ADDICTION AND BRAIN DAMAGE

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Derek Richter

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PREFACE

Alcoholism and drug addiction are presenting serious problems in many parts of the world today. In a number of countries there is evidence of a steady rise in alcohol consumption in recent years and the rising morbidity rate, together with the increased involvement of young people, is a matter for growing concern. It has long been known that heavy drinking can lead, not only to general physical ill health, but also to conditions such as delirium tremens and dementia involving the brain. Addiction is itself a form of mental disorder and recently there have been a number of reports suggesting that heavy drinking, even at socially acceptable levels, can give rise to brain damage in individuals who are susceptible to the effects of alcohol. However, the nature of the brain damage, and the reasons why some individuals are more vulnerable than others, are still far from clear.

In this situation the Helping Hand Organisation, which was founded by Barry Richards in the UK in 1964, organised an international Symposium on 'Addiction: Biochemical Aspects of Dependence and Brain Damage' at Magdalen College, Oxford in September 1979. During the past fifteen years the Helping Hand Organisation has carried out pioneer work on the treatment and rehabilitation of alcoholics and drug addicts in a chain of residential homes and day centres set up by them in different parts of the country. They have also engaged in research on social and other aspects of addiction. The primary purpose of the 1979 Oxford Symposium was to bring together a group of leading scientists and clinical research workers engaged in the study of addiction in different countries so as to provide an opportunity for them to assess the implications of the recent findings on the biochemical and physiological mechanisms involved in dependence and in the causation of brain damage. The papers given at the Symposium are presented in this volume.

The scientific programme of the Symposium was arranged by Dr G.K. Shaw. We are indebted for their generous financial support for the meeting to Astra Chemicals Ltd, Aspro Nicholas Ltd, Barclay's Bank Ltd, Mr Barry Richards, The Brewer's Society, Mr P. Collins, EMI Medical Ltd, The Jurgens Trust, The Medical Research Council, The Mental Health Foundation, The Rank Group Charity, Roche Products Ltd, Sandoz Products Ltd and The Wellcome Trust.

Derek Richter

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PART ONE

BIOCHEMICAL AND PHYSIOLOGICAL MECHANISMS

1 THE EFFECTS OF ALCOHOL ON METABOLIC PROCESSES

Hans Krebs

The chief target organs of ethanol are the nervous system and the liver. From the biochemical point of view the mechanism of action of alcohol on the nervous system is still mysterious in many ways. We are only just beginning to get some understanding of the manner in which ethanol interferes with the function of the nervous system. This applies to the mechanism of acute intoxication as well as to the chronic manifestations such as polyneuritis, the deterioration of personality, delirium tremens and Korsakoff's psychosis.

By contrast, we do have some biochemical information on the mechanism of the effects of alcohol on the liver. Above all, it is clear *why* the liver is one of the major target organs. The liver is the only organ which possesses a highly active enzyme capable of breaking down alcohol. This specific role of the liver in disposing of ingested alcohol was discovered by the Danish physiologist, Einar Lundsgaard (1938). He discovered that alcohol is not metabolised by the eviscerated animal but is readily oxidised by the isolated perfused liver. The specific enzyme responsible for the removal of alcohol is alcohol dehydrogenase, an enzyme which has been crystallised and obtained in a pure form. It brings about the reaction:

$$\text{Ethanol} + \text{NAD} \longrightarrow \text{acetaldehyde} + \text{NADH}_2$$

By a further dehydrogenation the acetaldehyde is converted to acetic acid, and the acetic acid can then be completely burned either in the liver or in a variety of other tissues, particularly in cardiac muscle and kidney. Although some alcohol appears in the breath and in the urine, probably more than 90 per cent of ingested alcohol undergoes complete oxidation in the body. The oxidation of alcohol is an effective source of energy, because it is coupled with the synthesis of ATP.

Why should the occurrence of this reaction of alcohol in the liver be harmful and, in cases of chronic alcoholism, eventually lead to liver cirrhosis? There is something special about the location of alcohol dehydrogenase within the liver cell, in that this enzyme is located in the cytosol, in contrast to the majority of dehydrogenases which are located within the mitochondria. The rapid dehydrogenation of alcohol to acetic acid causes a unique kind of upset of the intracellular chemical balance. The rate of conversion of NAD into $NADH_2$ causes a shift in the ratio of the concentrations of NAD and $NADH_2$, primarily in the cytosol where most of the synthetic activities of the liver cells are located (see Krebs, 1968; Krebs *et al.*, 1969). This ratio is of great importance to many metabolic processes because most dehydrogenases share with ethanol the coenzymes NAD and $NADH_2$ and their effectiveness depends on the relative concentrations of NAD and $NADH_2$, i.e. the $NAD/NADH_2$ ratio (referred to as the 'redox state' of the NAD-couple).

The shift caused by alcohol in the redox state of this couple is taken to be a major reason for metabolic disturbances in the liver, showing themselves early in the accumulation of fat in the liver of alocholics and ending in cirrhosis. The acetaldehyde formed by alcohol dehydrogenase has also been suspected of being harmful because aldehydes readily interact with amino groups of proteins. However, as the acetaldehyde is rapidly converted to acetic acid, it is doubtful whether its steady-state concentration rises sufficiently to combine with proteins.

Biochemical studies have thus contributed an explanation of why alcohol is toxic to the liver, but this has not led to new ideas about a cure for liver damage.

One of the secondary effects of alcohol on liver metabolism should be mentioned. Under certain conditions, i.e. when alcohol is taken in the fasting state or when carbohydrate intake is low, hypoglycaemia can develop. The mechanism of hypoglycaemia is as follows: the shift in the redox state of the $NAD/NADH_2$ couple inhibits glucose synthesis in the liver. In the fasting state the sources of glucose are limited. Adequate glucose levels in the blood can be maintained only by the resynthesis of glucose from lactic acid formed during exercise and from protein broken down during starvation. Gluconeogenesis is inhibited by alcohol because the pathway of gluconeogenesis is effective only if the $NAD/NADH_2$ ratio is within the normal range. Gluconeogenesis, like the opposite metabolic process, glycolysis, depends on reactions involving this ratio. Therefore changes in the ratio also upset gluconeogenesis. The liver is the main site of gluconeogenesis.

As for the effects of ethanol on the second major target area, the nervous system, the mechanism of the damaging action must be quite different from that in the liver because of the absence of alcohol dehydrogenase from nervous tissue.

The older biochemical approaches to organ function (based on the study of enzymes and metabolic pathways of degradation and synthesis of cell constituents) which have been successful in the study of liver diseases have provided only limited answers to the problems of brain physiology and brain pathology. We must therefore explore whether other areas of biochemistry might be helpful.

In recent years new, more subtle effects of ethanol on metabolic processes have come to light. Work by Mϕrland and Bessesen (1977), Mϕrland (1979), Badawy *et al.* (1979), Rothschild *et al.* (1971, 1975) has shown that ethanol inhibits protein synthesis in the liver and other tissues. Tewari and Noble (1979) have demonstrated inhibitory effects of ethanol on protein synthesis in the brain. The experimental basis of this demonstration is the observation that the incorporation of radioactive amino acids into the tissue protein (which can easily be measured) is inhibited by ethanol. What does this mean?

We know from discoveries made by Schoenheimer and Rittenberg (1938) and Schoenheimer (1942) that many tissue proteins and other macromolecules are not stable; they are constantly being broken down and resynthesised, a phenomenon referred to as the 'dynamic state of body constituents'. This phenomenon is especially marked in the liver, where an explanation can be offered for the significance of this 'turnover'. Most liver proteins are enzymes taking part in metabolic processes. These processes are not always the same but depend upon the nutritional state of the organism and other physiological circumstances. When the diet contains much protein then the liver must degrade the excess and either burn it or convert it into fat or carbohydrate, for the body cannot store much protein. When the diet is low in carbohydrate the liver must synthesise glucose by gluconeogenesis.

When the body is exposed to drugs or poisons, for example barbiturates, enzymes are produced which detoxicate the drugs and poisons. The liver cannot at any one time be equipped with all the enzymes it might need in different physiological situations. For this it would have to be a very much larger organ. The liver deals with variable requirements by removing, through proteolysis, enzymes which it does not need in a given situation and by synthesising, from the amino acids released, the enzymes it requires. Thus the dynamic state of proteins is part of a process, adapting the organism to changing physiological circumstances.

Another function of the continuous protein synthesis/degradation is taken to be the elimination and replacement of faulty material. Tissue proteins may become denatured, for example, by the irreversible oxidation of SH-groups, or faulty protein molecules may arise either from mutations or from errors of the translation and transcription of genes. Thus, the turnover of cell constituents serves to maintain a fully functioning cell (Goldberg and Dice, 1974; Goldberg and St. John, 1976).

If ethanol inhibits the turnover of proteins it means that it inhibits the capacity of the organism to adapt itself and to maintain a state of efficiency. Examples of adaptive enzymes are the enzymes for degradation of tryptophan and tyrosine. These are essential amino acids and when the diet is low in protein they must be preserved and not degraded. The body deals with this by getting rid of the enzymes which degrade tryptophan and tyrosine. It is essential that these amino acids are removed when there is an excess. If they are not removed they may give rise to physiologically active substances in excess, such as tyramine and tryptamine. This adaptation of the enzymes is interfered with by ethanol (Mørland and Bessesen, 1977; Mørland, 1974; Badawy *et al.*, 1979; Rawat, 1974).

While, then, for the liver, the significance of the rapid turnover of some proteins can be understood, we cannot yet be quite sure of an analogous explanation for this turnover of cell constituents in the brain. The fact is that protein synthesis does occur in the brain, though not at the same rate as in the liver, and that in brain, as in liver, ethanol can slow down protein synthesis (see Tewari and Noble, 1979; Lindholm and Khawaja, 1979). Presumably in the brain, as in the liver, protein synthesis is an aspect of adaptation. The brain synthesises biologically active polypeptides such as the opioids and also a number of peptide hormones.

There are two aspects of special interest in the present context. The need for these peptides is not constant. Their quantities must therefore vary with time and this is achieved by a rapid turnover. The need also varies in different parts of the brain. In the past it has been difficult, from the biochemical point of view, to account for the mechanism of action of drugs and of toxic agents on the nervous system because such information as was available did not explain the differential effects of the substances in different areas of the brain, as for example, that anaesthetics affect only those areas connected with consciousness, but leave large portions of the brain in a normally functioning state.

I am very hopeful that the exploration of this new field of biologically active peptides, and of the specific receptor sites (see Snyder, 1979; Kosterlitz, 1976) will add greatly to the understanding of the specific neural function.

Finally, let us look at alcohol or drug dependence generally. Regular intake of a drug, or any 'unphysiological' substance, can cause habituation. It follows from what I have said that habituation is, at the biochemical level, the adjustment of the enzymic equipment of the tissues to the intake of special substances, and that this adjustment or adaptation involves continual protein synthesis. If this adjustment is not readily reversible when the stimulus is withdrawn, then the chemical organisation of the tissues – or of some tissues – becomes unbalanced, and to restore the balance the stimulus must be provided again. In other words, the organism has become addicted, has become drug-dependent. Thus we can translate the phenomenon of addiction into the language and concepts of biochemistry. This does not mean that we can now suggest a cure for drug dependence. Perhaps in the long run useful practical measures concerning the management of drug dependence may emerge. At present, prevention and treatment can be based only on practical experience, not on profound theoretical understanding. It needs no biochemistry to come to the conclusion that we must aim at limiting alcohol intake and we must provide intensive treatment for the rehabilitation of addicts.

Abbreviations

NAD = nicotinamide adenine dinucleotide
NADH$_2$ = reduced nicotinamide dinucleotide

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2 CATECHOLAMINE-DERIVED ALKALOIDS IN DEPENDENCE

Virginia E. Davis, Jesse L. Cashaw and Kenneth D. McMurtrey

Theories of Ethanol-evoked Formation of Alkaloids

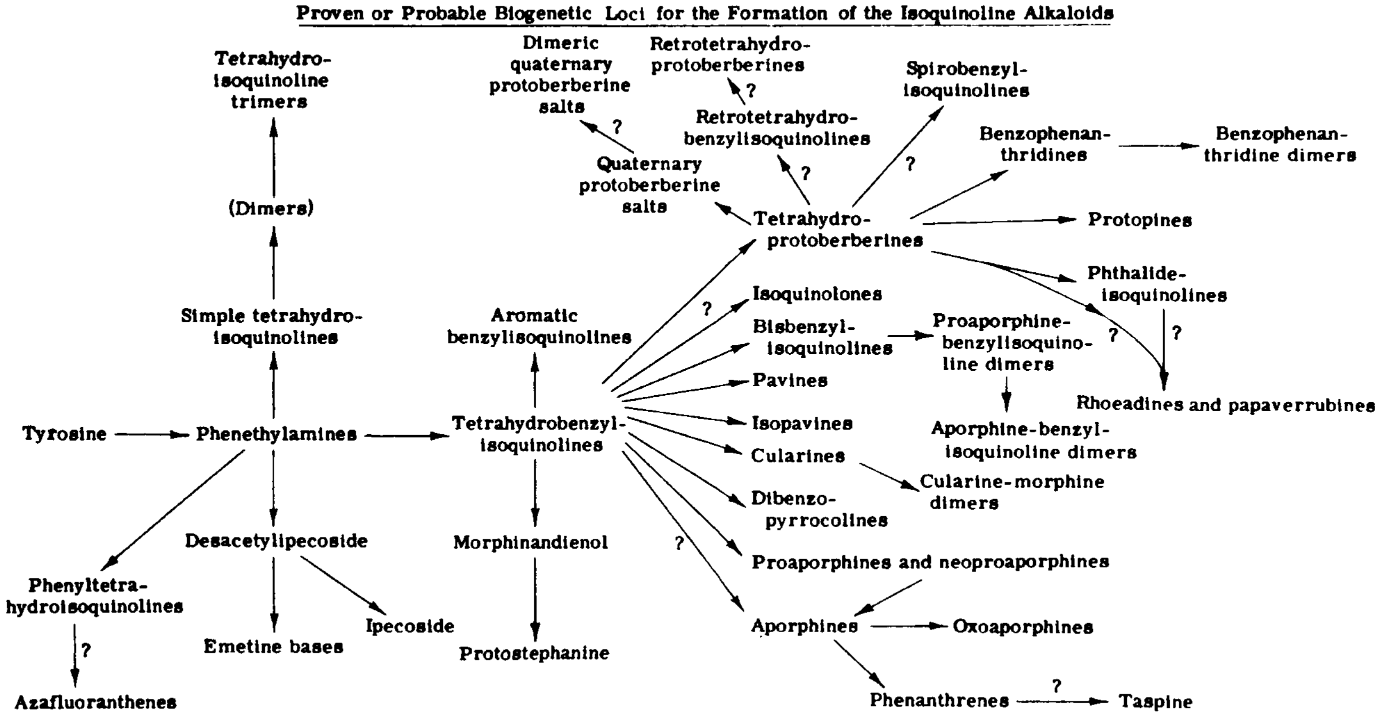
The original premise of ethanol-induced formation of pharmacologically active neuroamine-derived alkaloids (mediated by acetaldehyde, the proximal metabolite of ethanol) in mammalian species was simultaneously and independently advanced by our laboratory (Davis, 1971) and by Cohen (1971a). A common feature of both theories is the Pictet-Spengler condensation (Whaley and Govindachari, 1951) of β-arylethylamines with aldehydes to form Schiff base intermediates. These substances subsequently undergo cyclisation, yielding the tetrahydroisoquinoline alkaloids that may be involved in some aspects of alcoholism.

Cohen and his colleagues proposed that acetaldehyde, derived from ethanol, condenses directly with endogenous catecholamines to form biologically active l-methyltetrahydroisoquinolines (Cohen and Collins, 1970; Cohen, 1971a, b; Cohen, 1976). This laboratory also suggested that l-methyltetrahydroisoquinolines may be formed (Yamanaka *et al.*, 1970).

We further theorised that metabolism of ethanol evokes a more intricate chain of events leading to the production of complex 1-benzyltetrahydroisoquinolines as aberrant metabolites of catecholamines (Davis *et al.*, 1970; Davis and Walsh, 1970 (a); Davis, 1971). In this case, ethanol-generated acetaldehyde increases the availability of the dopamine-derived aldehyde for condensation with the parent amine by competitively inhibiting the oxidative disposition of the biogenic aldehyde. The result is an enhanced formation of tetrahydropapaveroline (THP, norlaudanosoline), a pharmacologically active benzyltetrahydroisoquinoline alkaloid.

Further metabolism of the simple tetrahydroisoquinolines formed by direct condensation of acetaldehyde or formaldehyde with catecholamines appears to be relatively limited. But in plants, benzyltetrahydroisoquinoline alkaloids are the biogenic precursors of a diverse array of even more complex alkaloids, including the tetrahydroberberine

Figure 2.1: Biogenesis of Isoquinoline Alkaloids



Source: Shamma, 1972.

(protoberberine), pavine, isopavine, aporphine, morphinane, cularine and protopine groups (Battersby, 1961; Bentley, 1965; Spenser, 1966; Kirby, 1967; Robinson, 1968; Santavy, 1970; Shamma, 1972), as shown in Figure 2.1.

We have proposed that the biosynthesis of complex alkaloids is a capability that is not entirely unique to plants. Certain of these reaction sequences may be evoked in man and animals, as well, which in turn might subserve some of the mechanisms brought into play in the misuse of ethanol and related drugs (Davis *et al.*, 1970; Davis and Walsh, 1970(a); Davis, 1971). The possibility that tetrahydropapaveroline (THP) may follow similar metabolic transformations in mammalian systems is thus an integral part of our hypothesis (see Figure 2.2). The indication in this figure of specific groups of alkaloids (aporphine, morphine and tetrahydroberberine) as emanating from THP is not, however, intended to be an exhaustive and all-inclusive statement of possibilities.

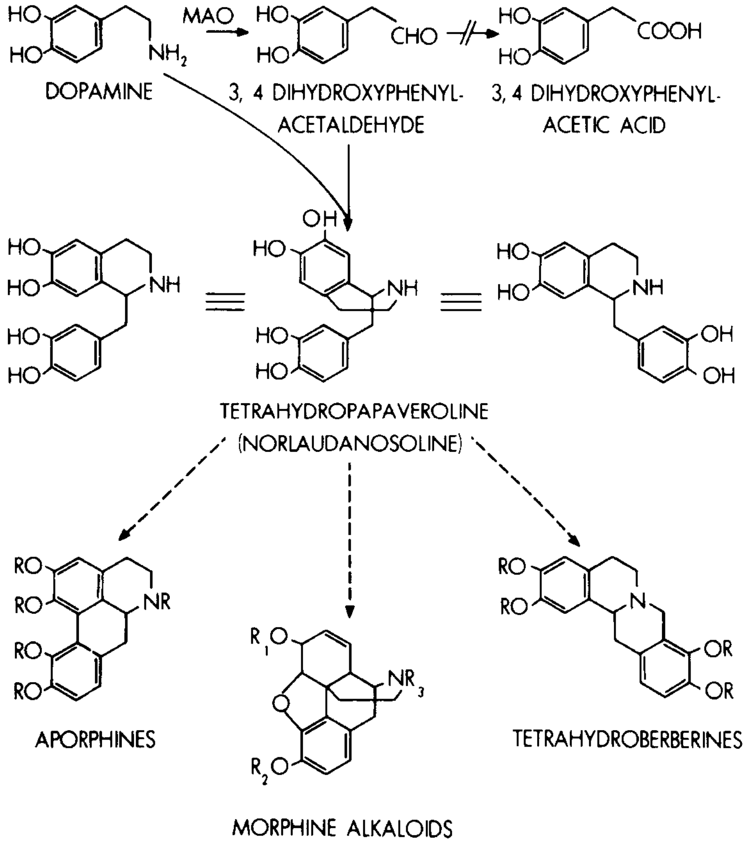
Our hypothesis regarding alcohol-induced alkaloid formation did not arise spontaneously: rather, it was derived from investigations into the effects of ethanol on the metabolism of neurotransmitters in both human subjects and experimental animals. Additionally, they were extended to include other drugs that produce dependence of the alcohol-barbiturate type, e.g. chloral hydrate, paraldehyde and barbiturates.

This is an important consideration for several reasons. Ethanol and related drugs produce a characteristic withdrawal syndrome. Drugs within this class can be substituted for ethanol in preventing or ameliorating alcohol withdrawal symptoms, and these drugs are cross dependent. Finally, the action of these drugs is potentiated when they are taken in combination with ethanol. It thus seems reasonable that common biochemical mechanisms may be responsible for producing the dependence state associated with prolonged and excessive use of ethanol and these pharmacologically equivalent drugs. Although this consideration is usually ignored, studies of the underlying mechanisms in alcohol abuse and dependence should also attempt to resolve the addiction liability of these pharmacologically equivalent drugs.

Common Features of Ethanol and Biogenic Amine Metabolism

It is well known that the primary reaction product of ethanol metabolism is acetaldehyde. It is also generally recognised that the

Figure 2.2: Structural representation of the ethanol-evoked modifications of dopamine metabolism. Known pathways, either *in vitro* or *in vivo*, are indicated by (——►). The site of inhibition of dopamine metabolism by acetaldehyde, the metabolite of ethanol, is shown by (——//——►). The proposed possible consequence of this inhibition resulting in the formation of diverse classes of complex alkaloids is illustrated by (- - - - →). The formation of tetrahydroprotoberberines in mammalian systems has now been established.



	R_1	R_2	R_3
NORMORPHINE	H	H	H
MORPHINE	H	H	CH_3
NORCODEINE	H	CH_3	H
CODEINE	H	CH_3	CH_3

Source: Davis *et al.*, 1970.

major portion of ethanol oxidation is catalysed by NAD-dependent alcohol dehydrogenase (ADH). The ethanol-derived acetaldehyde is oxidised to acetate by NAD^+-linked aldehyde dehydrogenase (ALDH), which has a broad substrate specificity.

Oxidative deamination of biogenic amines such as dopamine, norepinephrine and serotonin by monoamine oxidase results in the formation of the corresponding biogenic aldehydes. The aldehydes derived from these amines may be oxidised to the corresponding acid metabolite by aldehyde dehydrogenase (ALDH) (Deitrich, 1966) or they may be reduced to the alcohol metabolite by alcohol dehydrogenase (Raskin and Sokoloff, 1970) or by NADPH-linked aldehyde reductases (ALDR) (Tabakoff and Erwin, 1970; Ris and von Wartburg, 1973; Anderson *et al.*, 1976). The probability of oxidation or reduction depends to a large extent on the structural characteristics of the amine-derived aldehydes. The β-hydroxylated aldehyde analogs of norepinephrine, normetanephrine and octopamine are primarily reduced to the corresponding glycol metabolites by brain and both oxidised and reduced in the periphery, whereas the primary route of aldehydes without the β-hydroxyl group – such as the aldehydes of dopamine and serotonin – is oxidation to acid metabolites (Rutledge and Jonason, 1967; Breese *et al.*, 1969; Duncan and Sourkes, 1974; Tipton *et al.*, 1977). Because the amine-derived aldehydes and ethanol share common enzyme systems, the assumption is reasonable that the metabolism of ethanol can result in modifications in the catabolism of biogenic amines.

Effect of Alcohol on Amine Metabolism

Evidence has been provided for specific alcohol-induced alterations in the metabolic disposition of neuroamines in man. It was found that ingestion of ethanol by man significantly shifts the metabolism of the aldehyde derivatives of both serotonin (Davis *et al.*, 1967a) and norepinephrine (Davis *et al.*, 1967b, c; Smith and Gitlow, 1967) from an oxidative to a reductive pathway.

Two possible mechanisms may be responsible for the alcohol-evoked modifications in neuroamine metabolism. Decreased availability of NAD^+, with the accompanying increased $NADH:NAD^+$ ratio occurring during the metabolism of ethanol, may divert the metabolism of amine-derived aldehydes from an oxidative to a reductive pathway. Additional evidence indicates that the competitive inhibition of aldehyde dehydrogenase by the primary metabolite of ethanol – namely acetaldehyde – both *in vitro* (Lahti and Majchrowicz, 1967; Lahti and Majchrowicz, 1969) and *in vivo* (Walsh *et al.*, 1970b) plays a major role in the alcohol-induced modifications in amine metabolism. These mechanisms are not necessarily mutually exclusive, but the latter is strongly favoured.

The fact that acetaldehyde is a strong competitive inhibitor of the oxidation of amine-derived aldehydes by aldehyde dehydrogenase is important in this context. For example, the inhibition constant (K_i of 2.6×10^{-6} M) for acetaldehyde in inhibiting the oxidation of 5-hydroxyindoleacetaldehyde by rat brain aldehyde dehydrogenase is low (Lahti and Majchrowicz, 1969). This fact suggests that the oxidation of amine-derived aldehydes may be exquisitely sensitive to inhibition by the acetaldehyde formed during ethanol metabolism, even if the actual concentrations of acetaldehyde are extremely low.

Species Differences

There is a notable species difference in the sensitivity to ethanol-induced modifications in amine metabolism. In the case of human subjects, even moderate doses of ethanol produce dramatic changes in the metabolism of serotonin and norepinephrine (Davis *et al.*, 1967a, b, c; Smith and Gitlow, 1967). In marked contrast, administration of large, even hypnotic, doses of ethanol to rats is relatively ineffective in altering the metabolism of biogenic amines in the periphery; and in this species, administration of large amounts of the ethanol metabolite, acetaldehyde, is required to modify amine metabolism significantly (Walsh *et al.*, 1970b).

The inability of even hypnotic doses of ethanol to shift the metabolism of the norepinephrine-derived aldehyde from an oxidative to a reductive route in the intact rat, in comparison with the marked effect in man, may be related to the fact that the metabolic fate of norepinephrine is different in the two species. In man the major excretion product is the acid metabolite, 3-methoxy-4-hydroxymandelic acid, whereas in the rat the predominant product is the glycol metabolite, 3-methoxy-4-hydroxyphenylglycol. The greater propensity of man, therefore, to oxidise the biogenic aldehyde derivative to the acid metabolite in the periphery may make this pathway more sensitive to competitive inhibition by ethanol-generated acetaldehyde. Furthermore, the affinity of aldehyde dehydrogenase for acetaldehyde may differ markedly in the two species.

The differences in the response of man and rats to ethanol-evoked alterations in biogenic aldehyde metabolism appear to parallel the relative sensitivity of the two species to the pharmacological effects of alcohol. Alcoholism is uniquely a human problem but, because of limitations of techniques for research involving human subjects, certain aspects of biochemical research related to alcoholism must of necessity utilise experimental animals. It is obviously preferable to study the effect of alcohol in a species other than the rat, in a species that would more closely parallel man in his sensitivity to the reinforcing, intoxicating and addicting actions of alcohol. Nevertheless rodents are for economic reasons extensively chosen for such experimentation. Although rodents must often be used in the laboratory, it is important to remain aware of the differences among species in their response to ethanol, and to temper interpretations and extrapolation of any results obtained with experimental animals to man.

Effect of Ethanol and Related Drugs on Formation of Benzyltetrahydroisoquinoline Alkaloids

A pivotal consideration in the metabolism of biogenic amines is the relative propensity of aldehydes derived from specific amines to be further metabolised by the oxidative or the reductive route. Dopamine is an excellent substrate for monoamine oxidase. In contrast to the aldehyde derivative of norepinephrine, the 3,4-dihydroxyphenylacetaldehyde generated is preferentially oxidised to 3,4-dihydroxyphenylacetic acid by aldehyde dehydrogenase (Alivisatos and Tabakoff, 1973), particularly in brain. The aldehyde derivatives of the biogenic amines are highly reactive compounds. Holtz and colleagues (1964) reported that incubation of dopamine with guinea pig liver mitochondria preparations of monoamine oxidase – which lacked the cofactor for further oxidative metabolism of 3,4-dihydroxyphenylacetaldehyde formed on deamination of the amine – resulted in the formation of tetrahydropapaveroline. Tetrahydropapaveroline (THP, norlaudanosoline) is the benzyltetrahydroisoquinoline alkaloid produced by the nonenzymatic condensation of 3,4-dihydroxyphenylacetaldehyde with the parent amine.

Conditions that interfere with the normal enzymatic disposition of the dopamine-derived aldehyde – for example, a limited cofactor availability or the inhibition of aldehyde dehydrogenase – could be expected to increase the relative proportion of dopamine converted to THP. When this possibility was examined (Walsh *et al.*, 1970a), it was found that the relative formation of THP *in vitro* by rat liver and brain preparations was dependent on the substrate concentration and upon the availability of the aldehyde dehydrogenase cofactor, NAD^+, for further oxidation of the aromatic aldehyde to 3,4-dihydroxyphenylacetic acid. Tetrahydropapaveroline was the major metabolite of dopamine in the absence of exogenous coenzyme in both tissues.

Incorporation of the cofactor into incubation mixtures with liver essentially abolished tetrahydropapaveroline production and markedly enhanced the formation of the acid metabolite. With brain preparations, however, the addition of cofactors decreased tetrahydropapaveroline generation and only modestly increased dihydroxyphenylacetic acid formation. Tetrahydropapaveroline remained the major metabolite of dopamine in the brain tissue preparation under all conditions because of the limiting aldehyde dehydrogenase activity in comparison with monoamine oxidase activity in this *in vitro* preparation. Thus the relative amount of dopamine diverted to tetrahydropapaveroline assumed appreciable importance under conditions of limiting aldehyde oxidising capacity.

Ethanol and Acetaldehyde

The effect of ethanol and acetaldehyde on THP formation from labelled dopamine in rat liver and brain preparations was determined in the presence of exogenous NAD^+ (Davis *et al.*, 1970). The cofactor was incorporated to provide unlimited coenzyme for aldehyde dehydrogenase. Ethanol or acetaldehyde suppressed the oxidation of the intermediate aldehyde derivative of dopamine to the corresponding acid, and it concurrently augmented the conversion of dopamine to tetrahydropapaveroline. Competitive inhibition of the oxidation of 3,4-dihydroxyphenylacetaldehyde by acetaldehyde, therefore, facilitated the diversion of the amine-derived aldehyde to the alternative condensation route.

Substantial formation of the direct condensation product of dopamine with acetaldehyde was also observed when rat liver or brain preparations were incubated with ethanol or acetaldehyde (Davis *et al.*, 1970; Yamanaka *et al.*, 1970). The product of this reaction is the simple tetrahydroisoquinoline alkaloid, salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline).

Ethanol administration increases urinary excretion of catecholamines and stimulates release of these amines from adrenal medullary stores, effects that have been attributed to acetaldehyde derived metabolically from ethanol (Perman, 1958; Duritz and Truitt, 1966; Walsh and Truitt, 1968). Thus, if dopamine is similarly affected and freed from protected storage sites, it may then be more available for alkaloid-forming condensation reactions. Furthermore, the normal oxidative route for disposition of the dopamine-derived aldehyde is also impaired during the metabolism of acetaldehyde.

Chloral Hydrate

The well-established cross dependency and synergy between chloral hydrate and ethanol is of interest in relation to the possible drug-induced formation of amine-derived alkaloids. Incidentally, similar to the changes seen with ethanol, administration of chloral hydrate or paraldehyde to rats decreased the amount of serotonin metabolised in the periphery and excreted as 5-hydroxyindoleacetic acid, and increased 5-hydroxytryptophol excretion (Davis *et al.*, 1969; Huff *et al.*, 1971).

It is noteworthy that chloral hydrate has the potential to modify the disposition of amine-derived aldehydes for two reasons. Chloral hydrate is a potent inhibitor of aldehyde dehydrogenase (Erwin and Deitrich, 1966; Kramer and Deitrich, 1968), and it also competes for the reductive pathway of biogenic aldehyde metabolism (Tabakoff *et al.*, 1974). As with the primary metabolite of ethanol, acetaldehyde, it was found that the presence of chloral hydrate in incubation mixtures of brain stem homogenates supplemented with NAD also markedly decreased formation of 3,4-dihydroxyphenylacetic acid from dopamine and increased the generation of THP (Davis, 1973). Thus, the augmented formation of the alkaloid, THP, as a result of blocking the oxidation of the aldehyde metabolite of dopamine by alcohol, acetaldehyde and chloral hydrate, offers a common pharmacologically-induced aberration of dopamine metabolism.

Barbiturates

There is no evidence that barbiturates and certain tranquillisers chemically unrelated to ethanol inhibit the oxidation of biogenic amine-derived aldehydes. Barbiturates, however, have been shown to be potent inhibitors of the aldehyde reductases of brain (Tabakoff and Erwin, 1970; Erwin *et al.*, 1971; Ris and von Wartburg, 1973; Ris *et al.*, 1975). The aldehyde derivative of norepinephrine, unlike the dopamine-derived aldehyde, is not appreciably oxidised to the corresponding acid by brain aldehyde dehydrogenase. Rather, it is primarily reduced to the glycol by pyridine nucleotide-dependent aldehyde reductases. It is thus conceivable that barbiturate intervention may, to an important extent, evoke aberrant metabolism of the aromatic aldehydes derived from β-hydroxylated phenylethylamines by brain tissue. Consistent with this suggestion are the observations that the primary (i.e., reductive) metabolic pathway of the norepinephrine-derived aldehyde in brain tissue is disrupted by barbiturates. This disruption leads to elevated aldehyde levels and enhanced formation of a putative norepinephrine-derived benzyltetrahydroisoquinoline alkaloid (Davis *et al.*, 1974).

Barbiturates thus are capable of suppressing the reductive disposition of β-hydroxylated phenylacetaldehydes by inhibiting the aldehyde reductases, whereas ethanol, via its metabolite acetaldehyde, affects the metabolic oxidative disposition of phenylethylamine-derived aldehydes by inhibiting aldehyde dehydrogenases. The consequences of these interactions are increased levels of the biogenic aldehydes and a metabolic diversion which result in the formation of aberrant benzyltetrahydroisoquinoline alkaloids derived from the parent amine.

Pharmacological Actions of Tetrahydroisoquinolines

The hypotheses for possible involvement of amine-derived alkaloids in alcoholism generated an explosion of interest in the pharmacology of these compounds. The alkaloids receiving the most attention have been:

(1) 1-(3′,4′-dihydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (tetrahydropapaveroline, THP, norlaudanosoline) formed by condensation of dopamine with its intermediate aldehyde, 3,4-dihydroxyphenylacetaldehyde;
(2) 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol), the condensation product of dopamine and acetaldehyde; and
(3) the condensation products of formaldehyde with the catecholamines, norepinephrine and dopamine, i.e., 4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline and 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, respectively.

Interaction with Aminergic Systems

The tetrahydroisoquinoline alkaloids share molecular geometry with the parent catecholamines. It is not surprising, therefore, that these compounds interact with a variety of aminergic systems. The pharmacological properties of the tetrahydroisoquinoline alkaloids have been the subject of several recent comprehensive reviews (Hirst *et al.*, 1977; Cohen, 1978; Cohen, 1979; Deitrich and Erwin, 1980) and detailed recounting of the enormous body of literature related to this subject is beyond the scope of this presentation. But as an overview, investigations have demonstrated that these alkaloids act as 'false neurotransmitters' and interact with aminergic uptake, storage, transport and metabolism.

It has been reported that the tetrahydroisoquinolines are:

(1) transported into catecholamine neurons, where they are released on stimulation and are able to block the uptake of catecholamines in nerve terminals (Heikkila *et al.*, 1971; Cohen *et al.*, 1972; Greenberg and Cohen, 1972; Cohen, 1973a, b; Greenberg and Cohen, 1973; Locke *et al.*, 1973; Tennyson *et al.*, 1973; Cohen *et al.*, 1974; Mytilineou *et al.*, 1974; Alpers *et al.*, 1975);
(2) inhibitors of monoamine oxidase (Yamanaka 1971; Collins *et al.*, 1973; Cohen and Katz, 1975; Giovine *et al.*, 1976; Katz and Cohen, 1976; Meyerson *et al.*, 1976; Renis *et al.*, 1978);
(3) substrates for and competitive inhibitors of COMT (Collins *et al.*, 1973; Rubinstein and Collins, 1973; Meyerson *et al.*, 1979);
(4) inhibitors of ATP-phosphohydrolases (Meyerson *et al.*, 1978);
(5) either direct agonists or receptor antagonists of amine-sensitive adenylate cyclases (Miller *et al.*, 1974; Sheppard and Burghardt, 1974; Feller *et al.*, 1975; Sheppard *et al.*, 1976; Nimitkitpaisan and Skolnick, 1978; Clement-Cormier *et al.*, 1979);
(6) able to stimulate prolactin secretion by their ability to act as dopamine antagonists (Britton *et al.*, 1979); and
(7) inhibitors of tyrosine hydroxylase (Collins and Weiner, 1977).

Effect of Tetrahydroisoquinolines on Voluntary Consumption of Ethanol

Although evidence of strong interactions of tetrahydroisoquinoline alkaloids with many important neuronal regulatory systems emerged from these investigations, a direct connection between these compounds and alcohol-related behaviour was missing. The direct link was recently furnished by the remarkable findings of Myers and colleagues, which have been described in a series of publications (Melchior and Myers, 1977a, b; Myers and Melchior, 1977; Myers and Oblinger, 1977; Myers, 1978a, b; Melchior, 1979). Because the results are most significant, they can best be summarised by a direct quotation from a review by Myers (1978b):

> In a recent series of experiments, the CNS portion of the amine-metabolite theory has been tested directly. In order to stimulate the chronicity of the condensation product's presumed action within the

> brain during the prolonged ingestion of alcohol, THP has been infused around the clock into the cerebral ventricle of the rat. When animals of the nonalcohol drinking Sprague-Dawley strain are offered alcohol and water in a free-choice paradigm, as THP is infused every 30 min. in a dose as low as 100 pg/μl the rat's volitional intake of 3-9% alcohol suddenly rises (Myers and Melchior, 1977). Surprisingly, when the solution of alcohol is systematically increased in strength to aversive concentrations ranging from 11-30%, the THP-infused rats drink even more alcohol – as much as 13 to 16 g/kg on a given day. Marked ataxia, an elevated blood level, and other intoxication-like symptoms characterize this unusual drinking (Melchior and Myers, 1977a). If the intracerebral applications of THP are cut back to a once per day infusion, thereby reducing drastically the total dose of the TIQ to which the brain is exposed, the rat's normal aversion to alcohol nevertheless disappears. Again, a marked preference develops for the fluid (Myers and Oblinger, 1977), with the rat voluntarily consuming up to 10 g/kg per day of alcohol in concentrations of 15 or 20%.

This powerful pharmacologic effect of incredibly low doses of tetrahydropapaveroline in increasing voluntary alcohol intake appears to be permanent. The exaggerated self-selection of alcohol was found to persist when retesting was carried out one, six and nine months after the chronic infusions of the tetrahydroisoquinoline alkaloid had ceased (Melchior and Myers, 1977a; Melchior, 1979).

Replication of these findings by Deitrich and co-workers (Duncan and Deitrich, 1978; Deitrich and Erwin, 1980) soon followed, with the report that intraventricular infusion of small amounts of tetrahydropapaveroline increased the rats' preference for ethanol. Additionally, the effect was still present even ten months after infusion of the alkaloid.

This group also stressed another important consideration (Melchior *et al.*, 1978): 'In the chronic infusion of THP, the calculated average level for the lowest effective dose is 0.6 ng/g brain. This suggests that published reports indicating an inability to detect these compounds *in vivo* following the ingestion of ethanol . . . are not sufficiently sensitive to detect levels of the compounds that are physiologically efficacious.' In view of the remarkable potency of tetrahydropapaveroline delivered in picogram quantities into the brain in evoking abnormal drinking, Myers (1978a) also suggested that the endogenous formation of this alkaloid in alcohol-treated animals may be extremely difficult to detect.