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CENTRAL NORADRENERGIC MECHANISM OF ACUTE AND CHRONIC ETHANOL WITH OBSERVATIONS ON THE ROLE OF ACETALDEHYDE

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

by

Pushpa Varumal Thadani, M. Phil.

* * * * * *

The Ohio State University

1973

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ACKNOWLEDGMENTS

I heartily dedicate this dissertation to my parents, Varumal and Parpati, especially to my mother and to my sisters and brothers in acknowledgment of her understanding and moral support, without which this research would not have been possible.

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VITA

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PUBLICATIONS AND PRESENTATIONS


FIELDS OF STUDY

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Autonomic Pharmacology. Professor B.H. Marks

Neuroendocrinology Pharmacology. Professor H. Goldman

Drug Metabolism. Professor D. Couri

Pharmacology Related to Anesthesia. Professor E.B. Truitt, Jr.

Psychopharmacology. Professor H. Fisher

Minor Fields:

Advanced Physiological Chemistry. Professors G. Brierley, J. Rieske and H. Sprecher

Advanced Organic Chemistry. Professor G. Fraenkel

Advanced Organic Chemistry. Professor J. Swenton

Neurochemistry. Professor L. Horrocks

General Pathology. Professor W.G. Venzke
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INTRODUCTION

General Concepts

Alcoholic beverages have been used since the dawn of history and the abuse of alcohol has increased with the advent of distilled spirits. The excessive use of alcohol has long been regarded as a moral, psychic and physical problem, but only in recent years has a disease concept of alcoholism been accepted (Jellinek, 1960). Due to the predominance of alcoholism in the children of alcoholic parents, several investigators have considered that alcoholism might be an inheritable disorder like phenylketonuria, galactosemia, homocystinuria, etc., where a missing enzyme causes an inborn error of metabolism (Kalow, 1968; Lester, 1969). However, the familial tendency can be explained as easily by environmental as well as genetic factors.

At present it is difficult to prove a cause and effect relationship between alcohol's pharmacologic, pathologic and biochemical effects and the changes produced in the mood and behavior due to various contributory factors (i.e., social, cultural, psychological). The psychic alterations can vary from sedation, hypnosis, and
depression, to stimulation, mania and aggressive behavior and therefore it is difficult to correlate biochemical findings with a single central nervous system response. Chronic alcoholism also produces its own typical behavioral changes like emotional disturbances, depression, increased anxiety, loss of motor activity and sedation.

In recent years numerous studies have related the actions of psychotropic drugs, like tranquilizers, anti-depressants and hallucinatory drugs, to the changes in certain biogenic amines (norepinephrine, dopamine and serotonin) in the central and peripheral nervous systems (Schildkraut et al., 1967b, 1969a, 1970a). The role of biogenic amines in normal and abnormal behavioral states has been reviewed by Bryson (1971), Schildkraut (1969b), Schildkraut and Kety (1967a) and Sulser and Sanders-Bush (1971).

Functional disturbances of these amines have been shown in Parkinson's disease, depression, mania, and the schizophrenic reaction. A functional decrease in dopamine level in or near the substantia nigra and corpus striatum appears to be a biochemical cause of Parkinson's disease as L-dopa, a precursor of dopamine has been found to be more effective therapeutically than any other drug treatment. L-dopa presumably increases the concentration of dopamine in basal ganglia. Similarly in depression, a deficiency of norepinephrine (NE) at certain brain receptors may be a
cause as tricyclic antidepressants appear to potentiate NE at the receptor site by inhibiting its reuptake at the post-synaptic site (Schildkraut et al., 1967b). An increase in NE levels at certain brain receptors appears to be the cause of manic and hypomanic states and also in the agitation of anxiety states. Lithium salts have been effective in manic and hypomanic states, probably by decreasing the level of NE at critical receptor sites in the central nervous system, whereas chlorpromazine, an alpha-adrenergic blocker, is effective in the treatment of agitation or anxiety states. Many changes similar to the emotional effects produced by tranquilizers and stimulant drugs are also seen in alcoholics.

Among the biochemical factors contributing to ethanol action are the interlocking reactions coupled to its oxidation and the activity of its metabolites, especially acetaldehyde. It is generally accepted that ethanol is metabolized mainly in the liver but a small amount can be oxidized by other tissues such as kidney, heart (Fazekas and Rengei, 1968), lungs (Masoro et al., 1953) and brain (Sutherland et al., 1958, 1960; Raskin and Sokoloff, 1968). The metabolism of ethanol has been reviewed by Wallgren and Barry (1971), Von Wartburg (1971), Von Wartburg and Papenburg (1970) and Hawkins and Kalant (1972).
It has been shown that more than one oxidative system is involved in hepatic ethanol metabolism (Westerfeld, 1961; Von Wartburg and Papenberg, 1966). However, the most significant pathway, as shown in Figure 1, involves two dehydrogenase enzymes (Von Wartburg and Papenberg, 1966).

Figure 1 Metabolism of Ethanol

As shown above, the primary step involves the oxidation of alcohol to acetaldehyde by alcohol dehydrogenase (ADH), which is a zinc-containing enzyme that utilizes nicotinamide-adenine dinucleotide (NAD\(^+\)) as a hydrogen acceptor. Several studies have demonstrated that the
metabolism of acetaldehyde in vivo proceeded at a much greater rate than its formation from ethanol indicating that the initial oxidative step normally was the rate limiting step (Westerfeld, 1961; Jacobsen, 1952). Orme-Johnson and Ziegler (1965) reported that the mixed function oxidase system in mammalian liver microsomes, which plays a major role in hepatic metabolism of many drugs, could also catalyze the reduced nicotinamide-adenine dinucleotide phosphate (NADPH) and oxygen-dependent oxidation of methanol and ethanol to their corresponding aldehydes in vitro.

In recent studies, Lieber and DeCarli (1968, 1970a) have confirmed that hepatic microsomes were capable of actively oxidizing ethanol to acetaldehyde. This microsomal-ethanol oxidizing system (MEOS) required NADPH as a coenzyme rather than NAD and was inhibited by carbon monoxide. It was fully active at a physiological pH (7.4) and resembled the microsomal drug detoxifying enzyme system. This system was inducible by alcohol. Therefore, they suggested that it could be related to the cross-tolerance seen in alcoholics.

Rubin et al. (1971) have also reported that in female rat, chronic ethanol feeding induced changes in the absorption spectra of microsomal hemoprotein which resembled those induced by phenobarbital. Mallov and Basel (1972) have demonstrated that acute ethanol intake
caused an induction in the microsomal enzymes as the rate of elimination of some drugs metabolized by this system, was increased, but only when no ethanol was present in the system. When ethanol was present in vivo, it decreased the rates of drug elimination due to competitive inhibition of this system.

However, the significant role of MEOS in ethanol metabolism is still doubtful as a number of investigators observed a lack of parallelism between the fall of MEOS activity and the rate of disappearance of ethanol from blood (Mezey, 1971; Kalant et al., 1972). They concluded that some other factor than MEOS activity was responsible for the increased rate of ethanol metabolism.

To explain the mechanism of increased tolerance of alcohol in alcoholics, various biochemical alterations have been repeatedly sought. It seems reasonable to assume that an increase in ethanol oxidation would be reflected by a change of the corresponding enzyme activities.

Investigations on an adaptive increase of liver dehydrogenase in experimental animals exposed to alcohol for long periods of time have yielded conflicting results. Several authors have found no such increase (Figueroa and Klotz, 1962; Greenberger et al., 1965; Videla and Israel, 1970), while others have reported enhanced enzyme activities under similar conditions.
Raskin and Sokoloff (1970) have reported an adaptive increase in ADH activity during chronic ethanol ingestion in brain even though at present no concrete evidence is available that this enzyme plays a significant role in central effects of ethanol.

Videla and Israel (1970) observed no change in liver ADH activity in rats treated chronically with ethanol, yet an enhanced rate of metabolism of ethanol was observed in liver slices. However, an increase in ethanol metabolism has been reported by a number of investigators in alcoholic subjects employing either the disappearance rate of blood ethanol or the appearance of C\textsuperscript{14}O\textsubscript{2} (Truitt, 1971; Kater et al., 1969; Mendelson et al., 1965). It has been shown also that at higher blood ethanol concentrations the enzyme ADH was saturated and the disappearance curve was linear (Marshall and Fritz, 1953; Nelson et al., 1957) whereas at low blood alcohol levels (0.1mg/ml), the disappearance curve was exponential because ADH was not saturated. The discrepancy observed in ADH activity could be due to species, sex, and strain differences in the experimental animals. The observed changes were relatively small considering that large doses of ethanol administered for long periods and never reached a doubling of activity. Hence they may not be comparable.
with true enzyme induction, as can be observed for microsomal-ethanol system after prolonged administration of ethanol (Lieber and DeCarli, 1968, 1970). This latter inducibility has been offered as an explanation of alcoholic tolerance. These workers also found that chronic administration of ethanol to rats increased blood alcohol clearance in vivo, with a parallel rise in MEOS activity in vitro, but no change in ADH and catalase activity (Lieber and DeCarli, 1972). The increase in activity of MEOS in vitro corresponded to 20-25% of the in vivo rate of metabolism in the rat and could account for most of the residual ethanol metabolism after inhibition of ADH by pyrazole. They also found that one dose of barbiturate slowed blood ethanol clearance and abolished its increase induced by ethanol pretreatment, suggesting that barbiturates interfered with the metabolism of ethanol. They concluded that MEOS played a significant role in ethanol metabolism in vivo. Narrod et al. (1971) have also demonstrated increased MEOS activity after chronic ethanol ingestion. Rubin et al. (1968, 1970) have demonstrated a proliferation of the smooth endoplasmic reticulum by chronic ethanol treatment in rats and an induction of certain microsomal drug metabolizing enzymes in both man and rat. They suggested that this effect might explain why chronic alcoholics when inebriated were
less tolerant than normal individuals to barbiturates but when sober were more resistant.

However, a number of other investigators suggested a different interpretation than that of Lieber and his associates. Mezey (1971) reported that MEOS, cytochrome P-450 and rates of ethanol disappearance from blood were increased by chronic ethanol feeding whereas ADH activity remained unchanged. After withdrawal, the rates of ethanol disappearance from the blood returned to control values in 2 days, while the activities of MEOS did not fall to control values until 7 days. They suggested that due to a lack of parallelism in the fall of the rates of ethanol disappearance from the blood and microsomal enzymes, some other factor than the enhanced activity of MEOS was responsible for the increased rate of ethanol metabolism after ethanol feeding.

Khanna et al. (1972) have corroborated the findings of increased MEOS activity and faster clearance of blood ethanol in rats after chronic ethanol feeding in a liquid diet similar to that used by Lieber and DeCarli (1968). However, when the protein content of the diet was increased to provide 25% rather than 18% of total calories, the rate of ethanol metabolism remained elevated, but MEOS activity in vitro did not. Finally the chronic administration of phenobarbital and other inducers of drug metabolism and of MEOS activity were found to have no
effect on the disappearance of ethanol from the blood and the whole body (Khanna et al. 1970; Khanna et al., 1972). Chronic administration of ethanol similarly failed to affect the rate of phenobarbital metabolism in vivo or by liver slices (Kalant et al., 1970b). These investigators (Khanna et al., 1971) have also studied the effect of carbon tetrachloride (CCl₄) alone and with a single dose of ethanol. They observed a 50% decrease in MEOS activity 6 hours after administration of CCl₄. This decrease amounted to 60% at 20 hours with identical CCl₄ treatment, but there was no change in ADH activity. At 20 hours, there was no significant effect of CCl₄ on the rate of ethanol uptake by liver slices or on the rate of metabolism in vivo as measured in whole body or as estimated from the rate of decrease of blood ethanol concentration. Therefore they concluded that MEOS did not play a significant role in ethanol metabolism in vivo or in the cross-tolerance between ethanol and other drugs (Khanna et al., 1970, 1971).

The evidence for a significant quantitative role of the MEOS system after acute or chronic ethanol administration is yet to be determined and the discrepancy observed may be due to the differences in species or strains used.

In chronic ethanol drinking, the role of catalase in ethanol metabolism has also been considered. Its role
in acute ethanol metabolism was negligible as the rate of ethanol metabolism was virtually unaffected by treatment with the catalase inhibitor, 3-amino-1,2,4-triazole (AT). However, Aebi and Von Wartburg (1960) did observe an increase in catalase activity after chronic ethanol administration, but a number of other investigators have found no increase in hepatic levels after similar treatment (Kinard and Hay, 1960; Hawkins et al., 1966). It has been suggested that the increase observed in MEOS activity in vitro after chronic alcohol administration was due to contamination with catalase activity. Lieber and DeCarli (1970) have shown that the increase in MEOS produced by ethanol intake was not abolished by AT (catalase inhibitor). They concluded that this further differentiated MEOS activity from catalase. The quantitative importance of this pathway still remains doubtful.

Multiple metabolic pathways are open to acetaldehyde but the oxidative metabolism through aldehyde dehydrogenase predominates in the liver. In the second step, acetaldehyde is converted to acetyl CoA by aldehyde dehydrogenase which is an NAD-dependent enzyme. This enzyme has been found in many mammalian tissues such as liver, brain, kidney (Deitrich and Hellerman, 1963). The enzyme was present in the soluble, mitochondrial and microsomal fraction (Erwin and Deitrich, 1966). They have
calculated that 60% of the total activity of acetaldehyde dehydrogenase resided in the mitochondrial fraction in brain.

In a recent study, it has been shown that 80% of the total activity of aldehyde dehydrogenase in liver was present in the mitochondrial fraction and only 20% in the cytoplasm (Marjanen, 1972). He observed no activity in the microsomal fraction of liver.

It has been demonstrated that tetraethylthiuram disulfide (disulfiram or Antabuse) inhibited aldehyde dehydrogenase activity (Jacobsen, 1950; Erwin and Deitrich, 1966). This inhibition was due to competition for NAD\(^+\).

It is not yet known whether the formation of acetyl CoA from acetaldehyde is through a direct pathway or by means of acetate. A small percentage of resulting acetyl CoA undergoes an intermediate metabolism but most of the acetyl CoA formed after ethanol administration was oxidized through citric acid cycle to CO\(_2\) (Himwich, 1956; Casier and Polet, 1958). Using labeled ethanol it has been shown by a number of workers that within 1 or 2 hours after the disappearance of free ethanol from the organism, about 85% of the ethanol administered had been oxidized to CO\(_2\) (Bartlett and Barnet, 1949; Masoro et al., 1953).
Fatty infiltration of liver as a complication of alcoholism has been known for a long time. About 15 years ago, Mallov (1957) reported that administration of a single intoxicating dose of alcohol caused a significant increase in the lipid content of the liver in rats.

Since then many investigators have demonstrated that the liver triglycerides were increased after a single large dose of ethanol in rats (DiLuzio, 1958; Horning et al., 1960; Ammon et al., 1966). The interactions of ethanol and lipid metabolism has been reviewed by Lieber (1971) and Wallgren and Barry (1971).

Isselbacher (1966) demonstrated that when alcohol was administered to man, the first change observed was an increase in the plasma triglycerides. This was followed, as the blood alcohol levels increased, with a gradual decrease in triglycerides to normal levels and a concomitant and often marked increase in plasma free fatty acids which appeared to be derived from adipose tissue. Furthermore, he found that the lipids which accumulated in the liver under these circumstances were triglycerides.

Domanski et al. (1971) and Johnson et al. (1971) studied the effect of a lethal dose of ethanol (6 g/kg) and pyrazole on liver triglycerides. They observed that this
dose of ethanol caused an accumulation of hepatic triglycerides but when pyrazole was injected prior to ethanol administration, it prevented accumulation of triglycerides. However, these results have not been confirmed by Bustos et al. (1971) as pyrazole did not prevent the fat accumulation in liver.

The effect of chronic administration of ethanol on hepatic triglycerides has been studied by Mendenhall et al. (1969), Rubin et al. (1968) and Kalant et al. (1972). They showed that the chronic administration of ethanol caused accumulation of triglycerides in the liver. Kalant et al. also reported that pyrazole administration increased the hepatic triglyceride accumulation. They concluded that as pyrazole by itself decreased hepatic triglycerides, the mechanism of synergism between ethanol and pyrazole could not be ruled out. Ylikhari (1970) reported that the hepatic alpha-glycerophosphate (α-GP) concentration and α-GP/dihydroxyacetone phosphate ratio were significantly increased 1 to 8 hours after ethanol administration. They suggested that the increased α-GP concentration, which is known to favor esterification of fatty acids to triglycerides played a role in the pathogenesis of acute ethanol-induced fatty liver.

Studies were performed by Gordon (1972) to show that ethanol directly enhanced the synthesis of fatty acids from two carbon sources in the perfused rat liver preparation
and their esterification to triglycerides. Bustos et al. (1971) studied the effects of chronic administration of ethanol on α-GP levels. The concentration of α-GP was not elevated even though triglyceride levels were increased. They concluded that the primary factor responsible for stimulation of esterification was something other than the raised levels of triglyceride precursors.

It has also been shown that in rats given one large sublethal dose of ethanol, moderate hepatic accumulation of triglycerides was observed, with a composition resembling that of adipose tissue (Brodie et al., 1961; Lieber and DeCarli, 1966). This has been reviewed by Lieber (1967). Poggi and DiLuzio (1964) have demonstrated that in rats, transfer of fatty acids from epididymal fat labeled in vivo was markedly enhanced by ethanol and epididymal fat depots decreased simultaneously with an increase in liver triglycerides (Ammon et al., 1966). Chronic administration of ethanol decreased oxidation of unsaturated fatty acids in isolated mitochondria (Toth et al., 1970).

Kudzma and Schonfeld (1971) have shown that in chronic alcoholics, hypertriglyceridemia was ethanol-dependent, whereas in nonalcoholic subjects, it was carbohydrate inducible and not by ethanol.

Ylikahri (1970) reported that a single dose of ethanol in rats caused an increase in serum triglyceride levels. Ethanol also increased lipoprotein lipase activity
(Mallov and Cerra, 1967) which accelerates the breakdown of triglycerides to free fatty acids and glycerol. Sympathetic and adrenal stimulation are known to increase plasma free fatty acids (FFA), therefore this lipolytic action of ethanol may be attributed to the release of catecholamines (Klingman and Goodall, 1957; Perman, 1961). Mallov and Cerra (1967) demonstrated the beta-adrenergic blocking agents inhibited the effect of ethanol on lipoprotein lipase activity whereas several alpha-adrenergic blocking agents did not prevent this effect. Also this increase in lipase activity was not seen in reserpine-treated adrenalectomized rats. It has also been shown that both mobilization of FFA and accumulation of triglycerides in liver were blocked by adrenalectomy (Mallov and Gierke, 1957) and by alpha-adrenergic blocking agents (Brodie et al., 1961) and by beta-adrenergic blocking agents (Estler and Ammon, 1967). Brodie et al. (1961) observed that dibenamine in 50 mg/kg given 24 hours and 48 hours prior to the alcohol dose blocked both the mobilization of FFA and the accumulation of triglycerides in the liver.

Hawkins and Kalant (1972) have recently reviewed the metabolic actions of ethanol. Ethanol also induces hyperglycemia. It has been reported that human subjects had initially showed a hyperglycemia after drinking 150 ml of whiskey but hypoglycemia was encountered later on
(Vartia et al., 1960). It was concluded that an initial rise in blood sugar after ethanol probably reflected glycogenolysis, while later on, when glycogen stores were depleted, ethanol ingestion caused hypoglycemia. Perman (1962a,b) has demonstrated that in rabbits, infusion of low doses of ethanol induced hyperglycemia and the elevation could be prevented by ganglionic blockade, suggesting that hyperglycemia after ethanol was probably due to increased catecholamine secretion from the adrenal medulla.

The other metabolic changes seen after acute or chronic administration of ethanol in liver were the decrease in $\text{NAD}^+/\text{NADH}$ ratio (Kalant et al., 1972; Krebs, 1968; Mendelson, 1970; Videla et al., 1970; Veech et al., 1972). This caused a rise in lactate-pyruvate ratio (Krebs, 1968). Kalant et al. (1972) reported that chronic intake of ethanol decreased the ratio of $\text{NAD}^+/\text{NADH}$ as occurred on acute treatment. However the change produced by chronic administration of ethanol was not adaptive as the ratio returned to normal after ethanol withdrawal.

Ammon and Estler (1968) showed that intravenous injection of ethanol in mice caused a decrease in brain and liver glycogen and glycogenolysis in liver was inhibited by beta-adrenergic blocking agents. This effect of ethanol can be attributed to release of catecholamines
which increases glycogenolysis by stimulation of the adenyl cyclase system.

Many investigators have shown that in rats and in mice, ethanol produced changes in the respiratory metabolism in brain. This has been reviewed by Israel (1970a). Heim et al. (1965) showed that in mice when an intravenous dose of ethanol (1.5 g/kg) was given and brain ethanol levels were 0.126%, it caused an increase in cerebral contents of creatine phosphate and glucose, and a decrease in glycogen, pyruvate, lactate, and coenzyme A. These same authors found further that with a larger dose of ethanol, there was an increase in fructose-1-6-diphosphate and adenosine monophosphate (AMP) and a decrease in adenosine diphosphate (ADP). During severe ethanol intoxication, adenosine triphosphate (ATP) and the ratio of ATP/ADP was increased in the brains of mice and rats (Ammon, 1968; Ammon et al., 1966). This suggested a decrease in the rate of utilization of energy-rich phosphate (in glycolysis and in citric acid cycle). Truitt et al. (1956) have demonstrated that ethanol was not an uncoupling agent for rat brain mitochondria whereas acetaldehyde in concentrations equal to those produced in man during the disulfiram-alcohol reaction, did lower the ratio of oxidative phosphorylation to oxygen consumption (Rehak and Truitt, 1958).
Ethanol effects are also seen on amino acid metabolism which is closely related to the TCA cycle. The findings on gamma-aminobutyric acid (GABA) concentration are still controversial as some workers find an increase in GABA content (Hakkinen and Kulonen, 1959, 1961) whereas Ferrari and Arnold (1961), Flock et al. (1969) and Gordon (1967) observed no change or a decrease in GABA content. These discrepancies have not yet been resolved concerning possible differences between strain and species. Hakkinen and Kulonen (1959, 1961, 1972) reported that in rats ethanol intoxication caused an increase in the content of glutamate and aspartate but a decrease in glutamine and GABA catabolism.

Adrenergic Actions of Acetaldehyde

Certain symptoms of "hangover" after drinking such as headache, sweating, nausea and vomiting have been attributed to acetaldehyde (Himwich, 1956). The blood acetaldehyde levels were reported to be higher in alcoholics than in nonalcoholics (Truitt, 1971). Brain concentrations of acetaldehyde or ethanol may be more significant for the central nervous system effects of alcohol than blood levels although they are undoubtedly proportionate. Duritz and Truitt (1966) demonstrated that in disulfiram pretreated rats, both acetaldehyde and
ethanol levels in the brain reached the peak value at 90 minutes but acetaldehyde levels decreased more slowly than did ethanol levels.

It has been shown that the highest concentration of acetaldehyde was in cerebellum which also contained mitochondria with the greatest sensitivity to acetaldehyde in vitro (Kiessling, 1962a,b). To explain the mechanism of ethanol-induced hyperglycemia, Kohei (1967a) studied the action of acetaldehyde which is the first metabolite of ethanol. He showed that an intravenous injection of acetaldehyde in rabbits caused a marked rise in blood glucose, pyruvic acid and alpha-ketoglutarate levels. In rats, a hyperglycemia and glycogen depletion in the liver was produced 10 minutes after acetaldehyde injection. He suggested that acetaldehyde effects on catecholamines could be responsible for this effect. Further studies showed (Kohei, 1967b) that propranolol blocked the acetaldehyde-induced hyperglycemia, whereas phenoxybenzamine did not. Rabbits that had been treated with reserpine did not show a hyperglycemic response to acetaldehyde.

Truitt et al. (unpublished) demonstrated that relatively small doses of acetaldehyde (200-300 mg/kg) produced a rise in plasma FFA levels in 15 minutes that was effectively blocked by 1 hour pretreatment with propranolol. Similarly, they found that acetaldehyde injection could
significantly increase hepatic triglyceride levels, as did ethanol (Truitt et al., 1966).

Acetaldehyde has been shown to interfere selectively with pyruvate-stimulated oxidative phosphorylation in mitochondria (Truitt et al., 1956; Rehak and Truitt, 1958; Beer and Quastel, 1958a,b). Ammon et al. (1969) demonstrated that acetaldehyde in low concentration inactivated coenzyme A in brain but much higher concentration of acetaldehyde was required for liver. Ethanol had no effect on brain coenzyme A activity. They concluded that the action of ethanol on the coenzyme was mediated by acetaldehyde.

Akabane et al. (1965) and Schneider (1969) perfused the adrenal medulla with acetaldehyde. They observed a release of norepinephrine (NE) and epinephrine (Epi) which was closely related to the action of acetaldehyde on the membrane storage sites. This action of acetaldehyde was not blocked by hexamethonium or atropine. Acetaldehyde and ethanol plus disulfiram elevated plasma levels of $\text{H}^3$-NE to a greater extent than ethanol alone (Walsh and Truitt, 1968). In the reserpinized cat depleted of catecholamines, acetaldehyde did not elicit its normal sympathomimetic effect (Akabane et al., 1965; Eade, 1959).

More recently, Schneider (1971) demonstrated that in isolated perfused cow adrenal medullae that catecholamine secretion induced by acetaldehyde was dose-dependent and
was not prevented by hexamethonium, tetracaine or by depletion of calcium from the perfusion fluid. Acetaldehyde did not release the protein from chromaffin granules or other cell constituents. They concluded that acetaldehyde caused catecholamine release was by a direct action on the chromaffin granules and that the catecholamines then diffuse from the cell into vascular system.

Actions of acetaldehyde on heart have been studied by Nakano and Kessinger (1972a) and Nakano and Prancan (1972b). They observed that in dogs acetaldehyde always increased heart rate, systemic arterial pressure and myocardial contractile force. This action was dose-related and similar changes were found with low blood ethanol levels (3 mg/ml or less) but with higher levels of ethanol, these parameters decreased. They observed results similar to acetaldehyde when higher doses of synthetic bourbon were used. These actions of acetaldehyde were blocked by propranolol (positive chronotropic and inotropic action) and phenoxybenzamine (pressor action). They concluded that these actions on the cardiovascular system were due to the release of catecholamines.
Effects of Ethanol or Acetaldehyde on Biogenic Amine Metabolism

Historically the first change observed in biogenic amines after ethanol ingestion was the increased excretion of catecholamines in alcoholics (Olson et al., 1960; Gursey and Olson, 1960). They also observed an decreased NE level in rabbit brain after ethanol infusion. The changes caused by ethanol on biogenic amine metabolism were studied later.

It was shown that in man, acute ethanol intoxication resulted in a decrease in urinary excretion of 5-hydroxyindoleacetic acid [5-HIAA] (Rosenfeld, 1960). He also demonstrated that ethanol caused a decrease in the oxidative metabolism of injected serotonin (5-HT) in the mice treated with ethanol (4-5 g/kg) prior to killing.

Maynard and Schenker (1962) found that ethanol inhibited decarboxylation of 5-hydroxytryptophan (5-HTP) in mice in vivo and suggested that the formation of 5-HT might be inhibited by ethanol. Feldstein et al. (1964) reported that in man, ethanol produced a change in serotonin metabolism. In 1965 these workers studied the metabolism of C\textsuperscript{14}-labeled 5-HT in rat liver homogenate with added NAD or NADH (Feldstein et al., 1965). They observed that 80% of 5-HT appeared as 5-HIAA and less than
10\% as 5-hydroxytryptophol (5-HTOH) when NAD$^+$ was present whereas in the presence of NADH (coenzyme for alcohol dehydrogenase), the metabolism was reversed. Davis et al. (1968) studied the distribution of metabolites in the urine after C$^{14}$-5-HT injection in man. The total excretion of 5-HIAA plus 5-HTOH was unchanged but the proportion of C$^{14}$ excreted as 5-HTOH was increased from 2\% in controls to 42\% after ethanol.

Studies were performed by Maynard and Schenker (1962) on the action of ethanol on monoaminoxidase (MAO) activity. They reported that in vitro 0.1 to 4\% ethanol inhibited liver MAO only slightly but did not affect the mouse brain MAO activity. In 1964 Towne demonstrated that both ethanol and acetaldehyde inhibited rat liver and brain MAO, but the degree of inhibition was marked only in the presence of acetaldehyde in the brain homogenate (36\% with acetaldehyde and only 1\% with ethanol). Lahti and Majchrowicz (1967) studied the effect of acetaldehyde on MAO activity using rat liver preparation. They observed that an initial concentration of 1 mM of acetaldehyde was required to inhibit oxidation of 5-hydroxyindolacetaldehyde to 5-HIAA. They also observed that an excess of NAD$^+$ in the system did not shift the oxidative pathway and there was no effect on MAO activity. They postulated that the shift in 5-HT metabolism was due
to competitive inhibition of aldehyde dehydrogenase by acetaldehyde, the metabolite of ethanol.

In 1967, Feldstein et al. demonstrated marked decrease in $^{14}$C-5-HIAA excretion after administration of various alcohol doses in man. They assumed that the observed decrease in 5-HIAA was due to an acceleration of reductive pathway for the formation of 5-HTOH and its conjugates which they did not measure. They believed that the most plausible explanation for this effect was the redox shift as a result of ethanol oxidation. This redox shift in liver as a consequence of acetaldehyde and acetate formation has been established by many investigators (Cherrick et al., 1965; Kalant et al., 1970a, 1972; Veech et al., 1972). Davis et al. (1967a) confirmed the findings of Feldstein and co-workers (1967) as they observed a definite increase in urinary 5-HTOH formation and concomitant decrease in 5-HIAA after ethanol consumption in man.

In rat liver preparation (both homogenates and slices) ethanol induced the formation of 5-HTOH (Feldstein et al., 1965; Eccleston et al., 1969) but observed no such change in brain slices (Tyce et al., 1968; Eccleston et al., 1969). These authors suggested that the changes seen by Davis and her co-workers were due to peripheral changes in 5-HT metabolism. Tyce et al. (1968) showed that ethanol had no effect on the metabolism
of labeled 5-HT injected directly into the brain of rats. However, Feldstein and Sidel (1969) demonstrated that in rat brain, multiple doses of ethanol increased the ratio of 5-HIAA and/or 5-CTOH to 5-HIAA. Also high doses of ethanol, 6 g/kg slightly inhibited decarboxylase and MAO activity but not with lower dose of ethanol (4 g/kg). They suggested that acetaldehyde might be the responsible agent. It is also been shown that the alteration in 5-HT metabolism was not necessarily due to changes in NADH levels in brain mitochondria but could be due to inhibition of aldehyde dehydrogenase by acetaldehyde (Lahti and Majchrowicz, 1969).

Palaic et al. (1971) have reported that the brain 5-HIAA level was decreased during acute and chronic ethanol treatment. However, Ahtee (1972) has shown that 7 g/kg of chronic ethanol ingestion in free choice situation in two selected strains of rats (with a preference for ethanol or water) did not cause increase in brain 5-HIAA.

A number of investigators have studied the alteration in NE metabolism after ethanol in man and in animals as it seems to cause a stress-like effect which increases the urinary excretion of catecholamines. In 1958, Perman observed that an ingestion of moderate doses of ethanol increased Epi in man while NE excretion did not differ from that obtained in control subjects. Similar increases in Epi level was observed in intact animals after acute
sublethal alcohol intoxication (Perman, 1960; Klingman and Goodall, 1957). However, they also observed an increased excretion of NE which was not effected by adrenalectomy. Smith and Wortis (1960a,b) and Smith and Gitlow (1967) were the first to show that ethanol altered the metabolism of infused dl-7-H\textsuperscript{3}-NE in man and animals, as it was shifted from the oxidative towards the reductive pathway resulting in decreased vanillyl mandelic acid (VMA) and increased 3-methoxy-4-hydroxyphenylglycol (MHPG). They also observed that when large amounts of ethanol were given, the excretion rate of unchanged NE during the first hour collection was nearly twice that of controls. They postulated that catecholamines released by ethanol "spare" degradation of the isotopic species resulting in its increased excretion. Anton (1955) has also reported a significant increase in urinary NE, dopamine and metanephrine but not in the levels of normetanephrine (NMN). Amine excretion in alcoholic patients has been studied by Schenker et al. (1967), Mendelson (1967) and Mendelson et al. (1969). Mendelson et al. found a significant increase in excretion of NE, NMN, Epi, and metanephrine during alcohol ingestion with a dose-response relationship to blood ethanol level. Schenker et al. (1967) observed that alcoholics excreted a significantly lower amount of tryptamine after ingestion of a standard dose of ethanol.
than non-alcoholics. However, Davis and her co-workers (1967) observed similar results with C\textsuperscript{14}-NE metabolism, but failed to find an alteration in excretion pattern of C\textsuperscript{14}-NE, C\textsuperscript{14}-NMN or total C\textsuperscript{14} after ethanol ingestion.

To show whether the alteration in catecholamine metabolism was due to ethanol per se or its metabolite acetaldehyde, Walsh and Truitt (1969), Walsh et al. (1970) studied in vivo the metabolic degradation of C\textsuperscript{14}-NE after ethanol or acetaldehyde in the rats. Ethanol by itself caused only a slight and insignificant shift in C\textsuperscript{14}-NE metabolism. Since it has been shown in vivo that ethanol administration decreased rat liver NAD\textsuperscript{+}/NADH ratios as a consequence of its oxidation, the absence of an effect of ethanol on C\textsuperscript{14}-NE metabolism strongly indicated the implication of an acetaldehyde-mediated mechanism. This was confirmed when acetaldehyde alone or ethanol plus disulfiram was injected as both of these compounds caused a marked decrease in C\textsuperscript{14}-VMA excretion, with compensatory rise in C\textsuperscript{14}-MHPG. These authors attributed the shift towards formation of reduced metabolites from catecholamines more to the competitive inhibition of aldehyde dehydrogenase by acetaldehyde than to the redox shift.

Majchrowicz et al. (1967) have estimated that the concentration of acetaldehyde present in blood after ethanol is sufficient to cause this shift.
Effects of Ethanol on Active Transport of Na\(^+\) and K\(^+\)

It has been shown that rat brain microsomal Na\(^+\)-, K\(^+\)-activated adenosine triphosphatase (ATPase) activity was inhibited by ethanol (Israel et al., 1965, 1966, 1967, 1970b; Sun and Samorajski, 1970). Israel et al. (1965) observed that the inhibition of microsomal Na\(^+\)-, K\(^+\)-activated ATPase activity by ethanol was dose-dependent and it was competitive with respect to K\(^+\) whereas the increase in Na\(^+\) concentration led to increased inhibition by ethanol. They also found that an injection of KCl solution counteracted the intoxicating effect of ethanol in rats. They suggested that inhibition of active transport played an important role in ethanol intoxication in vivo. Later on these workers studied the effect of ethanol on K\(^+\) transport in cerebral cortex slices. Ethanol inhibited the uptake of K\(^+\) in cortex slices and at the same concentration, it caused inhibition of Na\(^+\)-, K\(^+\)-activated ATPase activity in brain microsomes. Other alcohols and general depressants have been shown to produce the same effects as ethanol on the active transport of K\(^+\) and on the (Na\(^+\) + K\(^+\))-ATPase. Furthermore, their inhibitory potencies were correlated with their activities as depressants in vivo (Israel et al., 1966, 1967).
It has been reported that nerve ending fractions from guinea-pig cerebral cortex contained more than one-half of the Na\(^+\) + K\(^+\)-stimulated ATPase which was inhibited significantly to a greater extent than Mg\(^{2+}\)-activated ATPase or acetylcholine esterase (AChE) by ethanol in the concentration ranging from 0.043 to 2.57M (Sun and Samorajski, 1970). They suggested that these enzymatic changes at the synaptic level might be responsible for behavioral changes commonly seen after alcohol drinking.

Chronic administration of ethanol to rats, resulted in an increase in the rate of K\(^+\) and Rb\(^{86}\) accumulation by brain cortex slices in vitro (Israel et al., 1970b). They also observed that there was an increase in Na\(^+\) + K\(^+\)-stimulated ATPase in brain homogenates which returned to normal values with alcohol cessation. Ethanol added in vitro had no inhibitory effect on cation transport. They observed similar changes in ATPase activity in chronic alcoholics. They concluded that changes in cation transport and ATPase activity were the adaptive changes due to chronic ethanol intake.

Interactions of Ethanol or Acetaldehyde on Neurotransmitters

Kalant et al. (1967a,b) studied the effect of ethanol on synthesis, and release of acetylcholine (ACh) in rat or guinea-pig cerebral cortex slices. They observed that the
concentration of ethanol that could produce moderate to severe intoxication in vivo, did not affect the synthesis of acetylcholine but caused a significant reduction in the release of ACh from unstimulated cortex slices. Ethanol had no additional effect on release of ACh when 15 or 27 mM K\(^+\) was present in the medium. They concluded that the effect of ethanol in vivo might be due in part to an inhibition of ACh release at central cholinergic synapses. They also observed that brain slices from animals made tolerant to alcohol were refractory to the in vitro effect of ethanol on ACh release.

The effect of ethanol on biogenic amines in brain is still controversial as some investigators reported a decrease in amine levels whereas others showed an increase or no change. Gursey and Olson (1960) observed a decrease in 5-HT and NE in the brain stem of rabbits after ethanol infusion and on chronic administration whereas Bonnycastle et al. (1962) observed an increase in brain 5-HT of rats. Gursey and his co-workers claimed that the effect of ethanol lasted several days, therefore they compared this action to reserpine. They proposed amine depletion as a mechanism for the sedative, hypnotic and mood depressant actions of ethanol. However, these findings were not confirmed in the brains when both rabbit and rats were used (Efron and Gessa, 1963; Pscheidt et al., 1961; Duritz and Truitt, 1963, 1966;
Corrodi et al., 1966; Bhagat et al., 1970). Pscheidt et al. observed that peroral administration of 4 or 6 g/kg ethanol in rabbits decreased NE content of the brain stem only by 20% and five daily injections of 3 g/kg ethanol decreased 5-HT by 19%.

Tyce et al. (1970) studied the effect of intoxicating dose of ethanol on 5-HT turnover in rat brain. Ethanol had no effect on the concentration of 5-HT but the turnover of cerebral 5-HT was modestly decreased during ethanol intoxication.

Recently Kuriyama et al. (1971) studied the effect of acute and chronic administration of ethanol on 5-HT turnover and tryptophan hydroxylase activity in mouse brain. Their results clearly indicated that the effect of chronic ethanol administration on the cerebral 5-HT biosynthesis was distinct from acute ethanol effect. Chronic administration of ethanol increased the rate of biosynthesis of 5-HT and tryptophan hydroxylase activity in the brain whereas with a single dose of ethanol, there was a significant decrease in 5-HT biosynthesis rate and no change in tryptophan hydroxylase activity. They suggested that the increase of 5-HT biosynthesis might be partially a reflection of the continued stimulatory effect of ethanol on the pituitary-adrenal system as corticosterone levels were increased.
In the same year, Palaic et al. (1971) observed a significant increase in rat brain 5-HT levels after acute ethanol treatment but it remained unchanged during chronic treatment. Their subcellular distribution studies showed that acute ethanol treatment decreased 5-HT in high-speed sediment fraction, while increasing it in the supernatant. The content of 5-HT in nerve ending fractions was also lowered from 72% to 26% of the total 5-HT found in the brain homogenate. They suggested that ethanol depleted endogenous 5-HT from its storage site. Reichle et al. (1970) also showed that when rats were given multiple doses of ethanol at 12-hour intervals, the brain 5-HT levels were significantly increased and dopamine content was decreased whereas NE concentration remained unchanged.

In 1966, Corrodi et al. observed no significant change in brain catecholamines after ethanol administration, but in the animals receiving alpha-methyl-para-tyrosine, an inhibitor of NE and dopamine synthesis, along with ethanol there was a significant decrease in NE but not in dopamine in the brain when compared with its control. They concluded that the central NE neurons were specifically activated, directly or indirectly after acute ethanol administration; and this increased release of NE was revealed only after inhibition of synthesis. At the same time, Duritz and Truitt (1966) also reported that ethanol caused a small decrease in 5-HT and NE levels in
rat brain whereas acetaldehyde administered alone or produced in a high concentration from ethanol in the presence of disulfiram, caused a much greater decrease in NE content. They also showed that the effect of ethanol plus disulfiram on the brain NE could be blocked by chlorpromazine administration prior to ethanol injection. They suggested that acetaldehyde might be responsible for the depletion of NE in the brain.

Scope of the Present Work

There is enough evidence to indicate that ethanol or acetaldehyde interacts with biogenic amines stores. The increase in urinary excretion of catecholamines after ethanol ingestion, the marked changes in brain catecholamines seen in the presence of synthesis inhibitors and the indication that some adrenergic effects on heart can be blocked by beta-adrenergic blocking drugs are examples of such interactions. As the literature regarding the equilibrium level of biogenic amines in brain is still controversial, it seems reasonable that a study of turnover rate under steady state conditions could clarify some of this confusion.

The turnover study often permits the recognition of significant changes in biogenic amine synthesis and catabolism even when no change in equilibrium levels is seen. This type of study has shown that acute ethanol
treatment can reduce serotonin turnover slightly when a MAO inhibitor is used, whereas chronic intake of ethanol can elevate turnover rates of serotonin. These reports led us to investigate the acute and chronic effects of ethanol on catecholamine turnover rates in the brain. Since acetaldehyde has been shown to be directly responsible for catecholamine depletion in the brain, therefore its action on catecholamine turnover rates has been included in the present study.

The changes seen in liver, i.e., a fat deposition after chronic ethanol ingestion, have been attributed to an increase in fat mobilization from adipose tissue. Since it is possible that acetaldehyde is involved in this interaction, it was decided to study this possible mechanism by measuring the changes in plasma free fatty acids and liver triglycerides after chronic ethanol intake.
METHODS

In Vivo Release and Metabolism of $^3$H-Norepinephrine

A. General Procedures

Male Wistar rats weighing 140-150 g were obtained from Laboratory Supplies Farm, Indiana. The rats were given Purina Chow diet and water ad lib for at least 5-7 days before they were used for any experiments.

B. Treatment Schedules

Figure 2 shows the outlines of treatment schedules. The rats, fasted for 1 hour or non-fasted, were divided into two groups and anesthetized lightly with ether. One group of rats received 0.114 µg dl-7-$^3$H-norepinephrine (3.0 µc, specific activity 4.18 C/mM, obtained from New England Nuclear) in 20 µl Merle's solution intracisternally by the method described by Schanberg et al. (1967), whereas in the second group 0.40 µg (10 µc) dl-7-$^3$H-norepinephrine was administered by intracisternal injection (Glowinski and Axelrod, 1965; Schanberg et al., 1967).

The rats which were injected with 3.0 µc of $^3$H-NE, were further divided into four groups. One hour after the
FIGURE 2
Schematic treatment schedules for release and metabolism of $^3\text{H}$-NE \textit{in vivo}. 
Groups of Non-Fasted or 1 hour Fasted Rats

Received an intracisternal injection of 0.114 μg of dl-7-H\textsuperscript{3}-NE (3.00 μc, specific activity 4.18 C/mM) in 20 μl Merle's solution.

Received an intracisternal injection of 0.41 μg dl-7-H\textsuperscript{3}-NE (10 μc, specific activity 4.18 C/mM) in 20 μl Merle's solution.

Fasted

1 hr

EtOH Saline

4 g/kg

1 hr later

Non-Fasted

1 hr

Ach Saline

300 mg/kg

30 min

Ach Saline

300 mg/kg

30 min later

3 hrs

Fasted

5 hrs

Non-Fasted

19 hrs

Fasted

21 hrs

Saline

3 hrs

Saline

5 hrs

AcH Saline

300 mg/kg

30 min

AcH Saline

300 mg/kg

30 min later

EtOH Saline

4 g/kg

1 hr later

AcH Saline

300 mg/kg

30 min

AcH Saline

300 mg/kg

30 min later

Animals Killed

1. Brains analyzed for H\textsuperscript{3}-NE, H\textsuperscript{3}-NMN, and endogenous NE.

2. Blood samples analyzed for ethanol, and acetaldehyde levels (2 and 22 hours).
administration of $^{3}H$-NE, one group of fasted animals received an oral dose of ethanol, 4 g/kg as 20% w/v in saline whereas the control group received an oral dose of saline.

A second group of non-fasted animals were injected intraperitoneally with acetaldehyde, 300 mg/kg as 2% solution in saline. The control group received saline by an intraperitoneal injection. Thirty minutes later a second dose of acetaldehyde or saline was given. One hour from the first dose of acetaldehyde or after the oral dose of ethanol, the rats were killed by decapitation and the brains were rapidly removed, rinsed in ice cold saline, damp dried on filter paper, and then frozen in liquid $N_2$. The frozen brains were stored at $-20^\circ C$ until ready for analysis. The blood samples were collected in cold heparinized tubes and blood ethanol and some acetaldehyde levels (2 and 22 hours) were determined as described on page 40.

The remaining two groups of animals which had received 3.0 $\mu$c of $^{3}H$-NE were given the following treatment:

Three hours from the administration of $^{3}H$-NE, the rats in one group were fasted for 2 hours, then given an oral dose of ethanol or saline as described above. Five hours from the administration of $^{3}H$-NE, the second group of non-fasted animals were injected intraperitoneally with two doses of acetaldehyde or saline 30 minutes apart as
described previously. All these animals were killed by decapitation at the end of 1 hour from the drug treatment. The brains and blood samples were collected as described previously.

Nineteen hours from the administration of 10 μc H^3-NE, the rats were divided into two groups. One group was fasted for 2 hours and then given either an oral dose of ethanol or saline as described previously. The second group of non-fasted rats were injected with two doses of acetaldehyde intraperitoneally 30 minutes apart at the end of 21 hours from H^3-NE injection. The control rats were injected with saline. All these animals were killed 22 hours from the administration of H^3-NE. The brains and blood samples were collected as described previously.

Gas Chromatographic Determination of Ethanol and Acetaldehyde

A. General Procedures

Gas-liquid chromatography was used for the simultaneous determination of ethanol and acetaldehyde in the biological fluid. The method was originally published by Duritz and Truitt (1964), and has been modified both to avoid the problem of spontaneous acetaldehyde release (Truitt, 1970) and to analyze tissues as well as blood. An F-M Model 400 programmed gas chromatograph was used for the analysis with a Model 1609 hydrogen flame
ionization detector and a 1 mV Honeywell Brown Electronik recorder. The column measuring 0.25 inches in diameter and four feet in length was U-shaped and packed with 5% Carbowax 1500 on Haloport 60-F. New columns were always preconditioned for at least 18 hours at a temperature of 130°C.

The instrument was operated isothermally under the following conditions:

1. Column oven temperature 70°C.
2. Flame monitor temperature 120°C.
3. Injection port temperature 105°C.
4. Detector temperature 100°C.

Helium was used as the carrier gas at a flow rate of 35 ml/minute; the flow rate of H₂ was 23 ml/minute, while that for compressed air was 330 ml/minute; all conditions are 50 p.s.i.

Standards were prepared from stock solutions of ethanol (absolute alcohol in ampoules, Abbott, list no. 3772) and acetaldehyde (redistilled in nitrogen-sealed ampoules, Matheson, Coleman and Bell) prepared as a 2% solution in isotonic saline. Daily solutions were prepared in isotonic saline to obtain concentrations of ethanol, 2000 µg/ml, and acetaldehyde, 20 µg/ml, which were used as standards and were interspersed among experimental samples to correct for any changes in the detector sensitivity are read at the beginning of the
samples and the second set at the end of the samples. The peaks were obtained on range 10 attenuation 32 for ethanol and range 1 attenuation of 2 for acetaldehyde. All samples for analysis were deproteinized with 5% ZnSO₄ and 0.3 N Ba(OH)₂. The samples for analysis were prepared in 5 ml glass vials fitted with serum rubber stoppers. Samples were equilibrated in an Aminco-Dubnoff constant temperature water bath at 55°C. After 15 minutes at 55°C, the head space air of the vials was sampled by a pre-warmed plastic tuberculin syringe. A 500 μl air sample was equilibrated in the syringe by moving the plunger up and down at least 8-10 times and was then rapidly transferred to the injection port of the gas chromatograph. This method has general applicability for the determination of several volatile substances including a separate peak for acetone. Using the column specifications reported here, it has been shown that ether would interfere with acetaldehyde peak, whereas isopropyl alcohol and chloroform would interfere with the ethanol peak. Therefore, acetaldehyde measurements were not made in etherized rats. There is excellent separation without any tailing when ethanol, acetaldehyde, and acetone are present in the same sample. All samples were read twice. The peak heights obtained for acetaldehyde and ethanol were so sharp that it was not necessary to measure peak area.
B. Analysis of Biological Fluids

To avoid the spontaneous production of acetaldehyde \textit{in vitro} in blood samples when ethanol was present (Truitt, 1970), blood samples were deproteinized with \( \text{ZnSO}_4 \) and \( \text{Ba(OH)}_2 \) in the following manner: Two ml of the blood was added to 1 ml ice cold saline and 0.5 ml of 5% \( \text{ZnSO}_4 \) contained in a rubber-stoppered plastic centrifuge tube. After addition of the blood, 0.5 ml of 0.3 N \( \text{Ba(OH)}_2 \) was added. The tubes were throughly mixed and then centrifuged for 10 minutes at 1500 x g in the cold room at 4°C. Duplicate 0.4 ml samples of protein-free filtrate above the packed, precipitated red cells were transferred to capped vials containing 0.4 g of dry NaCl by means of a syringe. The vials were stored in a freezer at -4°C for 3-4 hours. Inclusion of NaCl in the vials doubled the sensitivity of the method and gave excellent duplication for sub-microgram quantities of acetaldehyde. Saline or blood standards were also treated in a similar manner except that one set of standards were analyzed without freezing whereas the other set was stored in the freezer with the samples.

The samples were removed from the freezer and left at room temperature for 4 minutes and then equilibrated in a water bath at 55°C for 4 minutes. A 0.5 ml head space air sample was equilibrated in the syringe by moving the plunger up and down at least 8-10 times and was then
transferred to the injection port of the gas chromatograph. The peak heights for ethanol were measured, whereas in some of the experiments the level of acetaldehyde in the blood could not be measured as its peak coincided with that of ether which was used to anesthetize the animals. A correction factor was obtained when both saline and blood standards were used. This correction factor was used to correct acetaldehyde and ethanol concentrations in the whole blood.

Determination of $^{3}H$-Norepinephrine, $^{3}H$-Normetanephrine, $^{3}H$-O-Methylated Deaminated Metabolites and Endogenous Norepinephrine

The method used for the separation of $^{3}H$-norepinephrine (NE) and $^{3}H$-normetanephrine (NMN) was that of Davis et al. (1967b), whereas determination of endogenous NE was done by the method of Anton and Sayre (1962, 1964).

A cation exchange resin, Dowex-50 in Hydrogen-form (200-400 mesh) was converted to ammonium form with 3 N ammonium hydroxide treatment overnight. The resin was then washed with distilled water until the effluent was pH 7.0 and then resuspended in deionized water and refrigerated. "Woelm" neutral chromatograph grade of alumina oxide was used for catechol adsorption. The alumina oxide was washed according to the procedure described by Anton and Sayre (1962). Alumina was washed
once with 2 N HCl for 45 minutes and then 3 times more for 10 minutes. It was then washed with distilled water until the pH of effluent was 3.4. The washed alumina was dried at 300°C for 2 hours and then stored in a dessicator. The columns used were 6 x 120 mm tube with 16 x 150 mm reservoir.

Figure 3 outlines the procedure for the determination of H³-NE and H³-NMN. The frozen brains were thawed quickly at room temperature and then homogenized for 30 seconds in 9 ml of ice cold 0.4 N perchloric acid (PCA) in the cold room at 4°C or in an ice bucket. The homogenates were then transferred to a 50 ml polyethylene centrifuge tube, the homogenizing flask washed with 1 ml of ice cold PCA and this wash was added to the homogenate. The homogenates were left at room temperature for 20 minutes and then centrifuged at 30,000 x g for 10 minutes at 4°C. The supernatant was then transferred to another ice cold polyethylene tube containing 0.7 ml of 2% solution of sodium metabisulfite made in 0.4 N PCA. The tissue pellet was then transferred to another clean homogenizing flask and homogenized for 30 seconds in 9 ml of cold PCA. Again the homogenate was transferred to a polyethylene centrifuge tube, the homogenizing flask was rinsed with 1 ml PCA, and the wash added to the homogenate. The tubes were left at room temperature for 20 minutes and then centrifuged at 30,000 x g for 10 minutes at 4°C. The
FIGURE 3
Schematic method for the separation of H3-norepinephrine and H3-normetanephrine

Frozen Brains

\[ \text{0.4 N PCA} \]

Homogenizations I and II

Centrifugations I and II

Precipitate discarded

Supernatant I and II plus 0.7 ml of a 2% solution of sodium metabisulphite.

\[ \text{5M K}_2\text{CO}_3 \]

pH adjusted to 4-5, centrifuged and supernatant stored at -20°C until analyzed for endogenous NE.

\[ \text{5M K}_2\text{CO}_3 \]

pH adjusted to 8-8.5 and centrifuged.

From supernatant

Precipitate discarded

Two 5 ml aliquots

\[ \text{5 N HCl} \]

pH adjusted to 5.5

Dowex-50-NH\textsuperscript{4+} form

Elution with 3N NH\textsubscript{4}OH

H\textsuperscript{3}-NE and H\textsuperscript{3}-NMN

\[ \text{5 N KOH} \]

pH adjusted to 8.4

Alumina column

Effluent and acetate eluate

\[ \text{5 N HCl} \]

pH adjusted to 5.5

Dowex-50 (NH\textsubscript{4}\textsuperscript{+} form)

Elution with 3 N NH\textsubscript{4}OH

H\textsuperscript{3}-NMN
second supernatant was combined with the first supernatant. The polyethylene tubes containing supernatants were always kept in an ice bucket. The supernatant was divided into two aliquots and the perchlorate ion was precipitated by adjusting the pH of one aliquot to 4-5.0 with 5 M K₂CO₃. The tubes were centrifuged at 30,000 x g for 10 minutes at 4°C. The 10 ml of pH-adjusted supernatant was transferred to a 50 ml stoppered pyrex glass centrifuge tube and stored at -20°C overnight. The endogenous NE was determined in this supernatant the next day.

The pH of the second aliquot was adjusted to 8-8.5 with 5 M K₂CO₃ and the tubes were centrifuged at 30,000 x g for 10 minutes at 4°C. The two 5 ml aliquots from the supernatant were used for analysis of H³-NE and H³-NMN.

For one 5 ml aliquot, the pH was adjusted to 8.4 with 5 N KOH and then an aliquot was taken to measure the total radioactivity in the supernatant. It was then quantitatively passed through 1.5 g deactivated alumina column which removed NE and other free catechols. The column was then washed with 5 ml 0.2 M sodium acetate buffer, pH 8.4. Both the effluent and acetate wash were collected, mixed thoroughly and then an aliquot was taken for radioactivity measurement. The pH of the remaining effluent was adjusted to 5.5 with 5 N HCl and the effluent was quantitatively passed through a Dowex-50-NH₄⁺ which picked up NMN, the only free amine remaining. The columns were
rinsed with two consecutive 5 ml water washes and the $H^3$-NMN was eluted with 25 ml of 3 N $NH_4$OH. An aliquot was taken to measure the radioactivity of $H^3$-NMN.

For the second 5 ml aliquot, the pH was adjusted to 5.5 with 5 N HCl and then quantitatively passed through Dowex-50-NH$_4^+$ column which picked up both $H^3$-NE and $H^3$-NMN. The columns were washed with water and the amines were eluted as described above. An aliquot was taken to measure the radioactivity of the total amines. All samples for radioactivity measurement were added to the vials containing 10 ml of Bray's solution and were counted by liquid scintillation spectrometry. Bray's solution was made up by dissolving 60 g of naphthalene in 500 ml of dioxane and then adding 100 ml of methanol, 20 ml of ethylene glycol; 8 g of PPO (2-5 diphenyloxazole) and 400 mg of dimethyl POPOP (4-methyl-5-phenyloxazolyl-benzene) were dissolved in this mixture by continuous stirring and finally the volume was made up to one liter with dioxane.

For each sample, the value of $H^3$-NE was calculated by the difference between the results from the two Dowex columns. The labeled O-methylated deaminated metabolites were calculated by the difference in total radioactivity in brain homogenate supernatant and the radioactivity of $H^3$-NE and $H^3$-NMN. All results were corrected for percent recovery of a standard compound (NE) taken through the
same procedure. The recovery of $H^3$-NE from the ion exchange resin varied from batch to batch and was in the range of 60-90%, whereas the recovery rate for the alumina was consistently about 90%. All results were also corrected for counting efficiency.

**Identification of $H^3$-Norepinephrine and $H^3$-Normetanephrine**

The material (both standard $H^3$-NE and samples) isolated on the ion exchange resin and alumina columns was identified by thin layer chromatography. The prepared cellulose plates were obtained from Uniplate, Analtech, Inc. A non-radioactive standard of NE was obtained from Cal-Biochem. An aliquot from the column eluates was treated with 15 ml acetone which was evaporated to near dryness in a vacuum oven and then plated with a capillary pipette.

The solvent system used for the separation of NE and its metabolites was butanol: 5 N acetic acid (3:1) as was described by Giese et al. (1967). For sharp separation of substances it was important to saturate the cellulose plates for 30 minutes above the solvent. The zones of radioactivity and non-radioactivity were detected by spraying the plates with p-nitroaniline. A comparison of $R_f$ values for the various compounds, i.e., $H^3$-NE and $H^3$-NMN were done with the corresponding standards.
Determination of Endogenous Norepinephrine

The frozen supernatant was thawed at room temperature and thoroughly mixed on a vortex mixer. Four-hundred mg of activated alumina and 200 mg of EDTA (obtained from Sigma Chemical Co.) was added to each tube and the pH was rapidly adjusted to 8.5 with 5 N KOH. The tubes were then shaken on a mechanical shaker for 10 minutes. The supernatant was suctioned off and the alumina was washed first with 5 ml of 0.2 M sodium acetate buffer and then two consecutive 5 ml double glass distilled water or with deionized water. The NE was eluted from alumina with 3 ml of 0.2 M acetic acid by shaking for 15 minutes on the mechanical shaker. The eluate was then passed through a Millipore membrane filter, 0.45 μ in size. The eluate pH was adjusted to 6.5 with 5 M K₂CO₃.

A standard solution of NE was prepared daily from a stock solution of 1 mg/ml in 0.01 N HCl and a standard curve was obtained using 0.5 μg, 1.0 μg, and 1.5 μg. The volume for the standard samples was made up to 3 ml with 0.2 M acetic acid. The pH for all these samples was adjusted to 6.5 with 5 M K₂CO₃.

Two internal and two external standards were included on each day for calculating the percentage recovery. For the internal standard the brain supernatant was divided into two 5 ml portions and to one 5 ml portion 1 μg of standard NE was added. For external standards 1 μg of
standard NE was added to a tube containing 5 ml of 0.4 N PCA and 0.17 ml of 2% sodium metabisulphite solution. The pH of these standards was adjusted to 4-5 with 5 M K₂CO₃ and the tubes were centrifuged for 10 minutes. The NE was extracted from the supernatant as described above.

An aliquot (0.4 ml, pH 6.5) from the eluate and an aliquot of NE standard was oxidized and the fluorescence was measured as described below:

1. To each sample 0.2 ml of 0.5 M phosphate buffer, pH 6.5 was added.
2. Then 0.04 ml of 0.25% potassium ferricyanide was added and the tube was thoroughly mixed for 30 seconds.
3. Exactly 1 minute following the addition of potassium ferricyanide, 0.4 ml of freshly prepared alkaline ascorbate solution was added. The tube was mixed thoroughly for 15 seconds.
4. At the end of 15 seconds, 1 ml of double distilled water was added. The tube was mixed thoroughly for 1 minute and the fluorescence was measured in an Aminco Bowman spectrophotofluorometer with number 5 slit combination, at activation and emission wavelengths of 410 μm and 520 μm respectively (uncorrected wavelengths) and a sensitivity setting of 40. An acetic acid blank and a water blank was prepared in the same manner.
as described above. It was observed that these two blanks were no different from brain supernatant sample blank.

The brain NE content was corrected for its daily percentage recovery, which varied in the range of 50-70 percentage.

**In Vivo Uptake and Metabolism of H\(^3\)-Norepinephrine**

A. Single Dose Ethanol Treatment

On the test day, the animals were fasted for 12-14 hours before an oral dose. The fasted rats received an oral dose of ethanol, 4 g/kg as 20% w/v solution in saline whereas the control rats were given an oral dose of an equivalent volume of saline. According to schedules (30, 45, 90 or 105 minutes after an oral dose) the rats were anesthetized lightly with ether and received 0.082 μg dl-H\(^3\)-norepinephrine (6.6 μc, specific activity 13.8 C/mM) in 20 μl Merle's solution intracisternally as described by Schanberg et al. (1967). The rats were killed by decapitation 15, 30 and 90 minutes after the intracisternal injection and the brains were rapidly removed, rinsed in cold saline, damp dried on filter paper and then frozen in liquid nitrogen. The blood samples were also collected and analyzed for blood ethanol levels
as described on page 40. The frozen brains were stored at -20°C until analyzed for endogenous NE, $H^3$-NE and $H^3$-NMN as described above. Figure 4 outlines the schedules for various treatments.

B. Chronic Ethanol Treatment

In Vivo Uptake and Metabolism of $H^3$-Norepinephrine

General Procedures

In addition to Purina Chow diet, the animals were maintained for 2 days on the liquid diet only. The liquid diet consisted of commercial Metrecal.

The Metrecal-fed rats were divided into 6 groups. Five groups were fed ethanol in their diet whereas the sixth group was used as a control group. On the first day all animals on ethanol diet were given 4% w/v ethanol in their liquid diet which was increased to 5% w/v on the second day and 6% w/v on the third day. After the third day, all experimental rats were maintained on 6% w/v ethanol. The 5 groups were fed ethanol in the following manner: 3, 5, 9, 13 and 18 days.

The sixth group was given Metrecal supplemented with isocaloric amounts of sucrose dissolved in water. A second group of control animals was also used but they were fed Purina Chow diet and water ad lib. The
FIGURE 4

Schematic treatment schedule for uptake and metabolism of $^3$H-norepinephrine in vivo.

**Experimental Group**

A single oral dose of ethanol
(4 g/kg as 20% w/v in saline)

30 min 45 min 90 min

**Control Group**

An oral dose of saline

90 min 15 min 30 min

After an oral dose given an intracisternal injection of dl-7-$^3$H-norepinephrine
(0.082 μg, 6.6 μc) in 20 μl Merle's solution.

90 min 15 min 30 min 15 min

After the intracisternal injection, the animals were killed.
food consumption was measured daily for all groups and the animals were weighed every third day.

Treatment on Schedule Days

On the schedule days, the ethanol-treated rats were fasted for 2 hours and then given an oral dose of ethanol, 4 g/kg as 20% w/v in saline. Ninety minutes later the rats were injected with 0.082 μg of dl-7-H3-norepinephrine in 20 μl Merle's solution intracisternally as described by Schanberg et al. (1967). Thirty minutes later the rats were killed by decapitation, the brains were quickly removed and frozen in liquid nitrogen as described above. The blood samples were also collected and analyzed for blood ethanol levels as described above.

The control animals received the same treatment as ethanol-treated rats on the test day except the group was divided into two sub-groups. One group received an oral dose of ethanol whereas the other group was given an oral dose of saline. The rats were killed by decapitation, the blood samples were collected for blood ethanol level analysis and the brains were removed and frozen with liquid nitrogen as described above. Endogenous NE and H3-NE and its metabolite H3-NMN were determined in the brains as described above.
In Vivo Release and Metabolism of $H^3$-Norepinephrine

General Procedures

Male Wistar rats received the same treatment as described for the uptake of $H^3$-NE in chronic ethanol treatment except only two groups of rats were fed ethanol for either 13 or 18 days.

Treatment on Schedule Days

Two hours fasted rats were anesthetized with ether and then 0.114 µg dl-7-$H^3$-norepinephrine (3.0 µc, specific activity 4.18 C/mM) in 20 µl Merle's solution was injected intracisternally. One hour later the animals received an oral dose of ethanol, 4 g/kg as 20% w/v in saline. Two hours from the administration of $H^3$-NE, the animals were killed by decapitation and the brains and blood samples were collected as described above. The frozen brains were analyzed for both endogenous NE and $H^3$-NE and its metabolites $H^3$-NMN.

Determination of Triglycerides and Free Fatty Acids

General Procedures

Male Wistar rats were fed ethanol or rum (Meyers Planters Punch containing 140 mg acetaldehyde/liter among other congeners) in their diet for different number of days as described above. On the schedule days, two
hour fasted rats received an oral dose of ethanol, 4 g/kg as 20% w/v in saline. The rats were killed by decapitation and the liver was quickly removed, rinsed in cold saline, damp dried on filter paper and then frozen in liquid nitrogen. The blood samples were collected in non-heparinized tubes and centrifuged in the cold room at 4°C for 10 minutes at 2000 rpm. The plasma and frozen liver samples were stored at -20°C until ready to analyze for free fatty acids and triglycerides respectively.

Determination of Triglycerides in Liver

The triglycerides (TG) in the liver were determined by the method of Sardesai and Manning (1968) with some modifications.

The working standard TG was prepared in chloroform daily from a stock solution of TG 10 mg/ml. The standard used for TG was olive oil, obtained from Nutritional Biochemicals. A standard curve of TG was prepared daily in the concentration range of 10-250 μg/ml.

The frozen livers were thawed at room temperature and then approximately 500 mg of the tissue was weighed out and homogenized with 3 ml cold saline. The homogenate was transferred to a tube and the homogenizing flask was rinsed with 1 ml of cold saline. This wash was added to the homogenate and the volume was made up to 5 ml with
cold saline. A 0.5 ml aliquot of the homogenate was further homogenized with 5 ml chloroform-methanol mixture (2:1 v/v) and the final volume was made up to 10 ml with the chloroform-methanol mixture in a flask. The flasks were shaken well and the homogenates were filtered through a fat free paper (Whatman #43) in cold room at 4°C. Eight milliliters of the filtrate was added to a glass-stoppered tube containing 8 ml of distilled water. Tubes were mixed on the vortex mixer gently and then left overnight in the cold room. The next morning the upper layer was suctioned off and the lower layer was passed through a 16 mm x 150 mm column containing 2 g of activated silicic acid mixture. The silicic acid mixture was prepared by mixing one part silicic acid and one part of Hyflo SuperCel. The TG were eluted with chloroform until the volume of eluate was 10 ml. The flasks were thoroughly mixed and two 3 ml aliquots of the eluate were used for determining TG whereas the third aliquot was used as a blank.

The solvent in all the samples (standards and unknown) was evaporated by heating them at 90-95°C in a heating block and by a stream of nitrogen gas. To the dried samples 0.5 ml of 2% alcoholic KOH was added and the tubes were heated at 62-65°C for 20 minutes in a heating block. At the end of 20 minutes 0.8 ml of 0.2 N H₂SO₄ was added and the tubes were thoroughly mixed. Glycerides were oxidized by the addition of 0.1 ml of 0.05 M sodium
metaperiodate for 10 minutes. Exactly 10 minutes later 1 ml of 0.05 M sodium arsenite was added, the contents of the tubes were mixed thoroughly and left on the bench for 7-10 minutes. At the end of this period the volume was made up to 2.2 ml with distilled water and then 2 ml of acetyl-acetone reagent was added. The acetyl-acetone reagent was prepared by mixing 150 g ammonium acetate, 3 ml glacial acetic acid, and 2 ml acetyl-acetone and then diluting the mixture to one liter with distilled water. After mixing the contents of the tubes, the tubes were heated at 58°C for 10 minutes. The tubes were cooled for at least 40 minutes at room temperature and then read at 412 μ. Two reagent blanks were used except one was saponified and the other was not.

Determination of Free Fatty Acids in Plasma

Free fatty acids (FFA) in the plasma were determined by a colorimetric microdetermination method of Kvam et al. (1964). A 0.25 ml sample of plasma was transferred to a clean stoppered tube and 0.75 ml of distilled water and 5 ml of extraction solution were added. The extraction solution was made up by mixing 1N H₂SO₄, hexane and isopropyl alcohol in the volume ratio of 20:200:780. The tubes were thoroughly shaken and then allowed to stand for 5 minutes. At the end of 5 minutes, 2 ml of distilled water and 3 ml of hexane were added. The contents of the
tube were thoroughly mixed and then allowed to stand until two phases were separated. A 4 ml aliquot of upper phase was transferred to another clean stoppered tube and used for FFA determination.

A working standard of stearic acid was prepared daily in hexane from a stock solution of stearic acid 0.568 mg/ml. A standard curve (range 0.2 to 0.6 μmoles) was prepared daily using the amounts equivalent to plasma FFA for the best results. The solvent hexane was evaporated from standard and unknown samples using a nitrogen-gas stream and a heating block at 80-90°C. The residue was dissolved in 5 ml chloroform and then 2.5 ml cupric nitrate reagent was added. Cupric nitrate reagent was prepared by mixing 450 ml of 1 M triethanolamine, 50 ml 1 N acetic acid and 500 ml 5% cupric nitrate solution. The tubes were shaken and then centrifuged for 10 minutes at 1600 rpm. The upper aqueous layer was carefully removed by suctioning and 2 ml of chloroform phase was transferred to a tube. After the addition of 0.1% sodium-diethyldithiocarbamate, the absorption of the solution was measured at 435 μ. Three milliliters of hexane was used as a reagent blank and the entire procedure of extraction was carried through.
Release and Metabolism of Labeled Norepinephrine in Brain Slices

The brain slices were prepared by the method of O'Neill et al. (1963). Male Wistar rats, weighing 200-250 g were killed by decapitation and their heads were plunged into an ice beaker. After rapid removal from the brain case, the brains were freed from blood vessels and dura and dropped into cold incubation medium (see below), divided into two cerebral hemispheres, and the brain stem removed with small curved forceps. Each hemisphere of the brain was sliced to yield as many as two or three thin slices (0.35 mg ± 0.05) and each new slice was removed with a blunt forcep and placed in a Petri dish containing ice cold incubation medium. Routinely the outer slices were not used. A tissue slice, damp dried on a clean, dry glass surface, was suspended from a wire loop and weighed (apparent wet weight) on a Roller-Smith microtissue balance. Each weighed slice was then transferred with blunt forceps to a 50 ml Ehlenmyer flask containing 5 ml of cold incubation medium and 0.1 μc dl-7-H³-NE (specific activity 6.18 C/mM). The incubation medium consisted of 118 mM NaCl, 4.7 mM KCl, 2.2 mM CaCl, 1.18 mM MgSO₄, 11 mM dextrose, and 25 mM sodium phosphate (made up by mixing 392 ml of 25 mM NaH₂PO₄ and 608 ml of 25 mM Na₂HPO₄) at pH 7.0 as described in the method of Colburn and Kopin (1972).
The mid-brain section of the brain (hypothalamus, thalamus, corpus-striatum and hippocampus) was cut into small pieces. Each piece, free from fluid, was weighed and then sliced on McIlwain slicer. The tissue slices were transferred to a flask containing 5 ml incubation medium and 0.1 µc of dl-7-H\(^3\)-NE.

The rubber stoppered flasks were incubated for 20 minutes at 37°C in a water bath with continuous oxygenation to determine the uptake of H\(^3\)-NE. The uptake of NE was stopped by cooling the flasks in an ice bucket. After 5 minutes the contents of each flask were poured into an evaporating dish and the brain slice, free from loosely adhering fluid, was transferred to another flask containing 3 ml of ice cold incubation medium.

To allow equilibration, the tissue slices were pre-incubated for 5 minutes at 37°C in a water bath. Then 0.1 ml of ethanol or acetaldehyde solution in incubation medium was added to give the final concentration of ethanol 0.11 M, 0.5% w/v (Kalant et al., 1967; Israel et al., 1970), and for acetaldehyde 0.47 mM (20 µg/ml). To the control flasks, 0.1 ml of incubation medium was added. The flasks were stoppered and incubated for 5, 10, 20 or 30 minutes at 37°C in a water-bath with continuous oxygen supply. The reaction was stopped by cooling the flasks in an ice-bucket for 5 minutes. Again the contents of each flask were poured out into an evaporating dish and the tissue slice,
free from fluid, was immediately transferred into a tube containing 0.5 ml of 0.4 N PCA. The mid-brain samples were centrifuged for 5 minutes at 2000 rpm. After centrifugation, 1.5 ml of 0.4 N PCA was added to the tissue. The supernatant incubation medium was kept cold for subsequent analysis of total radioactivity, deaminated metabolites and NE. The tissue slices were homogenized in 0.4 N PCA and the homogenizing flask was rinsed with 0.5 ml PCA. The total homogenate was centrifuged for 10 minutes at 16,000 x g at 4°C. The supernatant was neutralized with 5 M K₂CO₃ and then an aliquot (0.2 ml) was added to 10 ml Bray's solution vials and the total radioactivity in the slices was measured in a liquid scintillation spectrometer.

The total radioactivity released into the incubation medium was also determined by taking a 0.4 ml aliquot from the medium and counting in Bray's solution. The labeled amines present in another 1.2 ml aliquot of medium were adsorbed by a suspension of Dowex-50-NH₄⁺ form (4-5 ml of suspension). The tubes were shaken on a vortex mixer and then centrifuged for 5 minutes at 2000 rpm. After centrifugation, the remaining radioactivity was measured in a 0.5 ml sample from the supernatant in a 10 ml Bray's solution vial. Finally the radioactivity of H⁻³-NE in the medium was determined in samples separated by the method of Anton and Sayre (1962, 1964) as described on page 50.
with some modification in the quantity of EDTA and alumina used. A 0.1 ml of 2% EDTA solution and 0.1 ml of 0.1% sodium metabisulfite solution were added to the aliquots. Only 200 mg of alumina was used for the catecholamine adsorption. An external $^3$H-NE standard was included daily for percentage recovery. All the results were corrected for efficiency and for percentage recovery. The total radioactivity present in the form of amines was calculated from the difference between the total radioactivity in the medium and the deaminated products which were not adsorbed on the ion-exchange resin. The radioactivity of $^3$H-NMN was obtained by calculating the difference between the total radioactivity of amines and the radioactivity of $^3$H-NE.

Propranolol Blockade in Brain Slices

The brain cortex and mid-brain slices were prepared and incubated for 20 minutes with $^3$H-norepinephrine (0.1 µc/flask) as described above (page 61). The slices were transferred to fresh 3 ml medium and equilibrated by pre-incubation as described above on page 63. To one set of flasks, 0.1 ml of either ethanol or acetaldehyde solution was added to give a final concentration of 0.11 M, 0.5% w/v (Kalant et al., 1967; Israel et al., 1970), and 0.47 mM respectively. To the second set of flasks, both acetaldehyde or ethanol was added with propranolol to give
a final concentration of propranolol 1 \times 10^{-5}\text{M}. To the control flasks, either 0.1 ml of incubation medium or both propranolol and medium was added. The flasks were incubated for 30 minutes at 37\degree C with continuous oxygenation. The release of catecholamines was stopped by cooling the flasks in an ice-bucket for 5 minutes. The slices and the incubation medium were analyzed for total radioactivity and separation of its metabolites as described on page 63.

Determination of Cyclic 3'-5' Adenosine Monophosphate (cAMP) in Brain Slices

The brain slices after incubation were homogenized with 0.4 N PCA and centrifuged as described above on page 61. The brain slice homogenate supernatant was adjusted to pH 6.4-6.8 with 1 M \text{K}_2\text{CO}_3 and then stored at -80\degree C until analyzed for cyclic 3'-5' adenosine monophosphate.

Cyclic 3'-5' adenosine monophosphate (cAMP) was measured by the method of Steiner et al. (1970), for assaying the levels of this compound in the supernatant of brain homogenates. For each assay, a standard curve was prepared incorporating a logarithmic dilution of standard concentration of cAMP. Each assay tube contained acetate buffer (anhydrous, 50 mM, pH 6.2) freshly prepared. Following microliter-sized additions of the
unknown samples (standard between 0.03 and 10.0 pmole) 0.1 ml of an anti-cyclic 3'-5' adenosine monophosphate antibody (prepared in rabbits) was added to all assay tubes with the exception of the total counts (see sample assay set-up, Appendix 1). A radio-labeled I$^{125}$cAMP derivative was added (0.1 ml, 6-7000 cpm) and all tubes were shaken on a vortex mixer. After 2 hours of incubation at 4°C, 0.1 ml of a goat anti-rabbit gamma-globulin was added to each assay tube. This was followed by the addition of 0.1 ml of 1/200 normal rabbit serum. All tubes were shaken again and the assay tubes were incubated at 4°C for 16 hours.

At the end of the incubation period, ice cold distilled water was added to all tubes with the exception of the total counts and blank tubes. The tubes were kept at 4°C in the cold room for 30 minutes and then centrifuged using a swinging bucket rotor at 4°C at 2500 rpm, for 30 minutes. The supernatant was discarded and the additional supernatant was decanted by placing the tubes at a downward angle for at least 15 minutes and then swabbing on a paper towel. The tubes containing precipitates and total counts were counted for 1 minute in a solid crystal scintillation counter.

A standard curve was drawn on a semi-log paper 3 cycles plotting % cAMP as a log function. The levels of
cAMP in unknown sample were calculated from the standard cAMP curve.
RESULTS

Preliminary Experiments

Preliminary uptake experiments using a single dose of ethanol were carried out to determine whether an introduction of $^{3}$-NE more than 20% (about 70%) of brain endogenous NE would change the endogenous NE pool significantly. The rats were given a single dose of ethanol, 4 g/kg as 20% w/v saline solution, 105 minutes before an intracisternal injection of 0.321 μg of $^{3}$-NE (20 μc) and 15 minutes later the animals were killed. This dose of ethanol produced symptoms of intoxication in all rats. The average blood ethanol levels were 376 mg% as shown in Table 1. The level of $^{3}$-NE was slightly decreased in ethanol-pretreated animals compared to controls. Similar results were obtained when exogenous NE injected was about 20% of endogenous NE (Table 7). The endogenous NE was decreased by about 15% in ethanol-pretreated animals compared to controls. The specific activity of NE was also slightly decreased in ethanol-pretreated rats compared to controls. These decreases were statistically non-significant.

The levels of $^{3}$-NE were also similar in these two experiments (Tables 1 and 7). These results indicate that
TABLE 1.—Effects of a Single Oral Dose of Ethanol (4 g/kg) after 2 hours on the Accumulation and Metabolism of H^3-NE and Endogenous NE 15 minutes after Intracisternal Injection of 0.32 μg of dl-7-H^3-Norepinephrine.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>H^3-NE (μc/g)</th>
<th>Endogenous NE (μc/g)</th>
<th>Specific Activity (μc/μg)</th>
<th>H^3-NMN (μc/g)</th>
<th>Total Radioactivity (μc/g)</th>
<th>Blood Ethanol Levels (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.038 ±0.288a</td>
<td>0.45 ±0.034</td>
<td>4.80 ±0.896</td>
<td>0.144 ±0.039</td>
<td>2.690 ±0.361</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.766 ±0.279</td>
<td>0.37 ±0.022</td>
<td>4.68 ±0.614</td>
<td>0.146 ±0.42</td>
<td>2.162 ±0.295</td>
<td>3765 ±321</td>
</tr>
</tbody>
</table>

a±S.E.M.

bIndicates number of animals used.
the ethanol effect on $H^3$-NE metabolism is not dependent upon the size of the radiolabeled injection.

**In Vivo Release and Metabolism of $H^3$-Norepinephrine**

The rats were given either acetaldehyde (300 mg/kg x 2 doses, 30 minutes apart) or ethanol (4 g/kg) at 1, 5, or 21 hours after the injection of $H^3$-NE and allowed 1-hour period before sacrifice (thus making periods of 2, 6, and 22 hours for $H^3$-NE equilibration). This dose of ethanol produced symptoms of intoxication in all rats whereas with each dose of acetaldehyde the obvious intoxication symptoms lasted for 7-8 minutes only. The blood ethanol and acetaldehyde levels produced by these doses are shown in Table 2.

In acetaldehyde-treated rats the level of $H^3$-NE in the brain was decreased as compared to the controls at 2, 6, and 22 hours after the intracisternal administration of dl-7-$H^3$-norepinephrine (Table 2, Figure 5). The concentration of $H^3$-NE in the acetaldehyde-treated rats after 2 hours was less than one third of the control value and the difference was highly significant. After 6 hours, the change was about one half the control value, but was still significantly different. After 22 hours, the concentration of $H^3$-NE was decreased in acetaldehyde-treated rats but was not statistically significant. The level of endogenous NE was significantly decreased in all
TABLE 2. — Specific Activities and Turnover Rate of $^3$-Noradrenaline in Rat Brain 1 hour after a Single Oral Dose of Ethanol (4 g/kg) or after two IP Doses of Acetaldehyde (300 mg/kg, 30 minutes apart) at Various Times after the Intracisternal Administration of 3 μc of dl-$^3$-Noradrenaline (or 10 μc for 22 hours Determination).

<table>
<thead>
<tr>
<th>Drug</th>
<th>$^3$-NE (μc/g)</th>
<th>Endogenous NE (μc/g</th>
<th>Specific Activity (μc/μg)</th>
<th>Blood Ethanol/ Acetaldehyde Levels (μg/ml)</th>
<th>$^3$-NE (μc/g)</th>
<th>Specific Activity (μc/μg)</th>
<th>Turnover Rate (ng/g/hr)</th>
<th>2-6 hours</th>
<th>22 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.097±0.0026</td>
<td>0.420±0.030</td>
<td>0.238±0.047</td>
<td>0.019±0.001</td>
<td>0.015±0.005</td>
<td>0.415±0.021</td>
<td>0.021±0.11</td>
<td>9.59</td>
<td></td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>0.031±0.004</td>
<td>0.342±0.028</td>
<td>0.097±0.016</td>
<td>7.93±0.998</td>
<td>0.011±0.004</td>
<td>0.360±0.018</td>
<td>329±0.001</td>
<td>6.64</td>
<td>7.6</td>
</tr>
<tr>
<td>Control</td>
<td>0.076±0.013</td>
<td>0.488±0.049</td>
<td>0.154±0.016</td>
<td>0.016±0.001</td>
<td>0.009±0.002</td>
<td>0.425±0.012</td>
<td>0.033±0.003</td>
<td>14.38</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.110±0.015</td>
<td>0.373±0.024</td>
<td>0.321±0.054</td>
<td>2806±292</td>
<td>0.020±0.003</td>
<td>0.340±0.029</td>
<td>0.19±0.002</td>
<td>1954</td>
<td>0.059</td>
</tr>
</tbody>
</table>

$^a_h = rate\ constant = slope/0.434$

$^b_± standard\ error.$

$^cIndicates\ number\ of\ animals\ used.$

$^d_p < .05.$

$^e_p < .001.$

$^f_p < .01.$
FIGURE 5

Effect of a single oral dose of ethanol (4 g/kg) or acetaldehyde (300 mg/kg, 2 doses ip, 30 minutes apart) on the $H^3$- and endogenous-norepinephrine content of whole rat brain at various times after intracisternal injection of 3 μc of dl-7-$H^3$-norepinephrine.
acetaldehyde-treated animals. The specific activity was calculated to show the interaction of endogenous and exogenous NE in the 2 groups. At the earlier time, the ratio of specific activity of norepinephrine in acetaldehyde-treated animals to controls was about 0.4 whereas at 6 and 22 hours it was increased to 0.8 and 0.9 respectively. The specific activity of NE in acetaldehyde-treated rats was significantly decreased at 2 hours but a non-significant reduction was observed at 6 hours and at 22 hours when compared with controls.

In acute ethanol-treated rats (1 hour) the concentration of $H^3$-NE in the brain was increased when compared with the control group at 2 and 6 hours after the administration of dl-7-$H^3$-norepinephrine intracisternally whereas 22 hours after catecholamine administration it was almost unchanged (Table 2, Figure 5). Also, the endogenous NE level was decreased at 2 and 6 hours but was only significantly different at 2 hours in ethanol-treated rats when compared with the control group. The significance of this change is questionable however, because of the unusually high control value. At 22 hours the concentration of endogenous NE was slightly increased. As a result of the large difference in the endogenous NE level, the specific activity of norepinephrine was about two times higher at 2 hours and was significantly different whereas it was only slightly increased at 6 hours in ethanol-treated rats when compared
with controls. At 22 hours a slight decrease in the specific activity was observed in ethanol-treated rats. The ratio of specific activity of norepinephrine in ethanol-treated rats to controls at 2 hours was 2.0 whereas at 6 and 22 hours it was reduced to about 1.4 and 0.8 respectively.

When acetaldehyde-treated rats were compared with ethanol-treated rats, it was observed that a greater percentage of endogenous NE and radioactive norepinephrine was released in acetaldehyde-treated rats at 2 hours, but only radioactive NE was decreased at 6 hours. The level of $H^3$-NE retained in the brain was significantly decreased in acetaldehyde-treated rats at 2 and 6 hours. The specific activity of norepinephrine was significantly reduced to one third and about half at 2 and 6 hours respectively. The ratio of specific activity of norepinephrine in ethanol-treated rats to acetaldehyde was 3.3 and 1.8 at 2 and 6 hours after $H^3$-NE injection.

The metabolites of $H^3$-NE present in the brain were also examined after the administration of ethanol or acetaldehyde in these experiments. Two hours after the administration of labeled catecholamines, the total radioactivity in the brain of acetaldehyde-treated rats was about one third of the control value (Table 3, Figure 6) and was significantly decreased. However, at 6 and 22 hours it was not significantly lowered (Tables 4 and 5, Figure 6).
TABLE 3.--Effects of a Single Oral Dose of Ethanol (4 g/kg) for 1 hour or two IP Doses of Acetaldehyde (300 mg/kg, 30 minutes apart) on the Metabolites of $^3$H-Norepinephrine in Rat Brain 2 hours after Intracisternal Injection of 3 $\mu$C of dl-7-$^3$H-Norepinephrine.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Total Radioactivity ($\mu$C/g)</th>
<th>$^3$H-NE Radioactivity ($\mu$C/g)</th>
<th>% of Total</th>
<th>$^3$H-NMN Radioactivity ($\mu$C/g)</th>
<th>% of Total</th>
<th>O-Methylated Deaminated Radioactivity ($\mu$C/g)</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$0.168 \pm 0.035^a$</td>
<td>$0.097 \pm 0.020$</td>
<td>59.9</td>
<td>$0.006 \pm 0.003$</td>
<td>3.4</td>
<td>$0.065 \pm 0.017$</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>(8)$^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>$0.058^c$</td>
<td>$0.031^c$</td>
<td>57.1</td>
<td>$0.005 \pm 0.003$</td>
<td>5.7</td>
<td>$0.022 \pm 0.004$</td>
<td>37.2</td>
</tr>
<tr>
<td></td>
<td>$\pm 0.009$</td>
<td>$\pm 0.004$</td>
<td>$\pm 3.4$</td>
<td></td>
<td>$\pm 2.6$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$0.161 \pm 0.030$</td>
<td>$0.076 \pm 0.013$</td>
<td>51.3</td>
<td>$0.014 \pm 0.004$</td>
<td>8.0</td>
<td>$0.072 \pm 0.010$</td>
<td>40.7</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>$0.205 \pm 0.027$</td>
<td>$0.110 \pm 0.015$</td>
<td>53.1</td>
<td>$0.017 \pm 0.003$</td>
<td>8.6</td>
<td>$0.078 \pm 0.012$</td>
<td>38.3</td>
</tr>
</tbody>
</table>

$^a \pm$ S.E.M.

$^b$ Indicates number of animals used.

$^c P < .001$.  

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FIGURE 6

Brain concentrations of $^3$H-norepinephrine and its metabolites 1 hour after a single oral dose of ethanol (4 g/kg) or two ip doses of acetaldehyde (300 mg/kg, 30 minutes apart) in rats 2, 6 and 22 hours after intracisternal injection of 3 µc of dl-$^3$H-norepinephrine (10 µc for 22 hours determination).
The concentration of $H^3$-NMN was not different from the controls at any of the time periods. The $H^3$-labeled O-methylated deaminated metabolites were calculated by the difference between the total radioactivity in the brain and the concentration of $H^3$-NE and $H^3$-NMN. The radioactivity of these metabolites was markedly decreased in acetaldehyde-treated rats at 2 hours but it was unchanged at 6 and 22 hours. The percentage of total radioactivity represented by O-methylated deaminated metabolites, normetanephrine, and unchanged $H^3$-NE was calculated and it was observed that $H^3$-labeled O-methylated deaminated metabolites accounted for about 40% of the total metabolites at 2 and 6 hours after the injection of $H^3$-NE in both control and in acetaldehyde-treated rats. At 22 hours after the injection of catecholamines, the percent of these metabolites was lower in acetaldehyde-treated rats. The percentage of $H^3$-NE was slightly less than 60% of the total radioactivity in both groups at 2 and 6 hours but at 22 hours it accounted for 90% because $H^3$-NMN was present in too low a concentration to be separated.

At 2 and 6 hours after catecholamine administration, the total radioactivity in the brains of ethanol-treated rats was somewhat increased but the increase was not significantly different (Tables 4 and 5, Figure 6). The unchanged $H^3$-NE retained in the brain was higher in ethanol-treated rats but as a percent of total
TABLE 4.—Effects of a Single Oral Dose of Ethanol (4 g/kg) for 1 hour or two IP Doses of Acetaldehyde (300 mg/kg, 30 minutes apart) on the Metabolites of H3-Norepinephrine in Rat Brain 6 hours after Intracisternal Injection of 3 μc of dl-7-H3-Norepinephrine.

<table>
<thead>
<tr>
<th></th>
<th>6 hours</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>H³-NE % of Total</td>
<td>H³-NMN % of Total</td>
<td>O-Methylated Deaminated % of Total</td>
</tr>
<tr>
<td></td>
<td>Radioactivity (μc/g)</td>
<td>Radioactivity (μc/g)</td>
<td>Radioactivity (μc/g)</td>
<td>Radioactivity (μc/g)</td>
</tr>
<tr>
<td>Control</td>
<td>0.035±0.005a (5)b</td>
<td>0.019±0.001±5.52</td>
<td>0.002±0.002±4.13</td>
<td>0.014±0.005 ±37.1</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>0.025±0.007 (7)</td>
<td>0.011±0.002±4.7</td>
<td>0.002±0.001±3.0</td>
<td>0.012±0.006 ±41.9</td>
</tr>
<tr>
<td>Control</td>
<td>0.039±0.008 (6)</td>
<td>0.016±0.004±1.3</td>
<td>0.006±0.001±0.88</td>
<td>0.017±0.003 ±42.0</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.052±0.009 (7)</td>
<td>0.020±0.003±2.57</td>
<td>0.009±0.002±1.57</td>
<td>0.023±0.004 ±42.0</td>
</tr>
</tbody>
</table>

a±S.E.M.

bIndicates number of animals used.
radioactivity it was not different from the control group. At 22 hours the total radioactivity was almost unchanged in ethanol-treated rats compared to controls and so was the concentration of $^{3}H$-NE (Table 5, Figure 6). The concentration of $^{3}H$-NMN and $^{3}H$-labeled O-methylated deaminated metabolites were slightly elevated but when expressed as percentage of total radioactivity, the two metabolites were similar in the two groups. The O-methylated deaminated metabolites accounted for 40% of the total radioactivity at 2 and 6 hours, whereas they were present in much smaller percentages at 22 hours. The percentage of $^{3}H$-NMN was about 8% at 2 hours which increased to 17% at 6 hours. At 22 hours the concentration of $^{3}H$-NMN present was too low and therefore it could not be separated.

In comparing the acetaldehyde effect to the ethanol treatment, the total radioactivity was significantly decreased in acetaldehyde-treated rats and so was the labeled normetanephrine and other metabolites at 2 and 6 hours (Tables 3 and 4). The percent of total radioactivity represented by O-methylated deaminated metabolites, normetanephrine and unchanged $^{3}H$-norepinephrine was the same in two groups. Both drugs decreased the concentration of endogenous NE to the same extent at 2, 6, but not at 22 hours (Table 6). At 22 hours only acetaldehyde treatment released endogenous NE. However, it was only
TABLE 5.—Effects of a Single Oral Dose of Ethanol (4 g/kg) for 1 hour or two IP Doses of Acetaldehyde (300 mg/kg, 30 minutes apart) on the Metabolites of H3-Norepinephrine in Rat Brain 22 hours after Intracisternal Injection of 10 µc of d1-7-H3-Norepinephrine.

<table>
<thead>
<tr>
<th></th>
<th>22 hours</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>H3-NE</td>
<td>H3-NMN</td>
<td>O-Methylated</td>
<td>Deaminated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Radioactivity</td>
<td>% of Total Radioactivity</td>
<td>% of Total Radioactivity</td>
<td>Radioactivity</td>
<td>Radioactivity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(µc/g)</td>
<td>(µc/g)</td>
<td>(µc/g)</td>
<td>(µc/g)</td>
<td>(µc/g)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.017 ±0.005</td>
<td>0.015 ±0.005</td>
<td>88.6 ±6.31</td>
<td>0.002 ±0.002</td>
<td>11.4 ±9.00</td>
<td></td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>0.012 ±0.002</td>
<td>0.011 ±0.001</td>
<td>96.3 ±3.0</td>
<td>0.001 ±0.001</td>
<td>3.7 ±9.00</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.011 ±0.002</td>
<td>0.010 ±0.002</td>
<td>91.0 ±3.83</td>
<td>0.001 ±0.001</td>
<td>9.0 ±9.00</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.010 ±0.002</td>
<td>0.009 ±0.001</td>
<td>83.8 ±9.00</td>
<td>0.001 ±0.001</td>
<td>16.2 ±9.00</td>
<td></td>
</tr>
</tbody>
</table>

aH3-NMN could not be separated as it was present in too low concentration.

b±S.E.M.

cIndicates number of animals used.
with acetaldehyde that a marked reduction in percentage of radioactive NE was observed and the greatest effect was seen at 2 hours (Table 6). With ethanol treatment a greater percent of $^3$H-NE was retained in the brain at earlier time but the level of $^3$H-NE decreased at 6 and 22 hours (Table 6).

The disappearance of unchanged $^3$H-NE was multiphasic and the curve could be resolved into two major compartments in both ethanol- and acetaldehyde-treated rats (Figures 7 and 8). The initial phase being most rapid which was followed then by a slower disappearance of unchanged $^3$H-NE. The specific activity values were converted to log values and then the slope of decline with its standard error was calculated by the method of least squares analysis for 2 to 6 hours (Table 2) and then for 6 to 22 hours (Table 2).

The rate constant after acetaldehyde was increased by about two and a half times as compared to its control group in the initial phase (between 2 and 6 hours), but not at the later time. The absolute turnover rate was markedly increased in acetaldehyde-treated rats compared to controls. Even though the rate constant after acute ethanol treatment was slightly increased compared to its control group, the rate of turnover of NE was decreased slightly due to the lower endogenous NE content in ethanol-treated rats.
TABLE 6.—Percentage Change in $H^3$- and Endogenous-Norepinephrine Content in the Rat Brain after a Single Dose of Ethanol (4 g/kg) or two IP Injections of Acetaldehyde (300 mg/kg, 30 minutes apart). Data Calculated from Table 2.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>% of $H^3$-NE Change</th>
<th>% Endogenous NE Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/kg</td>
<td>2 hrs</td>
<td>6 hrs</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>0.3</td>
<td>-68.9</td>
<td>-39.2</td>
</tr>
<tr>
<td>(2 doses)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>4.0</td>
<td>44.1</td>
<td>24.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FIGURE 7

Disappearance of $^{3}$H-norepinephrine from the whole rat brain 1 hour after acetaldehyde (300 mg/kg, two ip doses, 30 minutes apart) or saline at various times after intracisternal injection of 3 μc of dl-7-$^{3}$H-norepinephrine (10 μc for 22 hours determination).
[H^3] Norepinephrine Specific Activity μc/μg in Brain

- ■ Acetaldehyde
- ▲ Control (saline)

I = S.E.M.

TIME - HOURS

0.6
0.4
0.2
0.1
0.05
0.01

0.1
0.05
0.01

0.1
0.05
0.01

4 8 12 16 20 24
FIGURE 8

Disappearance of $^{3}$H-norepinephrine from the whole rat brain 1 hour after a single oral dose of ethanol (4 g/kg) or saline at various times after intracisternal injection of 3 μc of dl-7-$^{3}$H-norepinephrine (10 μc for 22 hours determination).
[H$^3$] Norepinephrine Specific Activity $\mu$c/µg in Brain

- **Ethanol**
- **Control (saline)**

$\bar{I}$ = S.E.M.
The rate constant between 6 and 22 hours was also calculated as shown in Table 2. The rate constant and the turnover rate of NE was unaffected by acetaldehyde treatment whereas after acute ethanol treatment, both the rate constant and the turnover rate of NE was slightly increased compared to control.

**In Vivo Uptake and Metabolism of H$_3^3$-Norepinephrine**

**A. Single Dose Ethanol Treatment**

The rats were given ethanol by stomach tube (4 g/kg) and then they received dl-7-H$_3^3$-NE intracisternally at various intervals. This dose of ethanol produced symptoms of intoxication within 10-15 minutes after the oral dose. Blood samples were analyzed for ethanol level at 1 or 2 hours after the administration of ethanol. The ethanol concentration at these two time periods were essentially the same as the blood ethanol level reaches its peak between 1 and 2 hours (Table 7).

When the rats were given ethanol for 45 minutes and the brains exposed to H$_3^3$-NE for 15 minutes, there was a marked reduction in the concentration of H$_3^3$-NE in the whole brain compared to controls (Table 7, Figure 9). The endogenous NE content was not effected by ethanol treatment. The specific activity of norepinephrine was
TABLE 7.—Effect of a Single Oral Dose of Ethanol (4 g/kg) after 1 or 2 hours on the Uptake of 
H\textsuperscript{3}-Norepinephrine in the Rat Brain at Various Time Intervals after Intracisternal Injection of 
6.6 \(\mu\text{c}\) of dl-7-H\textsuperscript{3}-Norepinephrine.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Time Between H\textsuperscript{3}-NE and Kill (minutes)</th>
<th>Total Treatment Time (minutes)</th>
<th>H\textsuperscript{3}-NE ((\mu\text{c/g}))</th>
<th>Endogenous NE ((\mu\text{g/g}))</th>
<th>Specific Activity ((\mu\text{c}/\mu\text{g}))</th>
<th>Blood Ethanol Levels ((\mu\text{g/ml}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>60</td>
<td>0.765 ±0.140&lt;sup&gt;a&lt;/sup&gt;&lt;br&gt;(6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.40 ±0.03</td>
<td>1.94 ±0.35</td>
<td>0.40 ±0.03</td>
</tr>
<tr>
<td>Ethanol</td>
<td>15</td>
<td>60</td>
<td>0.552 ±0.130&lt;sup&gt;(8)&lt;/sup&gt;</td>
<td>0.43 ±0.037</td>
<td>1.387 ±0.417</td>
<td>3763 ±234</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>120</td>
<td>1.08 ±0.038&lt;sup&gt;(5)&lt;/sup&gt;</td>
<td>0.48 ±0.052</td>
<td>2.29 ±0.158</td>
<td>0.39 ±0.033</td>
</tr>
<tr>
<td>Ethanol</td>
<td>15</td>
<td>120</td>
<td>0.91 ±0.189&lt;sup&gt;(8)&lt;/sup&gt;</td>
<td>0.51 ±0.038</td>
<td>1.89 ±0.478</td>
<td>3748 ±288</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>120</td>
<td>0.584 ±0.036&lt;sup&gt;(6)&lt;/sup&gt;</td>
<td>0.39 ±0.033</td>
<td>1.58 ±0.260</td>
<td>0.39 ±0.033</td>
</tr>
<tr>
<td>Ethanol</td>
<td>30</td>
<td>120</td>
<td>0.621 ±0.049&lt;sup&gt;(8)&lt;/sup&gt;</td>
<td>0.38 ±0.038</td>
<td>1.70 ±0.194</td>
<td>3937 ±315</td>
</tr>
<tr>
<td>Drug</td>
<td>Time Between H&lt;sup&gt;3&lt;/sup&gt;-NE and Kill (minutes)</td>
<td>Total Treatment Time (minutes)</td>
<td>H&lt;sup&gt;3&lt;/sup&gt;-NE (μc/g)</td>
<td>Endogenous NE (μg/g)</td>
<td>Specific Activity (μc/μg)</td>
<td>Blood Ethanol Levels (μg/ml)</td>
</tr>
<tr>
<td>-------</td>
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<td>--------------------------------</td>
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<td>-----------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Control</td>
<td>90</td>
<td>120</td>
<td>0.271 ±0.032 (7)</td>
<td>0.46 ±0.025</td>
<td>0.60 ±0.073</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>90</td>
<td>120</td>
<td>0.381 ±0.051 (10)</td>
<td>0.39 ±0.038</td>
<td>1.07 ±0.18</td>
<td>3603 ±358</td>
</tr>
</tbody>
</table>

<sup>a</sup>S.E.M.

<sup>b</sup>Indicates number of animals used.

<sup>c</sup><sup>P</sup> < 0.05.
decreased in ethanol-treated rats when compared with control rats.

When the rats were given 2 hours of ethanol treatment a difference was found in the amount of $H^3$-NE taken up by the brain when compared to controls. This effect varied with the length of time allowed between the $H^3$-NE administration and the time of sacrifice. If only 15 minutes treatment with $H^3$-NE was allowed, a slight decrease in uptake of $H^3$-NE occurred (Table 7, Figure 9). After longer exposure of the brain to $H^3$-NE, a larger amount of the radiolabel was retained at 30 and 90 minutes treatment times. This increase was statistically non-significant at 30 and 90 minutes.

The endogenous NE concentration was almost unchanged in two groups of 2 hours ethanol-treated rats as compared to controls. However, it was significantly decreased in one ethanol-pretreated group (30 minutes) compared to its control (Table 7, Figure 9). The specific activity of NE remaining in the brain decreased with longer exposure of the brain to $H^3$-NE both in controls and in ethanol-treated rats but this decrease in specific activity of NE was less marked in ethanol rats than controls. This decrease in NE specific activity was statistically significant when the brain was exposed to $H^3$-NE for 90 minutes.

In the present study, metabolism of intracisternally administered $H^3$-norepinephrine in the rat brain was also
FIGURE 9

Effect of a single oral dose of ethanol (4 g/kg) after 1 or 2 hours on the concentration of $^3$H- and endogenous norepinephrine in the whole rat brain at various intervals after the intracisternal injection of 6.6 μc of dl-7-$^3$H-norepinephrine.
Norepinephrine Specific Activity

<table>
<thead>
<tr>
<th>ETHANOL</th>
<th>1 HOUR</th>
<th>ETHANOL</th>
<th>2 HOURS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>EtoH 15 min.</td>
<td>Control</td>
<td>EtoH 15 min.</td>
</tr>
</tbody>
</table>

- NE Specific activity
- H³-NE
- Endogenous NE

Intracisternal Injection Time

Endogenous NE (µg/g) or H³-NE (µc/g) in Brain

S.E.M.

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examined after a single dose of ethanol for 1 or 2 hours. The level of total radioactivity in the brain was lower in the animals treated with ethanol for 1 or 2 hours than in control animals except in one group where the time between intracisternal injection and kill was 90 minutes. The decrease was not statistically significant (Table 8, Figure 10). The concentration of unchanged H\textsuperscript{3}-NE varied more with the time of exposure of brain to the radiolabel than with the ethanol dose. As stated earlier, a small but non-significant decrease (15-25%) in H\textsuperscript{3}-NE level was found at shorter times (15 minutes) in ethanol rats as compared to control, whereas at longer exposure times, the level of H\textsuperscript{3}-NE was non-significantly higher in ethanol-treated rats. Ethanol caused a decrease as well as an increase in the levels of tritiated normetanephrine and O-methylated deaminated metabolites. It caused a statistically significant decrease in the concentration of labeled normetanephrine in the rats given ethanol for 1 hour only. In 2 hour ethanol-treated rats, the rate of formation of tritiated normetanephrine was initially faster but it was slower thereafter. The tritiated labeled O-methylated deaminated metabolites were calculated by the difference between the total radioactivity in the brain and the concentration of unchanged H\textsuperscript{3}-norepinephrine and normetanephrine. The percentage of total radioactivity represented by the labeled O-methylated deaminated
TABLE 8. -- Effect of a Single Dose of Ethanol (4 g/kg) after 1 or 2 hours on $^3$H-Norepinephrine Metabolism in the Whole Rat Brain at Various Intervals after the Intracisternal Injection of 6.6 µc of dl-$^3$H-Norepinephrine.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Minutes Between $^3$H-NE and Kill Ethanol Dose Time (hours)</th>
<th>Total Radioactivity (µc/g)</th>
<th>% of Total Radioactivity</th>
<th>H$^3$-NE (µc/g)</th>
<th>% of Total Radioactivity</th>
<th>H$^3$-NMN (µc/g)</th>
<th>O-Methylated Deaminated % of Total Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15 (1 hr)$^a$</td>
<td>1.043 ± 0.177$^b$</td>
<td>67.6</td>
<td>0.148 ± 0.043</td>
<td>16.1 ± 0.06</td>
<td>0.127 ± 0.06</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>(6)$^c$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ethanol</td>
<td>15 (1 hr)</td>
<td>0.801 ± 0.132</td>
<td>64.1</td>
<td>0.068 ± 0.013</td>
<td>10.2 ± 0.06</td>
<td>0.181 ± 0.06</td>
<td>25.7</td>
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<tr>
<td></td>
<td>(8)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>15 (2 hrs)</td>
<td>1.510 ± 0.054</td>
<td>72.4</td>
<td>0.100 ± 0.028</td>
<td>5.2 ± 0.05</td>
<td>0.33 ± 0.05</td>
<td>22.4</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td></td>
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</tr>
<tr>
<td>Ethanol</td>
<td>15 (2 hrs)</td>
<td>1.137 ± 0.193</td>
<td>72.1</td>
<td>0.144 ± 0.061</td>
<td>12.0 ± 0.03</td>
<td>0.083 ± 0.03</td>
<td>15.9</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Drug</td>
<td>Minutes Between H3-NE and Kill Ethanol Dose Time (hours)</td>
<td>Total Radioactivity (µc/g)</td>
<td>% of Total Radioactivity</td>
<td>H3-NE (µc/g)</td>
<td>% of Total Radioactivity</td>
<td>(µc/g)</td>
<td>% of Total Radioactivity</td>
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<td>------------------------</td>
</tr>
<tr>
<td>Control</td>
<td>30 (2 hrs)</td>
<td>0.913 ± 0.130 (6)</td>
<td>0.584 ± 0.033 (2.5)</td>
<td>55.2</td>
<td>0.226 ± 0.040 (2.8)</td>
<td>0.103</td>
<td>21.3</td>
</tr>
<tr>
<td>Ethanol</td>
<td>30 (2 hrs)</td>
<td>0.851 ± 0.105 (8)</td>
<td>0.621 ± 0.049 (2.6)</td>
<td>65.8</td>
<td>0.160 ± 0.030 (2.5)</td>
<td>0.070</td>
<td>18.4</td>
</tr>
<tr>
<td>Control</td>
<td>90 (2 hrs)</td>
<td>0.404 ± 0.060 (10)</td>
<td>0.271 ± 0.032 (8.5)</td>
<td>72.6</td>
<td>0.059 ± 0.012 (1.0)</td>
<td>0.074</td>
<td>13.2</td>
</tr>
<tr>
<td>Ethanol</td>
<td>90 (2 hrs)</td>
<td>0.576 ± 0.077 (7)</td>
<td>0.381 ± 0.051 (5.8)</td>
<td>69.1</td>
<td>0.075 ± 0.015 (1.2)</td>
<td>0.120</td>
<td>18.5</td>
</tr>
</tbody>
</table>

\[a\] Indicates total treatment time.

\[b\] ± S.E.M.

\[c\] Indicates number of animals used.

\[d\] p < .05.
FIGURE 10

Effect of a single oral dose of ethanol (4 g/kg) after 1 or 2 hours on the metabolism of $H^3$-norepinephrine in the whole rat brain at various intervals after intracisternal injection of 6.6 $\mu$g of dl-7-$H^3$-norepinephrine.
INTRACISTERNAL INJECTION TIME

- **ETHANOL 1 HOUR**
  - Control 15 min.: (6)
  - EtoH 15 min.: (8)

- **ETHANOL 2 HOURS**
  - Control 15 min.: (5)
  - EtoH 15 min.: (8)
  - EtoH 30 min.: (6)
  - EtoH 30 min.: (8)
  - Control 90 min.: (7)
  - EtoH 90 min.: (10)

- **H³⁻Norepinephrine**
- **H³O⁻methylated deaminated**
- **H³⁻Normetanephrine**

[^S.E.M.]: S.E.M.
metabolites was in the range of 18-26% in ethanol-treated rats whereas it was between 13-22% in controls. The labeled normetanephrine was about 12% of the total radioactivity in the brains of ethanol-dosed animals whereas it was between 5-23% in controls.

The effect of 2 hour ethanol treatment on retention of tritiated norepinephrine is shown in Figure 11. As stated earlier, the specific activity of norepinephrine remaining in the brains of animals pretreated with ethanol was lower than the control animals when the time between intracisternal injection and kill was short (15 minutes), but it was higher as the time increased. The increase was statistically significant at 90 minutes. The slope of these lines was calculated and it was 0.200 for ethanol-treated rats and 0.437 for control rats. The values were not statistically different. The turnover rate of NE was 198 ng/g/hr and 446 ng/g/hr for ethanol-treated rats and controls respectively.

B. Chronic Ethanol Treatment

Separate groups of rats were fed ethanol in their liquid diet for variable periods and their food consumption and body weights were recorded (Figures 12 and 13). The control rats gained weight steadily whereas the ethanol fed rats showed a lag in weight gain before increasing again at about the control rate (Figure 12). The ethanol
FIGURE 11

Effects of a single oral dose of ethanol (4 g/kg) on the retention of $^3$H-norepinephrine in the whole rat brains at various intervals after intracisternal injection of 6.6 $\mu$c of dl-7-$^3$H-norepinephrine.
$H^3$-Norepinephrine Specific Activity $\mu$c/$\mu$g in Whole Brain

Control (saline)

$\pm$ S.E.M.
FIGURE 12

Growth curve of rats fed either ethanol (6 g %) or isocaloric amounts of sucrose added to a nutritionally complete liquid diet (Metrecal) for 18 days.
FIGURE 13

Daily food and ethanol consumption (g/kg) of rats consuming a nutritionally complete liquid diet (Metrecal) containing ethanol or isocaloric sucrose. The concentration of ethanol was increased from 4 g % to 5 g % and 6 g % on the second and third days respectively.
dose consumed per day by the rats increased with the number of days on ethanol until reaching a plateau after 6-7 days at a level between 9 g/kg to 16.8 g/kg. The peak value of 16.8 g/kg for ethanol consumption was reached on the ninth day on the alcohol diet.

In Vivo Uptake and Metabolism of $^{3}$-Norepinephrine

On the test day, the alcohol diet was removed 2 hours before the experiment. All rats except the control group were given a single oral dose of ethanol (4 g/kg). During this fasting period, withdrawal symptoms were minimum in the animals fed ethanol for prolonged periods. This dose of ethanol produced obvious symptoms of intoxication such as loss of the righting reflex. The blood ethanol levels were measured in rats given a single dose of ethanol or chronic ethanol treatment at the time of sacrifice. The blood ethanol levels were markedly lower in the animals given a single dose of ethanol than when fed ethanol for a number of days (Table 9). The blood ethanol levels were similar whether the animals had ethanol for 3 days or longer.

The level of $^{3}$-norepinephrine was similar in the animals pretreated with only a single dose of ethanol or given a dose of saline (Table 9, Figure 14). However, it was decreased when the rats were kept on the Metrecal liquid diet for 12-18 days and then given the single
<table>
<thead>
<tr>
<th>Drug</th>
<th>Days on Ethanol</th>
<th>H\textsuperscript{3}-NE (μc/g)</th>
<th>Endogenous NE (μg/g)</th>
<th>Specific Activity (μc/μg)</th>
<th>Blood Ethanol Levels (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.526 ±0.035&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.35 ±0.029</td>
<td>1.65 ±0.216</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single Dose of Ethanol</td>
<td>0.624 ±0.093</td>
<td>0.33 ±0.024</td>
<td>1.95 ±0.332</td>
<td>2368 ±388</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metrecal + Single Dose of Ethanol</td>
<td>0.340 ±0.152</td>
<td>0.33 ±0.028</td>
<td>1.09 ±0.537</td>
<td>2169 ±255</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td></td>
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</tr>
<tr>
<td>Ethanol  3</td>
<td>0.583 ±0.140</td>
<td>0.32 ±0.023</td>
<td>1.65 ±0.45</td>
<td>3757 ±424</td>
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</tr>
<tr>
<td></td>
<td>(5)</td>
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</tr>
<tr>
<td>Ethanol  5</td>
<td>0.350 ±0.060</td>
<td>0.39 ±0.039</td>
<td>0.81 ±0.151</td>
<td>3934 ±462</td>
<td></td>
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<tr>
<td></td>
<td>(7)</td>
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</tr>
<tr>
<td>Ethanol  9</td>
<td>0.609 ±0.117</td>
<td>0.27 ±0.037</td>
<td>2.43 ±0.567</td>
<td>3452 ±334</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 9—Continued

<table>
<thead>
<tr>
<th>Drug</th>
<th>Days on Ethanol</th>
<th>$^3$-NE (µc/g)</th>
<th>Endogenous NE (µg/g)</th>
<th>Specific Activity (µc/µg)</th>
<th>Blood Ethanol Levels (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>15</td>
<td>0.728 ±0.155</td>
<td>0.37 ±0.024</td>
<td>1.98 ±0.451</td>
<td>3654 ±247</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>18</td>
<td>0.652 ±0.026</td>
<td>0.30 ±0.030</td>
<td>2.24 ±0.226</td>
<td>3678 ±156</td>
</tr>
</tbody>
</table>

$^a$ ± S.E.M.

$^b$ Indicates number of animals used.

$^c$ P < .05 compared to single dose of ethanol.

$^d$ P < .01 compared to Day 5.

$^e$ P < .05 compared to Metrecal.
Effect of chronic ethanol feeding in a nutritionally complete liquid diet (Metrecal with 6% w/v ethanol added) for various periods on the concentration of H\(^3\)- and endogenous-norepinephrine in whole brain of rat 30 minutes after intracisternal injection of 3 μc of dl-7-norepinephrine.
**S.E.M.**

- NE Specific activity
- H³-NE
- Endogenous NE

H³-Norepinephrine Specific Activity $\mu$Ci/$\mu$g

- Combined Control
- Ethanol Single Dose + Ethanol Single Dose
- Metrecal Day-3
- Day-5
- Day-9
- Day-15
- Day-18

Endogenous NE $\mu$g/g or H³-NE $\mu$g/g in Brain

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dose of ethanol (Table 9, Figure 14). The endogenous NE content was not different in these groups when compared with control animals. The specific activity of norepinephrine was non-significantly decreased in chronic Metcreal rats receiving a single dose of ethanol when compared either with control or single dosed ethanol rats.

The concentrations of endogenous NE and tritiated NE were compared either with the rats given a single dose of ethanol or within the groups that had ethanol in their diet for different number of days. The level of $^{3}$H-norepinephrine was initially the same in the animals fed ethanol or given a single dose of ethanol but it decreased on the fifth day (Table 9, Figure 14). Thereafter it was higher in chronically ethanol-fed animals. The concentration of endogenous NE was similar in all groups except the one receiving 9 days on alcohol where it was slightly decreased.

The specific activity of norepinephrine was calculated to show the ratio between endogenous and exogenous NE. The specific activity was not significantly different in the groups fed ethanol for 3, 9, 15, or 18 days or when compared to the controls or the animals treated with a single dose of ethanol alone. However, it was significantly decreased in the brain of rats fed ethanol for 5 days.
The metabolism of tritiated norepinephrine was also studied after chronic ethanol treatment. The total radioactivity in the whole brain was increased by about 30% in the animals treated with a single dose of ethanol than in control rats. However, it was decreased in the rats fed Metrecal chronically and then given a single dose of ethanol (Table 10, Figure 15). The total radioactivity in rats treated chronically with ethanol showed initially no change, then a decrease when compared to rats treated with a single dose of ethanol. It was about the same on the third day of ethanol treatment, but decreased to its lowest level (about 60%) on the fifth day and then increased to its initial level. The decrease on the fifth day was statistically significant. When the rats given ethanol for different number of days were compared with the animals given the same liquid diet without alcohol but only a single dose of alcohol on the test day, the total radioactivity was increased in all groups of rats fed ethanol chronically except the one on the fifth day. This increase in total radioactivity in the whole brain was statistically significant on 3, 15 and 18 days of ethanol feeding. The concentration of labeled normetanephrine was increased by about 50% in animals treated with a single dose of ethanol than in controls. The concentration of $^{3}$H-NMN was about three to four fold higher in these two groups when compared with
TABLE 10.—Effect of Chronic Ethanol Feeding in a Nutritionally Complete Liquid Diet (Metrecal with 6% w/v Ethanol Added) for Various Periods on the Metabolism of H^3-Norepinephrine in the Rat Brain at 30 minutes after Intracisternal Administration of 6.6 μc of dl-7-H^3-Norepinephrine.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days on Ethanol</th>
<th>Total Radioactivity (μc/g)</th>
<th>H^3-NE % of Total Radioactivity</th>
<th>H^3-NMN % of Total Radioactivity</th>
<th>O-Methylated Deaminated % of Total Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.797 ±0.052a</td>
<td>0.526 ±0.035</td>
<td>63.1</td>
<td>0.096 ±0.009</td>
<td>0.175 ±0.020</td>
</tr>
<tr>
<td></td>
<td>(8)b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single Dose of Ethanol</td>
<td>1.052 ±0.092</td>
<td>0.626 ±0.093</td>
<td>61.2</td>
<td>0.135 ±0.019</td>
<td>0.291 ±0.082</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metrecal + Single Dose of Ethanol</td>
<td>0.414c</td>
<td>0.340 ±0.152</td>
<td>80.3</td>
<td>0.030 ±0.010</td>
<td>0.044 ±0.025</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
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<tr>
<td>3 Days</td>
<td>0.912 ±0.215</td>
<td>0.583 ±0.140</td>
<td>65.9</td>
<td>0.123 ±0.029</td>
<td>0.206 ±0.070</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Days</td>
<td>0.590d</td>
<td>0.350 ±0.060</td>
<td>62.6</td>
<td>0.081 ±0.021</td>
<td>0.159 ±0.035</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Days</td>
<td>0.718 ±0.110</td>
<td>0.609 ±0.117</td>
<td>88.4</td>
<td>0.054 ±0.014</td>
<td>0.055 ±0.028</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 10—Continued

<table>
<thead>
<tr>
<th>Treatment Days on Ethanol</th>
<th>Total Radioactivity (µc/g)</th>
<th>$^3$NE % of Total Radioactivity (µc/g)</th>
<th>$^3$NMN % of Total Radioactivity (µc/g)</th>
<th>O-Methylated Deaminated % of Total Radioactivity (µc/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 Days</td>
<td>0.956 ±0.139 (7)</td>
<td>0.728 ±0.155 ±9.06</td>
<td>0.111 ±0.030 ±1.84</td>
<td>0.117 ±0.052 12.2</td>
</tr>
<tr>
<td>18 Days</td>
<td>0.898 ±0.052 (4)</td>
<td>0.652 ±0.026 ±5.58</td>
<td>0.100 ±0.019 ±1.64</td>
<td>0.146 ±0.029 15.4</td>
</tr>
</tbody>
</table>

$^a$± S.E.M.

$^b$Indicates number of animals used.

$^c_{P} < .05$ compared to Days 3, 15, 18.

$^d_{P} < .05$ compared to Day 3, single dose of ethanol.
FIGURE 15

Effect of chronic ethanol feeding in a nutritionally complete liquid diet (Metrecal) with 6% w/v ethanol added for various periods on the metabolism of norepinephrine in whole rat brain 30 minutes after intracisternal injection of 3 μc of dl-7-H\(^3\)-norepinephrine.
Comb. Single Metrecal Day-3 EtoH EtoH dose

$\mu c/g$ in Whole Brain

$H^3$ - Norepinephrine
$H^3$ - Normetanephrine
$H^3$O-methylated deaminated

$S.E.M.$
the rats fed Metrecal chronically and then given an oral dose of ethanol. On the third day of ethanol feeding, the level of tritiated normetanephrine was about 90% of the acutely treated rats. However, it was markedly decreased (70% and 35%) on the fifth and ninth days. On the fifteenth and eighteenth day, the formation of normetanephrine increased but it was still only about 80% of the level in the brains of animals administered a single dose of ethanol.

The O-methylated deaminated metabolites were calculated by the difference between the total radioactivity and the unchanged $\text{H}^3$-NE and $\text{H}^3$-normetanephrine. The percentages of total radioactivity represented by $\text{H}^3$-NE and its metabolites were not significantly different in animals given an oral dose of ethanol or fed ethanol for the prolonged period. The unchanged tritiated norepinephrine accounted for 61-88% of the total radioactivity whereas its metabolites, O-methylated deaminated metabolites percentage varied from 4 to 25. The lowest percent of these metabolites was present on the ninth day of ethanol feeding. The metabolite normetanephrine was present in the range of 8-14% of the total radioactivity.

In Vivo Release and Metabolism of $\text{H}^3$-Norepinephrine

Two additional groups of rats were maintained for 13 and 18 days on the alcohol diet and were fasted for
2 hours on the test day. The lightly anesthetized rats received an intracisternal injection of $^3$H-NE and 1 hour later they received an oral dose of ethanol (4 g/kg). This dose of ethanol produced symptoms of intoxication even in chronically ethanol-treated rats. During this third hour of fasting, some of the animals fed ethanol for a prolonged period had withdrawal symptoms which disappeared after administration of ethanol dose. In the uptake experiments, the animals were fasted for 2 hours only, therefore only minimal withdrawal symptoms were seen. The blood ethanol levels were measured in some of the animals after chronic ethanol administration and were found to be increased by about 30% than those seen in animals given a single dose of ethanol (Table 11). The chronic ethanol administration for 13 or 18 days followed by the test dose of ethanol decreased the level of tritiated norepinephrine retained in the brain by about one third when compared with acute ethanol treatment (Table 11, Figure 16). This decrease was highly significant. However, the effect of chronic ethanol administration on the release of labeled norepinephrine was similar to acute acetaldehyde treatment as a similar change was observed in $^3$H-norepinephrine levels. The prolonged ethanol feeding had no effect on the endogenous NE content when compared to the rats given either a single dose of ethanol or acetaldehyde treatment. The specific activity of
TABLE 11.—Effect of Chronic Ethanol Feeding in a Nutritionally Complete Liquid Diet (Metrecal with 6% w/v Ethanol Added), Compared to 1 hour after a Single Oral Dose of Ethanol (4 g/kg) or Acetaldehyde (300 mg/kg, two ip Doses, 30 minutes apart) on the Specific Activity of H$^3$-Norepinephrine in Whole Rat Brain 2 hours after Intracisternal Injection of 3 μc of dl-7-H$^3$-Norepinephrine.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>H$^3$-NE (μc/g)</th>
<th>Endogenous NE (μg/g)</th>
<th>Specific Activity (μc/μg)</th>
<th>Blood Ethanol Level (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Dose of Ethanol</td>
<td>0.110 ±0.015$^a$</td>
<td>0.37 ±0.024</td>
<td>0.321 ±0.054</td>
<td>2806 ±292 (10)$^b$</td>
</tr>
<tr>
<td>13 Days on Ethanol</td>
<td>0.042 ±0.013$^c$</td>
<td>0.38 ±0.026</td>
<td>0.127 ±0.013</td>
<td>3600 ±82 (9)</td>
</tr>
<tr>
<td>18 Days on Ethanol</td>
<td>0.033 ±0.008$^c$</td>
<td>0.41 ±0.03</td>
<td>0.084 ±0.019</td>
<td>3500 ±300 (7)</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>0.031 ±0.004$^c$</td>
<td>0.34 ±0.028</td>
<td>0.097 ±0.016</td>
<td>7.93 ±0.998 (13)</td>
</tr>
</tbody>
</table>

$^a$± S.E.M.

$^b$Indicates number of animals used.

$^c$P < .001 compared to a single dose of ethanol.
FIGURE 16

Effects of chronic ethanol feeding in a nutritionally complete liquid diet (Metrecal with ethanol 6% w/v added) compared to a single oral dose of ethanol (4 g/kg) after 1 hour or two ip doses of acetaldehyde (300 mg/kg, 30 minutes apart) on the concentration of $^3$H-NE in whole brain of rat after the intracisternal injection of 3 μc of dl-7-$^3$H-norepinephrine.
Norepinephrine Specific Activity

\( \text{Endogenous NE} \)

\( \text{EtoH} \)

Single Dose

13-Day EtoH

18-Day EtoH

AcH

\( \mu \text{c/g} \) or \( \text{H}^3 \text{-NE} \)
norepinephrine was decreased to about 25-40% by the chronic ethanol treatment when compared with acute ethanol treatment. This decrease was highly significant. The specific activity of norepinephrine in the brains of rats fed ethanol for prolonged periods was not different from that of acetaldehyde-treated rats.

The metabolism of tritiated norepinephrine was also examined in the present study. The total radioactivity in the whole brain of rats fed ethanol for 13 or 18 days was decreased by about 55% when compared with acute ethanol treatment. This decrease was statistically significant (Table 12, Figure 17). However, the decrease was not as great as for a single acetaldehyde treatment. Thirteen days of ethanol feeding markedly reduced the concentration of labeled normetanephrine (50%) when compared with the single ethanol dosed rats. However, the concentration of labeled normetanephrine was decreased by only about 30% on the eighteenth day of ethanol feeding. When the effect of prolonged ethanol feeding was compared with acetaldehyde effect, the decrease in labeled normetanephrine formation was smaller. The labeled O-methylated deaminated metabolites concentration were also decreased (to about 40%) on the thirteenth day on ethanol compared to a single dose administration and to about 60% in the animals fed ethanol for 18 days. The chronic ethanol treatment produced a smaller decrease in
TABLE 12.—Effect of Chronic Ethanol Feeding in a Nutritionally Complete Liquid Diet (Metrecal with 6% w/v Ethanol Added) Compared to 1 hour after a Single Oral Dose of Ethanol (4 g/kg) or Acetaldehyde (300 mg/kg, two IP Doses, 30 minutes apart) on the Release and Metabolism of $H^3$-Norepinephrine in the Whole Rat Brain 2 hours after Intracisternal Injection of 3 $µ$c of dl-$H^3$-Norepinephrine.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Radioactivity ($µc/g$)</th>
<th>$H^3$-NE % of Total Radioactivity</th>
<th>$H^3$-NMN % of Total Radioactivity</th>
<th>O-Methylated Deaminated % of Total Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.164 ± 0.022$^a$ (8)$^b$</td>
<td>0.076 ± 0.013 51.3</td>
<td>0.014 ± 0.004 8.00</td>
<td>0.074 ± 0.010 40.7</td>
</tr>
<tr>
<td>Single Dose of Ethanol</td>
<td>0.205 ± 0.027 (10)</td>
<td>0.110 ± 0.015 53.1</td>
<td>0.017 ± 0.003 8.6</td>
<td>0.078 ± 0.012 38.3</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>0.058 ± 0.009 (13)</td>
<td>0.031 ± 0.004 57.1</td>
<td>0.005 ± 0.003 5.7</td>
<td>0.022 ± 0.005 37.2</td>
</tr>
<tr>
<td>13 Days on Ethanol</td>
<td>0.080 ± 0.018 (9)</td>
<td>0.042 ± 0.013 49.8</td>
<td>0.009 ± 0.002 11.9</td>
<td>0.029 ± 0.008 41.7</td>
</tr>
<tr>
<td>18 Days on Ethanol</td>
<td>0.090 ± 0.030 (7)</td>
<td>0.033 ± 0.008 39.6</td>
<td>0.012 ± 0.007 10.9</td>
<td>0.047 ± 0.011 49.5</td>
</tr>
</tbody>
</table>

$^a$± S.E.M.  
$^b$Indicates number of animals used.  
$^c$P < .05.
FIGURE 17

Effects of chronic ethanol feeding in a nutritionally complete liquid diet (Metrecal with 6% w/v ethanol added) 1 hour after an oral dose of ethanol (4 g/kg) or two ip doses of acetaldehyde (300 mg/kg, 30 minutes apart) on the concentration of H\textsuperscript{3}-NE and its metabolites in whole brain rat 2 hours after the intracisternal injection of 3 μc of dl-7-H\textsuperscript{3}-norepinephrine.
\[ \mu g/g \text{ in Whole Brain} \]

- **H}^3\text{-Norepinephrine}**
- **H}^3\text{ O-methylated deaminated}**
- **H}^3\text{-Normetanephrine}**

\[ \text{S.E.M.} \]
the formation of these metabolites when compared with acute acetaldehyde treatment.

The percentage of total radioactivity represented by unchanged $^3$H-norepinephrine, $^3$H-normetanephrine and labeled O-methylated deaminated metabolites was calculated. The O-methylated deaminated metabolites accounted for the major metabolites in the brain and were in the range of 40-50% of total radioactivity. Initially the rate of formation of these metabolites was slow in chronically ethanol-treated rats but increased thereafter. The percentage of extraneuronal metabolite normetanephrine was only in the range of 6-12% of total radioactivity. The unchanged $^3$H-NE accounted for about 40% in the animals on ethanol for 18 days whereas it was about 50-55% in the other three groups.

**Triglycerides and Free Fatty Acids in Liver and in Plasma**

In another chronic series of rats fed the ethanol liquid diet, measurements were made of the effects on total hepatic triglycerides and the plasma free fatty acid levels. These studies were conducted for both pure ethanol and rum (a high congener beverage with significant amounts of acetaldehyde). The concentration of liver triglycerides was not affected on the third day of ethanol administration when compared to the control rats (Table 13). However, the liver triglycerides were significantly
TABLE 13.— Effect of Chronic Ethanol or Rum Feeding for Various Periods in a Nutritionally Complete Liquid Diet (Metrecal with 6% w/v Ethanol Added) on Liver Triglycerides and Free Fatty Acids of Plasma 2 hours after a Single Oral Dose of Ethanol (4 g/kg).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Triglycerides (mg/g)</th>
<th>Free Fatty Acids (μmole/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
<td>Rum</td>
</tr>
<tr>
<td>Control</td>
<td>16.1 ±1.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.41 ±0.027</td>
</tr>
<tr>
<td></td>
<td>(±1.18&lt;sup&gt;a&lt;/sup&gt;)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(21)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3 Days</td>
<td>17.10 ±0.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.49&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>0.72&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>±0.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>±0.063&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 Days</td>
<td>7.85 ±1.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.50&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>0.39&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>±0.036&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>±0.058&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>9 Days</td>
<td>13.20 ±2.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.90&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td></td>
</tr>
<tr>
<td>13 Days</td>
<td>11.6 ±1.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.38&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>0.51&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>±0.031&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>±0.043&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>17 Days</td>
<td>17.9 ±3.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.62&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>0.43&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>±0.02&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>±0.08&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Treatment</td>
<td>Triglycerides (mg/g)</td>
<td>Free Fatty Acids (μmole/ml)</td>
</tr>
<tr>
<td>-----------</td>
<td>---------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Rum</td>
<td>Ethanol</td>
</tr>
<tr>
<td>21 Days</td>
<td>12.5 ±2.26</td>
<td>23.2C ±4.72</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(4)</td>
</tr>
</tbody>
</table>

\[ ^{a} \pm \text{ S.E.M.} \]

\[ ^{b} \text{Indicates number of animals used.} \]

\[ ^{c} p < .05 \text{ compared to control.} \]
increased in the animals given rum for the same number of days. On the fifth day of chronic administration of alcohol (ethanol and rum), the rate of formation or accumulation of triglycerides in the liver decreased markedly in both groups from its third day value but not significantly from the level of triglycerides found in the control animals. Thereafter the concentration of triglycerides was not effected by ethanol significantly to that of control levels. However, the triglycerides concentration was significantly increased on 17 and 21 days in the rum-fed rats.

The chronic ethanol feeding for 3 days increased the level of FFA present in plasma significantly when compared with the controls (Table 13). However, the administration of rum for the same period did not significantly increase the FFA levels in plasma. Beyond 3 days of ethanol administration, the levels of FFA in the plasma were not significantly effected by ethanol. However, a significant increase was produced by prolonged feeding of rum at 5, 13, and 21 days.

In Vitro Release of $^{3}$H-Norepinephrine by Ethanol or Acetaldehyde

By Rat Brain Cortex Slices

The brain cortex slices were incubated with ethanol or acetaldehyde in the incubation medium at 37°C for various
time periods. The total radioactivity taken up by the individual slice was calculated by combining the radioactivity remaining in the slice with the total radioactivity of the metabolites and $^3$H-NE in the incubation medium (Table 14, Figure 18). The results were expressed as percentage of this total radioactivity. Ethanol in a final concentration of 0.11 M, did not release $^3$H-NE into the incubation medium from cerebral cortex slices as compared to control slices when incubated for various time periods (Table 14, Figure 18). There was also no change in tissue amines after incubation for various times.

Acetaldehyde, in a final concentration of 0.47 mM (20 µg/ml) had no effect on the release of $^3$H-NE into the incubation medium when incubated for 5, 10, or 30 minutes but at 20 minutes the percentage radioactivity in the medium was slightly increased (about 10%) as compared to control slices. There was a concomitant decrease in tissue amines at this time. There was almost no change in the tissue amines at other incubation times. The tissue amines represented between 59-66% of the total radioactivity whereas the radioactivity represented by the medium was in the range of 34-41% of the total radioactivity.

Ethanol or acetaldehyde did not affect the formation of deaminated metabolites as these metabolites represented about 15% of total radioactivity in the incubation medium.
TABLE 14.—Effect of Ethanol (0.11 M) or Acetaldehyde (0.47 mM) on Release of 0.1 μc of d1-7-H3-Norepinephrine from Rat Brain Cortex Slices Incubated for Various Times.

<table>
<thead>
<tr>
<th>Incubation Time (minutes)</th>
<th>Total Radioactivity*</th>
<th>Incubation Medium</th>
<th>Norepinephrine</th>
<th>Deaminated Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medium + Slices</td>
<td>Slices</td>
<td>% of Total Radioactivity</td>
<td>dpm/50 mg</td>
</tr>
<tr>
<td></td>
<td>dpm/50 mg</td>
<td>dpm/50 mg</td>
<td>dpm/50 mg</td>
<td>dpm/50 mg</td>
</tr>
<tr>
<td>Control</td>
<td>12875 ± 755</td>
<td>8076 ± 136</td>
<td>65.7 ± 2.32</td>
<td>4398 ± 89</td>
</tr>
<tr>
<td>Ethanol</td>
<td>12472 ± 1165</td>
<td>8134 ± 995</td>
<td>63.4 ± 3.09</td>
<td>4116 ± 280</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>11007 ± 1158</td>
<td>7038 ± 1056</td>
<td>61.3 ± 3.68</td>
<td>8134 ± 1056</td>
</tr>
<tr>
<td>Control</td>
<td>13037 ± 976</td>
<td>8558 ± 571</td>
<td>65.8 ± 2.53</td>
<td>4245 ± 143</td>
</tr>
<tr>
<td>Ethanol</td>
<td>12387 ± 2976</td>
<td>8538 ± 844</td>
<td>61.0 ± 1.56</td>
<td>3993 ± 147</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>12875 ± 403</td>
<td>7905 ± 408</td>
<td>65.5 ± 1.03</td>
<td>4100 ± 143</td>
</tr>
<tr>
<td>Control</td>
<td>13349 ± 783</td>
<td>8582 ± 713</td>
<td>63.1 ± 2.34</td>
<td>3984 ± 147</td>
</tr>
<tr>
<td>Ethanol</td>
<td>12079 ± 904</td>
<td>7632 ± 900</td>
<td>60.6 ± 1.67</td>
<td>3643 ± 147</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>11440 ± 403</td>
<td>6730 ± 414</td>
<td>61.5 ± 2.59</td>
<td>3957 ± 140</td>
</tr>
<tr>
<td>Control</td>
<td>13988 ± 794</td>
<td>8897 ± 1404</td>
<td>62.2 ± 1.63</td>
<td>4083 ± 390</td>
</tr>
<tr>
<td>Ethanol</td>
<td>12949 ± 932</td>
<td>8487 ± 655</td>
<td>60.6 ± 2.25</td>
<td>4102 ± 376</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>12875 ± 856</td>
<td>8001 ± 931</td>
<td>61.5 ± 2.11</td>
<td>3957 ± 376</td>
</tr>
</tbody>
</table>

*The results are expressed as mean values, ± S.E.M. of 6 slice preparations. The brain slices were pooled together from 5 or 6 animals.
FIGURE 18

The effect of ethanol (0.11 mM) or acetaldehyde (0.47 mM) on the release and catabolism of 0.1 μc of dl-7-H³-norepinephrine from rat cerebral cortex slices after various time intervals.
Total dpm x 10^3 / 50 mg in Rat Cerebral Cortex Slices

- **Con- EtOH AcH**: Control
- **Con- EtOH AcH**: Control
- **Con- EtOH AcH**: Control
- **Con- EtOH AcH**: Control

**Incubation Time**
- 5 min
- 10 min
- 20 min
- 30 min

**Legend**
- NE - Medium
- Deaminated Metabolites - Medium

**I = S.E.M.**
which was similar to control after 5, 10 or 20 minutes incubation. However, the formation of these metabolites was increased when the slices were incubated for 30 minutes, as they represented 30% of the total radioactivity of the incubation medium whether ethanol or acetaldehyde was added or not. There was a corresponding decrease in $^{3}H\text{-NE}$ percent in the medium. The level of $^{3}H\text{-NE}$ in the incubation medium was about 90% at 5, 10 or 20 minutes but it was decreased to about 70-75% when slices were incubated for 30 minutes.

**By Rat Mid-Brain Slices**

The mid-brain slices were incubated with ethanol or acetaldehyde in the incubation medium, at 37°C for 20 or 30 minutes. The total uptake of $^{3}H\text{-NE}$ for each mid-brain slice preparation was calculated by addition of the total radioactivity in the incubation medium and radioactivity remaining in the tissue.

Ethanol, in a final concentration of 0.11 M, increased release of $^{3}H\text{-NE}$ into the incubation medium, when the slices were incubated for 20 minutes, but not at 30 minutes (Table 15, Figure 19). There was a concomitant decrease (about 16%) in percent radioactivity in tissue amines. This decrease in tissue amines was statistically non-significant. The decrease in tissue amines was almost unaffected by ethanol at 30 minutes.
TABLE 15.—Effect of Ethanol (0.11 M) or Acetaldehyde (0.47 mM) on Release of 0.1 μc of dl-7-H<sup>-</sup>Norepinephrine from Rat Mid-Brain Slices Incubated for 20 or 30 minutes.

<table>
<thead>
<tr>
<th>Incubation Time (minutes)</th>
<th>Total Radioactivity*</th>
<th>Slices % of Total Radioactivity</th>
<th>Incubation Medium</th>
<th>Norepinephrine dpm/50 mg</th>
<th>Deaminated Metabolites dpm/50 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medium + Slices dpm/50 mg</td>
<td>Slices dpm/50 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control Ethanol Acetaldehyde</td>
<td>Control Ethanol Acetaldehyde</td>
<td>Control Ethanol Acetaldehyde</td>
<td>Control Ethanol Acetaldehyde</td>
<td>Control Ethanol Acetaldehyde</td>
</tr>
<tr>
<td>20</td>
<td>8079 ±1256 7519 ±933 7892 ±877</td>
<td>5392 ±1196 4042 ±716 4694 ±694</td>
<td>66.0 ±1.36 55.8 ±3.22 59.5 ±2.58</td>
<td>2072 ±268 2394 ±151 2558 ±201</td>
<td>512 ±84 464 ±46 416 ±59</td>
</tr>
<tr>
<td>30</td>
<td>8522 ±1861 10591 ±2083 9107 ±1338</td>
<td>5645 ±1164 6757 ±1278 5745 ±799</td>
<td>66.5 ±3.8 64.5 ±0.71 63.2 ±1.23</td>
<td>1987 ±482 2648 ±522 2512 ±441</td>
<td>505 ±218 760 ±216 590 ±136</td>
</tr>
</tbody>
</table>

*The results are expressed as mean values, ± S.E.M. of 6 or 4 slice preparations. The mid-brain slices were pooled together from 5 or 6 animals.
FIGURE 19

The effect of ethanol (0.11 M) or acetaldehyde (0.47 M) on release and metabolism of 0.1 μc of dl-7-H$^3$-norepinephrine from rat mid-brain slices after various time intervals.
Total Deaminated Metabolites in Medium

NE - Medium

NE - Tissue

I = S.E.M.

Total dpm x 10^3/50 mg in Rat Mid-brain Slices

Incubation time:
- Control
- EtoH
- AcH

20 min
30 min

(4)
Acetaldehyde, in a final concentration of 0.47 mM (20 µg/ml), slightly increased (10-12%) the release of \(^3\text{H}^-\text{NE}\) into the incubation medium at both incubation times as compared to control slices (Table 15, Figure 19). The concomitant decrease in percent radioactivity in the tissue amines was slightly greater at 20 minutes than at 30 minutes. The radioactivity in the medium accounted for 34-44% of the total radioactivity whereas the radioactivity remaining in the tissue was in the range of 55-66%.

The percentage of total radioactivity in the medium represented by labeled NE was about 70% in the presence of ethanol and in controls whereas it was about 85% when acetaldehyde was added to the incubation medium. The formation of deaminated metabolites was not affected by ethanol or acetaldehyde and it accounted for about 15% of total radioactivity of the incubation medium. These results do not indicate the presence of a very active uptake or release mechanism as effected by either ethanol or acetaldehyde in isolated tissues.

**Propranolol Blockade**

**In Rat Brain Cortex Slices**

The cerebral cortex slices were incubated for 30 minutes in a medium containing either propranolol alone
or with ethanol or acetaldehyde. The final concentrations of propranolol, ethanol and acetaldehyde in the incubation medium were $1 \times 10^{-5}$M (2.5 μg/ml), 0.11 M and 0.47 mM (20 μg/ml) respectively. The total radioactivity in each slice was calculated by addition of radioactivity in the medium and radioactivity remaining in the tissue (Table 16, Figure 20). The addition of propranolol alone to the incubation medium produced a small decrease in tissue catecholamines as percent of total radioactivity when compared with control slices. There was a concomitant increase in release of catecholamines into the incubation medium. However, this decrease in tissue amines was statistically non-significant.

An addition of acetaldehyde or ethanol along with propranolol caused no further decrease in tissue catecholamines or increase in the release of $^{3}$H-NE into the medium (Table 16, Figure 20). The tissue amines represented about 56-58% of total radioactivity when only propranolol was present or when both the drugs were present in the medium, whereas it was about 61-62% when only acetaldehyde or ethanol was present.

The addition of propranolol alone or with the second drug increased the percentage of $^{3}$H-NE (15-25%) in the incubation medium whereas percentage of the deaminated metabolites remained almost unchanged. Thus it appears
TABLE 16.--Effect of Propranolol (0.01 mM) on the Release of 0.1 μC of dl-7-\[H\]^-Norepinephrine by Ethanol (0.11 M) or Acetaldehyde (0.47 M) from Rat Brain Cortex Slices Incubated for 30 minutes.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Incubation Medium</th>
<th>Total Radioactivity</th>
<th>% of Total Radioactivity</th>
<th>Incubation Medium</th>
<th>Norepinephrine</th>
<th>Deaminated Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medium + Slices</td>
<td>Slices</td>
<td></td>
<td>Norepinephrine</td>
<td></td>
<td>Deaminated Metabolites</td>
</tr>
<tr>
<td></td>
<td>dpm/50 mg</td>
<td>dpm/50 mg</td>
<td></td>
<td>dpm/50 mg</td>
<td></td>
<td>dpm/50 mg</td>
</tr>
<tr>
<td>Control</td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Ethanol</td>
<td>Ethanol</td>
<td>Ethanol</td>
<td>Ethanol</td>
<td>Ethanol</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>Acetaldehyde</td>
<td>Acetaldehyde</td>
<td>Acetaldehyde</td>
<td>Acetaldehyde</td>
<td>Acetaldehyde</td>
<td>Acetaldehyde</td>
</tr>
<tr>
<td>Control</td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>Ethanol</td>
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<td>Ethanol</td>
<td>Ethanol</td>
<td>Ethanol</td>
<td>Ethanol</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>Acetaldehyde</td>
<td>Acetaldehyde</td>
<td>Acetaldehyde</td>
<td>Acetaldehyde</td>
<td>Acetaldehyde</td>
<td>Acetaldehyde</td>
</tr>
<tr>
<td>None</td>
<td>1433 ±594</td>
<td>12929 ±856</td>
<td>8297 ±299</td>
<td>6997 ±672</td>
<td>2.25 ±1.43</td>
<td>597 ±2.19</td>
</tr>
<tr>
<td>Propranolol</td>
<td>13466 ±795</td>
<td>12469 ±485</td>
<td>12469 ±405</td>
<td>12469 ±405</td>
<td>12469 ±405</td>
<td>12469 ±405</td>
</tr>
</tbody>
</table>

*The results are expressed as mean values, ± S.E.M. of 6 slice preparations. The brain slices were pooled together from 5 or 6 rats.
FIGURE 20

The effect of propranolol (0.01 mM) in the presence of ethanol (0.11 M) or acetaldehyde (0.47 mM) on release and metabolism of 0.1 μc of dl-7-3H-norepinephrine from rat cerebral cortex slices after 30 minutes of incubation.
Total Deaminated Metabolites in Medium

NE - Medium

NE - Tissue

I = S.E.M.

Total dpm x 10^3/50mg in Rat Cerebral Cortex Slices

- Control + Propranolol
- EtoH + Propranolol
- AcH + Propranolol
that propranolol does not influence the release of biogenic amines from brain cortex slices.

**In Rat Mid-Brain Slices**

The rat mid-brain slices were incubated either with propranolol alone, ethanol and propranolol or acetaldehyde and propranolol. The concentrations used for these drugs were the same as in the previous experiment. The total uptake of $^3$H-NE by each mid-brain slice preparation was calculated by addition of total radioactivity in the incubation medium and the radioactivity remaining in the slices.

Propranolol, in a final concentration of $1 \times 10^{-5}$M (2.5 μg/ml), slightly increased the release of $^3$H-NE into the incubation medium as a decrease in percentage of total radioactivity in tissue amines was seen (Table 17, Figure 21). The decrease in tissue amines was about 10% as compared to control and was statistically non-significant. When ethanol (0.11 M) or acetaldehyde (0.47 mM) were added to incubation medium containing propranolol also, similar decreases in tissue catecholamines were observed as compared to ethanol or acetaldehyde alone. However, no further increase in the release of $^3$H-NE was observed when propranolol alone was present or both drugs were present together. The tissue amines represented about 61% of total radioactivity when either propranolol or both
TABLE 17.—Effect of Propranolol (0.01 mM) on the Release of 0.1 µC of
dl-7-H3-Norepinephrine by Ethanol (0.11 M) or Acetaldehyde
(0.47 M) from the Rat Mid-Brain Slices Incubated for 30 minutes.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Control</th>
<th>Ethanol</th>
<th>Acetaldehyde</th>
<th>Control</th>
<th>Ethanol</th>
<th>Acetaldehyde</th>
<th>Control</th>
<th>Ethanol</th>
<th>Acetaldehyde</th>
<th>Control</th>
<th>Ethanol</th>
<th>Acetaldehyde</th>
<th>Control</th>
<th>Ethanol</th>
<th>Acetaldehyde</th>
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<tr>
<td>Total Radioactivity*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium + Slices</td>
<td>dpm/50 mg</td>
<td>8522</td>
<td>10591</td>
<td>9107</td>
<td>5654</td>
<td>6757</td>
<td>5745</td>
<td>66.5</td>
<td>64.5</td>
<td>63.2</td>
<td>1987</td>
<td>2648</td>
<td>2512</td>
<td>585</td>
<td>760</td>
</tr>
<tr>
<td></td>
<td>±1861</td>
<td>±203</td>
<td>±1338</td>
<td>±1164</td>
<td>±1287</td>
<td>±799</td>
<td>±3.0</td>
<td>±0.71</td>
<td>±1.23</td>
<td>±482</td>
<td>±522</td>
<td>±441</td>
<td>±218</td>
<td>±216</td>
<td>±136</td>
</tr>
<tr>
<td>Slices</td>
<td>dpm/50 mg</td>
<td>9899</td>
<td>11367</td>
<td>6448</td>
<td>6104</td>
<td>6968</td>
<td>60.5</td>
<td>61.5</td>
<td>61.3</td>
<td>2790</td>
<td>2913</td>
<td>2993</td>
<td>804</td>
<td>633</td>
<td>751</td>
</tr>
<tr>
<td></td>
<td>±1395</td>
<td>±1678</td>
<td>±559</td>
<td>±660</td>
<td>±1025</td>
<td>±378</td>
<td>±2.21</td>
<td>±2.05</td>
<td>±1.78</td>
<td>±503</td>
<td>±974</td>
<td>±286</td>
<td>±184</td>
<td>±132</td>
<td>±147</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Incubation Medium</th>
<th>Norepinephrine</th>
<th>Deaminated Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpm/50 mg</td>
<td>dpm/50 mg</td>
</tr>
<tr>
<td>Control</td>
<td>10756</td>
<td>6448</td>
</tr>
<tr>
<td>Ethanol</td>
<td>9899</td>
<td>6104</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>11367</td>
<td>6968</td>
</tr>
</tbody>
</table>

*The results are expressed as mean values, ± S.E.M. of 4 slices preparation. The mid-brains were pooled together from 5 or 6 rats.
FIGURE 21

The effect of propranolol (0.01 mM) in the presence of ethanol (0.11 M) or acetaldehyde (0.47 mM) on release and metabolism of 0.1 μc of dl-7-H3-norepinephrine from rat mid-brain slices after 30 minutes of incubation.
Total Deaminated Metabolites (in medium)

□ NE-Medium

■ NE-Tissue

I = S.E.M.

Total dpm x 10^5/50 mg in Rat Mid-brain Slices

Control Control +
Propranolol

EtoH EtoH +
Propranolol

AcH AcH +
Propranolol
drugs were present whereas it was between 63-66% when no drug or ethanol or acetaldehyde alone was present.

The deaminated metabolites represented about 18% of the total radioactivity in the incubation medium whereas \( {^{3}\text{H}}\)-NE accounted for 70% when both propranolol and ethanol or acetaldehyde was present. Similar results were observed when only propranolol or one of the other drugs was added to the incubation medium.

**Cyclic 3'-5' Adenosine Monophosphate**

**in Brain Slices**

The level of cyclic AMP was measured in the rat brain slices incubated for 30 minutes in the medium containing either ethanol, acetaldehyde, propranolol, or when both ethanol or acetaldehyde and propranolol were present. The final concentrations of propranolol, ethanol, and acetaldehyde were \( 1 \times 10^{-5} \text{M} \), 0.11 M, and 0.47 mM respectively. The level of cyclic AMP in the rat cerebral cortex slices was increased in the presence of ethanol or acetaldehyde compared to the control slices but this increase was statistically non-significant (Table 18). However, the same concentrations of ethanol or acetaldehyde did not have an effect on the level of cyclic AMP in the mid-brain slices.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Brain Region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cerebral Cortex</td>
</tr>
<tr>
<td>None</td>
<td>0.358 ± 0.081&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.670 ± 0.178</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>0.663 ± 0.204</td>
</tr>
<tr>
<td>Propranolol</td>
<td>0.765 ± 0.280</td>
</tr>
<tr>
<td>Ethanol Plus Propranolol</td>
<td>0.398 ± 0.047</td>
</tr>
<tr>
<td>Acetaldehyde Plus Propranolol</td>
<td>0.701 ± 0.101</td>
</tr>
</tbody>
</table>

<sup>a</sup>The results are expressed as cyclic AMP pmoles/mg wet tissue ± S.E.M. The brain slices were pooled together from 5 or 6 animals.

<sup>b</sup>Indicates the number of slice preparations used.
Propranolol, a beta-adrenergic blocking agent, by itself caused a non-significant increase in the level of cyclic AMP in the cerebral cortex slices, but not in the mid-brain slices when compared to the control slices (Table 19).

When both propranolol and acetaldehyde were present in the medium, the level of cyclic AMP in the cerebral cortex slices was unchanged compared to propranolol alone or acetaldehyde alone (Table 18). In contrast to acetaldehyde, ethanol and propranolol caused a non-significant decrease in the cyclic AMP levels in the cerebral cortex slice.

The levels of cyclic AMP in mid-brain slices were almost unchanged whether propranolol was added with ethanol or acetaldehyde (Table 18).
DISCUSSION

The primary aim of this study has been to investigate the possible role of biogenic amines, particularly NE, in the central actions of ethanol and the possibility that this action may be mediated through acetaldehyde. Previous evidence has indicated that in the peripheral system, especially the heart, ethanol-mediated release of catecholamines was due to acetaldehyde (Walsh et al., 1970a). The present study appears to confirm these findings in the central nervous system (CNS).

In this investigation, the estimation of release, uptake and metabolism of NE was studied by turnover analysis by introducing a tracer dose of labeled amines into the endogenous pool by an intracisternal injection. This isotopic method was preferred to other methods because the results are more direct as the turnover of NE can be calculated from the declining slope of specific activity of NE, whereas with other isotopic methods, using NE precursors, the results could be complicated due to the persistence of the NE precursor. Another alternative to the isotopic methods has been to use biosynthesis inhibitors but these produce adverse and non-specific pharmacologic actions when
used in large doses. Another disadvantage of this method is the incomplete blockage of NE synthesis. Also depletion of NE may accelerate the normal control existing in the neurons, which might complicate the interpretation of results. Instead of biosynthesis inhibitors, metabolism inhibitors (MAO inhibitors) have also been used to study the rate of biogenic amines. The turnover rate of an amine is calculated by a decrease in the concentration of metabolite or an increase in amine concentration in the brain with time. Due to accumulation of biogenic amines in the brain, the normal control existing in the neuron may be altered which would complicate the interpretation of the results.

In the peripheral system, the action of ethanol has been studied on the metabolism of $^{14}$C-NE both in man and in animals (Smith and Wortis, 1960b; Smith and Gitlow, 1967; Davis et al., 1967b). A shift in metabolism of $^{14}$C-NE from an oxidative to a reductive pathway was observed after acute ethanol dose. Walsh et al. (1970a) have shown that these induced changes in $^{14}$C-NE metabolism after ethanol are mediated by acetaldehyde.

In chronic alcoholic subjects, the higher blood acetaldehyde levels seen (Truitt, 1971) have been attributed to an increase ethanol metabolism in the liver (Truitt, 1971; Kater et al., 1969; Mendelson et al., 1965). This increased acetaldehyde level in chronic alcoholics
may be responsible for some of the CNS action, i.e., behavioral and emotional changes, seen after alcohol. The other change seen after chronic ethanol intake is fat accumulation in liver but its mechanism is still not known. It is possible that increased acetaldehyde levels are responsible for deposition of fat by an increase in fat mobilization from adipose tissue through hypothalamus-anterior pituitary-adrenal cortex system.

Based on the evidence presented here, it is apparent that ethanol given as a single dose (1) slightly decreases turnover of NE in rat brain in vivo, (2) initially increases neuronal uptake of labeled NE. However when ethanol is administered chronically (1) it increases the release of NE and therefore, its turnover and (2) does not affect the uptake of NE.

Acetaldehyde when given in large doses can induce release of NE and thereby the turnover of NE is increased in vivo. The levels of acetaldehyde used in the present investigation can be produced in animals after disulfiram administration, chronic administration of ethanol in animals and in chronic alcoholics. The fact that in the present investigation chronic ethanol administration produces an increase in the release of labeled NE which is similar to acetaldehyde but not to acute ethanol, would indicate that this change produced by chronic ethanol may be due to increased acetaldehyde levels. The results will
be discussed in the order as they were presented in the Results section.

**In Vivo Release and Metabolism of $^3$H-Norepinephrine**

**A. After Acute Ethanol or Acetaldehyde Treatment**

The ability of ethanol or acetaldehyde to interact with catecholamine stores in the CNS was measured by injecting $^3$H-NE intracisternally in rats, as it has been shown that exogenous NE was bound in dense core vesicles at nerve terminals when injected intraventriculary (Aghajanian and Bloom, 1967) or intracisternally (Iversen and Simmonds, 1967) and it was mixed and released with endogenous NE (Glowinski and Axelrod, 1965b, 1966a; Glowinski et al., 1966b). They also showed that a substantial proportion (5-20%) of the injected dose was selectively accumulated and retained in the central catecholamine-containing neurons. In spite of these findings this method for the turnover rate determination has been criticized by some investigators for a lack of validity due to its physiological distribution pattern over the other isotopic method as exogenous NE can also be taken up by dopaminergic neurons. Whereas when NE precursors are used, labeled NE is being synthesized in the noradrenergic neurons. However, the population of dopaminergic neurons in the brain is small compared with the number of noradrenergic neurons. Other advantages
of this method over the other techniques (NE precursors, synthesis or metabolism inhibitors) are the cost and the sensitivity as turnover rate can be measured in small brain samples. The most important advantage of $^{3}$H-NE method is the measurement of turnover rates under steady state conditions without interfering with the size of the normal NE pool or with normal adrenergic function.

To study the effect of ethanol on the neuronal $^{3}$H-NE uptake, the drug is administrated prior to the radiolabel and the animals are killed shortly thereafter. In this case the brain content of tritiated NE primarily reflects initial neuronal uptake. However when the drug is administered after intracisternal injection of $^{3}$H-NE, the neuronal uptake of the amine is not influenced as it is already in a bound-form and the changes in the levels of NE and its metabolites reflect effects of the drug upon release and metabolism of the amine. To study the turnover rate of NE (i.e., rate of amine utilization and replacement by newly synthesized non-labeled NE), $^{3}$H-NE is injected intracisternally for different time periods and the slope of the disappearance curve is calculated. In these experiments, ethanol or acetaldehyde is given for 1 hour after the label.

It has been previously shown that $^{3}$H-NE is stored in multiple pools in the adrenergic nerve endings in rat brain (Glowinski et al., 1965a). The rate of disappearance
of $H^3$-NE from these pools was multiphasic, at first the
disappearance was rapid and then it became increasingly
slower (Glowinski et al., 1965a). The experiments described
in this report further support the concepts of a multiphasic
disappearance of NE after both drugs (Figures 7 and 8).

Under the experimental conditions described above, a
single dose of ethanol slows the disappearance of $H^3$-NE
with only a small decrease in endogenous NE, whereas two
doses of acetaldehyde facilitated the disappearance of both
the level of $H^3$-NE and endogenous NE content in the brain
(Table 2). Thus the effects of a single dose of ethanol
and acetaldehyde are basically different.

It has been shown that acetaldehyde caused a
significant decrease in endogenous NE only when increased
blood acetaldehyde levels were produced either by disulfiram
pretreatment before ethanol dose or by administration of
acetaldehyde itself (Duritz and Truitt, 1966). The present
study confirms these findings, with blood acetaldehyde
levels similar to those reported by these investigators
in rat brain pretreated with disulfiram before ethanol
administration. A number of investigators have shown that
ethanol alone caused a small decrease in endogenous NE in
the brain only when biosynthesis inhibitors were present
(Corrodi et al., 1966; Duritz and Truitt, 1966). A
decrease was found in the present study, but only at a
shorter time (2 hours) was it statistically significant
(Table 2). However this observed difference was probably due to an unusually high level of endogenous NE content in controls, or to difference in the age in this group of the animals.

The blood ethanol levels observed in the present investigation after 1 hour of acute ethanol treatment were in the range of 200-300 mg % (Table 2). Duritz and Truitt (1966) showed that the blood ethanol levels reached the peak value at 30 minutes (about 175 mg %) after 2 g/kg dose and were about the same even after 90 minutes (156 mg %) both in rats and rabbits. With a higher dose of ethanol, 4 g/kg, they observed an elevated level of blood ethanol (341 mg %). They also found that acetaldehyde did not reach its highest blood level until 90 minutes after ethanol dose. Tyce et al. (1970) measured the blood and brain ethanol levels by an enzymatic method and still found similar levels both in the brain and blood. The results shown here are in agreement with those observed by other investigators.

As the action of a single dose of ethanol differed from the acetaldehyde action on the rate of disappearance of $^3$H-NE in the brain, it is possible that these two drugs release NE from different pools in the brain. It has been previously shown that NE is stored in more than one pool and one of these pools appears to be labile, i.e., NE not firmly bound, with half life of 3-1/2 hours whereas the
other is firmly bound, referred as the "reserve" pool with half life of 17 hours. If the drug is affecting the release of NE from the "labile" pool, an increased rate of disappearance of $\text{H}^3$-NE would be observed. As ethanol caused a slow disappearance of $\text{H}^3$-NE at shorter time than at longer time (Table 6), it would suggest that ethanol is not affecting the release of NE from the "labile" pool. As a greater retention of $\text{H}^3$-NE was observed in the ethanol-treated rats at shorter time than at longer time, with almost the same percentage change in endogenous NE, the specific activity of NE remaining in the brain was calculated (Table 2). The specific activity of NE remaining in the brain after ethanol was higher than in the control animals. At early time periods, the ratio of specific activity of NE in ethanol-treated rats was about twice that of the control, but it was reduced at later times. A similar decrease in specific activity of NE has been observed with reserpine when the drug was given 1 or 21 hours after intraventricular injection of labeled catecholamines (Glowinski et al., 1965a). This indicates that the recently bound NE is more resistant to the depleting action of ethanol, or that there is more free $\text{H}^3$-NE in the brain at this time which cannot be released by ethanol. The latter possibility is less probable because unbound $\text{H}^3$-NE would be rapidly metabolized in axoplasm.
In contrast to ethanol, acetaldehyde releases a greater percentage of tritiated catecholamines the shorter it is stored but it affects the same percentage of endogenous NE irrespective of the time (Table 6). As the increase in disappearance rate of $^3$H-NE after acetaldehyde was similar to that of reserpine but not on endogenous NE, therefore the specific activity of NE remaining in the brain was much lower with acetaldehyde than with reserpine. This would indicate that acetaldehyde is releasing NE mainly from "labile" pool which has a much faster turnover rate than "reserve or non-functional" pool and it has probably no effect on its uptake or binding to vesicles which is believed to be affected by reserpine.

Even though ethanol is generally considered to be a depressant, it can produce both types of mood changes, i.e., euphoric and depressant action, therefore its action on catecholamine release in the CNS can be compared with antidepressant or stimulants and with sedatives. In recent years a number of investigators have shown that several tricyclic antidepressants slowed the rate of disappearance of $^3$H-NE when the drug was administrated after the intracisternal injection but not prior to treatment of the drug (Schanberg et al., 1967; Schildkraut et al., 1967, 1969; Schubert et al., 1970). The decrease in the rate of disappearance of labeled NE especially with desmethylimipramine was similar to that observed in
ethanol-treated rats in the present study. These workers did not measure endogenous NE. Our interpretation of these results is that ethanol's action on the release of NE is similar to antidepressant drugs but not on the reuptake of NE as this will be discussed in detail later on. It can be postulated from these results that a single dose of ethanol initially increases the release of NE at receptor site and this increase in NE causes a decrease in its synthesis by a negative feedback mechanism. Additional work needs to be done to elucidate this hypothesis.

Ethanol, after acute and chronic administration, is known to cause a stress-like effect which causes an increase in excretion of NE and changes in its metabolism (Klingman and Goodall, 1960; Davis et al., 1966, 1967a,b; Feldstein et al., 1967). Thierry et al. (1968) and Rosecrans (1969) have reported that in rats, an acute stress situation caused a decrease in endogenous NE but observed no change in the specific activity of NE. This indicates that ethanol in a single dose can cause a stress-like action in the CNS.

However the actions of acetaldehyde on depletion of NE may be quite different from ethanol in the CNS. Its action seems to be partly similar to amphetamine as both drugs cause a decrease in the levels of endogenous NE and tritiated NE at shorter (1 hour) and at longer (21 hours) time (Glowinski and Axelrod, 1965b; Schanberg et al., 1967).
It has been shown that acetaldehyde has an activating
effect on the EEG like amphetamine but acts as a depressant
in very high doses (Akabane et al., 1964). Recently it has
been shown that d-amphetamine released H$^3$-NE from cat brain
in situ when the amine was injected into the lateral
ventricle (Carr and Moore, 1970a,b). It has been suggested
that the mechanism by which amphetamine depletes NE in the
brain is similar to tyramine's action in the peripheral
nerve (Kopin and Gordon, 1962, 1963; Glowinski and Axelrod,
1965b). The actions of acetaldehyde on NE release in the
peripheral system are similar to tyramine (Walsh et al.,
1970). Tyramine releases and displaces bound H$^3$-NE
transiently from adrenergic neurons and has been suggested
to involve release of transmitter from extra-granular
stores and also inhibit the uptake of NE at the axon
membrane level. Therefore our interpretation of these
results would be that in the CNS acetaldehyde in moderate
doses, depletes loosely bound H$^3$-NE in a manner similar
to tyramine and amphetamine but its effect on the uptake
or reuptake are not yet known. On the other hand, ethanol's
action in CNS may be two fold (1) in some aspects, it is
similar to reserpine's action on catecholamine release but
less potent and (2) it is also similar to tricyclic
antidepressant on the mechanism of uptake.

The disappearance rate of H$^3$-NE is a net balance of
release, reuptake, storage and metabolism. An increase
in disappearance rate would indicate an increase in release and probably an increase in turnover rate of NE. However a slow disappearance of $^3$H-NE cannot be taken to indicate that release is unaffected since it is possible that the uptake process is affected. When turnover rates for both ethanol and acetaldehyde were calculated, there was a small but non-significant decrease in turnover of NE after acute ethanol treatment whereas after acetaldehyde, NE turnover was increased significantly (Table 2).

These results after a single dose of ethanol are compatible with the findings of Corrodi et al. (1966) and those of Duritz and Truitt (1966). Corrodi et al. (1966) studied the turnover of NE using the amine synthesis inhibitor (alpha-methyl-p-tyrosine) and observed a decrease in endogenous NE content. They concluded from their fluorescence studies that ethanol increased release and synthesis of NE however evidence was not provided for the latter statement. Duritz and Truitt (1966) studied the turnover of NE both in ethanol and acetaldehyde-treated rats. They observed that acetaldehyde given in large doses or produced by ethanol by disulfiram pretreatment depleted NE from the rat brain stem. The present observations after acetaldehyde are in agreement with their work. Ethanol has been shown to cause a small decrease in serotonin turnover rate after a single dose of ethanol.
(Tyce et al., 1970; Kuriyama et al., 1971). They used MAO inhibitor to study their turnover rate.

The turnover rate of NE in the whole brain is an indicator of the overall change in NE utilization and replacement of newly synthesized NE. The rate constants obtained in these experiments for acutely administered ethanol and its control were slightly higher (20%) than those obtained after acute stress under the same experimental conditions (Thierry et al., 1968) and when an NE precursor was used (Lin et al., 1969). However the turnover rates for NE were higher in the present investigation due to higher endogenous NE concentration compared to the other investigators.

B. After Chronic Ethanol Administration

The animals on the alcohol-containing liquid diet gained almost the same weight as controls on the prolonged ethanol feeding but not initially (Figure 12). This indicates that ethanol calories are being utilized as efficiently as sucrose and that there were no serious problems of food absorption or utilization.

The consumption of ethanol increased progressively and reached a maximum plateau on the ninth day (Figure 13). On the thirteenth and eighteenth days, withdrawal symptoms such as tremor and convulsion were seen within 3 hours after the removal of alcohol diet. Administration of the
test dose of ethanol promptly interrupted this syndrome in most of the rats. This indicates that physical dependence to ethanol can be induced in a relatively short time. Similar withdrawal symptoms, after ethanol and diluted bourbon whiskey, have been observed in mice, monkeys and dogs (Ellis et al., 1971, 1970; Goldstein et al., 1971).

The blood ethanol levels were only slightly higher after chronic administration compared to acute treatment (Table 11). This small difference in the level of blood ethanol observed after chronic ethanol treatment can be due to several factors such as length of fasting which affects the rate of absorption of ethanol. Secondly it reflects an increase in the metabolism of ethanol as prolonged ethanol consumption can induce changes in hepatic ethanol metabolizing enzymes (ADH and "MEOS" or catalase) in animals (Kalant et al., 1972; Lieber and DeCarli, 1970, 1972; Mezey, 1972). An increased metabolism of ethanol is also observed in alcoholic subjects (Mendelson et al., 1965; Kater et al., 1969; Truitt, 1971).

In habituated drinkers, ethanol produces quite different behavioral and emotional changes than those seen in naive drinker. In recent years changes in biogenic amines have been correlated with the alteration in mood and behavior found in certain mental diseases and produced by depression. In the affective disorders, patients treated with drugs such as antidepressants and MAO
inhibitors had decreased urinary excretion of catecholamines (NE and Epi) whereas there was an increased excretion of NMN (Schildkraut et al., 1967b, 1970a). Studies with animals have shown that the brain levels of $^3$H-NE were increased after tricyclic antidepressants. In some animal species the behavioral excitation produced by MAO inhibitors was temporally correlated with increased levels of NE whereas with an increase in serotonin, no behavioral excitation was seen (Spector et al., 1963). Changes in the biogenic amine metabolism have been shown after ethanol intake both in man and animals (Davis et al., 1967a,b; Feldstein et al., 1967, 1969).

An attempt was made to correlate the effect of chronic ethanol intake on catecholamine turnover rate in rat brain. The rate of disappearance of tritiated NE was increased in the brains of rats given alcohol for 13 or 18 days compared to acutely administrated ethanol rats (Table 11). However there was no change in the endogenous NE in chronically ethanol-treated rats (Table 11). In contrast to the acutely ethanol-treated rats, the rate of disappearance of $^3$H-NE in chronically ethanol-fed rats was similar to the rate seen after acetaldehyde-treated rats. This faster rate of disappearance of $^3$H-NE in chronically ethanol-treated rats is probably due to an increase in ethanol metabolism which has been shown both in man and in animals (Mendelson et al., 1965; Kater et al., 1969;
Lieber and DeCarli, 1970b; Truitt, 1971; Kalant et al., 1972). This increased ethanol metabolism probably produced increased levels of acetaldehyde. Truitt (1971) has shown that the blood acetaldehyde level was higher in alcoholics than in non-alcoholic subjects. Majchrowicz and Mendelson (1970) observed a greater increase in blood acetaldehyde levels in chronic subjects after bourbon than after ethanol drinking. In this laboratory, in another series of chronic experiments it has been shown that the blood and brain acetaldehyde levels were higher following chronic ethanol than in acutely ethanol-treated rats (Truitt, unpublished). It has also been observed that the brain acetaldehyde levels reached a peak between the fifth and ninth days after the start of chronic ethanol feeding in three series of rats received ethanol or in one series using rum in Metrecal.

The results from these experiments are compatible with the interpretation that the release of catecholamine (NE) in the brain after chronic ethanol administration is mediated by increased levels of acetaldehyde produced by increased metabolism of ethanol in liver and to some degree in brain. As an adaptive change in brain ADH activity has been reported after chronic ethanol administration (Raskin and Sokoloff, 1970).

The results shown here were not seen by Bhagat et al. (1971) in their preliminary study not yet fully published.
They observed a decreased rate of disappearance of \(^{3}\text{H}-\text{NE}\) after chronic administration of ethanol in rats. The observed difference is probably due to administration of a small dose of ethanol as it was administered orally three times a day (3 ml of 15% solution).

The turnover rate of 5-HT after chronic, continuous oral ethanol administration (in a liquid diet) was significantly increased in mice with an increase on tryptophan hydroxylase activity (Kuriyama et al., 1971). However in female rats, no change in 5-HT turnover rate was observed after chronic ethanol intake (Palaic et al., 1971). These results on 5-HT turnover indicate that ethanol effect varies with the species and sex of animals. Also the difference in the species found may be related to a sensitivity of serotonergic and noradrenergic systems. Therefore from our results, it can be interpreted that an increase in NE turnover may produce behavioral excitation after ethanol whereas an increase in 5-HT could produce depression.

Long term administration of imipramine appears to have similar effects on the rate of disappearance of \(^{3}\text{H}-\text{NE}\) as chronic ethanol treatment (Schildkraut et al., 1970b). However the decrease in the level of \(^{3}\text{H}-\text{NE}\) is not as great as that seen after chronic ethanol treatment. This is because the mechanism by which antidepressant affect the NE level at the receptor site is by blocking the reuptake
mechanism of NE and this increases NE at the receptor site and probably decreases its synthesis by a negative feedback mechanism. It is possible that the release or uptake mechanism of NE after long-term administration of ethanol is different from antidepressant agents especially if acetaldehyde is the causative agent. This will be discussed in the next section, i.e., with metabolism of H\(^{3}\)-NE.

Even though both ethanol and morphine appear to produce addiction on long-term administration and withdrawal symptoms upon abstinence, their effects on NE turnover rate appear to be different. Morphine dependence in the rats did not increase NE turnover rate (Neal, 1968) whereas chronic ethanol intake appears to increase H\(^{3}\)-NE disappearance, which indicates increased turnover rate of NE.

The increased disappearance of H\(^{3}\)-NE after chronic ethanol feeding could be due to increased formation of tetrahydropapaveroline alkaloids as it has been shown that both ethanol and acetaldehyde enhanced its formation in rat brain homogenates (Davis et al., 1970; Walsh et al., 1970b). The second group of alkaloids, tetrahydroisoquinolone, which are formed spontaneously by condensation of catecholamines and acetaldehyde, may also affect the release of NE in vivo after chronic ethanol feeding, as
these compounds have been shown to cause the release of $^3$H-catecholamines in vitro (Heikkeila et al., 1971).

From these results, it is postulated that after long-term feeding of ethanol an increase release of NE in the brain is mediated by acetaldehyde.

$^3$H-NE metabolism was studied to determine the release mechanism of NE by acetaldehyde or ethanol. According to the current concept, sympathomimetic drugs or nerve impulse release NE in physiologically active form or extra-neuronally, whereas drugs like reserpine release mainly by an intraneuronal mechanism. After both acetaldehyde and ethanol the major metabolites present in the brain were O-methylated deaminated products at 2 and 6 hours (Tables 3 and 4). This is in agreement with the results of other workers on various drugs like tyramine, tricyclic antidepressants and under acute stress conditions (Kopin and Gordon, 1962, 1963; Schanberg et al., 1967; Schildkraut et al., 1967b; Thierry et al., 1968). Even though ethanol administration resulted in a small increase in formation of $^3$-NMN compared to acetaldehyde (Tables 3 and 4), no alteration in deaminated metabolites was found. This difference in metabolic fate of $^3$-NE at synaptic site by acetaldehyde or ethanol indicates the ethanol is affecting the reuptake of NE when compared with acetaldehyde or somehow acetaldehyde inactivates the enzyme catecol-O-methyltransferase (COMT). However the second possibility is
unlikely as the control animals given a dose of saline also had a decrease level of \( H^3\text{-NMN} \). An alternate explanation of these results would be the variation that is observed in the percentages. The percentages of total radioactivity represented by \( H^3\text{-NMN} \) for both drugs is within the range observed by other investigators for shorter time (2 hours).

Amphetamine and tricyclic antidepressants increase the formation of \( H^3\text{-NMN} \) under similar conditions and in cat brain \textit{in situ} but the increase observed in NMN formation was much higher than that seen with ethanol (Schanberg et al., 1967; Schildkraut et al., 1967b, 1969a; Carr and Moore, 1970a,b). Therefore ethanol's action on uptake of NE will receive additional discussion later.

At 22 hours, only a small amount of deaminated metabolites were present whereas a large percentage of radioactivity in the brain accounted for unchanged \( H^3\text{-NE} \) (Table 5). The radioactivity of labeled NMN was too low for separation. Glowinski et al. (1965a) and Iversen and Glowinski (1966) have also reported higher percentage of \( H^3\text{-NE} \) in the rat brain 6 and 24 hours after intraventricular injection of \( H^3\text{-NE} \). Our results are in agreement with their results.

The effect of ethanol or acetaldehyde on the metabolism of labeled NE is different from that of reserpine as the latter drug increases the level of deaminated metabolites both in the peripheral and in central nervous system.
(Kopin and Gordon, 1962, 1963; Glowinski and Axelrod, 1966b). They suggested that reserpine released NE intraneuronally and not extraneuronally as there was no change in NMN level. Whereas studies with tyramine and amphetamine have shown that these two drugs release physiologically active NE into the circulation (Kopin and Gordon, 1963) and from brain (Glowinski and Axelrod, 1966b) as the deaminated metabolites are not altered. Since the deaminated metabolites in the brain are not effected by ethanol or acetaldehyde, this would indicate that both ethanol and acetaldehyde are releasing NE in physiologically active form or extraneuronally in the manner similar to indirect sympathomimetic drugs like tyramine or amphetamine.

However after chronic administration of ethanol, the metabolism of labeled NE was more similar to the pattern of acetaldehyde than of ethanol given in a single dose. The concentration of brain $^3$H-NMN was not altered in the rats addicted to ethanol (Table 12). This indicates that the reuptake mechanism of NE is not affected after chronic ethanol administration. However a greater percentage of O-methylated deaminated metabolites were present on the eighteenth day in ethanol addicted rats compared to non-addicted rats receiving a control dose of ethanol. This would indicate that NE is probably being released intraneuronally and is metabolized by MAO. This increase in deaminated metabolites in the rats fed ethanol for
18 days is similar to reserpine effect on $^{3}\text{H}-\text{NE}$ metabolism (Glowinski and Axelrod, 1966b). It has been suggested by these workers that reserpine releases NE in a physiologically inactive form. The results from the present investigation are compatible with the interpretation that release pattern of NE in the CNS after chronic administration of ethanol is probably similar to reserpine.

As the percentage of deaminated metabolites in the brain of ethanol addicted rats is higher than acetaldehyde treated rats, it is possible that due to the persistence high concentration of acetaldehyde in the brain after the fifth to ninth days of alcohol feeding, more NE is being released intraneuronally than after two doses of acetaldehyde. This would also explain the increased rate of disappearance of $^{3}\text{H}-\text{NE}$ after prolonged feeding of ethanol.

It is believed that the inactivation of released NE is mainly by its neuronal reuptake mechanism but a small percentage is metabolized by COMT at the synaptic site. This metabolite, NMN is then further metabolized by MAO at nerve terminals. It has been shown in vivo that acetaldehyde but not ethanol produced profound changes in $^{14}\text{C}-\text{NE}$ (from oxidative to reductive pathway) metabolism when $^{14}\text{C}-\text{NE}$ was injected in tail vein (Walsh et al., 1970a). Other investigators have observed that in man ethanol ingestion produced similar changes in $^{14}\text{C}-\text{NE}$ metabolism (Davis et al., 1967a,b; Feldstein and Sidel, 1969). These
workers did not find any alteration in $^{14}\text{C-}\text{NMN}$ levels in the peripheral system, but observed an increased excretion of $^{14}\text{C-}\text{MPGH}$ and a concomitant decrease in $^{14}\text{C-}\text{VMA}$. These two metabolites were not separated in the brain as it was beyond the scope of this investigation, therefore no conclusion on the change in these pathways in amine metabolism in the brain can be made at this point.

From the release and metabolism of labeled NE experiments in the rat brain, it can be postulated that acute ethanol's actions are different than those of chronic ethanol in the CNS. The action of a single large dose of ethanol on catecholamines seems to be two fold in the CNS (1) in some aspects it is similar to reserpine's releasing action on the depletion of NE from "reserve" pool but it may not be such a potent drug as reserpine is. Also it does not seem to affect the binding of amines to its storage vesicles as reserpine does. (2) It probably increases the release of NE initially by acetaldehyde formation, but then by negative feedback (i.e., decrease uptake mechanism) it decreases NE synthesis. Therefore a slight decrease in its turnover rate is seen. In this manner its actions are similar to tricyclic antidepressants. Further work needs to be done on this aspect of ethanol's action.

In contrast to acute ethanol treatment, prolonged feeding of ethanol causes an increased disappearance rate
of $H^3$-NE which indicates an increased turnover rate of NE in the brain. This action seems to be similar to acetaldehyde when given in large doses. However the metabolites of $H^3$-NE after long-term ethanol feeding indicate that NE is released probably in a different manner than after acute ethanol or acetaldehyde treatment. NE is probably released in an inactive form like after reserpine or intraneuronally.

Acetaldehyde, when given in large doses, causes
(1) a depletion of NE similar to indirect sympathomimetic agents like tyramine or amphetamine, (2) releases the newly bound or synthesized amine from labile pool which has a rapid turnover rate. It causes the depletion of NE by displacement from its storage vesicles without directly affecting its binding sites or competing for its storage site. The metabolism of $H^3$-NE both after acetaldehyde and acute ethanol treatment suggests that the release of NE is in physiologically active form, i.e., extraneuronally. But additional work needs to be done to elucidate the exact mechanism by which NE is released at the nerve terminals that is by exocytosis or by some other mechanism.
Uptake and Metabolism of $^{3}$H-Norepinephrine

A. Single Dose Ethanol Treatment

To study the effects of a single dose of ethanol on uptake of NE, the drug was administered prior to intracisternal injection of $^{3}$H-NE. If animals were killed shortly (15 minutes or less) thereafter, then the brain content of tritiated NE primarily reflects initial neuronal uptake.

Prior treatment with a single dose of ethanol for 45 or 105 minutes and 15 minutes exposure to labeled NE, caused a similar decrease in $^{3}$H-NE level in the rat brain which reflects an inhibition of neuronal uptake (Table 7). There was no change in endogenous NE. These results indicate that ethanol pretreatment for shorter (1 hour) or longer times (2 hours) has the same effect on the neuronal uptake of labeled NE. Various tricyclic antidepressants like imipramine, desmethylinipramine, protriptyline, and cocaine have been shown to cause inhibition of neuronal uptake of intracisternal administered $^{3}$H-NE (Schanberg et al., 1967; Schildkraut et al., 1967a, 1969a, 1970). These investigators also showed that the effect of cocaine appeared to be dose related. These tricyclic antidepressants also did not change endogenous NE content in the brain. Amphetamine has also been shown to decrease retention of labeled NE in the brain (Schanberg et al., 1967). In this
respect ethanol's action on neuronal uptake of $H^3$-NE appears to be similar to these drugs, but not when the formation of labeled NMN is compared. The tricyclic antidepressants and cocaine increase the formation of NMN significantly whereas with ethanol, both an increase and a decrease of NMN levels was observed. The difference observed in the present data could be due to the time of exposure of radiolabel, as these investigators killed the rats 6 minutes after the intracisternal injection.

However the effect of ethanol on neuronal uptake at longer exposure of brain to $H^3$-NE was quite different than when the brains were exposed to radiolabel for 15 minutes. The level of $H^3$-NE retained in the brain was increased with time (Table 7). The endogenous NE content was decreased significantly only in one group of ethanol-treated rats which was probably due to a higher concentration of endogenous NE seen in the controls. This would indicate that ethanol increases amine neuronal uptake at longer time either by decreasing its synthesis, release or by changes in membrane permeability. Recently Schildkraut et al. (1970) have shown that a single dose of imipramine inhibited the neuronal uptake of $H^3$-NE in the brains of animals killed 6 minutes after intracisternal injection as reflected by lower concentration of $H^3$-NE whereas when the animals were killed 270 minutes after intracisternal injection, there was a slow disappearance
of labeled NE in the brain. Therefore our results of uptake experiment can be interpreted that ethanol's action is similar to the action of imipramine on the disappearance of tritiated NE.

It has been suggested that imipramine and other tricyclic antidepressants produce their effect by increasing the level of NE at the receptor site by either blocking or inhibiting its reuptake as the membrane pumps of both NE and 5-HT neurons appear to be sensitive to these drugs. It is possible that ethanol, like antidepressants, affects the membrane pump of NE and 5-HT neurons as synaptosomal Na⁺, K⁺-activated ATPase is inhibited by ethanol in the concentrations that would produce mild to severe intoxication in vivo (Sun and Samorajski, 1970; Israel et al., 1967, 1970). This would decrease the NE reuptake initially (or an increase in NMN level) thereby increasing NE level at the receptor site initially. But then by a negative feedback mechanism, ethanol decreases NE synthesis or its turnover rate. This would be reflected by the slower disappearance rate of H³-NE as observed in the present experiment when the brain was exposed to radiolabel for longer time (Figure 11). The turnover rate was calculated even though the drug was given prior to H³-NE injection as the ratio of the specific activity remaining in the brain of ethanol-treated rats to control at longer time (90 minutes) was similar to that
observed in the release experiment at 2 hours (Table 2). A decrease in the turnover rate of NE is observed after ethanol which indicates either a decreased release or utilization of NE at the nerve terminals in the CNS.

The percentage of deaminated metabolites of H\textsuperscript{3}-NE was unaffected by ethanol pretreatment.

The results of uptake and metabolism of H\textsuperscript{3}-NE after acute ethanol treatment are compatible with the interpretation that initially ethanol increase the neuronal uptake of NE but it decreases NE turnover rate in the "labile" pool either by a decreased release or utilization of NE at later time.

B. After Chronic Ethanol Administration

After 3 days administration of ethanol, the neuronal uptake of H\textsuperscript{3}-NE into the brain was almost unchanged compared to a single dose of ethanol, however it was decreased on the fifth day (Table 9). This indicates that the effects of chronic administration of ethanol develop gradually and probably reaches its peak effect about the fifth day. After this period no further changes are seen in the retention of H\textsuperscript{3}-NE probably due to adaptive changes in the enzymes in the brain. It has been reported in chronic ethanol studies that brain MAO and indoleacetaldehyde aldehyde dehydrogenase (AID) were unaffected whereas after an initial decrease brain NADPH linked aldehyde
reductase (AIR) increased markedly as compared to controls. These changes in enzyme levels were observed on the fifth day of ethanol feeding (Collins et al., 1971). An alternate explanation of these changes in the disappearance of rate of $\text{H}^3\text{-NE}$ on the fifth and ninth days may be due to an increase in acetaldehyde levels in the brains as observed after chronic ethanol feeding in rats (Truitt, unpublished). He also observed that acetaldehyde levels in the rat brain reached its peak in several other chronic alcohol feeding studies between the fifth and ninth days.

It can also be pointed out that ethanol consumption reached its peak on the ninth day (Figure 13). Acetaldehyde levels were not measured in this study because ether anesthesia, required for the intracisternal injection, interfered with the retention time of the acetaldehyde.

An effect similar to the fifth day on alcohol was also seen in rats fed Metrecal (Figure 14) chronically. This group had only a few animals and all these animals were more irritable than the ethanol-fed rats. Therefore the decrease in the retention of $\text{H}^3\text{-NE}$ seen in the brain of rats fed Metrecal chronically may be due to a stress-like effect. From these results, no conclusion can be drawn as the data is insufficient and more work needs to be done.

The specific activity of NE remaining in the brain was decreased significantly on the fifth day but increased
on the ninth day in the ethanol addicted compared to non-addicted rats given a control dose of ethanol. These results indicate that more newly bound NE is released on the fifth day whereas on the ninth day probably both pools are being affected by ethanol per se or by increased concentration of acetaldehyde accumulated after chronic ethanol intake.

To our knowledge, only one study of this type has been done after long-term administration of imipramine (Schildkraut et al., 1970). They observed similar decreases in the retention of H3-NE after short- and long-term administration of imipramine when the brain was exposed to labeled NE only for 6 minutes. As we have shown earlier in this investigation that ethanol given as a single dose produced similar changes to imipramine at 15 minutes but not at later times, therefore a possibility must be considered that the increase in the release of NE at shorter time exposure or decreased reuptake of NE at postsynaptic site, might cause a feedback inhibition of NE biosynthesis from a postsynaptic neuron. Thus an increase in the retention of H3-NE was seen after 30 minutes exposure to radiolabel in long-term ethanol fed animals.

Tetrahydroisoquinolone alkaloids formed spontaneously by condensation of catecholamines and acetaldehyde may be affecting the uptake of H3-NE in vivo after chronic ethanol administration as it has been reported that these
compounds do inhibit uptake of $H^3$-catecholamines by crude synaptosomes from rat brain (Heikkila et al., 1971).

An alternate interpretation of these results is that acetaldehyde in high concentration is masking the effect of ethanol on neuronal uptake or reuptake of NE when fed for long periods of time.

The increased uptake of $H^3$-NE into the brain and changes in the concentration of its metabolites (no change in $H^3$-NMN level and a decrease in $H^3$-deaminated metabolites) were qualitatively similar after short-term and long-term administration of ethanol but the changes in deaminated metabolites were more pronounced after the ninth day on alcohol (Table 9).

As there was no change in the percentage of labeled NMN of total radioactivity irrespective of the number of days fed ethanol, this would indicate that the reuptake mechanism for NE is not affected. The other possibility that must be considered is that the adaptive change in membrane pump of NE in noradrenergic neurons might occur shortly after ethanol intake. This hypothesis is compatible with the results of Israel et al. (1970b) as they observed an adaptive change in Na$^+$-, K$^+$-activated ATPase after chronic ethanol feeding.

The changes in deaminated metabolites have been correlated with the release of NE in physiologically active or
inactive form at the nerve terminals. As the percentage of deaminated metabolites was decreased with prolonged feeding of ethanol, this would indicate that NE in a physiologically active form is being released after chronic ethanol. However on the ninth day, the per cent of deaminated metabolites is decreased to its lowest percentage of total radioactivity. These changes in deaminated metabolites may be interpreted that noreadrenergic nerve terminals are probably not releasing actively NE on nerve impulse due to changes in membrane. This interpretation would also explain the high percentage of H$^3$-NE retained in the brain on this day. It is also possible that a gradual increase in acetaldehyde after ethanol feeding is affecting membrane permeability to ions at nerve terminals but as the ethanol feeding continues the adaptive changes to acetaldehyde may take place. Additional work needs to be done to elucidate this hypothesis.

**In Vitro Release of H$^3$-Norepinephrine by Ethanol or Acetaldehyde**

Experiments were performed to see whether the action of ethanol or acetaldehyde seen on the brain catecholamine release and metabolism in vivo, could also be seen in brain slices. Release of H$^3$-NE into the incubation
medium has been observed with other drugs such as amphetamine, tyramine, reserpine, both with synaptosomes and cerebral cortex preparations (Colburn and Kopin, 1972; Ziance et al., 1972a,b).

In other drug experiments of this type, the release of NE from cerebral cortex slices appears to occur from nerve endings since the results were similar to those obtained from synaptosomes (Colburn and Kopin, 1972; Ziance et al., 1972a). The accumulation of exogenous NE appears to be in nerve endings containing catecholamines since histochemical fluorescence studies have shown a marked increase in fluorescence of catecholamine-containing nerve terminals after the addition of NE or alpha-methyl-NE to the cerebral cortex slices (Hamberger and Masuoka, 1965). Coyle and Snyder (1969) have observed that $^{3}$H-amine uptake was localized in synaptosomes enriched fraction of homogenate from different regions of rat brain.

Both acetaldehyde and ethanol in the concentration used had no significant effect on the release of $^{3}$H-NE into the incubation medium at various incubation times except at 20 minutes incubation with acetaldehyde (Table 14). A concomitant decrease in tissue amine was observed at this time with acetaldehyde (Figure 18). These findings at this incubation time are qualitatively similar to those observed after acetaldehyde in vivo. The lack of effect
may be due to insufficient concentration of acetaldehyde used in vitro. An alternate possibility is that the increase in \( ^3 \text{H}^- \text{NE} \) released into the medium is not observed due to a probable quenching effect of a metabolite of acetaldehyde as the level of tissue amines, as a constant percent decrease in dpm/50 mg is found irrespective of the incubation time (Figure 18).

In contrast to the ethanol's action on the cerebral cortex slices, a slight release of \( ^3 \text{H}^- \text{NE} \) from the mid-brain slices was found at 20 minutes but not at 30 minutes (Table 15). Release of \( ^3 \text{H}^- \text{NE} \) by ethanol was associated with a decrease of \( ^3 \text{H}^- \text{amine} \) in the tissue (Figure 19). These results can be interpreted that (1) ethanol's action varies with the regions of the brain, (2) this difference may be due to the white matter present in the slices. The latter possibility is a more likely explanation for the difference observed for various times. An alternate possibility is that this regional difference observed with ethanol is due to ADH activity in the brain. Additional work needs to be done to elucidate this hypothesis.

However the action of acetaldehyde on the release of \( ^3 \text{H}^- \text{NE} \) from mid-brain was similar to the cerebral cortex slices. A possibility must be considered that acetaldehyde may be evaporated by this time, therefore its concentration is not sufficient to produce the effects seen in vivo.
In summary, although slight release of $^{3}$H-NE appears at some time periods with a concomitant decrease in tissue amines, it is our conclusion that in vitro, the release of $^{3}$H-NE from the rat cerebral cortex and mid-brain slices is not significantly affected by ethanol or acetaldehyde.

**Propranolol Blockade in Brain Slices**

This experiment was designed to evaluate whether (1) a block of receptor at the adrenergic nerve terminal by a beta-adrenergic blocking drug would affect the release of catecholamines in the brain slices or (2) a change in the release of $^{3}$H-NE would influence the level of cAMP in the brain slices.

The most clear-cut finding of this experiment was the significant decrease in cerebral cortex tissue amines which was associated with an increase in release of $^{3}$H-NE by propranolol alone. However no additional effect was seen on the release of $^{3}$H-NE when either acetaldehyde or ethanol was also added.

A similar but a non-significant decrease was also observed in tissue amines with propranolol when mid-brain slices were used. However the action of both ethanol and acetaldehyde in the presence of propranolol were not clear-cut. It is possible that propranolol is effectively blocking the beta-receptors at the nerve terminals in the control brain slices but its action in the presence of
ethanol or acetaldehyde is altered. The latter possibility is less likely as it has been shown previously that some of the action of ethanol and acetaldehyde are blocked by propranolol in the peripheral system especially in the heart (Walsh, 1969).

As it has been pointed out earlier that the variation in slice size, amount of white matter, and the concentration of ethanol or acetaldehyde used in vitro may have an effect on the release of H\(^3\)-NE from the tissue.

The experiment with propranolol indicates that propranolol by itself induces the release of H\(^3\)-NE from the brain slices probably by blocking beta-receptors, but the possible non-specific action of propranolol as a local anesthesia should also be considered. No additional effect on the release of H\(^3\)-NE is seen when both ethanol or acetaldehyde plus propranolol is present.

An increase in cerebral cortex cyclic AMP level was observed but not in the mid-brain slices with ethanol and acetaldehyde even though the same concentration of these drugs did not release labeled NE into the incubation medium (Tables 14 and 18). This increase in cyclic AMP is probably due to a variation seen in the samples and no conclusion is drawn at this point that this increase is only due to the drug effect. The level of cyclic AMP observed in the present study for control cerebral cortex slices is similar to that reported by Rall and Sattin (1970),
expressed as pmoles/mg protein and without the first incubation with $^{3}$H-NE. However the control level of cyclic AMP in the present study was much lower than those observed by Uzunov and Weiss (1971). The difference observed may be due to the method or the strain of animals used.

Recently it has been shown that ethanol given as a single oral dose causes a decrease only in rat cerebellum cyclic AMP level but not in the other regions of brain (Volicer and Gold, 1973). The level of cyclic AMP in mouse cerebral cortex slices was uneffected after 7 minutes incubation when a single dose of ethanol was administered prior to sacrifice (Israel et al., 1972). French et al. (1973) have also reported that chronic ethanol feeding to rats did not increase cyclic AMP in the incubated cerebral cortex slices when exposed to $10^{-5}$M NE. The results of the present investigation are qualitatively similar to those observed by the above investigators.

The addition of propranolol to the incubation medium increased the cerebral cortex cyclic AMP level probably due to the individual variation seen in the samples as the level of cyclic AMP in the mid-brain was unaffected by propranolol (Table 18). No conclusions are drawn at this point that this effect on cyclic AMP is due to propranolol alone.
When both acetaldehyde and propranolol were present in the medium, no further increase in cyclic AMP level was observed in the cerebral cortex or in mid-brain slices. These results indicate that propranolol is probably interfering with the action of acetaldehyde on catecolamine release from the brain slices. Therefore no further stimulation of adenyl cyclase system occurs. This interpretation would be consistent with the observation seen on the release of labeled NE from cortex slices (Table 14).

In contrast to the combined action of acetaldehyde and propranolol, ethanol and propranolol decreased the level of cyclic AMP in the cortex slices without affecting the mid-brain cyclic AMP levels. This decrease could be due to the additive depressant action of propranolol and ethanol as ethanol is considered as a depressant of CNS whereas propranolol is a local anesthetic agent. Additional work needs to be done to elucidate the above hypothesis.

Triglycerides and Free Fatty Acids in Liver and in Plasma after Chronic Ethanol or Rum Administration

The results obtained after chronic ethanol administration indicate that hepatic fat accumulation did not occur when the diet provided 30-40% of calories as
ethanol, except on the fifth day when a non-significant decrease was observed (Table 13).

Kalant et al. (1972) have shown that hepatic fat accumulation occurred only when the diet provided high calories both from ethanol and fat but not when ethanol alone was present. The present findings are in good agreement with these investigators and some others (Lieber and Decarli, 1966; Porta et al., 1967; Theuer et al., 1971) that ethanol alone may not be the sole causative agent for the fatty liver seen after chronic ethanol administration. However, in preliminary experiments some investigators observed increased TG content in the liver of monkey (Ruebner et al., 1971) and of mice (Forsyth et al., 1971) with ethanol alone. This difference in the hepatic TG levels may be due to the species used.

In contrast to ethanol feeding, an initial increase in the liver TG concentration was observed in rats fed rum for 3 days. As rum contains a higher percentage of acetaldehyde compared to the ethanol used in the present investigation, the data is compatible with the interpretation that the amount of acetaldehyde production from ethanol may influence the degree of fatty liver. However the levels of TG fell to the control values on the fifth day even though it has been shown that the blood acetaldehyde level reaches its peak between the fifth and ninth day after chronic ethanol and rum administration.
An increase in both liver MAO and NADPH-linked indolacetaldehyde aldehyde dehydrogenase (AID) has also been observed on the fifth day after ethanol ingestion which then fell to control (Collins et al., 1971). It can be hypothesized that this elevation in MAO activity would cause an increased metabolism of catecholamines released by the action of acetaldehyde in the peripheral system (Walsh et al., 1970) and therefore less change in the liver TG concentration was seen compared to control. A significant increase was seen in hepatic TG on the seventeenth and twenty-first day on chronic rum administration may be due to changes in nucleotides ratio (NAD⁺/NADH) resulting from additive effects of increased alcohol and acetaldehyde metabolism. Truitt et al. (1966) have shown that two acetaldehyde doses given 30 minutes apart produced a slight degree of fatty liver in female rats. The accumulation of hepatic TG after acute ethanol is greater in female rats than in male rats (Domanski et al., 1971) therefore no deposition of hepatic TG after chronic ethanol administration in this report may reflect the sex difference or the diet difference as mentioned earlier.

The increase in hepatic TG level observed after chronic rum intake may be due to one of several possible mechanisms. These mechanisms are (1) a decreased utilization of TG in liver, (2) an increased synthesis of
TG in liver, (3) an increased mobilization of TG from adipose tissue (Isselbacher et al., 1964). A change in plasma FFA would also indicate the change in fat mobilization from adipose tissue.

An initial increase in the plasma FFA occurred at 3 days on ethanol but prolonged feeding had no further significant effect. In contrast, the plasma FFA levels were elevated irrespective of the number of days on the diet which provided rum. These results strongly indicate that an initial increase in fat mobilization (FFA) from adipose tissue is occurring which increases the deposition of TG in liver after rum feeding. This would be consistent with the hypothesis of Isselbacher et al. (1964). This increased FFA levels in plasma may be due to increased levels of acetaldehyde in rum as it has been observed that a small dose of acetaldehyde elicited a prompt rise in the plasma FFA (Truitt and Twardowicz, unpublished). The action of acetaldehyde on the fat mobilization from adipose tissue may be mediated through hormones and adrenergic neurotransmitters released from the hypothalamic-anterior pituitary-adrenal cortex system as cited by Brodie et al. (1961) for ethanol action.

The increase in plasma FFA observed on the third day after ethanol without any deposition of TG in liver may be explained by the increased lipoprotein lipase activity seen after a single dose of ethanol (Mallov et al., 1967).
The increased lipoprotein lipase activity would produce an elevated plasma FFA as seen in the present study. It has also been reported that hepatic TG are elevated by a large dose of ethanol after 15 hours (Brodie et al., 1961) and not earlier. Therefore it is possible that the lack of an effect in hepatic TG reflects the effect of a single dose of ethanol given to animals 2 hours prior to killing. More work needs to be done to explain the action of ethanol on plasma FFA and its mechanism of deposition of TG in the liver.

It may be summarized that acetaldehyde in high concentration does mobilize FFA from the adipose tissue probably by stimulation of hypothalamus–anterior pituitary–adrenal cortex system. Therefore an increase in the accumulation of liver TG is observed after rum feeding but not after ethanol.

General Discussion

An attempt has been made to correlate the blood and brain concentrations of ethanol and acetaldehyde after acute and chronic administration to changes in NE synthesis, utilization and metabolism in rat brain. The difference seen in NE turnover in the brain between the single dose and chronic administration of ethanol has led us to propose that (1) the effects on brain NE of ethanol, in
a single large dose, are quite different from its chronic administration and (2) the effects of chronic drinking on NE in the CNS are probably mediated through increased acetaldehyde.

This hypothesis would be compatible with the difference seen in the behavioral and emotional changes in naive and chronic drinkers. Generally after a large drink of alcohol, a non-alcoholic feels initially stimulated by a drink but this is followed by sedation due to increased consumption (larger intake) whereas an alcoholic is unaffected by a small drink of alcohol but with larger consumption of alcohol he becomes more irritable and often emotionally disturbed.

It is also possible that central actions of ethanol after acute administration are being antagonized by its metabolite, acetaldehyde, and as the concentration of acetaldehyde is low after a single dose of ethanol, only the depressant action of ethanol is seen. Therefore only a slight decrease in turnover rate for both NE and 5-HT is seen after a single ethanol intake.

It is the belief of this investigator that ethanol in a large single dose increases the release of NE at the noradrenergic receptor site, therefore initially its stimulant actions are seen. But as the biochemical changes at the cellular levels occur such as the alteration in the
coenzyme ratio \((\text{NAD}^+ / \text{NADH})\), oxidative phosphorylation then only its depressant actions are seen.

In contrast to the single dose of ethanol, chronic administration of ethanol produces the changes in NE which are similar to acetaldehyde. However the metabolism of NE suggests that the mechanism by which NE is released, may be different. It must be emphasized that in chronic ethanol feeding, the levels of both acetaldehyde and ethanol are high therefore the interactions seen with catecholamine pools in the CNS may be complex.

In the affective disorders, such as manic-depressive disorders, the relative increase or deficiency in NE at functionally important adrenergic receptor sites in the brain may be of crucial importance in the regulation of the type of emotional effect seen. Similar behavioral (excitation) and emotional changes are seen after chronic drinking and these are associated with the relative sensitivity of noradrenergic and serotonergic receptors at the nerve terminals in the CNS. However the physical dependence seen in alcoholic subjects may be related to the levels of acetaldehyde as a single dose of ethanol appears to relieve the symptoms of excitation, tremors and convulsions in the animals whereas chronic ethanol often exacerbates these problems. It should be emphasized that besides the genetic or constitutional differences, the psychological and environmental states may have an
important effect on the sensitivity of alcoholics to the biogenic amines in the CNS.

Acetaldehyde by itself acts as a stimulant and its interactions with catecholamine stores in the CNS appear to be similar to the indirect acting sympathomimetic drugs like tyramine or amphetamine.

Ultimately from our findings a concept is formulated that some of the aspects of behavioral and emotional changes seen in chronic alcoholism are correlated with the changes in NE biosynthesis and metabolism in the CNS which are probably acetaldehyde-mediated.
SUMMARY

1) A single dose of ethanol slightly decreased the release of NE from the "labile" pool and slowed its turnover rate in rat brain.

2) Acetaldehyde, in large doses released NE from the "labile" pool and increased its turnover rate in rat brain.

3) Chronic ethanol feeding increased the release of NE in rat brain in a manner similar to acetaldehyde. The metabolism of $^{3}$-NE was different from that seen after a single dose of ethanol or acetaldehyde.

4) A single dose of ethanol initially increased neuronal uptake of $^{3}$-NE without altering the pattern of metabolite formation.

5) On chronic ethanol feeding, the consumption of ethanol was increased until it reached its peak on the ninth day. This coincided with a peak in acetaldehyde production on about the fifth to ninth days.

6) Chronic administration of ethanol did not affect the neuronal uptake of $^{3}$-NE except on the fifth day of feeding when a decrease in the retention of $^{3}$-NE was observed. The deaminated metabolites of $^{3}$-NE were decreased on the ninth day of alcohol feeding without any change in $^{3}$-NMN levels.
7) *In vitro*, acetaldehyde and ethanol did not have an effect on the release and metabolism of $^3$-NE from the brain slices.

8) Propranolol, a beta-adrenergic blocker, increased the release of $^3$-NE from the brain slices without any change in its metabolism. But no additional effect on the release of $^3$-NE was observed when either ethanol or acetaldehyde was also added.

9) The level of cyclic AMP in the rat cerebral cortex slices was increased by ethanol, acetaldehyde and propranolol when added by itself to the incubation medium. Because of the individual variation seen in the sample, no conclusion is drawn that this effect is due to the drug alone.

10) Chronic feeding of rum increased the levels of liver triglycerides and plasma free fatty acids but ethanol produced an increase in plasma free fatty acids only on the third day of ethanol feeding.
APPENDIX

(1) Materials: Cyclic AMP radioimmunoassay kit purchased from Schwarz/Mann, Division of Becton, Dickinson and Co., Orangeburg, N.Y. Cyclic AMP radioimmunoassay standard curve set-up:

<table>
<thead>
<tr>
<th>Standard Cyclic AMP</th>
<th>Tube No.</th>
<th>Acetate Buffer</th>
<th>I^{125}_{cAMP}</th>
<th>Antibody (anti-cAMP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>1,2</td>
<td>300 µl</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>(Total Counts)</td>
<td>3,4</td>
<td>300 µl</td>
<td>100 µl</td>
<td>None</td>
</tr>
<tr>
<td>Trace (% binding)</td>
<td>5,6</td>
<td>300 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>0.03 pmoles</td>
<td>7,8</td>
<td>200 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>0.1 pmoles</td>
<td>9,10</td>
<td>200 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>0.3 pmoles</td>
<td>11,12</td>
<td>200 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>1.0 pmoles</td>
<td>13,14</td>
<td>200 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>3.0 pmoles</td>
<td>15,16</td>
<td>200 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>10 pmoles</td>
<td>17,18</td>
<td>200 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

(2) Calculations for total radioactivity, H^{3}-NE, H^{3}-NMN and endogenous NE in rat brain.

Total volume of brain homogenate supernatant = 20.7 ml.

Total radioactivity:

\[
\mu\text{c/g} = \frac{\text{cpm} \times 20.7 \text{ ml} \times 100}{\text{aliquot (ml)} \times \text{brain weight} \times 22.2 \times 10^5 \times \text{efficiency}}
\]
H<sup>3</sup>-NE μc/g =

\[
\frac{\text{Total cpm} \times 20.7 \text{ ml} \times 100}{5 \text{ ml} \times \text{brain weight} \times \text{efficiency} \times 22.2 \times 10^5 \times \% \text{ recovery on Dowex-50}}
\]

H<sup>3</sup>-NMN μc/g = \[
\frac{\text{Total cpm} \times 20.7 \text{ ml} \times 100}{5 \text{ ml} \times \text{brain weight} \times \text{efficiency} \times 22.2 \times 10^5}
\]

Endogenous NE μg/g =

\[
\frac{\mu g \times 3 \text{ ml} \times 20.7 \text{ ml}}{0.4 \text{ ml} \times 10 \text{ ml} \times \text{brain weight}} \times \% \text{ recovery on alumina oxide}
\]

Specific activity of NE μc/μg = \[
\frac{\text{H}^3-\text{NE} \text{ μc/g}}{\text{Endogenous NE} \text{ μg/g}}
\]

(3) Calculations for turnover rate of NE in rat brain.

Rate Constant = \( k = \frac{\text{Slope}^*}{0.434} \)

*Slope of the curve was calculated by the method of least square analysis for 2 and 6 hours and also for 6 and 24 hours (log specific activity vs. time in hours).

Absolute rate of NE turnover = \( K (\mu g/g/hr) = \)

\[
\text{rate constant (k/hr)} \times \text{endogenous NE} (\mu g/g).
\]
REFERENCES


Sutherland, V.C., Burbridge, T.N. and Simon, A.: Metabolism of C\textsuperscript{14}-Ethanol to C\textsuperscript{14}O\textsubscript{2} by Cerebral Cortex in Vitro. Fed. Proc. 17: 413, 1958.


