonin rhythm is under sympathetic nervous control. Reversal of environmental illumination reverses the 5-HT rhythm and re-synchronization to a new schedule requires about 6 days. This rhythm is already present in 6-day old rats (544), i.e. prior to complete innervation of the pineal (545). Denervation did not abolish this rhythm in rats up to twenty days old (546, 547), but no rhythm was demonstrated in pineals of rats that were between 23 and 25 days or 60 days after denervation.

Only the synthesis of serotonin, catalyzed by 5-hydroxytryptophan decarboxylase, is high during daytime and low during darkness (530, 531). Since the activity of tryptophan hydroxylase is high

at night, it is probable that the synthesis of 5-hydroxytryptophan from tryptophan occurs during one single night and serotonin is synthesized from 5-hydroxytryptophan during the following day. Serotonin is converted to N-acetyl-5HT and melatonin on the next night. It is therefore probable that the synthesis of melatonin from tryptophan is realized in 36 hours.

Weiss and Costa (463) have proposed that serotonin regulates its own synthesis and conversion to N-acetyl serotonin (NAS) and melatonin via a feedback regulation mechanism. It is believed that high concentrations of 5-HT have an inhibitory effect on N/A induced activity of adenylcyclase. This results in decreased activity of tryptophan hydroxylase and N-acetyl-transferase and hence a reduction in the synthesis of 5-HT, NAS and MEL. During a long photoperiod the β-receptors become more sensitive to N/A and this results in higher activity of tryptophan hydroxylase (508) and N-acetyltransferase (459, 460, 523, 524). As a result of the increased tryptophan hydroxylase activity, a greater amount of 5-HT is synthesized during a long photoperiod. This larger amount of 5-HT serves as a substrate for the synthesis of a large amount of NAS and MEL and also exerts an inhibitory effect on the noradrenaline induced activation of adenylcyclase, thereby regulating its own synthesis and the synthesis of NAS and MEL.

Philo and Reiter (548) have demonstrated a circannual rhythm in pineal 5-HT content, with high values at the winter solistices and low values at summer solistices.

1.22113 MELATONIN

In the rat, serotonin-N-acetyltransferase regulates changes in the synthesis of melatonin (510, 549). Since the activity of SNAT shows marked circadian rhythms in the pineals of various animal species (460, 510, 550 - 554), it is not surprising that melatonin undergoes a diurnal rhythm. The maximal activity of N-acetyltransferase during the night results in a high synthesis of NAS (510). Axelrod et al (534) have shown that HIOMT activity is maximal during the night. Consequently, in rat pineal the level of MEL during the night is 7 to 10 times greater than during the day (555, 556). This rhythm persists in darkness (557), suggesting that it is endogenous. Human urinary melatonin content was shown to be several times higher between 23H00 and 07H00 than between 15H00 and 23H00 (558). Arendt (493) has reported that serum levels of melatonin are several fold higher at night.

There are numerous other reports of a nyctohemeral rhythm in MEL in human plasma (493, 559 - 565), human urine (560, 565 - 568) and human pineal (569). Nyctohemeral melatonin rhythms have also been demonstrated in various other species (Table 4). Pang et al ((592) demonstrated the existence of a diurnal rhythm in rat retinal MEL content and proposed that the diurnal rhythm of eye pigmentation in vertebrates could be regulated by melatonin.

Factors such as the human female menstrual cycle (593), seasons (559), hypothalmic lesions (594), changes in

TABLE 4

NYCTOHEMERAL MELATONIN RHYTHMS IN VARIOUS SPECIES

SPECIES	PLASMA	CSF	PINEAL	URINE	REF
Rat			++		555
Rat			++		556
Rat			++		570
Rat	++				571
Rat				++	572
Rat	++		++	++	573
Rat	++			++	574
Rat			++		575
Rat	++		++		576
Rat	++				577
Rat				++	575
Rat	++		++		578
Rat	++		++		579
Rat			++		580
Rat	++				581
Sheep	++				582
Sheep	++	++			583
Sheep	++				584
Monkey	++	++			495
Hamster			++		585
Hamster			++		586
Hamster			++		587
Trout	++				588
Trout	++				589
Cattle	++	++			591
Chicken			++		555
Chicken	++				584
Chicken	++				590
Donkey	++				584
Pig	++				584
Camel	++				584
Turtle	++				591
Quail			++		555

longitude (560) and manic-depressive disease (595, 596) have been reported to affect the melatonin rhythm.

Vollrath et al (597) have reported the possibility of the existence of a weekly melatonin rhythm.

Figure 9 has a schematic representation of the day/night rhythms of substances discussed above.

1.2212 OTHER FACTORS INFLUENCING NEURAL CONTROL

Westfall (598) has suggested that prostaglandins (PGs) which are released in the vicinity of the autonomic neuroeffector junction influences both the release of transmitter and the cellular response to the neurotransmitter. The following observations support a role for PGs in the sequence of events linking N/A release and increased indole production:-

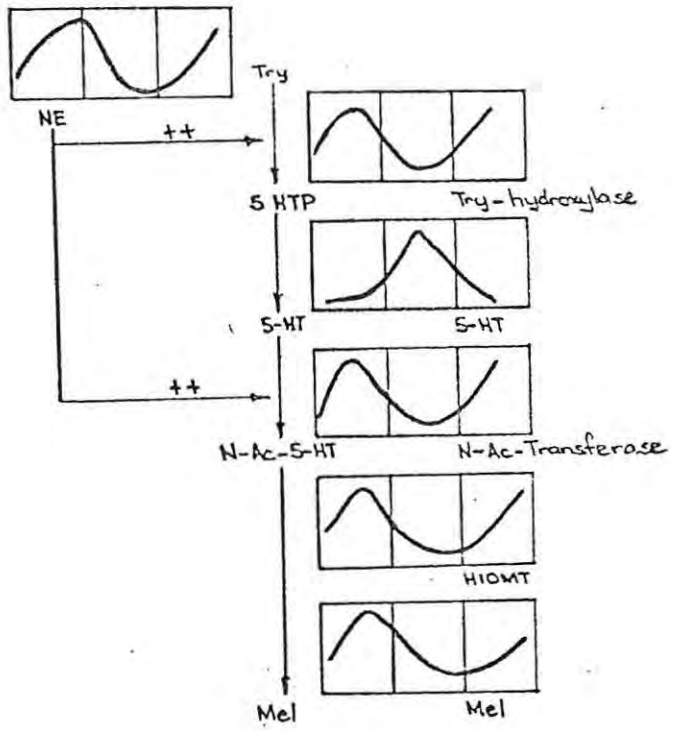
(a) N/A releases PGs from pineal gland in a dose-dependent manner (599);

(b) addition of PG E_2 to the culture medium of rat pineal glands increases cAMP content, its binding to protein kinase and the activity of SNAT (599, 600);

(c) blockade of PG synthesis in vivo impairs the increase of SNAT, HIOMT and pineal melatonin content occuring at night (601).

Taurine, an amino acid which is present in high concentration in the pinealocytes, is released by N/A, apparently through a β-adrenergic mechanism (602). Taurine increases the de novo

FIGURE 9



Scheme of day/night rhythms of noradrenaline , tryptophan hydroxylase, serotonin, N-acetyltransferase, HIOMT and melatonin, and the influence of norepinephrine on tryptophan-hydroxylase and N-acetyltransferase.

Balemans , M.G.M (870)

synthesis of MEL from tryptophan by cultured pineal glands in vitro and stimulates SNAT activity by an interaction with β -adrenergic system (603).

1.222 HORMONAL CONTROL OF PINEAL INDOLE BIOSYNTHESIS

The association between hormones and pineal indoleamines is well illustrated by the variation of pineal indole levels on different days of the estrus cycle. The effects of hormones on pineal enzymes and the 5-HT and MEL content are discussed.

1.2221 ENZYMES

The existing reports suggest that SNAT is relatively insensitive to hormonal changes while HIOMT is very sensitive.

1.22211 SNAT

Quay (605) suggested that SNAT does not regulate changes found in pineal MEL content during the estrus cycle. This theory was supported by the reports of other investigators (604) who demonstrated that SNAT activity did not show modifications as a function of the stage of the estrus cycle, nocturnally or diurnally.

Estradiol has no significant effect on pineal SNAT activity in ovariectomized rats (605, 606) and also in ovariectomized and intact female coturnix quail (607).

Testosterone, 5α-dihy rotestosterone, 3α-17β androstanediol and 3β-17β-androstanediol were administered to intact and castrated male rats to determine their effect on the nocturnal rise in SNAT activity (608). Castration resulted in a reduction of the nocturnal rise in SNAT activity when compared with intact rats. Pavlinov and Isachenkov (608) have reported a stimulated nocturnal rise of SNAT activity within 2 to 4 hours of administration of the androgen while Rudeen and Reiter (609) have reported no change in nocturnal pineal enzyme activity. Differences in experimental techniques and drug dosage could account for the differences in these results.

1.22212 HIOMT

In rats (604, 610), sheep (604) and equines (611), HIOMT activity was found to vary during the estrus cycle. One group of researchers (610) found that HIOMT activity was greatest during diestrus and lowest during proestrus and estrus while others (606, 612) have observed the highest HIOMT activity during estrus. The differences in the time at which these HIOMT assays were performed could account for the discrepancy in results.

Estradiol administration to rats results in a biphasic, dose-dependent effect on HIOMT; low doses (i.e. 0,05 - 1 mg/day) are stimulatory while high doses (i.e. more than 5 mg/day) are inhibitory (606, 613). Using cultured pineal glands, Hori et al (614) showed a dose-dependent increase in HIOMT activity in the range 0,1 to 15 nM which is the physiological concentration range of estradiol in the ovarian venous blood of rats. Similar effects were reported by other researchers (615). Hori et al

(614) also observed that the protein synthesis inhibitors, cyclohexamide and puromycin abolish the stimulatory effect of HIOMT, suggesting that the stimulatory effect is due to an increase in the synthesis of the enzyme protein.

Castration results in reduced HIOMT activity in male rats (608) and also in male coturnix quail (607). These authors have also reported that administration of testosterone in doses ranging from 0,1 to 5 mg/day stimulates HIOMT activity in these castrated animals. Nagle et al (616) reported that HIOMT activity was inhibited by 5 mg/day testosterone propionate. Noradrenaline reversed the effects of both dosage ranges of testosterone, suggesting the possibility of antagonism between testosterone and N/A for induction of HIOMT activity.

Various dosages of progesterone have appeared to inhibit HIOMT activity in female rats (617).

Hypophysectomy reduces HIOMT activity in male rats (618 - 620). Alexander et all (621) have demonstrated that the stimulatory effect of the pituitary upon HIOMT may decrease with age.

Follicular stimulating hormone (FSH) and luteinizing hormone (LH) increase HIOMT activity (60). Antonou et al (622) have reported that i.v. administration of synthetic LH releasing hormone (LH-RH) to rats of both sexes increases pineal HIOMT activity.

1.2222 SEROTONIN

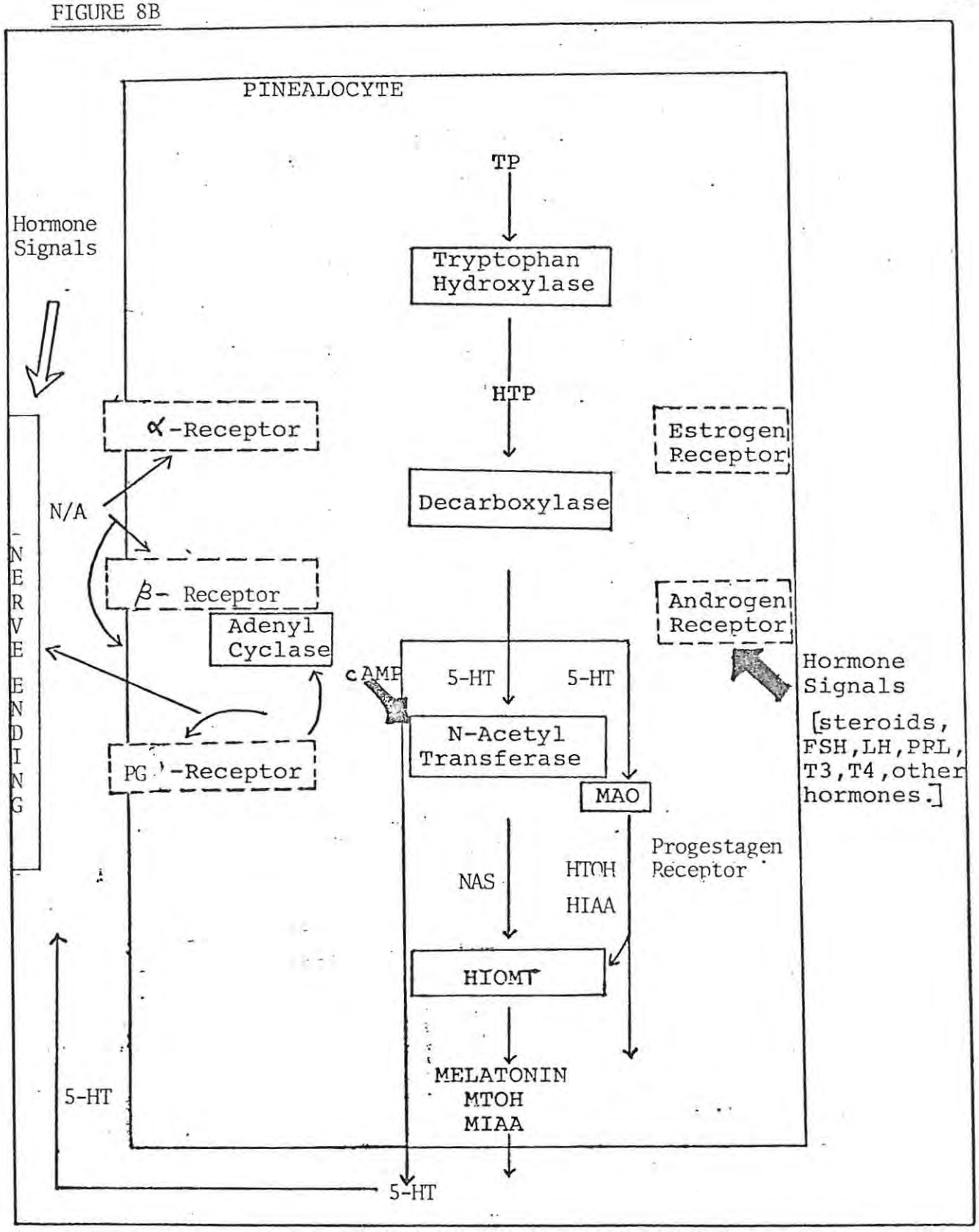
Estradiol treatment affects 5-HT and N/A turnover rate (604, 623). Bovine pineal 5-HT content is high during the winter months, while fertility is decreased. Testosterone administration to 3-day old female rats depresses pineal serotonin levels and abolishes the circadian 5-HT rythm up to 20 days of age (544). In male rats testosterone accelerates pineal 5-HT and N/A turnover rates (623). FSH and LH also modify the turnover rates of 5-HT and N/A in castrated rats.

1.2223 MELATONIN

A number of hormones which affect HIOMT, also affect melatonin synthesis.

Estradiol affects MEL synthesis (604, 606, 610) as well as its release in vivo (624) and in vitro (625). Estradiol shows a biphasic dose-dependent effect on MEL synthesis, similar to its effect on HIOMT. When estradiol is added to the culture medium at physiological concentration (10^{-8} - 10^{-9} M), it induces the synthesis of a specific pineal protein. This protein resembles the estradiol-induced protein found in the uterus (626) and stimulates melatonin production (613). Clomiphene acetate, an antiestrogenic substance which competes with estradiol for receptor binding sites, blocks this stimulating effect of estradiol.

Progesterone treatment reduces melatonin synthesis and release in vivo and in vitro (624, 625).



SCHEMATIC REPRESENTATION OF THE MECHANISMS INVOLVED IN NEURAL AND HORMONAL CONTROL OF MELATONIN SYNTHESIS.

CARDINALI D.P. (630)

Castration of male rats depress MEL synthesis. Administration of testosterone shows a dose-dependent effect on MEL synthesis (606); low doses increase MEL synthesis while high doses depress MEL synthesis.

The observation that various hormones influence pineal function prompted many investigators to examine pinealocytes for the presence of hormone binding sites. To date, putative receptors for estradiol (627, 628, 629), testosterone (628, 629), 5 α - dihydrotestosterone (628, 629) and progesterone (736) have been reported.

Hormone effects have been found to be subsidiary to the major circadian driving force coupled to pineal activity by the sympathetic nerves (630). N/A released from the pineal nerve endings induces the synthesis of receptor proteins for estrogens and androgens (627, 629), indicating that the activity of the afferent sympathetic neurons controls the sensitivity of the gland to circulating hormones.

Refer to Figure 8B for a schematic representation of the mechanisms involved in neural and hormonal control of pineal indole biosynthesis and metabolism.

1.23 EFFECT OF DRUGS ON PINEAL INDOLES

Although the pineal lies within the cranial vault, it is outside the blood-brain barrier, which makes it accessible to pharmacological agents in vivo (550).

1.231 SEROTONIN

Various substances affect the serotonin content and/or the serotonin rhythm. In vivo pineal 5-HT levels are increased by the administration of 5-hydroxytryptophan (5-HTP) (631) or tryptophan (631 - 633). A similar effect was observed when 5-HTP or tryptophan were added to the medium in which rat pineals had been cultured (634). α-methyl-p-tyrosine enhances the effect of tryptophan while N/A blocks it (632). Mescaline increases the conversion of ^{14}C-tryptophan to ^{14}C-5HT by cultured pineals but this effect is not mimicked by LSD or psilocybin (635).

L-N/A decreases the serotonin content of cultured pineals (636). An increased conversion of 5-HT to N-acetyl-5HT is a possible explanation for this effect. The effect of L-N/A is blocked by propranolol (458). Inhibition of N/A synthesis with α-methyl-p-tyrosine, a tyrosine hydroxylase inhibitor, causes an elevation of 5-HT (632). In vivo 5-HT levels are decreased by p-chlorophenylalanine (634), which inhibits tryptophan hydroxylase and catechol synthesis (637). Serotonin levels are also decreased by RO4-4602, an inhibitor of aromatic-L-amino acid decarboxylase (638). Bensinger et al (639) have reported that a high tryptophan concentration can inhibit total tryptophan hydroxylation in cultured pineals.

Administration of reserpine or β-phenylisopropylhydrazine (a MAO inhibitor) prevents the nocturnal decline in 5-HT

levels in rats (631, 640). Similar effects are exerted by N-methyl-3-piperidylbenzoate, an anticholinergic agent (641). The daytime increase in 5-HT levels is prevented by N/A, dopamine (642) or actinomycin(640). The mechanism by which actinomycin acts has not been established and the effect of the catecholamines has been attributed to the accelerated N-acetylation of 5-HT. Bretylium or guanethidine had no effect on 5-HT rhythms (641).

1.232 MELATONIN

The synthesis of ^{14}C-MEL from ^{14}C-tryptophan in cultured rat pineals is increased by L-N/A and structurally related substances e.g. D-NA, dopamine, L-adrenaline, tyramine, octopamine and tryptamine (453) and amphetamine (642). This increase is blocked by propranolol but not phenoxybenzamine (458). Dibutyryl cAMP stimulates the formation of ^{14}C-MEL from ^{14}C-tryptophan. This effect is blocked by cyclohexamide and actinomycin D (469, 457). Addition of dimethyltryptamine (DMI) to pineal homogenates accelerates the o-methylation of N-acetylserotonin (643).

In vivo experiments have shown that administration of L-dopa causes a rapid and major increase in pineal melatonin content (644). The mechanism by which L-dopa acts could involve either the release of N/A from pineal sympathetic nerve terminals or the intravascular conversion of the catechol amino acid to dopamine, which acts directly on the pineal parenchymal

cells to stimulate melatonin synthesis (453). The latter is more consistent with the finding that pineal sympathetic denervation potentiates the dopa-induced increase in pineal MEL (644).

There have been reports that the monoamineoxidase inhibitors Catron® (453) and harmine (645, 646) increase MEL production. Illnerova (647) has shown that pargyline, administered during the day, produces an initial decrease and thereafter an increase in MEL, by affecting the activity of N-acetyl transferase. Other researchers (648, 649) have produced further evidence for the elevation in MEL production after pargyline administration to rats.

Acute administration of both noradrenaline (DESI, maprotiline) and/or 5HT (IMI, CLOMI, ROll-2465) uptake inhibitors cause elevation of pineal melatonin (649). Parfitt et al (650, 651) have reported that DESI increases pineal MEL in isolated rat pineals. Chronic CLOMI results in a reduction in the elevated MEL seen with the acute dose (649). However, RO 11 - 2465, a selective 5HT uptake inhibitor (652) did not cause a reduction of the acute elevation of MEL (649). It is possible that the effects of chronic CLOMI administration on MEL production results from reduced β-adrenergic sensitivity..

Localized irradiation of the head of rats with 450 R increased ^{14}CMEL biosynthesis from ^{14}C-tryptophan but irradiation of the body only had no such effect (653).

Increasing 5HT availability by precursor loading with L-5-HTP in both acute and chronic dosage had no significant effect on MEL production (649). Morphine had no effect on MEL synthesis (654).

Cyclohexamide (453) or histamine phosphate (653) inhibit the synthesis of MEL in rats.

1.233 ENZYMES

1.2331 TRYPTOPHAN HYDROXYLASE

Pineal tryptophan hydroxylase differs from the enzyme present in the brain (655). The activity of the pineal enzyme is 100-fold higher than that in the brain (656).

Shibuya et al (509) reported that the activity of pineal tryptophan hydroxylase increases 1,5 fold during the first 3 hours of darkness and persists until the lights come on. L - propranolol and cyclohexamide, injected immediately before onset of darkness, blocked the increase in activity in animals placed in darkness for 3 hours after injection.

N/A and cAMP stimulate the activity of this enzyme in cultured pineals (508).

α -propyldopacetamide inhibits tryptophan hydroxylase activity (549). p-chlorophenylalanine (p CPA) inhibits tryptophan hydroxylase in vivo, in vitro or when added to organ cultures (473, 639, 656).

1.2332 SNAT

The addition of N/A to the medium in which rat pineals were cultured increased SNAT activity (459, 467, 470, 510 524, 551, 657). This effect is blocked by cyclohexamide and propranolol (460). SNAT activity in cultured pineals is also increased by adrenaline L-Dopa and octopamine (510). Deguchi and Axelrod (459) have reported that isoprenaline, pargyline and Catron (R) increase SNAT activity. The magnitude of the increase in SNAT activity caused by isoprenaline depends upon the time of day that the animal is sacrificed (521). Cocaine and procaine increase SNAT activity in cultured pineals (658). This effect is blocked by propranolol and phentolamine and also by bilateral superior cervical ganglionectomy of animals. These observations suggest that the effect of cocaine and procaine is mediated by release of N/A from surviving sympathetic nerve terminals.

Hypoglycaemia induced by insulin administration or stress from immobilization cause a rapid increase in SNAT activity in rats (659). These increases are believed to be mediated by the release of catecholamines from 3 loci viz:-

(a) Sympathetic nerve terminals in the pineal;

(b) sympathetic nerve terminals in other organs that are not susceptible to damage by 6-hydroxydopamine

(c) adrenal medulla.

Propranolol blocks these effects.

SNAT is activated by disulfide containing peptides (660). It has been suggested that a disulfide containing peptide with high reactivity towards SNAT may participate in the intracellular regulation of the enzyme.

Administration of reserpine, propranolol or cyclohexamide to rats results in the suppression of the nocturnal rise in pineal SNAT activity (460). Phenoxybenzamine, p CPA or actinomycin D do not cause a suppression of the nocturnal rise in pineal SNAT activity (460). Isoprenaline elevates the levels of N-acetylserotonin and the activity of SNAT in vivo; this effect is reversed by propranolol but not by phenoxybenzamine (469) or phentolamine (661, 662).

If pargyline is administered during the light period of the diurnal cycle, it first decreases then increases SNAT activity in vivo (647). Parfitt and Klein (650, 651) have reported that DESI can increase the activity of SNAT in vivo and in vitro. According to a recent report (663), chronic administration (5 weeks) of lithium chloride causes a significant decrease in dark-induced activity of rat pineal SNAT. In vitro, various concentrations of lithium chloride did not affect SNAT activity. Lithium-induced subsensitivity of β-adrenergic receptors is suggested. Environmental lighting can prevent or quickly reverse the nocturnal elevation of SNAT activity in the rat pineal. I.V. injections of carbachol mimic the acute effect of light while injections of α-bungarotoxin,(a specific cholinergic blocker (664)), near the suprachiasmatic nucleus of the hypothalamus, prevents the effects of light (665).

These results suggest the involvement of acetylcholine in the effects of light on the circadian rhythms in the rat pineal.

1.2333 HIOMT

Klein (456) reported that the addition of L-N/A in a concentration of 10^{-5} M to rat pineal culture media increases HIOMT activity, while Weiss (666) reported that 10^{-3} M N/A inhibits HIOMT activity.

Psychomimetic agents such as mescaline, LSD, amphetamine, methoxybufotenin and dimethyltryptamine (DMT) increase HIOMT activity in pineal homogenates (643).

Various compounds have been reported to inhibit HIOMT activity in vitro, without affecting melatonin synthesis (451). Such inhibitors include substituted N-benzoyltryptamines and N-phenylacetyltryptamines (667), neuroleptics like haloperidol (668, 669), fluphenazine, GABA (668) and oxypertine (670). S-adenosyl-homocysteine (SAH) inhibits MEL production by inhibiting HIOMT (670, 671). Compounds structurally similar to SAH, such as cysteic acid and homocysteic acid were also found to inhibit HIOMT, but at a much higher concentration. However, adenosine, methionine and homocysteine had no effect on HIOMT activity (671).

Serotonin and histamine have also been associated with the inhibition of HIOMT activity, but only in high concentrations of 10^{-3} M and above (672). Weiss (666) reported that MEL inhibits HIOMT but Karahasanoglu and Ozanol (671) found melatonin to be ineffective even in very high concentration.

Variations in assay conditions may account for this difference.

It has been reported that the naturally occuring vitamin, pyridoxal-5 -phosphate, strongly inhibits HIOMT activity in vitro (673). These authors have suggested that the inhibition was competitive with respect to the methyl donor S-adenosyl methionine but non-competitive as regards the substrate N-acetylserotonin. N/A abolished the inhibitory effect of the vitamin on HIOMT.

Many researchers (673, 674, 675) have suggested that pteridines affect HIOMT activity. Ebels et al (675) have reported that neopterin stimulates HIOMT activities for the substrates 5-HTP, 5HT and 5-hydroxyindoleacetic acid (5-HIAA) during the night. HIOMT activities for the combination N-acetylserotonin (NAS)/ 5-hydroxytryptophol (5-HTOH) are shifted to a later hour in the dark period whereas HIOMT activities for the substrates 5HTP, 5HIAA are shifted towards an earlier period.

1.234 PINEAL MORPHOLOGY

N/A is stored within granular vesicles of postganglionic sympathetic neurons (436). Administration of reserpine decreased the granularity while iproniazid increased the granularity of those vesicles (676). Many drugs which affect the N/A concentration at sympathetic nerve terminals in the brain, act similarly in the pineal e.g. DESI (677), 6-hydroxydopamine (678), tyramine (679), metaraminol (680) and anti-nerve growth factor (681).

Serotonin is found in pinealocytes as well as within sympathetic nerve endings (680). Parenchymal serotonin is depleted by p CPA (682) and α-methyltyrosine (439). Inhibition of tryptophan hydroxylase by α - propyldopacetamide results in depletion of neural 5HT (439). Nialamide increases the density of the granular vesicles containing 5HT while p CPA and reserpine reduces the density of these vesicles (683).

1.24 PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS OF PINEAL INDOLEAMINES

"Secretions from the pineal participate in several neural and neuroendocrine mechanisms, including control of gonadal, adrenal and thyroid functions, sleep and various biological rhythms" (630). This statement can be substantiated by an examination of the physiological effects of pinealectomy and the effects of pineal secretions on the physiology and biochemistry of mammals.

1.241 EFFECTS OF PINEALECTOMY

Pinealectomy (PLM) in rats blocked the effects of short photoperiods on the length of the estrus cycle (684). PLM also blocked the reproductive collapse caused by short day periods in hamsters (685). In rats, earlier vaginal opening (686, 687) and earlier ovulation (689) was also observed. These observations are indicative of precocious puberty. An increase in ovarian (689, 690) and uterine mass (691) have also been reported after PLM in rats.

LH surge in normal adult female rats is unaffected by PLM (692,693). The LH surge in ovariectomised, steroid-treated females was also unaffected (685m 696). Whereas the LH surge in adrenalectomized female rats was modified by PLM (693). PLM increases the LH and FSH response to visual stress (696). PLM also increases plasma LH, PRL, progesterone and estradiol during late pregnancy (697). Alonso and coworkers (698) reported that pinealectomy increases the pituitary content and concentration of LH and FSH, suggesting that the pineal gland exerts an inhibitory action on the hypothalmic-pituitary axis, that seems to be independent of testicular function. In contrast, other investigators have reported that PLM does not influence daily pituitary LH content and reverses the hypophyseal FSH values in the dark period.

PLM causes an increase in the seminal vesicle, prostate (699) and testicular masses (691). Transient increases in plasma testosterone were also observed after pinealectomy (700, 701).

In rats, PLM resulted in increased feed consumption and thyroid function (702 - 705). Csaba et al (704) have shown that adult pinealectomized rats take up 50% more I_2 than the controls. PLM has also been associated with an increase in thyroid mass (706). In hamsters, PLM reversed the depression in plasma thyroxine and free thyroxine index (707).

PLM was found to increase adrenal activity as measured by the RNA to DNA ratio, irrespective of ovarian involvement (706). PLM increased ACTH release in adrenalectomized blinded-anosmic rats (708). Corticosteroid (819, 820) and aldosterone

(710) secretion was increased by PLM but the diurnal rhythm in plasma corticosterone was unchanged (711). In rats, PLM increased plasma renin and produced hypertension (712, 713). It has been reported that PLM depresses the 5 ∝ -reductase pathway (709).

PLM has been associated with an increase in body mass in rats (714). The decrease in pituitary and plasma Growth Hormone (GH) and the retarded body growth in animals exposed to constant darkness was counteracted by pinealectomy. Ronnelkeive and McCann (715) have reported that pinealectomy reduces the level of GH during the day but not during the night.

It has been reported that pinealectomized male rats show an increase in pituitary prolactin levels but a decrease in plasma prolactin levels, implying an inhibition of prolactin release by PLM (716). Nir et al have reported that PLM in rats results in a 13% decline in milk yield during the second half of the nursing period. (717).

Philo and Rieter (718) have reported that pinealectomy induces convulsions in gerbils. They suggest that this effect may be due to a depression of the noradrenergic system.

PLM in female fowls results in an increase in heterophil and monocyte counts; lymphocyte counts are decreased while basophil and eosinophil counts are unchanged (719).

Many of the above effects can be reversed by melatonin and/or other pineal constituents.

1.242 ENDOCRINE EFFECTS OF PINEAL INDOLES

1.2421 PITUITARY GLAND

1.24211 GONADOTROPIN SECRETION

Evidence that MEL administration affected gonad function in rats was first presented in 1963 by Wurtman et al (720). These investigators showed that a low dose of MEL (1 mg/day) administered subcutaneously could decrease the incidence of estrous vaginal smears and suppress the growth and functional activity of female rats exposed to continuous illumination.

Subsequently, numerous researchers investigated the anti-gonadal effects of MEL in rats and other mammals. MEL administration to rats has been associated with a decrease in the incidence of estrous smears (687, 720, 721), delayed vaginal opening (722), decreased ovarian and uterine mass (720) as well as increased gonadal involution in underfed rats when melatonin is injected at the end of the light phase (723 - 725).

MEL induces LH release in anovulatory rats continuously exposed to light (726). LH release and ovulation is inhibited by MEL in cycling rats (727). Pituitary LH and FSH response to LHRH in prepubertal rats is inhibited by MEL in vitro (728) but no effect is seen on the LHRH response in adults (728, 729). These authors have suggested that MEL can act directly on the

neonatal pituitary gland and suppress the response to LHRH, which in turn suppresses pituitary LH release. The rat pineal LHRH content shows a circadian rhythm with maximum levels at 6.00 p.m. and minimum levels at 6.00 a.m. (730). These reports, together with the observation that the pineal gland and the superior cervical ganglia accumulate intravenously injected radiolabelled LHRH to a greater extent than other brain areas, has prompted Trenitini et al (731) to suggest that the pineal gland is a possible target organ for LHRH.

MEL administration has been shown to block the pre-ovulatory surge in serum LH (732, 733) as well as decrease serum FSH (734, 735). Some authors (736, 737) reported an increase in the pituitary LH content following administration of MEL to immature rats, suggesting LH suppression. However, other researchers did not observe any change in pituitary LH levels (738 - 740). Roche et al (740) reported that the castration-induced rise in serum LH was suppressed by MEL but other researchers (741) did not observe this effect. In man, MEL administration resulted in a reduction in LH release in one study (742) while no effect on LH release was observed in other studies (743, 744). Thus, it can be seen that many of the above reports are conflicting. Some of the differences in the above studies include dosage differences and differences in the routes of administration. These variables could account for the differences in results. The time of administration of MEL is also important. Tamarkin et al (745) found quite

fortuitously that administration of MEL to male hamsters in the late afternoon duplicated the effect of a non-stimulatory photoperiod on the sex organs. This effect was later reproduced in both male and female hamsters (746 - 748). The importance of the route of administration is illustrated by the finding that MEL implants in the median eminence significantly reduce pituitary content of LH while implants in the cerebral cortex or the anterior pituitary had no effect on pituitary LH stores (749).

Some of the other pineal indoles have also been associated with antigonadal properties. Methoxytryptophol (MTOH) is reported to have a more pronounced effect than MEL in reducing the frequency of estrus smears (721). MTOH has no effect on pituitary FSH stores but 5-HT increases them (750). 5-MTOH and 5-HT do not modify pituitary LH stores, while 5-hydroxytryptophol (5-HTOH) reduced the pituitary LH content in one study (749) but not in the other (751). The essential difference between these two studies was the manner of administration.

It has been reported that the LH release by MEL and 5HT is not due to a direct action on the anterior pituitary. MEL injected iv causes a reduction in serum LH concentration, supporting the view that indoles and methoxyindoles act indirectly via the hypothalamic-hypophyseal complex (752).

Serotonin inhibits the synthesis of FSH-releasing factor (FSH-RF) in vitro (753). Serotonin administration also causes ovarian hypertrophy (754). It is possible that this is due to inhibition of FSH-RF and LH-RH.

Recently, pineal peptides possessing antigonadotropic activity have been isolated (753, 754). The presence of a gonadotropin resembling LH has been demonstrated in bovine pineal glands (755).

MEL inhibited androgenization of female rats when injected simultaneously with testosterone (756). MEL administration also caused reduction in spermatogenesis (867), plasma testosterone (700), androgenic effects of testosterone (758) and testis mass (759). Testicular steroidogenesis was inhibited by MEL in vitro (760). Exogenous MEL also decreases the masses of the rat seminal vesicle and ventral prostate (700, 761 - 764). MEL suppresses testosterone synthesis in vitro (765) and also accelerates testicular and prostatic regression in hypophysectomised rats (766). These observations suggest that Mel may act directly on the testes as well as indirectly, via the brain.

1.24212 GROWTH HORMONE (GH)

Serotonin enhances GH release (767). High doses of MEL (80 mg/kg) suppressed the secretion of GH in response to 5-HT while 5-HTP administration enhanced it (768, 769).

MEL pre-medication reduced GH response to L-tryptophan (770) and insulin induced hypoglycaemia (771). It has been suggested that MEL is a competitive inhibitor of 5-HT at the 5-HT receptor and consequently, MEL blocks GH stimulation via serotoninergic pathways.

1.24213 PROLACTIN

Acute injections or short treatment with MEL has been associated with an increased release of prolactin (695, 772 - 774) while chronic daily administration to anosmic rats inhibited prolactin release (724 - 725). MEL increased prolactin release in pituitary cells from fetal rats (775) but had no effect on prolactin release from bovine pituitary cells (776).

It has been reported that injection of MEL or 5-HT into the third ventricle of rats increases serum prolactin levels (735). MEL and 5-HTP increase serum prolactin levels when injected intravenously into rats but 5-HT has no effect (773). A diurnal prolactin rhythm, with high levels during the dark period, has been reported (773, 777). Neutralization of plasma MEL and N-acetylserotonin causes a significant reduction in prolactin levels, although the diurnal rhythm persists.

After examining the effects of various pineal compounds on prolactin secretion in cultured rat pituitary clonal cells, Hanew et al (775) have concluded that the pineal (and MEL in particular) has a modulatory effect on prolactin release.

1.2422 ADRENAL GLANDS

Pineal extracts have been reported to contain both substances that stimulate aldosterone secretion (778 - 780) as well as substances that inhibit aldosterone (781 - 783).

It has been suggested that the aldosterone stimulating activity results from the high concentration of 5-HT in pineal extracts (778, 779). An infusion of angiotensin II induced an increase in 5-HT content of the pineal, hypothalamus and brain stem in dogs. These observations suggest the possibility of the existence of positive and negative feedback relationships between the brain isorenin-angiotensin systems and 5-HT metabolism in the pineal (784).

The inhibitor of aldosterone secretion that was isolated from pineal extracts was reported to show close resemblance to MEL in structure (782, 783). Acute administration of MEL significantly reduces aldosterone production, while hypophysectomy abolishes this effect (785, 786). The combined effect of the above factors on aldosterone secretion has not been investigated.

Reports on the effects of pineal indoleamines on glucocorticoid secretion are conflicting. Intrahypothalamic implants of MEL have been associated with a reduction of adrenal mass in adult castrated male rats (787). Acute or chronic subcutaneous administration of MEL did not modify plasma levels of corticosterone, ACTH or pituitary stores of ACTH (788). However, IV administration of MEL has been associated with reduced

plasma corticosterone levels (789). This effect was not reproduced by IV administration of 5-MTOH and 5-HTOH. In vitro experiments have suggested that MEL inhibits adrenal corticosterone production (790). It is possible that the differences in the results obtained in the above experiments can be explained by the differences in the administration of MEL.

Ogle and Kittay (791) have reported that intraperitoneal administration of MEL for one week stimulates adrenal 5-α - reductase activity in ovarectomized and hypophysectomized rats.

The above reports suggest that the pineal could have a modulatory effect on adrenal function.

1.2423 THYROID GLAND

The administration of pineal extract to rats has been associated with a decrease in thyroid mass and ^{131}I accumulation by the thyroid gland as well as the inhibition of thiouracil-induced goiter (792). It has been reported that MEL administration has an inhibitory effect on thyroid function (703, 793, 794). Baschier, et al (793) and Narang et al (703) also reported a reduction in thyroid mass but this effect was not evident in the results obtained by De Aospo et al (794). The large doses (75 mg, 100 mg and 150 mg) used by the first two research groups as opposed to the small dose (10 mg) used by the third group, might account for the differences in observations.

Some investigators have shown that MEL administration results in a depression of ^{131}I uptake rate (793 - 795) and TH secretion rate (703). In contrast, other researchers(796, 797) have associated MEL administration with increased thyroid activity, whilst Naber et al (798) found MEL to have neither stimulatory nor depressant effects on ^{131}I uptake into the thyroid.

The presence of a TSH inhibitor has been demonstrated in the rat pineal gland (799). It is now evident that the pineal exerts some influence on the functioning of the thyroid gland.

1.2424 PANCREAS

MEL administration increased blood glucose levels in monkeys (800). The release of insulin from the rat pancreas was blocked by MEL in vitro (801).

1.243 PINEAL GLAND

MEL administration was found to have numerous effects on the pineal. It modified the diurnal rhythm in rat pineal 5-HT content (802). In rats (803), mice (804) and hamsters (805), morphological signs of pineal activation was observed after MEL administration. MEL also increased rat pineal tubulin levels (806) and enhanced cat pineal arginine vasotocin release (806).

Juillard et al (807) have suggested that the indoleamine (presumably 5-HT) in the granular vesicles of the pineal, functions in the metabolism, storage, and/or release of proteinaceous compound(s) in the granules.

1.244 CENTRAL METABOLIC EFFECTS

The administration of MEL influences the metabolism of various central neurotransmitters.

It has been reported that MEL administration increases hypothalamic 5-HT and γ-aminobutyric acid (GABA) content (808 - 810). MEL administration increases urinary 5-HIAA levels in Parkinsonian patients (808). Carman et al (811) have reported increased 5-HIAA levels in the CSF of depressed patients, following MEL administration. All these reports suggest that MEL exerts an influence on the serotonergic system.

MEL, administered intra-peritoneally, had no significant effect on the brain DA content in mice (812). However, intra-arterial or intra-cisternal administration of MEL produces significant increases in DA and N/A levels in rat brain (813). It has been suggested that the rapid conversion of MEL to 6-hydroxymelatonin, following the intraperitoneal administration, might be responsible for the conflicting results.

Cardinali et al (814) reported that MEL inhibits the uptake of N/A, 5-HT, DA and glutamate into synaptosome-rich homogenates of the rat hypothalamus. This inhibition is thought to be non-competitive. The effect is dose-dependent. The release of transmitter substances is also increased by MEL in a dose-dependent manner. It has been reported that MEL appears to interfere with the uptake and release processes in 5-HT-containing neurons more readily than in catecholamine-containing neurons. The observation that MEL depresses 5-HT uptake by

the rat hypothalamus without affecting the uptake or accumulation of N/A, DA or glutamate (815), lends support to the above proposal.

MEL administration depresses hypothalamic MAO activity (816).

The activity of pyridoxal phosphokinase, an enzyme involved in the synthesis of DA, 5-HT and GABA, is increased rapidly by intraperitoneal injections of MEL (817).

There have been reports that MEL inhibits the release of prostaglandins from the medial basal hypothalamus, in vitro (818, 819).

MEL administration decreases the melanocyte-stimulating hormone (MSH) content of the pituitary gland (820, 821). This observation is of interest since MSH produces an exaggerated response to noxious stimuli in animals (822 - 823) and it produces feelings of nervousness and anxiety in man (824). A double-blind study has shown that an inhibitor of MSH, viz melanocyte-stimulating-hormone-release-inhibitory factor (MIF), is a more effective antidepressant than placebo in four of five patients (825).

1.245 PROTEIN SYNTHESIS

Protein synthesis (826, 827) and microtubule protein content (828) is decreased by MEL administration. This effect of MEL has been associated with its effect on transmitter uptake and release (see setion 1.244) because brain microtubule protein (829) or actin-like protein (830, 831) is implicated in the process of neurotransmitter uptake and release by nerve endings (814).

MEL inhibits thromboxane synthesis in platelets (832).

There have been reports that pineal indoleamines impair contractile-protein dependent processes such as axonal transport (828, 833) and movement of pigment granules (834).

1.246 BEHAVIOURAL EFFECTS OF MELATONIN

MEL administration reduces locomotor activity in blind anosmic rats (835) as well as in rats deprived of food (836). No effect on locomotor activity was observed after MEL administration to pinealectomised rats (837) or hypophysectomised rats (822).

There have been reports that MEL induces and prolongs sleep in humans (809, 820, 838 - 841) and in animals (841 - 843). EEG changes have been reported in cats, humans, rats and chicks (838, 842, 843). EEG studies have shown that melatonin-induced sleep resembles natural sleep.

In cats, MEL abolishes the sensory evoked activation of epileptiform activity (807). Application of an anti-melatonin antibody on the surface of the rat brain was followed by seizures. The possible use of MEL in the treatment of epilepsy should therefore be evaluated.

MEL administration to Parkinsonian patients produces general improvement in some patients (810) and mild tremor control without general improvement in others (812, 844). MEL has also increased urinary 5-HIAA levels in parkinsonian patients (809).

Aqueous extracts of pineal glands have been shown to produce clinical improvement in some patients with chronic schizophrenia (845, 846). In contrast, an infusion of MEL has been associated with marked hallucinations and exacerbation of symptoms lasting 18 to 36 hours (847). One investigation (848) revealed a higher excretion of an unidentified acidic metabolite of MEL by schizophrenic patients.

Carman et al (811) have reported that two patients suffering from Huntington's chorea showed depression and psychomotor retardation following MEL administration. These investigators tested the efficacy of MEL as an antidepressant and reported that symptoms of dysphoria were exacerbated. Loss of sleep and mass and a drop in oral temperature were also observed. There was an increase in 5-HIAA and calcium in the CSF of 3 of the 4 patients studied. In a more recent study, Skene (849) evaluated the use of MEL in a variety of animal models of depression. It was suggested that MEL might be useful in the treatment of "agitated" depressions.

The available information on the physiological effects of pineal indoleamines suggest that they might be potentially useful therapeutic agents. Until recently, most studies had been restricted to the endocrine effects of the pineal compounds. More investigations into the other effects of these compounds are needed.

CHAPTER 2

THE EFFECT OF TRICYCLIC ANTIDEPRESSANTS ON THE UPTAKE OF ^{3}H-5HT FROM BOVINE PINEAL SLICES

2.11 INTRODUCTION

The mammalian pineal contains a higher concentration of 5-HT than reported in any other part of the brain (535 - 537). However, the actual role of 5-HT in the pineal remains to be elucidated. Melatonin, a metabolite of 5-HT, is thought to be the most important pineal hormone. Therefore, the intra-pineolocyte regulation of the concentration of 5-HT might influence the concentration of melatonin and hence the functioning of the pineal.

From their research on bovine pinealocyte suspensions, Ducisand Distefano (850) have suggested that a transport system for 5-HT might exist in the pineal gland. These authors later (851) attempted to characterize the mechanism of uptake of 5-HT into bovine pinealocytes. It was suggested that high concentrations of 5-HT (10 μM to 5 mM) appear to use amino acid transport systems e.g. L-lysine, L-leucine and L-alanine. Uptake is partially sodium dependent, temperature dependent and is sensitive to inhibitors such as chlorpromazine and imipramine. Low concentrations (0,005 - 1 μM) of 5-HT appear to use a highly specific allosteric carrier for transport. This uptake system is sodium dependent and inhibited by drugs such as chlorpromazine, cocaine, metaraminol reserpine and imipramine.

Administration of TADs has been associated with changes in MEL production rate (649, 650, 651). This effect of TADs is believed to be due to increased SNAT activity brought about via the β -receptors. However, if TADs exert a marked inhibitory effect on 5-HT uptake in the pineal (as in the brain and in blood platelets), it is possible that this might have a modulatory effect on MEL production.

Therefore, the purpose of this study was to determine the effect of varying doses of various TADs on the uptake of ^{3}H-5HT in bovine pineal slices.

2.12 MATERIALS AND METHODS

2.121 MEDIUM:

The composition of the Kreb's Henseliet medium used was as follows:

NaCl	6,89g/ℓ
KCl	0,35g/ℓ
CaCl	0,28g/ℓ
$MgSO_4$	0,14g/ℓ
KH_2PO_4	0,16g/ℓ
$NaHCO_3$	2,0g/ℓ
Glucose	2,0g/ℓ
ascorbic acid	0,02g/ℓ
EDTA $2H_2O$	O,015g/ℓ

Ascorbic acid and EDTA $2H_2O$ were included as chemical stabilizers. The pH of the medium was adjusted to pH 7,4 with carbogen (5% CO_2 in O_2).

2.122 TISSUE

Fresh pineal glands of oxen (of Hereford origin) were obtained from the local abbatoir on the morning of each experiment. The animals were approximately eighteen months old. The animals were decapitated between 06H00 and 07H00 each morning and the glands were excised 20 to 50 minutes post-mortem and immediately placed in ice-cold Krebs Henseliet medium.

The tissue was rapidly sliced with a sterile scalpel into pieces that were roughly 1 mm^3. During this entire procedure, the tissue was kept cold and unnecessary handling of the tissue was avoided.

2.123 DETERMINATION OF ^{3}H-5HT UPTAKE

The procedure involved incubation of pineal slices with ^{3}H-5HT, termination of the incubation by centrifugation at 0°c and determination of the amount of ^{3}H-5HT taken up into the slices.

One hundred and fifty milligrams of bovine pineal slices was placed in a 10 ml, flat-bottom, quick-fit flask. The flask contained 3 ml of chilled medium. The medium for the

control contained 5 x 10^{-4} M pargyline while the medium for the test samples contained 5 x 10^{-4} M pargyline and the required concentration of the test drug. The medium was equilibrated with carbogen (5% CO_2 in O_2). The flask was then incubated in a waterbath at 37°C Mixing was ensured by constant shaking. The pre-incubation period was 3 minutes. 5 x 10^{-7} M ^{3}H-5HT (5-Hydroxy [G-^{3}H] tryptamine creatinine sulphate; specific activity 14, 7 curies/mmol - obtained from Amersham International) was then added to the reaction vessel and it was incubated for a further 3 minutes.

The contents of the flasks were rapidly transferred to plastic centrifuge tubes and centrifuged in an MSE Highspeed 18 Refrigerator centrifuge at 0°c and 12 000 g for 8 minutes. The supernatant was then decanted and the slices were rinsed twice with 5 ml ice-cold normal saline.

The tissue was then transferred to a testtube containing 6 ml 0,4 N perchloric acid. The tissue was sonicated for 45 seconds, using the Braunsonic 1510. Ice had to be used to cool the system during this process.

The mixture was then vortexed on a rotamixer for 30 seconds and centrifuged for 10 minutes at 3 000 rpm. Hereafter, 2 ml of the supernatant was transfered to a glass scintillation vial containing 6 ml of Instagel (R) (Packard Instrument Company), after which the vials were counted for their radioactivity.

A Beckman LS 3150T liquid scintillation counter was used. Counting time was 20 minutes.

Inhibition of ^{3}H-5HT uptake was calculated by the method used by Sugden (852) i.e.

$$\% \text{ inhibition of amine uptake} = \frac{100}{1} \times \frac{\text{Mean cpm control} - \text{mean cpm test}}{\text{Mean cpm control} - \text{mean cpm background}}$$

Experiments were done in duplicate.

2.124 VALIDATION OF THE PROCEDURE

Certain tests had to be performed before the above procedure (section 2.23) was adopted.

2.1241 TIME OF INCUBATION WITH ^{3}H-5HT

The uptake of ^{3}H-5HT as a function of time was determined by incubating 150 mg of pineal slices in 3 ml medium, at 37°c, for varying periods. The pineal slices were then centrifuged and the radioactivity was extracted and counted as before. Efficiency was calculated using the internal standard method. Experiments were done in duplicate.

2.1242 TEMPERATURE

Although 37°C is the physiological temperature and therefore a suitable temperature for incubation, it had to be determined whether 37°C was actually the optimum temperature for studying 5-HT uptake into bovine pineal tissue.

150 mg pineal slices were incubated in 3 ml medium for 3 minutes, at various temperatures. After the incubation period, the tissue was treated as described in section 2.241.

Experiments were done in duplicate

2.1243 INCLUSION OF PARGYLINE

In 5-HT uptake studies using brain tissue, a MAO inhibitor such as nialamide or pargyline is added to prevent the metabolism of the 5HT which is taken up by the cell. This precaution is taken to avoid an error which may arise if the 5HT is metabolised.

In the pineal, the problem is more complex because metabolism by both MAO and SNAT have to be considered. It has been reported that pargyline initially inhibits and thereafter stimulates SNAT activity (647). It was therefore essential to determine the effects of pargyline on the experimental system to be used.

150 mg pineal tissue was incubated at 37°c in 3 ml medium containing 5×10^{-4} M pargyline. The medium used for the controls did not contain the pargyline. After incubation, the tissue was treated as described in section 2.241.

Experiments were done in duplicate.

2.13 RESULTS AND DISCUSSION

2.131 TIME OF INCUBATION

Results are presented in Figure 10 and Table 5 The initial rate of uptake (i.e. between 1 minute and 4 minutes) appeared to be rather rapid. A linear increase in uptake occurred in the first 8 minutes of incubation. Thereafter, a drop in the uptake was observed. This effect was unusual since a "plateau" effect was expected. A possible explanation for this observation could be MAO and/or SNAT activity. Results in section 2.33 show that 5HT uptake dropped after 30 minutes despite the presence of pargyline in the incubation medium. It is possible that metabolites such as MEL and HIAA were formed and released into the incubation medium.

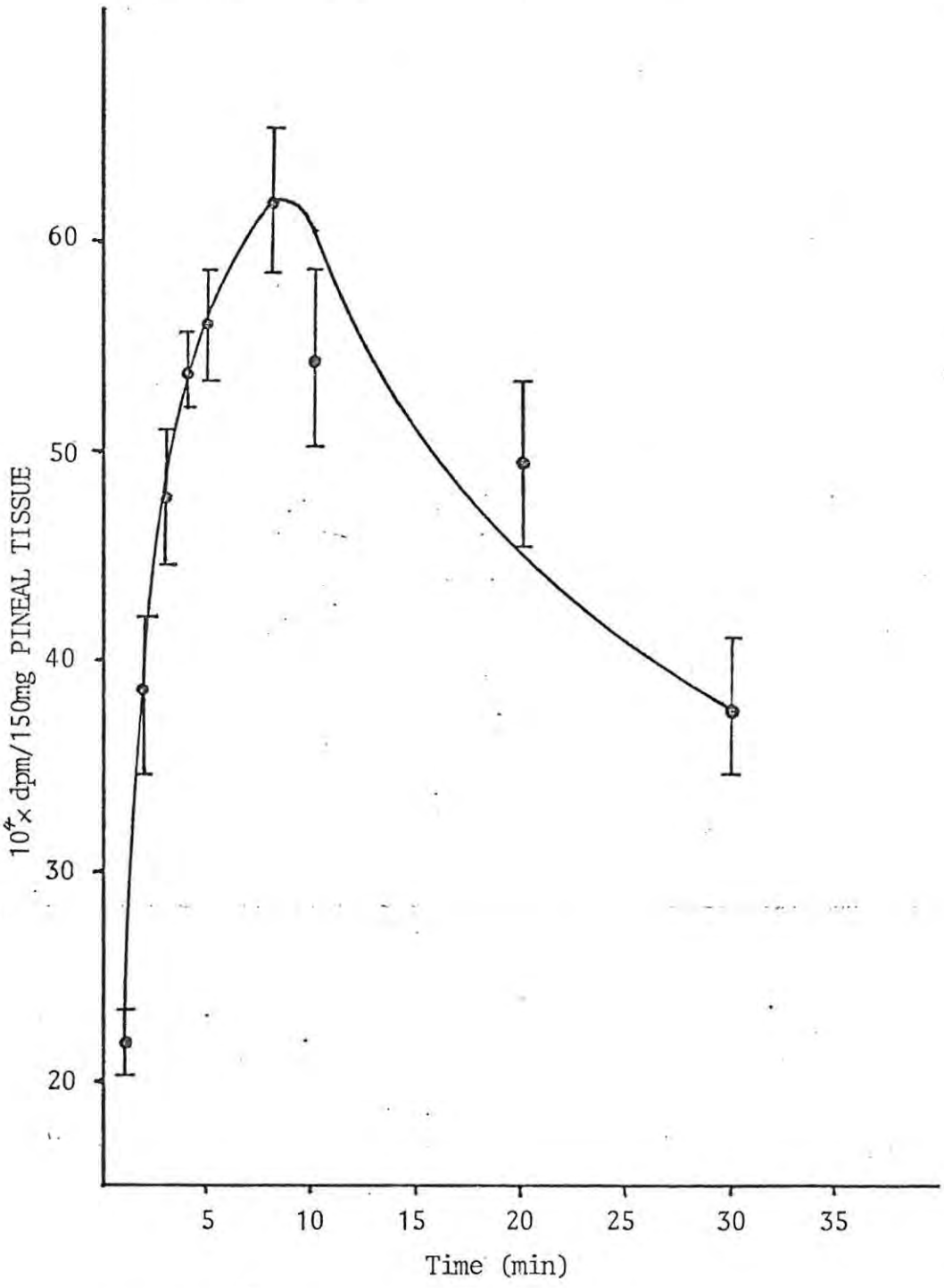
In view of the above observations, it seemed reasonable to incubate the test samples at 37°c for 3 minutes.

2.132 INCLUSION OF PARGYLINE

Results are presented in Table 6

The uptake of ^{3}H-5HT into pineal slices was higher in the presence of pargyline during both incubation periods. Uptake of ^{3}H-5HT in the presence of pargyline was lower after 30 minutes than after 5 minutes. It is possible that pargyline stimulates SNAT after a certain period of incubation. The metabolism of 5-HT, together with the inhibitory effect that MEL might exert on 5-HT uptake (814), might be responsible for the observed effect.

FIGURE 10



UPTAKE OF ^{3}H-5HT AS A FUNCTION OF TIME

TABLE 5

TIME (min)	UPTAKE (10 dpm/150mg pineal tissue)
1	24 ± 2
2	38 ± 3
3	48 ± 3
4	54 ± 1
5	56 ± 2
8	62 ± 3
10	55 ± 4
20	49 ± 3
30	37 ± 1

EFFECT OF TIME ON THE UPTAKE OF ^{3}H-5HT IN BOVINE PINEAL SLICES

TABLE 6

ADDITION	TIME (min)	UPTAKE	SIGNIFICANCE
none	5	50 ± 2	
pargyline	5	53 ± 3	NS
none	30	22 ± 4	
pargyline	30	40 ± 6	$p < 0,05$

Effect of pargyline on the uptake of ^{3}H-5HT in pinealocytes at two different incubation times

Uptake expressed in 10^4 dpm/150gm pineal ± SEM.

Since the uptake of ^{3}H-5HT appeared to be higher in the presence of pargyline, pargyline was used in the uptake studies.

The use of a β -blocker (as a SNAT blocker) did not seem to be necessary because pargyline appeared to inhibit the metabolism of 5-HT in the first 5 minutes of the studies.

2.133 TEMPERATURE

Results are presented in Figure 11 and Table 7 The 5-HT uptake process in the bovine pineal is temperature-dependent. Initially, uptake increased linearly. Maximum uptake was observed at 37° C. Thereafter, uptake decreased rapidly with an increase in temperature. It is possible that this rapid decrease in uptake is due to denaturation of protein at the high temperatures.

These observations suggest that 37°c is the most suitable incubation temperature for the 5-HT uptake process.

2.134 INFLUENCE OF TADs ON THE UPTAKE OF ^{3}H-5HT

Results are presented in Figure 12.

TADs appear to exert some influence on the uptake of 5HT into bovine pineal slices. The effect of TADs on the 5HT uptake system is dose dependent. The maximum inhibition of uptake was only 51%. Although DESI, CLOMI and IMI did inhibit the uptake of 5HT, this effect was observed within a limited concentration range for each drug. It is therefore difficult to predict the consequences of these effects if they occurred in vivo.

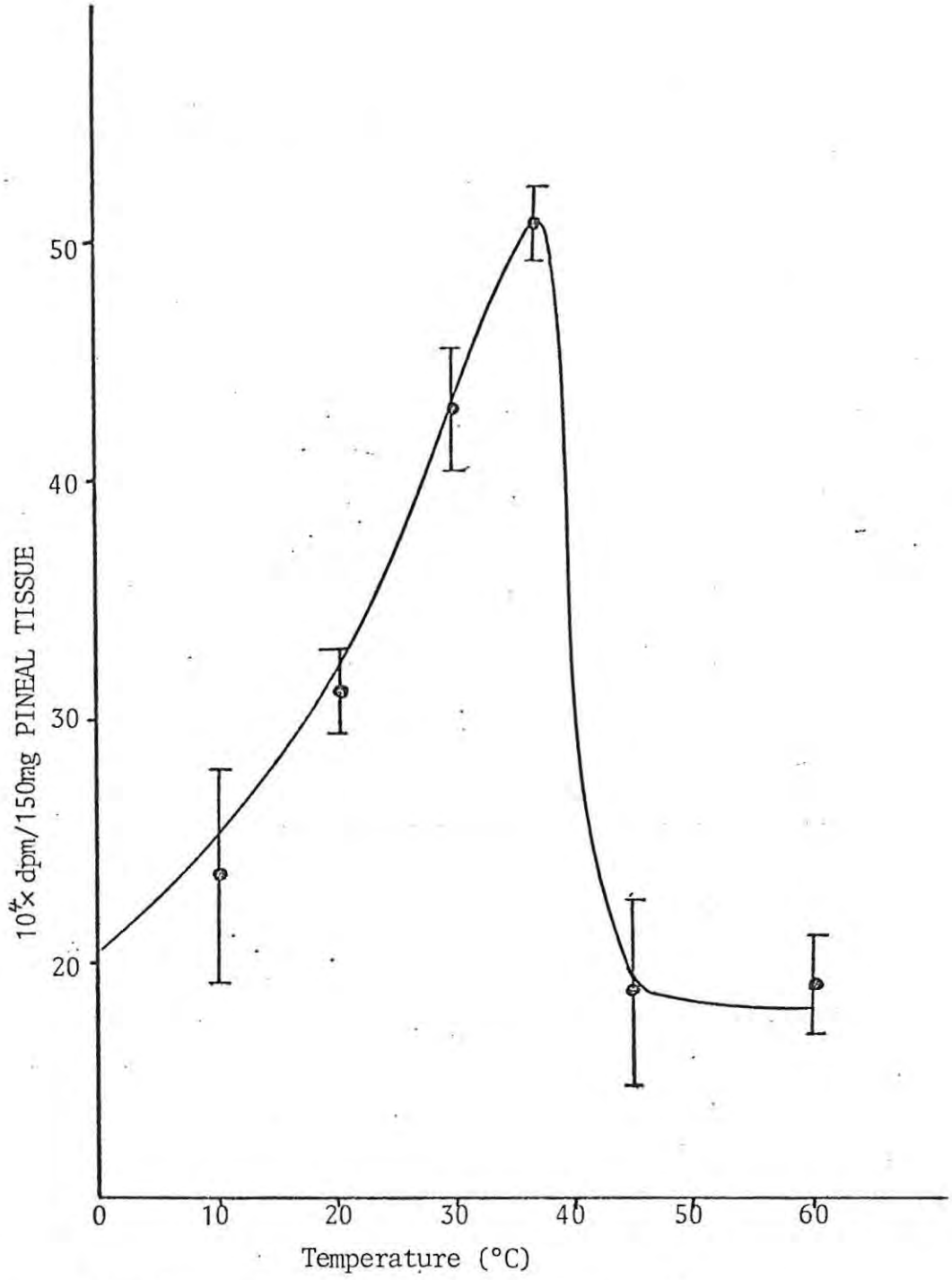
TABLE 7

TEMPERATURE (°C)	UPTAKE
0	20 ± 4
10	27 ± 9
20	32 ± 2
30	46 ± 5
37	51 ± 2
45	17 ± 8
60	18 ± 4

Effect of temperature on the uptake of ^{3}H-5HT from bovine pineal slices

Uptake expressed as 10^{4}dpm/150mg pineal ± SEM.

FIGURE 11



EFFECT OF TEMPERATURE ON THE UPTAKE OF ^{3}H-5HT INTO BOVINE PINEAL SLICES.

TABLE 8

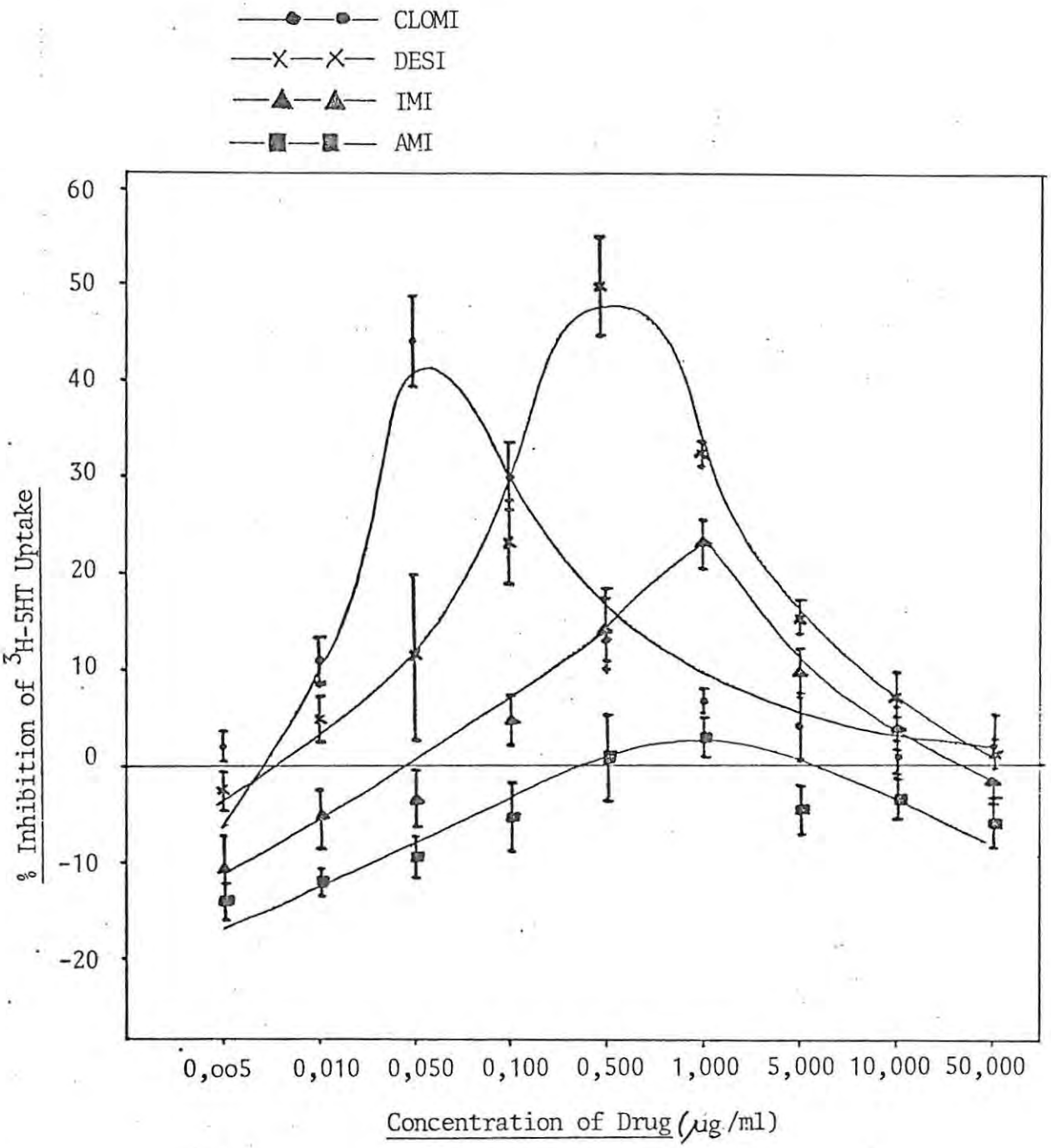
CONCENTRATION (ug/ml)	DRUG			
	CLOMI	DESI	IMI	AMI
0,005	4,5 ± 1,6	-2,0 ± 2,3	-10,0 ±2,0	-13,0 ± 3,2
0,010	11,3 ± 3,2	5,0 ± 4,5	-4,5 ± 3,8	-11,0 ± 2,9
0,050	45,0 ± 5,0	11,5 ± 3,5	-3,0 ± 3,2	-9,0 ± 2,4
0,100	29,8 ± 2,1	23,0 ± 4,8	5,0 ± 1,6	-5,0 ± 3,6
0,500	13,0 ± 2,5	51,0 ± 4,6	13,8 ± 4,0	0,8 ± 2,0
1,000	7,0 ± 3,2	32,0 ± 3,4	23,0 ± 4,8	2,8 ± 5,3
5,000	3,6 ± 2,0	15,0 ± 2,4	9,8 ± 2,1	-4,5 ± 2,5
10,000	1,0 ± 1,8	7,0 ± 2,8	3,7 ± 1,8	-3,6 ± 3,0
50,000	1,8 ± 2,0	1,0 ± 1,4	-1,5 ± 2,5	-6,0 ± 2,2

Effect of TADs on the uptake of ^{3}H-5HT into bovine pineal slices

Results are expressed as % inhibition of uptake of ^{3}H-5HT ± SEM

FIGURE 12

THE EFFECT OF TADs ON THE UPTAKE OF ^{3}H-5HT IN BOVINE PINEAL SLICES



In vitro studies on tissue slices and synaptosomes from the hypothalamus and cerebral cortex have been used to demonstrate the inhibition of uptake of ^{3}H-5HT by TADs (see section 1.146). The order of activity of TADs in inhibiting 5-HT uptake into brain tissue was as follows:- CLOMI > IMI ≳ AMI > DESI. Thus, DESI is the weakest inhibitor of 5-HT uptake in brain tissue. This is in contrast to the observations made in the study on bovine pineal slices. There are two possible reasons that might explain this difference.

It is possible that the 5-HT uptake system into bovine pinealocytes is different from the 5-HT uptake systems into brain tissue and platelets. Owman (440, 441) has reported that adrenergic nerves in the pineal accumulate 5-HT that is actively synthesized by the parenchymal cells. N/A and 5-HT are not only simultaneously present in these nerves, but as suggested by indirect evidence, they may even coexist within the same vesicles (442). It has been suggested that both vesicular 5-HT and N/A might be released by impulses reaching sympathetic nerves in the pineal. Thus, it appears that 5HT uptake systems in the pineal might differ from that in brain tissue and platelets.

Fuller and Perry (853) have reported that the specificity of neurotransmitter uptake inhibitors resides in the uptake pump rather than the substrate. This idea is believed to be mechanistically sound because inhibition is thought to result from a combination of the drug molecule with the receptor macromolecule so as to compete with the substrate attachment, rather than by

combination with the substrate molecule. Fuller and Perry (963) supported this theory with in vitro studies that suggested that DESI effectively blocked the uptake of 5-HT into noradrenergic nerve terminals whereas fluoxetine, which is a highly selective inhibitor of 5-HT uptake in brain and heart (307), did not inhibit 5-HT uptake in the pineal. The observation that desipramine is a relatively potent inhibitor of 5-HT into bovine pineal slices is in accordance with the above theory. However, according to the above theory, CLOMI, which is a very weak inhibitor of N/A uptake in brain tissue should be the weakest inhibitor of 5-HT uptake into bovine pineal slices. This contrasts with the observations made in this experiment. It is therefore possible that the interaction between the uptake inhibitor and 5-HT uptake system in the pineal is more complex than that proposed by Fuller and Perry (963).

A second possible explanation has been advanced by Jaim-Etcheverry and Zieher (677) who reported a significant increase in pineal N/A concentration after DESI administration. This observation could present an alternative explanation for the relatively high activity of DESI against 5-HT uptake into pinealocytes. If DESI produces an increased concentration of N/A in vitro, the increased N/A concentration might overcome the initial inhibitory effect of pargyline on SNAT. MEL would then be produced. It is possible that the MEL produced might inhibit 5-HT uptake in pineal slices, as in the case of hypothalamic synaptosomes (814).

Another interesting observation made in these experiments was that an increase in drug concentration appeared to inhibit the uptake of 5-HT up to a particular point and thereafter an increase in the 5-HT uptake was seen. Maximum inhibition occured at one particular concentration.

The apparent increase in 5-HT uptake after the initial inhibition of 5-HT uptake in these experiments might be due to permeability changes in the pinealocyte membrane resulting from the high concentration of TADs (see section 2.2). In erythrocytes and hepatocytes TADs have been shown to have both membrane stabilizing and lytic effects that were concentration dependent and temperature dependent (854 - 857). At 37°C, Yasubarc et al (855) found that the degree of cytotoxicity produced by the TAD on hepatocytes was CLOMI > DESI > IMI. These effects were correlated with their surface activities. The same order of activity (i.e. based on concentration at which an apparent increase in uptake was seen) of TAD was found in these studies. It has been reported that the rank order of surface activity of drugs correlated with the rank order of their degree of uptake by the hepatocytes and erythrocytes (857). The difference between the membrane stabilizing and lytic effect is believed to be directly related to the extent of the drug concentration.

Ducis and Distefano (851) have suggested that the 5-HT uptake system in the pineal is dependent on Mg^{2+}-dependent $(Na^{+} + K^{+})$-ATPASE. Chlorpromazine has been associated

with inhibition of this enzyme system in a dose dependent manner (858). Since TADs are similar in structure to chlorpromazine, it is possible that the TADs also affect this system at higher concentrations, and thereby affect permeability of the pinealocyte membrane.

These studies suggest that the effect of TADs on 5-HT re-uptake in the pineal differs from the effect that these drugs have on 5-HT reuptake systems in the brain and in platelets. The significance of the effects of TADs on 5-HT uptake in the pineal is unclear. However, the possibility of a correlation between these observations and the "therapeutic window" observed with antidepressant drugs, should be investigated.

2.2 ELECTRON MICROSCOPIC STUDIES

2.21 INTRODUCTION

These electron microscopic studies were designed to determine whether high concentrations of TADs interfere with the integrity of the pinealocyte membrane.

2.22 MATERIALS AND METHODS

The procedure followed was that employed by Mr Cross of Rhodes University,(Department of Electron Microscopy)

2.221 COLLECTION AND PRIMARY FIXATION OF MATERIAL

The source of the tissue was the same as in section 2.122. The tissue was incubated as described in section 2.123.

DESI, in concentrations of 2×10^{-7} M and 5×10^{-5} M, was used. The incubation medium of the control did not contain a TAD.

On termination of the incubation period, the pieces of tissue were rinsed and placed in labelled specimen tubes (4 per tube) containing 10 ml cold buffered glutaraldehyde solution (5%) and allowed to fix at 4°c for 24 hours.

2.222 SECONDARY FIXATION AND DEHYDRATION

After 24 hours, the gluteraldehyde was decanted and the tissue pieces were rinsed twice in 0,1 M phosphate buffer (pH 7,3). The pieces of tissue were then placed in 5 ml osmium tetroxide solution and this fixation process required ninety minutes.

Osmium tetroxide was then decanted and the tissue was rinsed with PO_4 buffer. The tissue was then placed in 10 ml of 30% ethanol for five minutes. The tissue was rinsed with buffer and placed for 5 minutes in 10 ml of 50% ethanol. This procedure was repeated for 80% ethanol, 90% ethanol and two changes of absolute alcohol.

2.223 EMBEDDING

Absolute alcohol was decanted and 10 ml of propylene oxide was poured into the specimen tube. The tissue was exposed to the propylene oxide for 15 minutes. The propylene oxide

was then decanted and the tube was refilled with a 75 : 25 propylene oxide : resin (Epon 812) mixture. This mixture was allowed to infiltrate the tissue for 30 minutes. The mixture was then decanted and replaced by a 50 : 50 propylene oxide : resin mixture. A further 30 minutes was allowed for infiltration. The 50 : 50 propylene oxide : resin mixture was replaced by a 25 : 75 mixture. Another 30 minutes was allowed for infiltration. The tissue was then transferred to specimen tubes containing pure resin and the infiltration period was 30 minutes.

After 30 minutes, the tissue was transferred to specimen moulds that were 2/3 full with fresh resin. The specimen moulds were transferred to an oven and the contents were allowed to polymerize at 60°c for 36 hours.

After polymerization the moulds were removed from the oven and cooled. The blocks (i.e. solidified resin containing the tissue) were then removed from the moulds and stored in labelled tubes.

2.224 CUTTING AND COLLECTION OF ULTRATHIN SECTIONS

Ultra-thin sections of the embedded tissue were cut using an ultramicrotome. Ribbons of 5 - 6 sections were then transferred onto specimen grids which were placed on filter paper in a petri dish and allowed to dry.

2.225 HEAVY METAL STAINING OF ULTRA-THIN SECTIONS

A drop of uranyl acetate was placed on a layer of wax in a petri dish. Each grid was inverted and placed on the surface of the uranyl acetate. After 30 minutes the grid was removed and 3 drops of distilled water was allowed to flow gently over the grid, ensuring that the sections were not washed off. Excess water was removed by touching the edge of the grid with a piece of filter paper. The grid was then inverted in a drop of lead citrate and allowed to strain for five minutes. The grid was then washed with 3 drops of 0,2N sodium hydroxide, followed by a wash in 3 drops of distilled water.

The excess water was removed using filter paper and the grid was replaced in the storage petri dish.

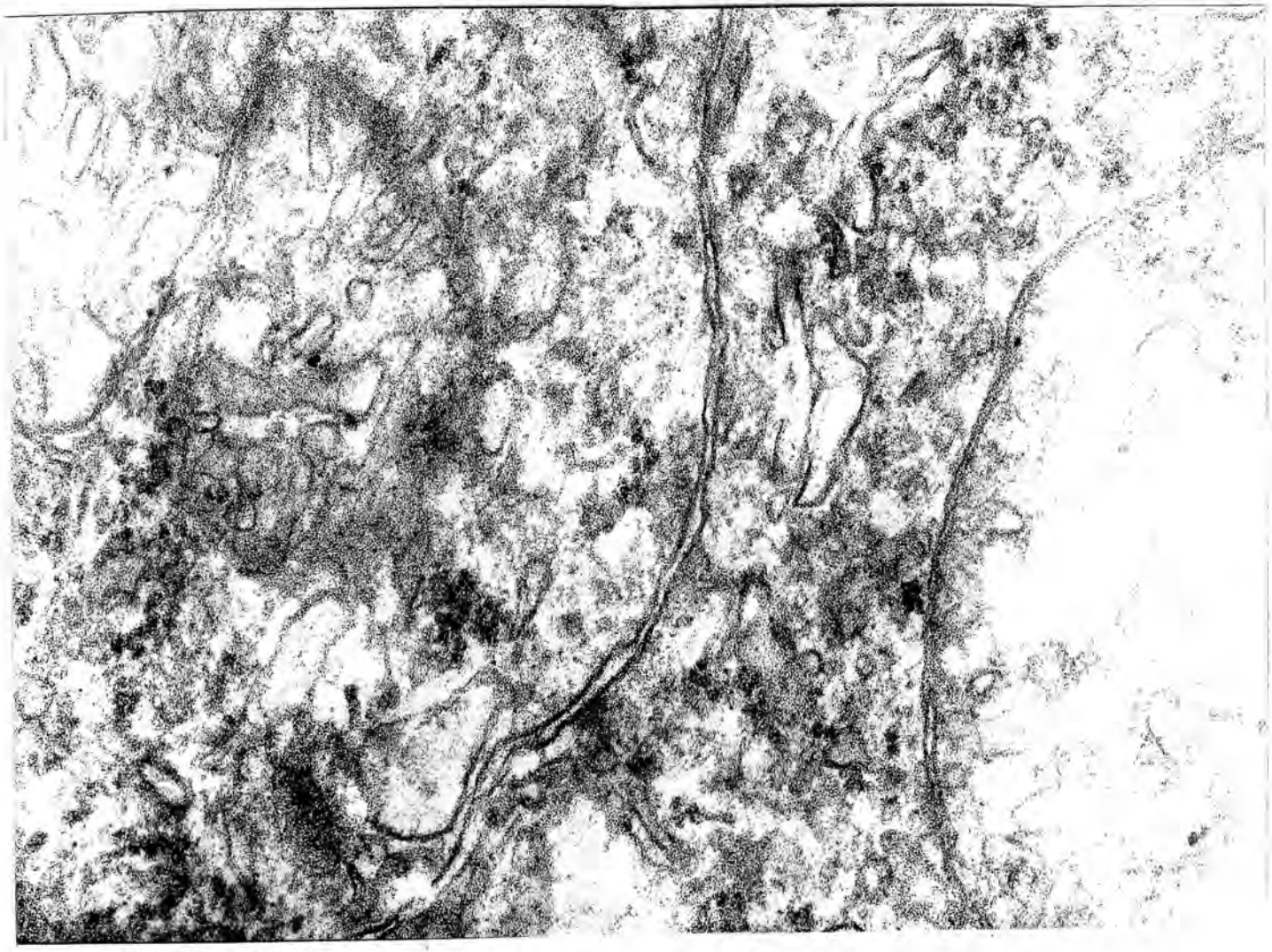
Slices were then viewed in an electron microscrope.

2.23 RESULTS AND CONCLUSION

The electronmicrographs (Fig. 13 - Fig. 15) were interpreted by Prof. Theron of Pretoria University (Physiology Dept.)

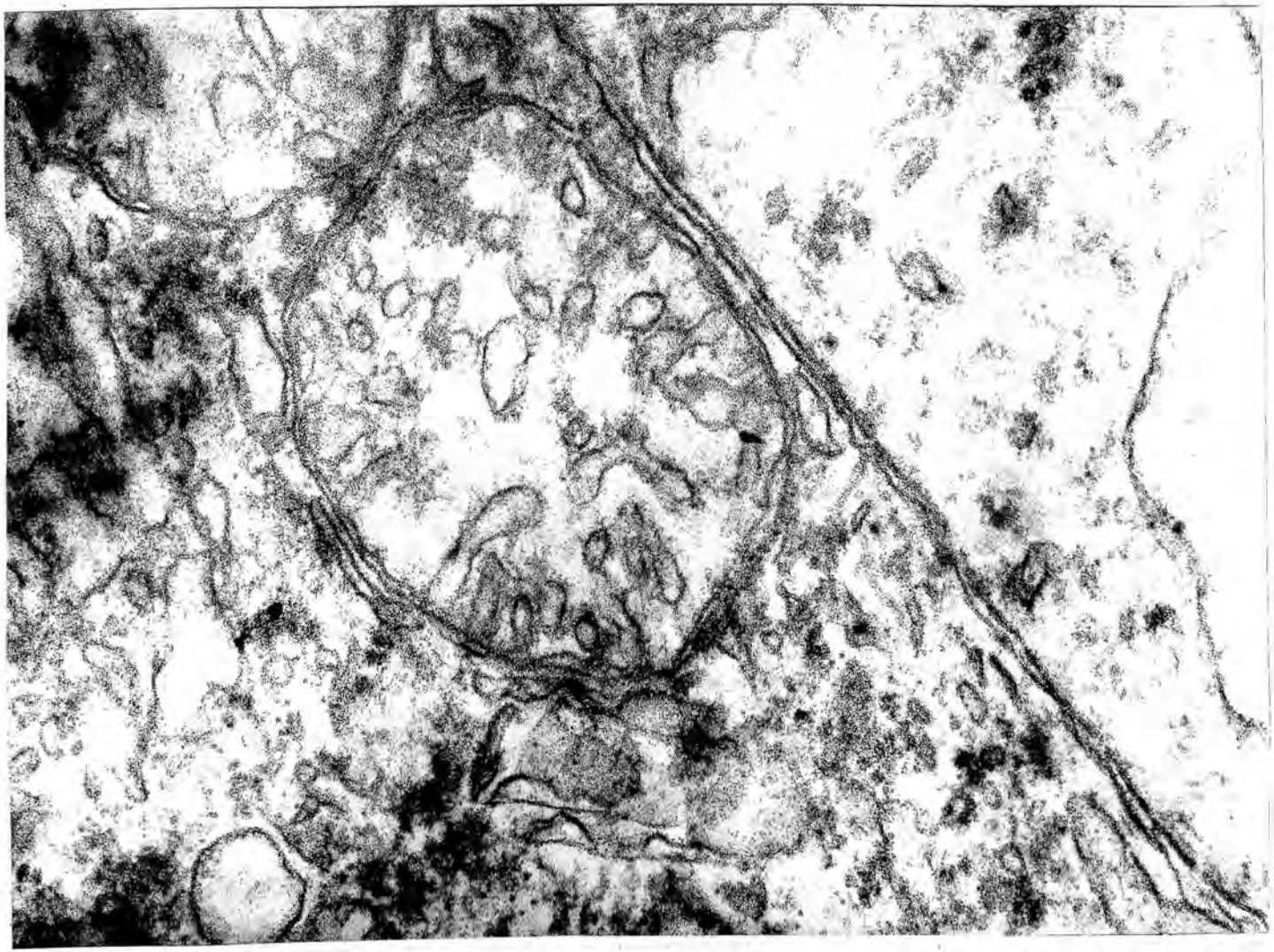
Figure 13 represents a section of tissue that was treated without drug. The tissue used to prepare the micrograph in Figure 14 was treated with 2×10^{-7} M DESI while the tissue used to prepare the micrograph in Figure 15 was treated with 5×10^{-5} M DESI.

FIGURE 13: MICROGRAPH OF A SECTION THROUGH UNTREATED BOVINE PINEAL TISSUE MAGNIFICATION: 96000X



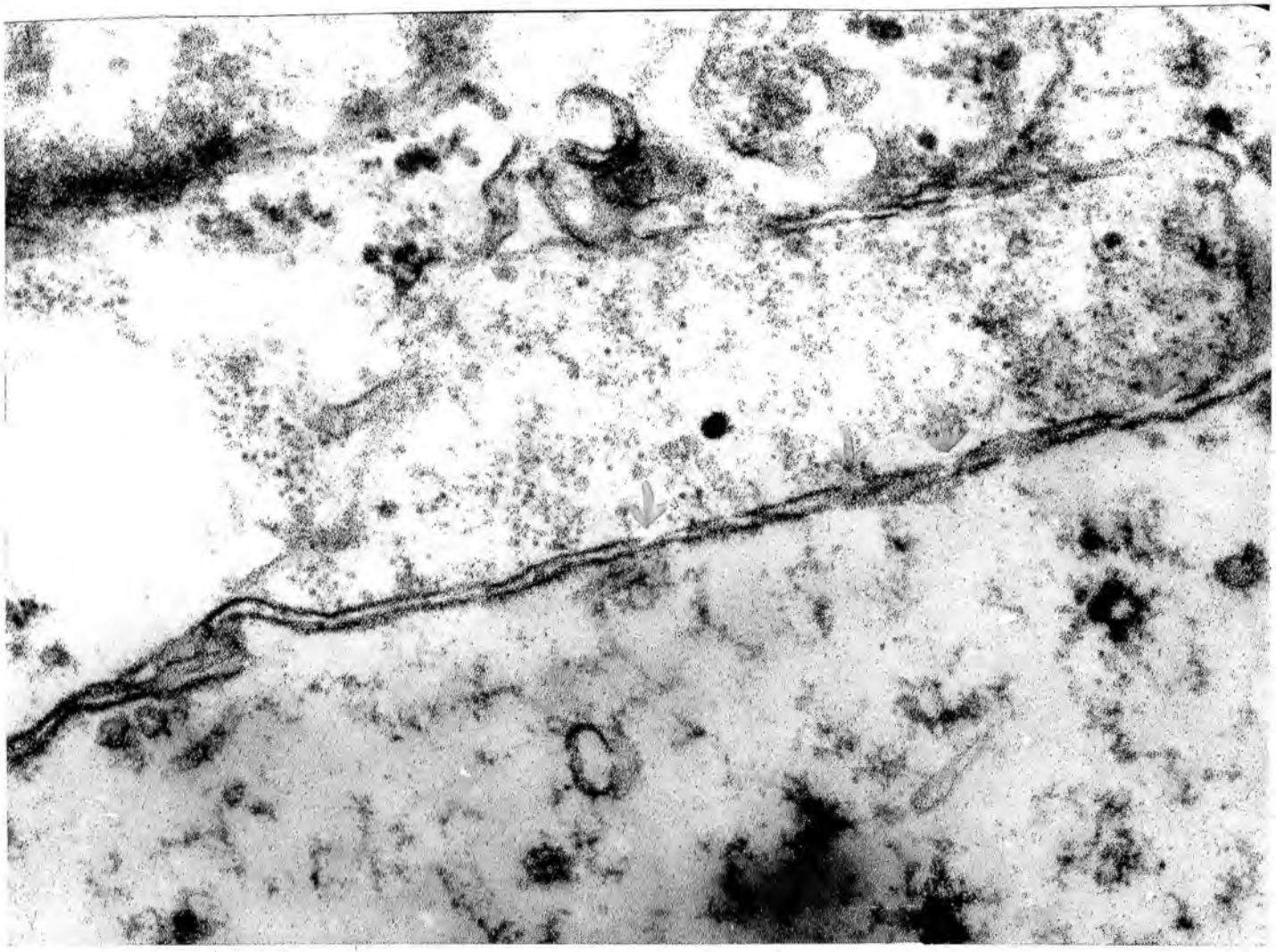
NOTE: THE MEMBRANES APPEAR TO BE INTACT

FIGURE 14: MICROGRAPH OF A SECTION THROUGH BOVINE PINEAL TISSUE TREATED WITH 2 x 10^{-7} M DESI MAGNIFICATION: 96000×



NOTE: THE MEMBRANES APPEAR TO BE INTACT

FIGURE 15: MICROGRAPH OF BOVINE PINEAL TISSUE THAT HAD BEEN TREATED WITH 5×10^{-5} M DESI MAGNIFICATION: 96000×



NOTE: THERE APPEAR TO BE "INDENTATIONS" OR "BREAKAGES" IN THE MEMBRANES. (SEE ARROWS).

The micrographs seem to suggest that "breakages" occur in the membrane under the influence of high levels (i.e. 5 x 10^{-5} m) of DESI (Figure 15). The membranes in Figure 13 and Figure 14 appear to be intact. However, this observation should be verified by using other systems.

If the above findings can be confirmed after the use of other systems, it can be concluded that the sudden increase in 5-HT uptake seen in Figure 12 is due to "breakages" in the pinealocyte membrane.

CHAPTER 3

THE EFFECT OF TRICYCLIC ANTIDEPRESSANTS ON THE METABOLISM OF RADIOLABELLED SEROTONIN BY THE RAT PINEAL GLAND, USING ORGAN CULTURE

3.1 INTRODUCTION

Many clinical studies support the hypothesis of an altered N/A and/or 5-HT metabolism in depressed patients (859 - 863). The pineal gland is unique in that an altered N/A concentration can influence the metabolism of 5-HT via the enzyme SNAT. SNAT is responsible for the synthesis of MEL, the most widely studied secretion of the pineal.

MEL has been associated with a subjective state of relaxation and well being when administered to normal man (811). It has been found to induce sleep in animals (841 - 843). Altschule (842) has suggested that MEL might induce an increase in hallucinatory activity in schizophrenic patients. MEL administration has been reported to lead to an enhanced dysphoria as well as loss of sleep and mass in depressed patients (811). In contrast, MEL appeared to have potential use in "agitated" depression, when animal models of depression were used (849). An altered MEL rhythm in depressed patients has been reported (864).

The above reports have evoked interest in the possible effects that TADs could have on 5-HT metabolism in the pineal and whether these effects could be implicated in the therapeutic actions of TADs

In this study, the effects of chronic TAD administration on the metabolism of ^{3}H-5HT by rat pineals in organ culture were studied. The TADs used were desipramine (a potent N/A reuptake inhibitor), clomipramine (a weak N/A reuptake inhibitor), imipramine and amitriptyline (the latter two TADs affect both N/A and 5-HT reuptake). Mianserin a tetracyclic antidepressant, was used to represent an "atypical" drug in this study.

3.2 MATERIALS AND METHODS

These studies were based on a modification of the method used by Cardinali et al (613). The technique involved incubating pineal glands from rats that had been pretreated with the test drug, in a medium that contained the nutrients necessary to keep the glands viable for twenty four hours. Incubation temperature was 37°c, to simulate physiological conditions. ^{3}H -5HT was added to the medium. The pineals were expected to take up the ^{3}H-5HT and to metabolise it. The metabolites would be released into the incubation medium, which could be analysed using thin layer chromatography (TLC) and scintillation counting.

3.21 MEDIUM

BGJb medium (Fitton-Jackson modification-obtained from Gibco Europe) was used. The composition of this medium was as follows:-

Components	mg/ℓ
Inorganic Salts	
Dihydrogen sodium ortho phosphate	90,00
Magnesium sulphate 7 H_2O	200,00
Potassium chloride	400,00
Potassium dihydrogen phosphate	160,00
Sodium bicarbonate	3 500,00
Sodium chloride	5 300,00
Amino Acids	
L-alanine	250,00
L - aspartic acid	150,00
L - cysteine HCl	90,00
Glycine	800,00
L - histidine	150,00
L - isoleucine	30,00
L - leucine	50,00
L - lysine	240,00
L - methionine	50,00
L - phenylalanine	50,00
L - proline	400,00
L - serine	200,00
L - threonine	75,00
L - tryptophane	40,00
D^c - valine	65,00

Vitamins	mg/ℓ
Alpha tocopherol phosphate	1,00
Ascorbic acid	50,00
Biotin	0,20
Calcium panthothenate	0,20
Choline chloride	50,00
Folic acid	0,20
Inositol	0,20
Nicotinamide	20,00
Para amino benzoic acid	2,00
Pyridoxal phosphate	0,20
Riboflavin	0,20
Thiamine hydrochloride	4,00
Vitamin B_{12}	0,04

Other Components	
Benzyl penicillin	100 units/ml
Streptomycin sulphate	0, 1mg/ml
Amphotercin B	2,5 mg/ml
L - glutamine	200 mg/ℓ
Calcium lactate	555,00
Glucose	10 000,00
Phenol red	20,00
Sodium acetate	50,00

The mean tonicity of this medium is 390 milliosmoles.

3.22 ORGAN CULTURE SYSTEM

This was based on the techniques developed by Daya (606). The organ culture vessels consisted of the bottom part of 1 ml glass ampoules that had been cut about 1,2 cm from the base. A lip was made on each of these vessels to facilitate handling. These vessels were sterilized by dry heat, before use.

The culture chamber was a sterile medical flat (100 ml) bottle with a sterile piece of filter paper that had been saturated with water for injection. The moist filter paper was used to maintain a humid atmosphere within the culture chamber.

The required volume of BGJb medium (Fitton Jackson modification) was placed in the culture vessel. The pineal glands were transferred, aseptically, into these vessels. The required volume of ^{3}H -5HT was added. The culture vessel was then placed in the culture chamber, which was lying on its flat side. The atmosphere within the culture chamber was saturated with carbogen (5% CO_2 in 95% oxygen) and the bottle was closed. The culture chamber was then placed in an incubator at 37°c.

3.23 SOURCE OF PINEALS

Pineals were obtained from Albino rats of the Wistar strain. Mass of animals was between 120 g and 150 g. The animal room was kept at approximately 20°c. The environmental lighting was controlled (12 hour dark/12 hour light, light period extended from 6H00 to 18H00) by a time switch. White

fluorescent tubes provided a light intensity of 300 watts/cm^2 during the light phase. Animals were allowed to have food and water ad lib.

The drug administered to the animals was dissolved in normal saline and administered intraperitoneally between 10H00 and 10H30 each morning. DESI, IMI, AMI and CLOMI were administered in a dose of 50 mg/kg while mianserin was administered in a dose of 10 mg/kg.

The animals were sacrificed at 09H00 by neck fracture. After decapitation, the pineal was exposed by making an incision through the bone on either side from the foramen magnum to near the orbit, and removing the top of the skull with forceps. The pineals were rapidly removed using clean forceps. Precautions were taken to ensure that they were free from tissue or blood before they were placed in the culture vessel.

3.24 THIN LAYER CHROMATOGRAPHY

This technique was based on the method developed by Klein and Noticles (865). Glass plates 20 cm x 20 cm were used. They were coated with kieselgel G60 (Merck) to a thickness of 0,2 mm.

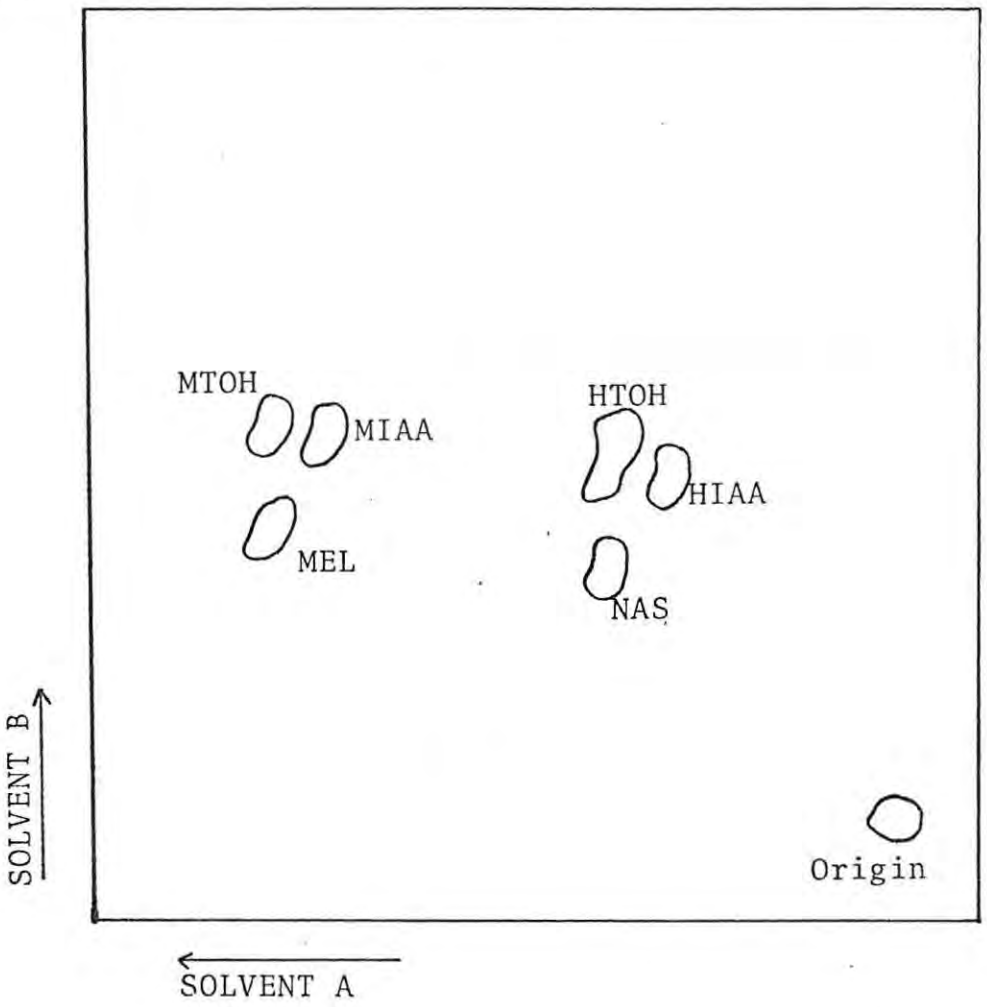
A solution of the reference substances (mixture A) was made and it consisted of 0,2 mg/ml of each of the following substances:- serotonin, methoxytryptamine, methoxyindole acetic acid, hydroxytryptophol, N-acetyl serotonin, methoxytryptophol, hydroxyindole acetic acid and melatonin. The solution was made by disolving 1 mg of each of the above substances in 2,5 ml 95% ethanol.

2,5 ml of a solution of 1% ascorbic acid (antoxidant) in 0,1 N HCl was then added to the drug solution. The mixture A was stored at -20°c in a light resistant container. The plates were spotted in subdued light. Nitrogen gas was used to dry the spots and prevent atmospheric oxidation of the indoles.

Two-dimensional TLC was used. The procedure was performed in the dark, to prevent decomposition of the metabolites. The first solvent system (A) consisted of chloroform: methanol: glacial acetic acid (93 : 7 : 1 by volume). This solvent was allowed to run up the plate to a height of 17 cm. The plate was removed from the tank and dried with N_2. It was then replaced in the same solvent system which was allowed to run up to a height of 17 cm for the second time. After the second run the plate was removed and dried with N_2. The plate was turned 90° and placed in a developing tank containing the second solvent (B), ethyl acetate. The solvent was allowed to run to a height of 10 cm. The plate was then removed and dried under N_2.

After spraying the plate with Erlicks reagent, it was placed in an oven at 60°C for 20 minutes to allow development of spots. The position of the spots on the TLC plate is represented in Figure 16.

FIGURE 16



Tracing of chromatographic separation of pineal indole compounds

Solvent A : chloroform/methanol/glacial acetic acid (93:7:1)

Solvent B : ethyl acetate

3.25 DETERMINATION OF THE EFFECT OF TADs ON ^{3}H-5HT METABOLISM

Four pineals were incubated as described above (section 3.22) with 2 x 10^{-5} M ^{3}H-5HT (specific activity 17,6 curies/mmol-Amersham) in 120 μl BGJb medium (Fitton Jackson modification--Gibco Europe). The incubation period was 24 hours. Pineals for the control were obtained from rats that had received normal saline intraperitoneally. A blank containing no tissue was included in each series of incubations.

At the end of the incubation period, the glands were removed from the medium to stop the reaction. 20 μl of the incubation medium and 20 μl of mixture A were chromatographed in duplicate, as described above. The radioactivity in each of the identified spots was determined by scraping off the spot and adding it to a glass scintillation vial containing 1 ml 95% ethanol. After the scrapings had dissolved in the ethanol, 7 ml Instagel $^{(R)}$ (Packard Instruments) was added. Radioactivity was counted in a Beckman LS3150T liquid scintillation counter for 20 minutes. Efficiency was calculated by the internal standard method. Results were expressed as dpm/pineal. Each value was corrected for the radioactivity of the blank.

3.3 RESULTS AND DISCUSSION

The results obtained in these experiments suggest that TAD administration has a marked effect on the metabolism of 5-HT and some of its metabolites.

3.31 EFFECT OF TADs ON SYNTHESIS OF MEL FROM 5HT

Results are presented in Tables 9 and 10 and Figures 17 and 18.

The two enzymes responsible for the conversion of 5-HT to MEL are SNAT and HIOMT. SNAT converts 5-HT to N-acetyl-5HT and this metabolite is converted to MEL by HIOMT. In these experiments, an increase in the synthesis of both N-acetyl-serotonin and MEL was seen in the first eleven days. Thereafter a slight reduction in the synthesis of both these compounds was observed. These effects are probably due to the influence of TAD's on enzyme activity.

The activity of SNAT, the first enzyme involved in the conversion of MEL from 5HT, is controlled by the concentration of N/A at the sympathetic nerve endings (refer to section 1.221111). Zatz (525) has reported that N/A interacts with the β -adrenergic receptors and activates adenyl cyclase. Adenylcyclase is responsible for the conversion of ATP to cyclic AMP. Cyclic AMP is implicated in the synthesis of the enzyme protein and also in the maintenance of SNAT in its active form. It is interesting to observe that DESI, a potent N/A reuptake blocker in brain and platelets (see section 1.146) appeared to produce the greatest increase in MEL synthesis while CLOMI, a relatively weak inhibitor of N/A reuptake (its metabolite inhibits N/A reuptake) appeared to cause a relatively smaller increase in MEL synthesis. This observation suggests that the influence of TADs on MEL synthesis is due (at least partly) to SNAT activation. The observation that both N-acetyl serotonin

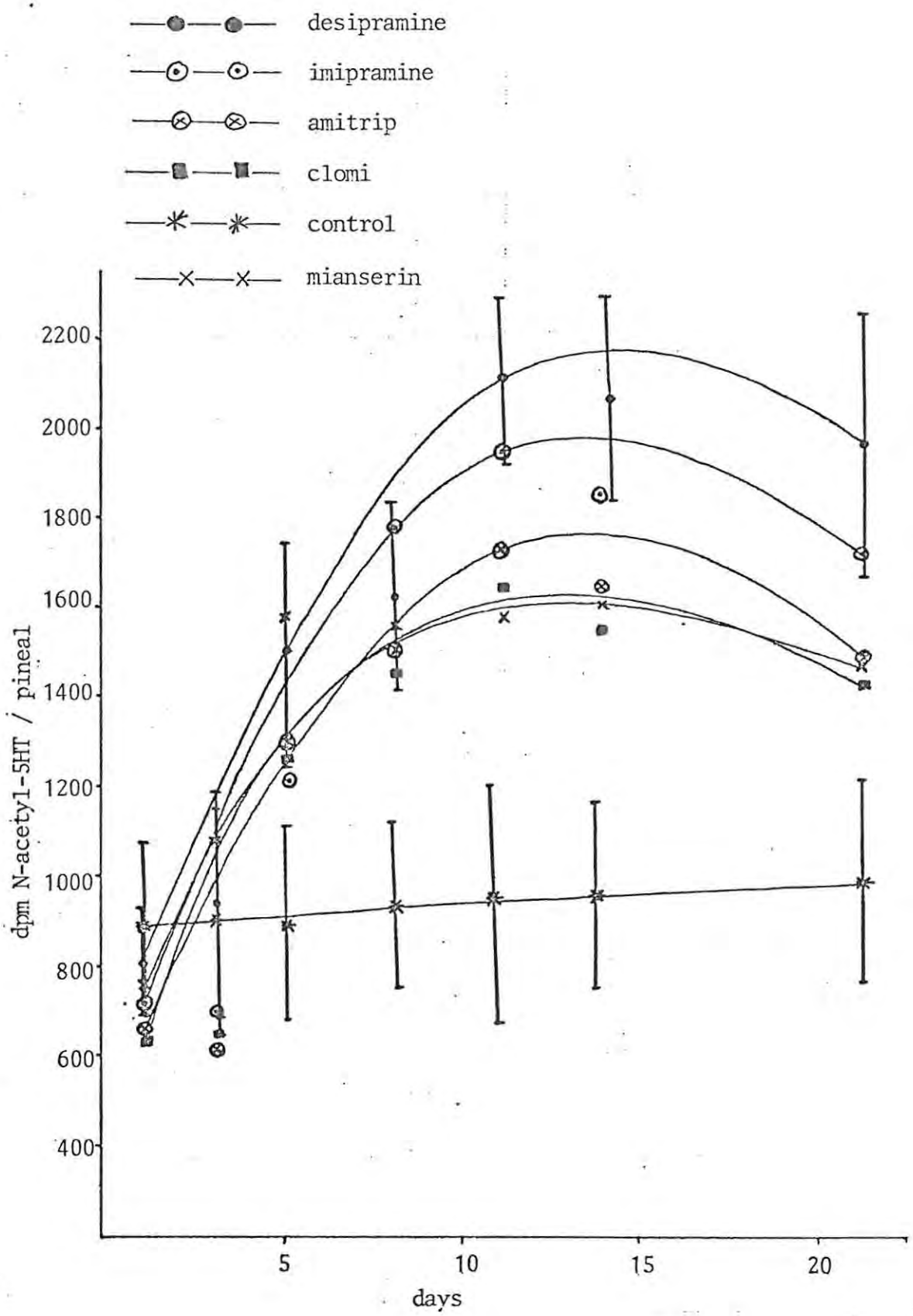
TABLE 9

	TREATMENT					
DAY	DESI	IMI	AMI	CLOMI	MIAN	CONTROL
1	1010±124	920±103	870±149	840±172	960±141	1090±123
3	1140±128	900± 98	820±127	850±131	1266±165	1100± 94
5	1700±135	1410± 96	1500±162	1460±145	1770±172	1090±107
8	1810±143	1970±138	1700±159	1650±153	1750±158	1116±106
11	2300±198	2120±195	1920±173	1840±164	1750±143	1150±142
14	2250±186	2040±162	1840±165	1740±129	1800±139	1150±178
21	2150±173	1910±187	1680±141	1620±112	1660±131	1180±130

Effect of TADs on the synthesis of N- acetyl - serotonin

Results are expressed as dpm N - acetyl 5HT/ pineal ± SEM

FIGURE 17



EFFECT OF TAD's ON N-ACETYL SEROTONIN PRODUCTION

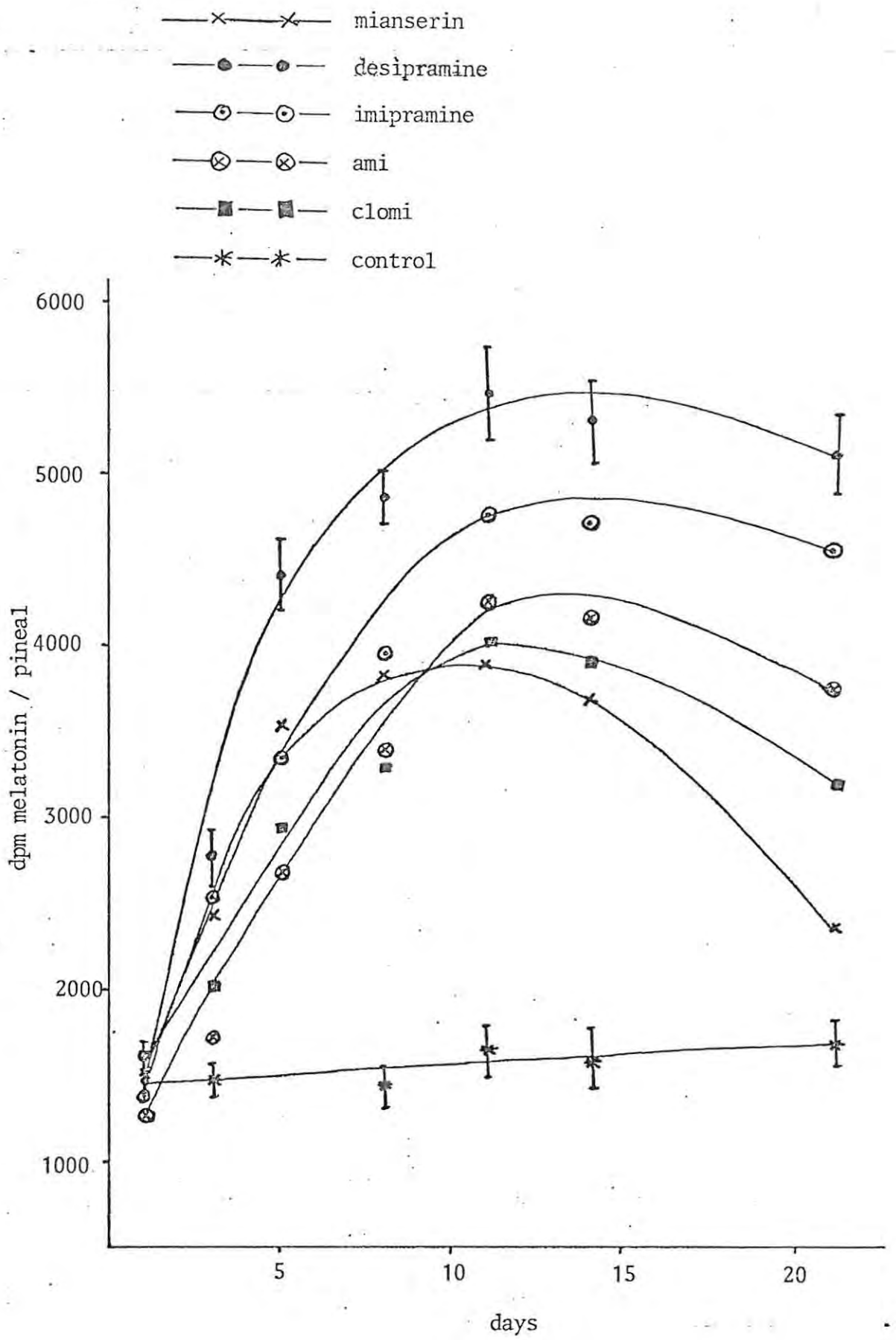
TABLE 10

	TREATMENT					
DAY	DESI	IMI	AMI	CLOMI	MIAN	CONTROL
1	1450±240	1360±270	1250±189	1600±305	1520±180	1450±208
3	2740±500	2500±430	1700±302	2000±298	2400±389	1470±265
5	4360±441	3300±480	2640±417	2800±490	3500±396	1500±290
8	4800±385	3900±347	3350±488	3570±520	3780±460	1430±290
11	5380±670	4720±395	4200±526	3970±683	3850±570	1640±351
14	5270±480	4650±401	4100±413	3850±571	3640±516	1570±298
21	5050±620	4500±490	3690±561	3140±415	2300±250	1660±280

Effect of TADs on Mel production

Results are expressed as dpm Mel/ pineal ± SEM

FIGURE 18



EFFECT OF TAD's ON MELATONIN PRODUCTION

and MEL synthesis was increased lends support to this deduction.

It has been suggested that increased SNAT activity produces a greater concentration of substrate for HIOMT which then synthesizes an increased concentration of MEL (525). However, it appears that TADs might have a stimulant action on both SNAT and HIOMT in this experiment, since the increase in the concentration of MEL produced was higher than that of N-acetyl serotonin. This observation is surprising in view of the report that sympathetic stimulation results in the inhibition of HIOMT activity (532). It is therefore necessary to determine the effect of TADs on SNAT activity and HIOMT activity individually. Such investigations should provide insight into whether TADs affect HIOMT activity directly or whether the apparent increase in HIOMT activity is a consequence of increased SNAT activity, i.e. the greater availability of NAS.

The effect of mianserin, the "atypical" antidepressant, was similar to that of the other drugs. Mianserin has been shown to increase the release of N/A by blocking presynaptic α_2 receptors (866). This action probably accounts for the increased MEL production.

It is interesting to observe that a slight but progressive diminution of MEL and N-acetyl serotonin synthesis appears to occur after $\pm$ 11 days. Previous reports on SNAT activity might hold an explanation for this observation. It has been reported that the

magnitude of the rise in SNAT activity may vary depending on the pinealocytes' previous exposure to stimulation (613). A period of stimulation by N/A is believed to produce a subsensitive response to subsequent N/A stimulation. Thus, the diminution in the synthesis of MEL and N-acetyl-5HT is probably due to reduced SNAT activity as a result of subsensitivity to stimulation by N/A.

There is a shift to the right in the dose response curve of SNAT induction after repeated administration of N/A (523). Either a change in the conformation of the β-receptor or a change in the number of available receptors can cause a change in the sensitivity of SNAT. Some investigators (459 - 460, 523 - 524) have shown that subsensitivity and supersensitivity can be due to a decrease or increase, respectively in the number of β-adrenergic receptors available. Wilkonson (625) has suggested that the sensitivity of the pineal β-receptors appear to be dependent upon Ca^{2+} ions, possibly by controlling enzyme induction at an intracellular site beyond the β-receptor.

Similar experiments performed over a longer duration of time should reveal whether MEL and N-acetylserotonin secretion eventually drops to control levels or below or whether the production of two compounds eventually reaches a steady state level that is significantly higher than the control value.

3.32 EFFECTS OF TADs ON HYDROXYINDOLEACETIC ACID, METHOXYINDOLEACETIC ACID, HYDROXYTRYPTOPHOL AND METHOXYTRYPTOPHOL

Results are presented in Tables 11 - 14 and Figures 19 - 22.

As shown in Figure 6, MAO converts 5HT to 5-hydroxyindole acetaldehyde. The latter compound is an unstable intermediate which is rapidly converted to 5-hydroxyindole acetic acid (HIAA) and 5-hydroxytryptophol (HTOH) by the enzymes aldehyde dehydrogenase and alcohol dehydrogenase, respectively. HIOMT then acts on HIAA and HTOH and converts them to 5-methoxyindoleacetic acid and 5-methoxytryptophol, respectively.

In these experiments, the production of both HTOH and HIAA were increased slightly for the first eight days. Thereafter, a decline in production was observed. This effect of TADs might appear to be due to the influence of the drugs on MAO and/or the dehydrogenases. However, it is also interesting to note that the increase in HIAA and HTOH synthesis is accompanied by a decrease in the production of their methoxy metabolites. Therefore, it is possible that the decreased metabolism of HIAA and HTOH could be reflected as an apparent increase in production. The effects of TADs on MAO and the dehydrogenases in the pineal are unknown and therefore should be investigated. Chapter 4 gives some insight into the effects of TADs on HIOMT and SNAT activity.

The observation that TADs increase MEL production with simultaneous decreases in MTOH and MIAA production is rather surprising since HIOMT catalyses all three conversions. There are two possible explanations for this observation.

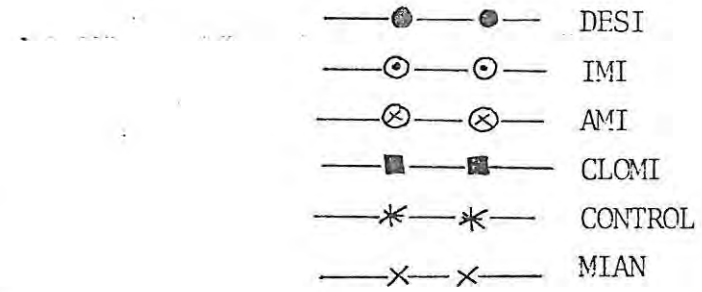
TABLE 11

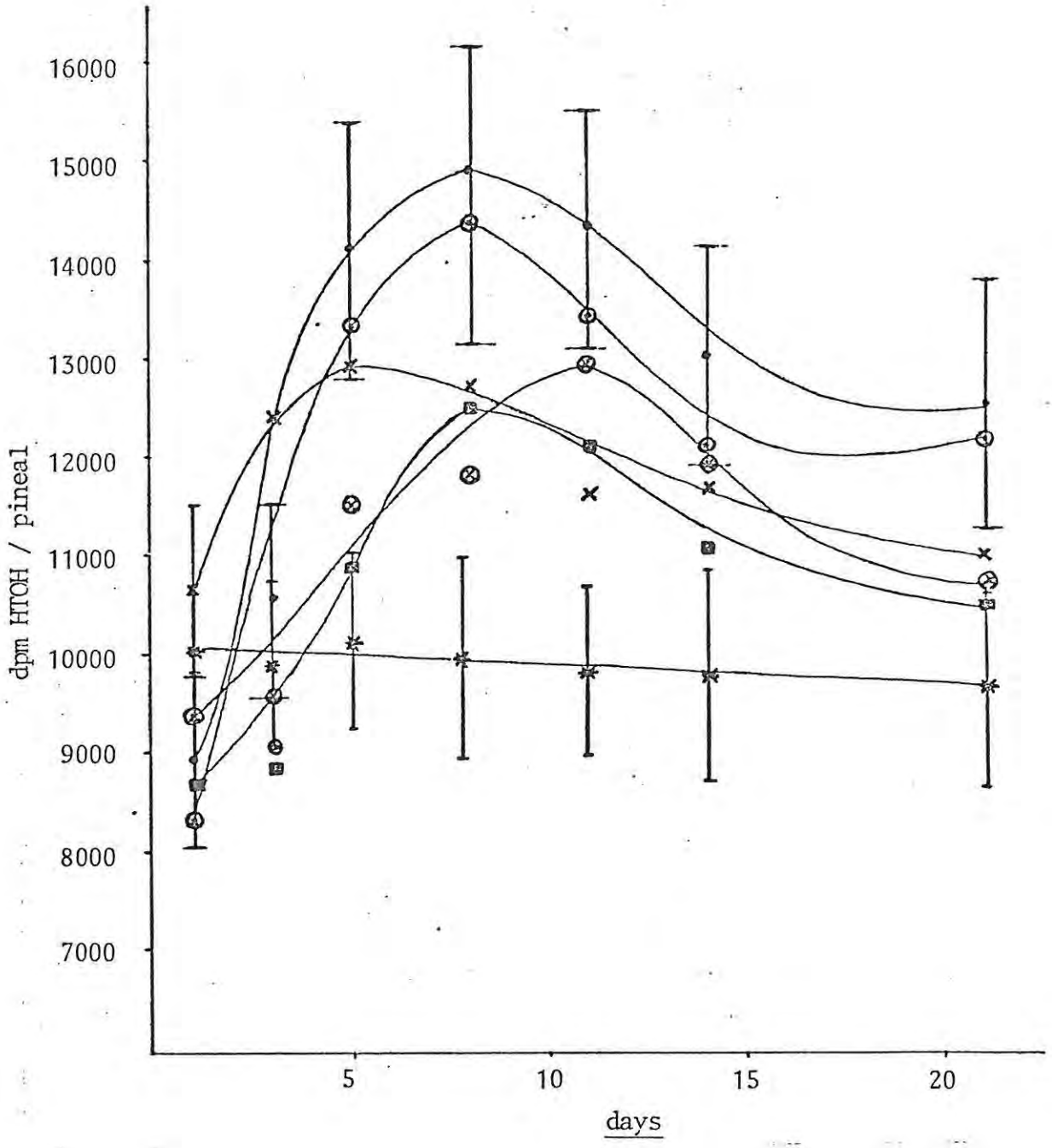
DAY	TREATMENT					
	DESI	IMI	AMI	CLOMI	MIAN	CONTROL
1	8950+874	8320+863	9400+896	8700+869	10650+838	8700+869
3	10600+986	9600+898	9100+904	8890+845	12350+963	8890+845
5	14100+1321	13300+1186	11500+987	10900+923	12900+1076	10900+923
8	14890+1275	14340+1213	12900+1130	12500+1051	12700+1015	12500+1051
11	14300+1206	13400+1017	11050+1076	12100+1148	11600+946	12100+1148
14	13000+1106	12100+1040	11C50+1076	11050+1007	11680+973	11050+1007
21	12500+1277	12150+1169	10700+985	10500+976	11000+870	10500+978

EFFECT OF TAD_5 ON HTOH SYNTHESIS

Results are expressed as dpm HTOH / pineal ± SEM

FIGURE 19





EFFECT OF TAD's ON HYDROXYTRYPTOPHOL PRODUCTION

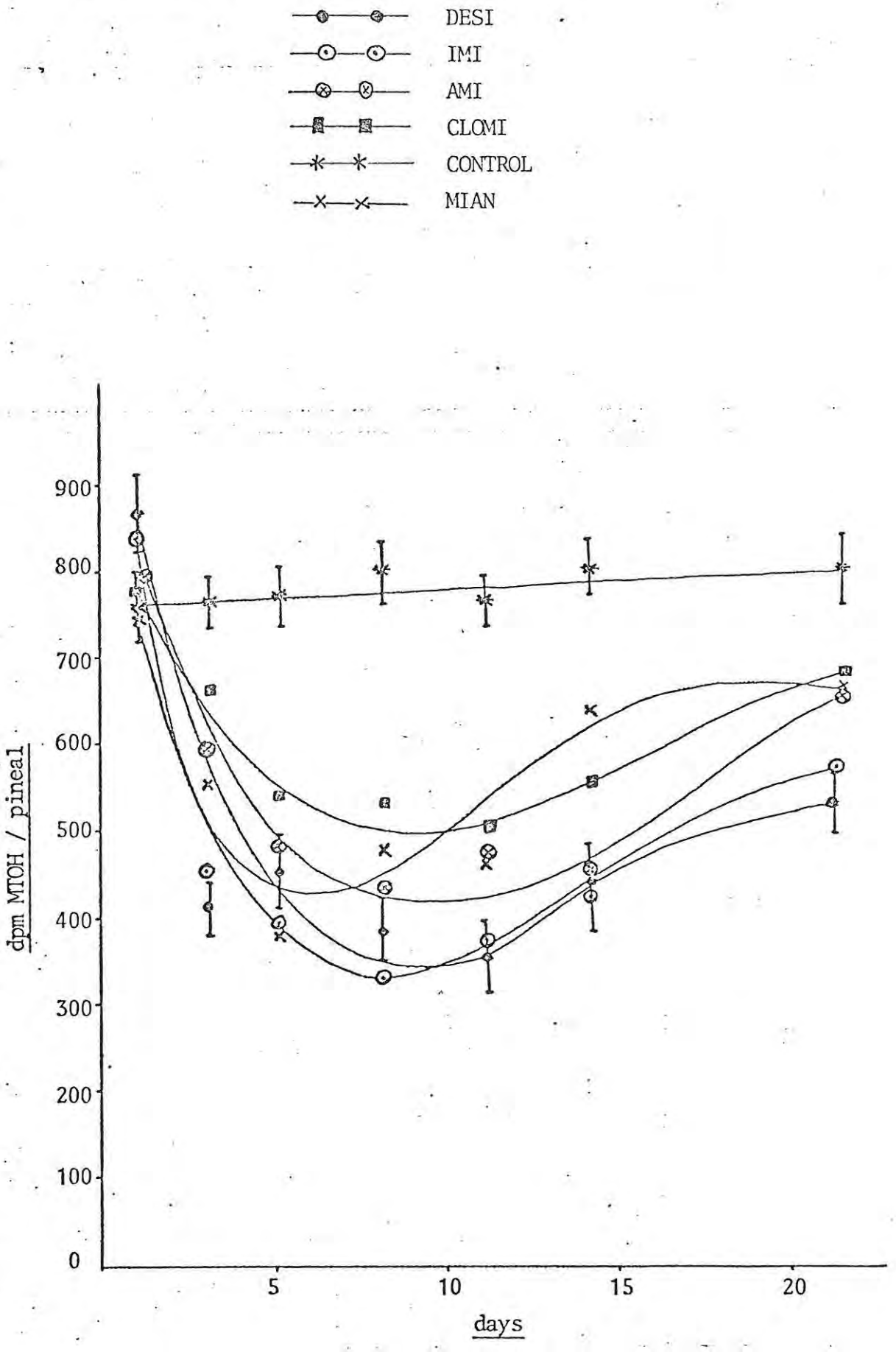
TABLE 12

	TREATMENT					
DAY	DESI	AMI	IMI	CLOMI	MIAN	CONTROL
1	860±98	831±84	788±80	770±85	735±90	750±80
3	410±62	450±42	590±48	655±62	550±58	760±68
5	450±48	391±40	477±51	534±61	376±42	765±71
8	380±42	330±38	430±46	530±54	472±45	797±73
11	351±39	370±39	469±48	500±47	460±53	760±61
14	438±46	420±48	450±31	551±50	635±59	798±69
21	529±38	562±43	649±56	680±59	657±64	799±78

Effect of TADs on MTOH synthesis

Results are expressed as dpm MTOH/ pineal ± SEM

FIGURE 20



EFFECT OF TAD's ON METHOXYTRYPTOPHOL PRODUCTION

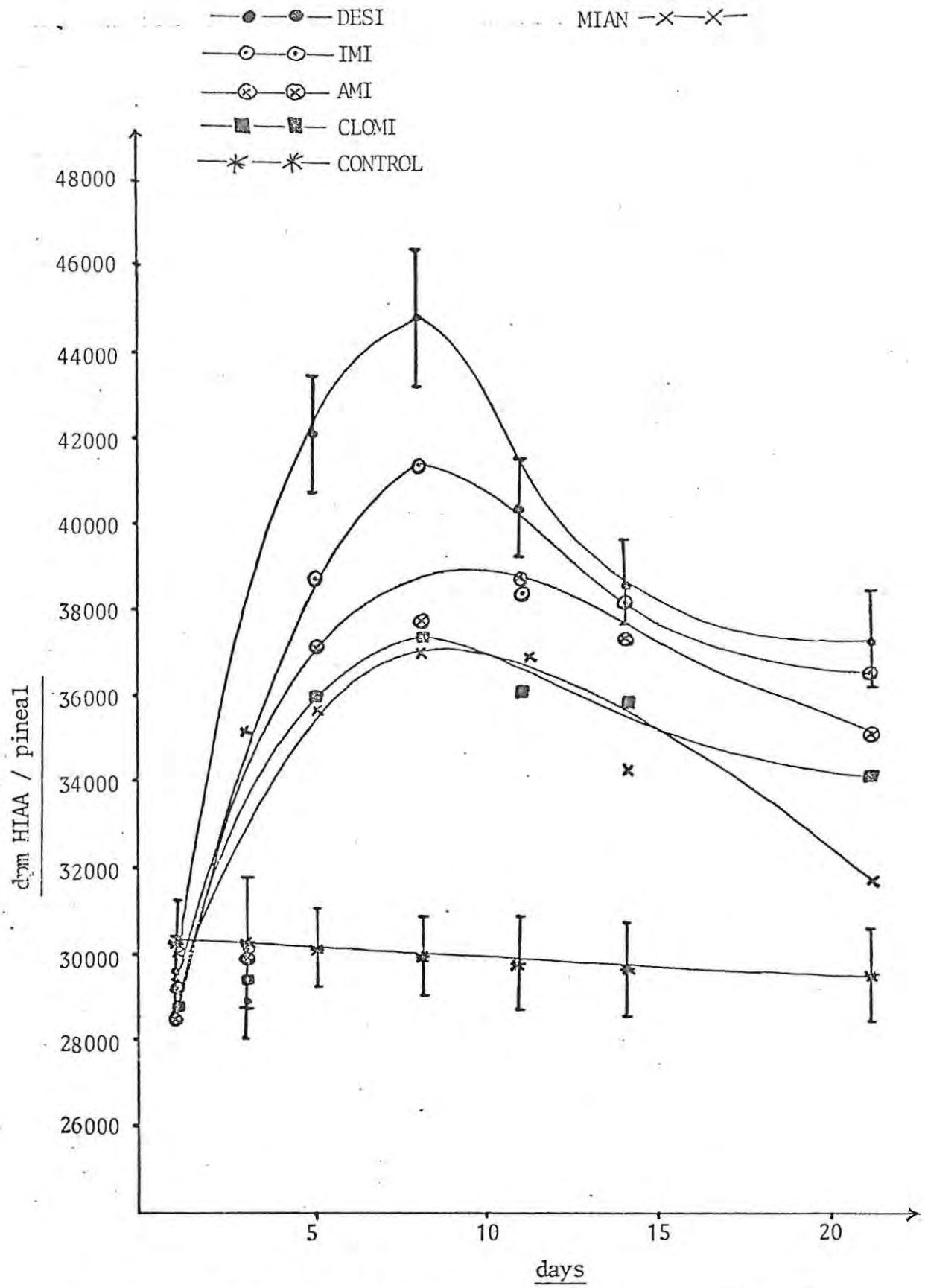
Table 13

DAY	TREATMENT					
	DESI	AMI	IMI	CLOMI	MIAN	CONTROL
1	29600±1836	28500±2000	29200±1763	28800±1621	29700±1780	33000±1800
3	28900±1715	29900±1973	30100±1987	29400±1730	35500±1987	30100±2013
5	41920±2971	37000±2001	38600±2115	36850±2307	35700±2010	30100±1998
8	44600±3013	37600±2134	41200±2998	37000±2518	36900±2136	29900±1976
11	40200±2010	38600±2710	38200±2440	36000±2016	36750±2096	29700±2000
14	38400±2036	37180±1874	38000±1876	35800±2111	34200±1995	29600±2010
21	37040±2144	35000±1851	36400±1599	34000±2009	31600±1870	29400±2121

Effect of TADs on HIAA synthesis

Results are expressed as dpm HIAA/ pineal ± SEM

FIGURE 21



EFFECT OF TAD's ON THE UPTAKE OF HYDROXYINDOLEACETIC ACID

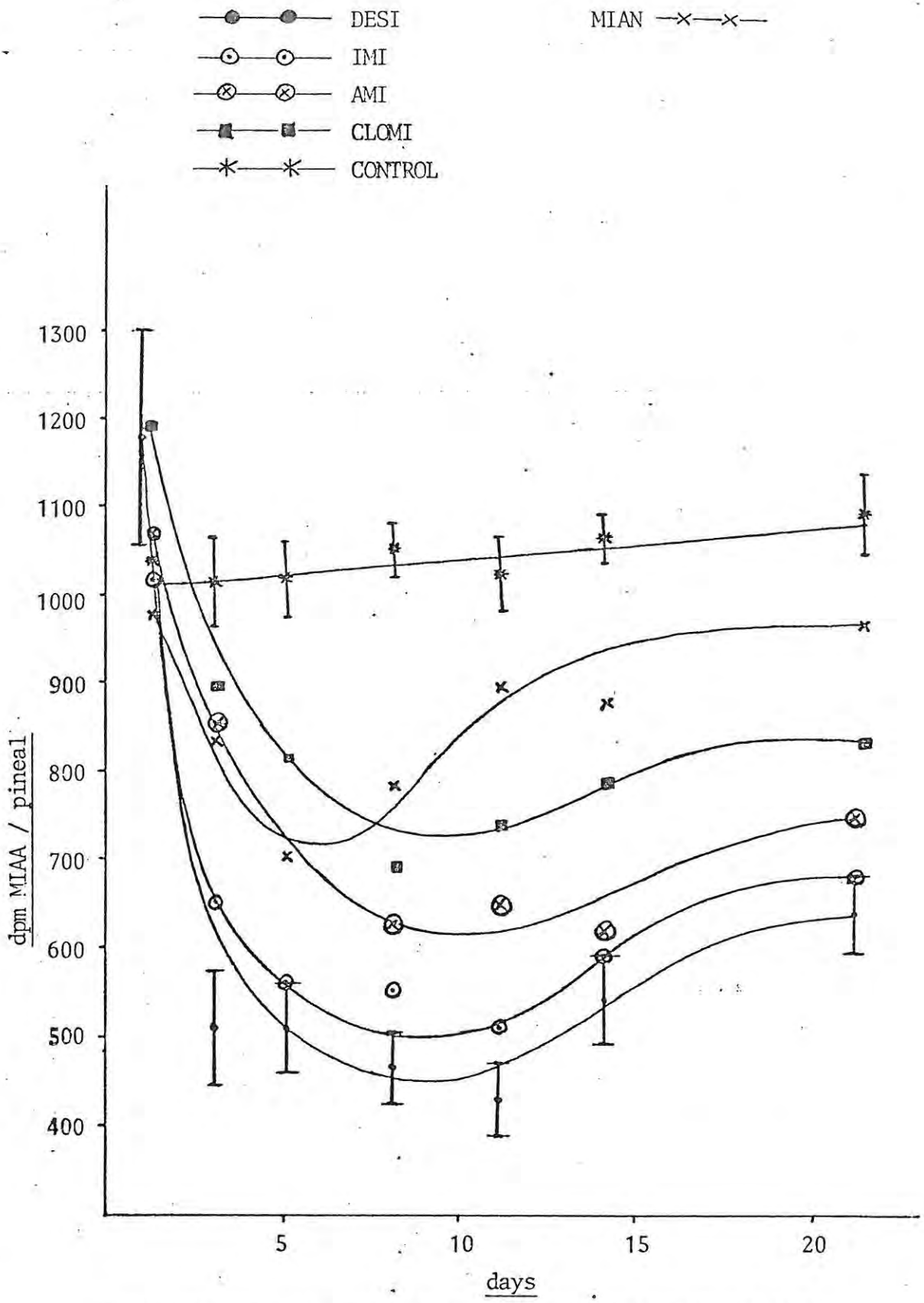
TABLE 14

	TREATMENT					
DAY	DESI	IMI	AMI	CLOMI	MIAN	CONTROL
1	1170±102	1090±92	1060±89	1180±99	970± 91	1030±101
3	510± 64	650±59	850±80	890±82	829±78	1005±97
5	510± 50	559±60	748±81	810±75	700±62	1010±63
8	465± 39	550±49	625±53	690±63	778±68	1040±85
11	430± 40	502±43	648±59	735±81	890±71	1015±90
14	540± 51	590±51	619±47	784±59	870±75	1050±52
21	638± 46	680±64	746±74	830±68	958±78	1086±98

Effect of TADs on MIAA synthesis

Results are expressed as dpm MIAA/ pineal ± SEM

FIGURE 22



EFFECT OF TAD's ON THE UPTAKE OF METHOXYINDOLEACETIC ACID

In 1971 Jackson and Lovenberg (867) reported that HIOMT exists as two differently charged molecular species. On examining the rat pineal HIOMT activity, Pevet et al (868) found that the synthesis of MEL is high at the end of the dark period and middle of the light period while HIOMT activity concerned with production of 5-MTOH is only observed during the light period. A comparison of peaks suggested that the pineal produces more MTOH than MEL. It was then suggested that different HIOMT enzymes are involved in the synthesis of MTOH and MEL. This proposal was confirmed by Balemans et al (869). If isomeric forms of HIOMT are present in the pineal, it is possible that one type metabolises N-acetyl serotonin while the other type metabolises HIAA and HTOH. It is also possible that TADs have different effects on different types of HIOMT. Increased MIAA and MTOH levels and reduced MEL levels could result from such effects.

If only one form of HIOMT is present in the pineal, it is possible that N-acetylserotonin has a greater affinity than HIAA and HTOH for the enzyme. In this situation, an increased production of MEL would be associated with an increased occupation of receptor sites by N-acetyl serotonin. Therefore, fewer molecules of HIAA and HTOH would be metabolised.

It is clear that many more studies involving enzyme activity and pineal function are required before the effects of TADs on serotonin metabolism in the pineal can be properly explained.

3.33 POSSIBLE IMPLICATIONS OF THESE OBSERVATIONS

MEL is the only metabolite in this study that has been intensively studied. Various drugs have been reported to affect MEL synthesis - (refer to section 1.232). Among the drugs that increase MEL production are the MAO inhibitors Catron (R) (430), harmine (645 - 646) and pargyline (647 - 649); the TADs DESI (649 - 651), IMI (649) and CLOMI (649) and the tetracyclic antidepressant, maprotiline (649). Thus, it appears that various antidepressant drugs influence MEL synthesis.

An interesting observation made in these experiments was the time required before MEL production could reach peak levels. This period corresponds with the lag period before the therapeutic effects of TADs become evident. All the TADs as well as mianserin appear to produce peak levels of MEL after approximately the same period in these studies. If these results can be extrapolated to the clinical situation, it is possible that little difference would be expected in the therapeutic efficacy of these drugs. The order in which the TADs appear to increase MEL production appears to be similar to the order of inhibition of N/A reuptake. This is interesting since maprotiline, which acts predominantly on the N/A system, is an effective antidepressant (20 - 22) while fluoxetine which is reported to be devoid of effect on N/A reuptake (307) but is a potent 5-HT reuptake inhibitor, has not been useful clinically.

The observations made in this experiment, together with observations made in previous investigations suggest the possibility that changes in MEL secretion could be implicated in the actions of TADs.

The possibility that HIAA, HTOH, MIAA and MTOH also have an influence on the actions of TADs should not be dismissed. Very little is known about the physiological actions of these compounds. There is a possibility that one or more of these compounds might also be implicated in the therapeutic actions of TADs.

Similar investigations using varied doses of the drugs used in this study, as well as other antidepressants such as lithium; the newer antidepressants such as trazodone and nomifensine, β_2 stimulants like salbutamol and also ECT should provide useful information. Studies of the effects of HIAA, HTOH, MTOH and MIAA on behaviour may also prove to be clinically useful.

CHAPTER 4

THE EFFECT OF TRICYLCLIC ANTIDEPRESSANTS ON THE ACTIVITY OF SNAT AND HIOMT IN THE RAT PINEAL GLAND

The need to determine the effects of TADs on SNAT activity and HIOMT activity individually is emphasized in Chapter 3. The drugs chosen for these studies were DESI (which appeared to be the most potent stimulant of MEL synthesis) and CLOMI (which appeared to be least active).

4.1 EFFECT OF TADs ON SNAT ACTIVITY

4.11 INTRODUCTION

The basis for this assay is the acetylation of tryptamine by NAT in pineal homogenates, using radiolabelled acetyl-coenzyme A. The resulting product is ^{14}C-acetyltryptamine. This product can be extracted from the aqueous incubation medium using an organic solvent. The radioactivity in the organic solvent is then counted using liquid scintillometry.

The amount of radioactive acetyltryptamine formed is taken to be an indication of SNAT activity. Deguchi and Axelrod (523) found that this radioactive product has the same Rf values as authentic acetyltryptamine, when analysed by TLC. Radioactive acetyltryptamine is used (by adding tryptamine as substrate) instead of radioactive acetyl-hydroxytryptamine because the former compound is more liposoluble and can be easily extracted in an organic solvent.

4.12 MATERIALS AND METHODS

The assay technique used is a slight modification of the method developed by Deguchi and Axelrod (523).

4.121 SOURCE OF TISSUE

Pineals were obtained from Albino rats of the Wistar strain. The mass of the test animals varied between 150 g and 180 g. They were housed in a room with controlled lighting (12 hour dark/12 hour light). The light intensity during the light phase was 300 watts/cm^2. The temperature of the room was approximately 20°c.

The drugs were administered intraperitoneally in a dose of 50 mg/kg. Normal saline was administered to control animals.

The animals were sacrificed by neck fracture, as described in section 3.23.

4.122 ASSAY TECHNIQUE

One pineal was homogenised in a glass homogenizer with 100 ml cold 0,05 m phosphate buffer (pH 6,5) and tryptamine hydrochloride (5×10^{-3} M). 5×10^{-4} M acetyl-l-^{14}C-coenzyme A (Amersham-specific activity 7,4 m Ci/mMol) was added and the tube was incubated for 20 minutes at 37°c. Blanks in which tissue was omitted were also incubated.

The reaction was stopped by removing the tube from the incubator and adding 10 ml 0,5 M borate buffer (pH 10).

The reaction mixture was then transferred into a glass-stoppered test tube containing 2,5 ml toluene/isoamyl alcohol (97 : 3), using a Pasteur pipette. It was mixed in a vortex mixer for 30 seconds and centrifuged at 300 rpm for 10 minutes. 2 ml of the organic phase was then transferred to a glass scintillation vial containing 6 ml Instagel (R) (Packard Instruments). The radioactivity in each vial was measured, using a Beckman LS3150T liquid scintillation counter. The vials were counted for 20 minutes. Quench curves were used to convert cpm to dpm. Readings were corrected for the blank.

4.13 RESULTS

Results are presented in Table 15

4.14 DISCUSSION

A progressive increase in SNAT activity was observed in the first eleven days and thereafter a diminution in activity was observed. The initial increase in activity was probably due to increased noradrenergic stimulation and the subsequent decrease was probably due to development of subsensitivity, as explained in section 4.3.

These results confirm a previous report (430) that an increase in SNAT activity is observed after TAD administration. It appears that the increase in N-acetyl serotonin synthesis and MEL synthesis in section 4.3 could be due to stimulation of SNAT by TADs.

TABLE 15

TIME (days)	dpm N-acetyltryptamine/pineal + SEM	
	DESI	CLOMI
1	453 ± 21	420 ± 35
3	561 ± 34	503 ± 28
5	684 ± 51	599 ± 34
8	729 ± 60	698 ± 41
11	863 ± 52	787 ± 50
14	800 ± 28	721 ± 39
21	708 ± 41	684 ± 52

Effect of TADs on SNAT activity

4.2 EFFECT OF TRICYCLIC ADNTIDEPRESSANTS ON THE ACTIVITY OF HIOMT IN THE RAT PINEAL GLAND

4.21 INTRODUCTION

In 1961 Axelrod and Weissback (486) developed a technique to assay HIOMT. It was based on the transfer of a radioactive methyl group from 5-adenosyl-l (methyl-^{14}C) methionine (the methyldonor) to N-acetylserotonin (the substrate). This reaction resulted in the formation of radioactive MEL, which was extracted from the reaction mixture and counted by liquid scintillometry. The measure of the reactivity was indicative of HIOMT activity. Subsequently, other hydroxyindoles (487) were added as substrates and information on the methylation of these substrates was obtained.

The above studies provided information about the methylation of N-acetyl serotonin (486) or other 5-hydroxyindoles (487), depending on the particular 5-hydroxyindole added as substrate. The possibility of the existence of several more or less specific HIOMT enzymes (867) was not considered.

Balemans et al (869) later developed a technique in which the 5-hydroxyindoles present in the pineal were used as a substrate and no N-acetylserotonin or any other 5-hydroxyindole was added to the incubation medium. The methylating capacity of HIOMT in the synthesis of MEL, MTOH, MIAA, 5-methoxytryptamine and 5-methoxytryptophan can be determined in the same experiment. These metabolites are separated by TLC and counted by liquid scintillometry.

4.22 MATERIALS AND METHODS

The source of material was the same as in section 4.121. The method developed by Balemans et al (869) was used.

One rat pineal was excised and slightly disrupted in a homogeniser tube containing 30 ml 0,1 M phosphate buffer (pH8.0). 10 ml 5-adenosyl methionine-^{3}H (Amersham-International) was added and the tube was incubated at 37°c for 60 minutes.

The reaction was stopped by the addition of 10 ml H_2SO_4 (pH 1,0). A borate buffer (pH 10.0) could not be used for this purpose because Balemans et al (869) found that 5-adenosyl methionine disintegrates rapidly in alkaline medium and causes complications if TLC is applied.

The incubated pineal was then homogenized in the incubation medium.

Two TLC plates were used to separate the methoxyindoles. Glass plates (20 cm square) were used. These were coated with kieselgel G60 (merck) to a thickness of 0,2 mm. The standard solution of reference substances contained 1 mg/ 20 ml of the following compounds: methoxytryptophol, methoxyindole acetic acid, methoxytryptamine, methoxytryptophan and melatonin.

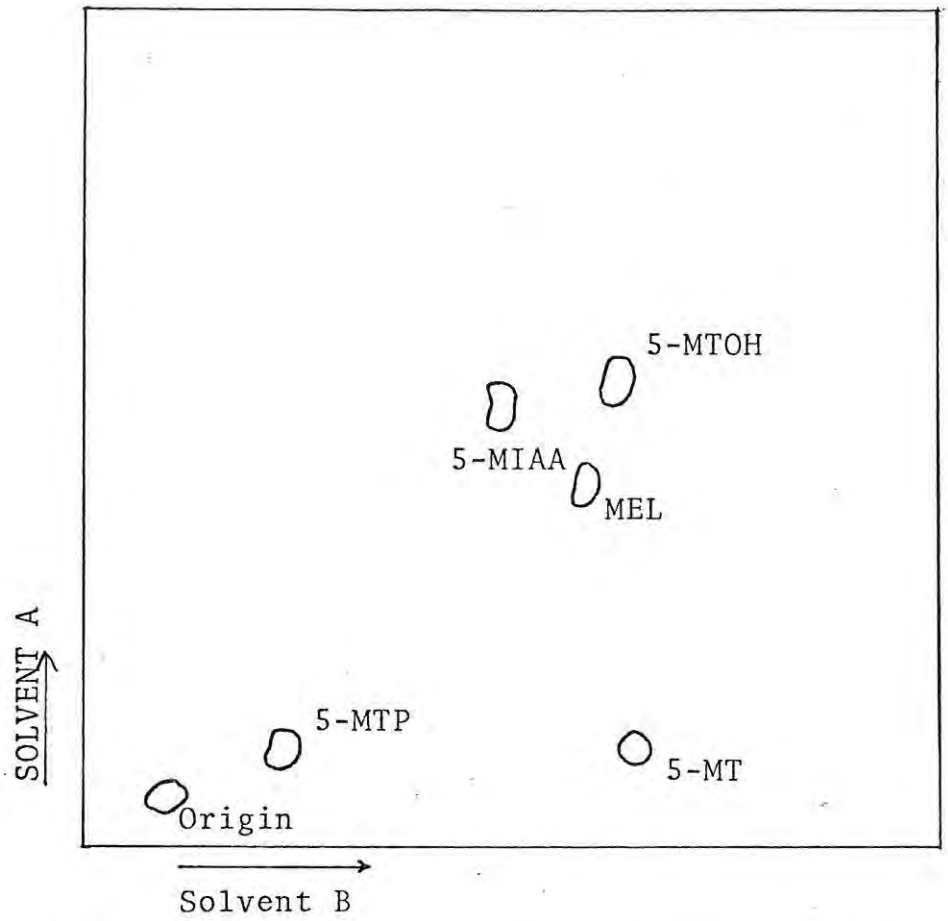
15 µl of incubation medium, together with 20 µl of the solution of standards was spotted onto the first plate. N_2 gas was used to dry the spots, to prevent decomposition.

A two-dimensional TLC technique was applied. As solvent system for the 1st direction, chloroform : methanol : acetic acid (93 : 4 : 3) was used. The plates were dried under N_2 and developed in the second direction (i.e. after turning the plate 90°) in chloroform; methanol: 25% ammonia (60:35:5). The plate was then dried under N_2 and sprayed with Ehrlichs reagent. The plates were then placed in an oven at 60°c for 20 minutes to allow development of the spots. The position of the spots on this plate is illustrated in Figure 23.

Although all the metabolites had separated on the first plate, 5-methoxytryptophan, 5-methoxytryptamine and 5-methoxyindole-acetic acid did not give reproducible results. Balemans et al (869) have suggested that this could be due to the influence of the solvent systems on the methoxyindoles. The radioactivity in the MEL and MTOH spots was measured as described in section 3.25.

15 µl of incubation medium with 20 µl of the solution of standards was spotted on a second plate. The chromatogram was developed in darkness in chloroform : methanol:25% ammonia (60 : 35 : 5). It was then dried under N_2, sprayed with Ehrlich's reagent and developed as before. The MEL and and MTOH spots were not clearly defined on this plate. The radioactivity in the spots containing 5-methoxytryptamine (5-MT), 5-MIAA and 5-methoxytryptophan (5-MTP) was determined as before. Refer to Figure 24.

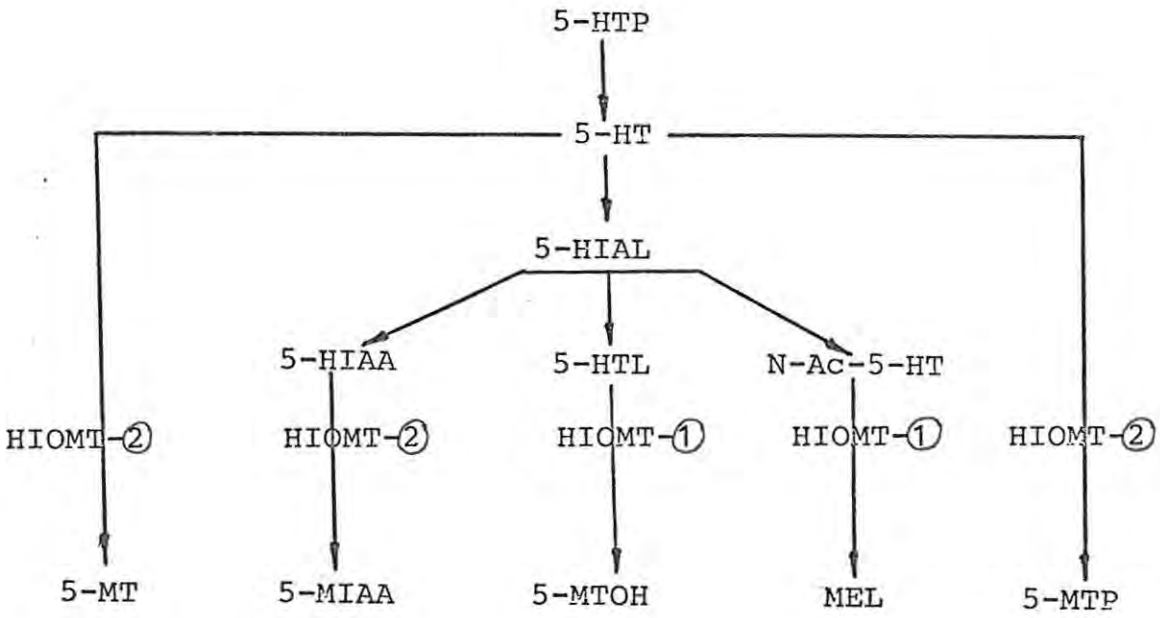
FIGURE 23



Tracing of chromatographic separation of pineal indole compound

Solvent A :	chloroform	:	methanol	:	acetic acid
	93	:	6	:	1
Solvent B :	chloroform	:	methanol	:	ammonia
	60	:	35	:	5

FIGURE 24



SCHEME OF 5-HYDROXYINDOLE METABOLISM IN RAT PINEAL

② - indicates that HIOMT activity was assessed from the second TLC plate.

① - indicates that HIOMT activity was assessed from the first TLC plate.

5-HTP = 5-hydroxytryptophan

5-HTP = serotonin

5-HIAL = 5-hydroxyindoleacetaldehyde

5-HIAA = 5-hydroxyindole acetic acid

5-HTL = 5-hydroxytryptophol

N-Ac-5-HT = N-acetylserotonin

5-MT = 5-mthhoxytryptamine

5-MIAA = 5-methoxyindole acetic acid

5-MTOH = 5-methoxytryptophol

5-MTP = 5-methoxytryptophan

MEL = melatonin

4.23 RESULTS

Results are presented in Tables 16 - 20.

4.24 DISCUSSION

The synthesis of 5-MT, 5-MIAA, 5-MTOH and 5-MTP appeared to be decreased initially and after a period of about 8 - 11 days, a slight decrease in sythesis was observed. The synthesis of MEL increased and thereafter a slight decline in synthesis was observed. Thus, the results obtained in these experiments in which the endogenous substrate was used, correlate with those in which a substrate (serotonin) was added (Chapter 3).

The results in these experiments seem to suggest that the major metabolic pathway of serotonin is its conversion to 5-MT. HIOMT is the only enzyme required for this conversion. The proportion of 5-MTP synthesized is also large when compared with 5-MIAA, 5-MTOH and MEL. Here again, HIOMT is the only enzyme required for the conversion of 5-hydroxytryptophan to 5-MTP. This implies that HIOMT activity in those conversions is very high. It therefore seems unlikely that an increase in the N-acetylserotonin content (due to SNAT stimulation) would have any marked effect on the synthesis of 5-MT and 5-MTP. Yet, a definite change in the synthesis of these compounds were observed. It is therefore possible that the HIOMT involved in the synthesis of MEL is different from the HIOMT that metabolizes 5-HT and 5-MTP. Another factor that supports this proposal is the observation that MEL production was

TABLE 16

TIME (days)	dpm 5-MT / pineal ± SEM	
	DESI	CLOMI
1	16053 ± 103	16154 ± 218
3	15873 ± 315	15857 ± 465
5	13731 ± 259	14321 ± 139
8	10931 ± 314	13852 ± 210
11	12561 ± 421	13979 ± 148
14	14325 ± 401	14471 ± 269
21	14879 ± 276	15034 ± 283

Effect of TADs on HIOMT activity, in the conversion of 5-HT to 5-MT

Note: Both CLOMI and DESI appear to inhibit HIOMT activity (slightly) and maximum inhibition appears to occur after 8 days of treatment. CLOMI and DESI appear to be equally effective in inhibiting HIOMT.

TABLE 17

TIME (days)	dpm 5-MIAA / pineal ± SEM	
	CLOMI	DESI
1	526 ± 18	603 ± 45
3	500 ± 20	571 ± 38
5	429 ± 34	414 ± 29
8	321 ± 26	330 ± 12
11	398 ± 15	358 ± 20
14	423 ± 31	395 ± 34
21	451 ± 40	409 ± 53

Effect of TADs on HIOMT activity in the conversion of 5-HIAA to 5-MIAA

Note: CLOMI and DESI appear to inhibit HIOMT activity. Maximum inhibition appeared to occur after 8 days of treatment. The drugs seem to be equally effective.

TABLE 18

TIME (days)	dpm MTP / pineal ± SEM	
	DESI	CLOMI
1	14320 ± 539	13760 ± 749
3	13415 ± 441	13364 ± 538
5	12006 ± 431	12874 ± 639
8	11777 ± 379	12009 ± 417
11	11999 ± 876	11635 ± 421
14	12138 ± 734	12041 ± 389
21	12398 ± 631	12721 ± 518

Effect of TADs on HIOMT activity in the conversion of 5-HTP to 5-MTP

* Note: There seems to be a slight decrease in HIOMT activity after treatment with DESI and CLOMI. Both drugs appear to be equally effective.

TABLE 19

TIME (days)	dpm MTOH / pineal ± SEM	
	CLOMI	DESI
1	498 ± 53	528 ± 43
3	434 ± 41	463 ± 50
5	379 ± 23	361 ± 38
8	301 ± 25	295 ± 22
11	365 ± 18	342 ± 31
14	379 ± 28	368 ± 40
21	402 ± 35	418 ± 41

Effect of TADs on HIOMT activity in the conversion of 5-HTOH to 5-MTOH

Note: There appears to be a slight decrease in HIOMT activity. Maximum inhibition appears to occur after 8 days of treatment.

TABLE 20

TIME (days)	dpm Mel / pineal ± SEM	
	DESI	CLOMI
1	763 ± 53	698 ± 61
3	791 ± 61	741 ± 52
5	842 ± 74	822 ± 73
8	1013 ± 81	987 ± 45
11	1164 ± 53	1004 ± 39
14	931 ± 59	941 ± 53
21	906 ± 81	911 ± 68

Effect of TADs on HIOMT activity in the conversion of N-acetyl serotonin to Mel

Note: Unlike the results presented in tables 16-19 these results suggest that DESI and CLOMI stimulate HIOMT activity. Maximum HIOMT activity is apparent after 11 days of treatment.

highest after about 11 days while 5-MTP and 5-MT reached their lowest levels by the 8th day. If the same enzyme was involved, the lag period before extreme levels were reached should correspond.

These studies seem to suggest that one type of HIOMT is concerned with the synthesis of MEL while another is involved in the synthesis of 5-MT, 5-MTP, 5-MTOH and 5-MIAA. TAD's seem to have a different effect on both these enzymes.

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