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LA THÈSE A ÉTÉ
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NEUROADRENAL RESPONSES TO OXYGEN TOXICITY

By

Ashok Kumar Singh

B.Sc. (1968), M.Sc. (1970), Ph.D. (1974)

Banaras Hindu University

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

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of

Kinesiology

SIMON FRASER UNIVERSITY

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Neuroadrenal responses to oxygen toxicity

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ABSTRACT

Although it is known that neuronal and adrenal factors exert considerable influence on the development of oxygen toxicity, the responses of these systems during the induction of toxic symptoms have not been extensively investigated. The present study, therefore, was conducted to investigate the neuronal, neuroadrenal and metabolic responses during exposure to oxygen at high pressure (OHP) in rats. Normal rats were subjected to OHP for different time intervals until convulsions were produced. Rats treated with 6-hydroxy dopamine (6-OHDA) (68 $mg.kg^{-1}$, i.v.), which induces "chemical sympathectomy", were subjected to OHP in the same manner, as also were adrenalectomized and shamoperated rats, and rats treated with hexamethonium (20 $mg.kg^{-1}$, i.v.) or α-methyl-p-tyrosine (MPT) 200 mg.kg, i.p.). Samples of blood and brain were collected and analysed for adrenaline, noradrenaline, total catecholamine, catechol-O-methyl transferase, ammonia and amino acids. Primary potentiators of convulsion

were sought from among these metabolites.

The result of these experiments show that Catecholamines in the brain of rats do not change significantly in response to exposure to OHP until after convulsions commence. Conversely alterations in the concentration of catecholamines caused by different drugs do not effect the latency of convulsion. These observations suggest that brain catecholamines are not important in the mechanism of convulsions induced by OHP. Most of the noradrenaline produced after convulsions induced by OHP comes from sympathetic nerves, since:

(a) no significant increase in noradrenaline concentration in blood was observed when rats treated with 6-OHDA were subjected to convulsions induced by OHP,

(b) the increase in the concentration of noradrenaline in blood in response to convulsions induced by OHP in normal rats was almost same as that in adrenalectomized rats.

In normal rats subjected to OHP for different time intervals, there was a significantly elevated brain ammonia and a decreased brain GABA. Concomitantly ammonia and catecholamine concentrations in blood were elevated. It is quite possible that these hormonal and metabolic changes interact to induce the toxic effects of OHP. Concentration of glutamate decreased and concentration of glutamine increased in blood and brain following convulsions, in all groups of rats, whether normal, adrenalectomized, shamoperated, or treated with 6-OHDA, hexamethonium or MPT.

Chemical sympathectomy of rats using 6-OHDA decreased the latency to convulsion, probably due to one or more of the following factors:

(i) low brain GABA.

(ii) elevated ammonia in brain and blood

(iii) decreased glutamate in brain and blood

(iv) an elevated adrenaline in blood.

Adrenalectomy protects rats against oxygen toxicity, i.e. latency to convulsion was increased from 43

minutes to 102.8 minutes. Ganglionic blockade with hexamethonium protected the rats against oxygen toxicity. Latency to convulsions increased to 98.8 minutes. Inhibition of brain catecholamine synthesis with MPT did not affect the concentration of catecholamines in blood as it did in brain, nor did it alter the time of the onset of convulsions due to the toxic action of oxygen.

The present study suggests that:

1. Concentration of ammonia in blood and brain, GABA in brain and adrenaline in blood are important in generating convulsions induced by OHP.

2. There is some evidence for an ammonia threshold in generation of the convulsions induced by OHP, in that the postconvulsive concentration of ammonia in blood and brain is constant among the different groups irrespective of the preconvulsive

concentration of ammonia, or the latency to convulsion.

ACKNOWLEDGEMENTS

I would like to thank Dr.E.W.Banister, my senior supervisor, for his invaluable advice and constructive criticism during the course of my research. I am thankfull to Dr.A.J.Davison, Dr.N.M.G.Bhakthan and Dr.J.S.Barlow for their interest and participation in this study. Also, Mr.M.Yim of Biological Sciences deserves credit for his technical assistance in the present study. I would especially like to thank my wife, Aruna, and friends, Badri, Mohan, Sundaram, Askok, Pillay, Unnithan and Bharath for their constant help.

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CHAPTER-1

INTRODUCTION

Oxygen at a concentration slightly greater than that present in air, becomes toxic to animals and plants. Several authors have reported that the toxic effects of oxygen are due to the production of free radicals and inactivation of enzymes (Bean 1945 and Stadie et al. 1944). However there is no conclusive study to demonstrate that these changes also occur in intact animals in response to oxygen at high pressure (OHP). In vivo, it is not possible to attribute the development of oxygen toxicity to any single chemical reaction or inhibitory mechanism, since numerous systems and components in the body tissue are sensitive to OHP. Ammonia toxicity (Banister et al., 1976) and reduction in brain gamma amino butyric acid (GABA) levels (Wood et al., 1965) have also been proposed as possible mechanisms for the induction of convulsions due to the toxic effects of OHP in intact animals.

Although it is known that neuronal and neuroadrenal factors exert considerable influence on the development of oxygen toxicity, the mechanism by

which the neuronal and neuroadrenal systems respond to OHP to induce toxic symptoms have not been extensively investigated.

The present studies have been conducted to investigate the neuronal and neuroadrenal changes occuring in response convulsions induced by OHP. The questions proposed for investigation are:

(1) What is the response of the neuronal and neuroadrenal systems to oxygen toxicity, subsequent to the production of convulsions by OHP, in particular, what proportion of the excess NA is produced by the adrenal gland and what by the sympathetic nervous system?

(3) How do metabolic changes in blood and brain tissue interact with neuroadrenal hormones to produce the toxic symptoms of OHP?

(4) How do sympathectomy, adrenalectomy, ganglionic blockade and blockade of catecholamine synthesis modify the toxic effects of oxygen?

An experimental schematic of the various experiments planned in these investigations together with the question proposed in each section and the consequences of their answers is shown in diagrams 1 - 5.

DIAGRAM-1: Showing the overall experimental approach to determine the contributory effects of the sympathetic nervous system and the hormonal system to the development of convulsion in animals exposed to oxygen at high pressure (OHP).

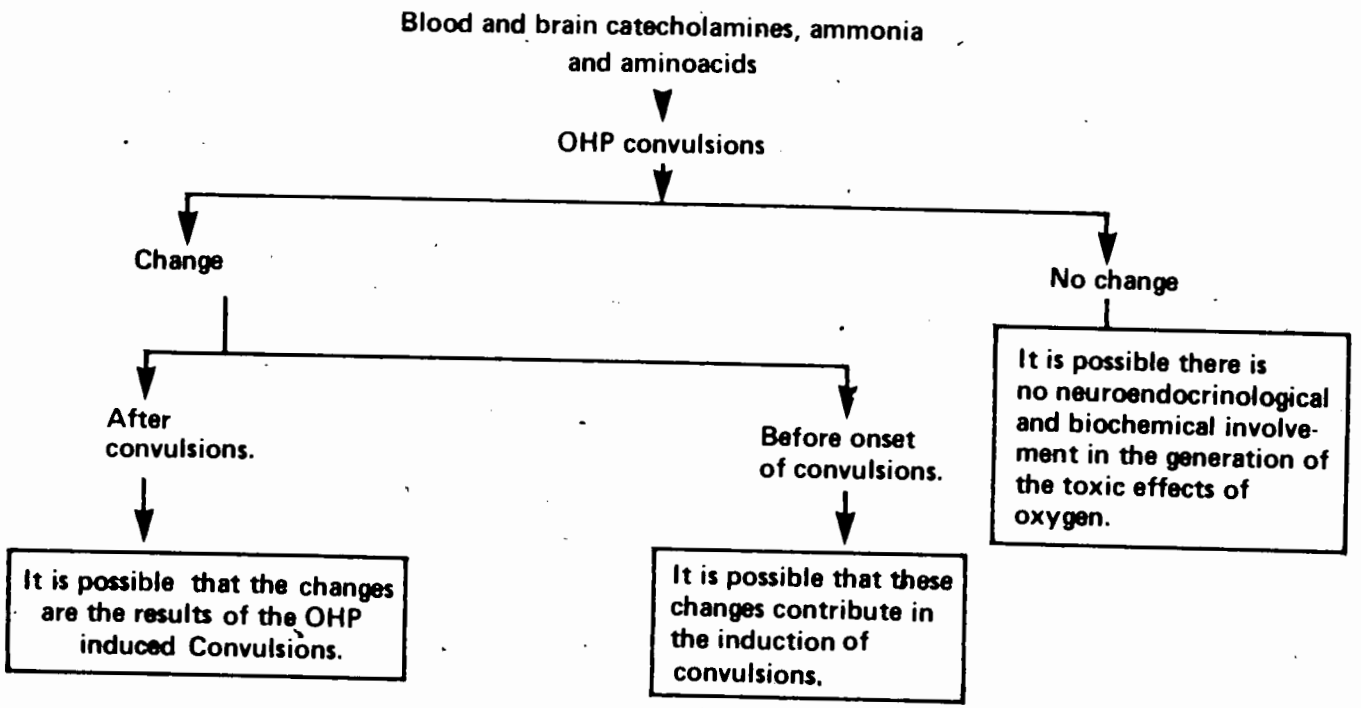

Blood and brain catecholamines, ammonia and aminoacids
OHP convulsions
Change
No change
After convulsions.
Before onset of convulsions.
It is possible there is no neuroendocrinological and biochemical involvement in the generation of the toxic effects of oxygen.
It is possible that the changes are the results of the OHP induced Convulsions.
It is possible that these changes contribute in the induction of convulsions.

DIAGRAM-2: Showing the overall experimental approach to determine the contributory effects of the adrenal gland to the development of convulsion in animals exposed to OHP.

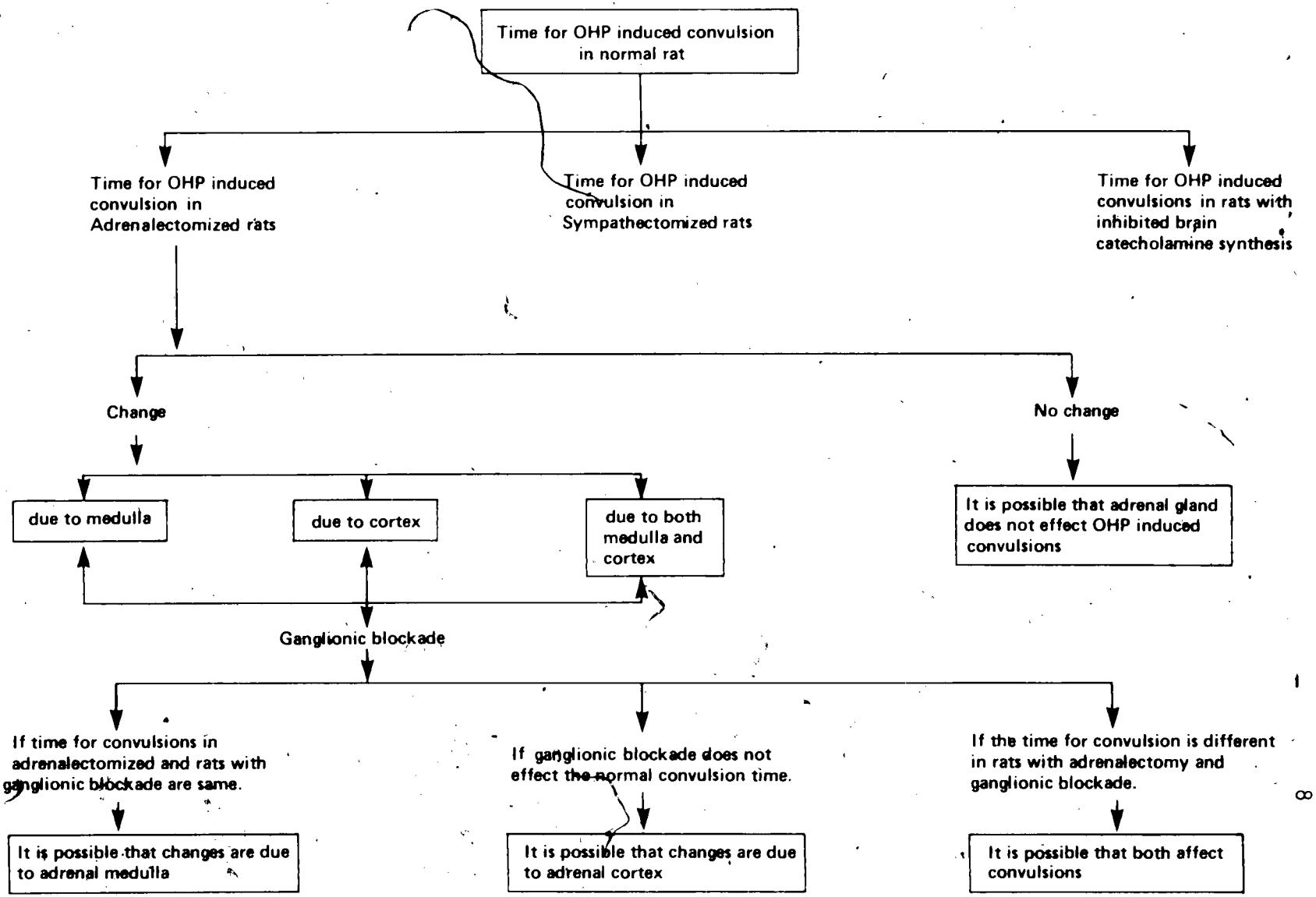

Time for OHP induced convulsion in normal rat
Time for OHP induced convulsion in Adrenalectomized rats
Time for OHP induced convulsion in Sympathectomized rats
Time for OHP induced convulsions in rats with inhibited brain catecholamine synthesis
Change
No change
due to medulla
due to cortex
due to both medulla and cortex
It is possible that adrenal gland does not effect OHP induced convulsions
Ganglionic blockade
If time for convulsions in adrenalectomized and rats with ganglionic blockade are same.
If ganglionic blockade does not effect the normal convulsion time.
If the time for convulsion is different in rats with adrenalectomy and ganglionic blockade.
It is possible that changes are due to adrenal medulla
It is possible that changes are due to adrenal cortex
It is possible that both affect convulsions

DIAGRAM-3: Showing the overall experimental approach to determine the contributory effects of the sympathetic system to the development of convulsion in rats exposed to OHP.

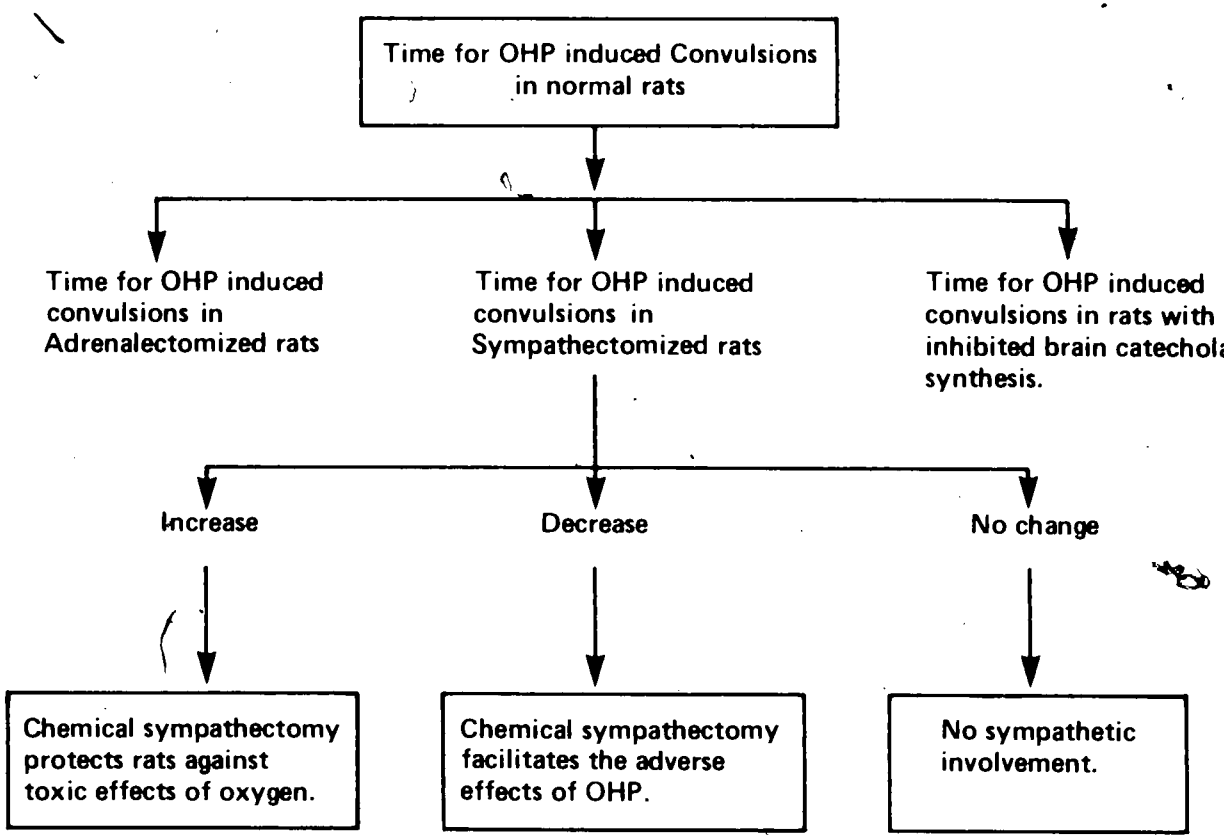

Time for OHP induced Convulsions in normal rats
Time for OHP induced convulsions in Adrenalectomized rats
Time for OHP induced convulsions in Sympathectomized rats
Time for OHP induced convulsions in rats with inhibited brain catecholamine synthesis.
Increase
Decrease
No change
Chemical sympathectomy protects rats against toxic effects of oxygen.
Chemical sympathectomy facilitates the adverse effects of OHP.
No sympathetic involvement.

DIAGRAM-4: Showing the overall experimental design to study the effects of inhibition of brain catecholamine synthesis to the development of convulsion in animals exposed to OHP.

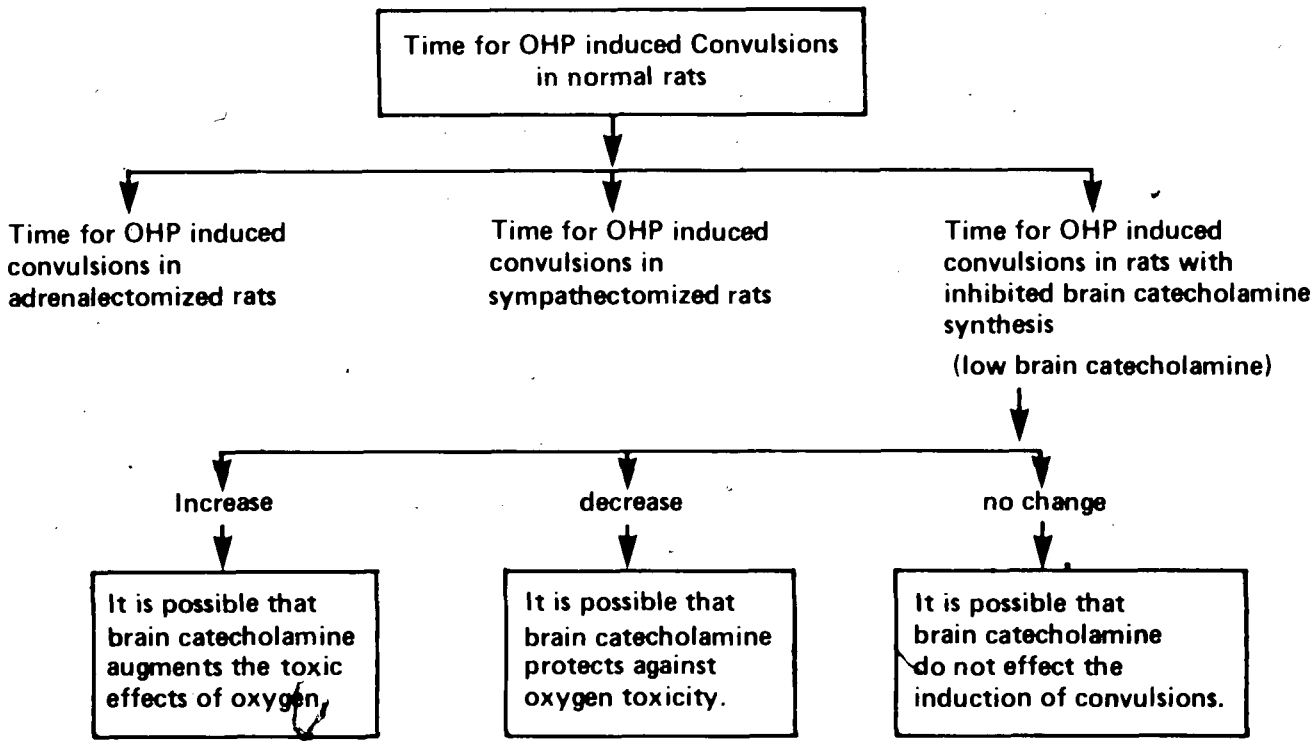
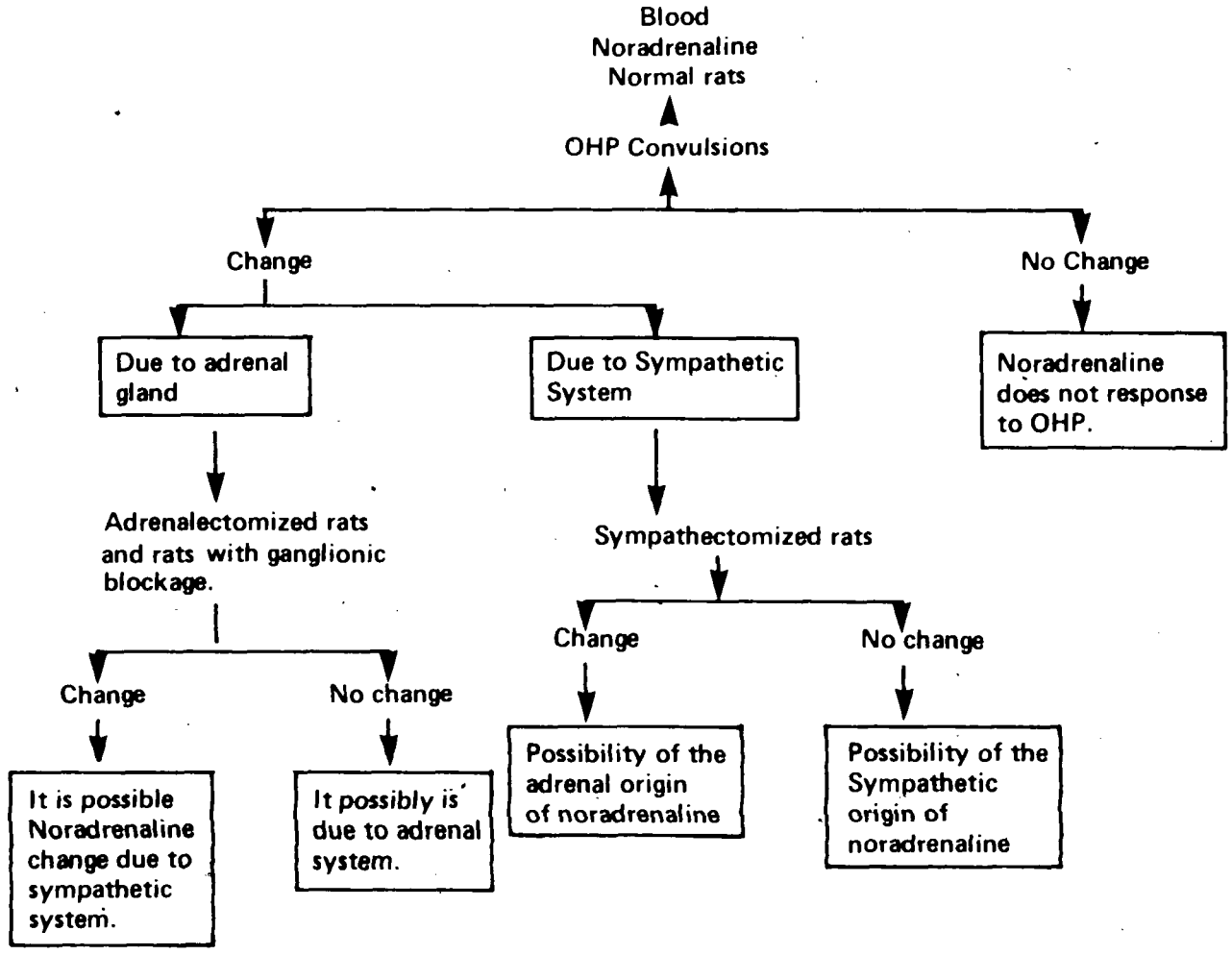

Time for OHP induced Convulsions in normal rats
Time for OHP induced convulsions in adrenalectomized rats
Time for OHP induced convulsions in sympathectomized rats
Time for OHP induced convulsions in rats with inhibited brain catecholamine synthesis
(low brain catecholamine)
Increase
decrease
no change
It is possible that brain catecholamine augments the toxic effects of oxygen.
It is possible that brain catecholamine protects against oxygen toxicity.
It is possible that brain catecholamine do not effect the induction of convulsions.

DIAGRAM-5: Showing the overall experimental approach to investigate the proportion of excess noradrenaline produced by the adrenal gland and by the sympathetic nervous system after the development of convulsion in normal rats.


Blood
Noradrenaline
Normal rats
OHP Convulsions
Change
No Change
Due to adrenal gland
Due to Sympathetic System
Noradrenaline does not response to OHP.
Adrenalectomized rats and rats with ganglionic blockage.
Sympathectomized rats
Change
No change
Change
No change
It is possible Noradrenaline change due to sympathetic system.
It possibly is due to adrenal system.
Possibility of the adrenal origin of noradrenaline
Possibility of the Sympathetic origin of noradrenaline

CHAPTER-2

REVIEW OF LITERATURE

All animals and plants which are not especially adapted to live under anaerobic conditions, need oxygen for the production of energy and maintenance of life. However oxygen becomes toxic if its concentration is slightly greater than that present in air. Scheele (1782) and Huber and Senebier (1801) preliminarily observed the toxic effects of oxygen on germination of seeds. A systemic study of the effects of oxygen toxicity, however, was undertaken a century later by Paul Bert (1878). In his book La Pression Barométrique(1878) he describes the important effects of oxygen poisoning, its nature and the involvement of the CNS in oxygen toxicity in mammals. The effects of oxygen at high pressure continued to be studied by Hill (1912), Behnke (1941), Stadie et al. (1945 a, b, c), and Haldane (1941)

Recently work on oxygen toxicity has progressed considerably due to its clinical use in:
(1) radiation of tumors (Van Den Brenk 1961)
(2) treatment of neoplastic diseases (Churchill-Davison et al. 1957)

(3) Surgery and Medicine (Boerema, 1961)

(4) treatment of carbon monoxide poisoning (Sluijter, 1963)

(5) diving.

In this review, selected physiological, biochemical and endocrinological effects of oxygen toxicity and possible mechanisms involved in the generation of these effects will be discussed.

OXYGEN REACTIVITY WITH CELL CONSTITUENTS

1. Electronic properties of molecular oxygen

In the ground state the oxygen molecule has 2 unpaired electrons (Pauling, 1960). Thus oxygen reacts very rapidly with molecules which have unpaired electrons. It also reacts with molecules which do not have an unpaired electron provided the first step during the reaction breaks an electron pair (Swartz et al., 1972). If only one of the electrons of the pair is transferred to oxygen, the remaining molecular fragment

will be a free radical.

2. Types of reaction

Molecular oxygen reacts with cell constituents in the following ways:

(a) The normal cellular oxidative reactions.

(b) Formation of hydrogen peroxide or organic peroxide which may ultimately enhance oxidative reactions.

(c) Chain reactions due to radical-radical reactions.

(d) reactions with free radicals converting them to more stable species i.e. termination of chain reactions.

3. Inactivation of enzymes by oxygen

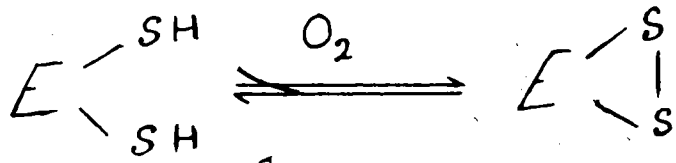
Bean (1945) and Stadie et al. (1945) observed that oxygen was capable of inactivating a number of enzymes. It was also observed that respiration of brain homogenates was markedly decreased by exposure of animals to OHP (Elliott and Libet 1942, Mann and Quastel 1946 and Van Goor and Jongblood 1942). Those

enzymes containing the sulfhydryl group are most sensitive to OHP. Haugaaurd et al. (1957) and Horn et al (1965) found that carbohydrate metabolism in heart muscle was inhibited by exposure to one atmosphere (atm) of oxygen without effecting the glucose utilization. They proposed that oxygen first inhibited glyceraldehyde phosphate dehydrogenase subsequently reducing tissue ATP, lactate and other metabolites. Thomas et al. (1963) observed that the toxic effects of oxygen on carbohydrate metabolism were caused mainly by inhibition of enzymes responsible for pyruvate metabolism. Various proposed mechanisms of enzyme inactivation are:

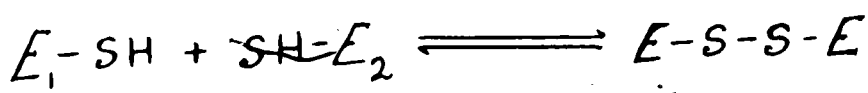
I. Inactivation of enzymes containing sulfhydryl groups

The important step in the inactivation of such enzyme involves the formation of the disulfide bond (Davies and Davies, 1965) in one of three ways:

1.By oxidation within the molecule: If 2 sulfhydryl groups are present adjacent to each other, oxidation may occur within the molecule to form a disulfide linkage



2.By oxidation between two enzyme molecule: A disulfide bond is formed between two enzyme molecules



3.By oxidation involving prior oxidation of nonprotein: A prior oxidation of a nonprotein such as glutathione is essential for inactivation of some SH groups

II.Inactivation of flavoprotein enzymes

The flavoprotein enzymes contain a free SH group and a nonhaem iron and these are all involved in the activity of the enzyme. Oxygen oxidises the sulphydryl group and changes the structure of the enzyme. Examples of such enzymes are lipoyl dehydrogenase, SDH,

xanthine oxidase, LDH, and cytochrome c.

Despite these conclusive demonstrations of enzyme inactivation by oxygen in vitro, it is not possible to attribute to them directly the symptoms of oxygen toxicity observed in intact animals exposed to OHP. The important reasons are (1) no conclusive study has demonstrated that the activity of these enzymes was inhibited in intact animals after exposure to OHP(Haugaard,1968), (2) the time course of in vitro enzyme inhibition is too slow to cause the fast appearing symptoms of oxygen toxicity after exposure of animals to OHP.

OXYGEN TOXICITY IN VIVO

The effects of oxygen at high pressure on organic materials or on isolated tissue constituents demonstrates the toxic action of excess oxygen. However, there are no data demonstrating that any of the effects shown in these systems are responsible for

the toxic effects of oxygen in living animals. Bean and Bohr (1944) observed that rat pyloric muscle in ringer solution began to relax when the oxygen pressure was increased to 5 atm. No relaxation occured when the same muscle was exposed to 5 atm air. They proposed that this relaxing effect of oxygen on muscle at high pressure resulted from the inhibition of tissue dehydrogenases. Riggs (1945) observed that sodium azide did not abolish muscle relaxation induced by OHP and proposed that sites of oxygen poisoning are glycolytic enzymes, possibly GDH. In addition to muscle relaxation, animals exposed to OHP suffer vasoconstriction, damage to blood vessels, damage to blood vessels of the eyes and direct damage to the retina (Noell 1958, and Hanson1966).

Jamieson et al. (1963) observed that rats subjected to OHP had low succinate dehydrogenase, lower concentration of SH groups and an increased concentration of disulfide groups in the lungs. Dixon et al.(1960) proposed, on the basis of their studies on oxidation of pyridine nucleotide, that hyperberic

oxygenation inhibits the utilization of high energy intermediates used for NAD reduction. They also suggested that the process is mediated by the oxidation of a labile SH group. Gordon et al (1963) demonstrated that rats exposed to 6 atm oxygen showed increased fructose 1,6 diphosphate and triose phosphate and decreased GPD in liver and muscle tissue but not in brain. They suggested that the effect of oxygen at the glycolytic level are caused by changes in concentration of cofactors. Dolezal et al.(1962) observed increased sugar in blood and decreased concentration of lactate and pyruvate in liver of rats exposed to oxygen at 1 atm. Sanders et al. (1966) observed that ATP contents of liver and kidney decreased when animals were exposed to OHP. Chance et al.(1966), however, failed to observe any change in brain ATP contents and observed very high ATP in liver when rats were exposed to OHP.

NEURONAL ELEMENTS AND OXYGEN TOXICITY

As discussed earlier, it is not possible to equate any one chemical reaction or inhibition, with the development of oxygen toxicity, since numerous systems and components in the body tissue are sensitive to OHP. It is more likely that oxygen poisoning is due to a variety of factors some related, and some unrelated to one another (Wood,1976). The most important effect of oxygen toxicity on the CNS is the manifestation of epileptiform convulsions (Bert,1943). OHP may effect the CNS in several ways by:

1. inactivating SH groups of enzymes (Stadie et al. 1945),
2. releasing toxic free radicals,
3. reducing brain gamma- aminobutyric acid (Wood 1971),
4. producing neuroendocrinological disturbances,
5. causing ammonia and carbon dioxide poisoning.

Inactivation of the sulphydryl groups of enzymes

As discussed earlier, oxygen at high pressure is known to inactivate certain enzymes. These enzyme inactivations lead to the inhibition of cellular metabolism (Stadie et al.1945) This ultimately decreased levels of ATP, lactate and pyruvate in tissue.

Release of toxic free radicals

As discussed earlier, oxygen at high pressure reacts with various constituents of tissue and produces toxic free radicals. These free radicals play an important role in producing the toxic symptoms of OHP.

Reduction in brain gamma aminobutyric acid (GABA)

GABA is a simple short chain amino acid present only in the vertebrate central nervous

system. It has two important function in the brain:

(1) as an inhibitor of nerve transmission (Krnjevic and Schwartz, 1967)

(2) as an intermediate in oxidative metabolism (Roberts and Eidelberg, 1960).

Otsuka et al(1966) demonstrated that GABA is selectively released by stimulating inhibitory neurones. The inhibitory action of GABA on mammalian cortical neurones was studied by Hayashi (1956). Studies of Obata et al.(1967) have shown that GABA is the inhibitory transmittor released in the medulla by endings of the cerebral purkinje cells.

Metabolism of GABA

GABA is formed from glutamate in the brain by the enzyme glutamic decarboxylase. It is uniformaly distributed in all central neurons. It is converted to succinate semialdehyde by transamination with α-ketoglutarate (αKG), a reaction catalyzed by GABA amino transferase (Kelly and Krnjevic 1969). Succinate semialdehyde is ultimately oxidized to succinic acid.

Since succinic acid is a component of the tricarboxylic acid cycle, GABA is constantly removed by this cycle.

GABA and oxygen toxicity

The role of GABA in the induction of convulsions has been studied by several workers. Gershenovich and Krichevskaya (1956) reported that in in brain of rats exposed to OHP there was an increased concentrations of glutamate and an inhibition of the enzyme system responsible for the synthesis of glutamine. Wood et al. (1965) observed that brain of rats convulsed by OHP exposure had decreased concentration of GABA and glutamate decarboxylase (GAD) activity. They noted that low GABA levels might be the major cause of seizures induced by OHP. The important causes of decreased GABA in brain in response to OHP might be:

1. inhibition of the enzyme responsible for the synthesis of GABA.
2. acceleration of membrane transport of GABA.
3. activation of the degrading enzyme GABA-T.

The inhibition of GAD might be due to inactivation of the SH groups at the active site. Since SH groups are common to many enzymes, it might be expected that those, other than GAD, which are directly involved in neurotransmittor metabolism will be similarly affected by OHP. As suggested by Wood et al. (1970), GABA seems to be the prime neurotransmitter regulating convulsive activity, it seems probable also that GAD is selectively inhibited by OHP.

Transport of GABA across the cell membrane is an important factor which may effect cerebral GABA levels. The synthesizing enzyme (GAD) is found only in nerve endings whereas the degrading enzyme GABA-T is present in mitochondria (Wood et al. 1971 and Weirstein et al. 1963). Thus GABA which is synthesized within the neurones has to cross one or more membranes to reach to a site of degradation, in the mitochondria. Wood et al. (1966) suggested that OHP could reduce the GABA in brain levels by altering membrane transport of GABA.

It is still not clear how a decreased concentration of GABA in brain would lead to the development of the symptoms of oxygen toxicity. It appears that a certain minimal level of GABA is necessary for proper functioning of the brain. When the level of GABA declines below this threshold, serious derangement in the brain activity is produced.

Relationships among GABA, glutamate, glutamine and ammonia

Banister et al.(1976) observed OHP significantly elevated blood and brain ammonia levels while reducing glutamate and elevating the concentration of glutamine. Leonard and Palfreyman (1972) observed that seizures induced by lepatzol, sound, and electric shock produced a pronounced increase in ammonia in the brain. However, these authors did not observe any change in concentration of glutamate in brain. The source of brain ammonia is not clear. Vrba (1955) suggested that it is formed by the breakdown of proteins whereas Muntz

(1953) proposed that ammonia accumulation is due to the breakdown of ATP and glu.

Glutamate is a major amino acid in brain, and acts in several ways:

1. Buffering ammonia via the glutamate/glutamine system.
2. Generating GABA and glutathione(Meister 1973).
3. Acting as a neurotransmitter.

1. Buffering ammonia:

Banister et al. (1976) observed that brain glutamine concentration increases and glutamate decreases following convulsions induced by OHP. They proposed that glutamate utilizes ammonia to synthesize glutamine. However, this procedure requires ATP and, overutilized, might cause serious depletion of ATP in brain.

2. Generation of GABA and glutathione:

Glutamate, reacting with GAD, is converted to GABA in the brain. It is quite possible that a low

glutamate level might decrease the synthesis of GABA during oxygen toxicity. Catabolism of GABA produces glutamate and succinic semialdehyde both of which may be utilized further by the Krebs cycle. Thus increased energy metabolism during oxygen toxicity could also lead to lower GABA concentration. Shcherbakova(1962) observed decreased glutamate decarboxylase activity in response to OHP exposure. This observation raises the possibility of direct action of OHP on the GABA system

Glutamate is also an important intermediate of the gamma- glutamyl cycle (Meister,1973). Meister's hypothesis of amino acid transport involves glutathione and membrane bound gamma- glutamyl transpeptidase. All common protein amino acids (except pro) are substrates for gamma- glutamyl trans peptidase. The amino acid taken into the cell by formation at the membrane of gamma- glutamyl peptide can be released intracellularly as free amino acid. This process requires three molecules of ATP to transport one molecule of amino acid.

Reduced glutathione also activates a number of enzymes by maintaining the SH groups of enzymes in the reduced state (Barron,1955). Jocelyn (1962) observed that the sulfhydryl groups of liver homogenates decreased when they are exposed to air. Jamieson et al. (1963) found a higher ratio of S-S/SH groups when lung preparations were exposed to OHP.

3.glutamate as a neurotransmitter:

Duggan and Johnston(1970) and Johnson and Aprison(1970) reported that the cat spinal and dorsal root ganglions have the capacity to store large ammounts of free glutamate. Baltistin et al.(1969) also have observed that the cerebral cortex of the cat has a very high concentration of glutamate and this high value is related to the functional integrity of the cortex. Busch(1955) observed that if labelled pyruvate is injected into the animal, labelled carbon is very rapidly incorporated into cerebral glutamate. Hayashi(1956) first observed the excitatory action of glutamate. This action is dependent on transport of sodium ions. It is

assumed that glutamate and sodium ions have the same carrier and, by conformational changes, the affinity of the carrier for sodium ions increases with addition of glu. Similarly the affinity of the carrier for glutamate itself is a function of sodium ions. However,the process of excitation by glutamate is not well elucidated.

NEUROENDOCRINOLOGICAL FACTORS IN OXYGEN TOXICITY

Neuroendocrinological factors exert a considerable influence on the development of oxygen toxicity. It has been shown that adrenalectomy (Taylor,1958), sympathetic blocking agents (Johnson and Bean,1957) and chlorpromazine (Bean,1956) have protective action against oxygen toxicity whereas A augments the toxic action of oxygen (Bean and Johnson,1955). However, little work has been undertaken to illustrate the response of the neuroendocrinological system to oxygen toxicity.

METABOLISM OF CATECHOLAMINES

SYNTHESIS: Tyrosine is the starting material for the synthesis of catecholamines by following pathway:

tyrosine-------dopamine--------NA------A.

DEGRADATION: Catecholamines are mainly inactivated by:

1. Catechol-O-methyl transferase (COMT)
2. Monoamino oxidase (MAO).

The process can be summarized as follows:

1. A----------metanephrine,
2. NA------normetanephrine
3. metanephrine and normetanephrine----------

---- 3-methoxy 4-hydroxy mandelic acid

Catecholamines in oxygen toxicity

Catecholamines in the brain mainly derive from central nerve endings. They include NA, dopamine, serotonin and A. NA is specifically stored in sympathetic nerve endings. It has been demonstrated that the amount of NA present in the splenic nerve increases towards the distal portion (Chidsey et al.,1963). Blood contains mainly A and NA. A is the major amine of the adrenal medulla.

When animals are exposed to OHP, abnormal activity of CNS inducing convulsions are associated with changes in concentration of NA and serotonin in brain (Faiman et al. 1971). Pfeifer and Galambos (1965) observed that inhibitors of MAO, which increase the concentration of brain amine, have potential anticonvulsive activity because depletion of brain amines decreases the threshold for convulsion. Faiman and Heble(1966) observed decreased NA and serotonin levels following seizures induced by OHP. Cross and Houlihan (1969) observed pronounced sympathetic out

flow, decreased hypothalamic NA, depletion of adrenal A and increased concentration of A and NA in blood following convulsions due to OHP. Voget(1959) showed that vigorous stimulation of the sympathetic system decreased the hypothalamic NA content and adrenal amines. The concentration of NA in brain decreases when rats and mice are exposed to OHP (Faiman and Heble,1966 and Haggendal,1967). Faiman et al.(1971) observed that regardless of the concentration of brain amines, there is no difference in the time for convulsion in rats. They also observed that increase in the concentration of NA or serotonin failed to serve any protective purpose against oxygen toxicity. They suggested that it is not the brain amine, but increased brain MAO activity which potentiates the toxic effects of oxygen. Changes in brain amines may be secondary to an alteration in the activity of MAO due to a combination of effects viz:- 1.raised oxygen tension which has been known to increase MAO activity, 2. the action of MAO which reacts directly with oxygen to form peroxides and other free radicals 3.increased peroxide and free radical activity which is responsible for the toxic

effects of oxygen (Zirkle et al., 1965 and Novick, 1966).

However, this hypothesis is based on the observation made in vitro using isolated cell constituents. No conclusive study has been made to demonstrate that MAO generates or even affects oxygen toxicity in the same manner in vivo. The role of brain amines in potentiating susceptibility to convulsions induced by OHP has been studied by Faiman and Heble (1966,69,70), Buckingham (1966), Haggendal (1967,68) and Diaz et al. (1968). Their studies suggest that brain amines contribute little to the onset of convulsions. However, it is not possible to reach a definite conclusion since most of the above studies used indirect methods. When a drug is used for such studies, it is difficult to determine whether OHP-drug interactions involve changes in brain amine concentration or whether they are mediated first by the drug either lengthing or abbreviating latency to oxygen toxicity.

6-HYDROXYDOPAMINE AND OXYGEN TOXICITY

6-hydroxydopamine (6-OHDA) is known to cause selective destruction of sympathetic nerve endings (Porter et al.,1963,65, Laverty et al.,1965, Thoenen and Tranzer,1968). Haeusler et al. (1969) observed that the drug decreases the stimulation of sympathetic nerve endings. Bloom et al.(1969) found that the effect of 6-OHDA on brain is specific to catecholaminergic neurones. It is also believed that this drug does not cross the blood brain barrier and affects brain catecholamines only when injected intracranially. However,systemic injection of this drug accelerates the turnover of NA in brain (Clark et al.1971). Tranzer and Thoenen(1968) observed that infusion of higher doses of 6-OHDA decreases the concentration of NA in brain as well as degeneration of the adrenergic nerve endings. They also observed that a critical dose of the drug is necessary to produce long lasting depletion.

Thoenen and Tranzer (1968) and Muller et al. (1969) observed that systemic injection of 6-OHDA results in markedly less blood NA and a reduction in blood tyrosine hydroxylase is produced. Thoenen et al.(1970) observed that response to 6-OHDA injection differs in different organs. Thus systemic injection of 1.0 mg.kg of this drug in rats reduced the NA contents in both heart and spleen tissue which soon returned to normal. However, injection of 3.0 mg.kg of the drug caused a long lasting effect in heart but to produce the same effect in the spleen, 30.0 mg.kg of the drug was needed. Systemic injection of 6-OHDA has no direct effect on the adrenal gland because of its relatively low blood supply. However, when the sympathetic system is destroyed and the neuronal vesicle NA contents have decreased considerably, secretions of catecholamines from the adrenal medulla are increased in order to compensate the fading sympathetic discharge. Axelrod et al.(1970) also reported that in addition to the reduction of tyrosine hydroxylase in sympathetic nerve endings, an elevation

in the concentration of this enzyme was observed in the adrenal gland. 6-OHDA also caused a reduction in the blood pressure which probably results in a reflex transistory stimulation of the sympathetic nervous system and increased activity of nerves to the adrenal gland.

Mechanism of action of 6-OHDA

Two properties of 6-OHDA seems to be of particular importance in destruction of nerve terminals:

1.the efficient accumulation of 6-OHDA in adrenergic nerve endings(Thoenen et al.,1970)

2.the extreme susceptibility of 6-OHDA to nonenzymatic oxidation.

Thus 6-OHDA first accumulates in adrenergic nerve terminals and is easily oxidized. Hydrogen peroxide produced subsequent to the oxidation, acts to produce damaging free radicals. The damage is maximum at the site of highest concentration of the drug i.e. at the nerve terminal.

Relationship between chemical sympathectomy and the hypophysial adrenal interaction

Kaplanski and Smelik (1973) observed reduction of catecholamines in brain when 6-OHDA was administered in the CNS. They did not find any correlation between catecholamine depletion and hypophysial activation of adrenal gland. Van Loon et al. (1971) also observed a negative correlation between catecholamines in brain and ACTH and corticosterone in both brain and plasma.

Ammonia poisoning

It has been observed earlier that the liberation of ammonia is closely associated with nervous activity(Quastel,1974). Banister et al.(1976) recently observed elevated ammonia in brain and blood of rats convulsed by OHP exposure. Although, the exact mechanism by which ammonia induces convulsions is not

known, the increase in concentration of ammonia in brains might be due to reduction in the brain buffering agents like glutamate and glutamine in OHP exposure.

EFFECT OF ADRENALECTOMY, GANGLIONIC BLOCKADE AND INHIBITION OF CATECHOLAMINE SYNTHESIS ON OXYGEN TOXICITY

Bean (1951) reported that exposure to OHP causes adrenocortical reactions in rats similar to those found in other forms of stress. Gerschman et al.(1954) observed that adrenalectomized rats survived OHP longer than normal rats. Bean (1955) observed that adrenalectomized rats had some protection against oxygen toxicity and that adrenocortical factors augmented the adverse effects of oxygen. The protection against oxygen toxicity observed after adrenalectomy was apparently related to the removal of both medullary and cortical hormones since administration of either of these hormones reversed the beneficial effects of adrenalectomy. Bean(1952) has suggested that the hypothalamus plays an important part

in the reaction to OHP. Bean and Johnson (1955) also indicated that the hypothalamus initiates a chain of reactions culminating in convulsive activity. They proposed that the protective action of adrenalectomy is not due to adrenocortical factors, but is due solely to decreased concentration of A.

Haeusler et al.(1968) and Davey et al.(1968) reported that hexamethonium inhibits the nicotinic excitation of cholinergic nerve endings. Nicotine is also known to produce an initial depolarization followed by a later hyperpolarization of the adrenergic ganglion(Brown 1966). The latter author also proposed that the degree of hyperpolarization was related to the degree of initial depolarization. Haefely(1971) proposed that an activation of the Na pump occurs very early in the initial depolarization of the ganglion and a ganglionic action potential characterstic of hyperpolarization is generated at a time when the depolarization is not completely decayed. Brown and Scholfield(1970) observed that blockade of nicotinic receptors at an early phase of depolarization by

hexamethonium also blocks the action of antidromic stimulation.

Brandon and Boyd(1962) observed that injection of acetyl choline releases NA in venous effluent from the spleen. Blakely et al.(1963) found that this release of NA by acetyl choline injection is prevented by hexamethonium, whereas hexamethonium does not effect the output of NA after nerve stimulation. Burn and Gibbons(1964) suggested therefore, that hexamethonium prevents the sympathomimetic effect of acetyl choline not by blocking cholinergic receptors but by preventing access of acetyl choline to them. The effect of this drug on convulsions induced by OHP is not well understood.

MPT is a potent inhibitor of tyrosine hydroxylase, the rate limiting enzyme in the synthesis of NA (Udenfriend et al.1965). The rate of depletion of NA after inhibition of this enzyme depends on its rate of utilization and thus is more rapid in tissue than in the blood (Udenfriend et al., 1965). Neff and

Costa (1967) observed that the net concentration of NA in tissue does not change but its turnover rate increased significantly in response to OHP. However, depletion of catecholamines in brain tissue by MPT does not affect the onset of convulsions in response to OHP (Faiman et al.1970). This study, therefore, will attempt to investigate the neuronal, neuroadrenal and metabolic changes occurring in response to convulsions induced by OHP.

CHAPTER-3

SECTION-I

EXPOSURE OF NORMAL RATS TO OHP FOR GRADED TIME INTERVALS UNTIL CONVULSIONS

MATERIALS AND METHODS

Thirty five rats(200-250 g) were included in this experiment divided into seven groups of five each. Each group was treated as follows:

GROUP-1: Rats of this group were maintained on purina lab chow and killed for control concentrations of blood and brain metabolites.

GROUP-2: Rats of this group were exposed to OHP for 10 minutes and sacrificed after decompression. Samples of brain and blood tissue were taken for analysis.

GROUP-3: Animals of this group were exposed to OHP for 15 minutes, sacrificed and treated as in group-2.

GROUP-4: These rats were exposed to OHP for 20 minutes and treated as in group-2.

GROUP-5: These rats were exposed to OHP for 25 minutes and treated as in group-2.

GROUP-6: Rats of this group were exposed to OHP for 30 minutes and treated as in group-1.

GROUP-7: All rats were exposed to OHP till convulsions

and treated as in group-2.

CHAMBER OPERATION FOR EXPOSURE OF RATS TO HIGH PRESSURE OXYGEN

A small chamber of about 100L was used for hyperbaric oxygenation of animals. All animals were exposed singly for the stated time and experimental condition. Oxygen was flushed through the pressure chamber after the animal was placed in it, at a rate of ($5L.min^{-1}$) and pressurized upto 72.5 psig.The pressurization was completed over a period of 6 minutes and thereafter gas flow was maintained at $5L.min^{-1}$ to minimize carbon dioxide accumulation. After specific time intervals or after convulsions, the rats were decompressed with stops of 4 minutes at 40 psig, 1 minute at 30 psig followed by 3 minutes continuous, slow decompression to ambient pressure(Banister et al.,1976).

Under these experimental conditions, 10 minutes elapsed between the beginning of decompression and

collection of blood and brain tissue. Thus a rat exposed for 10 minutes lived a further 10 minutes before being killed. The actual time from compression to death was 20 minutes. Since in this experiment interest was in the collection of blood as well as brain, a liquid nitrogen freezing technique could not be used. It was essential therefore to compare the concentrations of the parameters of this study under the different conditions of sacrifice and tissue collection exemplified by quick freezing after decompression or by decompression followed by ether anesthesia sacrifice.

Comparison of freezing and exsanguinated sacrifice methods:

Freezing the total rat immediately after its removal from the compression chamber prior to the sampling of brain tissue is not advantageous. Blood ammonia, glutamate and glutamine concentration estimated separately after sacrifice either by rapid freezing or by exsanguination techniques did not differ

significantly as shown below:

Killing method n=5	Ammonia $\mu g.g^{-1}$	glutamate $\mu mole.g^{-1}$	glutamine $\mu mole.g^{-1}$
Exsanguination	5.52±.27	9.42±.63	0.97±.164
Rapid freezing	5.1±.13	8.93±.71	1.03±0.49

Collection of blood and brain samples:

Blood samples were collected immediately after anesthesia and centrifuged for separation of serum. Part of the serum was processed immediately for estimation of ammonia and amino acids and the remainder was frozen with glutathione at -20°C for later estimation of catecholamine and COMT.

Brain samples were taken immediately and frozen rapidly in liquid nitrogen. These samples were analyzed for ammonia, amino acids, catecholamines and COMT. The methods are discussed later in the appendix.

RESULTS

Brain NA (Table-1, Figure-1):

The normal concentration of NA in brain was 128.9±3.6 $ng.g^{-1}$, which decreased significantly to 79.09±10.3 $ng.g^{-1}$ after 10 minutes of OHP exposure. A gradual increase was observed thereafter and values reached normal control concentrations after 30 minutes of exposure and then remained unchanged up to and after convulsions.

Brain A (Table-1, Figure-1):

The normal brain A concentration was 2.2±0.4 $ng.g^{-1}$. It decreased significantly to 0.32±0.03 $ng.g^{-1}$ after 10 minutes of OHP exposure . Brain A concentration then decreased further before rising to normal concentrations after 25 minutes of exposure. It then

remained unchanged up to 30 minutes of exposure. After convulsions induced by OHP brain A concentration decreased significantly to 1.35 ± 0.17 $ng.g^{-1}$.

Brain TC (Table-1, Figure-1):

The normal control concentration of TC was 491.37 ± 49.5 $ng.g^{-1}$. A significantly low value was noted after 10 minutes of OHP exposure. This concentration remained low for 15 minutes of exposure before the concentration rose close to normal again. No significant changes in concentrations of TC in brain immediately before and after convulsions were noted thereafter in normal rats.

Brain COMT (Table-1, Figure-1):

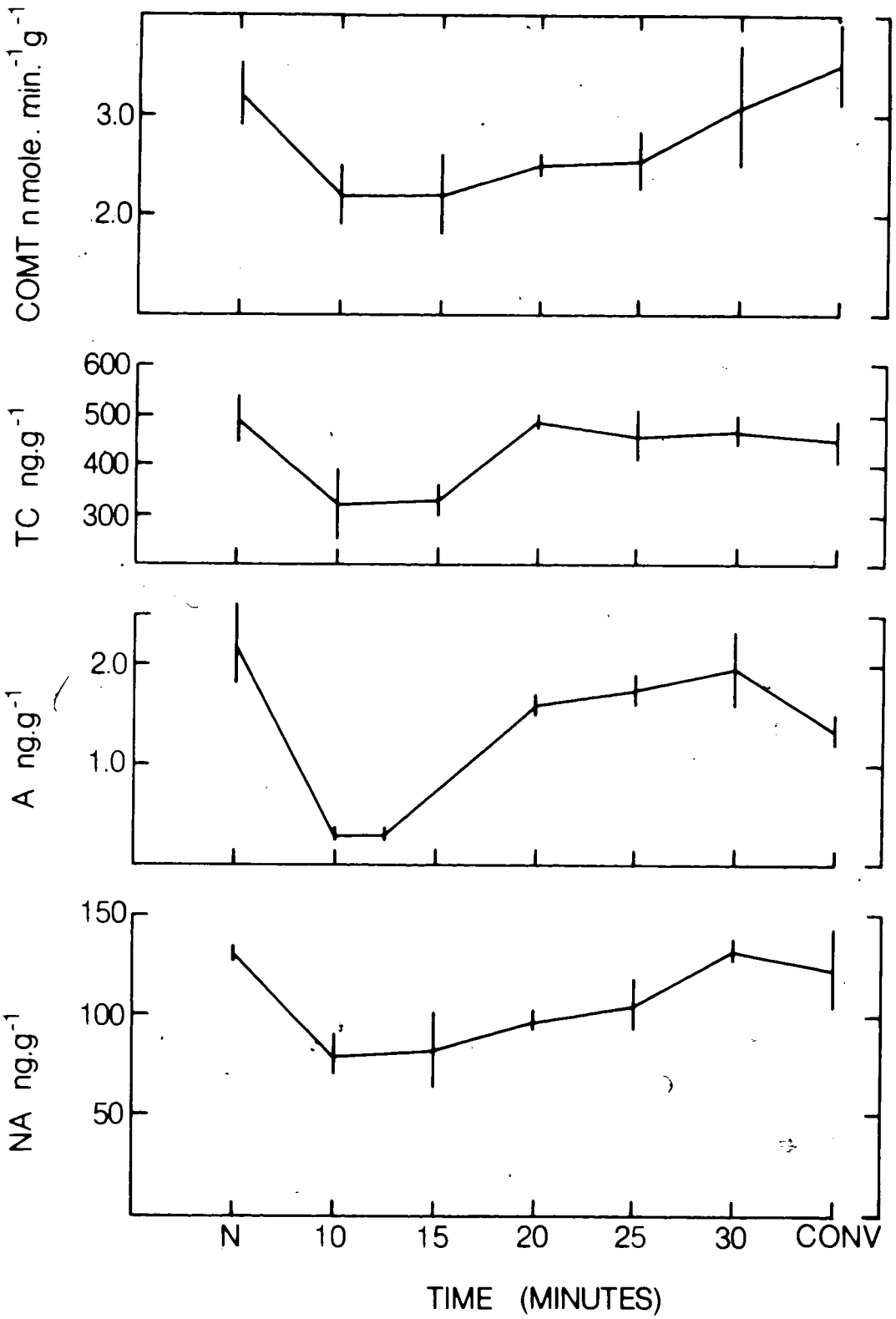
The normal activity of COMT in brain was 3.22 ± 0.28 $nmole.g^{-1}.min^{-1}$ which decreased significantly to 2.2 ± 0.31 $nmole.g^{-1}.min^{-1}$ after 10 minutes of exposure to OHP. A

TABLE I

Brain and blood noradrenaline (NA), adrenaline (A), total catecholamine (TC) and catechol-o-methyl transferase, brain only, (COMT) in normal and rats exposed to OHP for different time intervals. n = 5 in each group
$p < 0.05$ significant

	Control	10 min.	15 min.	20 min.	25 min.	30 min.	Convulsions
Brain NA	128.9±3.6	79.09±10.3	83.03±18.50	98.25±5.6	105.9±12.2	130.6±4.0	124.6±19.1
$ng.g^{-1}$	p	<0.05	<0.05	<0.05	<0.05	>0.1	>0.1
Brain A	2.[illegible]	0.32±0.03	0.36±0.03	1.64±0.09	1.75±0.17	1.97±0.38	1.35±0.17
$ng.g^{-1}$	p	<0.05	<0.05	<0.05	>0.1	>0.1	<0.05
Brain TC	491.37±49.50	321.27±69.15	330.9±31.1	489.3±7.8	461.6±47.7	468.0±30.1	448.9±44.1
$ng.g^{-1}$	p	>0.1	<0.05	>0.1	>0.1	>0.1	>0.1
Brain COMT	3.22±0.28	2.2±0.3	2.23±0.45	2.5±0.1	2.55±0.32	3.12±0.66	3.48±0.42
$nmole.g.^{-1}min^{-1}$	p	<0.05	<0.05	<0.05	<0.05	>0.1	>0.1
Blood NA	0.33±0.06	0.84±0.09	1.16±0.27	0.13±0.03	0.21±0.05	0.57±0.12	2.03±0.53
$ng.\ ml^{-1}$	p	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Blood A	0.19±0.03	0.62±0.08	0.60±0.08	0.09±0.01	0.12±0.03	0.54±0.23	2.62±0.81
$ng.\ ml^{-1}$	p	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Blood TC	0.57±0.04	2.08±0.22	2.36±0.60	0.39±0.1	0.4±0.1	1.30±0.21	5.75±0.47
$ng.\ ml^{-1}$	p	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

FIGURE-1: Brain noradrenaline (NA), adrenaline (A), total catecholamine (TC) and catechol-O-methyl transferase (COMT) concentrations in normal rats exposed to OHP for different time intervals until the onset of convulsions. (Mean ± SD, n=5)


COMT n mole. min.$^{-1}$ g^{-1}
3.0
2.0
TC ng.g^{-1}
600
500
400
300
A ng.g^{-1}
2.0
1.0
NA ng.g^{-1}
150
100
50
N
10
15
20
25
30
CONV
TIME (MINUTES)

gradual recovery was observed thereafter and values reached normal control concentrations after 30 minutes of OHP exposure. No significant change was noted after rats were subjected to convulsions induced by OHP.

Brain GABA (Table-2, Figure-2):

The normal concentration of GABA in rat brain was found to be 1.15 ± 0.12 $\mu mole.g^{-1}$. No significant change in the concentration of GABA in brain was noted upto 15 minutes of oxygen exposure. A significant decrease was noted thereafter and the lowest value reached prior to convulsion was 0.59 ± 0.06 $\mu mole.g^{-1}$ which was found after 30 minutes of OHP exposure. GABA levels in brain tissue remained significantly low after convulsions induced by OHP.

Brain ammonia(Table-2, Figure-2):

The normal concentration of ammonia in brain was $5.25 \pm 0.72\ \mu g.g^{-1}$. No significant change was noted up to 15 minutes of exposure. A significant increase in the concentration of ammonia in brain was then noted. The peak was $13.18 \pm 0.54\ \mu g.g^{-1}$ after 30 minutes of exposure. The concentration of ammonia in brain increased further to $17.7 \pm 1.1\ \mu g.g^{-1}$ after OHP induced convulsions.

Brain glutamate (Table-2, Figure-2):

The normal concentration of glutamate in brain was $9.42 \pm 0.63\ \mu mole.g^{-1}$. Successively after 15, 20, 25 and 30 minutes of OHP exposure, the levels of glutamate in brain decreased significantly to 5.7 ± 0.9, 4.56 ± 0.51, 4.36 ± 0.80 and $5.36 \pm 0.62\ \mu mole.g^{-1}$ respectively. After convulsions induced by OHP, the mean animal concentration of glutamate in brain was $5.18 \pm 0.49\ \mu mole.g^{-1}$.

TABLE II

Brain γ amino butyric acid (GABA μmole.g^{-1}), blood and brain ammonia (NH_3 μg.g^{-1} in brain, μg.ml^{-1} in blood) Glutamic acid (Glu. μmole.g^{-1} in brain, μmole. 100 $ml.^{-1}$ in blood), and Glutamine (including Aspargine; Glu.N, μmole.g^{-1} brain, μmole. 100 $ml.^{-1}$ blood) in normal and OHP exposed rats. n = 5 in each group.

	Control	10 min.	15 min.	20 min.	25 min.	30 min.	Convulsion
Brain GABA	1.15±0.12	1.36±0.35	1.04±0.66	0.93±0.05	0.89±0.05	0.59±0.06	0.75±0.10
	p	>0.1	>0.1	<0.05	<0.05	<0.05	<0.05
Brain NH_3	5.25±0.27	5.47±0.54	5.7±0.5	9.3±0.8	10.99±0.87	13.18±0.54	17.7±1.1
	p	>0.1	>0.1	<0.05	<0.05	<0.05	<0.05
Brain Glu	9.42±0.63	8.8±0.59	5.7±0.9	4.56±0.51	4.36±0.8	5.36±0.62	5.18±0.49
	p	>0.1	<0.05	<0.05	<0.05	<0.05	<0.05
Brain Glu.N	0.97±0.16	1.74±0.23	2.04±0.21	2.83±0.20	2.56±0.45	2.91±0.52	2.88±0.73
	p	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Blood NH_3	2.05±0.23	2.05±0.29	2.32±0.32	2.48±1.39	3.39±0.37	3.82±0.47	5.6±0.61
	p	>0.1	>0.1	>0.1	<0.05	<0.05	<0.05
Blood Glu	3.26±0.33	3.12±0.72	2.83±0.29	2.86±0.20	2.38±0.38	2.04±0.22	2.25±0.42
	p	>0.1	>0.1	>0.1	<0.05	<0.05	<0.05
Blood Glu.N	14.19±2.7	13.86±1.89	13.68±2.63	15.16±1.5	18.71±1.99	19.5±1.1	22.2±2.6
	p	>0.1	>0.1	>0.1	<0.05	<0.05	<0.05

p <0.05 significant

FIGURE-2: Brain gamma amino butyric acid (GABA), ammonia and glutamate (glu) concentration in normal rats exposed to OHP for different time intervals until convulsions. (Mean $\pm$ SD, n=5)

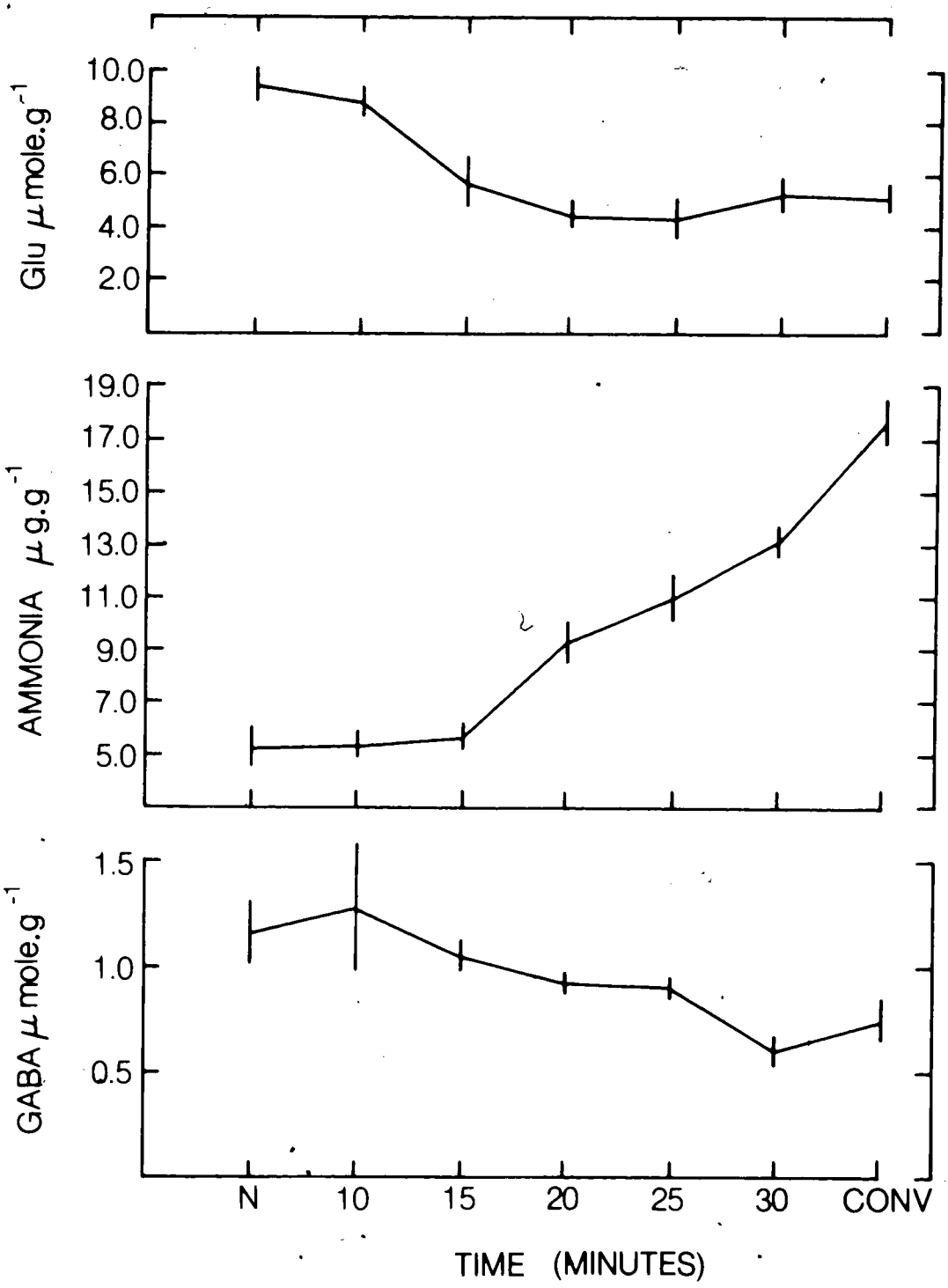
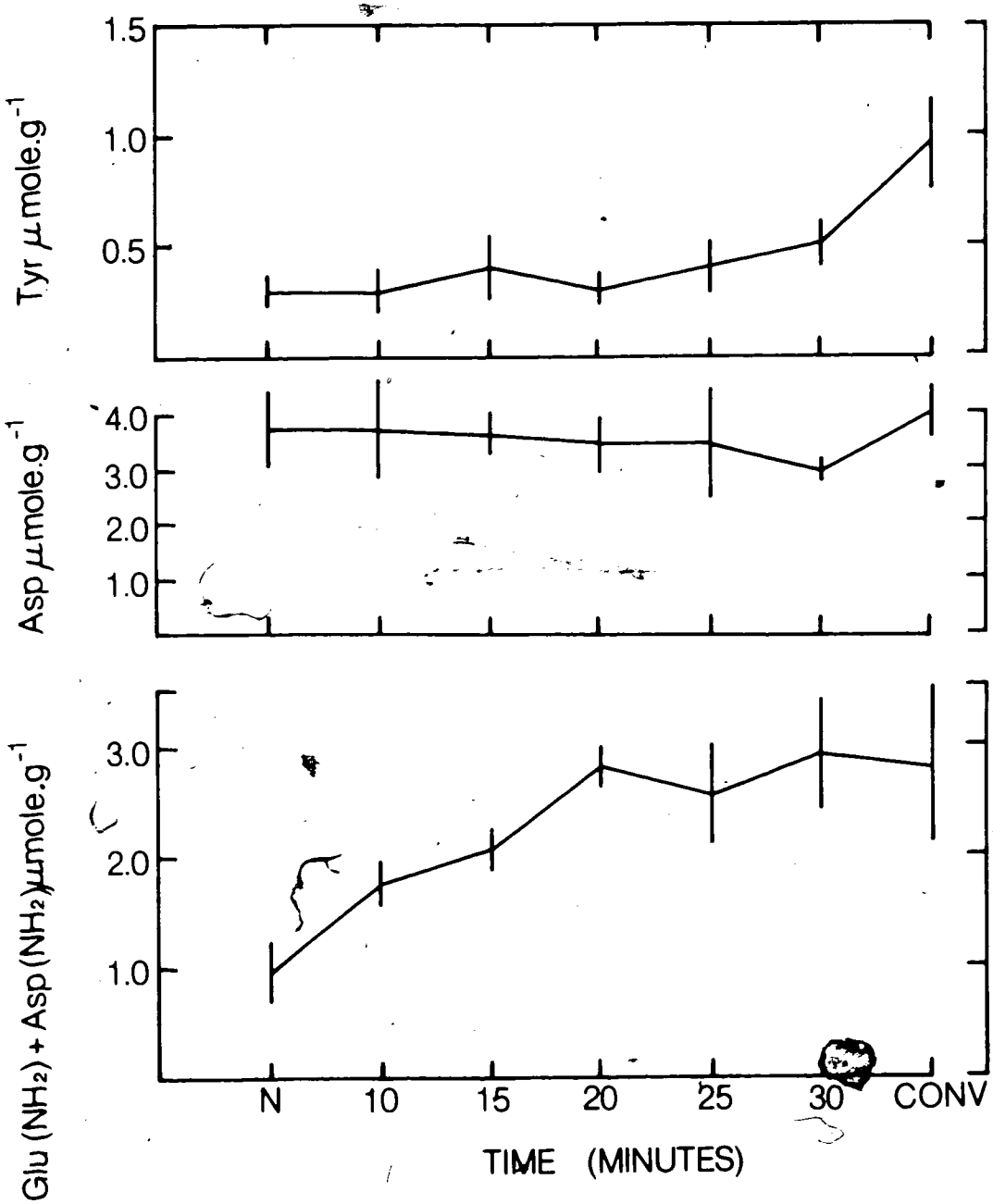

Glu μmole.g⁻¹
10.0
8.0
6.0
4.0
2.0
AMMONIA μg.g⁻¹
19.0
17.0
15.0
13.0
11.0
9.0
7.0
5.0
GABA μmole.g⁻¹
1.5
1.0
0.5
N
10
15
20
25
30
CONV
TIME (MINUTES)

TABLE III

Brain (μmole.g^{-1}) and blood (μmole.100 ml.$^{-1}$) amino acids in normal rats and rats exposed to OHP for different time intervals. n = 5 in each group.
* $p < 0.05$ = significant when compared with normal Control

	Control	10 min.	15 min.	20 min.	25 min.	30 min.	Convulsion
BRAIN Asp.	3.73±0.69	3.72±0.91	3.58±0.43	3.44±0.52	3.44±0.96	2.94±0.20	3.96±0.51
Ala.	0.61±0.11	0.64±0.11	0.64±0.15	0.53±0.05	0.55±0.18	0.65±0.12	0.60±0.11
Tyr.	0.31±0.08	0.28±0.10	0.39±0.15	0.33±0.07	0.41±0.13	0.51±0.12*	0.56±0.21*
Arg.	0.11±0.03	0.10±0.02	0.10 ±0.02	0.12±0.02	0.08±0.02	0.13±0.03	0.10±0.02
Thr.	1.03±0.20	1.05±0.08	1.00±0.13	1.12±0.06	1.04±0.13	1.03 ±0.10	1.02±0.20
Ser.	0.76±0.48	0.93±0.13	0.96±0.10	0.98±0.10	0.97±0.06	0.91±0.09	0.93±0.09
BLOOD Asp.	1.55±0.36	1.58±0.29	1.60±0.40	1.31±0.11	1.58±0.2	1.4±0.25	1.84±0.31
Ala.	10.3±1.4	10.41±1.10	10.24±1.84	10.8±1.0	11.4±0.9	20.9±1.5*	12.07±0.08
Tyr.	0.57±0.13	0.57±0.07	0.54±0.13	0.50±0.09	0.50±0.07	0.44±0.06	0.36±0.04*
Arg.	1.33±0.53	1.03±0.32	1.30±0.21	1.19±0.23	1.16±0.42	1.39±0.21	1.39±0.30
Thr.	0.81±0.10	0.78±0.10	0.76±0.10	0.86±0.09	0.8±0.1	0.82±0.13	0.79±0.11
Ser.	0.96±0.15	0.82±0.14	0.83±0.07	1.05±0.09	0.96±0.1	1.04±0.07	0.99±0.10

FIGURE-3: Brain glutamine+aspargine (glu.NH_2+asp.NH_2), aspartate (asp) and tyrosine (tyr) concentrations in normal rats exposed to OHP for different time interval until the convulsions were produced. (Mean ± SD, n=5)


Tyr μmole.g-1
1.5
1.0
0.5
Asp μmole.g-1
4.0
3.0
2.0
1.0
Glu (NH2) + Asp (NH2) μmole.g-1
3.0
2.0
1.0
N
10
15
20
25
30
CONV
TIME (MINUTES)

Brain glutamine and aspargine (glu.NH_2+Asp.NH_2) (Table-2, Figure-3):

The normal combined concentration of these amino acid amide in brain was 0.97 ± 0.16 μmole.g^{-1} which increased to 1.74 ± 0.23 μmole.g^{-1} after 10 minutes of exposure of rats to OHP. A gradual increase was then noted and after 30 minutes of exposure, the value was 2.91 ± 0.52 μmole.g^{-1}. After convulsions induced by OHP, combined concentration of these amino acids averaged 2.88 ± 0.73 μmole.g^{-1} in all animals exposed.

Brain tyrosine (Table-3, Figure-3):

The normal concentration of tyrosine in brain was 0.31 ± 0.08 μmole.g^{-1}. No significant change in this concentration was noted after 25 minutes of exposure. Concentration of tyrosine in brain however, increased significantly after 30 minutes of OHP exposure and remained elevated after convulsions induced by OHP.

Other brain amino acids (Table-3, figure-3):

No significant change in the concentration of any other brain aminoacid was noted throughout any of the experimental manipulations.

Blood NA (Table-1, Figure-4):

The normal concentration of NA was 0.33 ± 0.06 ng.ml^{-1}. The value of NA in blood increased significantly to 0.84 ± 0.09 and 1.16 ± 0.27 ng.ml^{-1} after 10 and 15 minutes of OHP exposure respectively. Values decreased to 0.13 ± 0.03 ng.ml^{-1} after 20 minutes of exposure and remained low up to 25 minutes. Thirty minutes after exposure, the concentration of NA in blood again increased to 0.57 ± 0.12 ng.ml^{-1}. After convulsions, the concentrations increased to 2.03 ± 0.53 ng.ml^{-1}.

Blood A (Table-1, Figure-4):

The normal concentration of A in blood was 0.19±0.03 $ng.ml^{-1}$ which increased significantly to 0.62+0.08 and 0.60±0.03 $ng.ml^{-1}$ at 10 and 15 minutes respectively after exposure to OHP. A significant decrease was noted after 20 minutes of oxygen exposure. The value increased again to 0.54±0.23 $ng.ml^{-1}$. after 30 minutes of OHP exposure. The concentration of A in blood after convulsions induced by OHP rose to 2.62±0.81 $ng.ml^{-1}$. in all animals.

Blood TC (Table-1, Figure-5):

The normal concentration of TC in blood was 0.57±0.04 $ng.ml^{-1}$. This concentration increased significantly to 2.08±0.22 and 2.36±0.60 $ng.ml^{-1}$ after 10 and 15 minutes of exposure respectively. Blood TC concentration was significantly low after 20 and 25 minutes of exposure of rats to OHP. After 30 minutes of exposure, the concentration of TC in blood increased significantly

FIGURE-4: Blood noradrenaline (NA), adrenaline (A) concentrations in rats exposed to OHP for different time intervals until the onset of convulsions. (Mean ± SD, n=5)

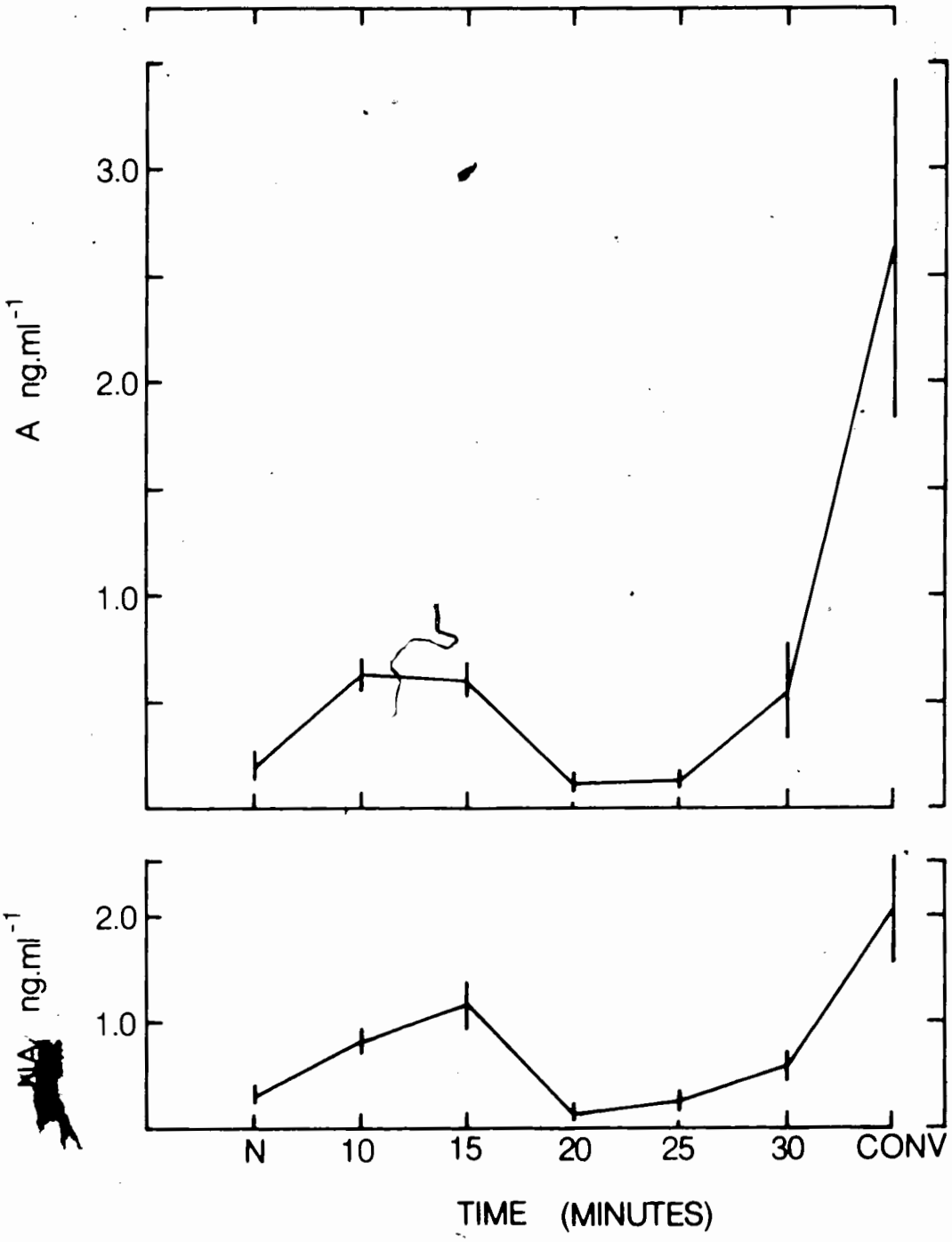
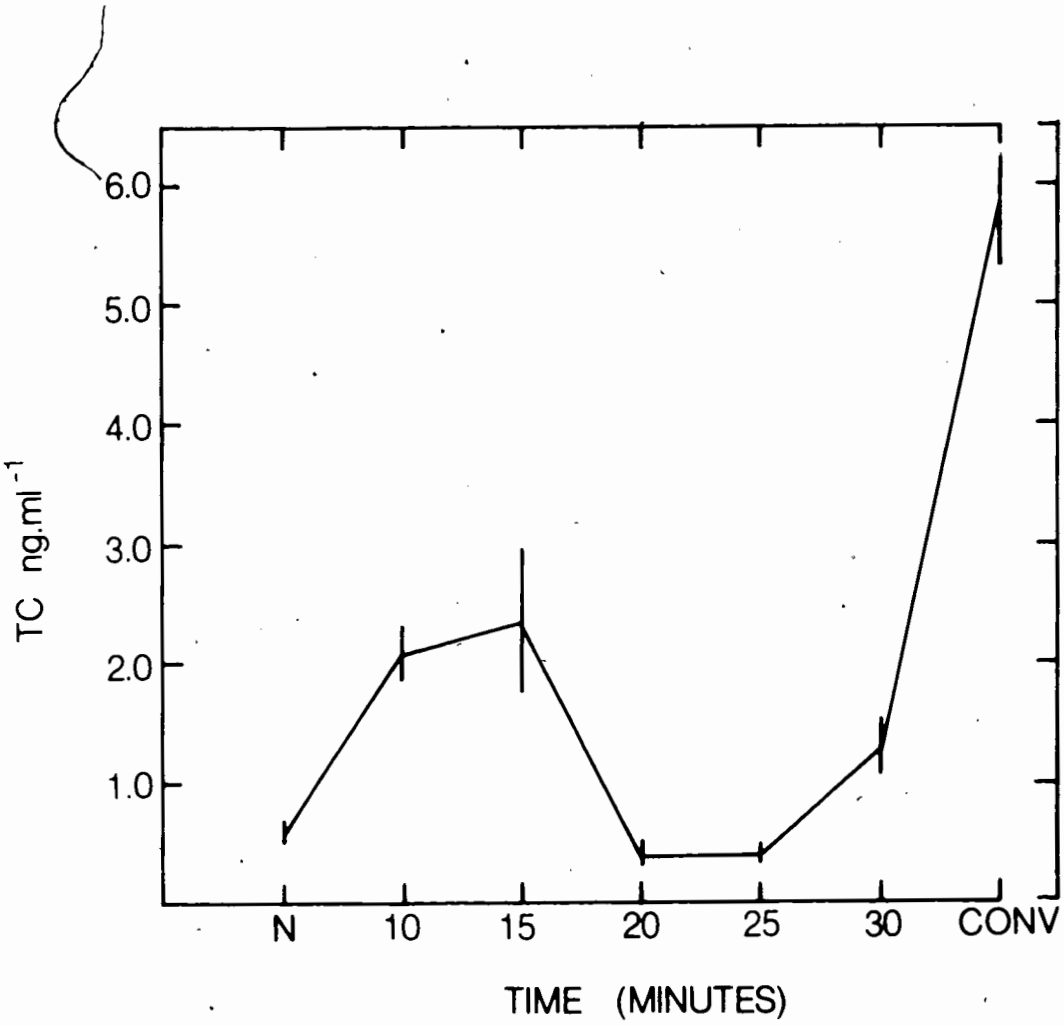

3.0
2.0
1.0
A ng.ml^{-1}
2.0
1.0
ng.ml^{-1}
N
10
15
20
25
30
CONV
TIME (MINUTES)

FIGURE-5: Blood total catecholamine (TC) concentration in normal rats exposed to OHP for different time intervals until the onset of convulsions. (Mean ± SD, n=5)


6.0
5.0
4.0
3.0
2.0
1.0
TC ng.ml-1
N
10
15
20
25
30
CONV
TIME (MINUTES)

again to 1.30 ± 0.21 ng.ml^{-1} After convulsion, the concentration of TC in blood increased further to 5.75 ± 0.57 ng.ml^{-1}.

Blood ammonia (Table-2, Figure-6):

The normal mean concentration of ammonia in blood was 2.05 ± 0.23 µg.ml^{-1}. No significant change in the concentration of ammonia in blood was noted up to 20 minutes of OHP exposure. A significant increase was then observed however, after 25 minutes of OHP exposure. After 30 minutes the concentration of ammonia in blood was 3.82 ± 0.47 µg.ml^{-1} which increased significantly to 5.6 ± 0.6 µg.ml^{-1} after convulsions induced by OHP.

Blood glutamate (Table-2, Figure-6):

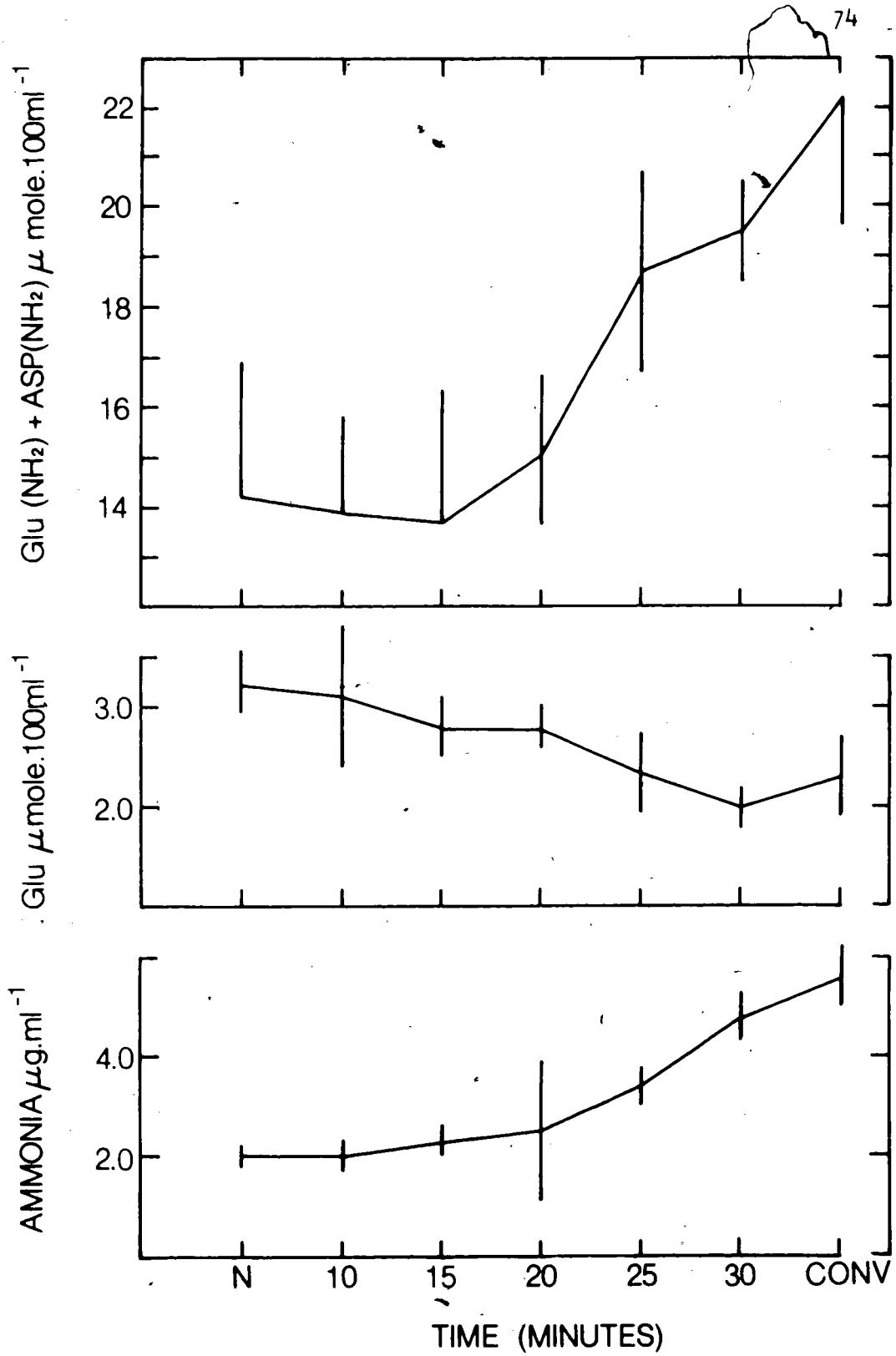
The normal concentration of glutamate in blood was 3.26 ± 0.33 µmole.100ml^{-1}. No significant change was noted up to 20 minutes of OHP exposure. A significant

decrease was found after this, the minimum concentration occuring after 30 minutes of exposure (2.04 ± 0.22 µmole.$100ml^{-1}$.). After OHP induced convulsions, the value of glutamate in blood was 2.25 ± 0.42 µmole.$100ml^{-1}$.

Blood glutamine+aspargine ($glu.NH_2 + asp.NH_2$) (Table-2, Figure-6):

The normal combined concentration for these amino acids in the blood was 14.19 ± 2.70 µmole.$100ml^{-1}$. No significant change in this value was noted for up to 20 minutes of exposure to OHP. After 25 minutes of exposure, the blood concentration of these amino acids increased significantly to 18.71 ± 1.99 µmole.$100ml^{-1}$ and remained elevated thereafter up to 30 minutes in oxygen exposed animals. After OHP induced convulsions, the $glu.NH_2 + asp.NH_2$ concentration increased further to 22.2 ± 2.6 µmole.$100ml^{-1}$ in the animals studied.

FIGURE-6: Blood ammonia, glutamate (glu) and glutamine+ asparagine (glu.NH_2+asp.NH_2) concentration in normal rats exposed to OHP for different time intervals until the onset of convulsions. (Mean ± SD, n=5)


Glu (NH2) + ASP(NH2) μ mole.100ml-1
22
20
18
16
14
Glu μmole.100ml-1
3.0
2.0
AMMONIA μg.ml-1
4.0
2.0
N
10
15
20
25
30
CONV
TIME (MINUTES)

Blood alanine (Table-3, Figure-7):

The normal concentration of alanine in blood was 10.29 ± 1.44 μmole.$100ml^{-1}$ in rats. No significant change in this value was noted when normal rats were exposed to OHP for up to 25 minutes. After 30 minutes of exposure, the levels of alanine in blood increased significantly to 20.91 ± 1.47 μmole.$100ml^{-1}$. After convulsions, the concentration of alanine in blood was 12.07 ± 0.08 μmole.$100ml^{-1}$ which were significantly higher than normal although lower than the 30 minute value.

Blood tyrosine (Table-3, Figure-8):

The normal concentration of tyrosine in blood was 0.57 ± 0.13 μmole.$100ml^{-1}$. No significant change in the concentration of this amino acid was noted up to 30 minutes of exposure of rats to OHP. However, after convulsions, its concentration decreased significantly to 0.36 ± 0.04 μmole.$100ml^{-1}$.

FIGURE-7: Blood alanine (ala) and aspartic acid (asp) concentration in normal rats exposed to OHP for different time intervals until convulsions. (Mean ± SD, n=5)

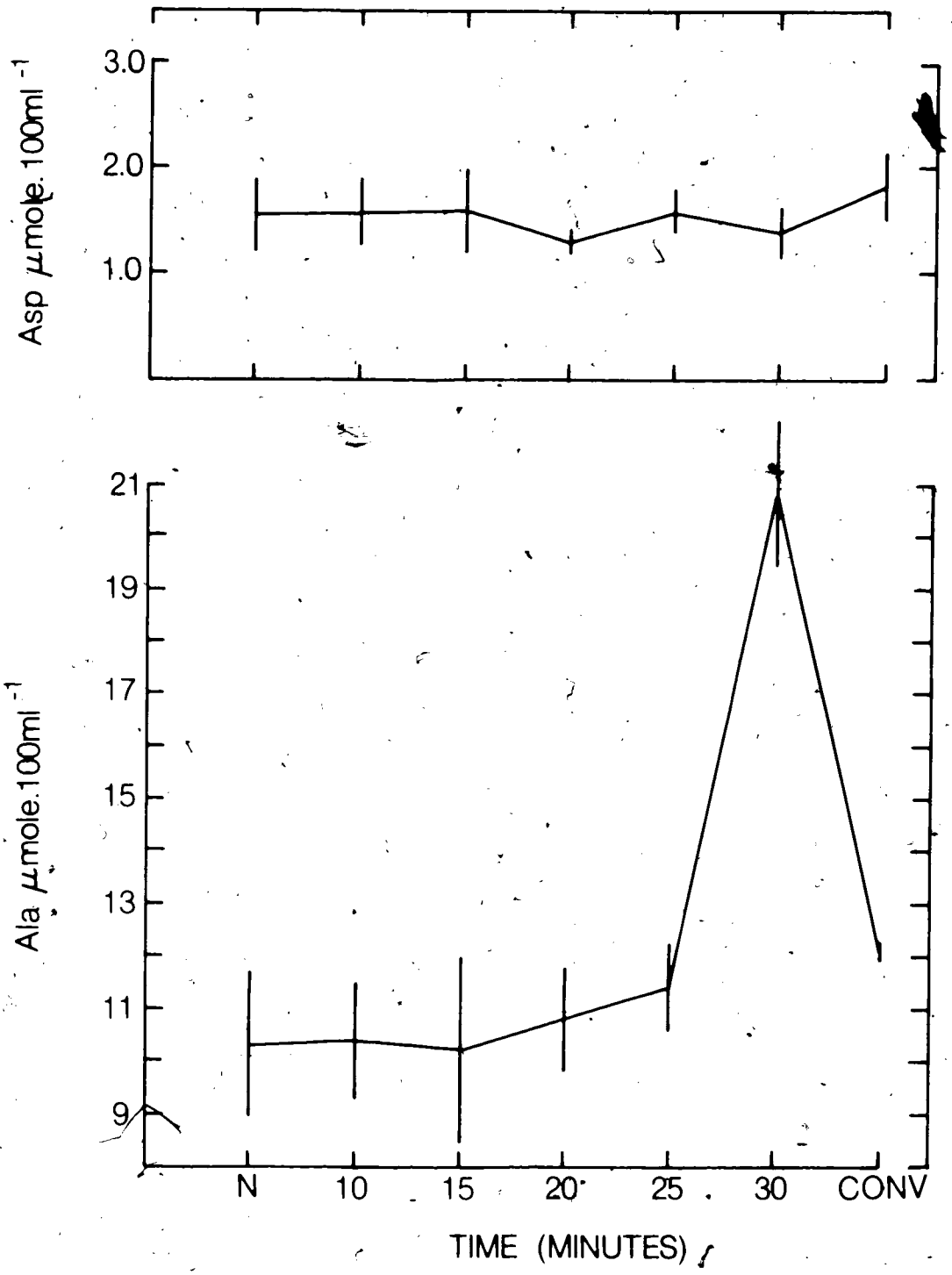
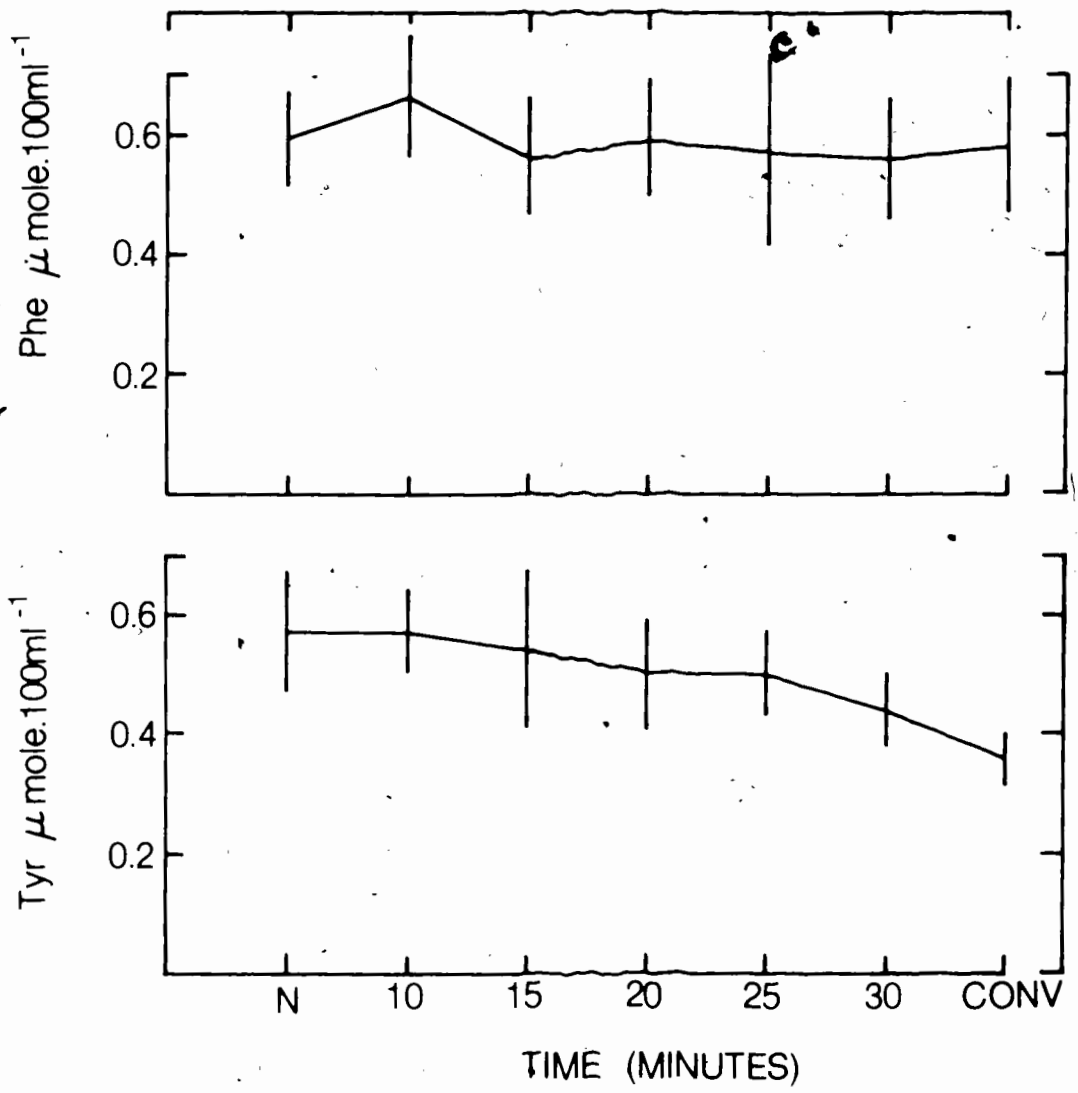

Asp μmole.100ml⁻¹
3.0
2.0
1.0
Ala μmole.100ml⁻¹
21
19
17
15
13
11
9
N
10
15
20
25
30
CONV
TIME (MINUTES)

FIGURE-8: Blood tyrosine (tyr) and phenylalanine (phe) concentration in normal rats exposed to OHP for different time intervals until convulsions. (Mean ± SD, n=5)


Phe μmole.100ml-1
0.6
0.4
0.2
Tyr μmole.100ml-1
0.6
0.4
0.2
N
10
15
20
25
30
CONV
TIME (MINUTES)

Other blood amino acids(Table-3, Figure-7 and 8):

No significant change in other blood amino acid concentration was noted either prior to or after OHP induced convulsions in normal rats.

DISCUSSION

Significantly decreased concentrations of A, NA, TC and COMT in brain were observed after 10 and 20 minutes of exposure. These concentrations returned to a normal baseline levels after 30 minutes and did not change significantly again even after convulsions induced by OHP. These observations suggest that decrease in the concentration of catecholamines in brain during the early phase of compression is not due to activity of the degrading enzyme,COMT, since the latter's activity was also suppressed during this period. Haggendal (1967) observed that when rats were exposed to pure oxygen at 6 atmospheres, the concentration of NA in brain decreased before any onset of convulsions. Decreased or normal concentrations of NA in brain were observed when convulsions in rats were produced by electric shock (Breitner et al.,1964)

Gibson and Wurtman (1977) proposed that the availability of tyrosine to the brain may be one of the important factors controlling the synthesis of catecholamines in brain. The fact that no significant change in concentration of tyrosine in brains were observed during the early periods of compression, suggests that the low catecholamines are not due to low tyrosine concentration. Probably there is some direct action of oxygen on catecholamine synthesis in brain, since higher concentration of tyrosine than normal were also observed during the later phase of compression.

As onset of convulsions becomes imminent, the concentration of catecholamines rise in brain from the initially depressed concentrations. It seems unlikely, however, that these altered concentrations of catecholamine in brain contribute to the induction of convulsions since their concentration never exceeds the control value, either immediately before or after convulsions are produced. Faiman et al. (1971) observed that alteration in the concentration of catecholamines in the brain by different drugs does not effect the

convulsion time in rats.

The time course of changes in concentration of ammonia, GABA, glutamate and glutamine suggests that an initial increase in brain ammonia is probably buffered by the glutamate/glutamine system. The aspartate/aspargine system does not seem to take part in such buffering. When production of ammonia is beyond the limits of this buffering system, the failure is reflected by an increased concentration of ammonia in brain (e.g. this occur 25 minutes after OHP exposure).

There are three possible mechanisms to explain the decrease in concentration of glutamate:-

(1) Increased ammonia inhibits the production of glutamate from α ketoglutaric acid and ornithine.

(2) Glutamate is rapidly converted to glutamine when there is an excess of ammonia.

(3) The mitochondrial glutamic-oxaloacetate transamination cycle of amino acid metabolism is used as an effective pathway through which the amino group of glutamate is

converted to urea (Katunuma and Okada, 1962).
The source of brain ammonia produced in response to OHP is not well known. There is some evidence that it is derived from cerebral protein, ATP and catecholamines (Vrba, (1955); Muntz, 1953; and Lowenstein, 1971).

In the present study, decreased concentrations of GABA in brain were observed both prior to and after convulsions induced by OHP. Very high ammonia and very low GABA before convulsion suggest that these substrates play an important reciprocal role in the etiology of convulsions. Banister et al. (1976) and Wood et al. (1970) have discussed the importance of increased ammonia and decreased GABA in potentiating convulsions induced by OHP. A low concentration of GABA in brain observed in the present study might be due to:

1. inhibition of the enzyme responsible for its synthesis (GAD),
2. activation of the degradative enzyme GABA-T,
3. acceleration of brain transport of GABA.

Wood et al. (1970) suggested that GAD is selectively inhibited by exposure of normal rats to OHP.

Catecholamines in blood were elevated during the first 15 minutes of oxygen exposure. They then decreased and remained low for 25 minutes of the compression. It is not possible to explain the sharp decrease in blood catecholamine concentrations between the 15th to 20th minutes of exposure. Possibly some interaction between changes induced by OHP and ether anaesthesia might cause such a sharp drop in concentrations of catecholamines in the blood. After 30 minutes, the blood catecholamine concentration again increased significantly and increased even further after convulsion. Cross and Houlihan (1969) observed a pronounced sympathetic outflow and depletion of adrenal medullary A with increased blood A and NA following convulsions induced by OHP. The initial increase in the blood catecholamine concentrations may be ascribed to a stress reaction whereas later increases may be due to the toxic effects of oxygen per se. However, the mechanism by which oxygen affects the adrenal and

sympathetic systems continues to remain obscure.

Catecholamine concentrations are very high in the blood before the onset of convulsions and might precipitate their induction as well as contributing to the lung damage usually associated with toxic oxygen exposures (Clark and Lambertsen,1971). The fact that exposure to OHP causes adrenocortical reactions similar to those found in other forms of stress and that adrenalectomy serves as a protective agent against oxygen toxicity (Bean,1956) supports the observation made in this study that increased activity of the adrenal gland contributes to the induction of convulsions by high pressure oxygen.

In blood, a significant increase in ammonia and glutamine+asparagine and a decrease in glutamate take place slightly after similar changes in the brain. These observations suggest either that ammonia production is delayed in blood or the blood has buffering systems other than glutamine/glutamate and alanine. Alternatively some ammonia may diffuse

continuously to the brain initiating the changes observed there. Increase in concentration of alanine in blood after 30 minutes of oxygen exposure suggests that alanine metabolism is also involved in buffering blood ammonia. However, a decrease in alanine in blood after convulsion is at present not explainable.

The concentration of tyrosine in blood decreased significantly after convulsions induced by OHP. It is not clear why this is so unless it reflects tyrosine utilization by a catecholamine synthesizing system in the adrenals or its diffusion into brain. Oldendorf and Szabo(1976) have suggested that tyrosine is carried from blood to brain by a common carrier for neutral amino acids.

In summary, this section of the study suggests that exposure of rats to OHP causes several hormonal and metabolic changes in brain and blood tissue and, these changes might be responsible for the induction of convulsion. It is not possible to conclude however that these are the sole causes because of the complexity of the system.

CHAPTER-4

SECTION-II

EFFECTS OF 6-OHDA ON CONVULSIONS INDUCED BY OHP IN NORMAL RATS

MATERIALS AND METHODS

Sixty rats(200-250 g) were divided into 12 groups of 5 animals in each and were each assigned to one of 12 treatments as follows:

GROUP-1: Control.

GROUP-2: Control rats subjected to OHP.

GROUP-3: One injection of 6-OHDA (68mg.kg^{-1}) intravenously 24 hours before being sacrificed.

GROUP-4: One injection of 6-OHDA (68mg.kg^{-1}) intravenously 24 hours before exposure to OHP and sacrificed immediately after convulsion.

GROUP-5: Two injection of 6-OHDA (68mg.kg^{-1}, each 24 hours apart), sacrificed 24 hours after the last injection.

GROUP-6: Two injections of 6-OHDA (as in 5) and subjected to OHP 24 hours after the last injection and sacrificed immediately after convulsion.

GROUP-7: Three injections of 6-OHDA (68mg.kg^{-1}, intravenously 24 hours apart), sacrificed 24 hours after the last injection.

GROUP-8: Three injections of 6-OHDA (as in 7) and subjected to OHP 24 hours after last injection and sacrificed immediately after convulsion.

GROUP-9: Two injections of 6-OHDA (as in 5), sacrificed 48 hours after last injection.

GROUP-10: Two injections of 6-OHDA (as in 5) and subjected to OHP 48 hours after last injection and sacrificed immediately after convulsion.

GROUP-11: Two injections of 6-OHDA (as in 5), sacrificed 72 hours after the last injection.

GROUP-12: Two injections of 6-OHDA(as in 5), exposed to OHP 72 hours after the last injection and sacrificed immediately after convulsion.

Operation of the chamber and the conditions of compression were same as in experiment 1. After convulsions, each animal was decompressed under the same schedule as in experiment 1, and blood and brain samples were collected and stored as discussed earlier. Each sample was analyzed for A, NA, TC, COMT, ammonia and amino acids (see appendix for methods).

RESULTS

Brain A (Table-4, Figure-9):

The normal concentration of A in brain was 2.22±0.40 $ng.g^{-1}$. This value decreased significantly to 0.51+0.10, 0.48+0.06 and 0.51±0.05 $ng.g^{-1}$ after 1, 2 and 3 separate equal injections of 6-OHDA two hours apart. An increase in A concentration was noted, 72 hours after a 2nd injection, to 0.79±0.07 $ng.g^{-1}$.

The concentration of A in brain decreased significantly to 1.35± 0.17 $ng.g^{-1}$ when normal rats were convulsed by OHP. However, no significant change from normal was observed when the 6-OHDA treated rats were exposed to OHP and convulsed.

Brain NA (Table-4, Figure-9):

The normal concentration of NA in brain was 128.90±6.0 $ng.g^{-1}$. Values after 1,2 and 3 equal injections of 6-OHDA were 86.5±9.2, 63.18±4.63 and 64.84±6.4 $ng.g^{-1}$ respectively. Forty eight and 72 hours after a 2nd injection, the concentration of NA in brain increased to 113.96±13.29 and 124.71±4.34 $ng.g^{-1}$ respectively.

No significant change was noted in the concentration of NA in brain when normal rats suffered convulsions induced by OHP . The pre and post convulsive concentrations of NA in normal rats were higher than those of 6-OHDA treated rats.

Brain TC (Table-4, Figure-9 and 10):

The normal concentration of TC in brain was 491.37±49.50$ng.g^{-1}$. Values after 1, 2 and 3 equal dose injections of 6-OHDA were 355.50±49.98, 259.75±31.44 and 271.20±34.39 $ng.g^{-1}$ respectively. Concentrations of TC in

brain 48 and 72 hours after a 2nd injection were 278.97±73.80 and 324.95±42.75 $ng.g^{-1}$. respectively.

No significant change from either the above normal or drugged control concentration was observed when normal and 6-OHDA treated rats were subjected to OHP and convulsed.

Brain COMT (Table-4, Figure-9 and 10):

The normal activity of COMT in brain was 3.22±0.28 $nmole.g^{-1}.min^{-1}$. Significantly lower concentrations were observed following 1,2 and 3 equal injections of 6-OHDA. The concentrations 48 and 72 hours after a 2nd injection were 2.64±0.40 and 2.50± 0.47 $nmole.g^{-1}.min^{-1}$ respectively and were not significantly different from normal.

No significant change fron either the above normal or drugged control concentration was observed when normal and 6-OHDA treated rats were subjected to OHP and convulsed.

TABLE IV

Brain catecholamines and catechol-o-methyl transferase in normal and 6 - OHDA treated rats before and after convulsions. n = 5 in each group.

c = Control, Conv. = Convulsed.
Values are mean ± SD.

Time (Hours) of sacrifice after 1st injection	Time (Hours) and number of injections		Adrenaline $ng.g.^{-1}$		Noradrenaline $ng.g^{-1}$		Total Catecholamine $ng.g^{-1}$		Catechol-o-methyl Transferase $nmole.g.^{-1} min^{-1}$	
	T	N	C.	Conv.	C.	Conv.	C.	Conv.	C.	Conv.
0	Control		2.22± 0.40	1.35±* 0.17	128.9± 6.0	124.6± 19.13	491.5± 49.5	448.9± 44.1	3.22± 0.28	3.48± 0.42
24	0	1	0.51±* 0.10	0.62±* 0.21	86.5±* 9.2	57.93±* 26.90	355.5±* 49.9	340.8± 61.5	2.46±* 0.45	2.56±* 0.35
48	24	2	0.48±* 0.06	0.48±* 0.16	63.1±* 4.6	41.82±*x 4.90	259.7±* 31.4	268.40±* 11.03	2.41±* 0.32	2.43±* 0.20
72	48	2	0.39±* 0.08	0.70±* 0.04	113.9±* 13.3	91.38±* 13.70	278.9±* 73.8	253.80±* 47.00	2.64± 0.40	1.65±* 0.38
96	72	2	0.79±* 0.07	0.45±*x 0.10	124.7± 4.3	121.79± 8.30	324.9±* 42.8	283.91±* 38.05	2.50± 0.47	2.10±* 0.35
72	24	3	0.51±* 0.07	0.67±* 0.10	64.8±* 6.4	91.38± 18.60	271.2±* 34.4	246.55±* 55.86	2.60± 0.51	1.9±* 0.7

* Significant when compared with normal control ($p < 0.05$)
x Significant when compared with corresponding drugged control ($p < 0.05$)

FIGURE-9: Brain adrenaline (A), noradrenaline (NA), total catecholamine (TC) and catechol-O-methyl transferase (COMT) concentrations in normal rats and in rats which received 1, 2 and 3 equal injections of 6-hydroxy dopamine (24 hours apart) and were exposed to OHP until convulsions. (Mean ± SD, n=5)

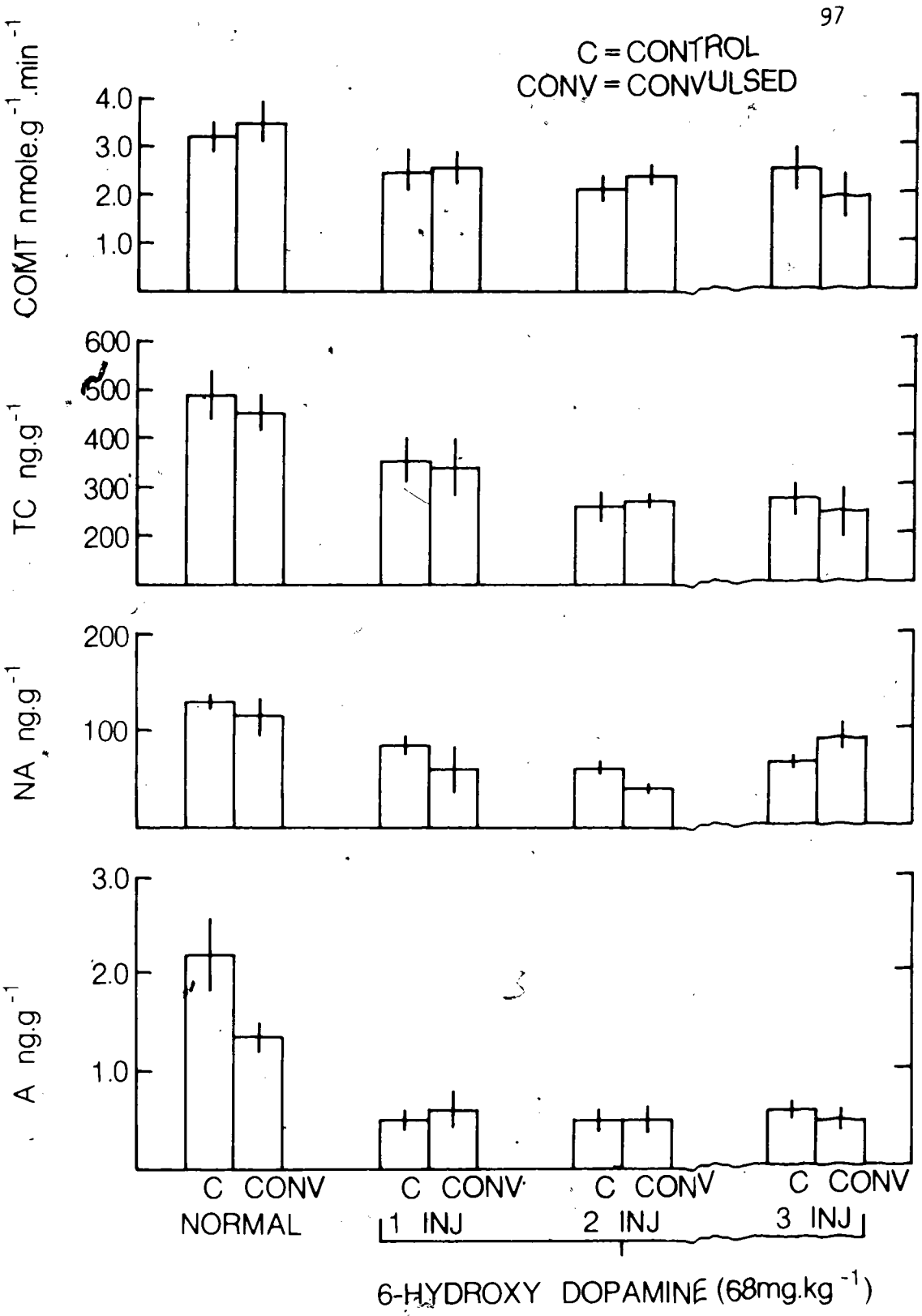
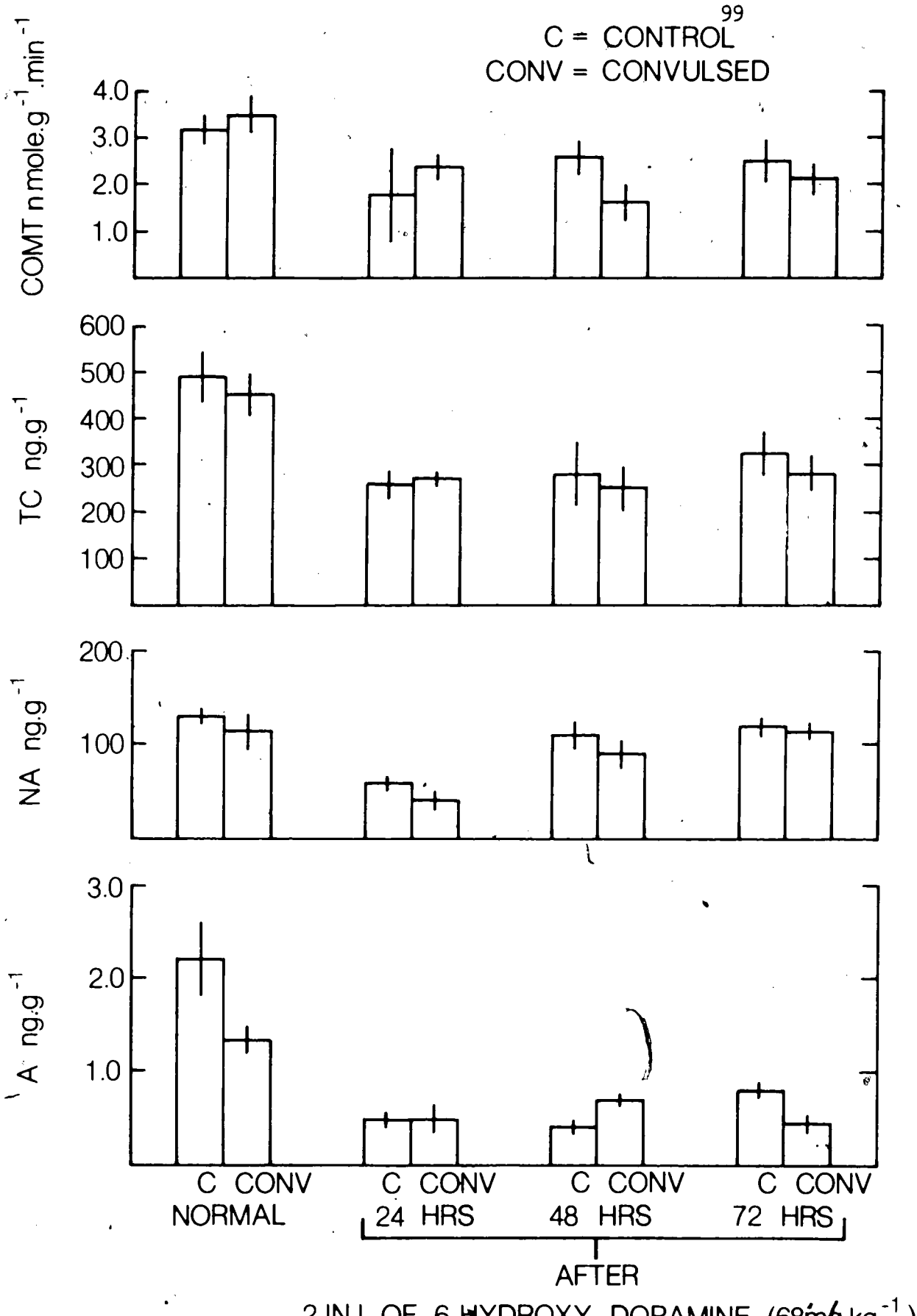

C = CONTROL
CONV = CONVULSED
COMT nmole.g^{-1}.min^{-1}
4.0
3.0
2.0
1.0
TC ng.g^{-1}
600
500
400
300
200
NA ng.g^{-1}
200
100
A ng.g^{-1}
3.0
2.0
1.0
C CONV
C CONV
C CONV
C CONV
NORMAL
1 INJ
2 INJ
3 INJ
6-HYDROXY DOPAMINE (68mg.kg^{-1})

FIGURE-10: Brain adrenaline (A), noradrenaline (NA), total catecholamine (TC) and catechol-O-methyl transferase (COMT) in normal rats and 24, 48 and 72 hours after receiving 2 equal injections of 6-OHDA 24 hours apart and exposed to OHP until the onset of convulsions. (Mean ± SD, n=5)


C = CONTROL
CONV = CONVULSED
COMT n mole.g^{-1}.min^{-1}
4.0
3.0
2.0
1.0
TC ng.g^{-1}
600
500
400
300
200
100
NA ng.g^{-1}
200
100
A ng.g^{-1}
3.0
2.0
1.0
C CONV
NORMAL
C CONV
24 HRS
C CONV
48 HRS
C CONV
72 HRS
AFTER
2 INJ OF 6-HYDROXY DOPAMINE (68mg.kg^{-1})

Brain ammonia (Table-5, Figure-12 and 13):

The normal concentration of ammonia in brain was 5.25 ± 0.73 $\mu g.g^{-1}$. Significantly increased concentrations resulted 24 hours after 3 separate equal injections of 6-OHDA, the concentrations being 7.36 ± 0.75, 7.51 ± 0.52 and 8.24 ± 0.67 $\mu g.g^{-1}$ respectively. At 48 and 72 hours after the 2nd injection, the levels of ammonia in brain decreased to 7.14 ± 0.84 and 6.02 ± 0.20 $\mu g.g^{-1}$ respectively.

The concentration of ammonia in brain increased significantly when normal and drugged rats were exposed to OHP. However, no significant difference were observed between concentrations for normal convulsed rats and 6-OHDA treated convulsed rats.

TABLE V

Brain ammonia ($\mu g.g^{-1}$) and amino acids ($\mu mole.g^{-1}$) in control and convulsed normal and 6 - OHDA treated rats. C = Control; Conv. = Convulsed. n = 5 in each group. Values mean ± S.D.

Time (Hours) of sacrifice after 1st injection	Time (Hours) and number of injections		Ammonia		GABA		Glutamate		Glutamine Aspargine		Aspartate		Tyrosine	
	T	N	C.	Conv.	C.	Conv.	C.	Conv.	C.	Conv.	C.	Conv.	C.	Conv.
0	Control		5.25± 0.72	17.8±* 0.9	1.52± 0.11	0.78±* 0.07	9.38± 0.67	5.18±* 0.48	0.97± 0.17	2.88± 0.73	3.73± 0.69	3.96± 0.51	0.31± 0.08	0.56±* 0.21
24	0	1	7.36±* 0.75	17.6±*x 1.2	1.44± 0.20	0.91±*x 0.08	6.26±* 0.51	5.03±*x 0.71	1.27± 0.18	2.9± 0.5	2.96± 1.03	3.12± 0.73	0.29± 0.05	0.41±*x 0.11
48	24	2	7.51±* 0.52	18.73±*x 1.07	0.95±* 0.11	0.74±*x 0.08	6.54±* 1.68	5.08±*x 0.84	1.59± 0.13	3.03± 0.63	3.79± 0.72	4.01± 1.03	0.57±* 0.07	0.57± 0.10
72	48	2	7.14±* 0.48	17.7±*x 1.0	0.98±* 0.09	0.66±*x 0.09	6.06±* 0.61	4.94±*x 1.05	1.00± 0.11	2.94± 0.61	3.11± 0.79	3.01± 0.89	0.59±* 0.04	0.53± 0.07
96	72	2	6.02± 0.20	18.98±*x 1.57	1.06± 0.12	0.74±*x 0.09	6.66±* 0.65	4.1±*x 0.8	1.11± 0.28	3.3± 1.0	4.01± 0.96	3.00± 0.36	0.4± 0.1	0.54± 0.09
72	24	3	8.24±* 0.67	19.9±*x 0.9	0.87±* 0.13	0.70±* 0.15	6.70±* 0.87	4.14±*x 0.65	1.20± 0.22	3.22± 0.55	3.76± 0.93	4.13± 0.92	0.60±* 0.03	0.59± 0.03

* significant (<0.05) when compared with normal control.

x significant (<0.05) when compared with corresponding control.

Brain GABA (Table-5, Figure-11 and 13):

The normal mean concentration of GABA in rat brain was 1.52 ± 0.12 $\mu mole.g^{-1}$. No significant change was observed in control rats injected with 1 dose of 6-OHDA. After a 2nd and 3rd injection of this drug, the mean concentration of GABA decreased significantly to 0.95 ± 0.11 and 0.87 ± 0.13 $\mu mole.g^{-1}$ respectively. Values returned to normal within 72 hours after a 2nd injection.

The concentration of GABA decreased significantly when normal and 6-OHDA treated rats were convulsed by OHP. The pre convulsive GABA concentration with 3 injections of 6-OHDA was not significantly different from the post convulsive concentration of GABA in convulsed normal rats.

Brain glutamate (Table-5, Figure-12 and 14):

The normal mean concentration of glutamate in brain was 9.38 ± 0.67 $\mu mole.g^{-1}$ which decreased significantly after 1, 2 and 3 separate equal injections of 6-OHDA. Values 48 and 72 hours after a 2nd injection of 6-OHDA were 6.06 ± 0.61 and 6.60 ± 0.65 $\mu mole.g^{-1}$ respectively.

Significantly reduced concentrations of glutamate in brain were observed when normal and 6-OHDA treated rats were subjected to convulsions induced by OHP.

Brain glutamine+aspargine ($glu.NH_2$+$asp.NH_2$) (Table-5, Figure-11):

The normal concentration of combined glutamine ($glu.NH_2$) and aspargine ($asp.NH_2$) in brain was 0.97 ± 0.17 $\mu mole.g^{-1}$. No significant change was observed after 1 injection of 6-OHDA but significantly elevated concentrations were observed after 2 injections of this

FIGURE-11: Brain GABA and glu.NH_2+asp.NH_2 in normal rats and those treated with 1, 2 and 3 equal injections of 6-OHDA 24 hours apart before and after being convulsed by exposure to OHP. (Mean ± SD, n=5)

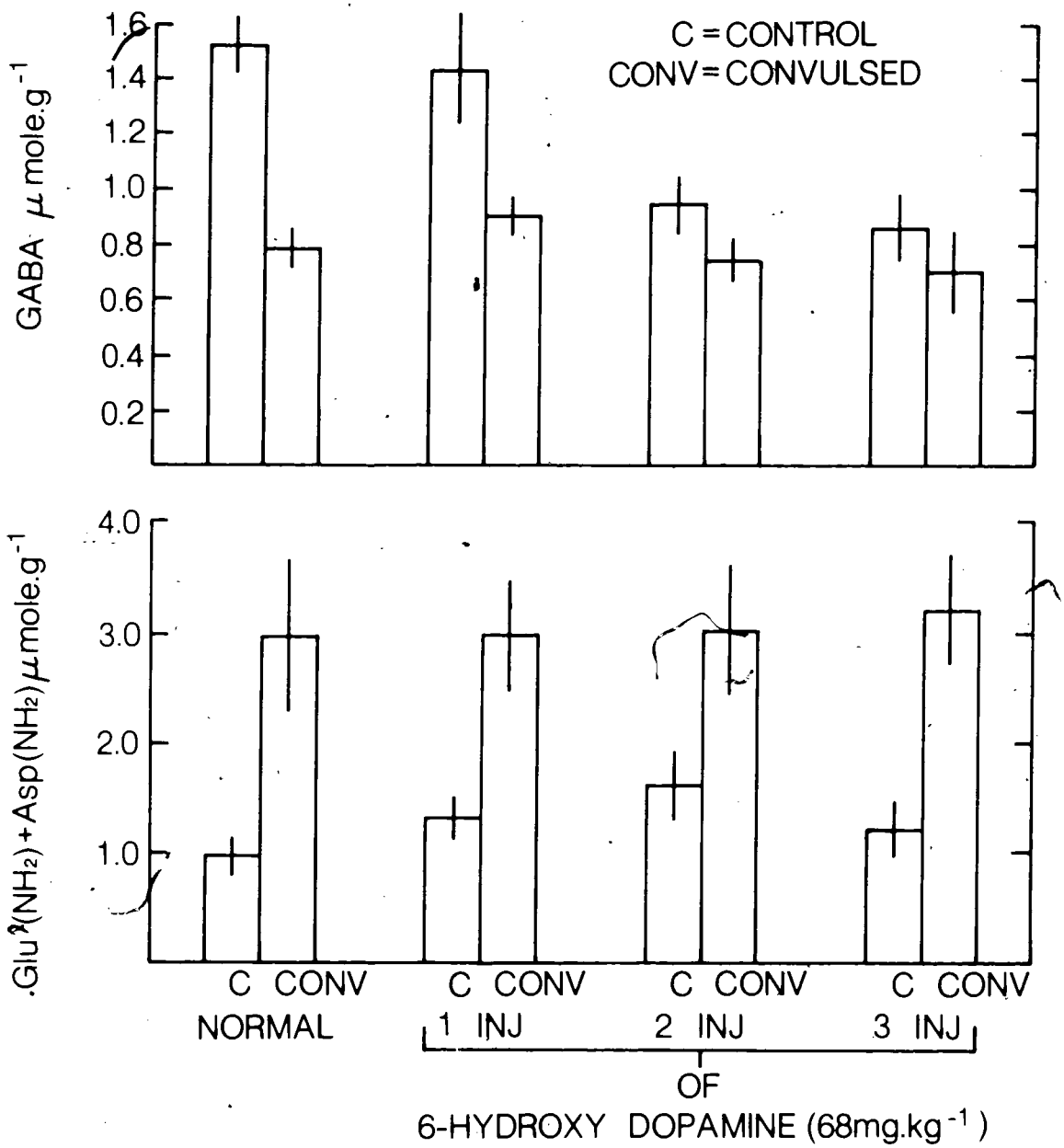

C = CONTROL
CONV = CONVULSED
GABA μ mole.g⁻¹
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
.Glu²(NH₂) + Asp(NH₂) μmole.g⁻¹
4.0
3.0
2.0
1.0
C CONV
C CONV
C CONV
C CONV
NORMAL
1 INJ
2 INJ
3 INJ
OF
6-HYDROXY DOPAMINE (68mg.kg⁻¹)

FIGURE-12: Brain ammonia and glutamate (glu) concentration in normal rats and in rats receiving 1, 2 and 3 equal injections of 6-OHDA respectively, 24 hours apart and convulsed by OHP. (Mean ± SD, n=5)

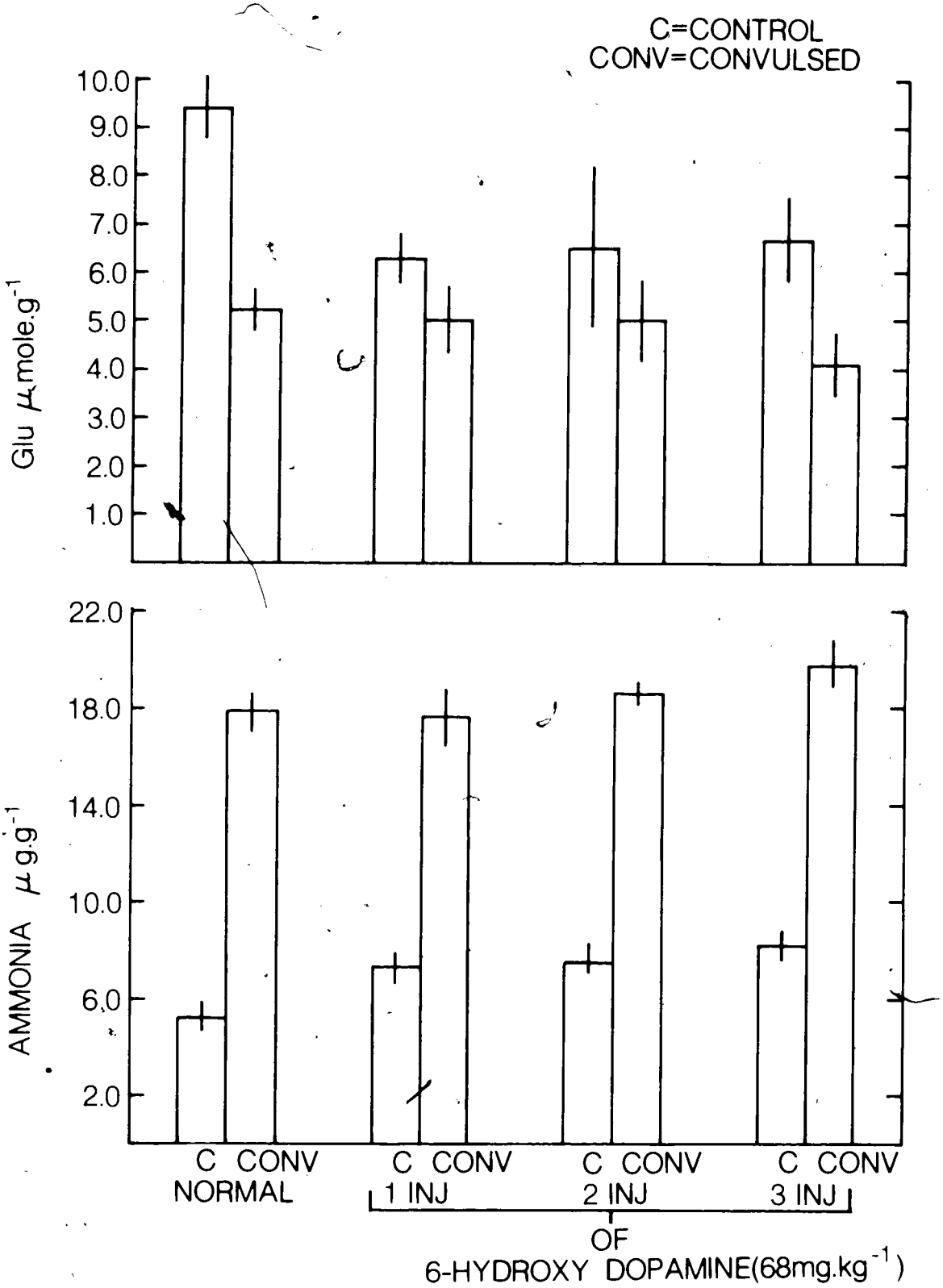

C=CONTROL
CONV=CONVULSED
Glu μmole.g-1
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
AMMONIA μg.g-1
22.0
18.0
14.0
10.0
6.0
2.0
C CONV
NORMAL
C CONV
1 INJ
C CONV
2 INJ
C CONV
3 INJ
OF
6-HYDROXY DOPAMINE(68mg.kg-1)

FIGURE-13: Brain ammonia and GABA concentration in normal rats and in 6-OHDA treated animals 24, 48 and 72 hours respectively after having received 2 equal injections of 6-OHDA 24 hours apart before and after being convulsed by OHP exposure. (Mean ± SD, n=5)

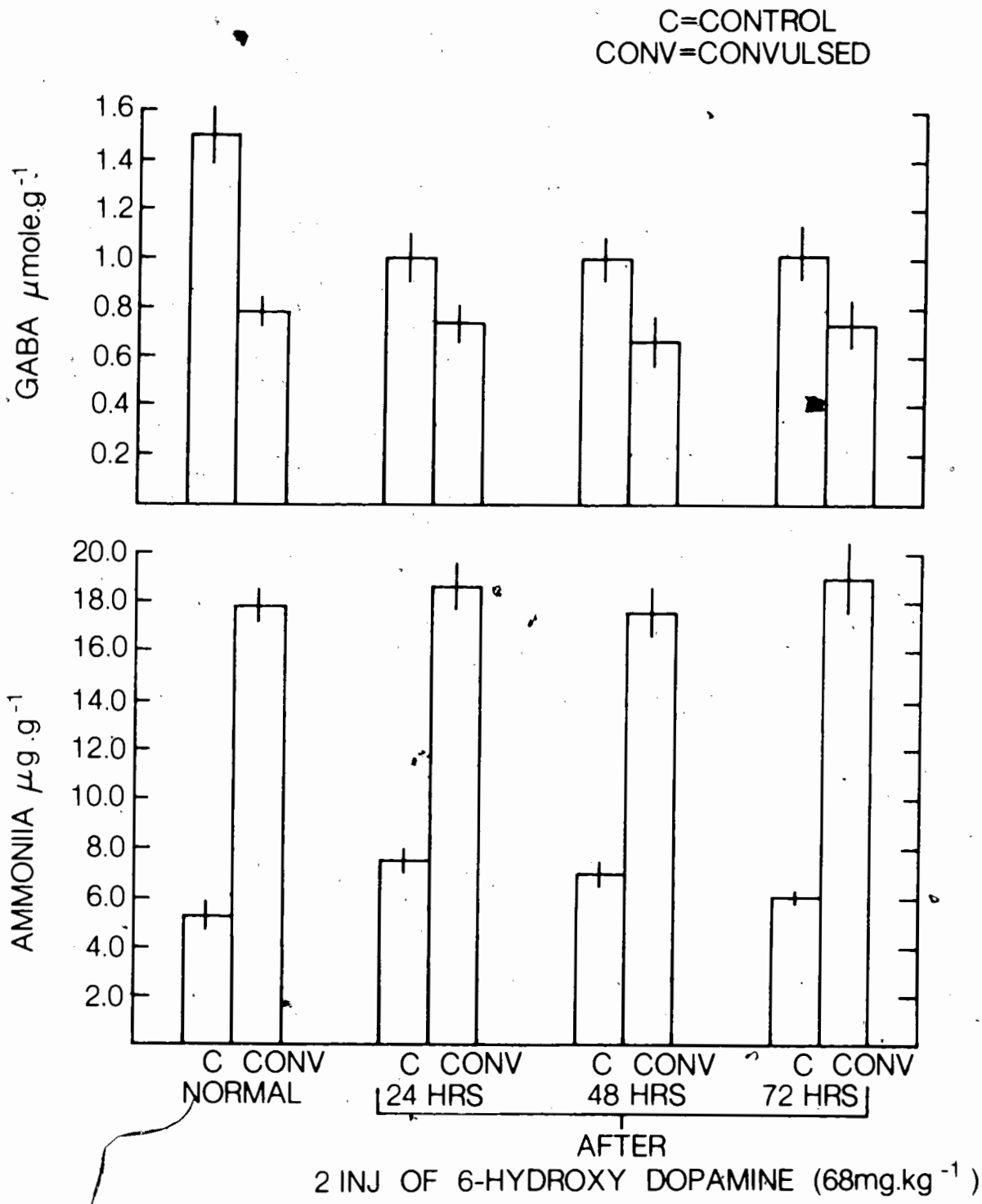
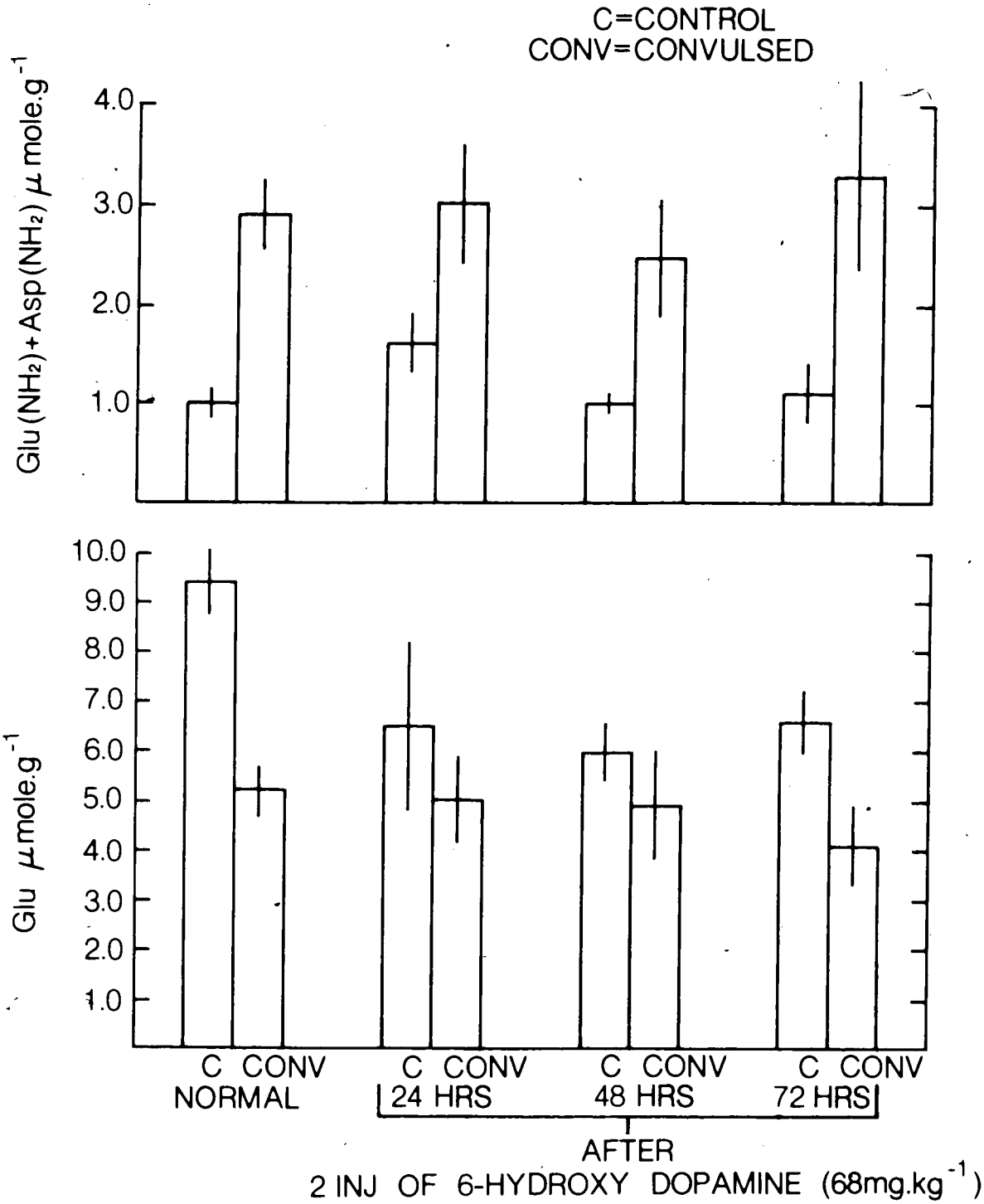

C=CONTROL
CONV=CONVULSED
GABA μmole.g-1
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
AMMONIIA μg.g-1
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
C CONV
NORMAL
C CONV
24 HRS
C CONV
48 HRS
C CONV
72 HRS
AFTER
2 INJ OF 6-HYDROXY DOPAMINE (68mg.kg-1)

FIGURE-14: Brain glutamate (glu) and glutamine+asparagine (glu.NH_2+asp.NH_2) concentrations in normal rats and 24, 48 and 72 hours after the normal rats received 2 equal injections of 6-OHDA 24 hours apart before and after being convulsed by OHP exposure. (Mean $\pm$ SD, n=5)


C=CONTROL
CONV=CONVULSED
Glu(NH2)+Asp(NH2) μ mole.g-1
4.0
3.0
2.0
1.0
Glu μmole.g-1
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
C CONV
NORMAL
C CONV
24 HRS
C CONV
48 HRS
C CONV
72 HRS
AFTER
2 INJ OF 6-HYDROXY DOPAMINE (68mg.kg-1)

drug. These altered concentrations returned back to normal control concentrations 48 hours after the 1st and 2nd injection and remained unchanged thereafter.

The brain content of these amino acids increased significantly when normal and drugged rats incurred OHP induced convulsions.

Brain tyrosine (Table-5):

The normal concentration of tyrosine in brain was 0.31 ± 0.08 $\mu mole.g^{-1}$. Concentrations of tyrosine in brain after 3 equal injections of 6-OHDA were 0.29 ± 0.05, 0.57 ± 0.07 and 0.63 ± 0.03 $\mu mole.g^{-1}$ respectively. No significant change from the elevated concentration was noted 48 hour after 2 injections of 6-OHDA but after 72 hours, the concentration of tyrosine in brain had returned to normal.

The concentration of tyrosine in brain increased significantly after non drugged rats were convulsed by OHP. No significant change was noted when 6-OHDA treated rats were similarly convulsed.

Other brain amino acids(Table-6):

No significant change in any other rat brain amino acid was observed prior to or after convulsions in either the non drugged or 6-OHDA drugged state of the animals.

TABLE VI

Brain amino acids ($\mu mole.g^{-1}$) in control and convulsed normal and 6 - OHDA treated rats. C = Control; Conv. = Convulsed. n = 5 in each group. Values = mean ± S.D.

Time (Hours) of sacrifice after 1st injection	Time (Hours) and number of injections T N	Alanine		Arginine		Threonine		Serine		Lysine		Leucine		Isoleucine	
		C.	Conv.	C.	Conv.	C.	Conv.	C.	Conv.	C.	Conv.	C.	Conv.	C.	Conv.
0	Control	0.61± 0.11	0.60± 0.11	0.11± 0.03	0.10± 0.02	1.03± 0.20	1.02± 0.20	0.76± 0.48	0.93± 0.09	0.15± 0.09	0.14± 0.02	0.69± 0.10	0.77± 0.13	0.61± 0.10	0.57± 0.10
24	0 1	0.55± 0.25	0.61± 0.21	0.07± 0.01	0.14± 0.02	0.96± 0.39	0.93± 0.37	0.79± 0.32	0.81± 0.05	0.11± 0.03	0.15± 0.06	0.66± 0.13	0.69± 0.23	0.55± 0.13	0.50± 0.10
48	24 2	0.56± 0.21	0.55± 0.22	0.08± 0.02	0.13± 0.04	0.97± 0.28	1.11± 0.34	0.69± 0.23	0.92± 0.33	0.18± 0.04	0.12± 0.04	0.60± 0.32	0.83± 0.31	0.47± 0.12	0.51± 0.03
72	48 2	0.54± 0.18	0.61± 0.17	0.08± 0.02	0.11± 0.03	1.11± 0.29	0.90± 0.40	0.77± 0.30	0.88± 0.13	0.13± 0.02	0.09± 0.05	0.62± 0.13	0.70± 0.13	0.51± 0.11	0.41± 0.10
96	72 2	0.56± 0.21	0.53± 0.10	0.14± 0.03	0.08± 0.16	1.17± 0.31	0.83± 0.35	0.87± 0.25	0.90± 0.51	0.16± 0.11	0.13± 0.09	0.67± 0.04	0.66± 0.20	0.66± 0.05	0.60± 0.07
72	24 3	0.53± 0.17	0.62± 0.05	0.08± 0.02	0.11± 0.04	0.97± 0.33	0.97± 0.33	0.81± 0.31	0.77± 0.30	0.197± 0.05	0.11± 0.05	0.67± 0.13	0.65± 0.11	0.59± 0.21	0.55± 0.09

Blood A (Table-7, Figure-15 and 16):

The normal concentration of A in blood was 0.19±0.03 $ng.ml^{-1}$. No significant change was observed after 1 injection of 6-OHDA but the concentrations increased significantly to 0.36±0.05 and to 0.34±0.10 $ng.ml^{-1}$ respectively after the 2nd and 3rd equal dose injections of 6-OHDA. Blood A concentrations returned to normal within 48 hours after the 2nd injection and remained unchanged thereafter.

The concentration of A in blood increased significantly when normal and drugged rats were convulsed by exposure to OHP. However, the magnitude of the increase in normal rats was much higher than in 6-OHDA treated rats. The concentrations after convulsion were 2.62±0.81, 0.72±0.19, 0.98±0.11 and 1.13±0.15 $ng.ml^{-1}$ in normal and in the rats which received 1, 2 and 3 injections of 6-OHDA respectively.

Blood NA (Table-7, Figure-15 and 16):

The normal mean concentration of NA in blood was 0.33 ± 0.06 ng.ml^{-1}. The mean value after 1 injection of 6-OHDA was 0.27 ± 0.10, after 2 injections, 0.11 ± 0.02 and after 3 injections, 0.11 ± 0.02 ng.ml^{-1}. Concentrations of NA in blood recovered substantially towards normal 72 hours after the 2nd injection of 6-OHDA.

When normal rats were convulsed by OHP, the mean NA level increased significantly from 0.33 ± 0.06 to 2.03 ± 0.53 ng.ml^{-1}. No significant change was observed when 6-OHDA treated rats were similarly convulsed.

Blood TC (Table-7, Figure-15 and 16):

The normal mean concentration of TC in blood was 0.57 ± 0.04 ng.ml^{-1}. The concentrations decreased to 0.40 ± 0.02 ng.ml^{-1} after the 2nd injection of 6-OHDA. No significant change was observed thereafter in response

TABLE VII

Blood catecholamines in normal and 6 - OHDA treated rats before and after convulsions. n = 5 in each group.

Values are mean ± SD

Time (Hours) of sacrifice after 1st injection	Time (Hours) and numbers of injections T N	Adrenaline $ng.ml^{-1}$		Noradrenaline $ng.ml^{-1}$		Total Catecholamine $ng.ml^{-1}$	
		Control	Convulsed	Control	Convulsed	Control	Convulsed
0	Control	0.19±0.03	2.62±0.81*	0.33±0.06	2.03±0.53*	0.57±0.04	5.75±0.47
24	0 1	0.21±0.04	0.72±0.19*x	0.27±0.10	0.21±0.08	0.51±0.01	0.89±0.12*x
48	24 2	0.36±0.05*	0.98±0.11*x	0.11 ±0.03*	0.11±0.02*	0.40±0.02*	12.0±0.20*x
72	48 2	0.27±0.11*	1.20±0.21*x	0.14± 0.05*	0.13±0.05*	0.41±0.05*	2.24±0.25*x
96	72 2	0.20±0.04	1.62±0.04*x	0.16±0.06*	0.37±0.08*x	0.45±0.07*	1.07±0.18*x
72	24 3	0.34±0.10*	1.13±0.15*x	0.11±0.22*	0.12±0.02*	0.40±0.05*	1.61±0.11*x

* Significant when compared with normal control ($p < 0.05$)

x Significant when compared with corresponding drugged control ($p < 0.05$)

FIGURE-15: Blood adrenaline (A), noradrenaline (NA) and total catecholamine (TC) concentration in normal rats and 6-OHDA treated rats respectively injected with 1, 2 and 3 equal injections of 6-OHDA 24 hours apart and exposed to OHP until convulsion. (Mean ± SD, n=5)

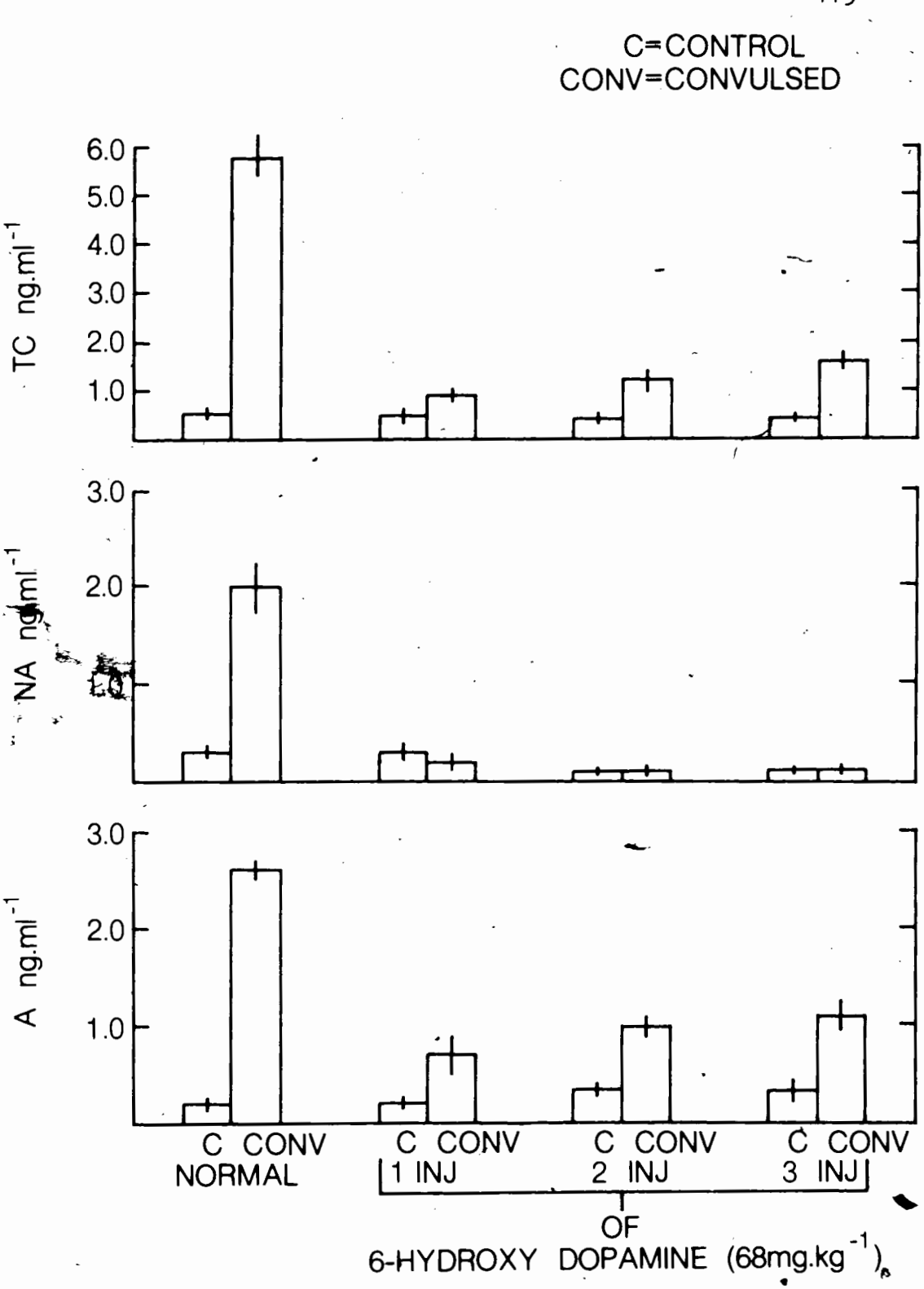
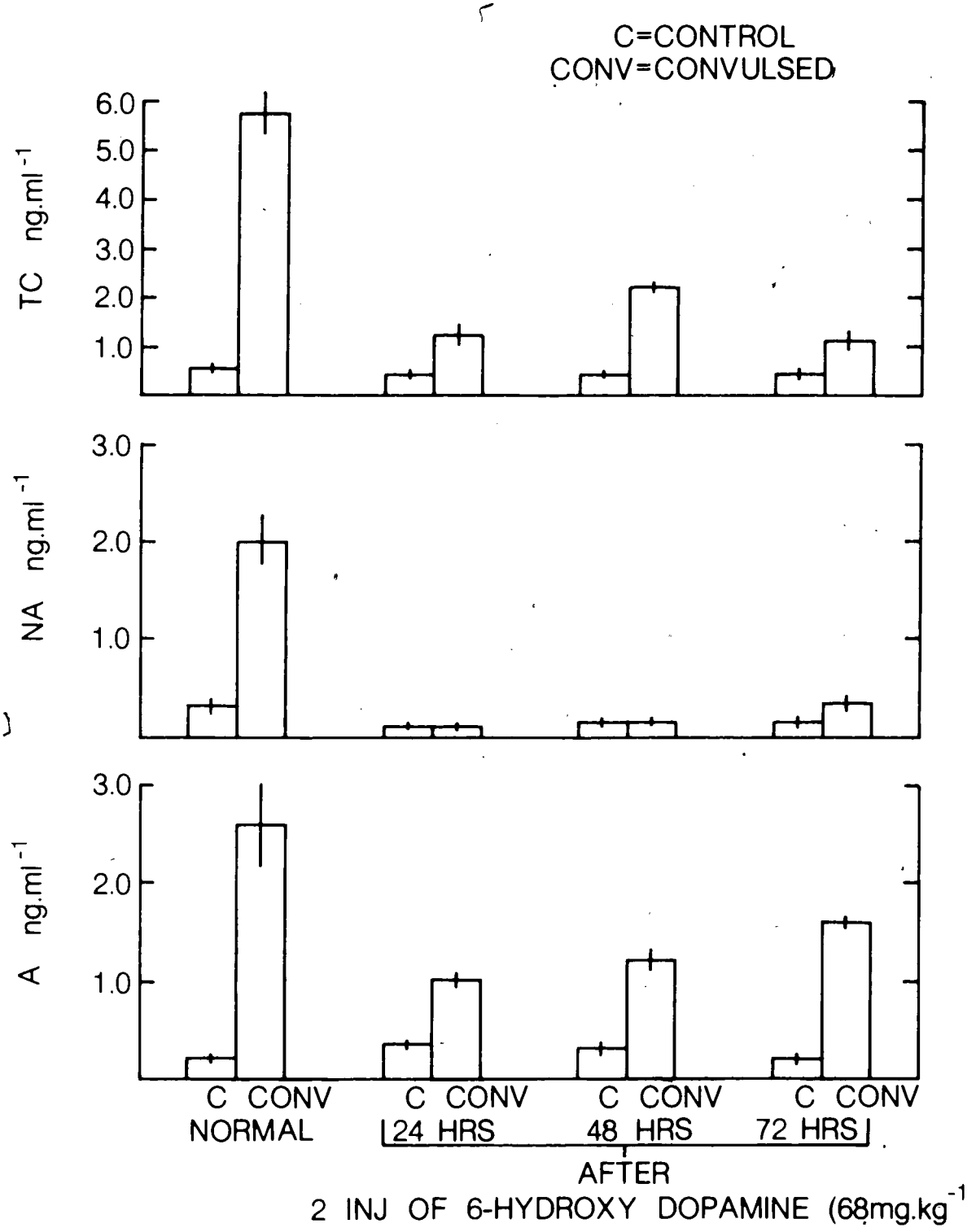

C=CONTROL
CONV=CONVULSED
TC ng.ml-1
6.0
5.0
4.0
3.0
2.0
1.0
NA ng.ml-1
3.0
2.0
A ng.ml-1
3.0
2.0
1.0
C CONV
NORMAL
C CONV
1 INJ
C CONV
2 INJ
C CONV
3 INJ
OF
6-HYDROXY DOPAMINE (68mg.kg-1)

FIGURE-16: Blood adrenaline (A), noradrenaline (NA) and total catecholamine (TC) concentration in normal and 6-OHDA treated animals 24, 48 and 72 hours respectively after they received 2 equal injections of 6-OHDA 24 hours apart and were convulsed by OHP exposure. (Mean ± SD, n=5)


C=CONTROL
CONV=CONVULSED
TC ng.ml-1
6.0
5.0
4.0
3.0
2.0
1.0
NA ng.ml-1
3.0
2.0
1.0
A ng.ml-1
3.0
2.0
1.0
C CONV
C CONV
C CONV
C CONV
NORMAL
24 HRS
48 HRS
72 HRS
AFTER
2 INJ OF 6-HYDROXY DOPAMINE (68mg.kg-1)

to an increased dose of 6-OHDA.

The concentration of TC in blood increased significantly after normal and 6-OHDA treated rats were subjected to OHP treatment. However, the magnitude of change was somewhat suppressed in the 6-OHDA treated animals.

Blood ammonia (Table-8, Figure-17 and 18):

The concentration of ammonia in the blood in normal animals was $2.04 \pm 0.22\ \mu g.ml^{-1}$. No significant change in this blood ammonia value was observed after 1 and 2 injections of 6-OHDA. Following the 3rd injection, however, the concentration of ammonia in blood increased significantly to $3.05 \pm 0.37\ \mu g.ml^{-1}$. Forty eight and 72 hours respectively after the 2nd equal dose injection of 6-OHDA, concentrations of ammonia in blood were 2.08 ± 0.18 and $2.13 \pm 0.21\ \mu g.ml^{-1}$ respectively.

convulsions induced by OHP caused the concentration of

ammonia in blood to increase significantly in both normal and in rats treated with 6-OHDA.

Blood glutamate (Table-8, Figure-17 and 18):

The normal glutamate concentration in blood was 3.26 ± 0.33 µmole.100ml^{-1}. No significant change in this concentration was observed during the standard 6-OHDA treatment.

Concentration of glutamate in blood decreased significantly when normal and 6-OHDA treated rats were exposed to oxygen at high pressure.

Blood glutamine+aspargine ($glu.NH_2 + asp.NH_2$) (Table-8, Figure-17 and 18):

The combined normal concentration for these amino acids was 14.19 ± 2.70 µmole.100ml^{-1}. No significant change in this concentration was observed following

6-OHDA treatment.

The above concentrations increased significantly in both normal and 6-OHDA treated rats when convulsions were induced by OHP.

Blood alanine (Table-8):

The normal alanine concentration in blood was 10.29 ± 1.44 μmole.$100ml^{-1}$. No significant change was observed following injection of 6-OHDA in normal rats.

Concentration of alanine in blood increased slightly to 12.07 ± 0.08 μmole.$100ml^{-1}$ when normal rats were convulsed by exposure to OHP. However, after 1, 2 and 3 injections of 6-OHDA, the concentration of alanine in blood increased significantly following convulsions induced by OHP to 20.72 ± 1.09, 20.60 ± 1.65 and 21.38 ± 1.41 μmole.$100ml^{-1}$ respectively.

Blood tyrosine (Table-8):

The normal concentration of tyrosine in blood was 0.57 ± 0.13 µmole.$100ml^{-1}$. No significant change was observed when normal rats were treated with 6-OHDA.

The concentration of tyrosine in blood decreased significantly when normal rats were subjected to oxygen convulsions. Tyrosine concentration in the blood increased significantly in the rats which received 2 and 3 doses of 6-OHDA and were exposed to OHP.

Other blood amine acids(Table-9):

No significant change in the other amino acids were noted either before or after convulsions induced by OHP in normal and 6-OHDA treated rats.

TABLE VIII

Blood ammonia ($\mu g.ml.^{-1}$) and amino acids ($\mu mole.100\ ml.^{-1}$) in control and convulsed normal and 6 - OHDA treated rats. C = Control; Conv. = Convulsed. n = 5 in each group. Values mean ± S.D.

Time (Hours) of sacrifice after 1st injection	Time (Hours) and number of injections T N	Ammonia		Glutamate		Glutamine Aspargine		Aspartate		Alanine		Tyrosine	
		C.	Conv.	C.	Conv.	C.	Conv.	C.	Conv.	C.	Conv.	C.	Conv.
0	Control	2.04± 0.22	5.58±* 0.62	3.26± 0.33	2.25±* 0.42	14.19± 2.70	22.23±* 2.60	1.55± 0.36	1.84± 0.31	10.29± 1.44	12.07±* 0.08	0.57± 0.13	0.36±* 0.04
24	0 1	1.83± 0.52	5.82±*x 0.51	3.11± 0.30	1.82±*x 0.20	13.48± 1.72	22.30±*x 1.94	1.53± 0.24	1.42± 0.19	10.9± 1.0	20.72±*x 1.09	0.62± 0.11	0.49± 0.07
48	24 2	2.88± 0.61	5.54±*x 0.44	3.20± 0.18	1.82±*x 0.19	14.4± 1.9	22.4±*x 1.3	1.30± 0.18	1.93± 0.15	10.28± 0.83	20.61±*x 1.65	0.57± 0.15	0.71±*x 0.03
72	48 2	2.08± 0.18	5.85±*x 0.49	3.04± 0.27	1.64±*x 0.54	12.16± 1.48	22.11±*x 1.06	1.52± 0.33	1.34± 0.14	10.99± 1.38	19.50±*x 4.28	0.51± 0.08	0.63±* 0.08
96	72 2	2.13± 0.21	5.60±*x 0.51	2.96± 0.44	1.46±*x 0.35	10.74± 1.57	22.61±*x 1.06	1.47± 0.31	1.53± 0.14	10.30± 0.93	20.97±*x .65	0.55± 0.10	0.55± 0.08
72	24 3	3.05±* 0.37	5.44±*x 0.48	3.07± 0.27	1.68±*x 0.22	15.20± 2.17	21.09±*x 1.78	1.45± 0.15	2.03± 0.35	11.15± 1.04	21.38±*x 1.41	0.56± 0.09	0.79±*x 0.07

* significant ($p<0.05$) when compared with normal control.

x significant ($p<0.05$) when compared with corresponding control.

FIGURE-17: Blood ammonia, glutamate (glu) and glutamine+ aspargine (glu.NH_2+asp.NH_2) concentrations in normal rats and in rats treated with 1, 2 and 3 equal injections of 6-OHDA and convulsed by OHP exposure. (Mean $\pm$ SD, n=5)

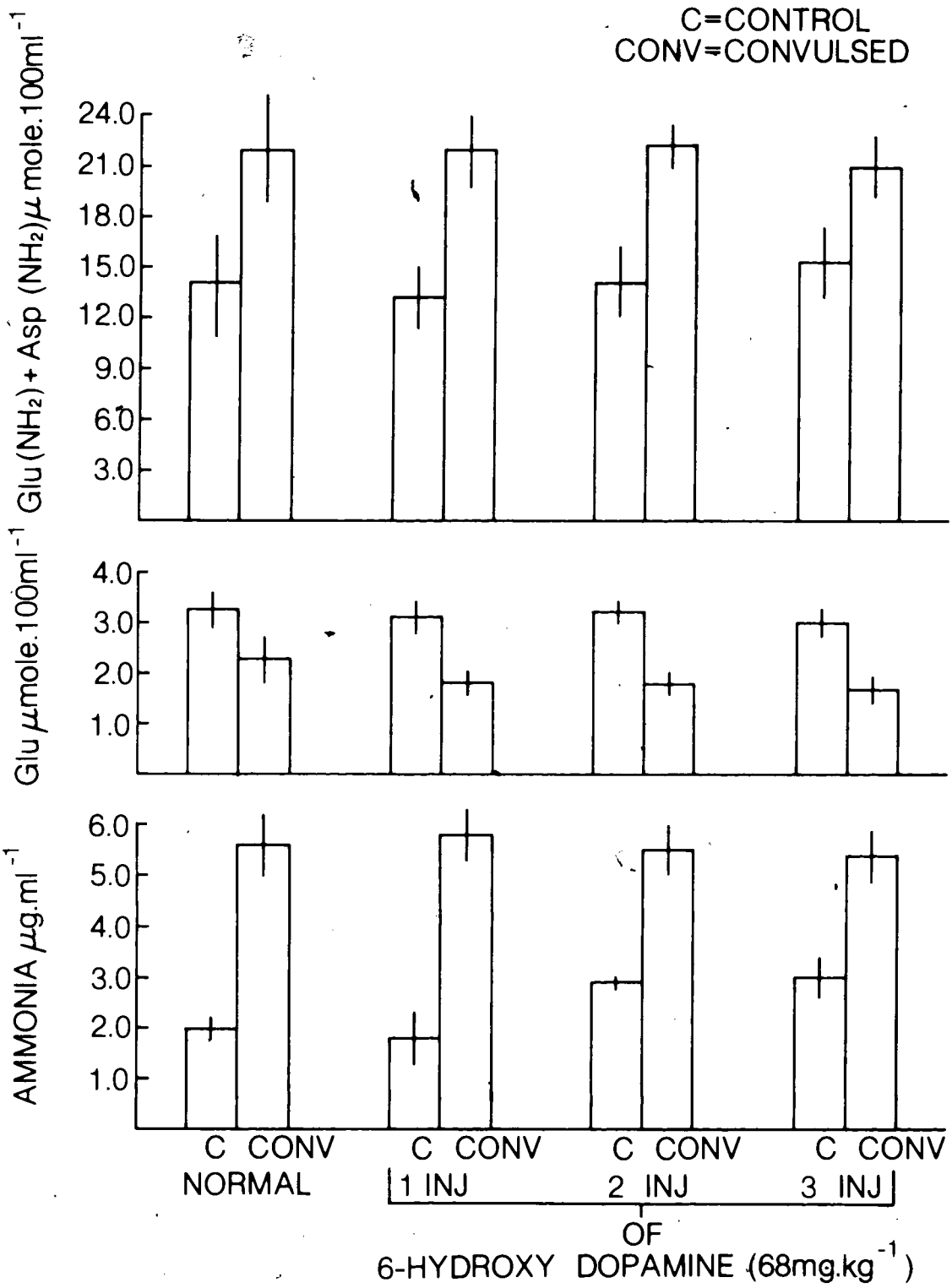

C=CONTROL
CONV=CONVULSED
Glu (NH2) + Asp (NH2) μ mole.100ml-1
24.0
21.0
18.0
15.0
12.0
9.0
6.0
3.0
Glu μmole.100ml-1
4.0
3.0
2.0
1.0
AMMONIA μg.ml-1
6.0
5.0
4.0
3.0
2.0
1.0
C CONV
NORMAL
C CONV
1 INJ
C CONV
2 INJ
C CONV
3 INJ
OF
6-HYDROXY DOPAMINE (68mg.kg-1)

FIGURE-18: Blood ammonia, glutamate (glu) and glutamine+ aspargine (glu.NH_2+asp.NH_2) in normal and 6-OHDA treated rats 24, 48 and 72 hours respectively after they received 2 injections of 6-OHDA and were convulsed by OHP exposure. (Mean ± SD, n=5)

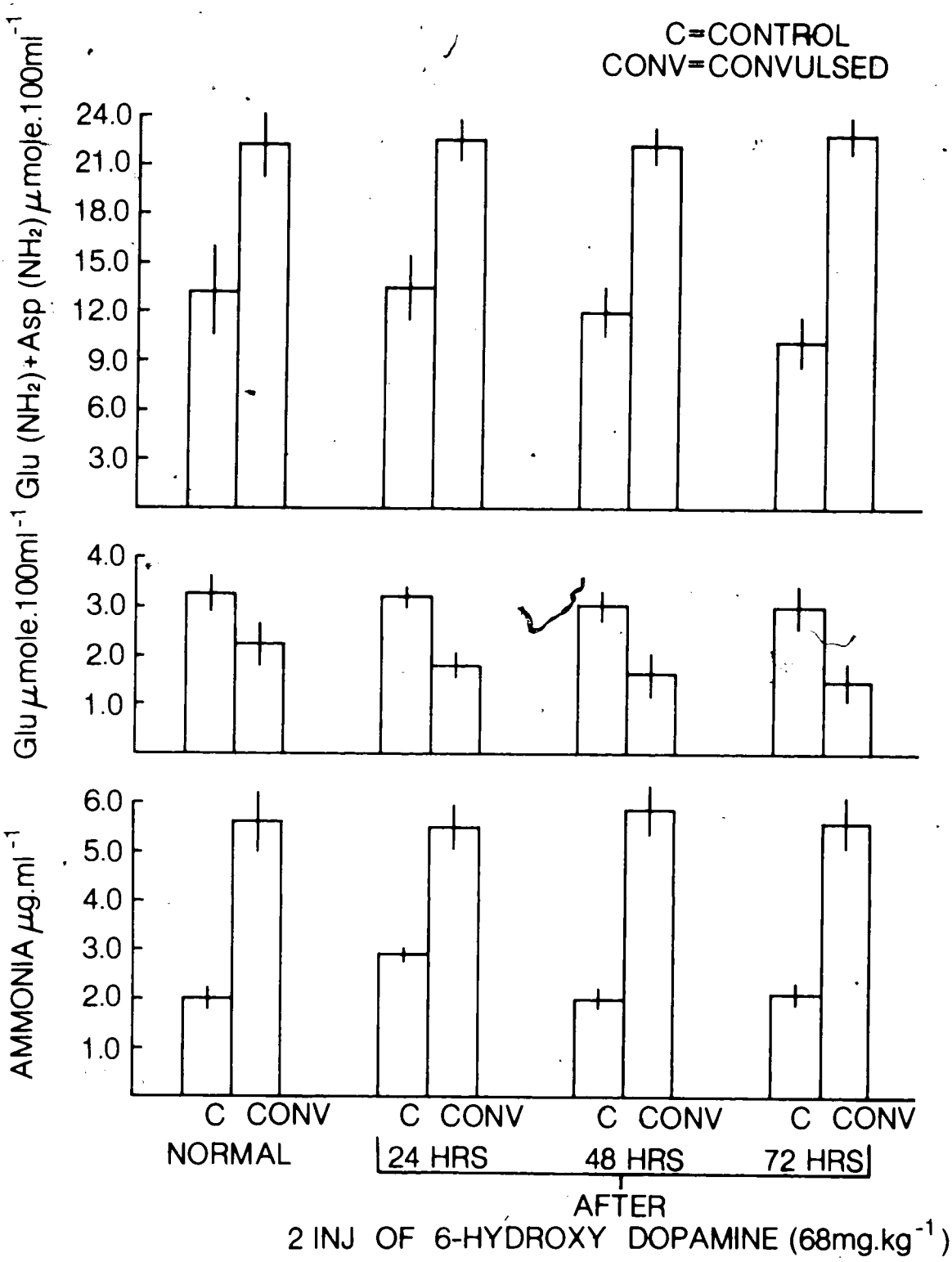

C=CONTROL
CONV=CONVULSED
Glu μmole.100ml⁻¹ Glu (NH₂)+Asp (NH₂) μmole.100ml⁻¹
24.0
21.0
18.0
15.0
12.0
9.0
6.0
3.0
4.0
3.0
2.0
1.0
AMMONIA μg.ml⁻¹
6.0
5.0
4.0
3.0
2.0
1.0
C CONV C CONV C CONV C CONV
NORMAL
24 HRS
48 HRS
72 HRS
AFTER
2 INJ OF 6-HYDROXY DOPAMINE (68mg.kg⁻¹)

TABLE IX

Blood amino acids (μmole.100 ml.$^{-1}$) in control and convulsed normal and 6 - OHDA treated rats. C = Control; Conv. = Convulsed. n = 5 in each group. Values mean ± S.D.

Time of sacrifice after 1st injection (Hours)	Time (Hours) and number of injections T N	Proline		Threonine		Phenyla-lanine		Serine		Leucine		Isoleucine		Valine	
		C.	Conv.	C.	Conv.	C.	Conv.	C.	Conv.	C.	Conv.	C.	Conv.	C.	Conv.
0	Control	2.06± 1.07	2.19± 0.44	0.81± 0.10	0.79± 0.12	0.59± 0.08	0.58± 0.11	0.96± 0.23	0.93± 0.23	4.01± 0.62	3.7± 0.9	3.69± 0.68	4.37± 0.38	0.81± 0.10	0.72± 0.21
24	0 1	2.11± 0.76	2.12± 0.79	0.79± 0.08	0.88± 0.15	0.57± 0.13	0.63± 0.12	0.92± 0.06	0.79± 0.33	3.73± 0.90	4.36± 0.52	4.00± 0.69	3.9± 0.5	0.79± 0.08	0.84± 0.36
48	24 2	2.01± 0.38	1.96± 0.32	0.80± 0.10	0.75± 0.23	0.56± 0.09	0.55± 0.13	0.97± 0.17	0.92± 0.19	3.91± 0.65	3.93± 0.77	4.00± 0.77	3.66± 0.48	0.80± 0.10	0.76± 0.18
72	48 2	1.96± 0.92	2.11± 0.52	0.83± 0.11	0.83± 0.20	0.55± 0.12	0.50± 0.23	1.02± 0.26	0.83± 0.21	3.76± 1.02	4.57± 0.33	3.02± 1.00	3.29± 0.36	0.83± 0.11	0.65± 0.28
96	72 2	1.92± 1.06	2.00± 0.73	0.78± 0.23	0.77± 0.21	0.58± 0.11	0.61± 0.21	0.90± 0.21	0.88± 0.17	4.00± 0.38	4.41± 0.25	4.01± 0.56	3.13± 0.71	0.78± 0.23	0.79± 0.15
72	24 3	2.05± 0.72	2.17± 0.36	0.77± 0.09	0.83± 0.21	0.60± 0.11	0.60± 0.20	0.94± 0.11	0.93± 0.32	3.76± 0.75	4.33± 0.35	4.08± 0.91	3.36± 0.51	0.77± 0.09	0.77± 0.13

Convulsion time:

The normal undrugged rats convulsed in 43.0±6.7 minutes after they were subjected to OHP. No significant change was noted when the rats were given 1 injection of 6-OHDA. After 2 and 3 equal concentration injections of 6-OHDA, however, the latency for convulsion successively decreased to 23.4±4.1 and 20.0+6.1 minutes respectively. The time for convulsions induced by OHP to develop 48 hours after a 2nd injection of 6-OHDA was not significantly different from the convulsion time observed 24 hours after two injections. However, 72 hours after a 2nd injection of 6-OHDA, the time for convulsions induced by OHP (33.0±3.7) recovered somewhat to its original latency of 43.0±6.7 minutes, although this was still significantly shorter than the normal time for convulsion in undrugged animals.

DISCUSSION

Systemically injected 6-OHDA produced significantly decreased concentrations of A, NA and TC in brain. These changes were reversible since brain catecholamines began to return to normal with passage of time after the injection of 6-OHDA. NA seems to be more seriously affected by increasing drug dose than A. A and NA account for only 25% of total brain catecholamines and 72 hours after a 2nd injection of 6-OHDA, A and NA are substantially recovered to control concentrations although the concentration of TC was still significantly depresssed. Seemingly other monoamine neurons are more sensitive to systemically injected 6-OHDA than A and NA neurones. Clark et al. (1971) reported that even a single injection of 6-OHDA accelerates the turnover of NA in brain. These data substantiate the observation of Clark et al. (1971) and several factors may account for them viz:-

1. There is a direct effect on brain from 6-OHDA in blood.
2. Blood borne factors, humoral or metabolic, increase or modify brain metabolism as a stress response.
3. There is a reflex, increased peripheral sympathetic response to falling blood pressure(Axelrod et al.1970).
4. There is a feedback inhibition of trans synaptic catecholamine synthesis in the cell body, peripherally and in the CNS.

Presently there is no evidence for (1) which is also contrary to the findings of Thoenen et al. (1970) that 6-OHDA does not cross the blood brain barrier and affects brain cells only if injected intracranially. Since this drug cause a decrease in blood pressure (Axelrod et al.,1970), factors (2) and (3) would presumably affect the adrenergic neurones. A significant decrease in brain COMT activity after serial injection of 6-OHDA suggests that a decrease in brain catecholamine concentration is not dependent on the enzyme. Perhaps a low catecholamine concentration decreases the activity of the degradative enzyme by a negative feedback mechanism.

When normal rats and rats treated with 6-OHDA were convulsed by OHP, no significant change was observed in brain NA (except 72 hours after a 2nd injection) and TC (except 72 hours after a 2nd injection). Haggendal (1967) observed a decreased concentration of NA in rat brain following convulsions induced by OHP. In the earlier experiments of this study a decrease in concentration of brain catecholamines was observed during the early phase of exposure to OHP but no significant change was noted either just before or after convulsions. In the present study, the concentration of catecholamines in brain was significantly lower than normal and convulsions were accelerated 24 hours after a 2nd injection of 6-OHDA. Within 48 hours after a second injection of 6-OHDA, the concentration of NA returned to normal and the time for convulsions remained abbreviated but was not as short as previously, 24 hours after the 1st injection. These observation suggest that the concentration of catecholamines in brain is not critical in determining the latency of convulsion. Faiman et al. (1971)

observed that increased concentration of catecholamines in brain is not a probable mechanism by which paragyline protects against oxygen toxicity since d-l DOPA also increases concentration of catecholamines in the brain but does not serve any protective purpose. However, the present study does seem to indicate that there may be some hierarchial, specific or additive effect in the character of the various monoamine neurones potentiating the convulsive state. It also suggests that there is a differential sensitivity of these neurones to whatever effect occurs in brain as a result of systemic injection of 6-OHDA.

The concentration of NA in blood decreased whereas concentration of A in blood increased significantly following 2 and 3 injections of 6-OHDA. These concentrations tended to return to normal 72 hours after a 2nd injection. Tranzer and Thoenen (1968) reported that 6-OHDA has the effect first of selectively destroying adrenergic nerve endings which is then followed by depletion of NA and reduction in

tyrosine hydroxylase activity (Muller et al.1969). The transient increase in concentration of A in blood observed in the present study, might be due to an activation of the adrenal gland in order to compensate a fading sympathetic discharge. Thoenen et al.(1970) and Levitt et al.(1965) also observed an increased catecholamine content of the adrenal gland concomitantly with an increased activity of tyrosine hydroxylase.

When rats in the present study were subjected to OHP, blood A and the concentration of TC in blood increased significantly in all groups whereas NA levels increased only in normal undrugged rats. Cross and Houlihan (1969) observed that oxygen toxicity in rats results in a pronounced sympathetic outflow and elevated A and NA. Tisala(1959) suggested that an atmosphere containing toxic concentrations of oxygen produces a stress reaction. Probably, it is due to this stress reaction, that blood A increases in response to OHP. As stated earlier, NA levels did not increase in rats treated with 6-OHDA and subjected to

OHP. This suggests that most of the NA produced in response to oxygen toxicity in normal rats comes from a sympathetic outflow. However, 6-OHDA also has some effect on the adrenal gland since the degree of increase in A in the blood of 6-OHDA treated animals in this study was significantly lower than in the normal undrugged animals. The mechanism for this relatively suppressed response of the adrenal gland to oxygen toxicity in rats treated with 6-OHDA is not known. Some recovery from 6-OHDA may be seen in the response to OHP 72 hours after injection (i.e there is an increased latency of convulsion).

In the present study, no significant change in the concentration of GABA was observed following one injection of 6-OHDA. The concentration of GABA however decreased after a 2nd and 3rd injection of this drug. 72 hours after a 2nd injection of 6-OHDA, the concentration of GABA in brain returned to normal. The concentration of this amino acid in brain decreased further after normal and drug treated rats were convulsed by exposure to OHP. Wood(1970) correlated

the susceptability to OHP seizures with the rate at which the brain content of GABA decreased. Rats with lower GABA in brain in this study also convulsed earlier in comparison with rats having higher GABA in brain (e.g. GABA in brain in normal rats was 1.52+0.11 $\mu mole.g^{-1}$ and convulsion time was 43.6 minutes whereas in rats treated with 2 injections of 6-OHDA, the concentration of GABA was 0.95+0.11 $\mu mole.g^{-1}$ and convulsion time was 23.4 minutes). A decreased concentration of GABA in brain following 6-OHDA treatment as observed in the present study suggests that 6-OHDA increases the state of excitability of the brain by decreasing the neurotransmitter inhibitor GABA. This then leads to early convulsion.

A significant reduction of GABA in brain as a direct or secondary effect of 6-OHDA may be the prime effector of convulsive activity. Convulsion times observed in the present study were inversely correlated with the GABA in brain. The fact that an initially low concentration of GABA in brain accompanied a shorter time to convulsion during OHP exposure is in contrast

to the findings of Faiman et al. (1971) who suggested that protection from oxygen convulsions occurs independently from changes in the concentration of GABA in brain. The correspondence in 6-OHDA treated animals, between low concentrationof GABA, depleted catecholamines and potentiation of convulsions, after exposure to OHP supports the finding of Schatz and Lal(1971) who found that MAO inhibitors increased GABA in brain and prevented convulsions.

There is a significant rise in concentration of ammonia and glutamine (with aspargine) and a decrease in glutamate in brain with repeated equal injections of 6-OHDA in normal rats. These changed concentrations return to normal as a function of time after 2 injections of the drug. Only brain glutamate remained significantly low for up to 72 hours after a 2nd injections of 6-OHDA. No significant change in other brain amino acids were observed in response to this drug.

The concentration of ammonia in blood increased only after a 3rd injection of 6-OHDA and glutamate and glutamine values in blood remained unchanged. The delayed increase in blood ammonia in comparison to brain ammonia might be due to (1) a poorer buffering mechanism in the brain than in blood (2) some unbuffered ammonia crossing the blood brain barrier. However, the exact source of ammonia in brain tissue is not yet known.

After animals were exposed to OHP, a further decrease in concentration of glutamate and an increase in concentration of ammonia and glutamine in blood and brain was observed. No further significant change in the concentration of ammonia in blood and brain was observed after convulsions developed in normal and 6-OHDA treated rats. This implies that a minimum concentration of ammonia has been reached before animals will convulse. Thus, the concentration of ammonia in blood and brain seems to be a limiting factor for inducing convulsions. It has been reported that an increase in ammonia concentration disturbs the

concentration of K^+ ions or glutamate in the glia and produces disturbed neuronal activity (Quastel,1974). A link between GABA, glutamate and ammonia concentration following convulsions induced by OHP in normal rats has also been indicated previously by Banister et al. (1976).

In the present study, the rats treated with 6-OHDA convulsed earlier than normal rats. The effect is also reversible since convulsion latency increase again as the time after injection increases. Based on the observations of this study, it appears that the ammonia and GABA contents of blood and brain are the more important factors determining the latency of convulsion. The concentration of catecholamines in brain does not seem to be as important in inducing convulsions since no change in convulsion latency may be observed 24 hours after one injection of 6-OHDA. Concomitantly the concentration of catecholamines was significantly low.

The similarity between the free radical producing activity of both 6-OHDA and MAO together with free radical activity normally associated with OHP itself would account for correspodence between brain catecholamines and GABA during oxygen toxicity (Malafors and Sachs (1968), stone et al. (1964), Thoenen et al.(1970) and Tunniclift et al.,(1973). Banister et al.(1976) also proposed that the integrity of GABA in brain may be linked to the metabolism of glutamate and ammonia, and to free radical activity through glutathione (a protective agent against free radicals) and the action of the gamma glutamyl cycle (Meister,1975).

CHAPTER-5

SECTION-III

EFFECT OF ADRENALECTOMY ON CONVULSIONS INDUCED BY OHP.

MATERIALS AMD METHODS

Twenty rats (200-250 g) were used in this study. They were divided into four groups of five rats in each. Each group was treated as follows:

GROUP-1: Adrenalectomized control: All these animals were adrenalectomized through an opening in the dorsolateral side just above the kidney. The adrenals were excised and the wound was closed with a sterlized suture. The wound was cleaned with alcohol to prevent infection. It was decided to perform the adrenalectomy one side at a time and let the animal recover for 24 hours before adrenalectomizing the other side. All animals of this group were killed 72 hours after total adrenalectomy. Estimation of blood A was selected as the criterion for establishing the efficacy of the adrenalectomy. Seventy two hours after adrenalectomy, the blood A level reduced to 0.006 ± 0.004 ng.ml^{-1} from a normal value of

0.19 ± 0.029 ng.ml^{-1}.

GROUP-2: Rats of this group were adrenalectomized as in group-1 and subjected to OHP 72 hours after adrenalectomy.

GROUP-3: Animals of this group were operated as in group-1 but the adrenals were not removed. These shamoperated rats were also killed 72 hours after operation.

GROUP-4: Animals of this group were shamoperated as in group 3 and subjected to OHP 72 hours after operation.

All these animals were exposed to OHP until convulsion. blood and brain tissue samples were collected and analyzed immediately or stored at -20C for subsequent batch analysis of catecholamines, COMT, ammonia and amino acids. All the chamber operations and conditions were the same in these experiments as have been described previously.

RESULTS

Brain NA (Table-10, Figure-19):

The concentrations of NA in brain in normal, shamoperated and adrenalectomized rats were 128.68±3.68, 133.42±12.99 and 130.82±15.88 $ng.g^{-1}$ respectively. No significant change in the concentrations were noted after these rats were subjected to convulsions induced by OHP.

Brain A (Table-10, Figure-19):

The concentrations of A in brain in normal, shamoperated and adrenalectomized rats were 2.22±0.4, 1.92±0.23 and 2.03±0.25 $ng.g^{-1}$ respectively. Values decreased significantly when these rats were convulsed.

Brain TC (Table-10, Figure-19):

The normal concentration of TC in brain was 491.18± 49.18 $ng.g^{-1}$. Values obtained after shamoperation and adrenalectomy were 427.88±72.26 and 438.1±44.9 $ng.g^{-1}$ respectively and were not significantly different from normal concentrations. No significant change was noted when these rats were convulsed by OHP.

Brain COMT (Table-10, Figure-19):

The activity of COMT in brain in normal, shamoperated and adrenalectomized rats were 3.22±0.28, 2.82±0.60 and 2.74±0.65 $nmole.g^{-1}.min^{-1}$ respectively. After convulsions induced by OHP, no significant change was noted in normal and shamoperated rats, but all concentrations decreased significantly in adrenalectomized rats.

TABLE X

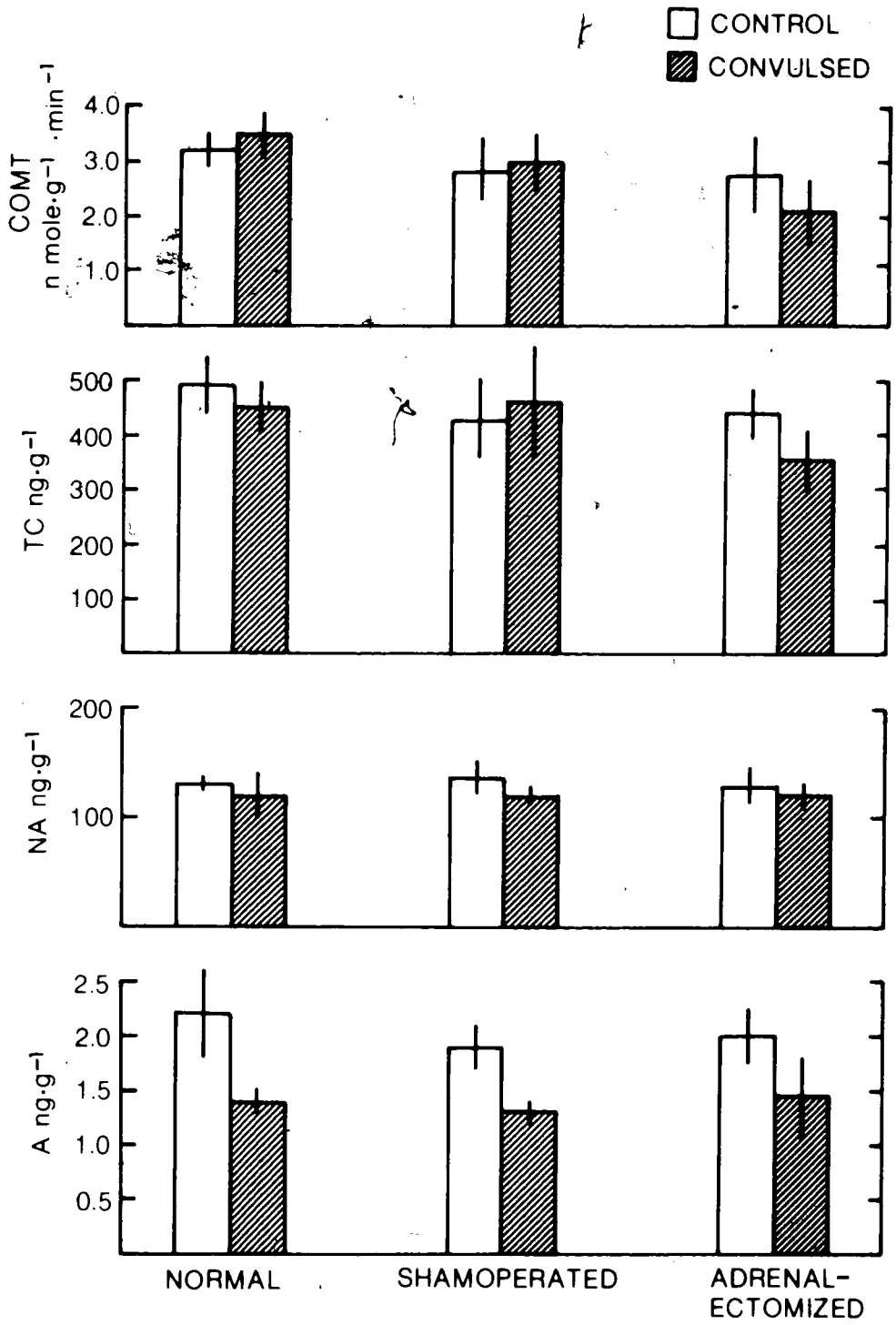
Brain catecholamines ($ng.g^{-1}$), COMT ($nmole.g.^{-1}min^{-1}$), blood catecholamines ($ng.ml^{-1}$) in normal, shamoperated and adrenalectomized rats before and after OHP induced convulsions.

	NORMAL		SHAMOPERATED		ADRENALECTOMIZED	
	Control	Convulsed	Control	Convulsed	Control	Convulsed
Brain Adrenaline	2.22±0.40	1.4±0.12*	1.92±0.23	1.13±0.10*x	2.03±0.25	1.5 ±0.4*x
Brain Noradrenaline	128.68±3.68	124.6±19.2	133.42±12.99	124.04±9.18	130.82±15.88	121.3±9.5
Brain Total Catecholamine	491.18±49.18	448.9±44.09	427.88±72.26	461.7±103.5	438.1±44.9	356.9±57.1
Brain COMT	3.22±0.28	3.48±0.62	2.82±0.60	3.00±0.52*	2.74±0.65	2.11±0.61*
Blood Adrenaline	0.19±0.03	2.62±0.82*	0.16±0.02	2.32±0.38*x	0.006±0.004*	0.012±0.005*
Blood Noradrenaline	0.33±0.06	2.03±0.53*	0.26±0.05	2.36±0.41*x	0.15±0.04*	1.69±0.46*x
Blood Total Catecholamine	0.57±0.04	5.75±0.46*	0.516±0.15	4.89±0.76*x	0.20±0.04*	2.51±0.81*x

* significant ($p < 0.05$) when compared with normal control.

x significant ($p < 0.05$) when compared with corresponding control.

FIGURE-19: Brain adrenaline (A), noradrenaline (NA), total catecholamine (TC) and catechol-O-methyl transferase (COMT) concentration in normal, shamoperated and adrenalectomized rats before and after OHP induced convulsions. (Mean ± SD, n=5)


CONTROL
CONVULSED
COMT
n mole·g⁻¹ ·min⁻¹
4.0
3.0
2.0
1.0
TC ng·g⁻¹
500
400
300
200
100
NA ng·g⁻¹
200
100
A ng·g⁻¹
2.5
2.0
1.5
1.0
0.5
NORMAL
SHAMOPERATED
ADRENAL-
ECTOMIZED

Brain GABA (Table-11, Figure-20):

The normal concentration of GABA in rat brain was $1.52\pm0.12\ \mu mole.g^{-1}$. No significant change in the concentrations of GABA were noted when the normal rats were shamoperated and adrenalectomized After convulsions induced by OHP, the brain GABA concentration decreased significantly to 0.78 ± 0.07, 0.66 ± 0.05 and $0.65\pm 0.12\ \mu mole.g^{-1}$ respectively in normal, shamoperated and adrenalectomized rats.

Brain ammonia (Table-11, Figure-20):

The normal concentration of ammonia in brain was $5.25\pm0.72\ \mu g.g^{-1}$. No significant change was noted when rats were subjected to shamoperation but the concentration of ammonia in brain decreased significantly to $3.67\pm0.37\ \mu g.g^{-1}$ after adrenalectomy. When normal, shamoperated and adrenalectomized rats were convulsed by OHP, the concentration of ammonia in brain increased significantly to 17.8 ± 0.90, 17.1 ± 2.5

and 16.98 ± 2.71 $\mu g.g^{-1}$ respectively. However, the post convulsive brain ammonia concentrations were not significantly different from each other.

Brain glutamate (Table-11, Figure-20):

The normal concentration of glutamate in brain was 9.40 ± 0.67 $\mu mole.g^{-1}$. No significant change in the brain glutamate concentrations was noted when rats were shamoperated but the concentration decreased significantly when rats were adrenalectomized. Brain glutamate levels decreased significantly to 5.18 ± 0.48, 4.1 ± 0.9 and 4.4 ± 0.6 $\mu mole.g^{-1}$ when the normal, shamoperated and adrenalectomized rats were convulsed by OHP.

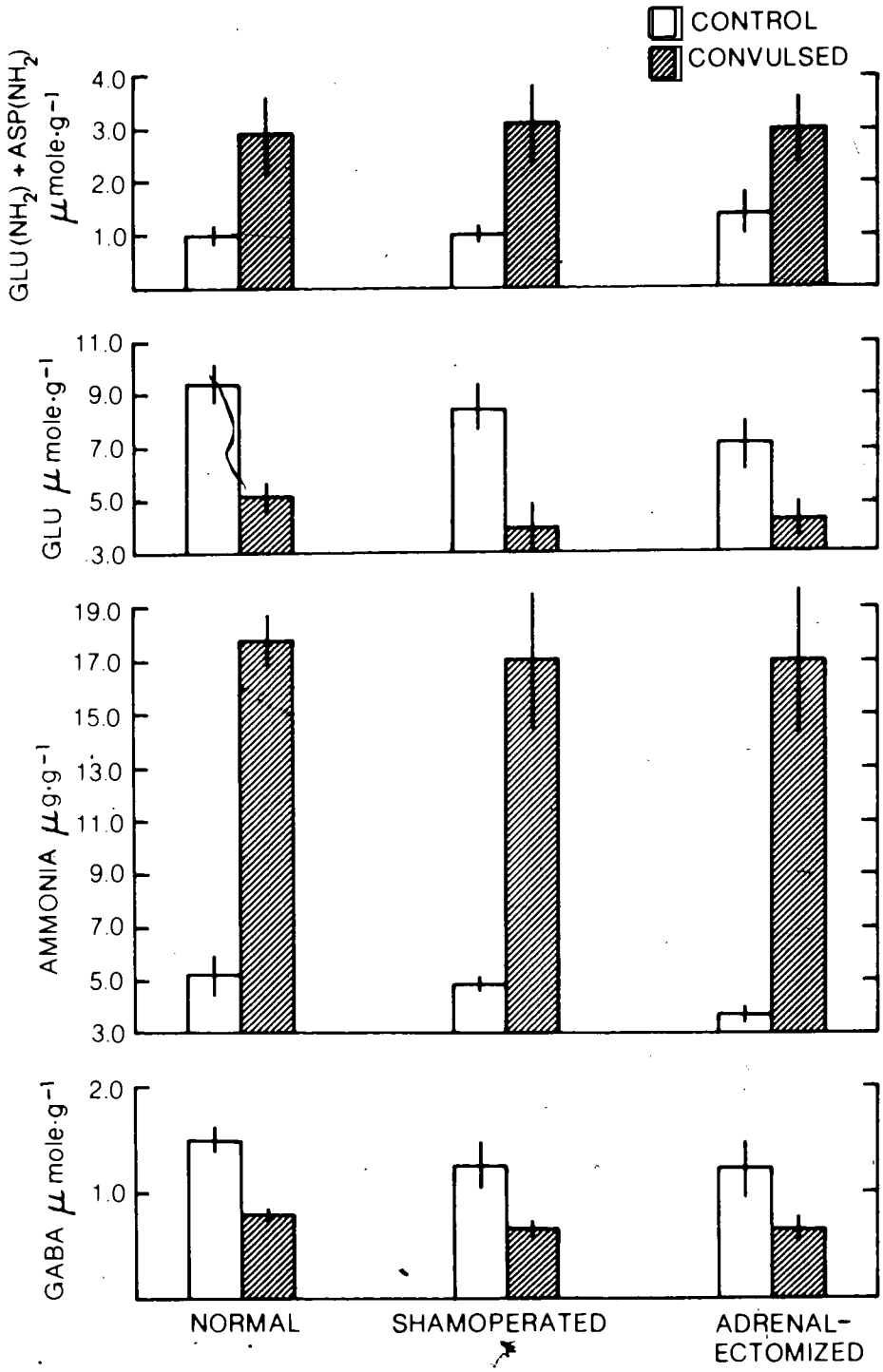
TABLE XI

Brain ammonia ($\mu g.g^{-1}$) and amino acids ($\mu mole.g^{-1}$) in normal, shamoperated and adrenalectonized rats before and after OHP induced convulsions.

	NORMAL		SHAMOPERATED		ADRENALECTOMIZED	
	Control	Convulsed	Control	Convulsed	Control	Convulsed
GABA	1.52±0.12	0.78±0.07*	1.26±0.19	0.66±0.05*x	1.20±0.26	0.65±0.12*x
Ammonia	5.25±0.72	17.8±0.9*	4.81±0.32	17.1±2.5*x	3.6±0.4*	16.98±2.71*x
Glu.	9.4±0.7	5.18±0.48*	8.73±0.83	4.1±0.9*x	7.2±0.81*	4.4±0.6*x
Glu. (NH_2) Asp. (NH_2)	0.97±0.16	2.88±0.73*	0.99±0.08	3.13±0.68*x	1.38±0.39	3.02±0.63*x
Asp.	3.47±0.77	3.90±0.69	3.61±0.76	3.71±0.49	3.53±0.47	3.52±0.93
Ala.	0.61±0.11	0.60±0.11	0.57±0.08	0.59±0.09	0.54±0.08	0.54±0.05
Arg.	0.11±0.03	0.10±0.02	0.12±0.02	0.13±0.03	0.126±0.02	0.12 ±0.02
Tyr.	0.37±0.10	0.56±0.21*	0.31±0.04	0.59±0.03*x	0.31±0.07	0.33±0.05
Thr.	1.03±0.21	1.02±0.20	1.13±0.11	1.02±0.08	1.04±0.11	1.04±0.08
Gly.	1.48±0.46	1.34±0.28	1.24±0.36	1.29±0.33	1.52±0.09	1.40±0.26
Ser.	0.95±0.13	0.99±0.11	0.90±0.14	0.92±0.18	0.92±0.15	0.94±0.13
Lys.	0.19±0.02	1.14 ±0.02	0.13±0.01	0.12 ±0.01	0.12 ±0.01	0.13 ±0.06

* significant ($p < 0.05$) when compared with normal control.
x significant ($p < 0.05$) when compared with corresponding control.

FIGURE-20: Brain GABA, ammonia, glu and glu$(NH)_2$+asp$(NH)_2$ concentration in normal, shamoperated and adrenalectomized rats before and after OHP induced convulsions. (Mean ± SD, n=5)


CONTROL
CONVULSED
GLU(NH2) + ASP(NH2) μmole·g-1
4.0
3.0
2.0
1.0
GLU μmole·g-1
11.0
9.0
7.0
5.0
3.0
AMMONIA μg·g-1
19.0
17.0
15.0
13.0
11.0
9.0
7.0
5.0
3.0
GABA μmole·g-1
2.0
1.0
NORMAL
SHAMOPERATED
ADRENAL-
ECTOMIZED

Brain glutamine+aspargine

(Glu.NH_2+Asp.NH_2)(Table-11, Figure-20):

The normal control combined concentrations for these amino acids were 0.97±0.16 μmole.g^{-1}. No significant change was noted when the normal rats were shamoperated or adrenalectomized. After convulsions induced by OHP in normal, shamoperated and adrenalectomized rats, the combined brain levels of these amino acids increased significantly to 2.88±0.73, 3.13±0.68 and 3.02±0.62 μmole.g^{-1} respectively.

Other brain amino acids (Table-11):

No significant change in concentrations of other amino acids in brain were observed before and after convulsions in normal, shamoperated and adrenalectomized rats.

Blood A (Table-10, Figure-21):

The normal concentration of A in blood was 0.19±0.03 $ng.ml^{-1}$. No significant difference was noted when the normal rats were shamoperated. In adrenalectomized rats, the blood concentration of A decreased significantly to 0.006± 0.004 $ng.ml^{-1}$. Blood A concentration increased significantly when normal and shamoperated rats were convulsed. However, when adrenalectomized rats convulsed, no significant change in concentration of A in blood was noted.

Blood NA (Table-10, Figure-21):

The concentration of NA in blood was 0.33±0.06 and 0.26±0.05 $ng.ml^{-1}$ in normal and shamoperated rats respectively. In adrenalectomized rats, the NA concentration decreased significantly to 0.15±0.04 $ng.ml^{-1}$. After convulsions induced by OHP, the concentration of NA in blood was less elevated after OHP induced convulsions in adrenalectomized rats than

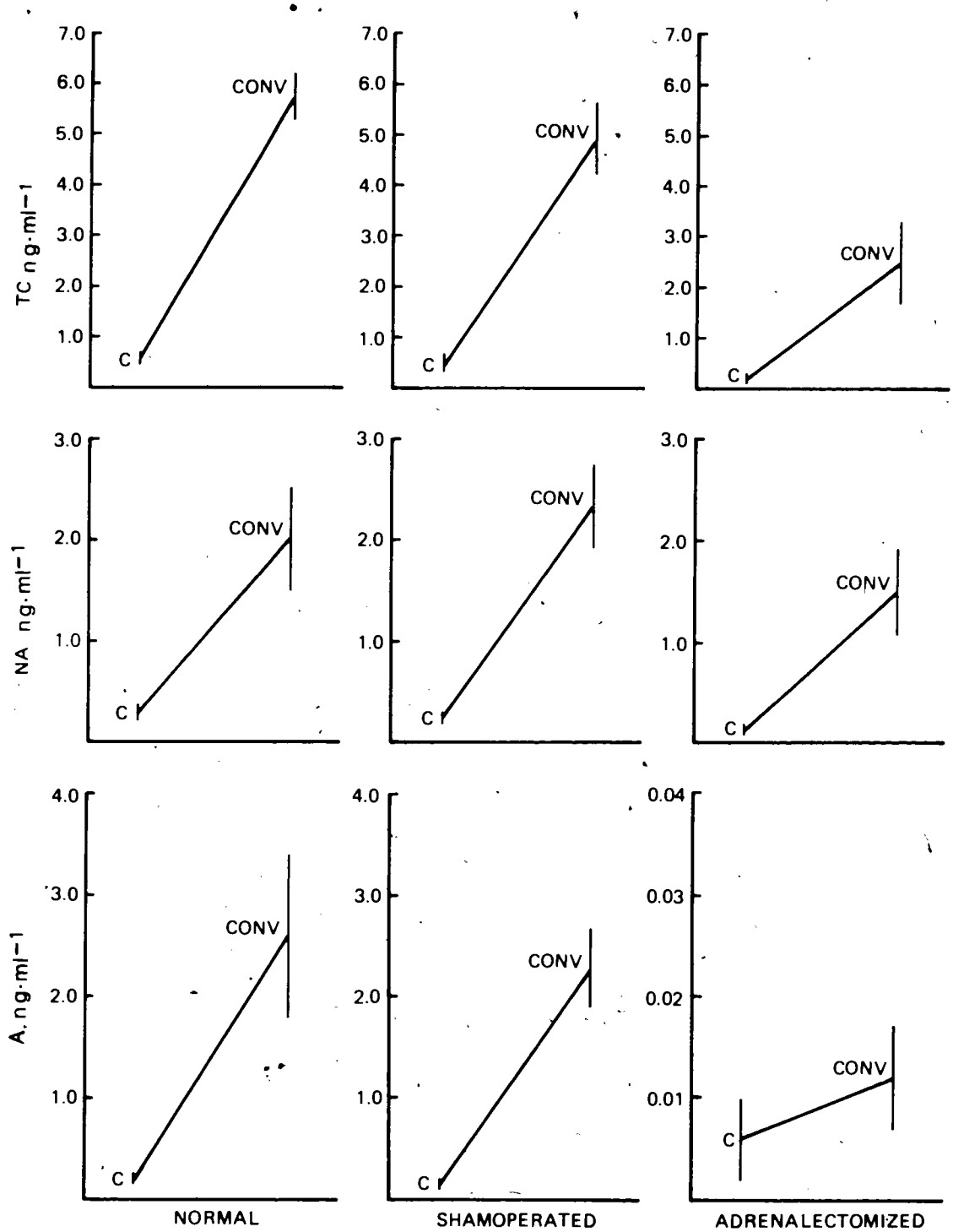
in normal or shamoperated animals. In all three convulsed groups, however, the concentration was significantly higher than in non convulsed animals. NA levels increased significantly in all three groups but adrenalectomized rats showed a significantly lower increase in comparison to normal and shamoperated rats.

Blood TC (Table-10, Figure-21):

The normal concentration of TC in blood was 0.57±0.04 ng.ml and did not change significantly when rats were shamoperated. The concentration decreased significantly to 0.20±0.04 $ng.ml^{-1}$ when the rats were adrenalectomized. When these normal, shamoperated and adrenalectomized rats were convulsed by OHP, the blood total concentration of catecholamine increased significantly to 5.75±0.46, 4.89±0.76 and 2.51±0.81 $ng.ml^{-1}$ respectively.

FIGURE-21: Blood adrenaline (A), noradrenaline (NA) and total catecholamine (TC) concentration in normal, shamoperated and adrenalectomized rats respectively, before and after convulsions. (Mean ± SD, n=5)

C = CONTROL
CONV = CONVULSED



Blood ammonia (Table-12, Figure-22):

The control concentration of ammonia in blood was 2.04±0.22 2.62±0.68 and 1.45±0.318 $\mu g.ml^{-1}$ respectively in non convulsed normal, shamoperated and adrenalectomized rats. These concentrations increased significantly when these rats were convulsed by OHP . However, the post convulsive blood ammonia concentrations were not significantly different in normal, shamoperated and adrenalectomized rats.

Blood glutamate (Table-12, Figure-22):

The normal concentration of glutamate in blood was 3.26±0.33 $\mu mole.100ml^{-1}$ which did not change significantly when rats were shamoperated. However, the blood glutamate levels decreased significantly when the rats were adrenalectomized, the value was 2.41±0.15 $\mu mole.100ml^{-1}$. After convulsions induced by OHP, the concentration of glutamate in blood decreased significantly to 2.25±0.42, 2.19±0.37 and 1.43±0.22

μmole.100ml^{-1} in normal, shamoperated and adrenalectomized rats respectuvely. Post convulsive glutamate concentration in adrenalectomized rats was significantly lower than postconvulsive concentrations in normal and shamoperated rats.

Blood glutamine+aspargine (glu.NH_2+asp.NH_2) (Table-12, Figure-22):

The normal combined concentration of these amino acids in blood was 14.20$\pm$2.70 μmole.100ml^{-1} and was not significantly different from the concentrations observed in shamoperated and adrenalectomized rats. After convulsions induced by OHP, the glu(NH_2)+asp(NH_2) concentrations increased significantly from control concentration to 22.23$\pm$2.64, 20.26$\pm$0.97 and 22.09$\pm$1.38 μmole.100ml^{-1} in normal, shamoperated and adrenalectomized rats respectively but were not significantly different from each other.

Blood alanine (Table-12):

The normal concentration of alanine in blood was 10.29 ± 1.44 μmole.100ml^{-1} and this increased slightly to 12.07+0.08 umole.100ml after convulsions induced by OHP. Concentrations of alanine in blood in shamoperated and adrenalectomized rats were 10.4 ± 0.7 and 8.86 ± 0.89 μmole.100ml^{-1} respectively. A significant increase was noted when these rats were convulsed.

Blood tyrosine (Table-12):

The control concentration of tyrosine in blood in normal, shamoperated and adrenalectomized rats were 0.57 ± 0.13, 0.70 ± 0.05 and 0.62 ± 0.07 μmole.100ml^{-1} respectively. A significantly low blood tyrosine value was observed when normal rats were convulsed. However, when shamoperated and adrenalectomized rats were convulsed by exposure to OHP, the concentration of tyrosine in blood remained unchanged.

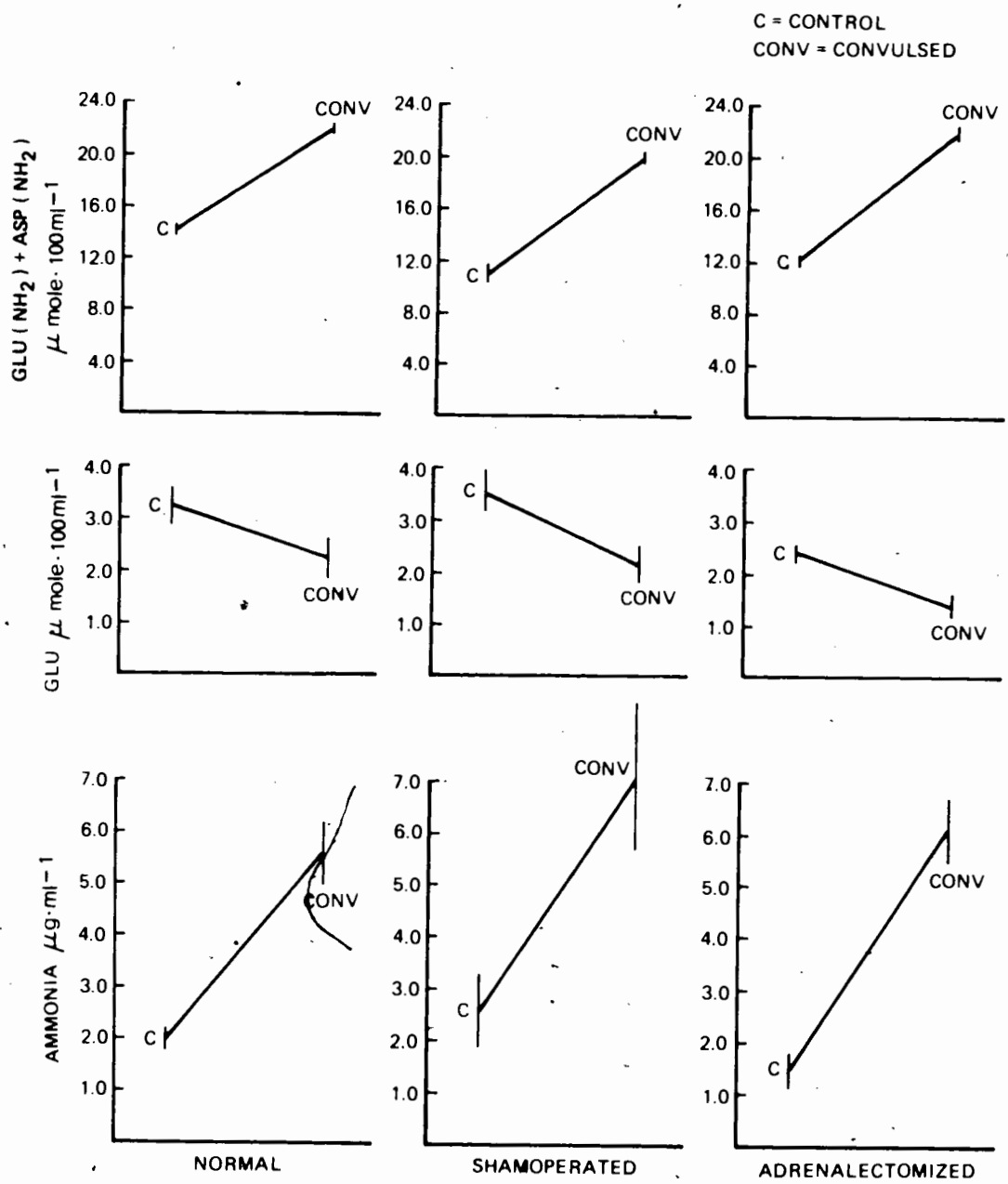
TABLE XII

Blood ammonia ($\mu g.ml^{-1}$) and amino acids ($\mu mole.100\ ml.^{-1}$) in normal, shamoperated and adrenalectomized rats before and after convulsions.

	NORMAL		SHAMOPERATED		ADRENALECTOMIZED	
	Control	Convulsed	Control	Convulsed	Control	Convulsed
Ammonia	2.04±0.22	5.58±0.62*	2.62±0.68	7.12±1.41*x	1.45±0.32*	6.17±0.66*x
Glu.	3.26±0.33	2.25±0.42*	3.54±0.41	2.19±0.37*x	2.41±0.15*	1.43±0.22*x
Glu & Asp (NH_2)	14.2±2.7	22.23±2.64*	11.40±5.38	20.26±0.97*x	12.31±0.90	22.09±1.38*x
Asp.	1.55±0.36	1.84±0.31	1.51±0.17	1.57±0.11	1.10±0.40	1.16±0.14
Ala.	10.29±1.44	12.07±0.08*	10.4±0.7	14.3±0.9*x	8.86±0.89	16.53±0.64*x
Tyr.	0.57±0.13	0.36±0.04*	0.62±0.07	0.57±0.06	0.70±0.05	0.64±0.09
Pro.	2.06±1.07	2.19±0.44	1.83±0.97	2.10±0.36	2.21±0.35	2.00±0.92
Thr.	0.81±0.10	0.79±0.10	0.76±0.21	0.88±0.30	0.73±0.25	0.85±0.36
Phe.	0.59±0.08	0.58±0.11	0.59±0.04	0.56±0.05	[illegible]3±0.07	0.50±0.05
Ser.	0.96±0.23	0.93±0.09	0.90±0.13	0.83±0.32	0.99±0.35	0.91±0.15
Leu.	4.01±0.62	3.7±0.9	3.79±0.72	3.84±0.82	2.33±0.50*	2.4±0.5*
Ileu.	3.69±0.68	4.39±0.56	4.39±0.38	4.32±0.74	3.02±0.26*	3.33±0.46*
Val.	0.72±0.21	0.61±0.19	0.74±0.13	0.77±0.09	0.69±0.08	0.81±0.08
Lys.	6.93±1.15	7.38±0.54	7.69±0.70	6.06±0.67	4.9±0.7*	5.1±0.4
Met.	0.47±0.21	0.50±0.12	0.41±0.14	0.58±0.19	0.32±0.21	0.41±0.17
His	2.13±0.31	2.11±0.36	2.09±0.25	2.09±0.04	1.24±0.19*	1.52±0.29*
Arg.	1.33±0.53	1.39±0.30	1.42±0.32	1.37±0.43	1.38±0.29	1.86±0.21
Gly.	3.19±0.93	3.43±0.59	3.71±0.61	3.17±0.51	3.28±0.73	3.20±0.82

* significant ($p < 0.05$) when compared with normal control.
x significant ($p < 0.05$) when compared with corresponding control.

FIGURE-22: Blood ammonia, glu and glu$(NH)_2$+asp$(NH)_2$ in normal, shamoperated and adrenalectomized rats before and after OHP induced convulsions. (Mean ± SD, n=5)


C = CONTROL
CONV = CONVULSED
GLU (NH_2) + ASP (NH_2)
μ mole · 100ml^{-1}
GLU μ mole · 100ml^{-1}
AMMONIA μg · ml^{-1}
C
CONV
NORMAL
SHAMOPERATED
ADRENALECTOMIZED

Other blood aminoacids (Table-12):

No significant change in the other amino acids was noted in this study except in leu, isoleucine and histidine which decreased significantly in adrenalectomized rats compared to control and shamoperated animals.

Time for convulsions:

The normal rats exposed to OHP convulsed after a mean time of 43.0+6.67 minutes. Shamoperated and adrenalectioized rats convulsed 76.4±3.0 and 102.8±5.0 minutes respectively after exposure to OHP.

DISCUSSION

In the present study no significant change was observed in the concentration of catecholamine in brain in normal, shamoperated and adrenalectomized rats. On the other hand, the concentration of catecholamine in blood was significantly low in adrenalectomized rats. The mean concentration of NA in blood in normal rats was 0.33 $ng.ml^{-1}$ and in adrenalectomized rats the concentration of NA in blood was 0.15 $ng.ml^{-1}$. These observations suggest that the blood NA concentration observed in adrenalectomized rats was produced from the sympathetic nerves. The brain COMT concentration also did not change significantly in normal, shamoperated and adrenalectomized rats.

After convulsions induced by OHP, brain NA and TC concentration did not change significantly whereas concentrations of A in brain decreased in all three groups. The activity of COMT in brain decreased only

in adrenalectomized rats. In adrenalectomized rats, the blood NA and TC concentration increased significantly but the concentration of A in blood remained unchanged after convulsions induced by OHP.

The concentration of GABA in brain did not change whereas concentrations of ammonia and glutamate decreased significantly when normal rats were adrenalectomized. The decrease in brain ammonia seems not to be due to its buffering via the glutamate/glutamine system since no significant change in the concentration of glutamine in brain was observed. Probably the low value is due to a diminished production of ammonia. No significant change in other brain amino acids was noted following adrenalectomy. In blood, significantly decreased concentrations of ammonia, glutamate, leucine, isoleucine and histidine were observed in adrenalectomized rats.

When normal, adrenalectomized and shamoperated rats were convulsed by OHP, the concentration of

ammonia and glutamine in blood and brain increased prior to convulsive behavior whereas the concentration of glutamate decreased significantly. The concentration of GABA in brain decreased significantly in all three convulsed groups in the pre convulsive period. However, after convulsion ammonia and amino acid concentrations in all three groups did not vary significantly. This supports earlier observations that when a critical concentration of ammonia is attained, the toxic action of oxygen is potentiated.

In the present study, it was also observed that adrenalectomized rats convulsed much later than shamoperated and normal rats exposed to OHP. The shamoperated rats attained some protection against oxygen toxicity since they convulsed much later than the normal rats but earlier than the adrenalectomized rats. Some explanation of this phenomenon may be found in the report that an increased concentration of catecholamine antagonists(i.e. insulin) are present in the blood of animals 48 hours after traumatic injury (Singh,1974). Leon et al.(1971) have also reported

significantly low plasma insulin levels in response to OHP exposure. These observations suggest that a depressed insulin value might potentiate the toxic effects of OHP. It appears possible therefore that delayed convulsive behaviour in shamoperated rats exposed to OHP might also be due to an elevated concentration of insulin and increased anabolic activity due to post operative wound healing reactions (Singh,1974).

Bean and Johnson (1954) and Gerschman et al.(1954) have found that adrenalectomy affords protection against oxygen toxicity. They proposed that exposure to OHP normally causes adrenocortical hypertrophy and an increased secretion of adrenocortical hormones which augment the toxic effects of OHP exposure. Earlier in this study, it has been observed that exposure of normal rats to OHP for graded time intervals results in elevated blood A and NA concentrations even before the onset of convulsions. These elevated concentrations of catecholamines thus might induce the toxic oxygen effects. In the present study it has been observed

that following adrenalectomy, the concentration of blood A was almost absent and NA concentration was reduced to half. Hale et al.(1973) proposed that oxygen tolerance in a given individual depends on their neuroendocrine state. Excess of catecholamine or 17-hydroxy corticoids potentiates or facilitate the adverse effects of oxygen. Observations of this study suggest that adrenalectomy protects rats against oxygen toxicity as a consequence of low concentration of catecholamines in blood. However, the present study also suggests that the catecholamines are not the only factor contributing protection against oxygen toxicity in adrenalectomized rats, since these adrenalactomized rats also had low concentration of ammonia in blood and brains. As stated earlier, a minimal threshold concentration of ammonia in brain and blood seems required to trigger the convulsions induced by OHP. Since, adrenalectomized rats have low basal concentration of ammonia in blood and brain, it follows that it might take a relatively longer time for ammonia to reach a hypothetical, convulsive, threshold concentrations in such animals. Hence adrenalectomized

rats might be expected to survive longer.

The observation of the present study suggests that the concentration of catecholamines in the brain does not play a significant role in protecting adrenalectomized rats against oxygen toxicity. The important reasons supporting this observation are: (I)that brain catecholamines and COMT do not differ significantly in normal, shamoperated and adrenalectomized rats. (2)that post convulsive concentration of catecholamines in brain do not differ significantly in any of the three groups of normal, shamoperated and adrenalectomized rats, whereas the latency for convulsive action was significantly different from control animals in the latter two groups.

In the earlier investigation of Bean (1954) and Taylor (1958), the onset of convulsive seizures and mortality was used as a criterion of the involvement of CNS in oxygen toxicity. Sometimes however, it is difficult to determine the precise onset of

convulsions. Similarly, with regard to mortality from OHP exposures, there are several factors other than dysfunction of the CNS such as lung damage and electrolyte imbalance which may contribute to observed mortality (Torbati et al.,1971). In order to avoid these confusing aspects of the problem, Torbati et al.(1971) proposed changes in brain electrical activity as an indicator of oxygen toxicity in the CNS. They observed that adrenalectomy does not protect rats against oxygen toxicity according to this criterion. In the present study, however, observations on the delayed onset of convulsion in adrenalectomized animals are supported by positive biochemical data and both suggest that adrenalectomy does have a protecting effect against OHP.

CHAPTER-6

SECTION-IV

EFFECT OF HEXAMETHONIUM AND α-METHYL-p-TYROSINE ON NORMAL RATS SUBJECTED TO CONVULSIONS INDUCED BY OHP.

MATERIALS AND METHODS

Hexamethonium treatment:

Gollnick and Ianuzro(1975) used this drug in rat exercise experiments intending to eliminate catecholamines usually produced from both the adrenal glands and the sympathetic nervous system. They observed that an injection of 20mg.kg^{-1} (intravenously) of hexamethonium produced general ganglionic blockade and decreased the blood pressure to 50-60mm.Hg. The drug was used in the present study with this intent in order to study the effects of ganglionic blockade on oxygen toxicity produced in rats by their exposure to OHP.

Ten rats(200-250 g) were included in the study, divided into 2 groups:

GROUP-1: 5 rats received 20mg.kg^{-1} of hexamethonium(i.v.).

All animals were sacrificed 25 minutes after injection.

GROUP-2: These rats were treated with hexamethonium(as in 1) and were exposed to OHP until convulsion 25 minutes after injection. They were decompressed and sacrificed under light ether anesthesia.

Methyl-p-tyrosine treatment:

Ten rats were used in this experiment and divided into 2 groups:

GROUP-1: Rats of this group were treated with 200 $mg.kg^{-1}$ α-MPT (i.p. in saline pH 9.0) as described by Faiman et al. (1971). Animals were killed 8 hours later.

GROUP-2: Rats of this group were treated with this drug as in group-1 and exposed to OHP 8 hours later until convulsions. They were decompressed and sacrificed under light ether anesthesia.

Blood and brain tissue samples were collected from both control and convulsed rats and stored at -20C for subsequent analysis as described later in the appendix.

RESULTS

Brain NA (Table-13, Figure-23):

The normal concentration of NA in brain was 128.86 ± 3.68 ng.g^{-1} which did not change significantly when normal rats were treated with hexamethonium. When normal rats were treated with MPT, the brain NA decreased significantly to 78.75 ± 11.38 ng.g^{-1}.

No significant change in the concentration of NA in brain was noted when normal and hexamethonium treated rats were convulsed by OHP. However, in MPT treated rats, brain catecholamines decreased in concentration significantly to 54.16 ± 14.45 ng.g^{-1} after convulsion.

Brain A (Table-13, Figure-23):

The normal brain A concentration was 2.22±0.4 $ng.g^{-1}$ which decreased to 1.53±0.37 and 1.28 ± 0.45 $ng.g^{-1}$ when normal rats were treated with hexamethonium and MPT respectively.

After convulsions induced by OHP, mean concentration of brain A was 1.35±0.17, 1.47±0.46 and 1.21±0.16 $ng.g^{-1}$ in normal, hexamethonium and MPT treated rats respectively.

Brain TC (Table-13, figure-23):

The normal mean concentration of TC in brain was 491.18+49.18 $ng.g^{-1}$. No significant change was noted when the normal rats were treated with hexamethonium whereas the concentration of TC in brain decreased significantly to 193.63±14.88 $ng.g^{-1}$ after the rats received MPT.

When normal, hexamethonium and MPT treated rats were exposed to OHP and convulsed, the mean concentrations of TC in brain were 448.97±44.1, 371.69±74.8 and 97.63±13.95 $ng.g^{-1}$ respectively.

Brain COMT (Table-13, Figure-23):

The activity of COMT in brain in normal, hexamethonium and MPT treated rats was 3.22±0.28, 3.04±0.40 and 2.58±0.35 $nmole.g^{-1}.min^{-1}$ respectively. No significant change in these activities were noted when normal and hexamethonium treated rats were convulsed by OHP exposure. However, when MPT treated rats were similarly treated, COMT activities decreased significantly to 1.90±0.25 $nmole.g^{-1}.min^{-1}$.

TABLE XIII

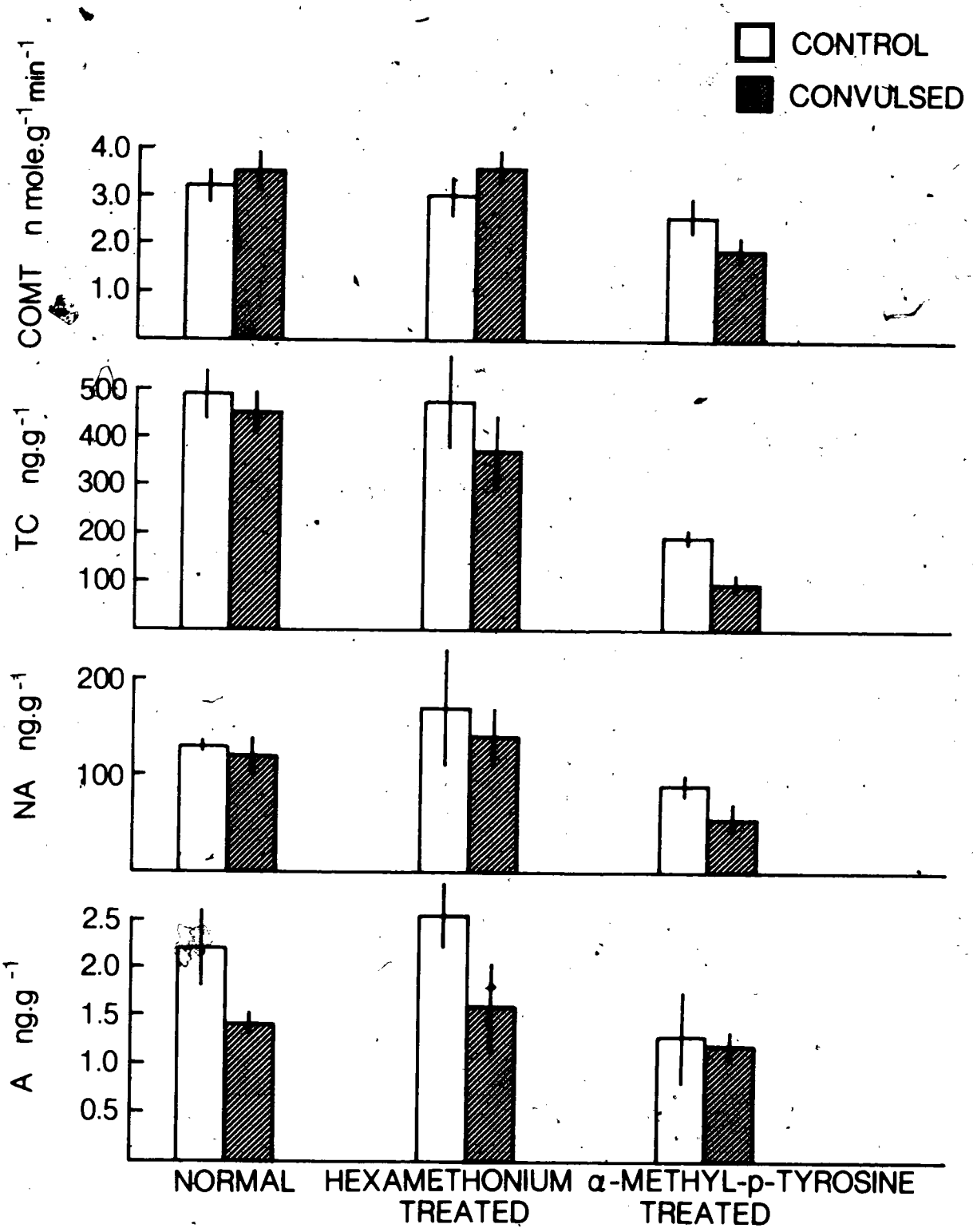
Brain catecholamines ($ng.g^{-1}$), COMT ($nmole.g^{-1}min.^{-1}$), blood catecholamines $ng.ml^{-1}$ in normal, hexamethonium and α-methyl-p-tyrosine treated rats before and after OHP induced convulsions.

	NORMAL		HEXAMETHONIUM		α-METHYL-p-TYROSINE	
	Control	Convulsed	Control	Convulsed	Control	Convulsed
Brain Adrenaline	2.22±0.40	1.53±0.17*	2.54±0.37	1.46±0.46*x	1.28±0.45*	1.21±0.16*
Brain Noradrenaline	128.86±3.68	123.0±17.9	172.6±60.2	141.4±31.9	78.75±11.38*	54.16±14.45*x
Brain Total Catecholamine	491.18±49.18	448.97±44.1	477.91±94.61	371.7±74.8	193.63±14.88*	97.63±13.95*x
Brain COMT	3.22±0.28	3.48±0.42	3.04±0.4	3.58±0.37	2.58±0.35*	1.9±0.3*x
Blood Adrenaline	0.19±0.03	2.62±0.81*	0.018±0.003*	0.05±0.02*x	0.12±0.03*	1.79±0.19*x
Blood Noradrenaline	0.33±0.06	2.03±0.53*	0.13±0.04*	0.49±0.16x	0.29±0.03	1.64±0.08*x
Blood Total Catecholamine	0.57±0.04	5.74±0.46*	0.28±0.04*	0.53±0.18x	0.47±0.12	2.48±0.31*x

* significant (p 0.05) when compared with normal control.

x significant (p 0.05) when compared with corresponding control.

FIGURE-23: Adrenaline(A), noradrenaline (NA), total catecholamine and COMT concentrations in normal, hexamethonium and MPT treated rats before and after convulsions. (Mean ± SD, n=5)


CONTROL
CONVULSED
COMT n mole.g⁻¹min⁻¹
4.0
3.0
2.0
1.0
TC ng.g⁻¹
500
400
300
200
100
NA ng.g⁻¹
200
100
A ng.g⁻¹
2.5
2.0
1.5
1.0
0.5
NORMAL
HEXAMETHONIUM TREATED
α-METHYL-p-TYROSINE TREATED

Brain GABA (Table-14, figure-24):

The normal mean concentration of GABA in brain was $1.52\pm0.11\ \mu mole.g^{-1}$ and this concentration did not change significantly when normal rats were treated with hexamethonium and MPT.

When normal, hexamethonium and MPT treated rats were convulsed by OHP exposures, concentrations of GABA in brain decreased significantly to 0.78 ± 0.07, 0.75 ± 0.07 and $0.77\pm0.10\ \mu mole.g^{-1}$ respectively.

Brain ammonia (Table-14, Figure-24):

The mean control concentrations of ammonia in the brain of normal, hexamethonium and MPT treated rats prior to oxygen exposure were 5.25 ± 0.72, 5.5 ± 1.13 and $4.74\pm0.73\ \mu g.g^{-1}$ respectively. Brain ammonia concentration increased significantly when these rats were convulsed. Post convulsive concentrations

observed in normal, hexamethonium and MPT treated rats were 17.87±0.89, 18.56±1.11 and 17.35±2.39 $\mu g.g^{-1}$ respectively.

Brain glutamate (Table-14, Figure-24):

The normal mean concentration of glutamate in brain was 9.38±0.67 $\mu mole.g^{-1}$. When the normal rats were treated with hexamethonium and MPT, concentration of glutamate in brain remained unchanged.

When normal, hexamethonium and MPT treated rats were exposed to OHP, the concentration of glutamate in brains decreased significantly to 5.18±0.48, 4.08±0.98 and 4.30±2.58 $\mu mole.g^{-1}$ respectively.

Brain glutamine+aspargine ($glu.NH_2$+$asp.NH_2$) (Table-14, figure-24):

The mean control concentration of combined glutamine and aspargine in normal brain was 0.97±0.16 $\mu mole.g^{-1}$.

The combined concentration of these amino acids did not change significantly when normal rats were treated with hexamethonium but decreased significantly when the rats were treated with MPT.

After convulsion in normal, hexamethonium and MPT treated rats, the brain glu.NH_2+asp.NH_2 concentrations increased significantly to 2.88±0.73, 2.72±0.73 and 2.10±0.69 $\mu mole.g^{-1}$ respectively.

Brain tyrosine (Table-14):

The normal concentration of tyrosine in brain was 0.31±0.10 $\mu mole.g^{-1}$ which increased significantly to 0.56±0.21 $\mu mole.g^{-1}$ after animals convulsed. The value of tyrosine in brain in hexamethonium treated rats was 0.33±0.11 $\mu mole.g^{-1}$ which did not change significantly when the rats were convulsed. However, mean control concentration of tyrosine in brain increased significantly when rats were treated with MPT and did not change further when these rats were convulsed.

TABLE XIV

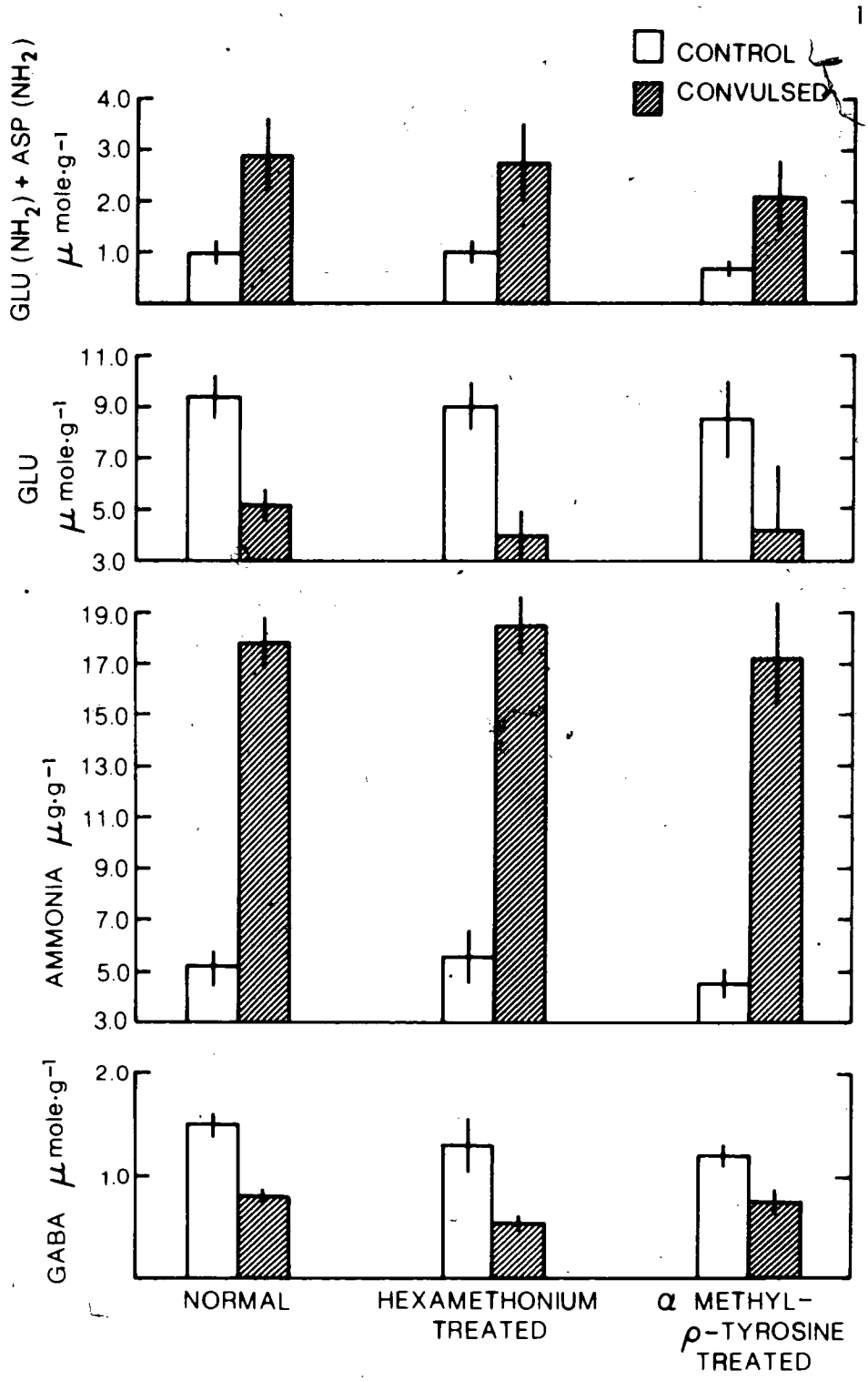
Brain ammonia ($\mu g.g^{-1}$) and amino acids ($\mu mole.g^{-1}$) in normal, Hexamethonium and α-methyl-p-tyrosine treated rats before and after OHP induced convulsions.

	NORMAL		HEXAMETHONIUM		α-METHYL-p-TYROSINE	
	Control	Convulsed	Control	Convulsed	Control	Convulsed
GABA	1.52±0.11	0.78±0.07*	1.33±0.26	0.75±0.07*x	1.20±0.10	0.77±0.10*x
Ammonia	5.25±0.72	17.87±0.89*	5.5±1.1	18.56±1.11*x	4.74±0.73	17.35±2.39*x
Glu.	9.38±0.67	5.18±0.48*	9.04±0.89	4.08±0.98*x	8.48±1.77	4.3±2.50*x
Glu & Asp (NH_2)	0.97±0.16	2.88±0.73*	0.99±0.19	2.72±0.73*x	0.66±0.11	2.1±0.70*x
Asp.	3.7±0.68	4.39±0.57	3.93±0.86	4.4±0.5	4.22±1.62	3.99±0.72
Ala.	0.61±0.12	0.0±0.11	0.62±0.08	0.62±0.18	0.50±0.06	0.51±0.24
Arg.	0.11±0.03	0.10±0.02	0.10±0.03	0.11 ±0.03	0.096±0.06	0.10±0.02
Tyr.	0.31±0.10	0.56±0.21*	0.33±0.11	0.29±0.15	0.71±0.11*	0.62±0.08*
Thr.	1.03±0.21	1.02±0.21	1.05±0.13	1.07±0.07	1.05±0.09	1.03±0.13
Gly.	1.48±0.46	1.34±0.28	1.30±0.28	1.21±0.33	1.31±0.07	1.13±0.19
Ser.	0.95±0.13	0.99±0.11	0.98±0.14	0.89±0.13	1.03 ±0.21	1.02 ±0.21
Lys.	0.19±0.02	0.14 ±0.02	0.16±0.09	0.17 ±0.05	0.18±0.09	0.18±0.08

* significant ($p<0.05$) when compared with normal control.

x significant ($p<0.05$) when compared with corresponding control.

FIGURE-24: Brain GABA, ammonia, glu and glu(NH_2)+asp(NH_2) concentration in normal, hexamethonium and MPT treated rats before and after OHP induced convulsions. (Mean ± SD, n=5)


CONTROL
CONVULSED
GLU (NH2) + ASP (NH2) μ mole·g-1
4.0
3.0
2.0
1.0
GLU μ mole·g-1
11.0
9.0
7.0
5.0
3.0
AMMONIA μg·g-1
19.0
17.0
15.0
13.0
11.0
9.0
7.0
5.0
3.0
GABA μ mole·g-1
2.0
1.0
NORMAL
HEXAMETHONIUM TREATED
α METHYL-ρ-TYROSINE TREATED

Other brain amino acids (Table-14):

No significant change in the other brain amino acids were noted in this study.

Blood NA (Table-13, Figure-25):

The normal concentration of NA in blood was 0.33 ± 0.06 $ng.ml^{-1}$. Hexamethonium and MPT treatment reduced the concentration of NA in blood to 0.13 ± 0.04 and 0.29 ± 0.03 $ng.ml^{-1}$ respectively.

After convulsions induced by OHP, the concentrations of NA in blood in normal rats increased significantly to 2.03 ± 0.53 $ng.ml^{-1}$. When hexamethonium treated rats were convulsed, NA concentration increased to 0.49 ± 0.16 $ng.ml^{-1}$ which was not significantly different from normal control concentrations. Exposure of MPT treated rats to OHP increased blood NA concentrations to 1.64 ± 0.08 $ng.ml^{-1}$.

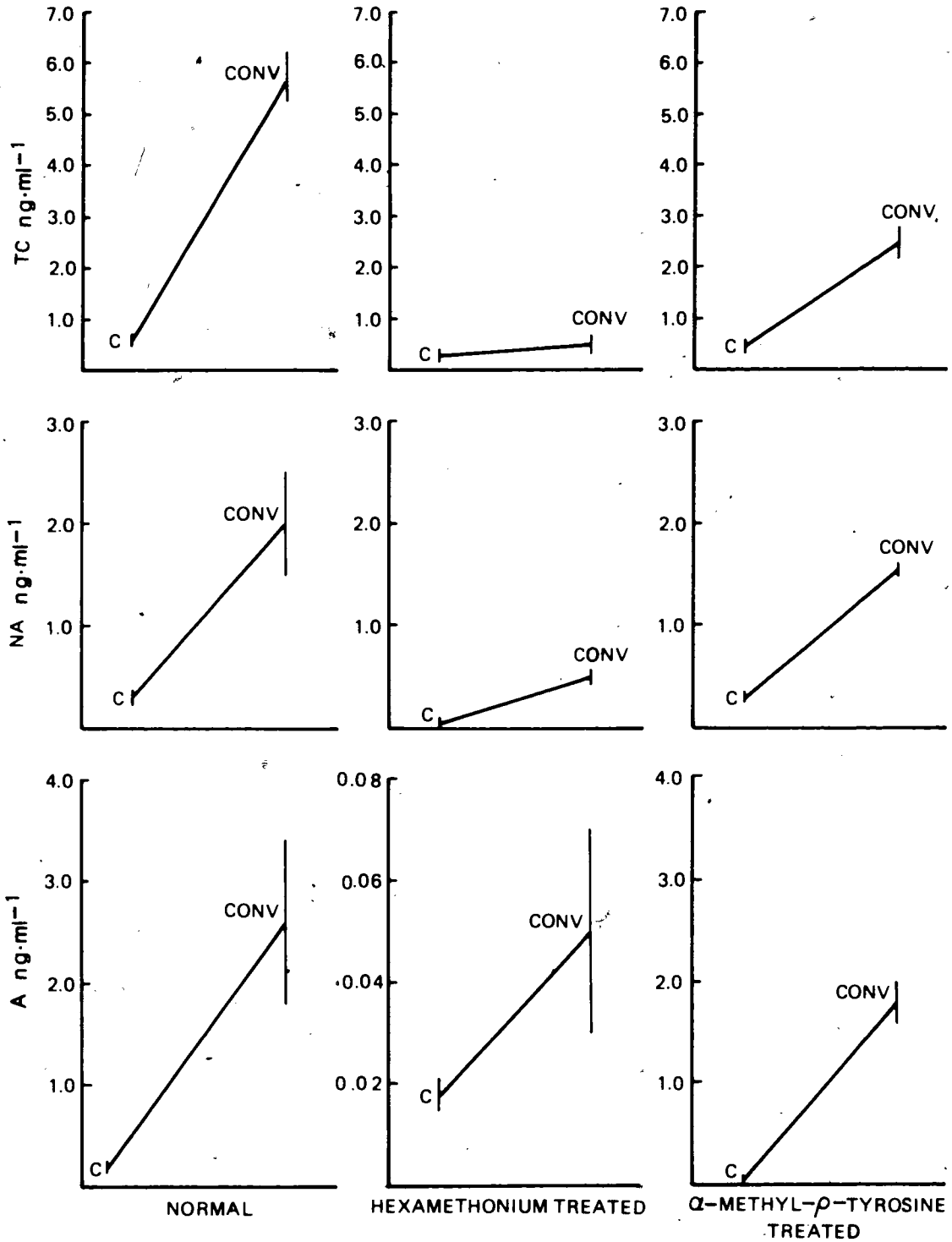
Blood A (Table-13, Figure-25):

The normal mean concentration of A in blood was 0.19 ± 0.03 ng.ml^{-1}. A significantly decreased A concentration was noted when rats were treated with hexamethonium. However, no significant change in concentration was found after MPT treatment in normal rats.

When these normal and MPT treated rats were convulsed, the concentration of A in blood increased significantly to 2.62+0.81 and 1.79 ± 0.89 ng.ml^{-1} respectively. When hexamethonium injected rats were convulsed, the blood A concentration was 0.05 ± 0.02 ng.ml^{-1} which was significantly lower than mean normal control concentrations.

FIGURE-25: Blood adrenaline (A), noradrenaline (NA) and total catecholamine (TC) concentration in normal, hexamethonium and MPT treated rats respectively prior to and after the onset of convulsions, produced by oxygen exposure. (Mean + SD, n=5)

C = CONTROL
CONV = CONVULSED



Blood TC (Table-13, Figure-25):

The normal concentration of TC in blood was 0.57±0.04 $ng.ml^{-1}$ which increased significantly to 5.74+0.46 $ng.ml^{-1}$ after rats were subjected to convulsions induced by OHP.

The concentration of TC in hexamethonium treated rats was 0.28±0.04 $ng.ml^{-1}$ and this increased significantly to 0.53±0.18 $ng.ml^{-1}$ after convulsions. This concentration was not significantly different from normal control concentrations.

When the rats were treated with MPT, the concentrations of TC in blood did not change significantly from normal. After OHP induced convulsions, the blood TC levels increased significantly to 2.48±0.31 $ng.ml^{-1}$.

Blood ammonia (Table-15, Figure-26):

The normal concentration of ammonia in blood was 2.04 ± 0.22 $\mu g.ml^{-1}$. No significant change in this concentration was noted when the normal rats were treated with hexamethonium or MPT.

When normal, hexamethonium and MPT treated rats were oxygen convulsed, concentrations of ammonia in blood increased significantly to 5.58 ± 0.62, 6.14 ± 0.21 and 5.47 ± 0.70 $\mu g.ml^{-1}$ respectively.

Blood glutamate (Table-15, Figure-26):

The normal mean concentration of glutamate in blood was 3.26 ± 0.33 $\mu mole.100\ ml^{-1}$ and decreased significantly to 2.25 ± 0.42 $\mu mole.100ml^{-1}$ after convulsion. No significant changes were noted when the normal rats were treated with hexamethonium and MPT. However, when hexamethonium and MPT treated rats were convulsed by OHP, concentrations of glutamate in blood

decreased significantly to 2.25±0.26 and 1.92±0.28 μmole.100ml^{-1} respectively.

Blood glutamine+asparagine (glu.NH_2+asp.NH_2) (Table-15, Figure-26):

The normal combined concentration for glutamine+asparagine acids was 14.20±2.70 μmole.100 ml^{-1} which increased significantly to 22.23±2.64 μmole.100ml^{-1} after convulsions.No significant change was noted when rats were treated with hexamethonium or MPT. However,when drug treated rats were convulsed, the blood concentration of these amino acids increased significantly to 21.71±2.16 and 21.57±1.16 μmole.100 ml^{-1} in hexamethonium and MPT treated rats respectively.These concentrations were not significantly different from the concentrations in normal convulsed animals.

Blood alanine (Table-15):

Blood alanine concentration in normal, hexamethonium and MPT treated rats were 10.29±1.44, 9.84±1.75 and 10.19±1.7 μmole.100 ml^{-1} respectively.

After convulsions induced by OHP, concentrations of alanine in blood increased to 12.07±0.08, 20.27±1.69 and 20.56±1.81 μmole.100 ml^{-1} in normal, hexamethonium and MPT treated rats respectively.

Blood tyrosine (Table-15):

The normal mean concentration of tyrosine in blood was 0.57±0.14 which decreased to 0.36±0.04 μmole.100ml^{-1} after OHP induced convulsions. No significant difference in the control and convulsed concentrations was noted when normal rats were treated either with hexamethonium or MPT.

TABLE XV

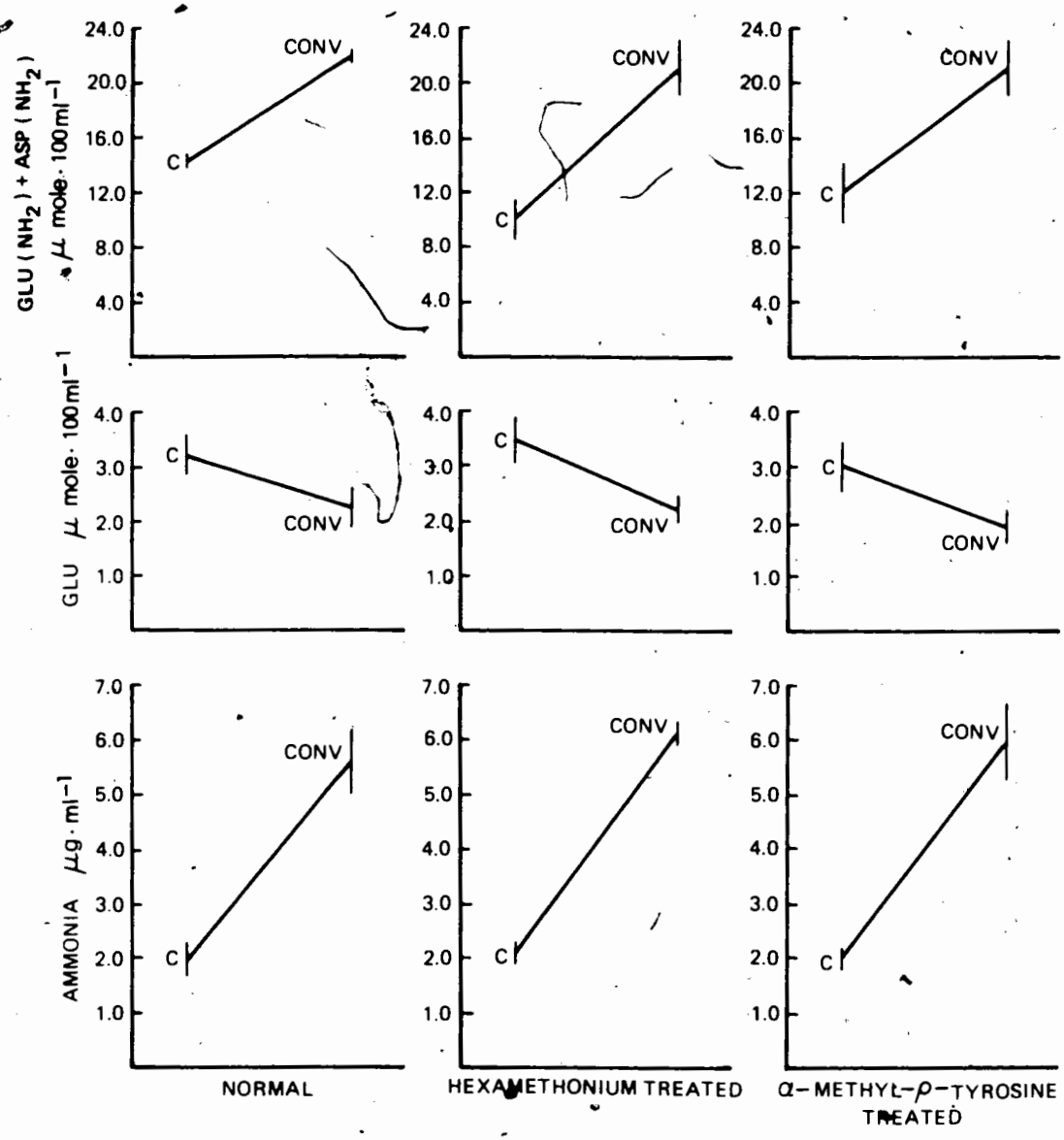
Blood ammonia ($\mu g.ml^{-1}$) and amino acids ($\mu mole.100\ ml.^{-1}$) in normal, Hexamethonium and α-methyl-p-tyrosine treated rats before and after convulsions.

	NORMAL		HEXAMETHONIUM		α-METHYL-p-TYROSINE	
	Control	Convulsed	Control	Convulsed	Control	Convulsed
Ammonia	2.04±0.22	5.58±0.62*	2.1±0.2	6.14±0.21*x	2.02±0.20	5.47±0.7*x
Glu.	3.26±0.33	2.25±0.42*	3.54±0.4	2.25±0.26*x	3.05±0.45	1.92±0.28*x
Glu & Asp (NH_2)	14.2±2.7	22.23±2.64*	11.06±1.52	21.71±2.16*x	10.95±2.25	21.57±1.16*x
Asp.	1.55±0.36	1.84±0.31	1.23±0.15	1.39±0.44	1.30±0.23	1.37±0.17
Ala.	10.29±1.44	12.07±0.08*	9.84±1.75	20.27±1.69*x	10.19±1.70	20.56±1.81*x
Tyr.	0.54±0.13	0.36±0.04*	0.51±0.09	0.54±0.13	0.47±0.16	0.56±0.14
Pro.	2.06±1.07	2.19±0.44	2.34±0.39	2.57±0.71	2.67±0.64	2.66±1.24
Thr.	0.81±.10	0.79±0.11	0.75±0.12	0.74±0.13	0.71±0.04	0.77±0.11
Phe.	0.59±0.08	0.58±0.11	0.54±0.12	0.54±0.13	0.54±0.14	0.55±0.15
Ser.	0.96±0.23	0.93±0.09	0.98±0.08	0.95±0.06	0.96±0.11	0.96±0.16
Leu.	4.01±0.62	3.7±0.9	3.8±0.7	3.14±0.45	3.61±0.78	4.0±0.79
Ileu.	3.69±0.68	4.39±0.56	3.93±0.92	4.11±0.36	3.48±0.44	3.56±0.68
Val.	0.72±0.21	0.61±0.19	0.67±0.32	0.79±0.13	0.77±0.19	0.71±0.13
Lys.	6.93±1.15	7.38±0.54	6.12±0.93	5.93±1.11	6.52±1.02	7.02±0.79
Met.	0.47±0.21	0.50±0.12	0.39±0.10	0.41±0.09	0.431±0.19	0.49±0.20
His.	2.13±0.31	2.11±0.36	2.02±0.36	2.19±0.42	1.92±0.83	2.01±0.73
Arg.	1.33±0.53	1.39±0.30	1.21±0.41	1.29±0.73	1.41±0.63	1.37±0.57
Gly.	3.19±0.93	3.43±0.59	3.16±0.79	3.23±1.02	3.71±0.93	3.49±0.76

* significant ($p<0.05$) when compared with normal control.
x significant ($p<0.05$) when compared with corresponding control.

FIGURE-26: Blood ammonia, glu and glu(NH_2)+asp(NH_2) concentration in normal, hexamethonium and MPT treated animals respectively prior to and after convulsions produced by exposure to OHP. (Mean ± SD, n=5)

C = CONTROL
CONV = CONVULSED



Other blood amino acids (Table 15):

No significant change in other blood amino acids were noted in the present study before or after OHP induced convulsions in normal and drug treated animals.

Time for convulsions:

When the normal rats were injected with hexamethonium, the latency for convulsion increased significantly to 98.8±7.3 minutes. When the normal rats were treated with MPT, the elapsed time to convulsion was 43.4±3.9 minutes which was not significantly different from the mean normal convulsion time.

DISCUSSION

In the present study no significant change in concentration of catecholamine in brain following treatment of rats with hexamethonium was observed. MPT however, decreased the concentration of catecholamines and COMT in brain significantly. Injection of normal rats with hexamethonium decreased all the catecholamines in blood whereas injection of MPT affected only A in blood.

Gollnick and Ianuzzo (1975) observed that hexamethonium treatment eliminates catecholamine both from the adrenal medulla and from sympathetic nerve endings. In the present study adrenalectomy reduced the concentration of A in blood from 0.19 $ng.ml^{-1}$ to 0.006 $ng.ml^{-1}$ whereas hexamethonium reduced it only to 0.18 $ng.ml^{-1}$. This suggests that hexamethonium treatment is not as effective as adrenalectomy in depleting the concentration of A in blood. However, in the present

study, hexamethonium pretreatment was used preferentially because:

(1) hexamethonium treatment decreased the concentration of catecholamine in blood without effecting the adrenal cortex and essentially effects an adrenalmedulloectomy,

(2) the same degree of depletion of catecholamine as produced by adrenalectomy could be obtained without the confusing hormonal and metabolic effects normally attendant upon surgical adrenalectomy.

MPT decreases the concentration of catecholamines by inhibiting tyrosine hydroxylase, the rate limiting enzyme of catecholamine synthesis (Levitt et al.,1965). After inhibition of tyrosine hydroxylase by MPT, the depletion of catecholamines depends on the rate of their utilization and thus is more rapid in tissue (Udenfriend et al., 1965). In the present study, MPT was observed to affect brain catecholamines more drastically than catecholamines in blood.

In this study no significant change in the

concentration of ammonia and amino acid in blood and brain was observed following treatment of normal rats with hexamethonium and MPT.

The concentration of A, NA and TC in blood increased significantly in hexamethonium treated rats exposed to OHP prior to convulsions. However, in hexamethonium treated rats, the magnitude of increase in the concentration of catecholamines was several times less than in either normal or MPT treated rats exposed to OHP. This probably results from the blockade of postganglionic acetylcholine receptor sites by hexamethonium(Burn and Gibbons,1964).

In rats treated with hexamethonium and convulsed by exposure to OHP, the concentrations of A in brain decreased significantly whereas NA, total catecholamine and COMT concentrations remained unchanged.

Brain ammonia and glutamine increased and GABA and glutamate decreased significantly after convulsions in rats treated with hexamethonium. In blood also,

ammonia and glutamine increased whereas the concentration of glutamate decreased following convulsions induced by OHP.

When rats treated with MPT were convulsed, the concentration of A, NA and COMT in brain decreased further from the depressed concentration in drugged animals but not exposed to oxygen. The concentration of A, NA and TC in blood however increased significantly. In these rats also, an increase in brain ammonia and glutamine and a decrease in GABA and glutamate was observed in response to convulsions induced by OHP. No significant change in the concentrations of other amino acids were noted after convulsions induced by OHP in hexamethonium and MPT treated rats.

The role of altered ammonia and amino acid concentrations in response to OHP has been discussed earlier. The important point in this experiment is that post convulsive concentrations of ammonia and amino acids in normal rats and in rats treated with

hexamethonium and MPT were not significantly different.

The latency for convulsion was significantly greater in in rats treated with hexamethonium than in normal rats whereas rats treated with MPT took the same time as normal rats to convulse in response to OHP exposures.

These observations suggest that:

1. Increased latency for convulsions induced by OHP in hexamethonium treated rats is probably due to the decreased concentrations of catecholamine in blood, since the concentration of other blood and brain parameters in control hexamethonium treated rats were the same as in normal undrugged animals.

2. Since the times for convulsions induced by OHP in hexamethonium treated and adrenalectomized rats were almost same, it might be proposed that the protection effected in adrenalectomized rats is due to removal of adrenomedullary hormones and is not due to the

influence of the cortical hormones.

3. The present study suggests that catecholamines in brain do not play an important role in inducing the convulsions due to OHP exposure. Supporting this conclusion are the facts that:

1. hexamethonium protects rats against convulsions induced by OHP without effecting brain catecholamines,
2. MPT reduces catecholamines in brain without effecting the time for the onset of convulsions.

Faiman et al. (1971) also observed that MPT decreased the catecholamines in brain almost to half without effecting the time for convulsions in normal rats.

4. Activity of COMT in brain changes parallel with the brain concentration of catecholamine i.e. low concentration of catecholamine was always attended by low COMT activity. If a decrease in concentration of catecholamine in rats treated with MPT after convulsions induced by OHP were due to the enzyme COMT, then its activity should be higher at a time when

catecholamines were low. However, this was not the case and suggests that:

1. oxygen probably inactivates COMT directly,
2. decrease in activity activity of COMT is probably due to a feedback mechanism, i.e. a reduction in concentration of catecholamine in brain reduces COMT activity by a positive feedback effect.

In summary, hexamethonium acts as protective agent against oxygen toxicity, probably by reducing the concentration of catecholamines in blood. MPT had no effect on convulsions although it reduced brain catecholamines significantly.

CHAPTER-7

GENERAL DISCUSSION

In the present study it has been observed that exposure of normal rats to OHP caused convulsions, increased the concentration of ammonia in blood and brain and decreased GABA in brain even prior to the onset of convulsive seizures. During the early phase of exposure of normal rats to OHP, the concentration of catecholamines in brain first decreased and later returned to normal remaining unchanged thereafter up to and after the animals convulsed due to exposure to OHP. Exposure of normal rats to OHP results in parallel changes in activity of COMT in brain. In response to OHP, the concentration of catecholamine in blood increased initially due to handling and exposure stress, but decreased thereafter showing adaptation to OHP before increasing again due to the toxic effects of oxygen. The concentration of catecholamines in blood was significantly increased even before convulsions began. The concentration of glutamine in blood and brain increased whereas the concentration of glutamate decreased in normal rats exposed to OHP. No significant change in other amino acids was noted when normal rats were exposed to OHP except in tyrosine and alanine

concentrations (Table-15).

These observations suggest that the convulsions occuring in response to OHP might be due to a low concentration of GABA in brain, elevated blood and brain ammonia and an increased concentration of catecholamines in blood. The concentration of catecholamines in brain do not seems to be important to inducing the toxic effects of oxygen, since MPT reduced the concentration of catecholamines in brain without effecting the latency period of convulsions. Hexamethonium treatment and adrenalectomy increased the latency for convulsions induced by OHP also without altering the concentration of catecholamines in brain. Faiman et al. (1971) also observed that an increased concentration of brain catecholamines failed to give any protection against oxygen toxicity. They suggested that an elevated MAO activity (Novick,1966) might cause a toxic reaction in brain after OHP exposures by producing toxic free radicals (Zirkle et al., 1955). In the present study, no increase in brain COMT activity (the other catecholamine degradative enzyme)

was observed after exposure of rats to OHP. This suggests that oxygen probably has some selective action in increasing MAO activity without altering the activity of COMT.

Bean et al.(1953) proposed that exposure of rats to OHP causes hypertrophy of the adrenal gland and increased secretion of adrenal hormones which augments the toxic effects of OHP in rats. Hale et al. (1973) also reported that oxygen tolerance in an individual depends upon their neuroendocrine state. Excess of catecholamine or 17-hydroxy corticoids would accentuate the toxic effects of oxygen. In the present study, the elevated concentration of catecholamines in blood were observed prior to the induction of convulsion in normal rats. Adrenalectomy and ganglionic blockade protected rats against oxygen toxicity and delayed convulsion in this study. These observations suggest that catecholamines in blood are important in in the induction of convulsions due to OHP exposure. In 6-OHDA treated rats, the latency for convulsions induced by OHP decreased concomitantly with low blood

NA and high A concentrations. However, this is probably due to:

1. a very high ammonia in blood and brain
2. a low GABA in brain
3. a low glutamate in blood and brain
4. an elevated blood A

after 6-OHDA injections in normal rats before OHP exposure.

The treatment of normal rats with 6-OHDA increased the concentration of ammonia in blood and brain and decreased their latency period to convulsions as shown in tables 6 and 8.

These results suggest that the period of exposure to OHP before convulsions are induced in rats decreases in some proportion to the increase in concentration of ammonia in in blood and brain. Similarly the concentration of ammonia in the blood of adrenalectomized rats was less than normal control concentrations. Adrenalectomized rats survived longer when exposed to OHP and convulsions were delayed. Postconvulsive concentrations of ammonia in blood and brain were not

significantly different in any of the groups of normal rats, adrenalectomized rats, and rats treated with 6-OHDA, hexamethonium or MPT. These observations suggests that a common critical elevation in the concentration of ammonia in blood and brain in all the different groups is necessary to potentiate convulsive seizures. If the control baseline of ammonia prior to oxygen exposure is already high, the concentration of ammonia will reach a critical level early and the onset of convulsion will be precipitated early. Banister et al.(1976) have also suggested that the concentration of ammonia in blood and brain is an important factors inducing convulsions during exposure of rats to OHP. They also observed that lithium protects rats against convulsions and suggested that ammonia may be removed from the system via an ammonia-lithium chelate complex described by Williams (1973).

When the concentration of ammonia in blood and brain increases in response to OHP, it is possible that glutamate is available to take up ammonia and form its amide glutamine. This is a potentially important

reaction buffering ammonia in the body. Alanine is also involved in buffering of ammonia in blood but not in brain. Due to these buffering reactions, one may observe an increased concentration of glutamine and decreased glutamate in blood and brain prior to and after convulsions induced by OHP.

In the present study, a decrease in the concentration of GABA in brain was observed when rats from all groups were convulsed by OHP. Wood et al. (1965) proposed that a decrease in concentration of GABA in brain after OHP exposure might be responsible for the induction of convulsions induced by OHP. In the present study also, there was a direct correlation between GABA in brain and latency for convulsions in rats treated with 6-OHDA as shown in table 6 and 8. Wood et al. (1970) proposed that OHP could reduce the concentration of GABA in brain by:

1. an inhibition of its synthesis due to an inactivation of the enzyme GAD.
2. an acceleration of membrane transport of GABA out of the cells of the CNS.

3. an acceleration of its degradation due to an activation of GABA-T.

Since glutamate is a precursor for the synthesis of GABA, a decrease in concentration of glutamate in brain by OHP or by injection of 6-OHDA would slow down the synthesis of GABA and decrease its concentration. Glutamate is also a substrate in the synthesis of glutathione and the latter also plays a role in the inactivation of free radicals by reducing them (Meister,1973). A decrease in concentration of glutamate would decrease glutathione formation which ultimately would increase the life of free radicals formed during the toxic effects of oxygen at high pressure. In the present study, treatment with 6-OHDA reduced the concentration of glutamate in brain. Thoenen et al.(1970) have also observed that this drug produces free radicals when accumulated in sympathetic nerve terminals. It is quite possible that low glutamate decreases glutathione synthesis and thus potentiates the life span of free radicals. Free radicals are also produced from MAO which is found to

be elevated after exposure of rats to OHP (Novick, 1966). A combined effect of these reactions together with low GABA and elevated ammonia might have led to the early convulsion of rats treated with 6-OHDA and exposed to OHP.

However, no significant change in concentration of GABA in brain was observed in this study when rats were adrenalectomized or treated with hexamethonium. These two groups of rats took longer to convulse than normal rats when exposed to OHP. As discussed earlier, these groups also had lower concentrations of ammonia in blood and brain before convulsions. These observations taken together suggest that a low concentration of GABA in brain is part of the mosaic of events inducing the act of convulsion but that of itself is not involved as a protective agent against oxygen toxicity.

CHAPTER-8

CONCLUSIONS

1. Catecholamines in the brain do not seem to be important for the induction of the toxic symptoms of OHP in rats. Injections of MPT reduced the concentration of catecholamines in brain without effecting the time for convulsions induced by OHP. Adrenalectomy and ganglionic blockade with hexamethonium on the other hand, provided protection to rats against oxygen toxicity without effecting the concentration of catecholamines in brain.

2. The concentration of ammonia in blood and brain increased and the concentration of GABA in brain decreased significantly even before the production of convulsion when normal rats were exposed to OHP for graded time intervals. Elevated brain ammonia causes serious electrolyte imbalance and hyperexcitation of the brain. Decrease in the concentration of GABA would also lead to hyperactivity of the brain since GABA acts as an inhibitor of nerve transmission.

DIAGRAM-6: Showing the response of brain and blood adrenaline, noradrenaline, ammonia and GABA (brain only) to the development of convulsion in animals exposed to OHP.

Normal Rats

↓

OHP Exposure

↓

	CONTROL	30 Minutes after OHP exposure	After Convulsion
BRAIN			
NA	128 .9 ± 3.6 $ng.g^{-1}$	No significant change	No significant change
A	2.2 ± 0.4 $ng.g^{-1}$	No significant change	Decrease significantly
GABA	1.52 ± 0.12 μ mole $.g^{-1}$	Decrease significantly	Decrease significantly
NH_3	5.25 ± 0.27 $\mu g.g^{-1}$	Increase significantly	Increase significantly
BLOOD			
NA	0.33 ± 0.06 ng. $m\ell^{-1}$	Increased significantly	Increased significantly
A	0.19 ± 0.03 ng. $m\ell^{-1}$	Increased significantly	Increased significantly
NH_3	2.05 ± 0.23 $\mu g.$ $m\ell^{-1}$	Increased significantly	Increased significantly

Brain catecholamines are not important in the induction of convulsion.

High brain and blood ammonia (NH_3) and low brain GABA just before convulsion seems to play important role in the induction of convulsion.

High blood A and NA also seem to induce the convulsion.

3. Blood catecholamines were also elevated before the onset of convulsions in normal rats exposed to OHP. Increased catecholamines in blood would potentiate the stress response and deplete the energy reserves.

4. These observations suggests that ammonia and GABA in blood and brain and catecholamines in blood play an important role in the induction of convulsive seizures following exposure of rats to OHP.

5. The brain COMT activity was either unchanged or decreased in response to convulsions induced by OHP.

6. Concentrations of glutamate in blood and brain decreased and concentrations of glutamine increased in response to OHP exposures in normal, 6-OHDA treated, adrenalectomized, hexamethonium and MPT treated rats. The glutamate/glutamine system is important in buffering ammonia in blood and brain. Since glutamate is a precursor for the synthesis of GABA, a decrease in concentration of glutamate in brain by OHP or by 6-OHDA

DIAGRAM-7: Showing the alterations in brain ammonia and GABA concentrations in response to OHP and the contributory effects of these changes to the development of convulsion in rats.

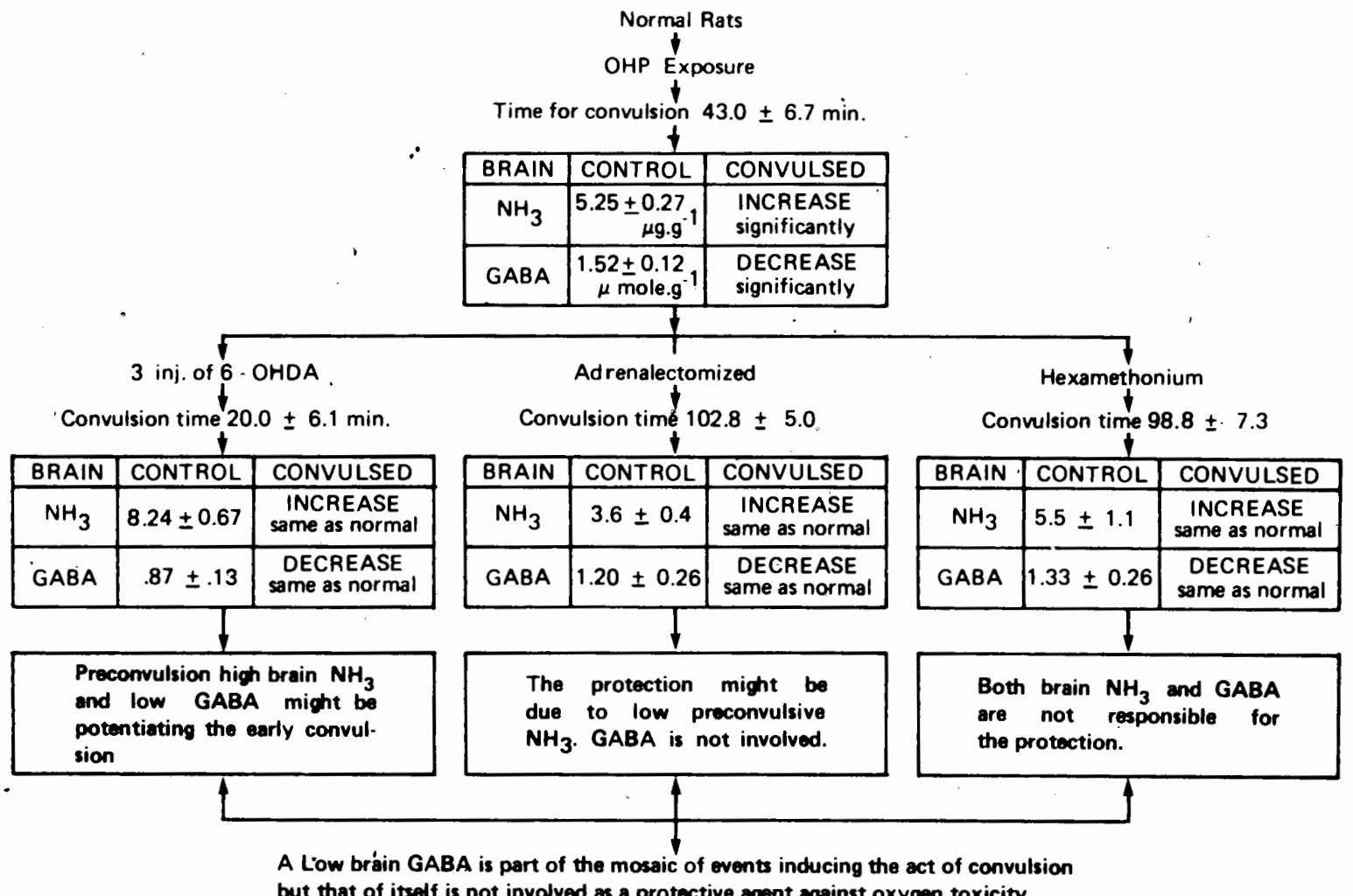
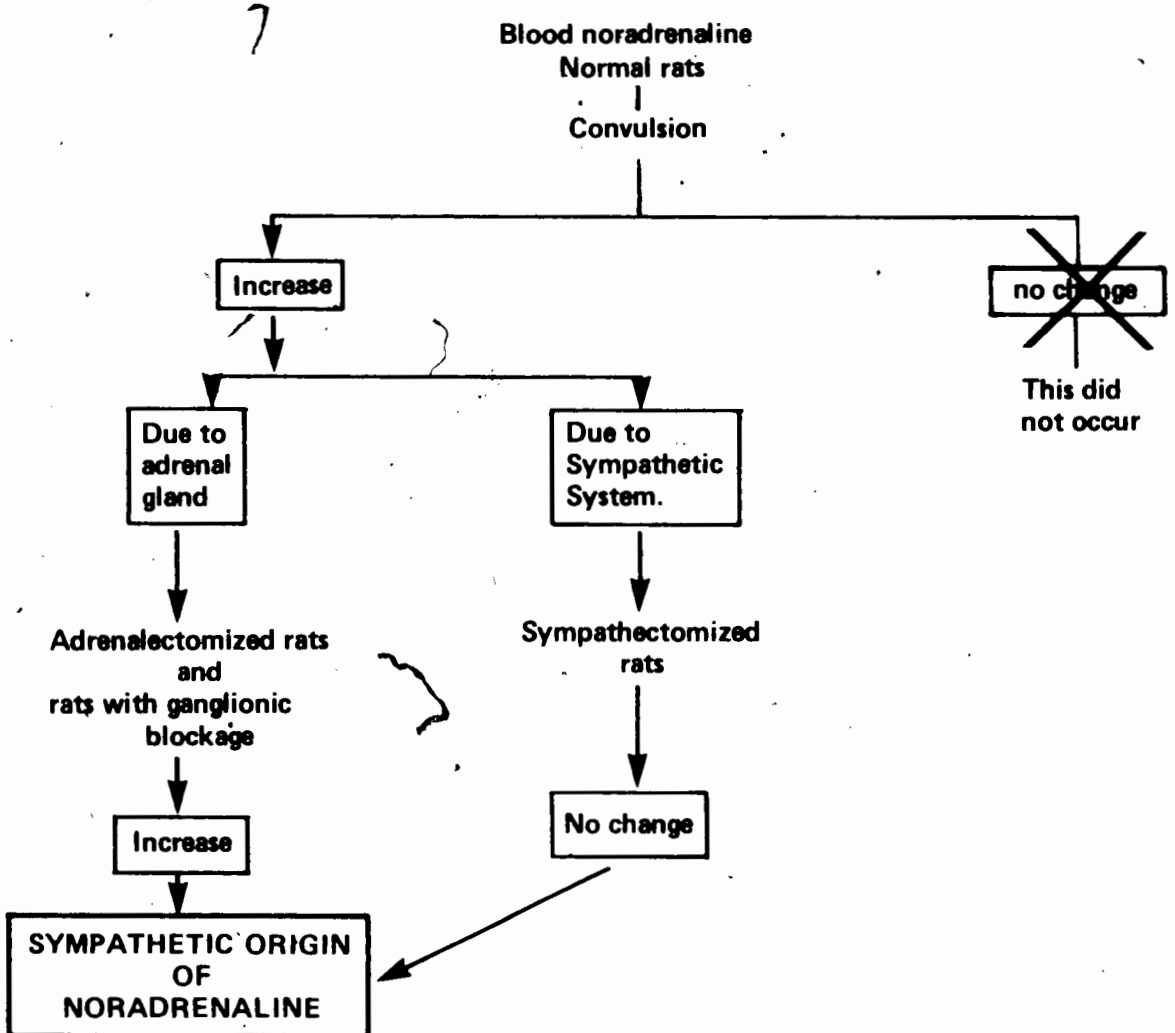

Normal Rats
OHP Exposure
Time for convulsion 43.0 ± 6.7 min.
BRAIN | CONTROL | CONVULSED
NH3 | 5.25 ± 0.27 μg.g-1 | INCREASE significantly
GABA | 1.52 ± 0.12 μ mole.g-1 | DECREASE significantly
3 inj. of 6 - OHDA
Convulsion time 20.0 ± 6.1 min.
BRAIN | CONTROL | CONVULSED
NH3 | 8.24 ± 0.67 | INCREASE same as normal
GABA | .87 ± .13 | DECREASE same as normal
Preconvulsion high brain NH3 and low GABA might be potentiating the early convulsion
Adrenalectomized
Convulsion time 102.8 ± 5.0
BRAIN | CONTROL | CONVULSED
NH3 | 3.6 ± 0.4 | INCREASE same as normal
GABA | 1.20 ± 0.26 | DECREASE same as normal
The protection might be due to low preconvulsive NH3. GABA is not involved.
Hexamethonium
Convulsion time 98.8 ± 7.3
BRAIN | CONTROL | CONVULSED
NH3 | 5.5 ± 1.1 | INCREASE same as normal
GABA | 1.33 ± 0.26 | DECREASE same as normal
Both brain NH3 and GABA are not responsible for the protection.
A Low brain GABA is part of the mosaic of events inducing the act of convulsion but that of itself is not involved as a protective agent against oxygen toxicity.

DIAGRAM-8: Integration of the evidence showing that most of the noradrenaline produced after OHP induced convulsion in normal rats comes from the sympathetic nervous system.


Blood noradrenaline
Normal rats
Convulsion
Increase
no change
This did not occur
Due to adrenal gland
Due to Sympathetic System.
Adrenalectomized rats and rats with ganglionic blockage
Sympathectomized rats
Increase
No change
SYMPATHETIC ORIGIN OF NORADRENALINE

would slow down the synthesis of GABA and decrease its concentration.

7. The concentration of alanine in blood increased whereas brain alanine concentration remained unchanged after normal, 6-OHDA treated, adrenalectomized, shamoperated, hexamethonium and MPT treated rats were convulsed by OHP.

8. The concentration of tyrosine in brain increased and blood tyrosine concentration decreased significantly when rats were exposed to OHP and convulsed. The actual cause of this change in tyrosine concentration is not known unless it reflects inhibition or acceleration of catecholamine synthesis.

9. No significant change in other amino acid concentrations was observed following convulsions induced by OHP.

10. Injection of 6-OHDA in normal rats reduced brain catecholamines, COMT, GABA and glutamate concentrations

and increased brain ammonia and tyrosine concentrations. Thoenen et al. (1970) reported that 6-OHDA does not cross the blood brain barrier and affects brain catecholamines only when injected in the brain. The present study suggests that systemic injection of 6-OHDA reduces brain catecholamines and the following factors may account for the present observation: (1) Blood borne humoral or metabolic factors modify the metabolism of brain as a stress response. (2) There is a reflex, increased peripheral sympathetic response to falling blood pressure (Axelrod et al., 1970).

11. 6-OHDA treated rats convulsed earlier than normal rats when exposed to OHP. This might be due to:

i. low GABA in brain

ii. high ammonia in blood and brain

iii. increased A in blood

iv. low glutamate in blood and brain

12. In blood, 6-OHDA increased the concentrations of A and ammonia whereas the concentration of NA and TC were significantly decreased.

13. Shamoperation provided some protection to rats against oxygen toxicity, though their hormonal and metabolic parameters, which were investigated in this study, did not differ significantly from normal rats.

14. Adrenalectomy and hexamethonium treatment of rats provided protection against convulsions induced by OHP probably by reducing concentrations of ammonia in blood and brain and concentrations of catecholamines in blood. Since adrenalectomy and hexamethonium treatment did not change the concentration of GABA and catecholamines in brain it might be suggested that the concentration of these latter parameters are not involved in any protective action against oxygen toxicity.

DIAGRAM-9: Integrating the results of adrenalectomy, sympathectomy and inhibition of brain catecholamine synthesis on OHP produced convulsions in rats.

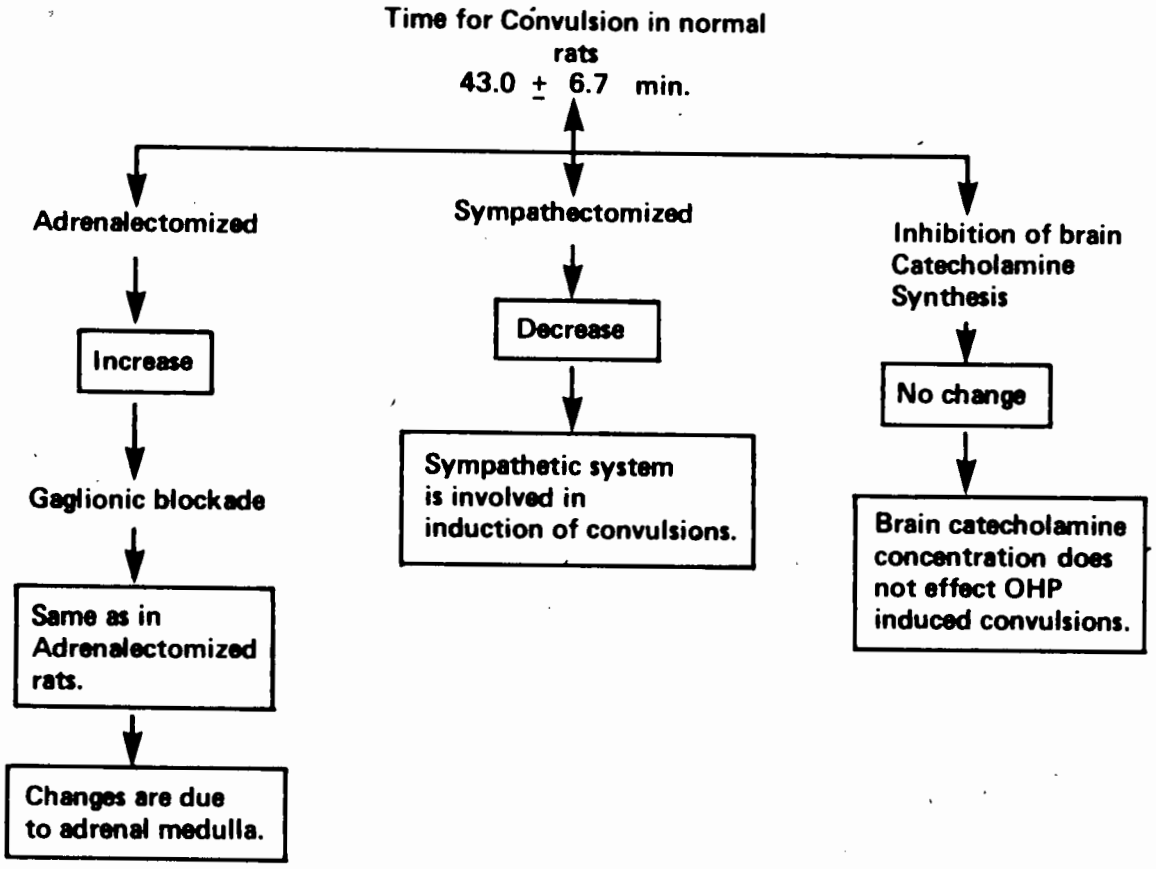

Time for Convulsion in normal rats
43.0 ± 6.7 min.
Adrenalectomized
Increase
Ganglionic blockade
Same as in Adrenalectomized rats.
Changes are due to adrenal medulla.
Sympathectomized
Decrease
Sympathetic system is involved in induction of convulsions.
Inhibition of brain Catecholamine Synthesis
No change
Brain catecholamine concentration does not effect OHP induced convulsions.

DIAGRAM-10: Showing a comparison of the effects of 6-OHDA injection, adrenalectomy and hexamethonium injection on blood ammonia, adrenaline, noradrenaline and convulsion time in normal animals and those convulsed by OHP.

Normal Rats

↓

OHP Exposure

↓

Convulsion time 43.0 ± 6.7 min.

BLOOD	CONTROL	CONVULSED
NH_3	2.05 ± 0.23 $\mu g/m\ell^{-1}$	INCREASE significantly
A	0.19 ± 0.03 $ng.m\ell^{-1}$	INCREASE significantly
NA	0.33 ± 0.06 $ng.m\ell^{-1}$	INCREASE significantly

↓

3.Inj. of 6-OHDA

↓

Convulsion time 20.0 ± 6.1 min.

BLOOD	CONTROL	CONVULSED
NH_3	3.05 ± 0.37	INCREASE same as normal
A	0.34 ± 0.10	INCREASE less than normal
NA	0.11 ± 0.02	No change

↓

Early convulsions might be due to high preconvulsive ammonia and adrenaline

Adrenalectomized

↓

Convulsion time 102.8 ± 5.0 min.

BLOOD	CONTROL	CONVULSED
NH_3	1.45 ± 0.32	INCREASE same as normal
A	0.006±0.004	No change
NA	0.15 ± 0.04	INCREASE less than normal

↓

The protection might be due to low preconvulsive ammonia, adrenaline and noradrenaline.

Hexamethonium

↓

Convulsion time 98.8 ± 7.3

BLOOD	CONTROL	CONVULSED
NH_3	2.1 ± 0.2	INCREASE same as normal
A	0.018 ± 003	INCREASE same as normal
NA	0.13 ± 0.04	INCREASE same as normal

↓

The protection might be due to low blood catecholamines.

DIAGRAM-11: Showing the relationship between the separate and integrated contributory effects of the catecholamines, ammonia and amino acids to the development of convulsion in animals exposed to OHP.

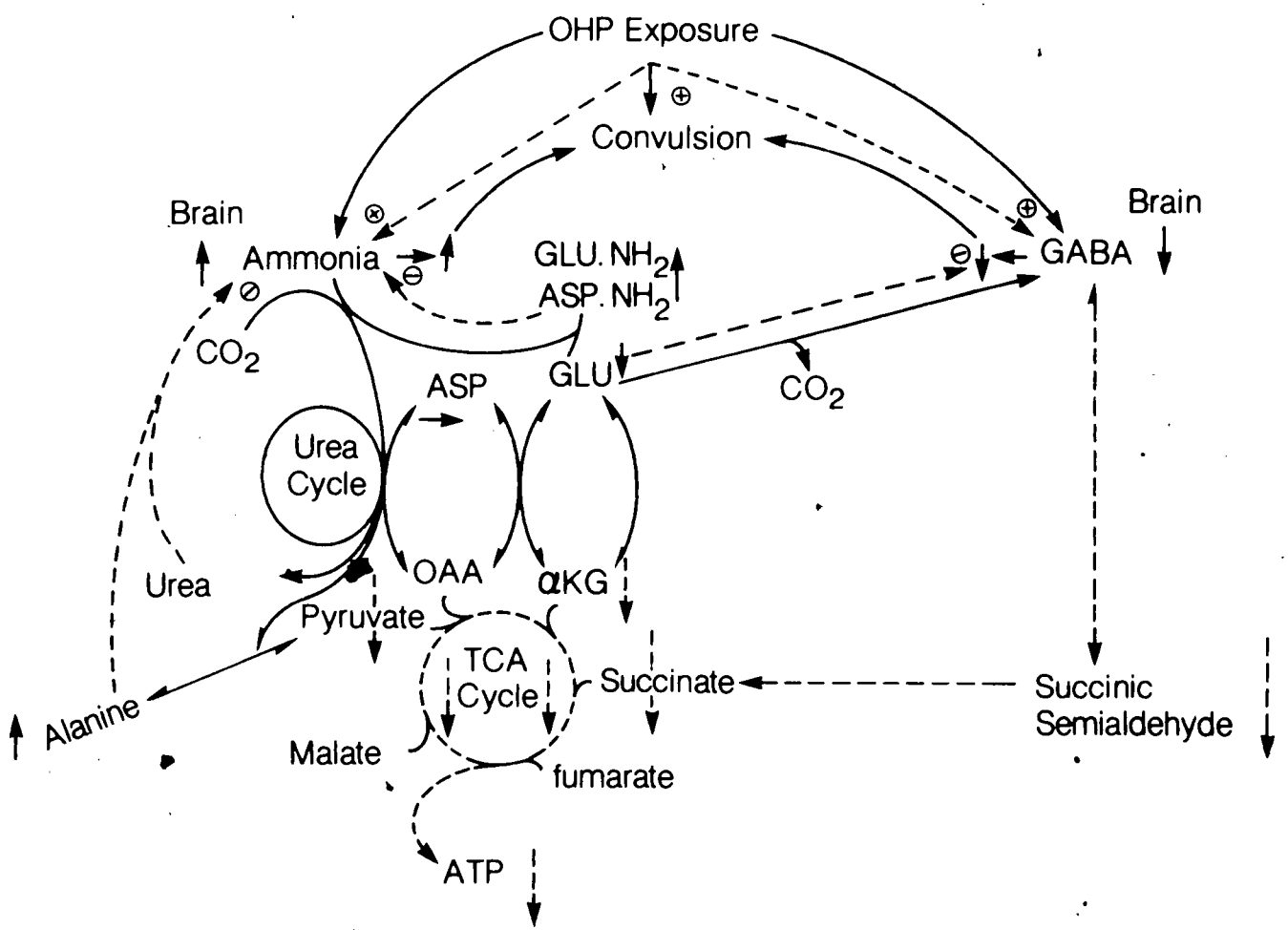
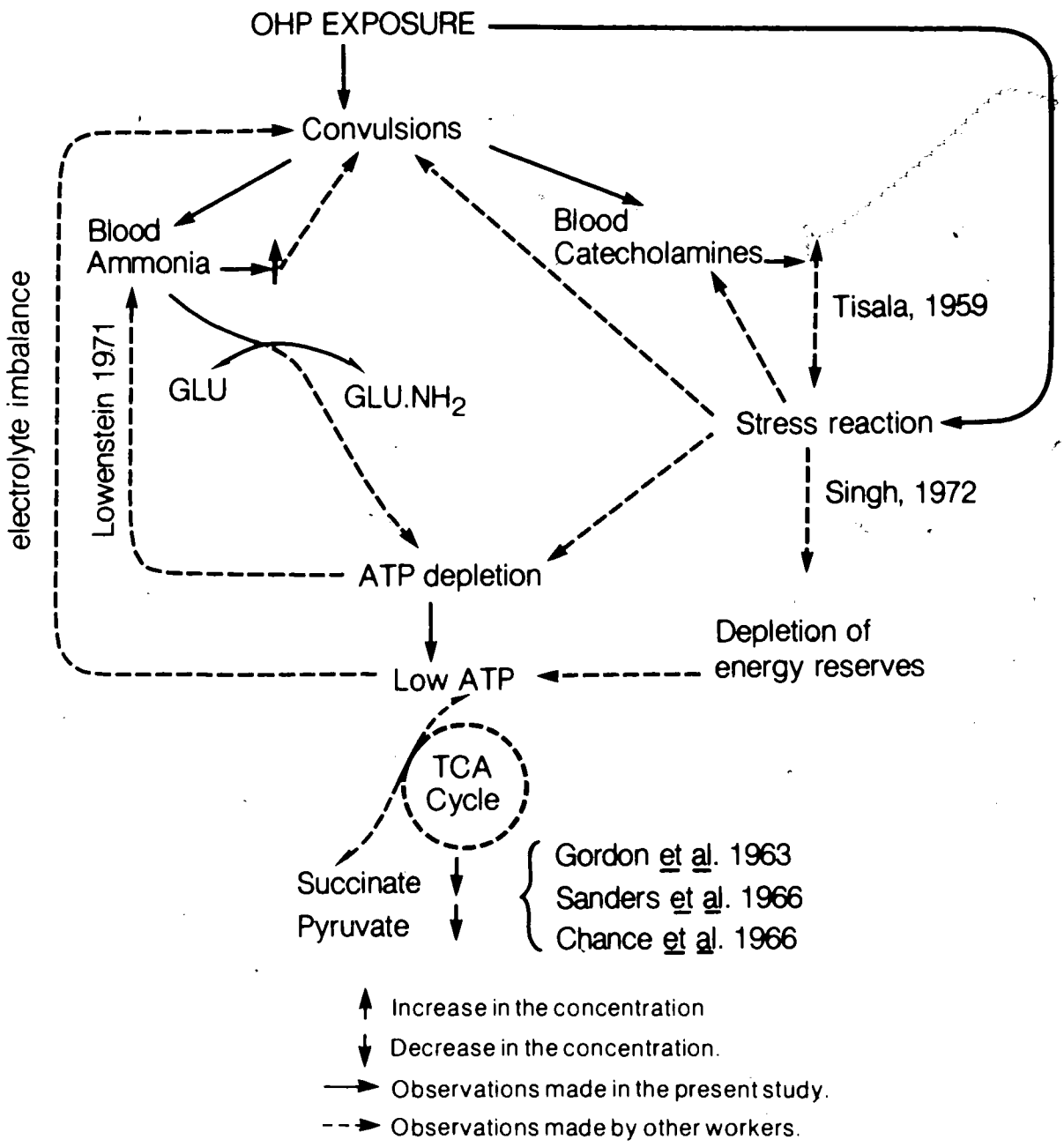

OHP Exposure
Convulsion
Brain
Ammonia
GLU. NH_2
ASP. NH_2
GABA
Brain
CO_2
ASP
GLU
CO_2
Urea Cycle
Urea
OAA
αKG
Pyruvate
TCA Cycle
Succinate
Succinic Semialdehyde
Alanine
Malate
fumarate
ATP
INCREASE in the concentration.
DECREASE in the concentration.
INHIBITORY ACTION.
EXCITATORY ACTION
Observations made in the present study.
Observations made by other workers.

DIAGRAM-12: Showing the relationship between the separate and integrated contributory effects of catecholamines, ammonia and amino acids to the development of convulsion in animals exposed to oxygen at high pressure.


OHP EXPOSURE
Convulsions
Blood Ammonia
Blood Catecholamines
Tisala, 1959
electrolyte imbalance
Lowenstein 1971
GLU
GLU.NH2
Stress reaction
Singh, 1972
ATP depletion
Depletion of energy reserves
Low ATP
TCA Cycle
Succinate
Pyruvate
Gordon et al. 1963
Sanders et al. 1966
Chance et al. 1966
Increase in the concentration
Decrease in the concentration.
Observations made in the present study.
Observations made by other workers.

CHAPTER-9

APPENDIX

DESCRIPTION OF TECHNICAL PROCEDURES

-CATECHOLAMINE ASSAY

-AMMONIA ASSAY

-AMINO ACIDS ANALYSIS

-STATISTICAL METHODS

CATECHOLAMINE ASSAY:

Serum and brain A, NA and total catecholamines were estimated by using a minor modification of the method of Passon and Peuler (1973). The original method is based on the conversion of A and NA to their metanephrines-H^3 by incubation with S-adenosyl-methionine-methyl-H^3 and catecholamine-O-methyl transferase. The metanephrines are isolated in toluene/isoamyl alcohol and separated by Silica Gel-GF thin layer chromatography.

Methodology:

Plasma:

In volume of 1.25 ml., 0.5 to 0.75 ml of plasma, 80 mM tris buffer pH 8.2, 10 umole SAM (including 2x10 counts SAM-H^3), 10 mM GSH, .22 mM paragyline and 24mM MgCl were incubated together. The reaction was started by adding COMT (2 to 3 mg protein) and was carried out

at 37C for 60 minutes. After incubation, 5.0 uM each of metanephrine and normetanephrine and 0.25 ml of borate buffer pH 11.0 were added to the mixture.

The metanephrines were extracted in toluene:isoamyl alcohol (3:2v/v) by shaking for 15 minutes. 0.1ml of acetic acid (0.1 N) was added to the organic layer and the mixture was shaken again for 15 minutes. The acetic acid layer was then frozen in a methanol-dry ice mixture and the organic layer was removed and discarded. A volume of 0.1 ml of methanol was added to the acetic acid extract to make it more volatile. It was then applied on a silica gel plate 2"x8" (Silica Gel-GF). The plates were developed with isopropanol: n-butanol:formic acid and water(60:20:1:19) for 3 hours. The plates were air dried at room temperature and the metanephrine bands were identified with short wave UV light. Each band was transfered to a 15 ml centrifuge tube and further extractions were carried out as described by Passon and Peuler(1973).

Assay of TC was the same as above, except the TLC separation was omitted (Passon and Peuler, 1973).

Treatment of brain:

Brain samples were homohenized with 0.2 N perchloric acid (1:4 w/v) and centrifuged in a cold centrifuge. The supernatant was decanted and pH was adjusted to 7 - 8 in order to bring the pH of the supernatant in the buffering range of the incubation buffer. After pH adjustment, the supernatant was centrifuged again and stored at -20C for batch analysis later. Before homogenization, 5mM reduced glutathione was added to prevent the oxidation of catecholamines during storage. The remaining procedures were the same as in plasma. If the catecholamine content was expected to be very high, the samples were diluted with incubation buffer.

Care was taken to conduct all extraction processes described above at or below 4C to avoid decay of these hormones.

CATECHOL-O-METHYL TRANSFERASE:

A radiometric assay was used for estimation of this enzyme (Axelrod,1962). The enzyme activity is determined by measuring metanephrine-H^3 from A and SAM-methyl-H^3 in the presence of Mg^{++}.

Enzyme preparation:

Serum was used directly for the enzyme assay. Brain tissue was homogenized with 0.15 M KCl (1:3 w/v) at 0C. The homogenate was centrifuged for 20 minutes and 20 µl of supernatant was used for assay.

Procedure:

Preparation of the incubation mixture was carried out

as shown below:

Incubation mixture	volume µl	amount µmole	final M
Enzyme	10-20		
Phosphate buffer, 1.0 M, pH 7.6	5	5	1×10^{-1}
Magnesium Chloride, 0.1 M	5	0.5	1×10^{-2}
Epinephrine-D-bitartarate 3 mM	5	0.015	3×10^{-4}
SAM-methyl-H^3, 50mCi/m mole	10	0.002	4×10^{-5}
SAM 1.0 mM	10	0.01	2×10^{-4}
Water	to 50 ul.		

Incubation of the reaction mixture was carried out at 37°C for 20 minutes. The reaction was stopped by adding 0.5 ml of borate buffer, pH 11.0. The metanephrine was extracted in 5 ml of toluene:isoamyl alcohol (3:5 v/v). Two ml of this organic phase was transfered to a counting vial containing 10 ml of Bray's scintillation solution and

counted in a scintillation counter (Beckman-Sc counter).

Calculation (Axelrod,1962):

The calculation of the enzyme activity was from the formula:

Activity ($nmol.g^{-1}.min^{-1}$) =

(SAM nmol/SAM cpm) X (experimental cpm - Blank cpm)

AMMONIA:

Ammonia was estimated by the Phenol-hypochlorate reaction, described by Weatherburn(1967). The ammonium ion in blood and brain tissue homogenate is converted to ammonia by alkalinization with a buffered potassium carbonate/potassium bicarbonate mixture. The ammonia is isolated and color is developed in solution by a phenol-hypochlorate method after which the optical density is measured spectrophotometrically.

AMINO ACIDS:

Treatment of blood:

Equal amounts of citrate buffer pH 2.0 was added to serum, mixed well and kept at room temperature for 30 minutes. Protein was separated with 80% alcohol and free amino acid was extracted twice. Alcohol was removed from the final extract by flash evaporation or on a water bath at 50C under a stream of nitrogen. The pH of the concentrate was adjusted to 2.0 and made up to 2.0 ml with distilled water. 0.05 - 0.1 ml of this extract was used for analysis by the procedure of Benson et al.(1967).

Treatment of brain:

The brain samples were homogenized in ice cold citrate buffer pH 2.0. The homogenate was left at room temperature for 10 minutes and then 80% ethanol was added and the samples were homogenized again. The final homogenate was centrifuged at 2000g for 15 minutes and the supernatant was separated. Alcohol was removed from the final extract by

evaporation at reduced pressure. The concentrated sample was adjusted to pH 2.0 and diluted to 2.0 ml. 0.05 - 0.1 ml of the extract was used for amino acid analysis as described above for the blood samples.

STATISTICS

An SPSS-6 computer program was used to determine the significance between two means by using the student t-test. Any difference with p less than 0.05 was taken as significant.

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