THE EFFECTS OF ETHANOL ON CATECHOLAMINE AND SEROTONIN METABOLISM IN MAN

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ABSTRACT

Chemical and gas chromatographic methods for the estimation of catecholamine and serotonin metabolites in normal urine have been investigated with the aim of applying them to the study of the effects of ethanol on biogenic amine metabolism. It was concluded that both methods would be incapable of accurately demonstrating any changes in urinary metabolite levels that were expected to occur as a consequence of ethanol ingestion.

A GCMS technique for quantitating five acidic catecholamine and serotonin metabolites was developed, and was found to exhibit excellent specificity and sensitivity. When applied to the analysis of alcoholic metabolites, the technique was subject to interference from extraneous compounds, and further development is required.

The GCMS technique was applied to the analysis of catecholamine and serotonin metabolites in the urine of normal male adults who had ingested ethanol. It was concluded that ethanol induces a shift in metabolism away from oxidative toward reductive pathways for adrenaline, noradrenaline and serotonin, but not for dopamine. An increased HVA excretion observed after ethanol ingestion was shown to be possibly due to the diuretic effect of ethanol.

This work provides a clarification of the diverse results previously reported in the literature, but it was, however, concluded that there are still several aspects of this field of alcohol research that require estensive investigation before a complete understanding of the ways in which ethanol influences catecholamine and serotonin metabolism can be achieved.

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ABBREVIATIONS

GENERAL

GC, GLC	gas chromatography, gas-liquid chromatography
MS	mass spectrometry
GCMS	gas chromatography-mass spectrometry
FID	flame ionisation detection
ECD	electron capture detection
MID	multiple ion detection
SIM	selected ion monitoring
HPLC	high performance liquid chromatography
REA	radioenzymatic assay

CHEMICALS, METABOLITES, ENZYMES

CA	catecholamine, catecholamine metabolites
IA	indoleamine, indoleamine metabolites
PG	propyl gallate
Res	rescorcinol
HGA	homogentisic acid
TMS	trimethylsilyl

All other metabolite and enzyme abbreviations are either explained in the text or given in the key to Figs. 1(b) and 1(c).

STATISTICAL

$\bar{x}$	mean
s.d.	standard deviation
s.e.	standard error
a	ordinate (y axis) intercept
b	regression coefficient
s_b	standard deviation of the regression coefficient (b)
n	sample number

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GENERAL INTRODUCTION

1.1 The Aim of This Study

The primary aim of this study is to evaluate the various methods used for the determination of urinary catecholamine and indoleamine (serotonin) metabolites, and to develop techniques for the analysis of samples obtained from human volunteers after consuming alcohol. The present investigation is part of a research programme that has been initiated to provide a greater understanding of the ways in which ethanol elicits changes in metabolic pathways.

1.2 The Physiological and Behavioural Roles of the Catecholamines and Serotonin

The distribution and the diverse behavioural and physiological roles that these compounds play, both as neurotransmitters and as neuroendocrine effectors, are outlined in Table 1(i). Because of the important part that the catecholamines and serotonin seem to play in the CNS, several speculations have arisen as to their role in modifying behaviour. It was, therefore, not surprising that in view of the effects of ethanol on behaviour, studies on the catecholamines and serotonin would be undertaken, hence this field of alcohol research currently represents one of the most intensive areas investigated.

1.3 The Mechanism of Neurotransmission by Catecholamines and Serotonin

1.3.1 Storage (Molinoff and Axelrod (1971), Seiden and Dykstra (1977))

Catecholamines in sympathetic nerves or in the adrenal medulla are stored in membrane-bound chrommaffin granules. This serves to inactivate the amines temporarily and to protect them from enzymatic destruction until they are released by an appropriate stimulus. The granules contain predominantly noradrenaline, but can also take up adrenaline, dopamine and serotonin. In serotonergic nerve cells serotonin is believed to be bound to similar dense-core granules. Evidence suggests that the granules are synthesized in the cell body, and migrate along the axon to the synaptic ending.

Table 1(i) Distribution and Physiological and Behavioural Roles of the Catecholamines and Serotonin

Compound	Distribution	Physiological and Behavioural Roles
Dopamine	Found in both the central (striatum, limbic structures, terminals ending in the functional neocortex) and peripheral nervous systems. Is a precursor of both NA and A.	Involved in prolactin secretion, is deficient in Parkinson's disease and is suggested to be involved in the pathogenesis of schizophrenia.
Dopamine and Noradrenaline		Involved in motor behaviour, aggression, food intake and schedule controlled behaviour.
Noradrenaline	Found in the adrenal medulla and in chrommaffin cells scattered throughout the body. NA is highly localized in peripheral postganglionic sympathetic nerves and is found in several areas of the CNS.	NA release from neuroeffector sites increases blood pressure, heart rate and stroke volume. Dilates the blood vessels supplying skeletal muscle, constricts those supplying the gut and skin with an overall increase in peripheral resistance. Get decreased GI tract motility and mydriasis (enlargement of pupils). Increases secretion from salivary and sweat glands and causes A and NA secretion from the adrenal medulla.
Noradrenaline and Adrenaline		Inhibit insulin secretion, increase mobilization of free fatty acids, stimulate the metabolic rate. NA and A both produce increased alertness, in humans A usually evokes more excitement and fear. Increased CA secretion is an important endocrine response to cold.
Adrenaline	Functions mainly as a hormone, being released into the circulation primarily from the adrenal medulla. Small amounts have also been found in mammalian brain and heart. Is found in chrommaffin cells.	Decreases peripheral resistance. Causes glycogenolysis by activating phosphorylase enzyme.
Serotonin	Centrally, is found mostly in a small part of the pons called the raphé system. Also found in many regions of the brain, and in cerebellar and spinal projections. Peripherally, 5-HT is found in blood platelets and in the GI tract (in enterochrommaffin cells and the mysenteric plexus).	Is a powerful smooth muscle stimulant, and a vasoconstrictor. 5-HIAA, the major serotonin metabolite is excreted in excessive quantities in metastatic carcinoid syndrome. Centrally, is purported to be involved in temperature regulation, sleep, seizure disorders, extrapyramidal function, mental deficiency, aggression and hypersexuality, affective disorders, pain perception and narcotic analgesics, and psychotic behaviour.

Ref: Seiden and Dykstra (1977), Molinoff and Axelrod (1971), Ganong (1975).

1.3.2 Release (Kelly et al. (1979))

It now appears likely that the catecholamines and serotonin are released directly from the granules by exocytosis. This release occurs as an all-or-none phenomenon with respect to any granule and all of the soluble contents are released. Calcium has been shown to have a role in release from vesicles in the adrenal medulla and in peripheral nerve terminals.

1.3.3 Synaptic Transmission (Seiden and Dykstra (1977))

Synaptic transmission by the neurotransmitters dopamine, noradrenaline and serotonin is represented diagramatically in Fig. 1(a). Neurotransmitters released by the nerve endings following presynaptic stimulation diffuse across the synaptic gap and interact with receptor sites on the post-synaptic membranes of nearby neurons. As a result of these interactions, a post-synaptic potential is developed.

1.3.4 Reuptake and Inactivation (Molinoff and Axelrod (1971), Seiden and Dykstra (1977))

Reuptake is the major mechanism for inactivation, particularly in tissues with rich adrenergic innervation. In sparsely innervated tissues, catechol-O-methyl transferase (COMT) has been shown to be important in terminating the effects of adrenaline. Catecholamines discharged into the blood stream either from the adrenal gland or by overflow from neuronal release are primarily inactivated by liver and kidney COMT and monoamine oxidase (MAO), or by reuptake by sympathetically innervated organs. Reuptake and oxidation by MAO are also thought to be involved in serotonin inactivation.

1.4 Effects of Ethanol on Catecholamine and Serotonin Turnover and Secretion

1.4.1 Animal Studies

Corrodi et al. (1966) using α-methyl-p-tyrosine to inhibit catecholamine biosynthesis, found that ethanol accelerates depletion of the noradrenaline content of rat brain, but not that of dopamine. These workers suggested that ethanol acts as a specific activator of noradrenergic neurons. In a further study, Carlsson et al. (1973) examined the synthesis of $[^3H]$-catecholamines from $[^3H]$-tyrosine in brain. They found an increase in $[^3H]$-dopamine and $[^3H]$-noradrenaline, and that

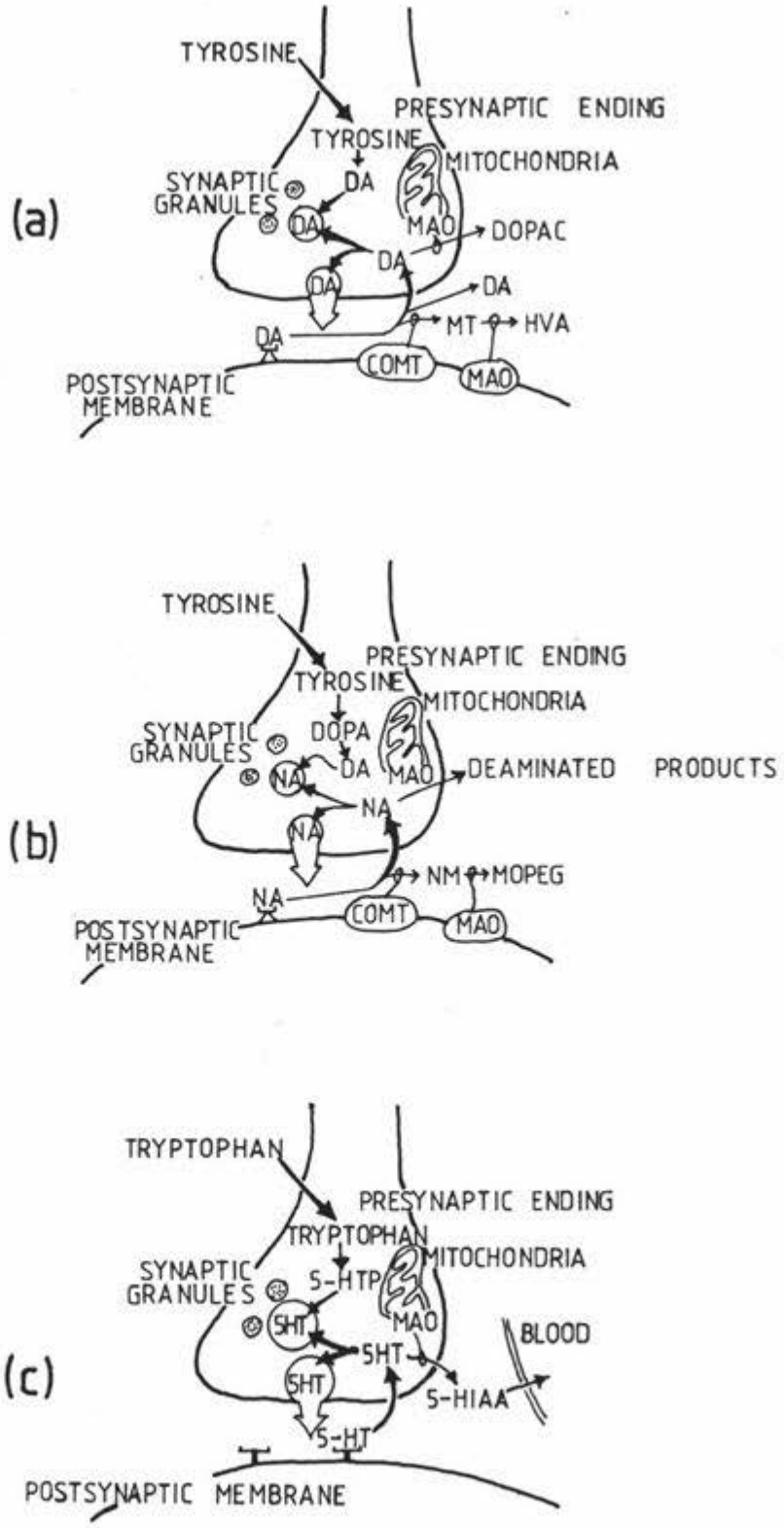


Fig. 1(a) Schematic models of the neuron; (a) dopamine, (b) noradrenaline and (c) serotonin, illustrating synthesis, storage in granules, release, and inactivation through reuptake and enzymatic degradation (from Seiden and Dykstra (1977)).

the ratio of [^{3}H]-dopamine/[^{3}H]-noradrenaline was significantly increased by ethanol. Hunt and Majchrowicz (1974a) found that in animals given a single dose of ethanol, noradrenaline turnover was increased, while dopamine turnover was unaffected during the first few hours of treatment. Afterwards, the turnover of both noradrenaline and dopamine was reduced, which they speculated may be in accordance with the known biphasic behavioural and physiological effects of ethanol on the CNS ie. initial stimulation and later depression.

Although the effects of ethanol on brain serotonin have received considerable attention, few consistent findings are available. Levels of brain serotonin following acute administration of ethanol have been measured by several investigators. Some have reported decreases (Gursey and Olsen (1960), Bonnycastle et al. (1962)), others have found increases (Reichle et al. (1971), Palaić et al. (1971)), and others have described unchanged levels (Efron and Gessa (1961), Tyce et al. (1968)). There is also conflicting data on the rate of biosynthesis following acute ethanol administration. Some show decreased rates, (Hunt and Majchrowicz (1974b)), others, increased rates (Palaić et al. (1971), while other investigators found no effects on brain serotonin turnover (Frankel et al. (1974)).

1.4.2 Studies on Normal Human Subjects

Kinzius (1950) reported transient elevations in blood noradrenaline levels 15-30 mins after ethanol ingestion. Abelin et al. (1958) noted that the urinary excretion of adrenaline increased 12-fold and noradrenaline 3-4 fold during the first hour after alcohol ingestion. Perman (1958), after giving normal subjects ethanol, noted that the rate of urinary catecholamine excretion increased relative to baseline values. He noted increased excretion of adrenaline, but not of noradrenaline. Anton (1965) reported that ethanol significantly increased urinary dopamine, noradrenaline and metanephrine, and slightly increased urinary adrenaline.

1.4.3 Studies on Alcoholic Subjects

Giacobini et al. (1960a) noted that hospitalized alcoholic subjects had significantly elevated excretions of adrenaline and noradrenaline during withdrawal. In a further study, Giacobini et al. (1960b) determined urinary catecholamine concentrations of sixteen male alcoholic patients in 24hr collections before , during and after ethanol

consumption. Despite markedly elevated blood ethanol levels, no change in the excretion of catecholamines was observed. The lack of adrenal medullary activation by ethanol in alcoholic subjects (in a convalescent phase), in contrast to the response seen in acute studies in normal non-alcoholic volunteers or experimental animals, may be a manifestation of the "tolerance" to alcohol seen in alcoholics (Gordon and Southren (1977)).

Carlsson and Haggendal (1967) noted increased arterial noradrenaline levels in 36 alcoholics studied at intervals from 6hr to 21 days after ethanol withdrawal. Withdrawal symptoms developed at about 12hr and elevated noradrenaline levels were noted between 13 and 24hr. The more striking withdrawal symptoms were associated with the highest levels of adrenaline.

Ogata et al. (1971) studied 4 alcoholic subjects who consumed ethanol on a "free-choice" programme (ad lib) and a programmed dose protocol. During free-choice ingestion, but not during programmed ethanol use, there was a significant increase in urinary adrenaline. There were no significant changes in noradrenaline excretion during the alcohol phase of the programmed study, although a small increase was observed. There were no significant changes in dopamine excretion. The authors noted that in the free-choice study, the progressive increase in adrenaline and noradrenaline values paralleled the upward movement of blood ethanol levels, and that during the post withdrawal period in a subject who experienced a symptomatically significant withdrawal syndrome, adrenaline and noradrenaline were maximally elevated. These studies suggest that enhanced urinary catecholamine excretion occurs throughout the course of long-term ethanol intake in alcoholics without any adaptational response (ie. sympathetic-adrenal medullary tolerance) (Gordon and Southren (1977)).

1.5 Biosynthesis and Metabolism of the Catecholamines and Serotonin

The biochemistry of catecholamines has been extensively reviewed by Sandler and Ruthven (1969), Weiner (1970), Molinoff and Axelrod (1971) and McIlwain and Bachelard (1971), and of serotonin by McIlwain and Bachelard (1971).

1.5.1 Biosynthesis

The pathway by which the catecholamines are derived from the dietary amino acids phenylalanine and tyrosine is shown in Fig. 1(b),

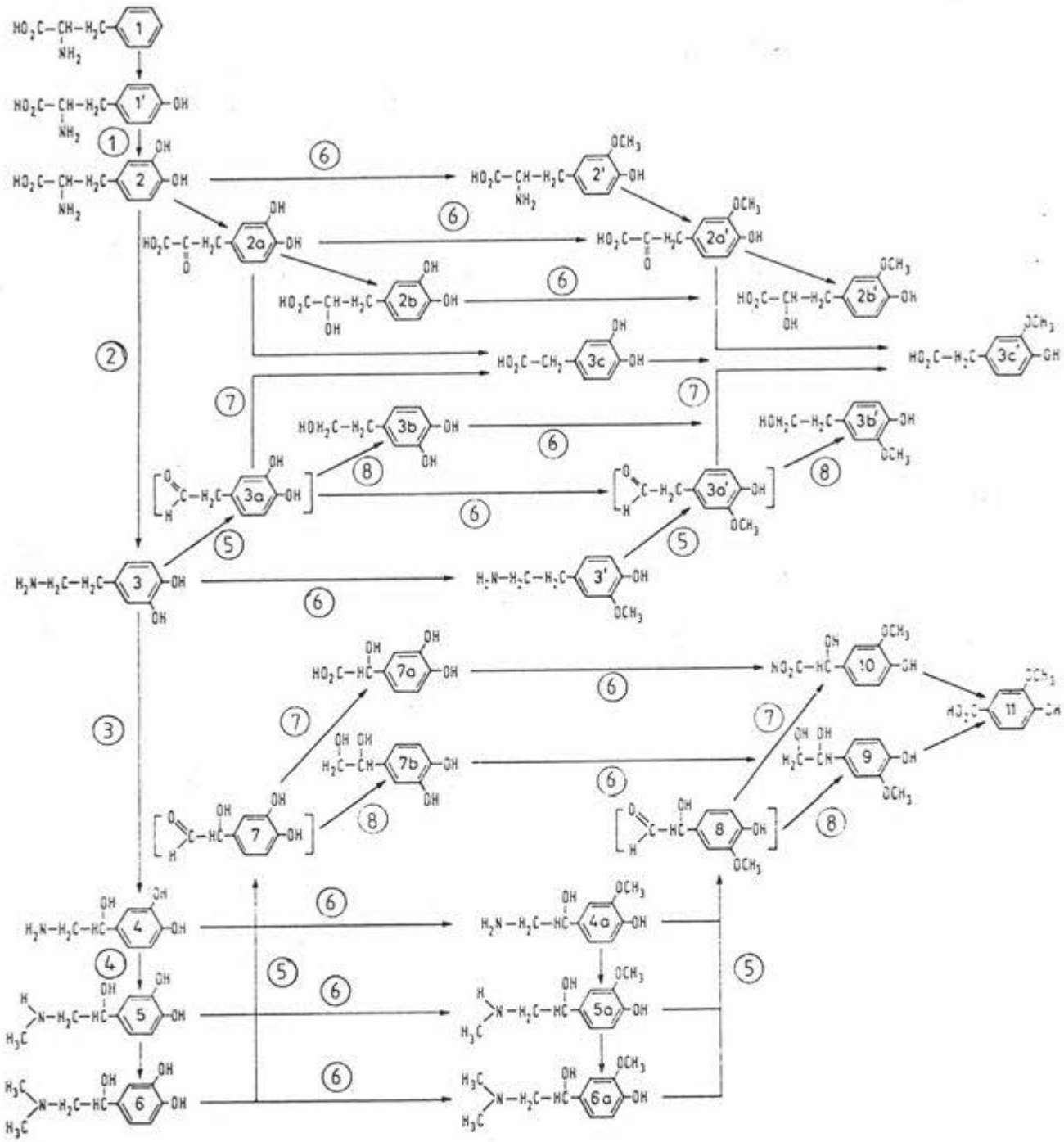


Fig. 1(b) The Biosynthesis and Metabolism of Catecholamines, (from Wisser and Knoll (1973)).

contd...

Fig. 1(b) Key

Index	Systematic Name	Trivial Name	Abbrev.
1	Phenylalanine	-	Phe
1'	4-Hydroxyphenylalanine	Tyrosine	Tyr
2	3,4-Dihydroxyphenylalanine	-	DOPA
2'	3-Methoxy-4-hydroxyphenylalanine	3-Methoxytyrosine	3-MTyr
2a	3,4-Dihydroxyphenylpyruvic Acid	-	DOPPA
2a'	3-Methoxy-4-hydroxyphenylpyruvic Acid	Vanilpyruvic Acid	VPA
2b	3,4-Dihydroxyphenyllactic Acid	-	DOPLA
2b'	3-Methoxy-4-hydroxyphenyllactic Acid	Vanillactic Acid	VLA
3	3,4-Dihydroxyphenylethylamine	Dopamine	DA
3'	3-Methoxy-4-hydroxyphenylethyl-amine	3-Methoxytyramine	3-MT
3a	3,4-Dihydroxyphenylacetaldehyde	-	-
3a'	3-Methoxy-4-hydroxyphenylacet-aldehyde	-	-
3b	3,4-Dihydroxyphenylethanol	-	DOPEt
3b'	3-Methoxy-4-hydroxyphenylethanol	Vanilethanol	MOPEt
3c	3,4-Dihydroxyphenylacetic Acid	Homoprocatechuic Acid	DOPAC
3c'	3-Methoxy-4-hydroxyphenylacetic Acid	Homovanillic Acid	HVA
4	1-(3,4-Dihydroxyphenyl)-2-amino-ethanol	Noradrenaline	NA
4a	1-(3-Methoxy-4-hydroxyphenyl)-2-aminoethanol	Normetanephrine	NM
5	1-(3,4-Dihydroxyphenyl)-2-methyl-aminoethanol	Adrenaline	A
5a	1-(3-Methoxy-4-hydroxyphenyl)-2-methylaminoethanol	Metanephrine	M
6	1-(3,4-Dihydroxyphenyl)-2-dimeth-ylaminoethanol	N-Methyladrenaline	N-MA
6a	1-(3-Methoxy-4-hydroxyphenyl)-2-dimethylaminoethanol	N-Methylmetanephrine	N-MM
7	3,4-Dihydroxyphenylglycolaldehyde	-	-
7a	3,4-Dihydroxymandelic Acid	-	DOMA
7b	3,4-Dihydroxyphenylethyleneglycol	-	DOPEG
8	3-Methoxy-4-hydroxyphenylglycol-aldehyde	-	-

contd...

Fig. 1(b) Key (contd.)

Index	Systematic Name	Trivial Name	Abbrev.
9	3-Methoxy-4-hydroxyphenylethylene-glycol	Vanilglycol	MOPEG
10	3-Methoxy-4-hydroxymandelic Acid	Vanilmandelic Acid	VMA
11	3-Methoxy-4-hydroxybenzoic Acid	Vanillic Acid	VA

Enzymes

1 Tyrosine Hydroxylase

2 DOPA Decarboxylase

3 Dopamine-β-Hydroxylase

4 Phenylethanolamine-N-Methyl Transferase (PNMT)

5 Monoamine Oxidase (MAO)

6 Catechol-O-Methyl Transferase (COMT)

7 Aldehyde Dehydrogenase

8 Aldehyde Reductase (Alcohol Dehydrogenase)

steps ①-④. Although this pathway represents the major route for catecholamine biosynthesis, the lack of specificity of several enzymes involved permits several alternative biosynthetic routes (Sandler and Ruthven (1969)). Serotonin biosynthesis from the dietary amino acid tryptophan is shown in Fig. 1(c), steps ①-②.

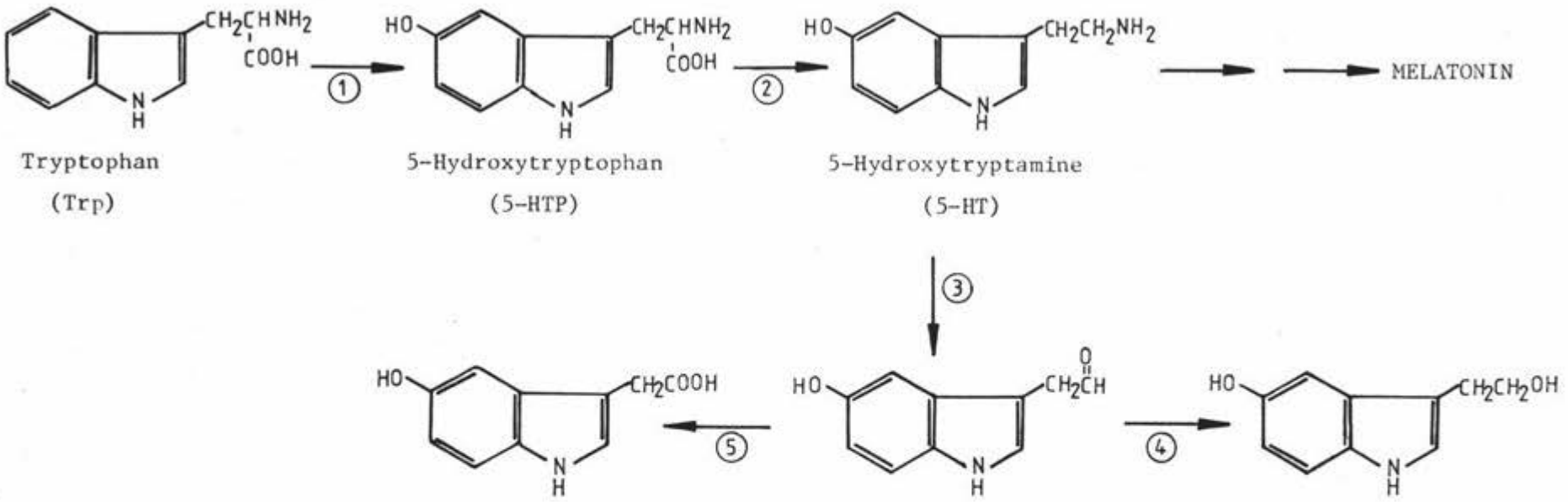
1.5.2 Metabolism

The metabolism of catecholamines (Fig. 1(b), steps ⑤-⑧) involves primarily two enzymes: catechol-O-methyl transferase (COMT, Fig. 1(b) ⑥) and monoamine oxidase (MAO, Fig. 1(b) ⑤). Both act on a wide variety of substrates and each is fully active on the products of the other. Thus an entire spectrum of catecholamine metabolites can be identified in urine - some acted on by MAO, COMT, or both. In addition to these two major enzymes, two others - an aldehyde dehydrogenase and an aldehyde reductase (Fig. 1(b) ⑦ and ⑧ respectively) are present, which act on the aldehyde products of MAO. The metabolism of serotonin (Fig. 1(c) steps ③-⑤) is much simpler than that of the catecholamines. Serotonin is acted on by the same MAO involved in catecholamine metabolism to give rise to an aldehyde product, which is in turn modified by either aldehyde dehydrogenase or aldehyde reductase.

Monoamine oxidase deaminates compounds in which the amine group is attached to the terminal carbon atom. N-methylation and β-hydroxylation decrease the susceptibility of phenylethylamines to MAO. Thus dopamine and tyramine are metabolized more readily than noradrenaline and adrenaline. MAO is widely distributed and, in addition to liver and the adrenal gland, has been shown to occur in skin, heart, kidney, salivary glands, intestine, and sympathetic nerve terminals where it is present mainly in the outer membrane of mitochondria.

The enzyme COMT is responsible for the 3-O-methylation of the catechol group using S-adenosylmethionine as a methyl donor. The enzyme can methylate catechols, but not monohydroxy derivatives of phenylethylamine. The enzyme preferentially O-methylates the 3-hydroxyl group of 3,4-dihydroxycatechols, although it does have activity on the 4-hydroxyl group to form 4-methoxy metabolites. It is broadly distributed in mammalian tissues, but the highest activity is found in the liver and kidney.

Aldehyde dehydrogenase is a NAD-dependent enzyme found in brain, liver and kidney. It oxidizes a wide range of aldehydes including those derived from catecholamines, tryptamines, tyramine and serotonin, to acid



Enzymes:

① Tryptophan-5-Hydroxylase

② 5-Hydroxytryptophan Decarboxylase

③ Monoamine Oxidase

④ Aldehyde Reductase

⑤ Aldehyde Dehydrogenase

Fig. 1(c) The Biosynthesis and Metabolism of Serotonin (5-HT).

end products.

Aldehyde reductase is also found in liver, brain and kidney, and has an equilibrium far to the side of alcohol production. There are two enzymes, a NAD oxidoreductase and a NADP oxidoreductase which have different substrate specificities. The NADP-linked enzyme will not reduce short chain aliphatic aldehydes while the NAD-linked enzyme will oxidize ethanol. The NADP-linked enzyme will reduce aldehydes derived from catecholamines, octopamine and tyramine.

In the central nervous system of man and throughout the body of rat and other species, the aldehydes derived from phenylethylamines (eg. dopamine) and indoleamines (serotonin) are oxidized to acids by NAD-dependent aldehyde dehydrogenase, whereas the aldehydes derived from β-hydroxylated phenylethylamines (eg. adrenaline, noradrenaline) are reduced to alcohols by NADP-dependent aldehyde reductase. However, in the liver and other peripheral tissues of man the aldehydes of adrenaline and noradrenaline are oxidized rather than reduced.

1.6 Excretion of Catecholamine and Serotonin Metabolites

1.6.1 Metabolite Conjugation

Catecholamines and their basic and neutral metabolites can be conjugated with sulphuric or glucuronic acids in position 4 (conjugation with sulphuric seems to be the predominant reaction in man) (Weil - Malherbe (1971)). This process occurs mainly in the liver, but conjugating enzymes and conjugated metabolites have also been found in the brain. The conjugated fraction of the acidic metabolites, if it exists, is small, and is usually disregarded during the assay of these metabolites.

There exists an enzyme - PAPS: serotonin sulphotransferase - found in brainstem, which catalyzes the transfer of sulphate from 3'-phospho-adenosine-5'-phosphosulphate to serotonin (Hidaka et al. (1969)). Sulphate conjugates of serotonin have been detected in urine, but conjugation does not appear to occur to a significant degree with serotonin metabolites.

1.6.2 Normal Levels of Urinary Catecholamine and Serotonin Metabolites

The normal physiological concentrations of neurotransmitter metabolites in urine vary widely amongst normal individuals. Tietz (1976) has described normal levels for 3 major metabolites using chemical

estimation techniques.

Metabolite	Normal Urinary Concentration ($\mu g\ mg^{-1}$ creatinine)
VMA	1.5 - 7.0
HVA	1.0 - 40
5-HIAA	0.8 - 7.3

Such wide ranging normal values may be partially due to the unreliability of the techniques used for their estimation. Probably more reliable normal values have been described in the literature using highly specific GCMS techniques for catecholamine (Muskiet et al. (1978b)) and serotonin (Domino et al. (1979)) metabolites in normal male subjects (Table 1(ii)).

Table 1(ii) Normal Levels of Urinary Catecholamine and Serotonin Metabolites Determined by GCMS Techniques

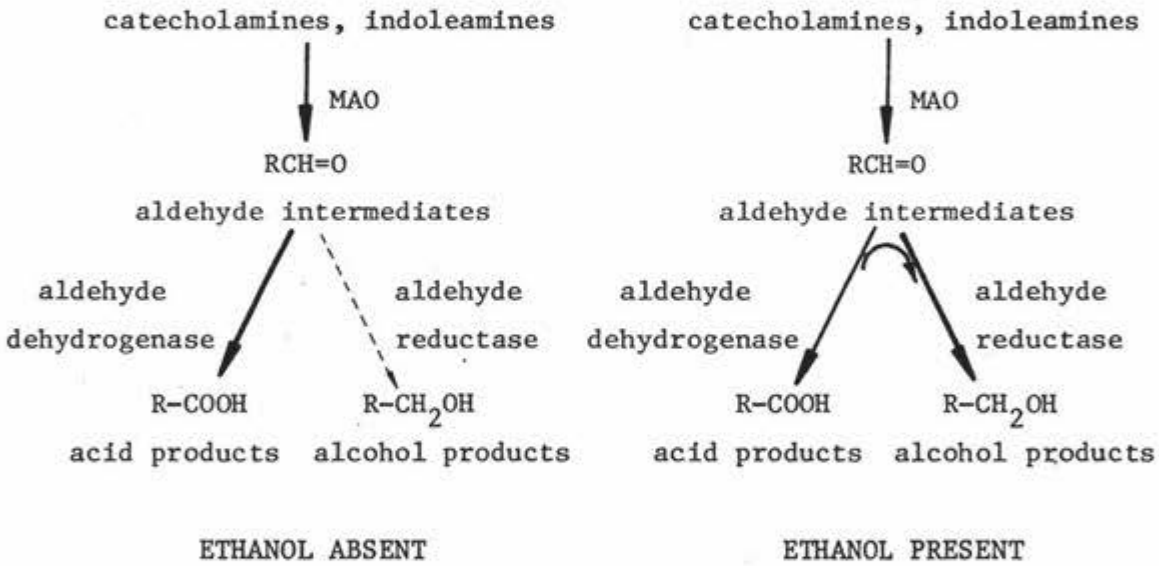
Metabolite	Normal Urinary Concentration		
	n	mean $\pm$ 1 s.d.	range
DOPPA	4	n.m.	-
VPA	6	0.74 $\pm$ 0.14	0.53 - 0.95
DOPLA	4	n.m.	-
VLA	5	0.18 $\pm$ 0.13	0.08 - 0.40
DOPAC	5	1.5 $\pm$ 0.5	0.6 - 2.0
HVA	25	4.0 $\pm$ 1.6	1.7 - 8.1
DOPEt	4	0.016 $\pm$ 0.009	0.005 - 0.026
MOPEt	4	0.032 $\pm$ 0.006	0.027 - 0.037
DOMA	4	0.118 $\pm$ 0.042	0.071 - 0.165
VMA	25	1.9 $\pm$ 0.8	0.8 - 4.4
DOPEG	7	0.33 $\pm$ 0.15	0.16 - 0.60
MOPEG	14	1.5 $\pm$ 0.5	0.7 - 2.5
5-HIAA	7	4.87 $\pm$ 0.32	3.88 - 5.64

Concentrations expressed as μg metabolite mg^{-1} creatinine, n.m. indicates not measurable.

1.7 Effects of Ethanol on Catecholamine and Serotonin Metabolism

The ingestion of significant quantities of ethanol alters the metabolism of the cell. Some of the alterations are due directly to the metabolism of ethanol (eg. a change in the lactate/pyruvate ratio, Papenberg (1971)), whereas others are due to its mere presence (eg. an increase in the corticosteroids, Jenkins and Connolly (1968)).

Ethanol, in addition to altering basic cellular biochemistry, affects the behaviour of the drinker. Behavioural events can possibly be related to neurotransmitter levels and metabolism. In the presence of ethanol, not only are the levels of transmitters altered, but the basic metabolic pathways are also changed (Feldstein (1971), Truitt and Walsh (1971)). In the presence of ethanol, the biogenic amine metabolites are diverted away from their oxidative pathways (which give rise to acidic products) into reductive pathways (which give rise to alcoholic products).



Effect of Ethanol on Catecholamine and Indoleamine Metabolic Pathways

Alterations in human biogenic amine metabolism due to ethanol have been investigated almost exclusively in urine samples due to their accessibility (as opposed to, for instance, CSF samples) and the fact that the immediate fate of the metabolised products is their excretion in the urine. The exact effects that an acute dose of ethanol has on the urinary levels of catecholamine and indoleamine metabolites in normal subjects so far described in the literature have been diverse and sometimes contradictory. This has been caused, in part, by the variety of ethanol doses, of distribution of dose over time, as well as the

inclusion or absence of controls for the diuretic effects of ethanol. The questionable adequacy of some of the methods used for the separation and identification of the amines and their metabolites is certainly one of the major contributing factors to these inconsistent findings (Hawkins and Kalant (1972)). The different results reported, and the various techniques employed are outlined in Table 1(iii).

The purpose of this study was to critically evaluate techniques previously used for the estimation of urinary catecholamine and serotonin metabolites. The techniques ultimately resolved as being the most accurate were then to be applied to alcohol studies in normal human volunteers in an attempt to resolve the diverse results of previous workers.

Table 1(iii) Techniques Used, and Results Attained by Previous Authors Investigating the Effects of Ethanol on Urinary Catecholamine and Serotonin Metabolites

Reference	Technique	Results
Smith et al (1960)	^{14}C-labelled precursors	Decreased VMA excretion
Perman (1961)	Chemical estimation	No significant change in 5-HIAA excretion
Feldstein et al. (1964)	^{14}C-labelled precursors, solvent extraction	Decreased 5-HIAA excretion
Anton (1965)	Chemical estimation	Increased VMA excretion, decreased 5-HIAA excretion
Davis et al. (1967a)	^{14}C-labelled precursors, solvent extraction	Decreased 5-HIAA excretion, increased 5-HTPh excretion
Davis et al. (1967b)	^{14}C-labelled precursors, column chromatography	Decreased VMA excretion, increased MOPEG excretion. No change in DOMA, DOPEG excretion
Feldstein et al. (1967)	^{14}C-labelled precursors, solvent extraction	Decreased 5-HIAA, postulated increased 5-HTPh excretion
Walsh et al. (1970)	^{14}C-labelled precursors, column chromatography	Acetaldehyde causes decreased VMA, DOMA excretion, increased MOPEG excretion in rats
Ogata et al. (1971)	Chemical estimation	Decreased VMA excretion, increased MOPEG excretion
Gitlow et al. (1976)	Paper chromatography (VMA), GLC (MOPEG, HVA)	Decreased VMA excretion, increased MOPEG excretion. Slightly increased HVA excretion