STUDIES ON RAT CEREBRAL GLUCOSE METABOLISM IN HYPERAMMONEMIA

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This is to certify that I, **L. RATNAKUHARI** have carried out the work embodied in the thesis for the full period prescribed under the Ph.D. Ordinances of the University.

I declare to the best of my knowledge that no part of this thesis was earlier submitted for the award of research degree of any University.

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INTRODUCTION

Ammonia, a normal constituent of blood, tissues and tissue fluids, originates from the catabolism of nitrogenous compounds. Unlike carbohydrates and lipids (which can be stored in any form without deleterious effects). Hence, various mechanisms have evolved in the animal kingdom to maintain and guard the body ammonia levels. As a result of this a wide range of tolerance to ammonia is seen in animal kingdom. Despite this, it must be emphasized that the tolerance is only of limited range and elevation in ammonia levels beyond this range is often lethal to organisms.

Very few instances are noticed wherein animals are directly exposed to toxic levels of ammonia (gaseous form) or to ammonium ions in nature. However, there are several abberations in the nitrogen metabolism which may result in an elevation in ammonia levels in the blood and/or tissues of animals. Many such abberations have been noticed in man. No record of such abberations exists for animals other than man. This may be partly because of the lethal nature of such metabolic derangements or that they highly incapaciate the animal that the animal may become highly vulnerable for predations. Hence much of the information, on the consequence of elevated ammonia levels, is available for man or in laboratory animals where in such conditions are experimentally induced.

The pathological states arising out of altered nitrogen metabolism resulting in an elevation of blood ammonia levels are known aa hyperammonemic states. As ammonia rapidly equilibriates between blood and tissues, the tissue ammonia levels are also elevated in hyperammonemic states. At elevated concentrations, ammonia affects different tissues in different manner. However, its influence at elevated concentrations on the central nervous system is of greater importance. In fact, elevation in blood and brain ammonia levels is a potentially complicating factor in the etiology of several neurological abnormalities observed in several pathological states. Voluminous literature is available on the effects of ammonia on the central nervous system and on the mechanism of ammonia toxicity. Several reviews have appeared from time to time to summerize and highlight the available literature.

A review of the literature suggested that ammonia might art on brain in more than one way. Earlier investigators from this laboratory have worked on the cerebral metabolism of glutamate and of branched amino acids in hyperammonemic states.

The chief objective of the present study is to investigate the influence of hyperammonemic states on cerebral energy metabolism. Before the finer details of the work are

described and discussed, an overview of metabolism, detoxification and influence of ammonia on cerebral energy metabolism may be helpful. It is also felt worthwhile to give brief description of cerebral energy metabolism.

CEREBRAL AMMONIA LEVELS:

The ammonia content of the brain depends on the functional state at the time of study. Under normal physiological conditions the cerebral ammonia levels are reported to be between 0.15 and 0.35 µmoles/gm wet weight of the tissue (Cooper and Plum, 1987). Post-mortem changes were also found to have a profound influence on the determination of cerebral ammonia levels (Thorn and Helmann, 1958; Warren and Schenker, 1964; Tews and Stone, 1965; Godin et al., 1967; Tsukada, 1971; Hawkins et al., 1973; Hindfelt, 1975). The cerebral ammonia content increases during heightened neuronal activity and declines with the suppression in the neuronal activity. Information on the regional heterogenity in the distribution of ammonia in brain, both in normal and pathological states, is completely lacking. Such a study would be of interest, since the ammonia levels are used as an "index of neuronal activity" (Folbergrova et al., 1969).

CONDITIONS INFLUENCING CEREBRAL AMMONIA LEVELS:

As mentioned earlier, cerebral ammonia levels fluctuate

in parallel to the functional atate. A reduction in functional activity due to anasthesia, during sleep and hibernation, was found to be associated with a decrease in cerebral ammonia content (Whistler et al., 1968; Oner, 1971). An elevation of cerebral ammonia levela was found to be always associated with increased cerebral activity as seen during convulsions (Vrba, 1957; Richter and Dawson, 1948; Tsukada and Takagaki, 1958;Hathway et al., 1965; Hathway and Mallinson, 1964; Tews and Stone, 1965; Tsukada, 1966; Folbergrova et al., 1969; Faimen et al., 1977), deprivation of sleep (Haulica et al., 1970) and painful shocks to extreemities (Tsukada et al., 1958; Tsukada, 1971). Pathological states such as anoxia (Richter and Dawson, 1948), hypercapnia (Folbergrova et al., 1972a), hypoglycenia (Agardh et al., 1978), ischemia (Thorn and Heimann, 1958), audiogenic seizures (in susceptible animals) (Leonard, 1965), hepatic encephalopathy (Schenker et al., 1974; Zeive and Nicoloff, 1975), portocaval anastamoses (Holman and Sieajo, 1974; Williams et al., 1975) and congenital disorders in the metabolism of amino acids and of urea cycle enzymes (Colombo, 1971; Hsia, 1974; Schenker et al., 1974) were found to enhance cerebral ammonia levela.

SOURCES AND PRODUCTION OF AMMONIA IN BRAIN:

Steady state level of ammonia in brain is governed by

the relative rates of its transport from blood and/or extracellular fluids into brain, release from brain into blood and/or extracellular fluids, its rate of formation from both endogenous and exogenous sources and finally by ammonia utilizing processes. However, in the ensuing description, the role of extracellular space and extracellular fluids would be ignored aa the contribution by this compartment to total cerebral volume was shown to be insignificant (accounting to 2-5* of total volume: Schultz, 1957; Luce, 1960).

TRANSPORT OF AMMONIA FROM BLOOD INTO BRAIN:

Though it is well known that blood ammonia levels are strictly guarded and maintained well below the tissue levels (blood ammonia < 0.1 mM), its contribution to tissue pools cannot be ignored. This process assumes a greater importance especially in hyperammonemic states, when the blood ammonia levels would be higher than usual. In blood, it was shown that the level of ammonia in red blood corpuscles was higher than plasma (Caesar, 1962; Hindfelt, 1975). Several lines of evidences indicated that either the blood or extracellular ammonia rapidly equilibrates with tissue pools for eg., systemic administration of ammonium salts increased the brain ammonia levels in a very short time (Hawkins et al., 1971; Lockwood et al., 1979; Duffy et al., 1983; Cooper et al.. 1988). Of the mechanisms proposed for ammonia transport

across the blood brain barrier, the pH gradient theory has been well accepted (Millene et al., 1958; Stabenau et al., 1959).

Ammonia can exist in two forms - the unprotonated NH_3 form and the protonated NH_4 form. These two forms are in a state of dynamic equilibrium in solution and their inter-conversion was found to be rapid and is dependent on pH of the medium (Cooper and plum, 1987). At alkaline pH, NH_4 dissociates into NH_3 and H and at acidic pH the NH_3 form would be converted to NH_4 form by accepting a proton. However, at physiological pH (7.4) at least 98% of ammonia is in NH_4^+ form. In this state the molecule has a limited permeability across the plasma membrane while the NH_3 form penetrates the plasma membrane with ease as it is more lipid soluble.

Though, brain ammonia concentrations were found to be lower than blood, roost of the blood ammonia enters the brain. It was observed that under physiological conditions, the pH of brain was lower than the blood pH (Hindfelt, 1975). The NH_3 form of ammonia from blood would then rapidly enter the brain as it is more freely permeable. This perturbs the equilibrium between protonated and unprotonated forms and favours the dissociation of additional NH_4^+ to NH in blood. Evidences for the pH dependent transport of ammonia were provided by the following observations. (a) The tissue (brain and muscle)

ammonia concentrations increased by 2-3 fold during metabolic and respiratory alkalosis (Stabenau et al., 1959). (b) Variation in the LD_{50} dose of various ammonium salts was directly related to their ability to induce changes in the blood pH (Warren and Nathon, 1958). Alkaline ammonium salts NH_4OH, $(NH_4)_2CO_3$ were found to be more lethal even at very low doses than acidic salts of ammonia (NH,C1) (SCOA, 1979). (c) Acidosis induced by injection of hydrochloric acid had a protective effect on the mice injected with ammonium chloride (SCOA, 1979).

PRODUCTION OF AMMONIA FROM **ENDOGENOUS AND EXOGENOUS** SUBSTRATES:

The inherent ability of brain to produce ammonia from ita endogenous substrates was conclusively demonstrated. In in vitro studies, cortical slices produced ammonia at a rate of 14 - *16* µmoles/gm wet weight. The results in these studies suggested that ammonia production in brain could be an aerobic process (Benjamin and Quastel, 1975). Such a high rate of endogenous ammonia production in brain elicited interest in the search for a precursor to this process. In summary, the three major precursors proposed to serve as substrates were proteins, amino acids and nucleic acids (Benjamin, 1982).

PROTEINS:

Proteins as a source for endogenous ammonia was sugge-

ated long ago (Weil-Malherbe and Green, 1955; Vrba, 1957). It haa been shown that of the total ammonia formed, during in vitro incubation of brain tissue in the absence of a substrate, atleast 25 - 35% was accounted by the deamidation of protein bound amide groups (Vrba, 1957; Wherrett and Tower, 1971) .

AMINO ACIDS:

The free amino acid pool of brain differs from that of other tissues by the presence of large quantities of glutamate family of amino acids (glutamate, glutamine, aspartate, alanine and γ-aminobutyric acid). The glutamate concentration in the brains of various mammals was found to be approximately 10 umoles/gm. wet weight while that of glutamine was 4 - 5 umolea (McIlwain and Bachelard, 1971). As these two amino acids occur in such large concentrations, much attention was focussed on ammonia production from these amino acids. It was indicated that glutamate contributes by about 30 - 50% to the total ammonia formed during in vitro incubation of cortical slices (Benjamin and Quastel, 1974,1975). It was proposed that ammonia liberation occurs majorly from the neurons (Benjamin and Quastel, 1974). Of exogenous glutamate taken up by brain, only 14% would be converted to ammonia, majorly *in* neurons (Benjamin and Quaatel, 1974). The glial cells as per these authors, do not produce ammonia. However, recent results suggest that glial cells are capable of oxidizing

glutamate completely to 2-oxoglutarate (Yu et al., 1982; Hertz et al., 1987; Lai et al., 1989).

During in vitro incubations of cortical slices, ammonia production was observed to be associated with a fall in the content of glutamine besides glutamate (Benjamin and Quastel, 1975). 30% of the ammonia formed could be accounted by the fall in the glutamine content. This value appears to be high as later studies indicated that ammonia production from exogenous glutamine might proceed at a lower rate, which may be due to the inhibition of the deamidation process by endogenous glutamate and ammonia (Benjamin, 1981). Recent evidence indicates that most of the nitrogen from glutamine is incorporated into amino acids such as alanine than into ammonia atleast in astrocytes (Yudkoff et al., 1988). The contribution made by amlno acids other than glutamate and glutamine apears to be negligible.

PURINE NUCLEOTIDES:

It was also postulated that the purine nucleotides, especially AMP and adenosine might serve as precursors for ammonia production by a process of deamination in the purine nucleotide cycle (Benjamin, 1982).

ENZYMES INVOLVED IN AMMONIA PRODUCTION:

As the major precursors in cerebral ammonia production

were shown to be proteins, glutamate, glutamine and purine nucleotides, a brief account of the enzymes involved in this process are given below.

Though, the contribution made by protein bound amide group is significant, it is surpriaing to note that no enzymatic mechanisms have been so far described in the literature. The only study that gives a clue to the enzymatic mechanism involved waa the inhibition of this process by methionine sulphoximine (Wherrett and Tower, 1971).

Normally, the primary step in the metabolism of most of the amino acids is the transamination wherein the α-amino group la transferred to an α-ketoacid to form a corresponding amino acid. Though both oxaloacetate and pyruvate have the ability to participate in these reactions, in most of the transamination reactions 2-oxoglutarate is the preferred substrate. Thi. results in the formation of glutamate which is later oxidized to produe ammonia in glutamate dehydrogenaae reaction. This pathway la known as transdeamination pathway. Of all the aminotransferases preaent in brain, the activity of aspartate aminotransferae waa found to be very high (Klingenberg and Pette, 1962; Balazs, 1965; Benuck et al., 1971a ; Lai et al., 1975; Dennia et al., 1976) followed by alanine aminotransferase. Both the enzymes were found to have a dual localization in the cells, i.e., both in mito-

chondria and cytosol. Because of the high activity of aspartate aminotransferase, it was shown that at least 80% *at* glutamate is metabolized through the transamination pathway (Balazs, 1965). This enzyme is also known to form stable macromolecular complexes with glutamate dehydrogenase, fumarase, malate dehydrogenase and citrate synthetase, though the significance of this process is yet to be understood (Churchich, 1978).

Hydrolytic cleavage of fllutamine would also ammonia from glutamine and the enzyme responsible for this process, glutaminase, was found to be present in brain. Two major isozymes of glutaminase have been identified in mammals (Katunuma et al., 1967). The phosphate independent glutaminase is supposed to be absent in brain (Weil-Malherbe, 1969,1972; Weil-Malherbe and Beall, 1970; Huang and Kerox, 1974; Kvamme and Olson, 1979,1981) or even if present it is less than 10% of the total glutamlnase activity (Temma, 1972, Nimmo and Tipton, 1979).

In the brain, 90% of the glutaminase activity was found to be due to phosphate activated form which is a mitochondrial enzyme and is subjected to feed back inhibition by ammonia and glutamate. Recent studies indicated a complex pattern of regulation of this enzyme in brain which depends on its cellular localization (Kvamme et al., 1982). The

enzyme localized in the nerve ending particles was found to be inhibited by both glutamate and ammonia while the astroglial enzyme was found to be inhibited by glutamate alone. The enzyme from both the sources is activated by calcium ions (Benjamin, 1981; Kvamme et al., 1982). Besides generating ammonia, this enzyme is supposed to play a major role in generating the neurotransmitters such as glutamate and GABA from glutamine (Bradford and Ward, 1976; Bradford et al., 1978). However, it is believed that the activity of thia enzyme under physiological conditions is not fully expressed due to high glutamate levels which are inhibitory to its activity (Benjamin, 1982).

AMMONIA PRODUCTION FROM PURINE NUCLEOTIDES:

Ammonia is also generated by the deamination o£ AMP mediated by the enzyme AMP-deaminase. AMP-deaminase is a cytosolic enzyme and exhibits allosteric properties. ATP acts as an allosteric activator and GTP as an allosteric inhibitor. It ia believed that under physiological conditions, the inhibitory effect by GTP is far more than the ATP activation. Its activity was also found to be stimulated by K^+ and thia inorganic ion could be substituted by Rb^+ and NH^+_4 (Setlow et al., 1966; Setlow and Lowenstein, 1967).

In addition to AMP, adenosine was also shown to be deaminated in the brain by the enzyme adenosine deaminase

(Buniatin, 1970). Hence, AMP might also be converted to adenosine by the enzyme 5'-nucleotidase and later deaminated. The presence of this latter enzyme was also demonstrated in brain and it was reported to be localized exclusively on the astroglial plasma membrane (Kreutzberg et al., 1978).

MINOR REACTIONS:

Besides the above three processes, ammonia is also generated in small quantities from other sources such as guanine and guanosine deaminases, NAD^+-deaminase, glucosamine-6-phosphate deaminase, monoamine oxidaae, serine dehydratase, histidase, etc.

As said earlier, the steady state levels of ammonia depends not only on the rate of transport and its endogenous synthesis, but also on the rate of its disposal or utilization.

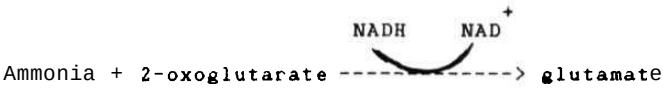
DISPOSAL OF AMMONIA:

Unlike the multitude of ammonia generating processes, only three pathways exist in animals sytems to dispose of ammonia. They are (a) carbomoyl phosphate biosynthesis (b) glutamate and (c) glutamine biosynthesis. As the carbomoyl phosphate synthesis is negligible in brain (Sadasivudu and Rao, 1974, 1976), the latter two processes would be

discussed.

GLUTAMATE BIOSYNTHESIS:

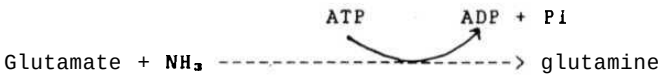
The enzyme involved, in fixing inorganic ammonia into organic linkage of glutamate, is glutamate dehydrogenase, the description of which was given earlier.



Under physiological conditions, the equilibrium of this enzyme catalyzed reaction was supposed to favour glutamate biosynthesis. However, the contribution of this pathway to the total pool of cerebral glutamate has been questioned recently (Cooper and Plum, 1987).

GLUTAMINE SYNTHESIS:

The major pathway of ammonia detoxification in brain is supposed to be glutamine biosynthesis (Weil-Malherbe, 1950; Cooper et al., 1979,1985; Waniweski and Martin, 1986).



This reaction is mediated by the enzyme glutamine synthetase. This enzyme is ubiquitously distributed in all animal cells. In brain it is believed that this enzyme is exclu-

sively localized in the astroglial cells (Martinez-Hernandez et al., 1977; Norenberg and Martinez-Hernandez, 1979). Though much work is done on the regulation of bacterial glutamine synthetase, not much information is available on the brain enzyme and the available evidences indicate that this enzyme from animal tissues is not subjected to regulation by various Uganda as the bacterial enzyme (Lehninger, 1984).

METABOLIC COMPARTMENTATION AND AMMONIA IN BRAIN

Studies on metabolic compartmentation revealed the existence of two metabolic pools of glutamate, of which one id larger and the other is smaller. It was postulated that the large pool of glutamate contains moat of the tissue glutamate pool and has a slow turnover rate while small pool of glutamate contains less glutamate and has a rapid turnover rate. These two pools do not mix with each other. Further, it was shown that the rate of synthesis of glutamine was less in the large pool than in the small pool. Glutamate in the large pool would be metabolized by oxidative deamination while in the small pool it would be majorly involved in glutamine synthesis. The precursor for glutamate in the large pool was shown to be glucose while in the small pool besides glucose, B-hydroxybutyrate, acetate, carbon dioxide, butyrate, propionate, citrate, leucine, etc., would serve as prepursors (Balazs et al., 1972; Van Den Berg, 1972; Clark et al., 1974;

Mohler et al., 1974; Van Den Berg et al., 1974). Further studies revealed that the large pool of glutamate is localized in the neurons and the small pool in the glia (Benjamin and Quastel, 1972; Van Den Berg et al., 1974; Garfinkel, 1966,1972; Clarke et al., 1974). This suggested that the metabolism of glutamate would be different in the neurons and glia. The neurons metabolize glutamate primarily by oxidative deamination while in glial cells major portion of glutamate would be converted to glutamlne and rest is transaminated to 2-oxoglutarate prior to the oxidation through citric acid cycle (Benjamin and Quastel, 1974). Higher activities of glutamate dehydrogenase in neurons than in the glia (Rose, 1968) and the presence of high affinity uptake systems for glutamate in glia (Henn et al., 1974; Henn, 1976; Ueiler et al., 1979) supported this concept. The glutamine so formed in the glia would be transported to nerve endings where it would serve as a precursor for releaseable pool of glutamate and GABA. This concept received support from the observation that exogenous glutamine would serve as a better precursor for releasable pool of both glutamate and GABA in synaptosomes (Bradford and Uard, 1976; Bradford et al., 1978) and in slices (Hamberger et al., 1979a,b) and the activity of glutaminase was high in synaptosomes than in the astrocytes (Ueiler et al., 1979). Further, histochemical and, biochemical localization of the enzyme glutamine synthetase exclusively

in astrocytea favoured this concept (Martinez-Hernandez et al., 1977; Norenberg and Martinez-Hernandez, 1979).

Thus, according to the the · y of metabolic comparmentation, ammonia would be generated by neurons and nerve endings from both glutamate and glutamine and liberated along with glutamate and GABA during functiona states. The exogenous ammonia, glutamate and GABA would be transported into the astrocytes where ammonia is converted to glutamine. The glutamine of the astroglial cells would be transported into nerve endings where it is reconverted to glutamate and related neurotransmitters. However, some of the recent obacrvations do not support this concept in toto (Kvamme et al., 1982; Yu et al., 1984, Fonnum 1984; Subbalakshmi and Murthy, 1985).

PATHOLOGICAL CONDITIONS ASSOCIATED WITH HYPERAMMONEMIA;

As mentioned earlier, in hyperammonemic states there would be an elevation in both blood and tissue levels of ammonia resulting in a metabolic and functional derangement. Such hyperammonemic states could be due to defects either in the synthesis, transport or disposal of ammonia. The hyperammonemic states known so far might be categorized into congenital or acquired disorders. A brief description of the known states of hyperammonemia would ensue below, but detailed descriptions are available in several reviews

(Schenker et al., 1974; Zeive and Nicoloff, 1975; Fischer and Baldessarini, 1976; Crossy et al., 1983).

CONGENITAL DISORDERS:

In congenital hyperammonemic states defects were found in the enzymes directly related to ammonia metabolism on one hand an on the other hand several defects in the enzymes involved in the metabolism of amino acids not related to ammonia were noticed.

One of congenital disorder with defective glutamate synthesis was reported to be Reye's syndrome. The defect in this syndrome is not in the enzymes of glutamate biosynthesis, but in the fragility of mitochondrial membranes which would be subjected to an acute insult (Partin et al., 1971; Bove et al., 1979). Consequently, the integrity of these membranes would be lost leading to the liberation of all the mitochondrial enzymes inclusive of glutamate dehydrogenase and carbomoyl phosphate synthetase which aggrevates the clinical condition (Brown et al., 1976; Robinson et al., 1978). Recently, it was shown that an endogenous inhibitor was bound to glutamate dehydrogenase which would lower the residual activity of glutamate dehydrogenase (Holt et al., 1983). Due to the loss of both glutamate dehydrogenase and carbamyl phosphate synthetase, ammonia accumulates in the blood and

tissues. Another set of disorders with defective gluamate dehydrogenase in brain is the Olivopontocerebellar atrophy. However, it is not known whether a hyperammonemic state prevails in this situation (Plaitakis et al., 1980, 1981a,b,1982,1983; Plaitakis and Berl, 1983).

No reports are available on congenital defects in glutamine biosynthesis which might be because of the premature death of the embryos as glutamine has several other roles to play besides serving as an ammonia trap.

Several congenital disorders of urea cycle enzymes have been reported. Clinical symptoms and magnitude of hyperammonemia varies from mild to severe depending on the enzyme affected and degree of metabolic derangement. Total or partial deficiency of carbamoyl phosphate synthetase, orthine transcarbamoylase and argininosucinic acid synthetase are some of the reported disorders associated with hyperammonemia (Hsia, 1974). Hyperammonemia was also observed in several other inherited disorders of amino acid metabolism such as ornithinemia, hyperlysinemia, maple syrup disease and hypervalinemia etc.,(Hsia, 1974).

ACQUIRED HYPERAMMONEMIC STATES:

A direct relationship between hepatic functioning and hyperammonemic states has been established. In several of the

reported acquired hypermmonemic states a severe deficiency of hepatic functioning was observed. The spectrum of neurological symptoms due to hepatic inadequacy was found to be highly variable and the term "hepatic encephalopathy" was coined to describe the changes. In the early stages of hepatic encephalopathy mental, personality and emotional changes were found to be very subtle. With the progress of encephalopathy, hypothermia, hyperventillation, confusion, drowsiness ensue which gradually lead to comatose condition. Neuromuscular changes like asterixis, hyper-reflexia, unsustained clonus were also noticed in early stages of hepatic encephalopathy which disappear along with pupillary and corneal reflexes with the progress of the disease. Convulsions, decerebrate rigidity and decortical posture were found occasionally (Schenker et al., 1974). Hepatic encephalopathy was found to be of two types. Exogenous hepatic coma or chronic liver disease due to decreased liver mass and gradual shunting of blood around liver (liver bypass) is one such condition. These conditions are seen usually in cirrhosis and portal systemic shunting and the onset of neurological and other clinical symptoms are less violent and precipitated by exogenous factors such as high protein diet or by drugs. Acute liver disease (endogenous hepatic coma) is the second one in which the decrease in functional liver mass is due to acute viral infections, drugs and other toxins and the onset

of clinical symptoms are very rapid and sudden.

Cerebral pathology varies with the duration and type of hepatic coma. In acute hepatic encephalopathy cerebral edema is a prominent feature (Ware et al., 1971), while in chronic hepatic encephalopathy, hypertrophy and hyperplasia of protoplasmic astrocytes are of common occurance. These **changes** are seen principally in cerebral cortex and in other regions of brain (Waggoner and Malamud, 1942). Neuronal cells do not show many of these chances in the acute hepatic encephalopathy while in chronic conditions degeneration and demyelination are exhibited (Zieve et al., 1960). However, the major change is usually seen in the protoplasmic astrocytes. In chronic hepatic encephalopathy, these cells proliferate, enlarge and finally undergo hydropic degeneration. The changes induced would result in the formation of Alzheimer type II astroglial cells (Adams and Foley, 1953). The Alzheimer type II cells were also found in experimental hepatic encephalopathy due to portocaval anastamosis in rats (Cavanagh and Kyu, 1971; Zamora et al., 1973; Cavanagh, 1974) and in induced hyperammonemias (Lapham, 1961, Mossakowski et al., 1970; Gibson et al., 1974; Norenberg et al., 1972; Norenberg and Lapham, 1974; Norenberg, 1977, 1981; Gregorios et al., 1985a,b). The development of Alzheimer cells were reported to be associated with the loss of filial acidic fibrillary protein in the grey matter (Sobel et al., 1981). The Alzheimer

changes in the glial cells under these conditions were believed to be due to abnormal biochemical events leading to altered electrolyte balance and other transport phenomena (Zamora et al., 1973 Cavanagh, 1974).

Ammonia was inculcated since a long time in the pathogenesis of hepatic encephalopathy. Increased levels of ammonia in blood and cerebrospinal fluid is a prominent laboratory finding in patients with hepatic encephalopathy. The major site of origin of ammonia in these patients was established to be intestine (Schenker et al., 1974; Zeive and Nicoloff, 1975; Onstad and Zieve, 1979). Dietary amino acids and urea (endogenous) are deaminated in the intestinal bacteria to generate ammonia. In the absence of functional liver, this ammonia enters the systemic circulation. Tissue ammonia levels rapidly equilibrate with blood ammonia levels and as a result an overall hyperammonemic condition sets In. Increased ammonia levels are supposed to deplete citric acid cycle intermediates and the energy states of the cell (due to glutamine formation) and this is believed to result in a broad spectrum of neurological changes (Schenker et al., 1974). Glial cells, as the seats of glutamine synthesis, were supposed to be involved in the detoxification process, and loose their energy resources which would be reflected in their morphology (Norenberg and Lapham, 1974; Norenberg,

1977). However, no direct relationship between the blood ammonia level and extent of coma has been established. Instead a direct relationship with a good correlation exista between glutamine levels in cerebrospinal fluid and clinical state of coma. Alternate hypotheses, with out the involvement of ammonia, have also been proposed to explain the pathogenesis of hepatic coma. These include the production of false neurotransmitters (Fischer and Baldessarini, 1976), involvement of short chain fatty acids (Zeive, 1982, 1985; Zeive and Brunner, 1985), mercaptans (Zeive, 1980; Zeive et. al., 1983) and altered GABAergic neurotransmission (Zenroli et al., 1982; Ferenci et al., 1982, 1983; Pappas et al., 1984)..

These hypothesis are proposed chelfly to explain the cerebral dysfunction in states of deranged hepatic function. Though such conditions are usually associated with hyperammonemia, the derangement of hepatic homeostatic mechansims also play a vital role. Presently only those hypotheses concerned with the direct action of ammonium ions are considered as in this study hyperammonemia was induced in animals by administering ammonium acetate. These animals had functionally intact livers at the time of the study. The hypotheses that were proposed to explain the direct action of ammonia are on the effects of this substance on the ionic fluxes and on cerebral energy metabolism. As the presnt work is on cerebral

energy metabolism, information available on this aspect has been reviewed in a greater detail than that on ionic fluxes.

EFFECTS OF AMMONIA ON IONIC FLUXES:

Maintainance of ionic gradients is essential for cerebral activity. It has been observed that about one-third of the energy synthesised in the brain is utilized for maintenance of the ionic gradients especially by Na^+ ,K^+ - ATPase (Berl, 1974). Changes in the ionic composition also has a wide range of effects on the cerebral metabolism (Lowry and Passonneau, 1966). In addition to these metabolic effects, ionic gradients also have a vital role in the release and uptake of the neurotransmitter and on the genesis and maintenance of resting membrane potential of neuron. Ammonium ions were shown to have a biphasic action on neuronal excitability. At low concentration they were shown to cause a stimulation while at higher concentration they suppress the neuronal function. As neuronal excitability is associated with the maintainance of ionic gradients, much attention was focussed on this aspect.

The ionic radii of the ammonium and K^+ ions (with hydration shells) were found to be similar. It was shown that ammonium ions can replace potassium ions for Na^+,K^+-ATPase. Activity of this enzyme was observed to increase in hyper

ammonemic states, which might alter the ionic gradients and the resting membrane potential (Hawkins et al., 1973; Sadasivudu et al., 1977,1975; Subbalakshmi and Murthy, 1981). As thia enzyme is also involved in the release of neurotransmitters, any changes in the activity of this enzyme in hyperammonemic states might also affect the release of neurotransmitters. Ammonium ions were also shown to inhibit the outwardly directed chloride pump which results in the disinhibition of the post synaptic neurons (Lux et al., 1970; Lux, 1971; Llinaa et al., 1974a,b; Raabe and Gunmit, 1975). Benjamin et al., (1978) reported an influx of chloride ions into the brain slices in the presence of ammonium ions. It is possible that these changes in the ionic fluxes may be associated with the reported changes in the fluidity of the neuronal membranes in the presence of ammonium ions (O'Connor et al., 1982,1984).

EFFECTS OF AMMONIA ON CEREBRAL ENERGY METABOLISM:

One of the very old and controversial concepts is that ammonia affects the cerebral energy metabolism. Acute administration of ammonium salts enhances the A-V difference for glucose across blood brain barrier suggesting an increase in the ceebral utilization of glucose in hyperammonemic states (Hawkins et al 1973). Under these conditions, a fall in cerebral glycogen levels was also observed (Hawkins et al.,

1973). However, glycogen content was found to increase in chronic hyperammonemic states (Norenberg, 1981). Ammonia is known to stimulate the activity of phosphofructokinase, a rate limiting enzyme in the glycolytic pathway thus channel ling more glucose into this pathway (Lowry and Passonneau, 1966). Hawkins et al., (1973) reported an increase in the levels of the glycolytic intermediates in brain in acute hyperammonemic condition. Pathophysiological concentration of ammonia was observed to elevate cerebral lactic acid both in vivo and in vitro conditions (Hawkin3 et al., 1973; Benjamin et al., 1978). It was postulated that the enhanced glucose utilization might account for the enhanced lactic acid production (Hawkins et al., 1973). However, studies in primary cultures of astrocytes and neurons indicated that ammonium ions in pathophysiological concentration (3mM) had no effect on the glucose oxidation (Lai et al., 1989).

Effects of ammonia on the operation of citric acid cycle is a subject of much controversy. It was postulated by Bessman and Bessman (1955) that ammonium ion promotes the synthesis of gluamate in the reaction mediated by glutamate dehydrogenase and thereby depletes cerebral 2-oxoglutarate levels and impairs the operation of the citric acid cycle and energy production in brain. In support of this hypothesis Eiseman et al., (1955) and Schenker and his associates (1967) observed a decrease in the cerebral 2-oxoglutarate levels

especially in the brain stem. McCandless and Schenker (1981) reported a decrease in the cerebral ATP levels in the micro-dissected areas of reticular activating system of the brain stem in hyperammonemic states. A similar observation was made by Bessman and Pal (1976, 1982). McKhan and Tower (1961) reported that high concentrations of ammonium chloride suppressed the oxidation of pyruvate and 2-oxoglutarate in the cerebral mitochondrial preparations. Additional support was provided by the observation of Lai and Cooper (1986) that ammonium ion inhibits the activity of 2-oxoglutarate dehydrogenase in the mitochondrial preparations.

Despite these evidences, this hypothesis is not accepted equivocally because of other contradictory observations. Hindfelt and Sieajo (1971) observed no changes in the contents of 2-oxoglutarate and ATP in brain in hyperammonemic states. Vergara et al., (1973) reported an increase in 2-oxoglutarate content in brain under these conditions. A similar observation was made by Hawkins et al., (1973). It is interesting to note that the oxidation of pyruvate was unaffected by the presence of pathophysiological concentrations of ammonia in primary cultures of astrocytes and neurons in the presence of glutamine (Hertz et al., 1987). Observations made in this laboratory also indicate that the activities of pyruvate dehydrogenase and the enzymes of

citric acid cycle are enhanced by the acute, subacute and durg induced hyperammonemic states suggesting an increased flux of the carbon skeleton through the citric acid cycle (Ratnakumari, 1984; Ratnakumari et al., 1985,1986; Ratnakumari and Murthy, 1989,1990). Thus, the effects of ammonia on the citric acid cycle and energy metabolism are yet to be resolved. These discrepancies on the effects of ammonia might be due to the differences in the experimental animals used in these studies, mode of induing hyperammonemia, methods adopted to arrest post-mortem changes and the regions selected for the analysis. Though controversial results were obtained in the levels of the metabolites of citric acid cycle and of ATP, it is quite possible that changes might occur in distinct cellular or subcellular metabolic compartments (Garfinkel and Hess, 1964; Garfinkel, 1966).

EFFECTS OF AMMONIA ON THE MALATE-ASPARTATE SHUTTLE:

Another hypothesis proposed to explain the neurotoxic effects of ammonia is its effects on the operation of the malate-aspartate shuttle (Hindfelt, 1975). This shuttle is responsible for the transport of reducing equivalents across the mitochondrial membrane. Of the several mechanisms regulating carbohydrate metabolism, the redox state of the cell, expressed as ratio of reduced pyridine nucleotides to oxidized pyridine nucleotides (NADH/NAD), is of prime impor-

tance. Several dehydrogenases participate in the oxidation of glucose, both in cytoaol and mitochondria, require a continuoua supply of NAD^+. It was shown that their activities are suppressed by excess of NADH, hence it is imminent that NADH generated must be reoxidized to NAD^+. In the mitochondria, NADH is oxidized in the elctron transport chain and thereby NAD is generated under aerobic conditions. Due to the lack of electron transport chain in the cytosol, NADH cannot be oxidized directly to NAD in this aubcellular compartment. Moreover, NADH is not exported from cytosol into mitochondria as the mitochondrial inner membrane is impermeable to these nucleotides. Therefore, it was proposed that NADH will bo converted to NAD in the cytosol itself and the reducing equivalents will be transported into the mitochondria in the form of metabolites. Several such shuttle mechansims have been proposed for the transport of reducing equivalents across the mitochondial membrane and of thes malate-aspartate shuttle is the major one in brain (Siesjo, 1978). This shuttle involves the participation of cytoaolic and mitochondi la] isozymes of aspartate aminotransferase and malate dehydrogenase, the mitochondrial dicarboxylic acid carriers and glutamate-aspartate carrier.

Operation of this shuttle la as follows. NADH generated in the cytoaol is utilized for the production of malate from oxaloacetate by the cytosolic malate dehydrogenase. Malate,

produced in the cytosol, is transported into mitochondria in exchange for mitochondrial 2-oxoglutarate. Mitochondrial malate dehydrogenase acts on malate and produces oxaloacetate which undergoes transamination with glutamate to produce 2-oxoglutarate and aspartate. 2-oxoglutarate transported into the cytoplasm, undergoes transamination with aspartate in a reaction mediated by cytosolic aspartate aminotransferase. As result, oxaloacetate is regenerated in the cytosol concomitant to the production of glutamate. Cytosolic glutamate is transported into mitochondria in exchange for mitochondrial aspartate and this process is mediated by the appropriate carrier protein (Siesjo, 1978). Importance of this shuttle for cerebral glycolysis has been repeatedly demonstrated. It has been shown that ihibition of aspartate aminotransferase activity with B-methylene aapartate would interfere with cerebral glucose metabolism, (Fitzpatrick et al., 1983). Elevation of $^{14}CO_2$ production from [1-^{14}C] pyruvate by aapartic acid in the primary cultures of astrocytes supports the concept that cytosolic oxaloacetate nerves as precursor for mitochondrial malate dehydrogenase (Murthy and Hertz, 1988). It has been postulated that in the presence of excess of ammonia cytosolic pool of glutamate is diverted from the malate-aspartate shuttle towards glutamine synthesis. Consequently operational rates of this shuttle will be affected and thus the cerebral glucose metabolism (Murthy and Hertz, 1988). Accumulation of

malate (Hindfelt and Siesjo, 1970) and decrease in levels of glutamate in brain (Cooper and Plum, 1987) in hyperammonemic states is in accordance with this concept. Similarly an increase in lactate production, lactate/pyruvate ratio, and cytosrlic NADH/NAD ratios provides support to this hypothesis (Hindfelt and Siesjo, 1970; Hawkins et al., 1973; Polli, 1971). It was also shown recently that the production of $^{14}CO_2$ from [1-^{14}C] pyruvate in primary cultures of astrocytes was suppressed by the addition of pathophysiological concentrations of ammonium acetate (3mM) in the absence of glutaraate or glutamine. This effect was abolished by the addition of gluatamate, aspartate and glutamine (Murthy and Hertz, 1988). Thus, precursors which replenish the cytosolic glutamate pool in hyperammonemic states are capable of supporting the operation of malate-aspartate shuttle even in the presence of ammonia. Thus, it appears to be true that ammonia affects cerebral energy metabolism, but the mechanism is different from that proposed by Bessman and Bessman (1955).

Thus it appears from the above that the effects of ammonia on cerebral dysfunction especially due to deranged cerebral energy metabolism is an enigma. The main reason for this appears to be lack of information on the effects of ammonia on specific metabolic compartments of brain. One way to approach this problem ia to understand the metabolism *at*

glucose in these compartments and the interrelationships between these compartments while they metabolize glucose in normoammonemic and in hyperammonemic states. As these metabolic compartments have distinct cellular localization, it may be worthwhile to study the above processes in the cells isolated from normal and experimental animals or conduct such studies in cells in primary cultures. The information gained from such in vitro studies may later be extended to in vivo situation. Though the availability of cell cultures has simplified this problem the results obtained from such studies must be cautiously interpreted as the cells in in vivo states are exposed to temporal and vectoral dynamic changes in the blood and brain and due to this metabolism might be different from that observed in vitro situation. Hence, in the present study, various cellular and subcellular fractions isolated from normal and hyperammonemic rats were used to study glucose metabolism. It has been assumed that whatever changes that have taken place in these metabolic compartments are retained while they are being fractionated. Experiments of the present study do support this assumption.

As was mentioned earlier, a brief review of the salient aspects of cerebral energy metabolism is given below.

It is well established that brain function is critically dependent on its energy metabolism. Any alterations in

the energy are known to be associated with neural disfunction and viceversa. Conditions auch as anoxia, ischemia, hypoglycemia which alter cerebral energy reserved usually results not only in neuronal dysfunction but also neuronal death. Moreover, brain has a very high metabolic rate when compared to other tissues. ihough brain is only 2* of the total body weight, its oxygen consumption is about 20% of total oxygen consumption of the body. The cerebral energy stores are utilized not only for synthesis of various molecules and transport of various ligands, they are also used for the maintenance of ionic gradients. In fact, it has been shown that about 1/3 of the total cerebral energy stores are spent tor this purpose (Berl, 1971). This is understandable, as maintenance of ionic gradients is very essential for electrical activity of the brain.

Despite such dependence on energy metabolism, brain can utilize very few subatrates for its energy metabolism. In fact, for long time it was believed that brain can utilize glucose for its energy metabolism. However, recent studies have indicated that brain has the capacity to utilize ketone bodies (during development and during prolonged starvation) and amino acids such as glutamate, aspartate, glutamine and branched-chain amino acids (Hawkins et al., 1971). However, glucose is the best known substrate for the maintenance of cerebral energy metabolism.

Cerebral glucose utilization, under normal physiological conditions, varies from species to species and even in a given species it varies with the physiological status of the animal. In addition, even under a particular physiological state it differs from region to region in brain. In general, the white matter regions have a lesser rate of glucose utilization than grey matter regions.

Despite such a critical dependence on glucose utilization, brain has very little free glucose content. In fact, free glucose can only be detected in the brain only if its metabolism is arrested or slow down considerably prior to the isolation of the brain from the animal. This is usually done eithe by rapid freezing of brain to subzero temperatures (liquid nitrogen temperature; -180°C) or by application of specific inhibitors of various intermediary reactions or by rapidly heating the brain to 60- 70°C with the help of the microwaves. Tissues like liver and muscle have adequate stores of glycogen which would be utilized if the need arises. However, in brain the glycogen stores are meager and are not adequate to support its metabolism for prolonged periods (Karnovsky et al., 1980).

For reasons mentioned above, brain glucose consumption is dependent on the supply of glucose through the blood. Glucose is transported from blood into brain by means of a

stereo specific saturable carrier mediated fecilitated transport mechanism (Fishman, 1964). Glucose transport into brain has been measured by using labelled glucose or deoxyglucose (Bachelard, 1971; Sokoloff, 1977; Bachelard et al. . 1973). In recent years, glucose labelled with positron emitting nucleids is also used to study glucose transport into brain. These studies indicated regional heterogenity in giucose transport in brain. The grey matter regions take up glucose much more avidly than the white matter regions. The Km of the glucose transporter across blood brain barrier for glucose appears to be between 6-10 umoles in several of the mammalian species studied (Growdon et al., 1971; Buschiazzo et al., 1970; Bachelard et al., 1973; Daniel et al., 197S; Pardridge and Oldendroff, 1977; Gilboe and Betz, 1970; Betz et al., 1973). Thus, the observed Km is close to plasma glucose concentrations. Any variations in plasma glucose concentrations may thus effect the transport of glucose across blood brain barrier. In fact, it has been shown that brain glucose levels were elevated rapidly when plasma fclucose levels are between 2-7 mmoles and much more slowly when plasma glucose levels are between 8-20 mmoles. The reported Vmax values for glucose transport across blood brain barrier is between 1-2 µmoles/gm/min. Glucose utilization by brain, determined by various methods is either less than or equal to the rate of glucose transport (0.6 to 1.0 µmoles/gm/min;

Hawkins et al., 1974; Sokoloff et al., 1977). Thus it appears that under normal physiological conditions, the transport of glucose across blood brain barrier ia not a rate limiting factor for cerebral glucoae utilization. However, any alterations either in blood supply or in glucose content (especially decrease as in hypoglycemla) would influence tin-availability of glucose to brain. Glucose uptake by brain wan also observed to change with fucntional states of brain and by drugs which alter these functional states such as depressants and convulsant .<.

Beyond blood brain barrier, glucose is avidly transported into various cell types and into nerve terminals by saturable carrier mediated mechanisms. The kinetic parameters of these celllar (as well as of synaptosomal) transport mechanism appear to be different from that of blood brain barrier. For example, the affinity of glucose transporter synaptosomes was reported to be 30 times higher than that of blood brain barrier (Diamond and Fishman, 1973). Thus these systems can operate even if extracellular glucose concentrations are lower than that of plasma. Similarly, astrocytes in primary cultures were shown to have a Km of 0.37 mmoles with Vmax of about 30 nmoles/mg protein/min (Cummins et al., 1979).

METABOLIC FATE OF GLUCOSE **IN BRAIN:**

The glucose that enters the brain and is taken up into cells and into nerve terminals has multiple metabolic fate. Thus in these cerebral components glucose may be utilized for glycogen synthesis, or it may enter into hexosemonophosphate shunt or into glycolytic pathway. In the former pathway glucose is converted into various other sugars ranging from tetroses to heptoses (if it is F type of pathway) or octoses (if it is L type of pathway). In the glycolytic pathway glucose is converted to lactate and pyruvatre and the latter would be oxidized in the citric acid cycle. A brief account of the metabolic fate of glucose in these pathways is **given** below:

GLYCOGEN METABOLISM:

Glycogen is an unique energy reserve which requires no ATP for the initiation of its metabolism. As was mentioned earlier, brain glycogen content is very low when compared to the tissues like liver and muscle. In brain glycogen content is about 2-4 µmoles/gm wet wt of tissue and it varies with plasma glucose content and functional status of the brain. As the cerebral glucose utilization is about 1-2 µmoles/gm/min the cerebral glycogen stores can sustain the brain energy metabolism for not more than 5 min after the cassation of the blood supply. Despite such a low content, brain glycogen is

dynamic as there is a rapid, continuous breakdown and synthesis of glycogen (17 μmoles/kg/min; Watanabe and Passonneau, 1973). Brain glycogen content also responds to variety of conditions. It increases during anesthesia (Passonneau et al., 1971), ethanol intoxication (Veloso et al., 1972), recovery from ischemia (Mrsulja et al., 1976), porto-caval anastomosis (Cavanagh and Kyu, 1971; Zamora et al., 1973; Norenberg and Lapham,1974), methionine sulphoximine intoxication (Phelps, 1975; Norenberg and Lapham, 1974; Hevor and Gayet, 1981) and conditions of traumatic injury to central nervous system (Farkas-Bargeton et al., 1972; Uatanabe and Passonneau, 1974). Brain glycogen turnover rate is increased by glucocorticoids (Uatanabe and Passonneau, 1973). Cerebral glycogen stores are depleted when energy utilization exceeds production as in ischemia (Folbergrova et al., 1970; Mrsulja et al., 1976; Uatanabe and Ishii, 1976; Kobayashi et al., 1977) and during enhanced electrical activity as in convulsions (King et al., 1967; Ferrendelli and McDougal, 1971a, b; McCandless et al., 1979). The cerebral localization of glycogen seems to be a controversy. Phelps (1972) reported that glycogen granules are in neurons and glial cells of immature animals while they are restricted to astrocytes in adults. These results are not in agreement with those of Passonneau and Lowry (1971), Guth and Uatson (1968) and Sotelo and Palay, (1968) who reported the presence of glycogen in neu-

rons and glial cells. Recently Inone et al., (1988) demonstrated the presence of glycogen synthetase in neurons, astrocytes, ependymal cells, oligodendrocytes and cells of choroid plexus. Further, they have also reported that immunoreactivity to glycogen synthetase antibody was more intense *in* neurons than in glial cells.

Enzymes responsible for the synthesis and utilization of glycogen and their regulatory properties have been demonstrated in brain (Goldberg and O'Toole, 1969). Several neurotransmitters and hormones and even ions like K^+ were shown to influence the activities of these enzymes (Quach et al., 1978). Rate of synthesis obtained in vitro and in vivo were similar at 1-2 μmoles/gm/hr.

Four of the separate enzymic stages were involved in the synthesis of cerebral glycogen from glucose. The first one of these is hexokinase. It phosphorylates glucose to glucose-6-phosphate with the expense of one ATP molecule. This is common route for glycolysis, glycogen synthesis and hexosemonophosphate shunt. The route specific for glycogen commences with the conversion of glucose-6-phosphate to glucose-1-phosphate by phosphoglucosemutase. This is a reversible reaction. The next enzyme catalyzes the interconversion of glucose-1-phosphate and UTP with organic pyrophosphate and UDP-glucose. The last step in which glucose unit

from UDP-glucose adds to preformed glycogen is catalyzed by glycogen synthetase. Out of all these four enzymes glycogen synthetase reaction was observed to be the slowest one. Two forms of the synthetase have recently been detected which differ in sensitivity in glucose-6-phosphate activation. Interconversion of these two forms "D" (dependent on glucose-6-phosphate) and "I" (independent of glucose-6-phosphate) occurs enzymically and play an important role in controlling the cerebral glycogen stores.

HEXOSEMONOPHOSPHATE SHUNT PATHWAY:

There are several controversies regarding the contribution of this pathway to cerebral glucose metabolism. This pathway provides two essential components viz. riboae sugars and NADPH. The former are required for nucleic acid synthesis and the latter mostly for lipid synthesis. Depending on the method and the cerebral preparation used the contribution of this pathway for cerebral glucose metabolism has been estimated to be 35% (Sacks, 1965; Hotta and Seventko, 1968; Appel and Parrot, 1970; Hostetler and Landau, 1967; Hostetler et al., 1970; Hakim and Moss, 1972, 1974). From these reports it appears that the shunt pathway is definitely active in brain. However, in recent years, two types of this pathway i.e., F type and L type have been demonstrated. F type pathway is supposed to be restricted to fat cells and L. type to liver

and other tissues (Williams, 1980). There is no definitive evidence as to which type of pathway is present in brain. The enzymes of the pathway have been demonstrated in the brain. The most interesting observation is that 6-aminonicotinamide (an inhibitor of the pathway) prevents the incorporation of glucose label into glutamate (Gaitonde 1981). It has also been shown that this pathway is during cerebral development (O'Neil and Duffy, 1966). It was also reported that the turnover of glucose in this pathway would decrease under conditions of increasd energy demand (Kauffmann et al., 1969).

The major metabolic fate of glucose in brain seems to be its entry into glycolytic pathway. In general, glucose can be metabolized anaerobically (glycolysis) or aerobically (respiration). In aerobic cells glycolytic pathway has three main functions: (1) it constitutes the first obligatory and preparatory step for the further oxidation of glucose molecule (2) it provides glucose-6-phosphate for the hexosemonophosphate shunt which yeilds pentose sugars for synthesis of nucleotides, nucleic acids and NADPH for reductive synthesis of lipids and other macromolecules (3) it contributes significantly to energy production in situations where oxygen is lacking.

Glycolysis can be mainly devided into two stages. First

stage leads to the formation of 2 phosphorylated 3-carbon intermediates from glucose at the expense of two ATP molecules. The second stage is that involving conservation of energy.

The initial step in the glycolysis is the phosphorylation of the glucose to glucose-6-phosphate which is catalyzed by hexokinase, is an irreversible reaction. Cerebral tissues show high hexokinase activity in keeping with their dependence on carbohydrate metabolism. It has been studied extensively in cerebral preparations and its properties indicate that it can constitute a controlling factor in the metabolism of the glucose by brain in vivo (McIlwain and Bachelard, 1971). Hexokinase is regulated by available levels of substrate and products. Hexokinase occurs partly particulate and partly in cytoplasm (Crane and Sol, 1983). The mitochondrial enzyme exhibit chromatographic properties and kinetic behaviour similar to those of cytoplasmic system (Thompson and Bachelard, 1970). The association of hexokinase to the mitochondria depends upon the ATP/ADP ratios present in the tissues. The rate of available Mg^{2+} and ATP is important for the activity of this enzyme. This glucose-6-phosphate is reveraibly converted to fructose-6-phosphate by glucosephos phate isomerase. This fructose-6-phosphate gets phoaphorylated to fructose-1,6-diphosphate by phosphofructokinase at

the expense of one more ATP molecule.

The mechanism of this enzyme action is particularly complex and is relevant to its role as a major control point of glycolysis. It undergoes a peculiarly complex series of interrelated modifications of its activity, involving groups of inhibitors and activators or deinhibitors, including its substrates and products, each one of which may effect the action of another (Passonneau and Lowry, 1963; Lowry and Passonneau, 1976). Like hexokinase, the ratio of Mg^{2+}/ATP is critical for the enzyme activity. This enzyme activity ia inhibited by excess ATP one of the substrates, is augmented by another inhibitor, citrate. Inhibition by both is decreased by the other substrate, fructose-6-phosphate. ATP inhibition is also reduced by inorganic orthophosphate, 5'-AMP, cyclic 3'-5'AMP and monovalent cations (potassium or ammonium ions). The inhibition by citrate provides a point of regulatory contact between the pathways of glycolysis and citric acid cycle in which accumulation of one intermediate of the cycle might decrease the rate of entry of metabolites from glycolysis to the cycle. Fructose-1,6-diphosphate is split up into dihydroxyacetone phosphate and glyceraldehyde-3-phosphate by aldolase. Aldolase is found in cerebral extracts from many species as a soluble enzyme of high activity (Lowry et al., 1954; Buell et al., 1958) yith a high affinity for its substrate. The enzyme of mammalian cerebral

cortex occurs atleast in 5 forma which differ in electrophoretic mobility from the aldolases of other tissues.

Glyceraldehyde-3-phosphate and dihydroxyacetonephosphate are freely interconvertible through the action of triosephosphate isomerase. Since, only glyceraldehyde-3-phosphate ia metabolized further along the main route of glycolysis it can be regarded that in the first stage of glycolysis two molecules of glyceraldehyde-3-phosphate were formed from one molecule of glucose. The glyceraldehyde-3-phosphate is oxidized to 1,3-diphosphoglycerate by glyceraldehyde-3-phosphate dehydrogenase with the reduction of NAD* to NADH. The 1,3-diphosphoglycerate ia a high energy intermediate which in a subsequent reaction (catalyzed by phosphoglycerate kinase) donates a phosphate group to ADP with formation of ATP and 3-phosphoglycerate. This ia converted to 2-phosphoglycerate by phoaphoglyceronutase. 2-phosphoglycerate is dehydrated to yield phoaphoenolpyruvate in a reaction catalyzed by enolase. Phosphoenolpyruvate la a high energy compound and can donate a phosphate group to ADP resulting in the formation of ATP and pyruvate. The reaction is catalyzed by pyruvate kinase. This also conaidered ae one of the regulatory points of cerebral glucose metabolism (Rolleston and Newsholme, 1967). The activity of this enzyme ia inhibited by free ATP; as with hexokinaae and phosphofructokinase, the

Mg:ATP ratio which is important (Rolleston and Newsholme, 1967; Wood, 1968). The enzyme is stimulated by potassium and ammonium iona and inhibited by sodium ions.

The sum of all reactions leading to formation of pyruvate from glucose will be

Glucose + 2Pi + 2ADP + $2NAD^+$ ——> 2Pyruvate + 2ATP + 2NADH

At this point, the aerobic and anaerobic pathways diverge. If oxygen *La* available, pyruvate is further oxidized to carbondioxide in citric acid cycle in the mitochondria. Under anaerobic conditions pyruvate is reduced to lactate in a reversible reaction catalyzed by lactate dehydrogenase.

Lactate dehydrogenase exists in atleast 5 different molecular forms or isozymes in higher animals (M_4; M_3H; M_2H; MH_2;H_4). The M_4 and M_3H isozymes are found to predominate in tissues highly dependent on glycolysis for energy , eg., white skeletal muscle and embryonic tissues. The MH_2 and H_4 isozymes predominate in tissues with a purely aerobic or respiratory metabolism (Lehninger, 1984).

Utilization of glucose by the brain in vivo proceeds normally at 18 umoles/gm/hr and of this 3 umoles/gm are converted to 6 umoles of lactic acid in each hour (McIlwain and Bachelard, 1971). The formation of lactic acid can however greatly increse when oxygen is limited or when cerebral

activity increases and rate of some 400 - 1000 μmoles/gm/hr are then reached during brief periods (King et al, 1967).

Under aerobic conditions the further metabolism of the glucose molecule occurs in the mitochondria where the pyruvate, (formed in the glycolysis) is further oxidized to CO_2 and water. Citric acid cycle is the main site to produce metabolically useful energy.

The reaction catalyzed by pyruvate dehydrogenase complex is not the part of the citric acid cycle but serve the purpose of the delivering two carbon units from the pyruvate molecule to the pool of citric acid cycle intermediates. The first carbon atom disappears as CO_2. The pyruvate oxidation mechanism is rather complicated, requiring three sets of enzyme bound reactions leading to COA S∿Ac and NADH. The three enzymes namely, pyruvate decarboxylase, lipoate acetyltransferase and lipoamide dehydrogenase are tightly bound together. Pyruvate dehydrogenase is inhibited by its products acetyl CoA, NADH and other ligands such as ATP. Ca^{++} stimulates the activity of this enzyme. Inhibition by ATP is through cAMP dependent protein phosphorylation (Jope and Blass, 1975, 1976; Randle et al., 1978). The interconversion of active and inactive forms were shown to be dependent on the energy state of the brain rather than the functional state (Booth and Clark, 1978).

As the rate of fatty acid oxidation were observed to be very low in brain, the acetyl CoA generated from pyruvate remains undiluted (Balazs and Haslam, 1965). However, the metabolic pool of pyruvate originating from glucose may be diluted by the pyruvate originating from the metabolism of amino acids such as alanine, serine, lysine and glycine.

The acetyl CoA, that is formed by the oxidation of the pyruvate condenses with oxaloacetate in a reaction mediated by citrate synthetase resulting in the formation of citrate. The citrate is converted to isocitrate via cis-aconitate by aconitase. At equilibrium, the distribution of these compounds are as follows: 91* citrate, 3* cis-aconitate and 6% isocitrate.

The next step in which isocitrate is oxidized to 2-oxoglutarate is catalyzer! by isocitrate dehydrogenase. Two types of isocitrate dehydrogenases occur in cerebral tissues and they differ in their coenzyme requirements and in subcellular localization. The major activity, NAD^+-linked is exclusively mitochondrial in occurence and is about 4 times more active in mitochondria than the $NADP^+$ linked enzyme which is found partly mitochondrial and partly soluble (Goebell and Klingenberg, 1964; Salgonicoff and Koeppe, 1968). NAD* - isocitrate dehydrogenase requires Mn^{2+} or Mg^{2+} and is activated by ADP which suggests it may perform a

regulatory role in citric acid cycle (Goebell and Klingenberg, 1964; Goldberg et al., 1966). The phosphorylation and dephosphorylation of this enzyme was shown to be a regulatory mechanism operative in bacteria (Garnak and Reeves, 1979; LaPorte and Koshland, 1983).

The oxidative decarboxylation of 2-oxoglutarate to succinyl CoA are high energy intermediate is catalyzed by the enzyme 2-oxoglutarate dehydrogenase. This has properties similar to those of pyruvate dehydrogenase. The further metabolism of succinyl CoA to succinate is catalyzed by succinyl-thiokinase, allows substrate level phosphorylation of GDP to GTP. Succinate is oxidized to fumarate by succinate dehydrogenase with FAD as the hydrogen acceptor. Succinate dehydrogenase is modulated by $CoQ_{10}/CoQ_{10}H_2$ ratio and indirectly by ADP and NADH. The inhibition exerted by oxaloacetate on this enzyme would be of physiological importance. This is followed by the conversion of fumarate to malate via the fumarase reaction. The malate thus produced, is oxidized to oxaloacetate in the presence of NAD^+ in a reaction catalyzed by malate dehydrogenase. This is a reversible reaction. Malate dehydrogenase is present both in cytoaol and mitochondria. So the sum of all the citric acid cycle including oxidative decarboxylation of pyruvate ia as follows:

Pyr + 4NAD⁺ + FAD⁺ + GDP⁺ + Pi——>

$3CO_2$ + 4NADH + $FADH_2$ + GTP

For one revolution of the cycle oxaloacetate condenses with acetyl-CoA and, since oxaloacetate reappears in the last reaction, the cycle is truely regenerative and allows, at least in theory, the oxidation of unlimited amount of acetyl groups from pyruvate.

OTHER REACTIONS ASSOCIATED WITH CITRIC ACID CYCLE:

Pyruvate can also enter the cycle after conversion to oxaloacetate and metabolites other than pyruvate can enter as acetyl CoA. There are several reactions in which some of the metabolites of citric acid cycle were generated.

Labelling of 2C and 3C of glutamate by using 2-[^{14}]-pyruvate implies the formation of oxaloacetate by addition of carbondioxide. The use of $^{14}CO_2$ or $H^{14}CO_3$ also showed the CO_2 to be rapidly incorporated into malate in mammalian brain. Glutamate and especially aspartate were also rapidly labelled (Cheng and Waelsch, 1963; Berl et al., 1962). Three of the enzyme systems catalyzing such incorporation were observed in cerebra preparations (Utter, 1959). These are carboxylation of pyruvate to form oxaloacetate (catalyzed by pyruvate carboxylase), carboxylation reactions which forms malate (catalyzed by malic enzyme) and carboxylation of phosphoenol-pyruvate to form oxaloacetate (catalyzed by phosphoenolpyru-

vate carboxykinase). All these enzymes were stimulated by biotin, acetyl CoA and ATP.

Oxaloacetate formation by this route proceeds at about only 12 μmoles of pyruvate carboxylated/gm/hr in rat cerebral cortex and in confined to the mitochondria (Salganicoff and Koeppe, 1968).

The second one `malic enzyme' which catalyzes NADPH linked formation of oxaloacetate from pyruvate and carbon dioxide is active in the brain and reacts at rates from 50-90 μmoles/gm/hr and occur mainly in mitochondria (Utter, 1959; Salganicoff and Koeppe, 1968).

The third one `phosphoenolpyruvate carboxykinase' would act mainly as a decarboxylating enzyme.

SCOPE OF THE PRESENT INVESTIGATION:

The main aim of the present study is to understand the alterations in the cerebral energy metabolism in hyperammonemic states. For this purpose, the following studies were made in the present investigation.

(1) Studies with homogenates:

This study will provide information as to the hyperammonemia induced changes in cerebral energy metabolism are uniform all over the brain or whether they are restricted to any particular region. This will further help in selecting the region(s) for the rest of the investigation. Only enzyme studies were performed in these preparations. The results were presented in Chapter 3.

(2) Studies with subcellular fractions:

The cerebral energy metabolism requires a close interaction between cytosol and mitochondria. Moreover, some of the enzymea of carbohydrate metabolism are present both in cytosol and mitochondria. Hence, it was felt that a study with subcellular fractions would help in a better understanding of changes in cerebral energy metabolism in hyperammonemic states. In this phase, studies were conducted on the enzymes of carbohydrate metabolism and on glucose oxidation. Further, selected metabolites of carbohydrate metabolism were also determined in the subcellular fractions

after they were incubated with glucose. Results of these studies were presented in Chapter 4.

(3) **Studies with cellular** fractions:

Brain is a cellular mosaic consisting of three major cell types viz., neurons, astrocytes and oligodendrocytes. These cells are structurally, and functionally different from each other and their metabolic profiles are highly characteristic to a given cell type. Under such circumstances, it is possible that ammonia (at a pathophysiological concentrations) may have a uniform effect on the three different cell types or it (ammonia) may have different effects on different cell types. Hence, these three cell types were isolated from normal and hyperammonemic rat cerebral cortex and the metabolic alterations were studied. In this study, as was done earlier, activities of the enzymes participating in carbohydrate metabolism were determined. In addition, alterations in glucose oxidation were also investigated. Further, these cells were incubated with glucose and the levels of various metabolites were determined.

In addition to the heterogenity between different types of cells, a new type of heterogenity was observed in the present study. This is between functionally distinct segments of the same cell, especially in neurons. Differences were observed in the distribution of enzymes and intermediates of glucose metabolism and in the rates of glucose oxidation in

synaptosomes and in neuronal perikarya. These results have also been discussed. Results obtained in this study were presented in the Chapter 5.

MATERIALS AND METHODS

MATERIALS & METHODS

MATERIALS

Acetylthiocholine iodide, adenosine diphosphate (ADP), adenosine triphosphate (ATP), acetyl coenzyme A, L-aspartic acid, alanine, aspartate aminotransferase (AAT), alanine aminotransferase (A1AT), aldolase, aconitase, butyrylthiocholine iodide, bovine serum albumin (BSA), coenzyme A (Co A), cocarboxylase (thiamine pyrophosphate; TPP), citrate, dithiothreotol (DTT), 5-5-dithiobis-2-nitro benzoic acid (DTNB), deoxyribonucleic acid (DNA), ethylenediaminetetraacetic acid (EDTA), enolaae, fructose-6-phosphate, fructose-1,6-diphosphate, glutamate, Y-glutamyl monohydroxamate, glutamate dehydrogenase (GDH), glyceraldehyde-3-phosphate, ∝-glycerophoaphate dehydrogenaae (∝-GDH), glucose-6-phosphate dehydrogenase (G-6-P DH), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), hexokinase (HK), isocitrate dehydrogenase (ICDH), lactate dehydrogenaae (LDH), lactate, malate dehydrogenase (MDH), nicotinamide adenine dinucleotide (NAD*), nicotinamide adenine dinucleotide reduced (NADH), nicotinamide adenine dinucleotide phoaphate (NADP*), oxaloacetate, 2-oxoglutarate, phosphoenolpyruvate, phenazine methosulfate (PMS), 2-phosphoglyceric acid, ribonucleic acid (RNA), pyruvate kinase (PK), phosphoglucose isomerase, pyridoxal-5-phosphate (P-5-P), sodium succinate, sodium pyruvate, triose

phosphate isomerase (TPI) were purchased from Sigma chemical co., St.Louia (USA). Ficoll-400 waa obtained from Pharmacia Fine Chemicals, Uppsala (Sweden). 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride (INT) waa purchased from Loba Chemie, India. Triton-X-100 was procured from Koch-Light Chemicals, U.K. Rest of the chemicals were of AR or GR grade and were purchased locally. Toluene, dioxane, naphthalene, diphenyl oxazole (PPO), 1,4-(bis-4-methyl-5-phenyl oxazole-2 yl benzene (POPOP) and Triton X-100 were of scintillation grade. Ethanol was distilled and treated over night with silver nitrate. Alcohol free of aldehydes was then redistilled and used. BW C8451 dibromide was a gift from Wellcome Ltd., Kent (UK). [U-^{14}C-GLUCOSE] with specific activity of 292 mCi/mmole and [U-^{14}C-GLUTAMATE] with specific activity of 240 mCi/mmole were supplied by Bhabha Atomic Research centre, Bombay, INDIA. Nylon meshes were purchased from Nybolt Co., Zurich.

ANIMALS:

Adult albino rats of Wistar strain were used as experimental animals. The animals were of either sex having a body weight of about 250 - 300 grams. Animals were maintained in air conditioned rooms (21$\pm$1° C) in groups of 6-8 per cage with free access to food (balanced pellet diet from Hindustan Lever Ltd.) and water.

INDUCTION OF HYPERAMMONEMIA:

Hyperammonemia was induced as per the method described earlier (Subbalakshmi, 1981, 1984; Subbalakshmi and Murt.hy, 1981, 1983, 1984, 1985; Jessy, 1988; Jessy and Murthy, 1985, 1989; Ratnakumari et al., 1985; Ratnakumari and Murthy. 1989).

Acute hyperammonemia:

Ammonium acetate (2.5 mmoles/100g body weight), dissolved in saline, was administered intraperitoneally, while the controls received none. Treated animals were decapitated during the convulsive phase which usually appeared between 25-50 min after the adminstration of ammonium acetate. In some experiments, animals were killed in preconvulaive period approximately 20 min after the administration of ammonium acetate.

Subacute hyperammonemia:

Ammonium acetate (0.35 mmoles/100gm. body wt), dissolved in saline, waa administered intraperitoneally. With this dosage there were no convulsions in th a animals even alter 18 hrs. However, animals in thie group were also decapitated between 25-30 min after the administration of ammonium acetate.

in **vitro** effects of **ammonia:**

Ammonium acetate waa added to the assay mixtures or incubation media containing either homogenates or aubcellular

or cellular fractions to a final concentration is either 1 or 5 or 10 roll.

TISSUE PREPARATION:

Brains were quickly removed from the heads of decapitated animals and transferred to ice-cold saline. Regional dissection of brain was performed at 0° C. Brain was dissected into cerebral hemispheres, cerebellum and brain stem (consisting of pons and medulla).

PREPARATION OF HOMOGENATES:

Different regions of brain were weighed separately and homogenates (10% weight/volume) of individual regions were prepared in 0.32 M ice-cold sucrose solution using Potter-Elvehjem homogenizer with a serrated teflon pestle. The motor-speed was maintained at 800 rpm and eight to ten up and down strokes were given during homogenization. Temperature was maintained at 0 - 4° C during this process. The 10% homogenates were diluted with 0.32 M sucrose to required strength as and when necessary. Triton X-100 was added to a final concentration of 0.1% (v/v) as a detergent to solubulize membranes.

PREPARATION OF SUBCELLULAR FRACTIONS:

Subcellular fractions, viz., mitochondria, synaptosomes and cytosol were prepared only from the cerebral cortex of normal and hyperammonemic rats. The method followed for this purpose was that of Cotman (1974) as described by Subba-

lakshmi and Murthy (1984, 1985a and b), Subbalakshmi (1984), Jessy (1988) and Ratnakumari and Murthy (1989, 1990). Cerebral cortex was homogenized in 0.32 M aucroae (pH 7.4) to obtain a 104 (w/v) homogenate and was centrifuged at 1,000g tor 5 min. The supernatant (S-1) waa aspirated and the pellet (P-1) waa discarded. Supernatant (S-l) was further centrifuged at 15,000 g for 12 min to obtain a pellet (P-2; consisting of myelin, synaptosomes and mitochondria) and supernatant (S-2). The pellet (P-2) was suspended in 5 ml of 0.32 M sucrose and layered over a preformed discontinuous density gradient of 4%, 6% and 13% Ficoll-400 in 0.32 M sucrose (pH 7.4) and was centrifuged at 63,580 g for 45 min. Simultaneously, the supernatant (S-2) was also centrifuged at the same centrifugal force. This reaulted in the separation of myelin (in 4% Ficoll layer) synaptosomes (at the interphaae of 6% and 13% Ficoll-400) and pellet (P-3) consisting of mitochondria. Myelin layer was discarded and the synaptosomal layer was aspirated with a Pasteur pipette and diluted with 0.32 M sucrose and centrifuged at 50,000 g for 20 min to sediment synaptosomes. Mitochondrial pellet was also subjected to similar treatment. The synaptosomes and mitochondria were suspended in 0.32 M sucrose and the protein content was adjusted to 1 mg/ml. The supernatant obtained from the centrifugation of S-2 waa taken as cytosol and the pellet (P-3, consisting of broken membranes and microsomes) was

discarded. Unless otherwise mentioned, these fractions were frozen overnight at -20° C and Triton X-100 was added to a final concentration of 0.1% before uae. All the centrifugal operations were carried out at 0 - 4° C in a high speed refrigerated centrifuge (Beckman J2-21 or Remi C-24) and the ultracentrifuge used for density gradient separation was MSE superspeed 700 or Beckman L8-80M.

CELL ISOLATION PROCEDURES:

Neurons and Astrocytes

Both neurons and astrocytes were isolated using the method of Farooq and Nort<.. (1978) with a few modifications (Jessy, 1988; Jessy and Murthy, 1989 and 1990). Cell isolation medium consisted of 8% (w/v) glucose, 5% (w/v) fructose and 2% (w/v) Ficoll-400 in 10 mM potassium phosphate-sodium hydroxide buffer, pH 6.0. Additional Ficoll was added to this medium to prepare solutions of increasing densities. Thus, a 7% Ficoll solution contained a total of *9i* Ficoll and so on. Cerebral cortex was isolated from the rats soon after decapitation. Grey matter was separated from the white matter and gently pressed on a wet Uhatman No.1 filter paper to remove meninges and surface capillaries. Free hand slices of approximately equal thickness were prepared and incubated at 37° C with constant shaking in isolation medium (5 ml/brain) consisting of 0.1% acetylated trypsin. After 60

min, trypsin containing medium was aspirated and the tissue was washed once with 5 ml of isolation medium and then with ice-cold isolation medium containing 0.1% soybean trypain inhibitor (5 ml/brain). This suspension was cooled to 4°C for 10 min and the tissue was washed once again with cull isolation medium. Tissue slices were then placed on ,00 u nylon screen and the cells were dissociated by gently tapping the tissue with a glass rod. The nylon screen was washed with isolation medium and the dissociated cells were collected. The crude cell suspension was then passed twice successively through monofilament nylon screens of pore diameter 300 u, 105 u, 80 u and 55 u to remove debri3 and broken processes. The crude cell suspension was centrifuged at 720g for 15 min at 4°C. The pellet (P-1), enriched with cells, was suspended in 7% Ficoll-400 and centrifuged at 720g for 10 min to separate a neuronal cell enriched pellet (P-2). Supernatant (S-1) was diluted in the ratio of 1:1.125 with the isolation medium and centrifuged at 1,120g for 20 min to get a pellet enriched with astrocytes (P-2). Both these cell enriched pellets (P-l and P-2) were suspended separately in cell isolation medium and layered separately on two different preformed density gradients of 15*. and 28% Ficoll-400 and centrifuged at 8,500g for 10 win. The pellet contained relatively enriched fraction of neuronal perikarya (refered hereafter as neurons) and the interface between 15% and 28%

Ficoll-400 contained astrocytes enriched preparation. This interface was aspirated and diluted with medium and centrifuged to obtain aatrocyte enriched pellet. Both the neuron and aatrocyte enriched pellets were washed with 0.32 M sucrose and were suspended in the same.

Cells from the experimental animals were isolated in a similar manner with slight modifications. The time for incubation with acetylated trypsin was reduced to 50 min and the crude cell suspension obtained after the disaggregation step was passed only through 300, 105, 80 u nylon meshes. The time for each differential centrifugation was increased by an additional 5 min.

Isolation of oligodendrocytes

Oligodendroglia were prepared essentially by the method of Snyder et al., (1980) with alight modifications. Cell isolation medium was Hank's balanced salt solution containing 25mM Hepes and the pH of the final solution was adjusted to 7.2 with sodium hydroxide. Normal and experimental animals were decapitated and the cerebral hemispheres were isolated. After the removal of blood capillaries as described above, slices of gray matter were transferred to 5 ml of Hank's balanced salt solution with 0.1% acetylated trypsin. After 30 min of incubation at 37°C, incubation medium was decanted, the tissue washed once with 5 ml of isolation medium and suspended in 5 ml of medium containing 0.1% soybean trypsin

inhibitor and chilled for 5 min on ice. The tissue was placed on 300 u nylon mesh stretched over a porcelain Hirsh funnel and gently tapped with a glass rod to aid dissociation of cells. The nylon screen was washed with isolation medium and the dissociated cells were collected. Crude cell suspension was then passed twice successively through monofilament nylon screens of pore diameter 300, 105, 80, 50 and 30 μ. The final cell suspension was centrifuged at l,120g for 10 min. The pellet, enriched with cells, was suspended in 35% sucrose and was layered over a discontinuous sucrose gradient of 8 ml of 53% sucrose and 5 ml of 45% sucrose in medium. The tubes were centrifuged at 3,065g for 15 min in a swinging bucket rotor. The pellet and 53% sucrose gradient containing oligodendroglia were diluted five fold with isolation medium and centrifuged to sediment cells. Oligodendroglial cells were suspended in 0.32 M sucrose.

Purity of the cell preparations was monitored with phase contrast microscopy and by determining the activities of specific marker enzymes.

Cell number

An aliquot (10-20 μl) of the call enriched preparation was diluted and stained with methylene blue (0.24) and the cell number was determined with a haemocytometer. Cells were counted at random in several of the RBC chambers and the cell number was calculated using the formula

Number of cells/RBC chamber X 1000

0.00625

Cell number of neurons, astrocytea and oligodendroglia were determined by this method. Synaptosomal number could not be determined by this method as their size was beyond the resolution of the light microscope.

Cell viability

Viability of the isolated cells was determined by dye exclusion method uaing 0.4V trypan blue.

BIOCHEMICALMETHODS

ENZYME ASSAYS:

All the enzyme assays, excepting those involving radioactive aubatratea or colorimentric methods were carried out in Gilford spectrophotometer (model 250), using a thermo-programmer to maintain the temperature at 37° C. Optimal concentrations of enzyme, substrate and cofactors and time of incubation (if necessary) were determined separately for each enzyme in homogenates, subcellular and cellular fractions (Fig no.1 to 16). These were adjusted in such a way to obtain a linear change in the rate of enzyme reaction during the assay. Changes in absorbancy were recorded at 15 sec interval for a period of 5 min and the results were plotted. From these plots, absorbancy changes in the linear kinetic zone were obtained and uaed for calculating the activities of

the enzymes. The final volume of the assay mixture was 250 µl, unless otherwise mentioned. Protein contents of the subcellular and cellular prepartations were adjusted to 1mg/ml before the commencement of the assay.

Blanks

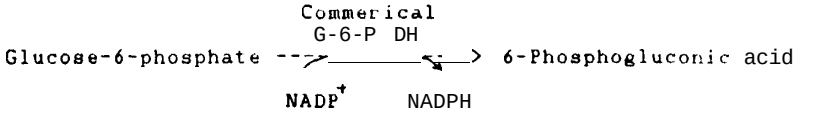
In all the enzyme assays, except for pyruvate and 2-oxo-glutarate dehydrogenases and glutamic acid decarboxylase, non specific changes in the absorbance were recorded in the blanks in which substrate was substituted with an equal volume of distilled water. Coenzyme A (CoA) was omitted in the assays of pyruvate and 2-oxoglutarate dehydrogenases. Enzyme activities were corrected for the nonspecific activities obtained from blanks.

ENZYMES OF GLYCOLYTIC PATHWAY

HEXOKINASE (HK; E.C.2.7.1.1):

Hexokinase activity was assayed by the method of Bergmeyer and Bernt (1974). Glucose-6-phosphate formed in the hexokinase reaction was reduced to 6-phosphogluconic acid with glucose-6- phosphate dehydrogenase in the presence of NAD. Change in the absorbancy at 340 nm, due to the formation of NADH.H in the course of the latter reaction, was followed.

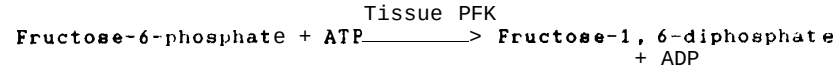
$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Tissue HK}} \text{Glucose-6-phosphate} + \text{ADP}$$

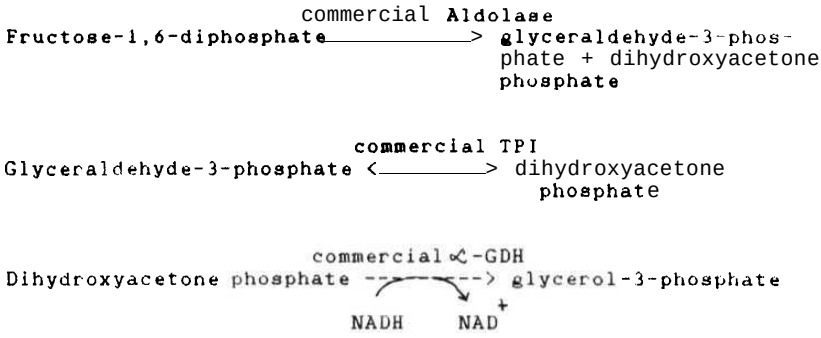


The reaction mixture consists of 80 mM triethanolamine-HCl (pH 7.6), 1.3 mM glucose, 7.40 mM magnesium chloride, 27.2 mM NADP , 19.2 mM ATP, 20 units of glucose-6-phosphate dehydrogenase/ml of reaction mixture and 10 ul of either 10% homogenate or subcellular fractions or 20 u1 of cell suspensions Reaction was started by the addition of ATP and the increase in absorbance at 340 nm was followed for 5 min at 15 aec intervals.

PHOSPHOFRUCTOKINASE (PFK; E.C.2.7.1.11)

The method of Foe and Kemp (1982) was adopted to assay the activity of this enzyme. Fructose-1,6-diphosphate formed in the course of the reaction waa converted to dihydroxyacetone phosphate by the addition of aldolase and triose phosphate isomerase (TPI). The added glycerophosphate dehydrogenase (GDH) converts the dihydroxyacetone phosphate to glycerol-3-phoaphate and in the course of the reaction NADH would be converted to NAD. Rate of this reaction waa followed by changes in the abaorbancy at 340 nm.



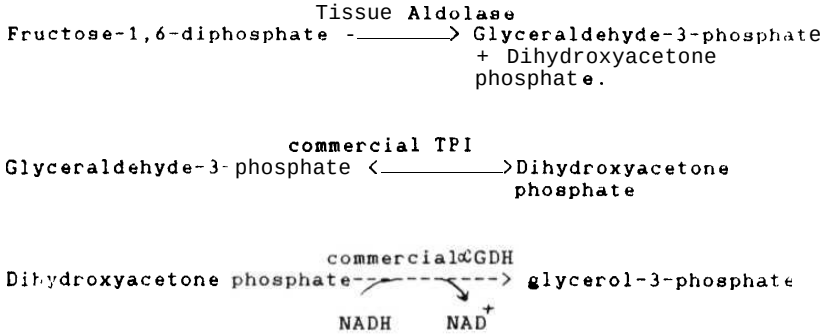


The assay mixture consists of 50 mM Tris-HCl buffer (pH 8.0), 5 mM magnesium chloride, 1 mM dithiothreitol, 1 mM EDTA, 0.2 mM NADH, 1 mM ATP, 25 mM fructose-6-phosphate, 40 units of aldolase/ml of reaction mixture, 20 units of triose phosphate isomerase/ml of reaction mixture, 20 units of α-glycerophosphate dehydrogenase/ml of reaction mixture and 10 µl of 10% homogenate or 2.5 ul of subcellular fractions and 5 ul of cell suspension. Reaction was initiated by the addition of fructose-6-phosphate and the decrease in absorbancy was measured at 340 nm.

ALDOLASE (E.C.4.1.2.13)

Aldolaae was assyed by the method of Bergmeyer and Bernt (1974). Trioae phosphate isomerase (TPI) was added to convert the glyceraldehyde-3-phosphate to dihydroxyacetone phosphate and the latter compound was reduced to glycerol-3-phosphate with the help of glycero-3-phosphate dehydrogenase in the

presence of NADH. Rate of formation of NAD was followed by the change in absorbance at 340 nm.

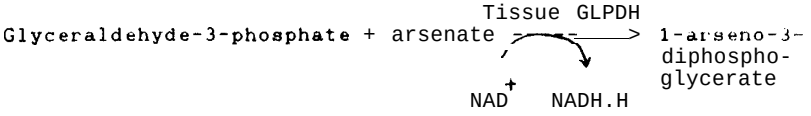


The reaction mixture contained 94 mM triethanolamine buffer (pH 7.6), 0.2 mM NADH, 1.1 mM fructose-1,6-diphosphate, 5 units of trioae phosphate isomerase/ml of reaction mixture, 5 units of glyero-3-phosphate dehydrogenase/ml ot reaction mixture, and 10 ul of 104 homogenate or 2.5 µ1 *ot* any one of the subcellular fractions or 5 ul of cell suspension. Reaction was started by the addition of fructose-1,6-diphosphate and the decrease in abaorbance was followed at 340 nm for S min at 15 sec intervals.

GLYCERALDEHYDE-3-PH0SPHATE **DEHYDROGENASE (E.C.2.7.1.40)**

The method of Allison and Kaplan (1964) was adopted for the assay of this enzyme. In the normal course of the reaction inorganic phosphate is incorporated into glyceral-dehyde-3- phosphate to form 1,3-diphosphoglyceric acid with

the concomitant reduction of NAD to NADH.H. However, in the assay mixture, equilibrium of the reaction was driven forward by substituting inorganic phosphate with arsenate, resulting in the formation of a nonmetabolizable product 1-arseno-3-phosphoglyceric acid. Reduction of NAD was followed at 340 nm.



The reaction mixture consisted of 45 mM sodium pyrophosphate buffer (pH 8.5), 0.24 mM NAD, 0.12 umoles of glyceraldehyde-3-phoaphate, 5 mM disodium araenate and 10 ul of 10% homogenate or 5 µl of one of the three subcellular fractions or 5 ul of cell suspension. Reaction was initiated by the addition of glyceraldehyde-3-phosphate and increase in absorbancy at 340 nm was followed for every 15 sec up to 5 min.

ENOLASE (E.C.4.2.1.11)

Enolase activity was assayed by the method of Bergmeyer and Bernt (1974). Phosphoenolpyruvate (PEP), formed by the action of enolase on 2-phosphoglycerate, was converted to pyruvate by pyruvate kinase (PK) and then to lactate with lactate dehydrogenase (LDH) which drives the reaction forward with the concomitant oxidation of NADH. Rate of formation at NADH.H. was followed at 340 nm.

$$\text{2-Phosphoglycerate} \xrightarrow{\text{Tieaue Enolase}} \text{Phosphoenolpyruvate}$$

$$\text{Phosphoenolpyruvate} + \text{ADP} \xrightarrow{\text{Commercial PK}} \text{Pyruvate} + \text{ATP}$$

$$\text{Pyruvate} + \text{NADH} \xleftrightarrow{\text{commercial LDH}} \text{Lactate} + \text{NAD}^{+}$$

The reaction mixture consists of 83 mM triethanolamine buffer (pH 7.6), 3.3 mM magnesium sulfate, 0.2 mM NADH, 0.09 mM 2-phosphoglycerate, 0.1 mM ADP, 5 units of pyruvate kinase/ml of reaction mixture, 5 units of lactate dehydrogenase/ml of reaction mixture and 2.5 ul of either 10% homogenate or any one of the three aubcellular fractions or 5 ul of cell suspension. Reaction was started by the addition of 2-phosphoglycerate and the decrease in the absorbance at 340 nm was followed for 5 rain at 15 sec intervals.

PYRUVATE KINASE (PK;E.C.2.7.1.40)

Pyruvate kinase was assayed by the method of Bergmeyer and Bernt (1974). This reaction was coupled to that of lactate dehydrogenaae (LDH) so that the pyruvate formed in the course of reaction is converted to lactate in the presence of NADH. Rate of formation of NAD was followed at 340 nm.

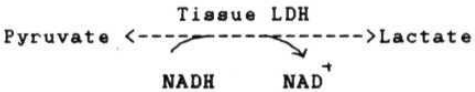
$$\text{Phoaphoenolpyruvate} + \text{ADP} \xrightarrow{\text{Tissue PK}} \text{Pyruvate} + \text{ATP}$$

$$\text{Pyruvate} + \text{NADH} \xleftrightarrow{\text{commercial LDH}} \text{Lactate} + \text{NAD}^{+}$$

The reaction mixture consists of 100 mM triethanolamine buffer (pH 7.6), 0.65 mM phosphoenol pyruvate, 3 rnM magnesium sulfate, 12 mM potassium chloride, 5.6 mM ADP, 0.24 mM NADH, 5 units of lactate dehydrogenase/ml of reaction mixture and 5 ul of either 10% homogenate or any one of the three subcellular or cellular fractions. Reaction was started by the addition of phosphoenolpyruvate and the decrease in optical density at 340 nm was followed for every 15 sec up to 5 min.

LACTATE DEHYDROGENASE (LDH;E.C.1.1.1.27):

Activity of this enzyme was determined by the method of Bergmeyer and Bernt (1974). The activity of this enzyme was assayed in both the directions by following the changes in absorbancy at 340 nm (pyruvate ——————> lactate) or at 500 nm in the presence of PMS and INT (lactate ——————> pyruvate).



The reaction mixture for measuring the lactate production contained 48 mM potassium phosphate buffer (pH 7.6), 0.6 mM pyruvate, 0.18 mM NADH and 0.2 ul of 10% honogenate or 0.5 µl of any one of subcellular fractions or 1 µl of cell suspension. The reaction was initiated by the addition of pyruvate and the fall in the absorbancy at 340 nm was followed up to 5 min at 15 sec intervals.

The reaction mixture for the assay in the directon of pyruvate formation contained 48 mM potassium phosphate buffer (pH 7.6), 1.2 mM lactate, 0.36 mM NAD, 6.5 mM PMS, 0.33 mM INT and 5 µl of any one of the three subcellular fractions or 1 µl of any one of the cell suspension. Reaction was started by the addition of lactate and was followed as the change in absorbancy at 500 nm at 15 sec intervals up to 5 min.

PYRUVATE DEHYDROGENASE **AND CITRIC ACID CYCLE** ENZYMES

Pyruvate **dehydrogenase** (PDH, **E.C.1.2.4.1)**

The method described by **Hinman** and Blase (1981) was used for assaying PDH.

$$\text{Pyruvate} + \text{CoA} + \text{NAD} \xrightarrow{\text{Tissue PDH}} \text{Acetyl CoA} + \text{CO} + \text{NADH.H}$$

$$\text{NADH.H} + \text{PMS} + \text{INT} \longrightarrow \text{formazan (reduced tetrazolium)} + \text{NAD}^{+} + \text{PITS}$$

The reaction mixture consists of 50 mM potassium phosphate buffer (pH.7.8), 2.5 mM NAD , 0.2 mM thiamine pyrophosphate, 60 mM CoA, 0.3 mM dithiothreltol, 5 mM pyruvate, 1 mM magnesium chloride, 6.5 uM (PMS), 300 µM INT and 10% homogenate or 20ul of of subcellular fractions or 10 ul of either of the cell suspension. After preincubation for 5 min at 37°C, CoA was added and change in absorbancy at 500 nm was followed.

Citrate synthase *(CS;* E.C.4.1.3.7)

The method of Shepherd and Garland (1969) was adopted for the assay of citrae synthetase.

$$\text{Acetyl CoA + Oxaloacetate} \xrightarrow{\text{Tissue CS}} \text{Citrate + CoA(SH)}$$

Free thiol groups of CoA (formed in the course of the reaction) were reacts ' with 5,5'dithio-bis(2-nitrobenzoic acid) (DTNB) and the rate of formation of DTNB-thiol complex was measu! d at 412 nm.

$$\text{Free thiol groups + DTNB} \longrightarrow \text{DTNB-thiol coloured complex}$$

Assay mixture consisted of 96 mM Tris-HCl buffer (pH.8.0), 97.2 uM DTNB, 0.24 mM oxaloacetate, 48 uM acetyl CoA and 10 ul of 10% homogenate or 20 ul of subcellular fractions or 2.5 ul of cell suspension. Reaction was initiated by the addition of oxaloacetate. Increase In absorbance at 412 nm was recorded at 15 sec. interval for 5 min. Enzyme activity was calculated using the molar extinction coefficient of DTNB-thiol complex (1 36 x 10*). (Srere, 1969).

ISOCITRATE DEHYDROGENASE (ICDH (NAD); E.C. 1.1.1.41)

This enzyme was assayed by the method of Plaut (1969)

$$\text{Isocitrate + NAD}^{+} \xrightarrow{\text{Tissue ICDH}} \text{2-oxoglutarate + } CO_2 \bullet \text{ NADH.H.}$$

NADH.H + PMS + INT ——————> formazan (reduced tetrazolium) + NAD + PMS

The reaction mixture contained 33.3 mM Tris-acetate buffer (pH 7.2), 1 mM magnesium chloride, 0.64 mM ADP, 333 uM NAD, 5.28 mM isocitrate, 6.52 µM PMS, 300 µM INT and 10 µl of 10% (w/v) homogenate or 20 µl aubcellular fracti • or 5 µl of cell suspensions. The reaction was initiated by the addition of isocitrate and change in absorbancy was followed at 500 nm aa described above.

2-OXOGLUTARATE DEHYDROGENASE (2-OGDH; E.C.1.2.4.2)

The method of Reed and Mukherjee (1969) was uaed for the assay of this enzyme.

2-oxoglutarate + CoA + NAD^+ —Tissue GDH——> Succinyl CoA + CO_2 + NADH.H

NADH.H + PMS + INT ——————> formazan (reduced tetrazolium) + NAD^+ + PMS

The reaction mixture consisted of 50 mM potassium phosphate buffer (pH.8.0), 1 mM magnesium chloride, 2 mM NAD, 0.2 µM thiamine pyrophosphate, 60 uM coenzyme A, 1 mM 2-oxoglutarate, 6.52 uM PMS, 0.3 mM INT and 10 ul of 10% homogenate or 20 µl of subcellular fractions or 10 ul of cell suspension. The reaction was initiated by the addition of CoA and changes in abaorbancy was followed at 500 nm.

SUCCINATE DEHYDROGENASE (SDH; E.C.1.3.99.1)

Succinate dehydrogenase was assayed as per the method of Veeger et al., (1969).

Succinate + FAD ——————> Fumarate + $FADH_2$

$FADH_2$ + PMS + DCPIP (blue) ——————> FAD + PMS
DCPIP (colourless)

The assay mixture consists of 50 mM potassium phosphate buffer (pH.7.6), 40 roM succinate, 6.52 uM PMS and 50 µM dichlorophenol indophenol (DCPIP) and 10 µl of 10% homogenate. Reaction was initiated with the addition of succinate and the reducion of DCPIP was followed at 600 nm for 5 min at 15 sec intervals.

Activity of this enzyme in subcellular fractions was determined as per the method of Nandakumar et al (1973).

succinate + FAD ——————> fumerate + FADH.

FADH, + PMS + INT ——————> formazan (reduced tetrazolium)
+ FAD + PMS

The assay mixture, in a final volume of 1 ml, contained 40 mM succinate, 100 mM Tris-phosphate buffer (pH.8.0), 4 mM INT, 1.63 µM PMS and 100 ul of subcellular fractions or 5 ul of cell suspensions. The tubes were incubated at 37° C for 15 min and 2 ml of glacial acetic acid waa added to arrest the reaction and the colour was extracted in to 5 ml toluene.

Optical density of the formazan formed was measured at 500 not.

MALATE DEHYDROGENASE (MDH; E.C.1.1.1.37)

Activity of this enzyme was studied in both the directions by the method of Yoshida (1969).

$$\text{L-Malate} + \text{NAD}^{+} \xleftrightarrow{\text{Tissue MDH}} \text{Oxaloacetate} + \text{NADH.H}$$

The reaction mixture for the assay in the direction of oxaloacetate formation, consisted of 83.2 mM Tris-HCl (pH.8.8), 3.? mM malate, 0.33 mM NAD, 6.52 µM PMS, 0.3 mM INT and 10 µl of 10% homogenate or 5 ul of subcellular fractions or 1 µl of cell suspension. Reaction was initiated by the addition of malate and was followed at 500 nm.

Reaction mixture for the assay in the direction of malate formation (oxaloacetate ⟶ malate) consisted of 84 mM Tris-HCl buffer (pH.8.8), 3.36 mM oxaloacetate, 160 µM NADH and 0.2 ul of 10% homogenate or 0.5 µl of subcellular fractions or 1 ul of cell suspension. The reaction was initiated by the addition of oxaloacetate and decrease in abaorbancy at 340 nm was followed at 15 sec intervals for 5 win. Activity was calculated using extinction coefficient (EmM • 6.22) of NADH.

ASPARTATE AMINOTRANSFERASE (AAT;E.C.2.6.1.1)

The method of Bergmeyer and Bernt (1974) was adopted

for the assay.

$$\text{L-Aspartate} + \text{2-Oxoglutarate} \xleftrightarrow{\text{Tissue AAT}} \text{Glutamate} + \text{Oxaloacetate}$$

The oxaloacetate formed in the reaction was converted to malate using purified malate dehydrogenase (MDH) and the NADH consumed in the course of reaction, which will be proportional to AAT activity, was measured.

$$\text{Oxaloacetate} \xrightarrow[\text{NADH}]{\text{commercial MDH}} \text{Malate} + \text{NAD}^{+}$$

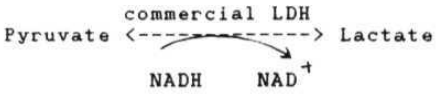
Assay mixture consisted of 80 mM potassium phosphate buffer (pH.7.4), 20 mM aspartate, 18 mM 2-oxoglutarate, 10 µg/ml of MDH (in 50% glycerol), 0.52 mM NADH and 1 ul of 10% homogenate or 0.5 ul of subcellular fractions or 5 ul of cell suspensions. Oxidation of NADH was followed at 340 nm at 15 sec intervals for 5 min after the addition of 2-oxoglutarate.

ALANINE AMINOTRANSFERASE (MAT; E.C.1.1.1.42)

A similar method was adopted for the assay of A1AT (Bergmeyer and Bernt 1974).

$$\text{Alanine} + \text{2-oxoglutarate} \xleftrightarrow{\text{Tissue A1AT}} \text{Glutamate} + \text{Pyruvate}$$

The pyruvate, so formed, was converted to lactate in the presence of NADH and purified lactate dehydrogenase (LDH).



The assay mixture consisted of 80 roll potassium phosphate buffer (pH 7.4), 40 mM alanine, 18 mM 2-oxoglutarate, 4 µg/ml of LDH (in 50% glycerol), 0.52 mM NADH and 1 ul of 10% homogenate or 0.5 µl of subcellular fractions or 5 ul of cell suspension. After a preincubation of 5 min at 37°C, 2-oxoglutarate was added and the rate of NADH oxidation was followed at 340 nm at 15 sec intervals for 5 min.

ACETYLCHOLINESTERASE (AChE; E.C.3.1.1.7) and PSEUDOCHOLINESTERASE (PChE; E.C.3.1.1.8):

The cholinesterases were assayed by the method of Ellman et al., (1961).

Assay mixture for acetylcholinesterase (250 µl) contained 40 pinoles of sodium phosphate buffer (pH 7.9), 0.15 umoles of acetylthiocholine iodide, 0.1 umole of DTNB and 10 ug of enzyme protein. Assay mixture for pseudocholinesterase (250 µl) contained 40 µmoles of sodium phosphate buffer (pH 7.9), 0.75 µmoles of butyryl thiocholine iodide, 0.1 µmoles of DTNB, 100 µM of BW 284 C51 (acetylcholineesterase inhibi-

tor) and 10 µg of enzyme protein.

The reactions were carried out at 37 °C and the increase in absorbancy was recorded at 412 nm. Enzyme activities were calculated by using the molar extinction coefficient of DTNB thiol complex (1.36 X 10^4) (Srere, 1969) and expressed as µmoles of acetylthiocholine or butyrylthiocholine hydrolyzed/mg protein/hr or nmoles of acetylthiocholine or butyrylthiocholine hydrolyzed/cell/hr.

GLUTAMINE SYNTHETASE (GS; 6.3.1.2):

Glutamine synthetase was assayed by the method described by Roue et al. (1970). In this reaction ammonium salts were replaced with hydroxylamine and the γ-glutamyl hydroxamate formed during the course of the reaction was determined colorimetrically after its reaction with ferric chloride.

The assay mixture (2 ml) contained 100 µmoles of imidazole-HCl buffer (pH 7.2), 40 umoles $MgCl_2$, 50 umoles 2-mercaptoethanol, 100 umoles L-glutamate, 20 umoles ATP, 200 umoles of hydroxylamine hydrochloride and 200 ug of enzyme protein. In the blanks both ATP and L-glutamate were omitted. At the end of 15 min incubation 3 ml ferric chloride reagent (6 gm $FeCl_3$, 3.35 gm trichloroacetic acid and 6.6 ml conc.hydrochloric acid in 100ml of water) was added and centrifuged. The absorbancy of the supernatant was measured at 535 nm. Using γ-glutamyl monohydroxamate as standard, the enzyme activity was calculated.

GLUTAMIC ACID DECARBOXYLASE (GAD; E.C.4.1.1.15) :

Glutamic acid decarboxylaae was assayed by the method of Murthy and Sadasivudu (1978) with some modifications as suggested by Rao and Murthy (1988).

^{14}C-GABA formed from [U-^{14}C]glutamate in the course of the reaction was separated by paper chromatography and the radioactivity of GABA spot was determined

The reaction mixture (0.3 ml) contained 250 mM potassium phosphate buffer (pH 6.6), [U-^{14}C] glutamate (100 mM, 3,300 dpm/nmole). 2.5 mM 2-mercaptoethanol, 1 mM ethylenediamine-tetraacetic acid, 0.1254 (v/v) Triton X-100 and 13 µM pyri-doxal-5-phosphate. Reaction was initiated by addition of enzyme protein (50 µgms of protein of subcellular or cellular preparation). Incubations were carried out at 37°C for 30 nun and was stopped by the addition of 2 ml of ethanol. The reaction mixture was centrifuged at 10,000 rpm for 5 min and the supernatant was dried overnight at room temparature. Residue was dissolved in distilled water, chromatographed along with authentic amino acid standards on Whatman No.1 chromatography paper using n-butanol : acetic acid : water (65:15:25) as solvent. Paper containing GABA spot (identified with the help of an authentic standard) was separated and transferred to a vial with Bray's scintillation fluid and the radioactivity was determined in a Beckman LS 1800 liquid scintillation spectrometer. Boiled enzyme was used in the

blanks.

ACTIVITY CALCULATIONS

In many of dehydrogenase assays described above, the terminal electron acceptor, INT, was reduced to formazan. Hence, the relationship between the amount of formazan formed and the NAD(P)H formed was established. This was carried out in two stages. In the first stage a formazan standard curve was prepared to establish the relationship between the optical density and the amount of formazan. In the second stage, INT concentration was fixed at a large excess and was reduced to formazan with varying NAD concentrations in an enzymatic reaction.

CHEMICAL REDUCTION OF INT

The reaction mixture (250 µl) contained 12.5 pinoles of phosphate buffer (pH 7.8), different concentrations of INT (20-100 nmoles) and 50 µl of 1% ascorbic acid with a trace of 0.1 N NaOH. The reaction was allowed to proceed till there was no further change in absorbancy (about 15 min) and the final absorbancy value at 500 nm was recorded.

ENZYMATIC REDUCTION OF INT

An assay mixture (250ul) consisting of 12.5 µmoles of phosphate buffer (pH 7.8), 75 nmoles of INT, 0.01 to 0.1 nmoles of NAD , and 0.8 µmoles of malate was incubated at

37 C. Reaction was initiated by the addition of 2.5 µg of purified MDH (commercial) and was allowed to proceed to completion. Final abaorbancy at 500 nm was recorded. Graphs were plotted with umoles of NAD on X-axis and umoles of formazan on Y-axia.

OTHER BIOCHEMICAL METHODS:

REMOVAL OP AMMONIA FROM COMMERCIAL ENZYMES

Enzymes supplied by Sigma were originally in ammonium sulphate (2M) solution. Removal of ammonium ions was essential, especially when studying the in vitro effect of ammonium ion on the enzymes of interest. Hence, the enzymes were dialyzed against several changes of 10 mM potassium phosphate buffer (pH 7.6), till the dialysate and the buffer were free of ammonia (tested with Nessler`s Reagent). Dialysis bags were washed with distilled water and the contents were diluted with an equal volume of glycerol to obtain a concentration of 0.5 mg protein/ml.

PROTEIN ESTIMATION:

Protein present in an aliquot of homogenates (100 µ l), cellular and subcellular fractions (20 µl) waa precipitated with two volumes of ice-cold 10% trichloroacetic acid (TCA) and kept at 0°C for 15 min. After centrifugation at 2,500 rpm for 15 min, the protein pellet was washed thrice with 3 ml of ethanaol, ethanol: ether (3:1; V:V) and finally with

ether. The delipidated protein pellet was air dried at room temparature and dissolved in 1 ml of 1N sodium hydroxide.

In the case of homogenates 4 ml of bluret reagent was added to the protein suspension and the absorbancy was measured at 540 nm against a sodium hydroxide blank (Varley, 1969).

In the case of subcellular and cellular fractions, a suitable aliquot of the above was used for protein determination by the method of Lowry et al., (1951).

AMMONIA DETERMINATION

Ammonia levels were determined in the extracts of whole brain. Rats were decapitated and the heads were allowed to fall directly into liquid nitrogen and were fixed for 10 min. Brains are quickly chiseled out and powdered in a stainless steel mortor pre-cooled with liquid nitrogen. Tubes containing 5 ml of 5% perchloric acid (PCA) were weighed and the powdered tissue was transferred and the tubes were reweighed. Tissue was homogenized and centrifuged at 5,000 rpm for 10 min. Supernatant was used for determining ammonia content after neutralization with 2M potassium bicarbonate and removal of precipitated potassium perchlorate.

Blood was collected from neck wound soon after decapitation and serum was separated. To 0.5 ml of serum, 1ml of 5% ice-cold PCA was added. The tubes were kept in ice for 30 min and centrifuged for 10 mln at 5,000 rpm. Supernatants

were neutralised to pH 7.0 with 2 M potassium carbonate and centrifuged to remove potassium perchlorate.

Ammonia was determined using phenol-hypochlorite reagent (Berthelot, 1859; Seligson and Hirahara, 1957; Chaney and Marbach, 1962; Giorgio, 1974). To 1 ml of supernatant, solution A (containing 50 g o£ phenol and 250 mg of sodium nitroprusside in 3.75 1 water) and 2ml of solution B (8.4 gms NaOH, 89.2 gms disodium hydrogen phosphate and 10 ml of 5% NaOCl/L) were added. After 20 min the intensity of the blue colour was read at 630 nm. Ammonium chloride (0.1-1.0 µmole) was used aa standard.

NUCLEIC ACID DETERMINATION:

Nucleic acids (RNA and DNA) were estimated by the procedure of Schmidt and Thanhausser (1945). Nucleic acids present in one ml of cell suspension (in 0.32 M sucrose; of predetermined cell number) were precipitated with 2 ml of 10% trichloroacetic acid (TCA). The pellet was washed once with 2 ml of 10% TCA and delipidated by washing twice with ethanol, ethanol:ether (3:1) and ether as suggested earlier. The final pellet was dried overnight at room temperature and digested in one ml of 1N potassium hydroxide at 37°C for 2.5 hours. Both DNA and protein were precipitated with 0.2ml of 6N HC1 and 0.6ml of 5% (w/v) TCA. The supernatant contained ribonucleotides. The pellet was hydrolyzed with 5% TCA at 80°C

for 30 miutes and centrifuged after cooling to room temperature. The absorbance of the nucleic acids was measured at 260nm (Hitachi spectrophotometer), using Calf thymus DNA and yeast RNA as standards.

PRODUCTION OF $^{14}CO_2$ FROM [U - ^{14}C] GLUCOSE:

Reaction mixture consists of 110mM NaCl, 1.2mM $MgSO_4$, 5mM KCl, 20mM HEPES, 10mM NaH_2PO_4 (pH. 7.4), 1mM $CaCl_2$, 2mH glucose, 0.1 uCi of [U - ^{14}C] glucose (500dpm/nmole) was placed in vials closed with Kontes rubber stoppers from which center well, with a strip of Whatman No.1 filter paper, was suspended. Viala were sealed with high vacuum grease and parafilm. The reaction was initiated by the addition of the subcellular/cellular fractions. The reaction mixture was incubated with shaking for 30 minutes at 37°C. To the zero time controls, perchloric acid was added before commencing incubation. After the incubation period, 0.2ml of hyamine hydroxide was injected in to the presuspended center wells. Reaction was terminated by injecting 0.2 ml of 3M perchloric acid in to the reaction mixture.The reaction mixture was further incubated for 1 hr with shaking at 37°C. At the end of incubation, the center well were removed and introduced in to scintillation vials containing 10 ml of Bray's scintillation fluid. Radioactivity was determined in Beckman Liquid Scintillation spectrometer. Optimal time and enzyme

concentrations were determined separately for each fraction (fig no. 17-18).

METABOLITE ASSAYS:

The subcellular and cellular prepartions (equivalent to 3 mg protein) were incubated aeperately with shaking in Kreb's Ringer-glucose-HEPES medium (121 mM NaCl ; 5 mM KCl ; 0.6 mM $CaCl_2$; 1.3 mM $MgSO_4$; 10 mM NaH_2PO_4 ; 10 mM HEPES and 5 mM glucose; Final volume 1 ml) for 30 min at 37°C. At the end of incubation, perchloric acid was added to a final concentration of 0.3 M and the the tubes were transferred to ice bath. After 10 min, the tubes were centrifused and the supernatant was neutralized with 2 M potassium carbonate till the pH is 7-7.4. The tubes were once again transferred to ice bath and centrifuged after 15 min at 10,000 g for 5 min. The neutralized supernatant was used for the determination of various metabolites by spectrophotometrlc methods. The final volume of the reaction mixtures for all the determinations was 0.5 ml and the reaction was initiated by the addition of supernatants (equivalent to 40 to 150 µgms of protein). All the reactions were carried out in a Hitachi spectrophotometer with a thermoprogrammer adjusted to 37°C. Changes in absorbancy were recorded at 1 min interval till 3 to 4 consequent values coincide with each other. Usually, each metabolite was assayed in 2 to 3 different volumes of

supernatant along with internal standards. Authentic standards (different concentrations) were used for preparing a standard curve and for the calculation of metabolite concentrations.

GLUCOSE-6-PHOSPHATE:

Glucose-6-phosphate content was determined by the method of Folbergrova et al., (1972b).

The glucose-6-phosphate present in the sample was converted to 6-phosphogluconate with glucose-6-phosphate dehydrogenase with the concomitant reduction of NADP to NADPH. The change in the absorbancy at 340 nm gives a measure of glucose-6-phosphate content.

$$\text{Glucose-6-phosphate} + \text{NADP}^{+} \xrightarrow{\text{G6PDH}} \text{6-phosphogluconolactone} + \text{NADPH} + \text{H}$$

$$\text{6-phospho-gluconolactone} + \text{H}_2\text{O} \longrightarrow \text{6-phosphogluconate}.$$

The assay mixture consists of 100 mM Trie (pH 8.0), 0.03 mM NADP, 0.2 mM DTT and glucose 6-phosphate dehydrogenase 0.5 µg/ml of reaction mixture. The reaction was completed in 3-5 min. Sensitivity of the assay method was 10 nmoles/ml.

FRUCTOSE-6-PHOSPHATE:

Fructose-6-phosphate was estimated according to the procedure of Folbergrova et al., (1972a). Fructose-6-phosphate was converted to glucose-6-phosphate with phospho-

glucose iaomerase and then to 6-phosphogluconolactone with glucose-6-phosphate dehydrogenase (G-6-P DH) with the concomitant reduction of $NADP^+$ to NADPH. Change in absorbancy gives a measure of fructose-6-phosphate.

$$\text{Fructose-6-phosphate} \xrightarrow{\text{phosphoglucose isomerase}} \text{glucose-6-phosphate}$$

$$\text{glucose-6-phosphate} + NADP^+ \xrightarrow{\text{G6PDH}} \text{6-phospho-gluconolactone} + NADPH + H$$

Reaction mixture consists of 50 mM Tris HC1 (pH 8.0), NADP 0.03 mM, phosphoglucoae iaomerase 20 µg/ml of reaction mixture, glucose-6-phosphate dehydrogenase 10 µg/ml of reaction mixture. Time taken for the reaction for completion was 8-10 min. Minimum concentration that can be detected by this method was *6* nmoles/ml.

PHOSPHOENOLPYRUVATE:

Phosphoenolpyruvate was estimated by the method of Lowry and Passonneau (1972).

Phosphoenolpyruvate in the sample waa converted to pyruvate with pyruvate kinase and then to lactate with lactate dehydrogenase (LDH) with the concomitant conversion of NADH to NAD. Change in abaorbancy at 340 nm givea a measure of phosphoenolpyruvate.

$$\text{Phoaphoenolpyruvate} + ADP \xrightarrow{\text{pyruvate kinase}} \text{pyruvate} + ATP$$

$$\text{pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{lactate} + \text{NAD}$$

Reaction mixture consists of 50 mM phosphate buffer (pH 7.0), 2 mM $MgCl_2$, 200 µM ADP, 100 µM NADH, pyruvate kinase 20 µg/ml of reaction mixture and lactate dehydrogenaae 5 µg/ml of reaction mixture. Time taken for the reaction for completion was 12-15 rain and the sensitivity was 2 nmoles/ml.

PYRUVATE:

Pyruvate was estimated as per the method of Lowry and Pasaonneau (1972).

Pyruvate was converted to lactate with the addition of lactate dehydrogenaae (LDH) and the accompanying change in absorbancy at 340 nm due to the conversion of NADH to NAD was taken as a measure of pyruvate content.

$$\text{pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{lactate} + \text{NAD}^{+}$$

Reaction mixture consists of 50 mM phosphate buffer (pH 7.0), 4 µM NADH and lactate dehydrogenase 5 jug/ml of reaction mixture. Reaction was completed in 5 min. Sensitivity of the method was upto 4 nmoles/ml.

LACTATE:

Lactate waa measured according to t'he method of Lowry and Passonneau (1972).

Lactate was converted to pyruvate with the addition of lactate dehydrogenaae (LDH) and NAD^+ and the change in absorbancy at 340 nm due to NADH formation was taken as a measure of lactate content.

$$\text{lactate} + NAD^+ \xrightarrow{\text{LDH}} \text{pyruvate} + \text{NADH}$$

Reaction mixture consists of 200 mM hydrazine buffer (pH 9.6), 2 mM NAD^+, and lactate dehydrogenase 10 µg/ml of reaction mixture. Time taken for the reaction to complete was 45-50 min. Minimum concentration that can be measured by this method was 10 nmoles/ml.

CITRATE :

Citrate was measured according to the method of Lowenstein (1969).

$$\text{Citrate} \xrightarrow{\text{aconitase}} \text{Cis-aconitate} \xrightarrow{\text{aconitase}} \text{Isocitrate}$$

$$\text{Isocitrate} + NADP^+ \xrightarrow{\text{ICDH}} \text{2-oxoglutarate} + CO_2 + \text{NADPH.H}$$

Citrate in the sample was converted to isocitrate with the addition of aconitase. The isocitrate was then converted to 2-oxoglutarate with isocitrate dehydrogenaae (ICDH) and NADP. The change in the absorbancy at 340 nm due to NADPH formation was taken as a measure of citrate content.

Reaction mixture consists of 100 mM triethonolamine buffer (pH 7.4), 300 μM NADP, 10 mM $MnSO_4$, aconitase 1 U/ml of reaction mixture, isocitrate dehydrogenase 1.8 U/ml of reaction mixture. Reaction was completed with in 5 min and the sensitivity was 10 nmoles/ml.

ISOCITRATE

Isocitrate was measured according to the method of Lowenatein (1969).

Isocitrate was converted to 2-oxoglutarate with the addition of isocitrate dehydrogenase (ICDH). The change in the absorbancy at 340 nm was taken as a measure of isocitrate content.

$$\text{Isocitrate} + \text{NADP} \xrightarrow{\text{ICDH}} \text{2-oxoglutarate} + CO_2 + \text{NADPH.H}$$

Reaction mixture consists of 100 mM triethanolamine buffer (pH 7.4), 300 μM $NADP^+$, 10 mM $MnSO_4$, isocitrate dehydrogenase 1.8 U/ml of reaction mixture. Reaction was completed in 8 min and the minimum concentration of isocitrate that can be estimated was 16 nmoles/ml.

2-OXOGLUTARATE:

The method of Folbergrova et al., (1972b) was adopted for the estimation of 2-oxoglutarate.

2-oxoglutarate in the sample was converted to glutamate in the presence of ammonium chloride, NADH and glutamate dehydrogenase (GDH) and the magnitude of change in absorbancy at 340 nm was taken as a measure of sample 2-oxoglutarate content.

$$\text{2-oxoglutarate} + \text{NADH} + \text{NH4} \xrightarrow{\text{GDH}} \text{glutamate} + \text{NAD}^{+}$$

Reaction mixture consists of 100 mM phosphate buffer (pH 6.8), 240 mM ammonium acetate, 3 µM NADH, 0.6 mM EDTA, GDH 120 µgs/ml of reaction mixture. Time taken for the reaction to terminate was 25-30 min. The minimum concentration that can be measured was 12 nmoles/ml.

MALATE:

Malate was estimated by the method of Folbergrova et al., (1972b).

Malate was converted to oxaloacetate by the addition of malate dehydrogenase (MDH) and NAD. The NADH formed (followed by the change in absorbancy at 340 nm) was taken as a measure of malate content.

$$\text{malate} + \text{NAD}^{+} \xrightarrow{\text{MDH}} \text{oxaloacetate} + \text{NADH.H}$$

Reaction mixture consists of 200 mM hydrazine buffer (pH 9.0), 0.2 mM EDTA, 0.2 mM NAD, MDH 25 µgms/ml of re-

action mixture. Time interval taken for the completion of the reaction was 45 - 50min. The sensitivity was upto 14 nmoles/ml.

ASPARTATE:

Aspartate was measured according to the method of Folbergrova et al., (1972b).

Aspartate in the sample was converted to oxaloacetate with the addition of aspartate aminotransferase (AAT) and 2-oxoglutarate. The oxaloacetate formed was converted to malate with malate dehydrogenase (MDH) in the presence of NADH. The change in absorbancy at 340 nn was proportional to the amount of aspartate in the sample.

$$\text{aspartate} + \text{2-oxoglutarate} \xrightarrow{\text{AAT}} \text{glutamate} + \text{oxaloacetate}$$

$$\text{oxaloacetae} + \text{NADH} \xrightarrow{\text{MDH}} \text{malate} + \text{NAD}^{+}$$

Reaction mixture consists of 100 mM Tris buffer (pH 8.0), 0.1 mM 2-oxoglutarate, 4 uN NADH, aspartate aminotransferase 5 µgms/ml of reaction mixture and malate dehydrogenase 2.5 µgms/ml of reaction mixture. Time taken for this reaction for completion was 55-60 min. The lowest concentration that can be measured by this method was 6 nmoles/ml.

ALANINE:

Alanine was estimated as per the method of Lowry and Passonneau (1972).

Alanine in the sample was converted to pyruvate with the addition of alanine aminotransferase (A1AT) and 2-oxoglutarate. The pyruvate formed was converted to lactate by the addition of lactate dehydrogenaae (LDH) and NADH. The change in the absorbancy at 340 nm was proportional to the amount of alanine present in the sample.

$$\text{Alanine} + \text{2-oxoglutarate} \xrightarrow{\text{A1AT}} \text{glutamate} + \text{pyruvate}$$

$$\text{pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{lactate} + \text{NAD}^{+}$$

Reaction mixture consists of 100 mM Tris buffer (pH 8.0), 0.1 mM 2-oxoglutarate, 4 µM NADH, lactate dehydrogenase 2.5 µgms/ml of reaction mixture and alanine aminotransferase 10 µgms/ml of reaction mixture . Reaction was completed between 50-55 min. The sensitivity of the assay method was upto 12 nmoles/ml.

GLUTAMATE:

Glutamate was measured according to the procedure of Folbergrova et al., (1972b).

Glutamate in the sample was converted to 2-oxoglutarate in the presence of NAD and glutamate dehydrogenase (GDH). The increase in the absorbancy at 340 was taken for the amount of glutamate present in the sample.

$$\text{glutamate} + NAD^{+} \xrightarrow{GDH} \text{2-oxoglutarate} + NADH + NH_4$$

Reaction mixture consists of 100 mM Tris buffer (pH 8.5), 0.3 mM NAD, 0.1 mM ADP, glutamate dehydrogenase 50 µgms/ml of reaction mixture. This reaction was completed with in 35 min. and the minimum concentration that can be measured was 8 nmoles/ml.

ADP:

ADP was estimated following the method of Folbergrova et al., (1972b).

ADP in the presence of pyruvate kinase (PK) gets phosphorylated resulting in the formation of ATP and pyruvate. This pyruvate was converted to lactate by the addition of lactate dehydrogenase (LDH) and NADH. The change in the absorbancy at 340 nm was proportional to the amount of ADP present in the sample.

$$ADP + \text{phosphoenolpyruvate} \xrightarrow{PK} ATP + \text{pyruvate}$$

$$\text{pyruvate} + NADH \xrightarrow{LDH} \text{lactate} + NAD^{+}$$

Reaction mixture consists of 50 mM phosphate buffer (pH 7.0), 0.2 mM DTT, 4 µM NADH, 0.02 mM phosphoenolpyruvate, 0.02 mM ADP, 2 mM $MgCl_2$, lactate dehydrogenase 8 µgms/ml of

reaction mixture and pyruvate kinase 0.5 µgms/ml of reaction mixture. Time taken for the reaction to complete was 10-15 min and the sensitivity of the assay procedure was up to 14 nmoles/ml.

ATP:

ATP was estimated as per the method of Folbergrova et al., (1972b).

ATP is converted to ADP with the concomitant phosphorylation of glucose to glucose-6-phosphate in the presence of glucose and hexokinase. This glucose-6-phosphate was converted to 6-phosphogluconate with G6PDH and in this process NADP is reduced to NADPH. The change in the absorbancy at 340 nm gives a measure of ATP content.

$$\text{ATP} + \text{glucose} \xrightarrow{\text{HK}} \text{ADP} + \text{G-6-PO4}$$

$$\text{G-6-PO4} + \text{NADP}^{+} \xrightarrow{\text{G6PDH}} \text{6-P-glucanolactone} + \text{NADPH}$$

Reaction mixture consists of 100 mM Tris buffer (pH 7.5), 1.0 mM glucose, 5 mM $MgCl_2$, 0.2 mM DTT, 0.03 mM $NADP^{+}$, hexokinase 1 µg/ml of reaction mixture, glucose-6-phosphate dehydrogenase 0.5 µgms/ml of reaction mixture. Time interval taken for this reaction for completion was 7-10 min and the least concentration that can be measured was 16 nmoles/ml.

STATISTICAL ANALYSIS OF DATA

Computer programmes were developed for calculating P values and to assess the statistical significance of the changes obtained in the course of experiment by Student's `T' test and Neumans & Kuel's multiple range test.

For all the figures legend is as follows:

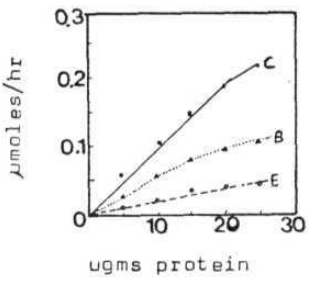
C : cerebral cortex; E : cerebellum; B : brain stem.

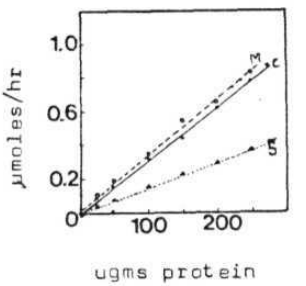
Cy: cytosol; M : mitochondria; S : synaptosomes.

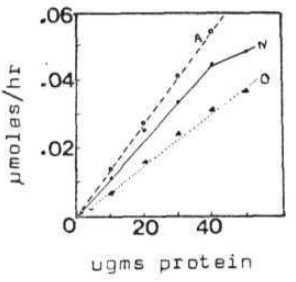
N : neurons; A : astrocytes; 0 : oligodendroglial cells.

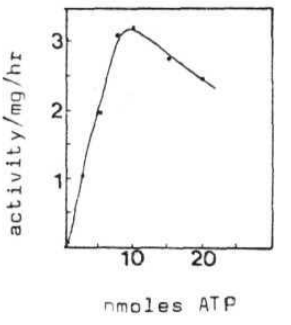
For substrates and other cofactors, optimal concentrations were checked only in cerebral cortex honogenates.

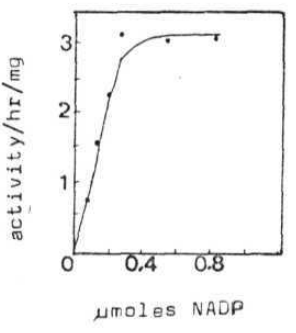
HEXOKINASE







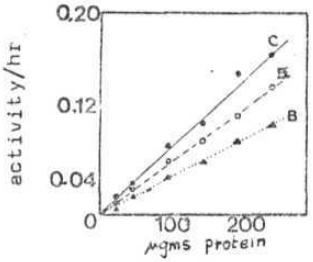


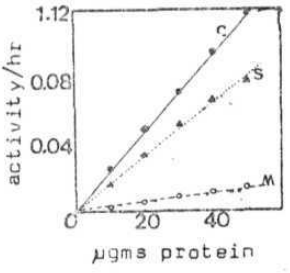


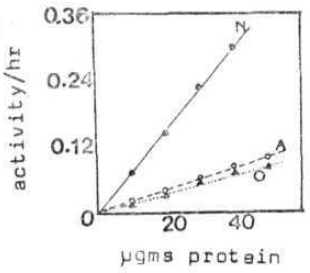
Activity units: µmoles of NADP reduced.

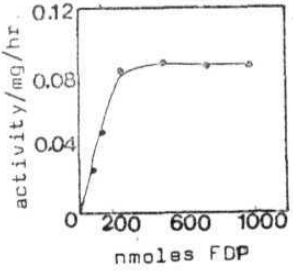
fig.1

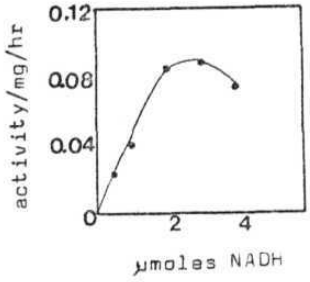
PHOSPHOFRUCTOKINASE







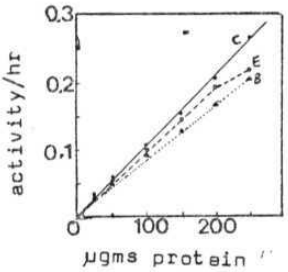


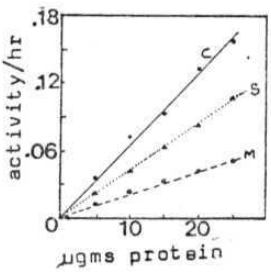


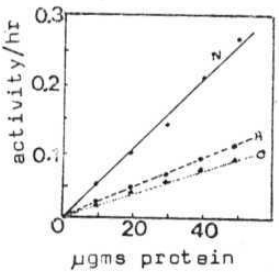
Activity: µmoles of NADP oxidized.

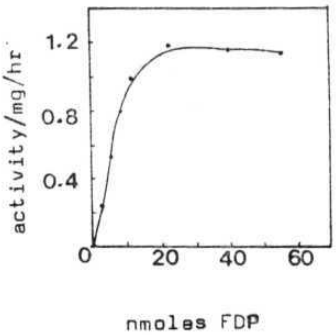
Fig.2

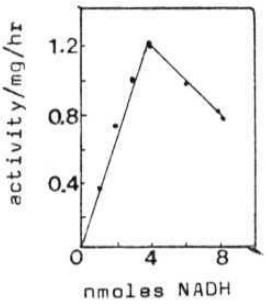
ALDOLASE







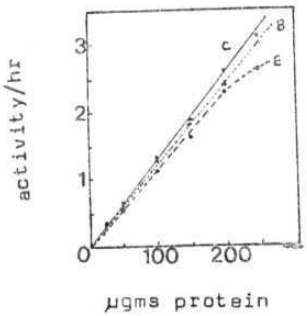


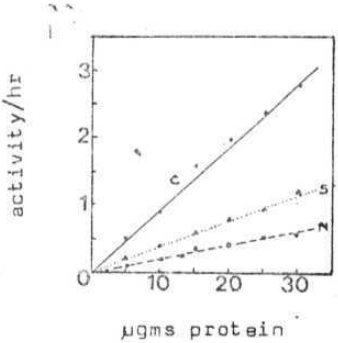


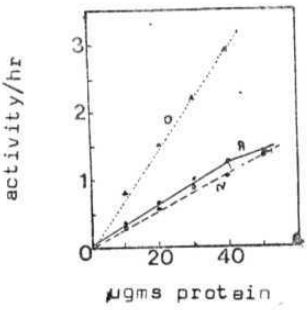
Activity units:
µmoles of NADH oxidized.

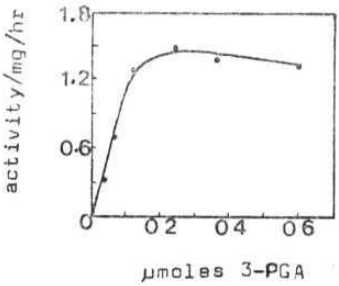
Fig.3

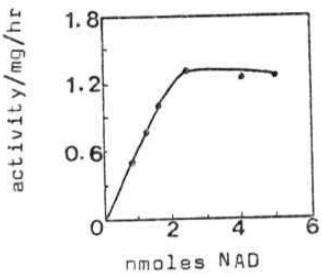
GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE











Activity units: µmoles of NAD reduced.

fig.4

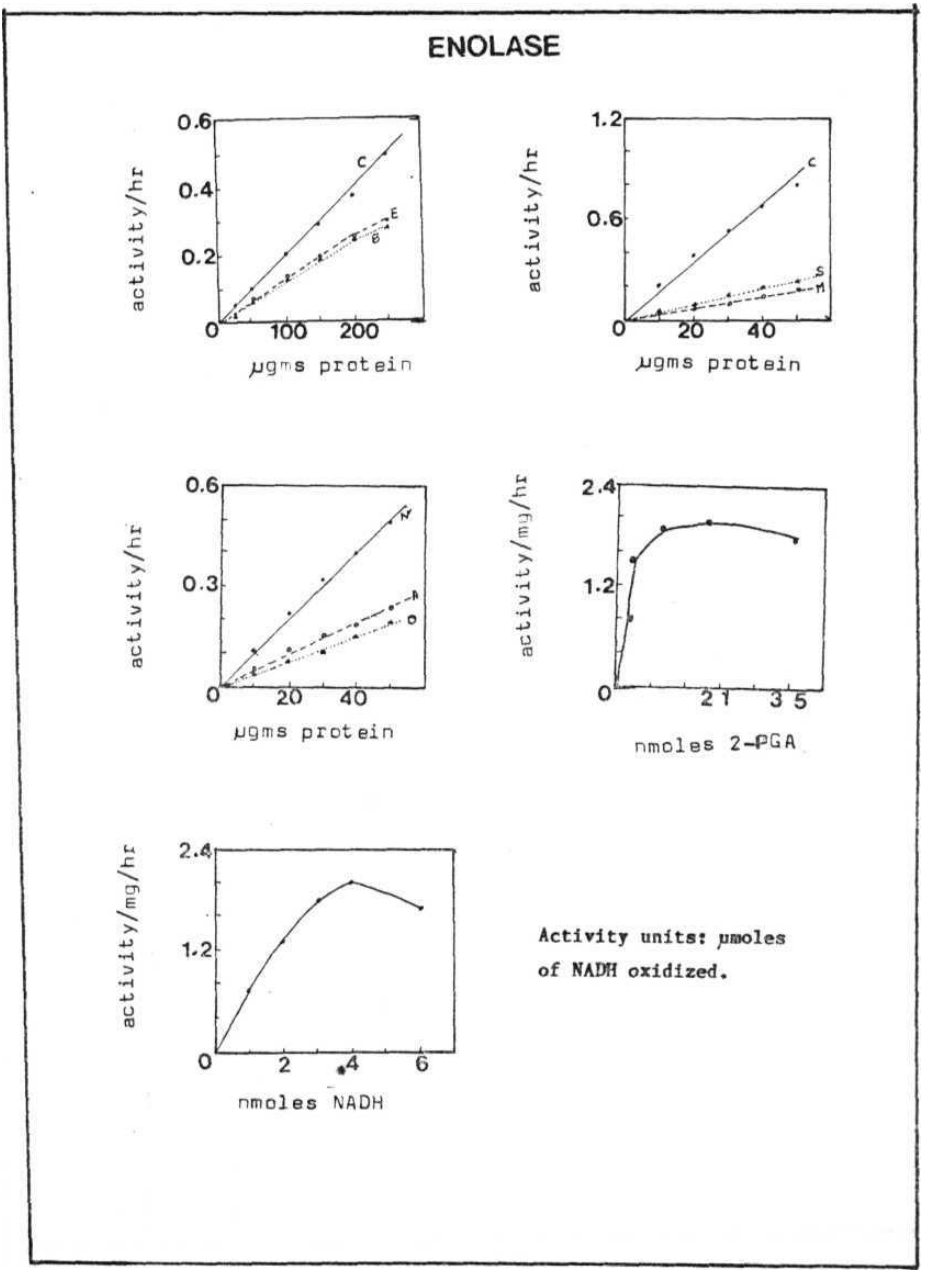
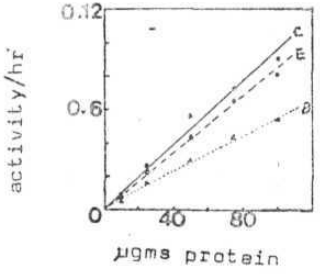
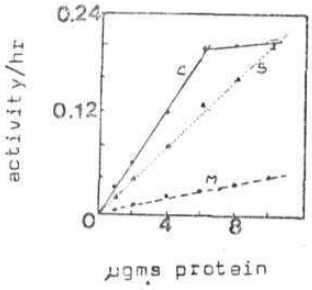
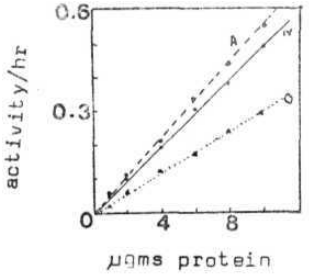

ENOLASE
activity/hr
0.6
0.4
0.2
0
100
200
µgms protein
C
E
activity/hr
1.2
0.6
0
20
40
µgms protein
C
S
activity/hr
0.6
0.3
0
20
40
µgms protein
activity/mg/hr
2.4
1.2
0
2 1
3 5
nmoles 2-PGA
activity/mg/hr
2.4
1.2
0
2
4
6
nmoles NADH
Activity units: µmoles
of NADH oxidized.

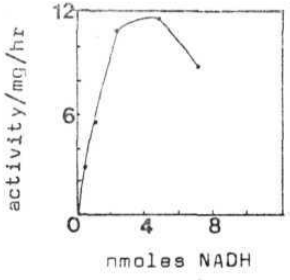
fig.5

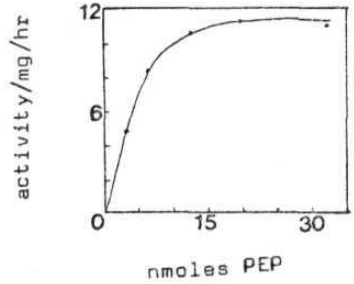
PYRUVATE KINASE







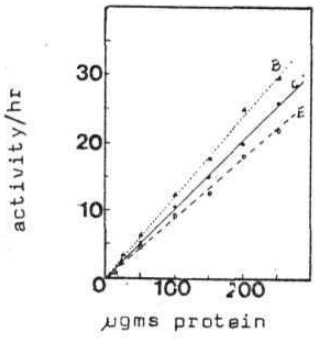


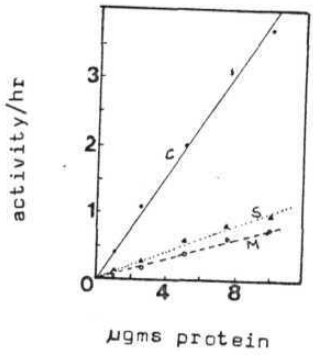


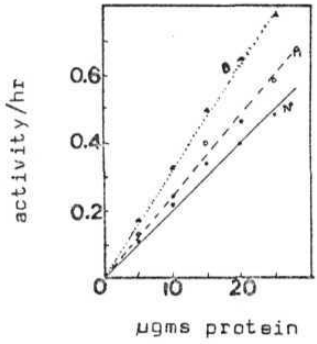
Activity Units: µmoles of NADH oxidized

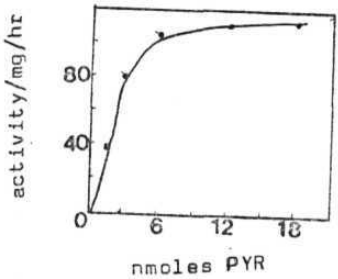
fig.6

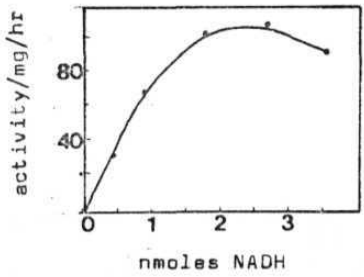
LACTATE DEHYDROGENASE (pyr → lact)







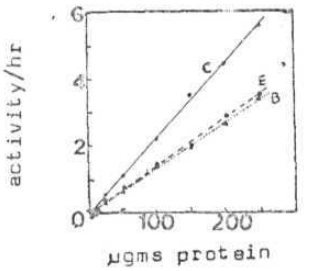


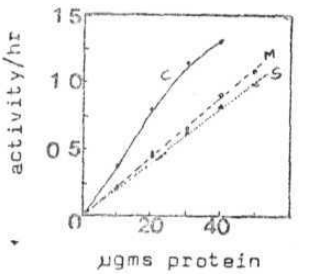


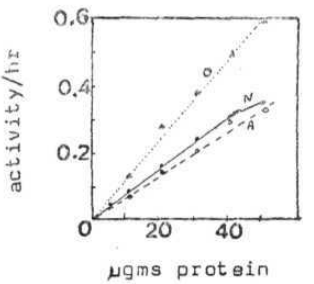
Activity units: µmoles of NADH oxidized.

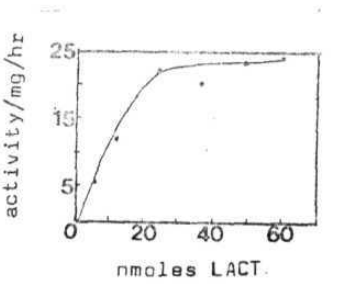
fig.7

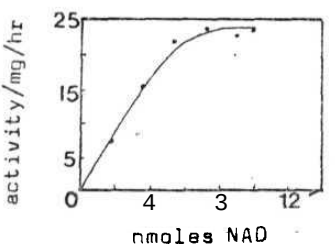
LACTATE DEHYDROGENASE (lac → pyr)







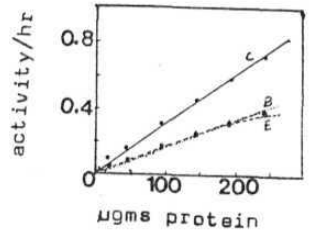


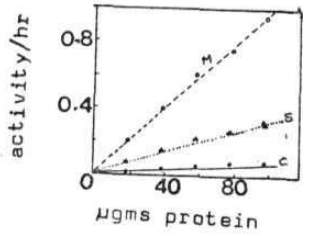


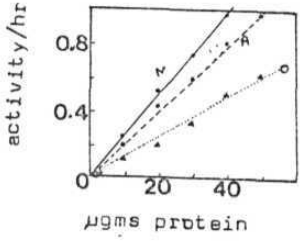
Activity units: µmoles of NAD reduced.

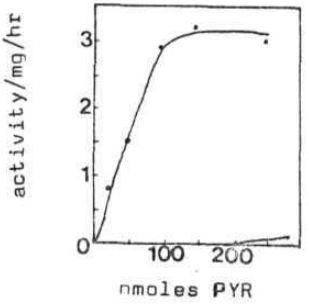
fig.8

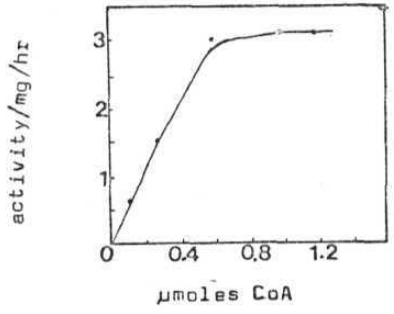
PYRUVATE DEHYDROGENASE







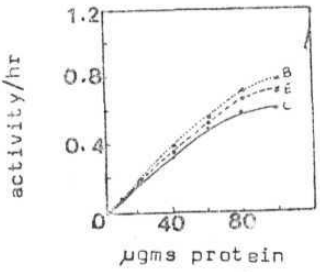


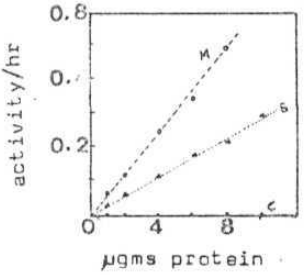


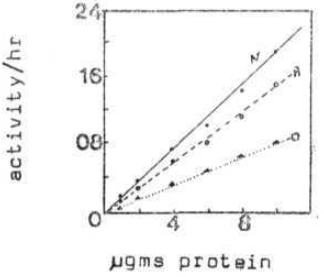
Activity units: µmoles of NAD reduced

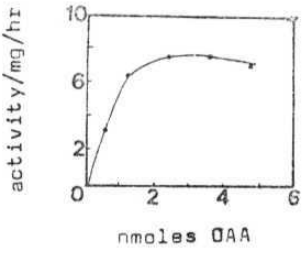
fig.9

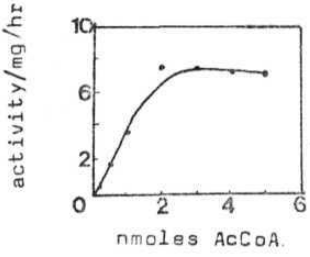
CITRATE SYNTHETASE







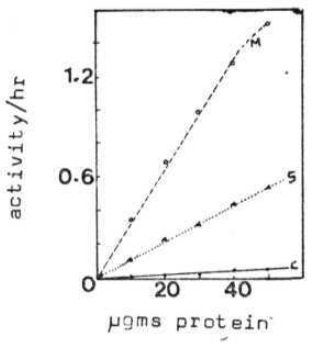


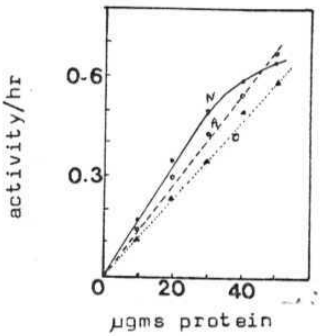


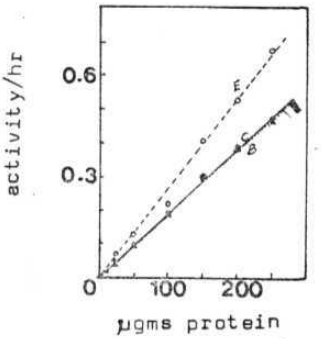
Activity units: µmoles of DTNB reduced

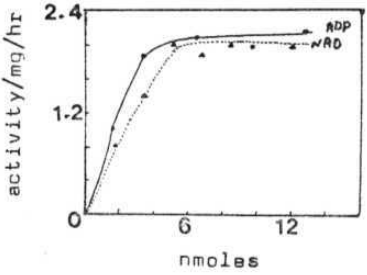
fig.10

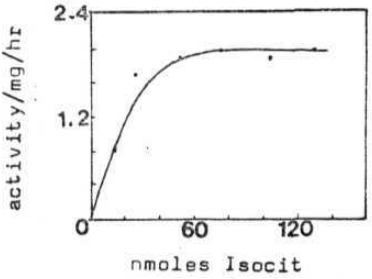
ISOCITRATE DEHYDROGENASE







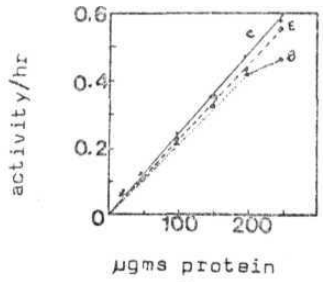


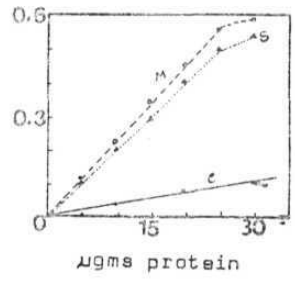


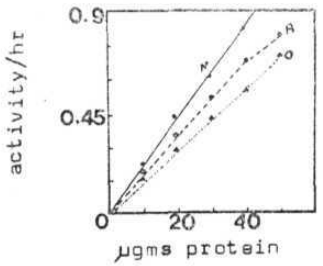
Activity units: µmoles of NAD reduced

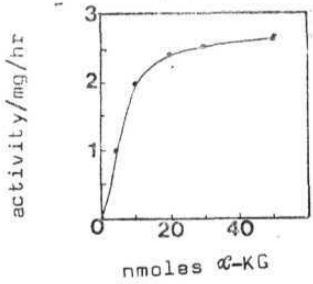
fig.11

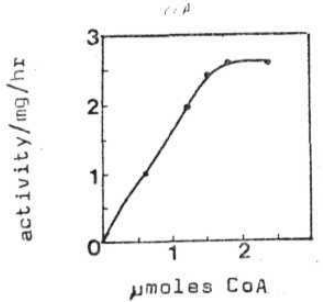
2-OXOGLUTARATE DEHYDROGENASE







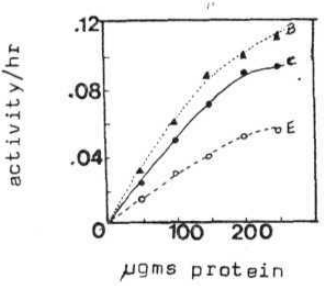


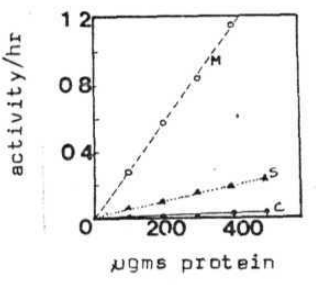


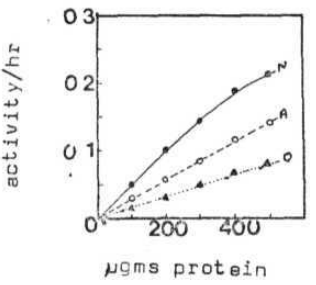
Activity units: µmoles of NAD reduced.

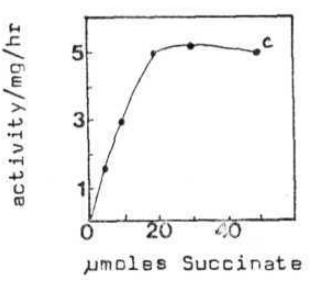
fig.12

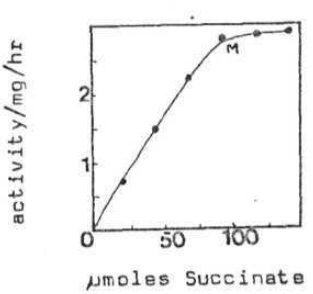
SUCCINATE DEHYDROGENASE

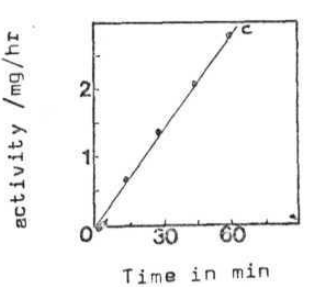








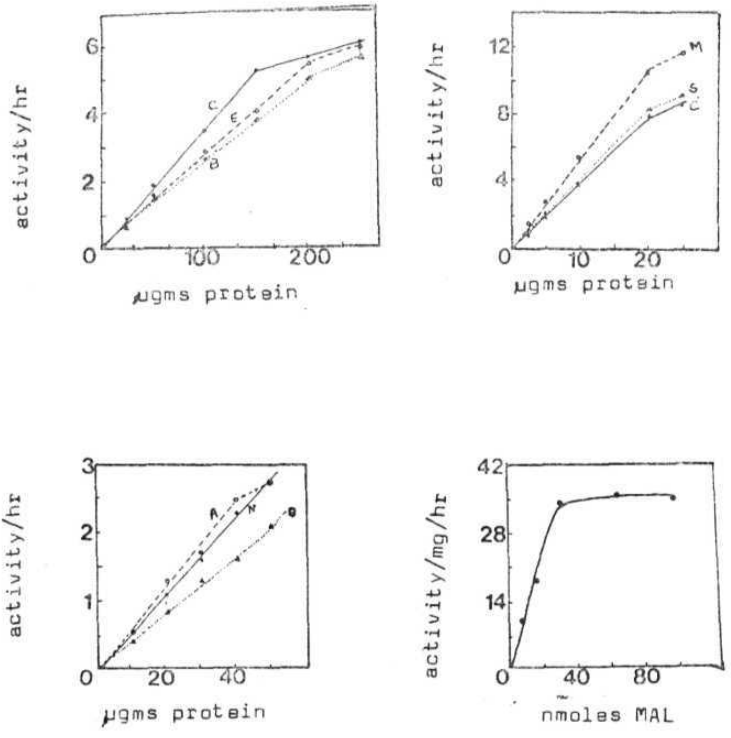




Activity units : µmoles of succinate oxidized.

fig.13

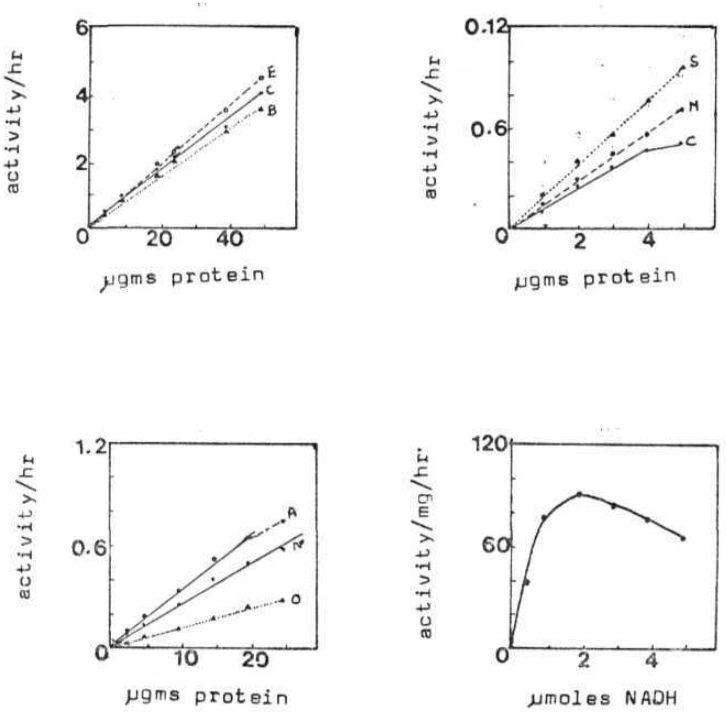
MALATE DEHYDROGENASE
(malate → oxaloacetate)



Activity units: µmoles of NAD reduced.

fig. 14

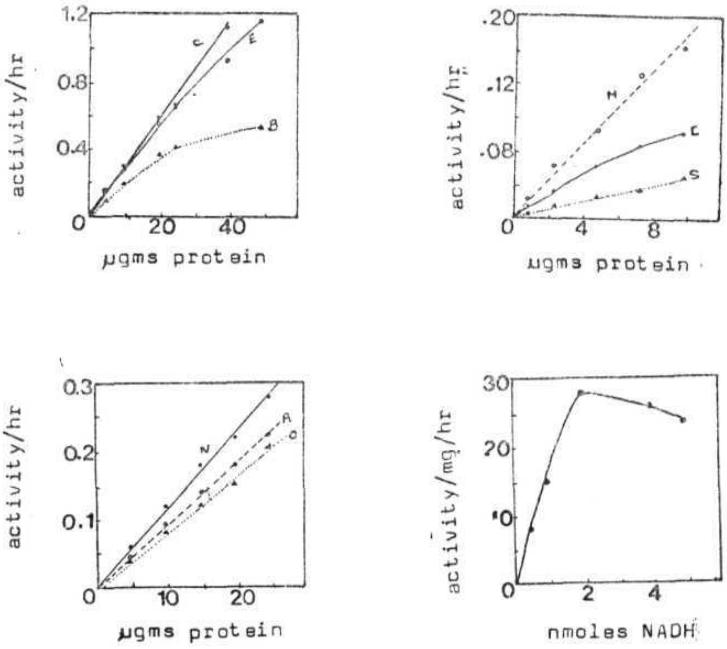
ASPARTATE AMINOTRANSFERASE



Activity: µmoles of NADH oxidized,

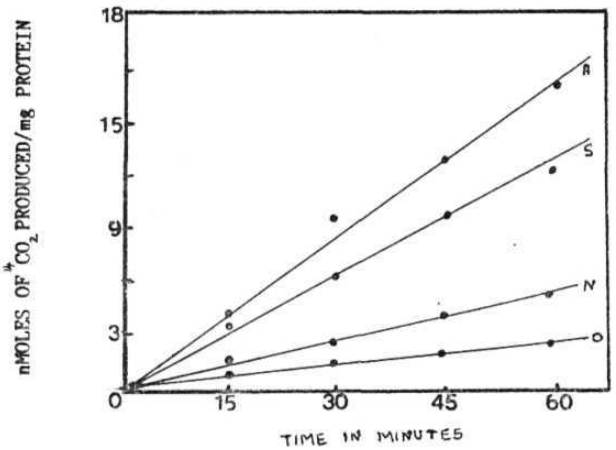
fig.15

ALANINE AMINOTRANSFERASE



Activity units: µmoles of NADH oxidized.

fig.16



TIME COURSE OF $^{14}CO_2$ PRODUCTION FROM [U-C] GLUCOSE

N: NEURONS A: ASTROCYTES

O OLIGODENDROGLIAL CELLS

S: SYNAPTOSOMES

fig.7

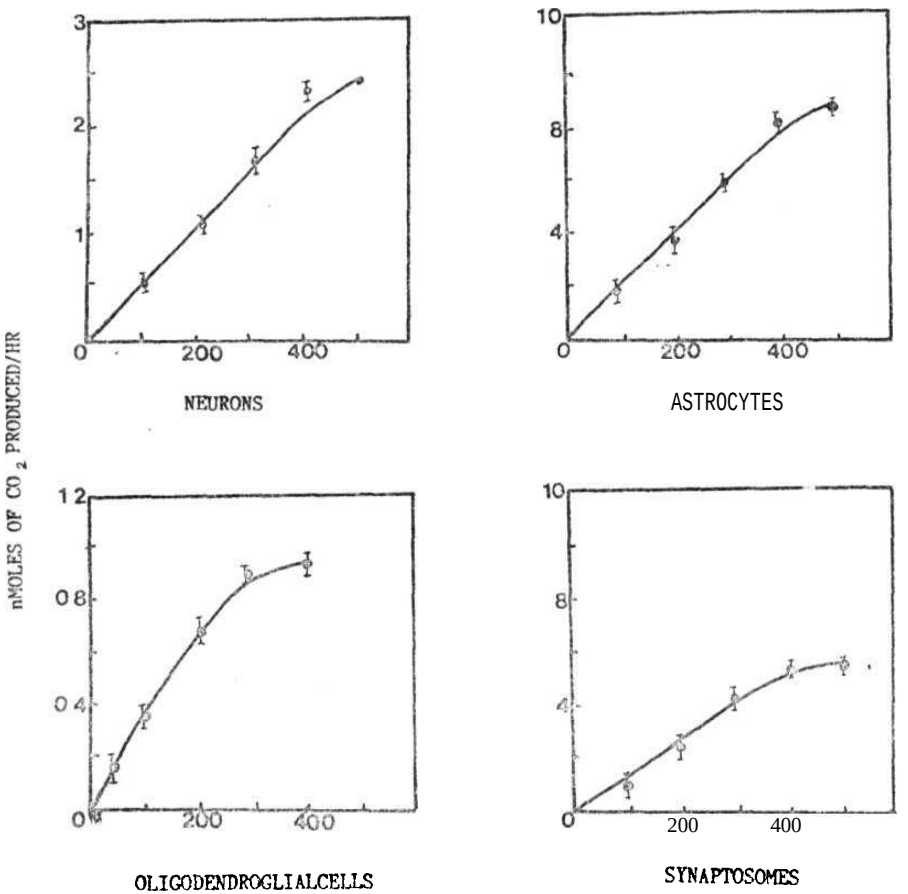

nMOLES OF CO_2 PRODUCED/HR
NEURONS
ASTROCYTES
OLIGODENDROGLIALCELLS
SYNAPTOSOMES
PRODUCTION OF $^{14}CO_2$ FROM [U-^{14}C]GLUCOSE
ACTIVITY/µG PROTEIN

fig.18

STU DIES WITH HOMOGENATES

Regional differences in the utilization of glucose carbon for the cerebral energy metabolism have been documented repeatedly in literature. These studies have been conducted by both invasive and non invasive techniques. For such studies whole animals, whole brain homogenates, subcellular fractions and brain slices have been frequently used. Techniques, such as administration of labelled 2-deoxyglucose followed by autoradiography, or usage of positron emission tomography have confirmed the earlier findings on the regional and topographical heterogenity of cerebral glucose metabolism (Sokoloff, 1977, 1980; Brownell et al., 1982; Phelps et al., 1982). As the cerebral glycogen stores are inadequate to support the cerebral glucose metabolism for prolonged periods, brain is dependent on the supply of glucose from the blood (Siesjo, 1978). Hence, cerebral glucose metabolism depends not only on the blood glucose content but also on the cerebral blood flow. However, earlier studies of Bachelard et al., (1973) have shown that the influx of glucose across the blood brain barrier is independent of blood glucose levels unless the latter is below 2 mM. This suggest that the cerebral glucose metabolism might be more dependent on the cerebral blood flow than on the blood glucose content unless it is $\leq$ 2mM.

Regional differences have been noticed in the cerebral vasculature as well as cerebral blood flow. In general, grey

matter regions of the brain receive a greater supply of blood than white matter regions (Freygand and Sokoloff, 1958). This is understandable as the grey matter contains the cell bodies of the neurons, astrocytes, dendritic and axonic arborisations and their terminals. These structures have a greater energy requirement as they are involved in the maintainance of the ionic gradients, synaptic transmission and biosynthesis of macromolecules. The white matter regions mostly consists of the axons where the synthetic activity is minimal and are cheifly involved in the nerve conduction or in the axonal transport. Moreover, the myelin lipida, which are the major constituents of these structures, have very low turnover rates (McIlwain and Bachelard, 1971). Hence, the energy requirements of these regions would naturally be minimal. It has also been noticed that the cerebral glucose metabolism of a given region would vary depending upon the activity state of that region. In general, neuronal excitation is associated with enhanced utilization of glucose while the depression of the cerebral activity is associated with a reduction in glucose metabolism (Sokoloff, 1977). This difference in the glucose metabolism during the activity states is usually associated with enhanced blood supply (Seisjo, 1978).

Besides the cerebral blood flow, other factors might also be responsible for the regional heterogenity of glucose metabolism. One such factor is the activities of the enzymes

involved in the cerebral glucose metabolism in different brain regions. Presently, these enzymes have been categorised, as is customary, into two groups: those enzymes involved in the conversion of glucose to pyruvate and lactate (glycolytic enzymes), (2) those involved in the oxidation of pyruvate (pyruvate dehydrogenase and citric acid cycle enzymes).

In this chapter attention will be focussed on the activities of the enzymes involved in the glucose metabolism in the homogenates of different brain regions, in normal and hyperammonemic states. It must be stated that studies have been carried out in the past on the regional distribution as well as the subcellular distribution of the glycolytic enzymes in rat brain under various experimental conditions (Knull, 1977, 1978, 1980; Knull and Khandelwal, 1982; Lai and Blass, 1984 a, b). However, no such studies have been conducted during hyperammonemic states.

In the present study activities of the enzymes of the glucose metabolism in the homogenates have been represented in two modes viz., activity per gm. wet weight of tissue and activity per mg protein (specific activity). Expression of the activity per gram wet weight of the tissue alone could be misleading if there are differences in the contribution of the water content and the dry matter to the total tissue weight of different brain regions in normal conditions.

Moreover, in the experimental conditions, if these parameters are altered, then the results will also be misleading. Similarly, if the activities are expressed only per mg protein, then any changes in the protein content will also result either in an underestimate or an overestimate of the enzyme activity. This is because of the fact, that the contribution of the enzyme protein to the total protein of that particular region is usually minimal and might also vary from region to region. Further, drug induced changes in the enzyme activity need not be asaociated with the changes either in the total protein content or in the content of the enzyme protein. Moreover, the change associated with any one of them need not be reflected in the other. Keeping these in mind, the activities have been represented for both gm wet weight and also for mg protein. However, it must be mentioned that there were no statistically significant differences in the protein contents of different regions of brain in normoammonemic animals (Table 3.1). The distribution of the activities of the enzymes of the glycolytic pathway in cerebral cortex, cerebellum and brain stem of normoammonemic rat brain are given in Table 3.2 (activities/gm wet weight of tissue) and in Table 3.3 (activities/mg protein).

Metabolism of glucose la initiated by phosphorylation of glucose to glucose-6-phosphate. The enzyme that mediates this reaction la hexokinase and the activity of this enzyme,

irrespective of its mode of expression, was similar in cerebral cortex and cerebellum. Its activity was lesser in the brain stem than that in the other two regions. Glucose-6-phosphate, thus formed, is at the cross roads of several metabolic pathways and its entry in to glycolytic pathway is facilitated by its conversion to fructose-6-phosphate which will be further phosphorylated to fructose-1,6-diphosphate. The earlier reaction is mediated by phosphohexose isomerase and the latter by phosphofructokinase. The latter enzyme is one of the regulatory point of the glycolytic pathway (Lowry and Passonneau, 1966; McIlwain and Bachelard, 1971; Sugden and Newsholme, 1975; Lehninger, 1984). Activity of this enzyme, irrespective of the mode of expression, was higher in cerebral cortex than in other two regions. Its activity in brain stem was lower than that of cerebellum.

Utilization of fructose-1,6-diphosphate in the glycolytic pathway depends upon its conversion to triose phosphates, a reaction mediated by aldolase. Unlike the above two enzymes, the activity of this enzyme was similar in all the three regions of the brain. Glyceraldehyde-3-phosphate dehydrogenase is the enzyme which converts glyceraldehyde-3-phoaphate (formed in aldolase reaction) to 1,3-diphosphoglyceric acid. Activity of this enzyme was similar in cerebral cortex and brain stem which was higher than that in the cerebellum. The 1,3-diphosphoglyceric acid will be converted

to 2-phosphoglyceric acid. This inturn will be converted into phosphoenolpyruvate in the reaction mediated by enolaae. Activity of this enzyme was higher in cerebral cortex than in the cerebellum and brain stem. There were no statistically significant differencea in the activity of enolase in the latter two regions. Activity levels of pyruvate kinase, an enzyme that converts phosphoenolpyruvate to pyruvate, was higher in the cerebral cortex than in the other two regions. Its activity was observed to be lower in the brain stem than in the cerebellum.

The pyruvate, so formed, may be converted to lactate by the enzyme lactate dehydrogenase. As the activity of this enzyme is reversible, the conversion of pyruvate to lactate is a reversible process. Lactate dehydrogenase activity, when measured in the direction of lactate formation, was higher in the cerebral cortex and brain stem when compared to the cerebellum. However, the profile of distribution was different when the enzyme activity was expressed per mg protein. Under such conditions, it was higher in brain stem than in cerebral cortex and cerebellum. There were no statistically significant differences in its activity in the latter two regions of brain. In the reverse direction (i.e., in the direction of pyruvate formation) lactate dehydrogenase activity was higher in cerebral cortex than in cerebellum and brain stem.

In summary, these reaults indicated that the activities of hexokinaae, phosphofructokinase and pyruvate kinase were lower in the brain stem than in the other two regions. Activities of other enzymes in this region were either similar or higher than those obtained in cerebral cortex and cerebellum.

When the activity levels of glycolytic enzymes were compared in a given region it was seen that the activity of lactate dehydrogenaae in the direction of lactate formation was much higher than the activities of all the other enzymes. Phosphofructokinase activity was leaser than that of hexokinaae and aldolase in cerebellum and brain stem but in cerebral cortex the activity of phosphofructokinase waa equal to that of aldolaae. Similarly, glyceraldehyde-3-phosphate dehydrogenaae activity waa much higher than that of the aldolaae only in brain stem but not in the other regions where as enolase activity waa higher than that of glyceraldehyde-3-phosphate dehydrogenaae in cerebral cortex but not in the other two regions. Lactate dehydrogenase activity in the direction of lactate formation waa higher than in the direction of pyruvate formation.

Of the enzymes of glycolytic pathway hexokinase, phoaphof ructokinaae and pyruvate kinase are supposed to be the key regulatory enzymes and are subjected to modulation by several ligands (McIlwain and Bachelard, 1971). Lower activity levels of these enzymes in the brain stem, which is a

predominantly white matter region, than cerebral cortex and cerebellum (predominantly grey matter regions) might be one of the factors responsible for the low rates of glucose utilization in this region. Other factors that influence glucose utilization in this region might be the cerebral blood flow and the nature and distribution of the transporter protein. Utilization of glucose, as measured by 2-deoxy-glucoae method, was reported to be higher in cerebral cortex than in the cerebellum (Duffy et al., 1980) though the activity levels of hexokinase were similar in these two regions. This suggests that other factors might be playing a key role in determining the metabolism of glucose in these two regions. Very low activities of phosphofructokinase in all the three regions supports the concept that this enzyme could be a major regulatory enzyme in the utilization of glucose. Higher activities of hexokinase than that of phosphofructo-kinase would result in the accumulation of glucose-6-phos-phate in the intracellular compartment. Such a notion is supported by several observations wherein the glucose-6-phosphate levels were reported to be several folds higher than that of fructose-6-phosphate (Carlson et al., 1975; Norberg and Seisjo, 1975a). This is understandable, as the former is utilized in several metabolic pathways other than glycolysis. For eg. glucose-6-phosphate may be utilized in the hexoae monophosphate shunt, for the synthesis of glycogen

and of amino sugars. Hence, glucose-6-phosphate that la formed in exceaa of ita utilization in the glycolytic pathway might participate in these pathways. Moreover, if the need arises for the increased utilization of glucose, glucose-6-phosphate may be drawn from this reserve.

In the cerebellum and brain stem, greater activity levels of aldolaae and glyceraldehyde-3-phosphate dehydrogenase, when compared with that of phosphofructokinase, would result in a greater conversion of fructose -1,6-diphosphate into triose phosphates thereby driving the reaction forward. Pyruvate kinase activity, which is several fold higher than those of other glycolytic enzymes might bring about the formation of large amounts of pyruvate. Moreover, as the activity of this enzyme is irreversible, all the other reactions of the pathway might be pulled forward which ensures the conversion of glucose into two molecules of pyruvate.

Greater activities of lactate dehydrogenase would facilitate the conversion of pyruvate to lactate and thereby regenerate the NAD which is required for the activity of glyceraldehyde-3-phosphate dehydrogenase. It has been shown that some of the lactate formed is exported from the cerebral tissue into the blood (Gibbs et al., 1942; Geiger et al., 1960) . However, it must be mentioned that the conversion of pyruvate to lactate is an uneconomical process as a result of which the cells would be restrained from using the pyruvate

in an effective manner. This effect would be more acute in an aerobic tissue such as brain. Though lactate can be converted back into pyruvate, this would result in the utilization of cytosolic pool of NAD which might have an adverse effect on glyceraldehyde-3-phosphate dehydrogenase activity. Infact, in aerobic tissues, such aa brain, alternate pathways are in operation to regenerate the cytosolic NAD . These mechanisms will be discussed later. Moreover, lactate formation would also bring about a change in pH of the intra and extracellular compartments which might adversely affect the activities of several enzymes.

The pyruvate formed during the glycolysis, is in the cross roads of several metabolic pathways. Pyruvate can be converted to lactate through lactate dehydrogenase activity or to amino acids in the reactions mediated by the several aminotransferases. Of these aminotransferases utilizing pyruvate as one of the substrates, the major one ia alanine aminotransferase. It mediates the formation of alanine and 2-oxoglutarate when glutamate and pyruvate are the substrates in a reversible reaction. Hence, the regional distribution of this enzyme in brain was investigated and compared with that of lactate dehydrogenase. When the activity of alanine aminotransferase was expressed per gram wet weight of tissue, the profile of distribution was observed to be cerebral cortex > cerebellum > brain stem. However, when the specific activity

was taken into consideration this profile was observed to be cerebral cortex = cerebellum > brain steal. Activity levels of this enzyme were observed to be several fold lesser than that of lactate dehydrogenase in all the brain regions. Hence, it appears that the transamination may not be a major pathway for pyruvate utilization in the brain (Table 3.4).

Another pathway for the metabolism of pyruvate is its transport from the cytosol into the mitochondria by a specific carrier mediated mechanism. The pyruvate which enters into the mitochondria may be converted to acetyl CoA. It has been shown that the pool of acetyl CoA formed from pyruvate is not diluted by the acetyl CoA originating from the fatty acid oxidation (Allweis et al., 1965). This may be because of the fact that, atleaat in adult animals, brain does not use fatty acids to meet its energy demands. Hence, it may be concluded that pyruvate is the major precursor for acetyl CoA formation in brain.

The acetyl CoA, so formed, may be used for the synthesis of fatty acids, cholesterol and acetylcholine or it may be further oxidized to CO_2 in citric acid cycle. However, the rate of lipid synthesis in the brain of adult animals is also very low (McIlwain and Bachelard, 1971). As a result very little amount of acetyl CoA would be diverted towards fatty acid synthesis. Another exclusive feature of the pyruvate metabolism in the brain is that it supports the synthesis of

acetylcholine which funtions as a neurotransmitter. It has been shown that the acetyl CoA, synthesized from the pyruvate provides acetyl moiety of acetylcholine (Quastel et al., 1936; Browning and Shulman, 1968; Grewaal and Quastel, 1973; Lefresne et al., 1973; Jope, et al., 1974; Tucek and Cheng, 1974; Jope et al., 1974; Gibson and Shimada, 1980; Benjamin and Quastel, 1982). However, the rate of this reaction i.e., the synthesis of acetylcholine is leaser than the rate of entry of acetyl CoA into citric acid cycle (Coxon and Peters, 1950; Hebb and Moris, 1969; McIlwain and Bachelard, 1971). These arguments suggest that most of the pyruvate carbon is converted to acetyl CoA and is oxidized in the citric acid cycle. Hence, the activitiea of pyruvate dehydrogenase and of the . nzymes of citric acid cycle were determined and are presented in Table 3.5 (activity/gm wet weight) and in Table 3.6 (activity/mg protein).

The entry of pyruvate carbon into citric acid cycle is initiated by the conversion of pyruvate to acetyl CoA in an irreversible reaction mediated by pyruvate dehydrogenase complex. Activity levels of thia complex were higher in cerebral cortex than in cerebellum and brain stem. However, its activity was same in the latter two regions of brain. This profile of distribution was independent of the mode of expression of the enzyme activity.

Metabolism of acetyl CoA in the citric acid cycle comme-

nces with its condensation with oxaloacetate to form citrate. Thia reaction is reversible and is mediated by citrate synthetase. Activity levels of this enzyme were similar in all the three regions of brain. Citrate, so formed, undergoes intramolecular reorganization to form isocitrate in a reaction mediated by aconitaae. The isocitrate is decarboxylated to form a five carbon compound, 2-oxoglutarate, in a reaction mediated by isocitrate dehydrogenase. This enzyme, in the mitochondria, requires NAD for its activity. There were no statistically significant differences in the activity levels of this enzyme in the cerebral cortex, cerebellum and brain stem. The next step in this metabolic pathway is the decarboxylation of 2-oxoglutarate to succinate. This is mediated by 2-oxoglutarate dehydrogenase complex which is similar to pyruvate dehydrogenase in several respects. Activity levels of this complex were also similar in all the three regions of the brain.

Succinate is converted to fumarate by the flavin dependent succinate dehydrogenase. Regional distribution of this enzyme was similar in cerebral cortex and brain stem which was greater than that in cerebellum. Fumarate undergoes intramolecular reorganization by the addition of a water molecule and malate is thus formed. The latter compound la dehydrogenated to yeild oxaloacetate. This reaction is mediated by NAD dependent malate dehydrogenase. As this is a

reversible reaction, activity of this enzyme was measured in both directions. In the direction of oxaloacetate formation, its activity was observed to be similar in cerebellum and brain stem which was lesser than that in cerebral cortex. However, in the reverse direction (oxaloacetate to malate), there were no statistically significant differences in its activity among the three regions of brain. When the specific activity of this enzyme is taken into account, it was higher in cerebral cortex than in brain stem but there were no statistically significant differences between cerebral cortex or cerebellum and brain stem.

When the activites of enzymes of citric acid cycle in a particular region were compared, it was observed that the malate dehydrogenase (assayed in the direction of malate formation) activity was greater than that of any other enzyme. This is followed by the activity of same enzyme in reverse direction (malate to oxaloacetate) and citrate synthase. Lowest activity levels were observed for succinate dehydrogenase in all the three regions of the brain.

Low activities of pyruvate dehydrogenase in the brain stem and cerebellum compared with that of cerebral cortex might be due to the lesser amount of enzyme and/or may be due to the differences in the states of phosphorylatlon (which inactivates) of the enzyme in these three regions. It is interesting to note that the activity of lactate dehydroge-

nase (pyruvate to lactate) was higher in brain stem than in other two regions. However, very high activities of citrate synthase in brain stem and in other regions would favour the condensation of acetyl CoA with the oxaloacetate to form citrate. Further, conversion of citrate to isocitrate and to 2-oxoglutarate might proceed at the same rate in all the three brain regions as the activity of isocitrate dehydrogenase is same in these regions. However, it must be mentioned that besides citric acid cycle, 2-oxoglutarate is also formed from glutamate and la also used for the synthesis of this amino acid. As the succinate dehydrogenase activity was very low when compared to 2-oxoglutarate dehydrogenase in all the brain regions, there might be a tendency for the accumulation of succinate.

Malate dehydrogenase (malate to oxaloacetate) activity, which is higher than that of citrate synthase, would provide adequate amounts of oxaloacetate for the condensation of acetyl CoA. Though malate dehydrogenase activity in the direction of malate formation was much higher than that in the reverse direction (oxaloacetate formation), it must be mentioned that malate is not the substrate for the citrate synthetase reaction. It has been shown that malate dehydrogenase is present both in the cytosol and in the mitochondria (Lehninger, 1984; Ratnakumari and Murthy, 1989,1990). In the cytosol this enzyme is responsible for the conversion of

oxaloacetate to malate while in mitochondria it will convert malate to oxaloacetate. The former reaction in the cytoaol plays an essential role in the operation of malate-aspartate shuttle. Besides malate dehydrogenase reaction, oxaloacetate ia also formed from aapartate by way of transamination with 2-oxoglutarate mediated by aspartate aminotransferase. Oxaloacetate has multiple fates in the mitochondria i.e., it may be used in transamination reactions, in citric acid cycle or for the formation of phosphoenol pyruvate.

Thus, it appears from these studies that the operational rates of citric acid cycle might be lower in brain stem and cerebellum due to lesser activities of pyruvate dehydrogenase when compared with that of cerebral cortex. Moreover, these results also indicate that the succinate dehydrogenase might also be one of the regulatory points of citric acid cycle. The latter suggestion is supported by several reports wherein succinate levels were observed to be higher than that of 2-oxoglutarate and fumarate (Goldberg et al., 1966; Folbergrova et al., 1974 a, b; Carlson et al., 1975; Norberg and Siesjo, 1975b; 1976).

EFFECTS OF AMMONIA:

As was mentioned earlier, cerebral glucose metabolism is a dynamic process and responds to alterations in the physiological states and also to pathological conditions. In general, increased cerebral glucose metabolism was observed in

the conditions where the cerebral activity was enhanced and a decrease was observed when the cerebral activity was depressed. Pathological states such as ischemia, hypoxia, hyperthermia, convulsions and depression of neuronal activity are known to result in alteration in cerebral glucose metabolism (McIlwain and Bachelard, 1971; Siesjo, 1978).

Several studies have been conducted in the past on cerebral glucose metabolism in hyperammonemic states. However, the results obtained were equivocal and were difficult to interpret due to the differences in the animals used in the study, mode of induction of hyperammonemia, degree and duration of hyperammonemia and the methods adopted for the study of glucose metabolism. In addition to these, complications are also due to the fact that the cerebral glucose metabolism is a result of a interplay of variety of factors. As was mentioned earlier, it depends upon the cerebral blood flow, transport of glucose across the blood brain barrier, activities of the enzymes of glucose metabolism and the rate and magnitude of flux of glucose carbon into various metabolic pathways.

The cerebral blood flow, which determines the amount of glucose available to the brain, depends upon the size of the blood vessels and the rate of the blood flow. It was reported that ammonia at higher concentrations dilates cerebral arterioles (Andersson et al., 1981). Administration of acute

doses of ammonium salts resulted in an increase in the cerebral blood flow with out any changes in the systemic haemodynamics and it vaa suggested that this increased cerebral blood flow might be due to the vasodilation of cerebral blood vessels in the presence of ammonium ions. However, results from other laboratories have indicated a fall in the cerebral blood flow in rats as well as in the dogs in hyperammonemic states (James et al., 1971, 1974; Gjedde et al., 1978). Results from another laboratory indicated no changes in the global cerebral blood flow or $CMRO_2$ in hyperammonemic states. However, it was shown by these investigators that ammonia can suppress the increase in cerebral blood flow especially in the pons and in the brain stem when the conditions are changed from normocapnia to hypercapnia (Barzilay et al., 1985). Chronic hyperammonemic situations, induced by portocaval anastomosis were shown to increase the cerebral blood flow in rats and man (Bianchi-Porro et al., 1969; Gjedde et al., 1978). Though the results are contradictory, it is evident that ammonia would affect the cerebral blood flow and thereby the amount of glucose available to the brain.

As glucose is not freely permeable across the blood brain barrier, it is transported by a specific carrier protein (Dick et al., 1984; Dick and Harrik, 1986). In other words, the availability of glucose to the brain depends not only on the concentration of glucose in the blood but also on

the rate of its transport across the blood brain barrier by the carrier protein. The rate of transport depends on the affinity of the carrier for glucose and the number of carrier protein molecules per unit area of the brain. The affinity between the glucose and the carrier protein may be altered by confirmational changes in primary and/or secondary structures of the protein or by changes in the surrounding membrane environment. Though the precise mechanics have not been worked out, it has been shown that the brain uptake index for glucose was decreased in portocaval anastomosis (Mans et al., 1983). However, administration of an acute dose of ammonium salts resulted in an increase in the arteriovenous difference of glucose across the brain thus suggesting an increased uptake of glucose into the brain under these conditions (Hawkins et al., 1973). Recently Jessy et al., (1990) suggested that the transport of glucose across the blood brain barrier might be unaltered in hyperammonemic conditions.

Subsequent to the transport of glucose across the blood brain barrier it encounters the membranes of different cell types i.e., neurons, astrocytes and oligodendrocytes. There is no information on the transport of glucose into the cell types in hyperammonemic states. Similarly, no information is available on the effect of ammonia on the endogenous pool size of glucose in these cells.

Once the glucose enters the cell, its fate depends upon

the operational rates of three different metabolic pathways, viz., glycogen synthesis, glycolysis and hexose monophosphate shunt. Though the glycogen content is low in the brain its turnover rate was shown to be very high (Karnovsky et al., 1980). Changes in the glycogen content were found to be variable with the mode of induction of hyperammonemic states. In chronic hyperammonemia, induced by portocaval anastomosis or by the administration of methionine sulphoximine, glycogen content was found to increase in the brain while a decrease in its content was reported when an acute dose of ammonium salts were administered (Folbergrova, 1973; Hawkins et al., 1973; Norenberg amd Lapham, 1974; Phelps, 1975; Hevor and Gayet, 1981; Hevor et al., 1985). Increase in the glycogen content in chronic hyperammonemic states especially due to methionine sulphoximine administration has been reported to be associated with the induction of enzymes involved either directly or indirectly in the synthesis of glycogen (Hevor and Gayet, 1981; Hevor et al., 1985). The flux of glucose carbon through hexose monophosphate shunt has been reported to be between 5-10% and significance of this pathway in adult animals has been controversial (Siesjo, 1978). However, there are no experimental results with respect to the effects of ammonia on the hexose monophosphate shunt pathway in hyperammonemic states. The above discussion implies that the major metabolic fate of glucose is its entry into glycolytic

pathway.

Several studies have been conducted on the levels of glycolytic intermediates during hyperammonemic states. But for the cerebral glucose content, in general, there was an increase in the levels of the glycolytic intermediates during acute hyperammonemic states (Hawkins et al., 1973). However, the precise mechanisms involved in bringing about such changes in the levels of these metabolites have not been specified by these investigators. Such changes in metabolites could be due to changes in the (1) activities of the enzymes involved in the metabolic pathway, (2) availability of the precursors for the metabolic pathway, and (3) in the rate of removal (by way of entry into another metabolic pathway or by way of transport into a different metabolic compartment which may be a subcellular organelle or even a whole cell). As many of the intermediates of the glycolytic pathway are in phosphorylated form, they are impermeable across the plasma membrane (Lehninger, 1984). Hence, the transport of these intermediates of the glycolytic pathway except pyruvate and lactate does not arise. However, it is quite possible that the removal of pyruvate or lactate in the cell can affect the rate of the flow of glucose carbon in the glycolytic pathway.

As mentioned above, changes in the activities of the enzymes involved could also affect the rate of flow of glucose carbon in the glycolytic pathway. In the present study,

the time interval between the administration of ammonium acetate and killing the animal is very short to account for altered rates of synthesis/degradation of enzyme protein as a mechanism for the changes in enzyme activity. Hence, the changes in the enzyme activity, if any, could be due to the direct or indirect action of ammonium ion on the enzyme. Under the latter category, ammonium ion might either enhance or decrease the rate of production of a ligand which might affect the activity of the enzyme. There are studies to indicate that the ammonium ions might also have direct effect on the activities of enzymes of glycolytic pathway. It has been observed that ammonium ion either disinhibits and/or activates phosphofructokinase both under in vivo and in vitro conditions dowry and Passonneau, 1966; Sugden and Newsholme, 1975). No information is available on the effect of ammonium ions, both under in vivo and in vitro conditions, on the activities of several of the glycolytlc enzymes in brain. Hence,the effects of ammonium ions on the activities of glycolytic enzymes in brain have been studied presently.

As in the case of the normal animals, activities of the fcn2ymea in the hyperammonemic animals were expressed per gm wet wt of tissue and also for tag protein. However, it must be mentioned that there were no atatlstlcally significant changes in the protein contents in the three different brain regions in normal and hyperammonemic animals (Table 3.1a). As

a result of this, the profile of changes in the activities of the enzymes of glycolytic pathway in all the three brain regions were same irrespective of the mode of the expression of the enzyme activity. Hence, the descriptions given below are equally applicable to both the cases, unless otherwise mentioned. After the administration of ammonium acetate there was an increase in blood and brain ammonia levels (Table 3.1b).

SUBACUTE CONDITIONS:

In subacute hyperammonemic state, hexokinase activity was observed to decrease in cerebral cortex and cerebellum while it was elevated in the brain stem. However, the changes observed in the activity of this enzyme were statistically significant only in the cerebral cortex and brain stem but not in cerebellum (Table 3.7). Under these experimental conditions, activities of phosphofructokinase and aldolase were elevated (Tables 3.8; 3.9). The magnitude of elevation in the phosphofructokinase activity was less in the brain stem when compared with that observed in cerebral cortex or cerebellum. In these two regions, the magnitude of change was more or less same irrespective of the mode of expression of activity (Table 3.8). The magnitude of change in aldolase activity was similar in all the three regions of brain (Table 3.9). There was a marginal elevation in the activity of glyceraldehyde-3-phosphate dehydrogenase in all the three regions of the brain

and it was observed that this change was not statistically significant in cerebral cortex and brain stem (Table 3.10). Enolase activity was elevated in these three regions of the brain but the magnitude of elevation in the cerebral cortex was only marginal and was statistically not significant (Table 3.11). Under these conditions, there was a two fold increase in the activity of pyruvate kinase in the cerebral cortex. Cerebellar activity of this enzyme was elevated by about 50i while the increase was marginal and statistically not significant in the brain stem (Table 3.12). Lactate dehydrogenase activity, measured in the direction of lactate formation, was unaltered in all the three regions of brain (Table 3.13). In the reverse direction activity of this enzyme was suppressed in the cerebral cortex, elevated marginally in the cerebellum and was unaltered in brain stem (Table 3.14).

ACUTE CONDITIONS:

Changes observed in the hexokinase activity in the rats administered with acute dose of ammonium acetate were similar to those in the subacute state i.e., a fall in the activity in cerebral cortex and cerebellum and an increase in brain stem. However, in this group of animals, unlike that of subacute group, the change noticed in the cerebellum was statistically significant (Table 3.7). Activities of phospho-

fructokinase, aldolaae, glyceraldehyde-3 phosphate dehydrogenase, enolase and pyruvate kinaae were elevated in the acute hyperammonemic state in all the three regions of the brain. In general, the magnitude of increase in the activity was much higher under the acute conditions than in the subacute state (Tables 3.8 to 3.12). The activity of lactate dehydrogenase, when assayed in the direction of lactate formation, was elevated in all the three regions of the brain in acute hyperammonemia. The magnitude of change observed was higher in cerebral cortex and cerebellum than in the brain stem (Table 3.13). Alterations in the activity of this enzyme measured in the opposite direction (pyruvate formation) were similar to those seen in the subacute state, especially in the cerebral cortex and cerebellum, i.e., its activity was suppressed in cerebral cortex and was elevated in cerebellum. In the brain stem of this group of animals lactate dehydrogenase activity was suppressed when compared to the controls. The magnitude of change was, of course, higher in the acute state than in the subacute state (Table 3.14).

The results obtained on the activities of glycolytic enzymes in the present study indicated a generalised increase in the activities of the enzymes of glycolytic pathway (upto pyruvate formation) with few exceptions. This would indicate that the cerebral glucose utilization in this pathway might be enhanced in hyperammonemic states.

Adequate literature is available on the levels of several intermediates of this pathway in hyperammonemic states. Some of these results are contradictory which may be due to the differences in the animals used and their age, methods adopted to induce hyperammonemic states, the mode of fixation of the brain to arrest the post-mortem changes and the region selected for the analysis. Hawkins et al., (1973) have induced hyperammonemic states by injecting the rats with ammonium acetate and after b minutes the brains were fixed by freeze blowing. By this method the supratentorial regions (brain excluding olfactory bulbs, cerebellum, pons and medulla oblongata) of the brain are immediately frozen (Veech et al., 1973; Veech and Hawkins, 1974). However, Bessman and Pal (1982) used mice and administered a different dose of ammonium acetate and the post-mortem changes were arrested by a 3 sec exposure to 1.5 KW microwaves. Though the latter method of arresting the brain metabolism is advantageous in the sense that regional studies can be made, the major problem will be redistribution of the metabolites within the brain regions and their diffusion into extracellular fluids. The method of Hawkins et al., (1973) though superior in the sense that no such redistribution takes place, the only problem will be lack of regional integrity. This aspect (regional integrity) is very essential as different regions of brain may exhibit temporal and vectoral differences in

their responses to the administered drug. Under such circumstances, if the metabolites were assayed in whole brain extracts it is quite possible that change in one region might either nullify or amplify the changes in other regions. However, until a better method is evolved with minimal post-mortem changes and a better of maintainance of regional integrity, caution must be exerted in interpreting the results.

Studies of Hawkins et al., (1973) indicated a marginal increase in cerebral glucose content in rats administered with ammonium acetate. In the same study, these investigators have reported no changes in the levels of cerebral glucose-6-phosphate. Thia was later contradicted by Bessman and Pal (1982) who reported an elevation in the levels of this metabolite. In the present study, a marginal decrease was observed in hexokinase activity in both cerebral cortex and cerebellum while an elevation was observed in brain stem. At the outset, it appears that the present results are contradictory to the reported changes in the levels of glucose-6-phosphate. However, it may be stated that the magnitude of inhibition ia very marginal and the increased availability of glucose might counter balance this inhibitory effect. Moreover, the results obtained in the present study are supportive of the observations made by both these investigators because the inhibitory effect of ammonium ions on hexokinase was observed only in

cerebral cortex and cerebellum. Increased activity of this enzyme in brain stem was in support of the observations made by Bessman and Pal (1982) on the whole brain.

In the present study, administration of ammonium salts resulted in an enhancement in the activity of phosphofructoklnase. This result was in concurrence with the earlier studies in which it was established that ammonium ions stimulate the activity of phosphofructokinase both under in vivo and in vitro conditions (Lowry and Passonneau, 1966; Sugden and Newsholme, 1975). Such a stimulation *in* the activity of this enzyme would result in the enhanced production of fructose 1,6-diphosphate in the brains of rats administered with an acute dose of ammonium acetate. However, results of the present study as well as of Hawkins et al.,(1973) do not agree with that of Bessman and Pal (1982) who reported a decrease in the levels of this intermediate.

Fructose 1,6-diphosphate, formed in the phosphofructokinase reaction, serves as a substrate for aldolase. Following the administration of either acute or subacute dose of ammonium acetate, an increase was observed in the activity of this enzyme suggesting an enhancement in the utilization of fructose 1,6-diphosphate. This suggestion is in accordance with the increased levels of dihydroxyacetone phosphate, one of the products of the aldolase activity reported in the rat brain in acute hyperammonemic states. Another triose phos-

phate, 3-phosphoglyceraldehyde that is formed in aldolase reaction, is the substrate for glyceraldehyde-3-phosphate dehydrogenase. Activity of this enzyme was also enhanced in hyperammonemic states, though the changes in subacute state were not statistically significant in cerebral cortex and brain stem. Such an increase in the activity of this enzyme would result in the enhanced production of 1,3-diphosphoglycerate as well as the reduction of NAD to NADH. Moreover, increased production of 1,3-diphosphoglycerate would also ensure an enhanced production of ATP in the subsequent reactions of the glycolytic pathway.

Though no reports are available on the levels of 1,3-diphosphoglycerate, it has been shown that the levels of 3-phosphoglycerate (which is produced by the phosphorolysis of 1,3-diphosphoglycerate by phosphoglycerate kinase) is enhanced in brain in acute hyperammonemic states (Hawkins et al., 1973; Bessman and Pal, 1982). This indicates an enhanced availability of 1,3-diphosphoglycerate, which is suggestive of an increased activity of glyceraldehyde-3-phosphate dehydrogenase. Subsequent to the formation, 3-phosphoglycerate is converted to 2-phosphoglycerate and the latter serves as substrate for enolase. The product of this reaction ia phosphoenolpyruvate, which is a high energy phosphate compound. Keeping in pace with the changes in the activities of all the other enzymes of glycolytic pathway, enolase activity was

also enhanced following the administration of ammonium salts (though this change in cerebral cortex in subacute condition was statistically not significant). Enhanced levels of phosphoenolpyruvate, reported by Hawkins et al., (1973) is in agreement with the enhanced activity of enolase observed in the present investigation. The ultimate step of glycolyais ia the formation of pyruvate which is mediated by pyruvate kinase. In this reaction phosphoenolpyruvate ia converted to pyruvate by transferring the phosphate to ADP resulting in the formation of ATP and pyruvate. Activity of this enzyme was also enhanced in three different regions of the brain in hyperammonemic states induced by the administration of either subacute or acute doses of ammonium acetate. This observation is supported by the enhancement in the levels of pyruvate observed in brain in hyperammonemic states of various etiologies (Hawkins et al., 1973; Jessy et al., 1990).

As was mentioned earlier, pyruvate is at the crossroads of several metabolic pathways. It can be converted to lactate or to alanine and other amino acids or to acetyl CoA which may be either oxidized in citric acid cycle or may be incorporated into lipids. One of the factors that determines the entry of pyruvate into these pathways is the activity levels of the initial enzymes of these metabolic pathways and the availability of the cofactors required for these enzymes. Much of the pyruvate may be converted to lactate as the

activity of lactate dehydrogenase is several folds higher than those of other pathways which utilize pyruvate. In hyperammonemic states induced by the administration of ammonium salts, it was observed that the lactate dehydrogenase activity especially in the direction of lactate formation is enhanced. Earlier reports on the enhanced levels of lactate in brain in hyperammonemic states support the present observation on the changes in the activity of this enzyme (Hawkins et al., 1973; Adams et al., 1979; Raabe and Lin, 1984; Jessy et al., 1990). Though an enhancement in lactate production would increase the rate of regeneration of NAD from NADH in hyperammonemic states, it would also result in the loss of pyruvate carbon. This is because of the fact that the lactate diffuses out of the cell into the extracellular space. In hyperammonemic states an increase in the arteriovenous difference for lactate was observed across the brain (Hawkins et al., 1973). Even if some lactate is retained in the brain, it still serves no purpose as lactate is a dead end product. However, lactate can be converted back to pyruvate and may be utilized but it was observed that the activity of lactate dehydrogenase in this direction was suppressed. This would prevent the reconversion of the much required NAD back to NADH.

In conclusion, studies on the enzymes of glycolytic pathway suggest an increased utilization of glucose in brain

in hyperammonemic states.

EFFECTS OF AMMONIA ON CITRIC ACID CYCLE ENZYMES:

The proposal of Bessman and Bessman (1955) that cerebral energy metabolism is affected in hyperammonemic states is centered around the impairment of citric acid cycle. As was mentioned earlier, these authors have proposed that ammonia detoxificaion through the reaction mediated by glutamate dehydrogenase would deplete the 2-oxoglutarate from citric acid cycle. This proposal sparked off several investigations on the content of 2-oxoglutarate and other citric acid cycle intermediates in brain in various animal models of hyperammonemic states. The results of such studies were controversial which may be due to the differences in the mode of induction of hyperammonemic and the brain regions analysed for this purpose. Despite of the enormous amount of literature available on the levels of citric acid cycle intermediates, very few reports were available on the activities of enzymes of citric acid cycle under these conditions (Ratnakumari, 1984; Ratnakumari and Murthy, 1985, 1986, 1989, 1990). Hence, in the present investigation activity levels of the enzymes of citric acid cycle were determined in hyperammonemic states.

Administration of subacute and acute doses of ammonium acetate resulted in an elevation in the activity of pyruvate dehydrogenase in all the three brain regions studied. The

magnitude of elevation in its activity was observed to be highest in the cerebellum followed by brain stem and was least in cerebral cortex. Moreover, the magnitude of change was maximal in the acute states than in subacute conditions (Table 3.15). The committed reaction of citric acid cycle, i.e., citrate synthesis is mediated by the enzyme citrate synthetase, was unaltered in the cerebral cortex and brain stem in the eubacute states while its activity was elevated in the cerebellum. However, under acute conditions, an elevation was observed in its activity in the cerebral cortex and cerebellum but not in the brain stem. The magnitude of elevation was higher in acute hyperammonemic conditions than in subacute states (Table 3.16). The activity of the next enzyme in the cycle i.e., isocitrate dehydrogenase was unaltered in both acute and subacute hyperammonemic states in all the three brain regions (Table 3.17).

Activity levels of 2-oxoglutarate dehydrogenase, an enzyme responsible for channelling of 2-oxoglutarate into citric acid cycle, was enhanced in both acute and subacute states of hyperammonemia in all the three regions of the brain. As in the case of pyruvate dehydrogenase the magnitude of elevation was observed to be higher in acute than in the subacute hyperammonemic states (Table 3.18). A similar change was also noticed in the activity of succinate dehydrogenase under these conditions and the magnitude of change was

higher in acute hyperammonemic state than the aubacute condition (Table 3.19). In contrast to the changes in the activities of above said enzymes, malate dehydrogenase activity (in the direction of oxaloacetate formation) was suppressed in all the three regions of brain in both acute and subacute states and the magnitude of suppression was higher in the acute than in the subacute state (Table 3.20). However, activity of this enzyme when measured in the reverse direction i.e., in the direction of malate formation, was unaltered in both subacute and acute states of hyperammonemia (Table 3.21).

A similar profile of changes were observed in the activities of the enzymes when expressed per mg protein (Table 3.15 to 3.21). The only exception was observed to be an increase in the citrate synthetase activity in the cerebral cortex in subacute hyperammonemic states (Table 3.16).

In the acute hyperammonemic states animals were sacrificed during convulsions. Hence, some of the changes observed in the activities of citric acid cycle enzymes could be due to the convulsive phenomenon rather than the effect of ammonia. Though, the results obtained after the administration of subacute dose of ammonia argue against such a possibility, it was felt to study the activities of the enzymes of citric acid cycle in non-convulsive phase of acute hyperammonemia. For this purpose, the animals were sacrificed 15 min after

the administration of an acute dose of ammonium acetate when animals have not entered the convulsive phase and the activities of these enzymes were determined (Table 3.15 to 3.21). Under these conditions, an increase was observed in the activity of pyruvate dehydrogenase in all the three regions of brain while the activity of citrate aynthetase showed no significant changes in the cerebellum and brain stem. However, its activity was elevated in the cerebral cortex. Changes observed in the isocitrate dehydrogenase activity, under these conditions, were similar to that seen in the convulsive phase of hyperammonemia. The elevation observed in the activity of 2-oxoglutarate and succinate dehydrogenases in the preconvulsive phase of acute hyperammonemia was similar to that seen during convulsions. Similarly, malate dehydrogenase activity in the direction of oxaloacetate formation was suppressed in the preconvulsive phase. It is worth pointing out that most of the changes observed during the preconvulsive phase were of higher magnitude than those observed during the convulsions.

A similar profile of changes were observed during preconvulsive phase in the activities of these enzymes when expressed per rog protein (Table 3.15 to 3.21). The only exception was found to be in the activity of isocitrate dehydrogenase in cerebral cortex wherein the elevation in the activity of this enzyme was statistically significant when it

was expressed per mg protein (Table 3.17).

In brief, administration of ammonium salts appears to enhance the activities of citric acid cycle enzymes except malate dehydrogenase. The enhancement observed in the activity of pyruvate dehydrogenase in hyperammonemic states might be due to enhanced availability of substrate or the changes in phosphorylation status of the enzyme. The former may be ruled out as the permeability barrier to the substrates were abolished by the addition of Triton X-100 to the homogenates of the brains of normal and hyperammonemic rats. It has been established that the phosphorylation of pyruvate dehydrogenase inactivates the enzyme and this process depends on the ATP/ADP ratio. Besides this, the ratio of NAD^+/NADH, the CoA availability, and the levels of oxaloacetate and acetyl CoA (feed back inhibitor) are also the factors that influence the activity of pyruvate dehydrogenase (Jope and Blass, 1975, 1976; Randle, 1981, 1983; Lehninger, 1984). It is possible that in hyperammonemic states, there might be an alteration in any one of the parameters which influence the pyruvate dehydrogenase activity. Whatever the mechanism may be, the elevation in pyruvate dehydrogenase activity promotes the decarboxylation of pyruvate and enhance the production of acetyl CoA in all the three regions of the brain.

Stimulation of citrate synthetase activity would result in enhanced condensation of acetyl CoA with oxaloacetate in

cerebral cortex and cerebellum in hyperammonemic states. However, it must be mentioned that though acetyl CoA availability (due to increase in pyruvate dehydrogenase activity) and citrate synthetase activity were enhanced in hyperammonemic states, the amount of oxaloacetate available under these conditions (see below) would also regulate the rate of its condensation with acetyl CoA and thus the production of citrate. However, such changes in the citrate production might not take place in brain stem where the citrate synthetase activity was not altered in hyperammonemic state.

Lack of changes in isocitrate dehydrogenase activity might not alter the rate of 2-oxoglutarate production in citric acid cycle in brain in hyperammonemic states. However, the increase in the activity of 2-oxoglutarate dehydrogenase under these conditions would rapidly decarboxylate 2-oxoglutarate to succinate and thus pulls the isocitrate dehydrogenase reaction forward. As there are many similarities in the kinetics and regulatory aspects of pyruvate and 2-oxoglutarate dehydrogenases (Randle, 1983; Lehninger, 1984), the stimulation of the latter enzyme may be due to the reasons mentioned earlier for pyruvate dehydrogenase. The increase in succinate dehydrogenase activity in hyperammonemic states would facilitate tha conversion of succinate to fumarate. Despite the stimulation of succinate dehydrogenase, it still exhibits least activity even under hyperammonemic states.

Suppression of malate dehydrogenase (in the direction of oxaloacetate formation) would retard oxaloacetate formation from malate. Lack of adequate amounts of oxaloacetate might restrict the synthesis of citrate in citric acid cycle. Under such conditions, acetyl CoA would accumulate, which might inhibit pyruvate dehydrogenase activity and thus its own production (Randle, 1983; Lehninger, 1984). Such changes would reduce the effectiveness of citric acid cycle in generating energy which is required in hyperammonemic states for the production of glutamine. However, it must be mentioned that acetyl CoA is also a stimulator for phosphoenolpyruvate carboxykinase which brings about the fixation of CO_2 into phosphoenolpyruvate and resulting the production of oxaloacetate (Cheng, 1971 a, b). In fact, an increase in this process, i.e., oxaloacetate production due to CO_2 production, has been reported in hyperammonemic states (Berl, 1971).

The results of the present study suggested that the synthesis and utilization of 2-oxoglutarate in citric acid cycle may not be altered in hyperammonemic states and the CO_2 fixation might be enhanced to restore the oxaloacetate in brain in hyperammonemic states.

STUDIES ON MALATE-ASPARTATE SHUTTLE:

One of the important reactions of glycolysis la the formation of 1,3-diphosphoglycerate which is mediated by the enzyme glyceraldehyde-3-phosphate dehydrogenase. The impor-

tance of this reaction is due to the fact that it paves a path for the conversion of inorganic phosphate to a high energy organic phosphate in the form of ATP. Concomitant to the conversion of 3-phosphoglyceraldehyde to 1,3-diphosphoglycerate, NAD^+ is reduced to NADH. It is believed that for the continuous synthesis of 1,3-diphosphoglyceric acid, NAD must be regenerated from NADH.

Usually NADH is oxidized and NAD^+ is regenerated in the electron transport chain of mitochondria. However, the mitochondrial inner membrane is impermeable to NADH or NAD (Lehninger, 1984). As a result of this, the NADH produced in the cytosol must be converted back to NAD with in this compartment. Malate-aspartate shuttle is supposed to play an important role in the regeneration of cytosolic NAD and in the transport of reducing equivalents across the mitochondrial membrane (Siesjo, 1978).

Results obtained in the present study indicated that cerebral glycolysis might be enhanced under hyperammonemic states. Under such conditions, there would be an enhancement in the production of NADH (through glyceraldehyde-3-phosphate dehydrogenase) in the cytosol. This implies that the operational rates of malate-aspartate shuttle might be affected under hyperammonemic states. As there are very few studies on the enzymes of malate-aspartate shuttle in brain in hyperammonemic states, this aspect was studied presently.

REGIONAL DISTRIBUTION OF ENZYMES OF MALATE-ASPARTATE SHUTTLE:

The regional distribution of malate dehydrogenase activity, measured in both the directions, has already been discussed (Table 3.5 and 3.6). Aapartate aminotransferase activity exhibited a profile similar to malate dehydrogenase in that its activity waa greater in cerebral cortex than in the other two regions. However, the magnitude of differences in the activity of this enzyme in the cerebral cortex and cerebellum was statistically not significant (Table 3.22).

EFFECTS OF AMMONIA :

The suppression in the malate dehydrogenase activity in both the directions in the three regions of brain in hyperammonemic states has already been discussed (Table 3.20 and 3.21). Aspartate aminotransferase activity was also suppressed in both these experimental conditions and its suppression was greater in the acute than in the subacute hyperammonemic state (Table 3.23).

The above results concur with the suggestion that cerebral malate-aspartate shuttle might be affected in hyperammonemic states. Suppression of malate dehydrogenase in the direction of malate formation would affect the production of oxaloacetate in the mitochondria which night have adverse effects on the operational rates of citric acid cycle and of malate-aspartate shuttle. Suppression in the activity of this

enzyme in the cytosol, in the direction of oxaloacetate formation, would be beneficial as it spares the cytosolic NAD . However, unchanged activity of thia enzyme in the direction of malate production has no influence on mitochondrial metabolism as malate is not the substrate for citrate synthetase reaction. But such a change in the cytoaol would maintain the rate of conversion of NADH to NAD . However, suppression of aspartate aminotransferase activity, whether it occurs in the cytosol or mitochondria, it would adversely affect the production of aspartate in the mitochondria and glutamate in the cytosol. Alteration in the production of either of these, would impede the exchange of glutamate and aspartate across the mitochondrial membranes. Thus it appears that the operational rates of malate-aspartate shuttle might be affected in hyperammonemic states.

The results obtained with the homogenates in hyperammonemic states may be summarized as follows:

(1) There is a generalized increase in the activities of glycolytic enzymes (with few exceptions) suggesting enhanced rate of glycolysis in brain in hyperammonemic states. This would result in increase in glucose consumption and production of pyruvate and/or lactate in brain in hyperammonemic states.

(2) There is a generalized increase in pyruvate dehydrogenase and the activities of enzymes of citric acid cycle excep-

ting malate dehydrogenase (malate to oxaloacetate). This would result in increased decarboxylation of pyruvate and of citric acid cycle activity upto oxaloacetate formation.

(3) Suppression in malate dehydrogenase activity might affect formation of oxaloacetate and thus citrate unless oxaloacetate is produced in alternate pathways (CO_2 fixation).

(4) Finally, the suppression of malate dehydrogenase (malate to oxaloacetate) and of aspartate aminotransferase would have an adverse effect on the operation of malate-aspartate shuttle. This might influence the regeneration of NAD^+ from NADH in the cytosol.

TABLE 3.1a

PROTEIN CONTENT IN DIFFERENT REGIONS OF RAT BRAIN IN NORMAL AND HYPERAMMONEMIC STATES

STATE	CEREBRAL CORTEX	CEREBELLUM	BRAIN STEM
NORMAL	115±12(11)	101±14(10)	109±14(10)
SUBACUTE	107+8(6)	103±10(6)	103±10(6)
ACUTE	110±9(10)	98±9(10)	102±11(10)

Values are mean+S.D. Protein content is expressed as mg.protein/gm.wet wt. of tissue. Number in parenthesis indicates number of experiments.

TABLE 3.1b

AMMONIA LEVELS IN BLOOD AND BRAIN OF NORMAL AND HYPERAMMONEMIC RATS

FRACTION	NORMAL	SUBACUTE	ACUTE
BRAIN	0.45+0.12(3)	1.4+0.4(3) +211* p<0.005	2.6+0.5(3) +487% p<0.001
BLOOD	0.0710.01(3)	1.110.2(5) +1420* p<0.001	1.8+0.1(4) +2352% p<0.001

Values are expressed in brain as μmoles of ammonia/gm. wet weight and in blood as μmoles of ammonia /ml. Values are mean+s.d. Numbers in parenthesis indicate the number of experiments.

TABLE 3.2

REGIONAL DISTRIBUTION OF GLYCOLYTIC ENZYMES IN RAT BRAIN

(ACTIVITIES. GM WET WEIGHT OF TISSUE"[1]. HR^{-1})

ENZYME	CEREBRAL CORTEX	CEREBELLUM	BRAIN STEM
1. HK	347±27	335±37	153±21 b,c
2. PFK	91±9	65±10 a	46+7 b,c
3. ALASE	106±20	94±9	88+5
4. GLPDH	140±11	111±7 a	132±9 c
5. ENASE	209±20	117±9 a	132+14 b
6. PK	1142±66	885±103 a	631±107 b,c
7. LDH(P->L)	11467±1622	9420±612 a	12890±961 c
8. LDH(L->P)	2634±210	1429+84 a	1537±21 b

HK : hexokinase; PFK : phosphofructokinase; ALASE : aldolaae; GLPDH : glyceraldehyde-3-phosphate dehydrogenase; ENASE : enolase; PK : pyruvate kinas«; LDH : lactate dehydrogenase. Activity units for HK are pinoles of NADP reduced; for GLPDH are poles of $NAD^{\cdot}$ reduced and for the rest activity units are umoles of NADH oxidized. Each value ia mean+s.d. of five individual experiments. Only thoae values which are significantly different ($p<0.05$) from other are indicated with letters. a: between cerebral cortex and cerebellum; b: between cerebral cortex and brainstem; and c: between cerebellum and brain stem. Neuman and Keul's multirange analysis was performed for stastical evaluation.

TABLE 3.3

REGIONAL DISTRIBUTION OF GLYCOLYTIC ENZYMES IN RAT BRAIN

(ACTIVITIES. MG PROTEIN"[1]. HR^{-1})

ENZYME	CEREBRAL CORTEX	CEREBELLUM	BRAIN STEM
1. HK	3.24±0.13	3.30±0.64	1.49±0.24 b,c
2. PFK	0.85+0.08	0.64±0.14 a	0.44+0.07 b,c
3. ALASE	1.0±0.16	0.92±0.03	0.85±0.03
4. GLPDH	1.31±0.11	1.09±0.12 a	1.28 + 0.15 c
5. ENASE	1.9+0.18	1.24±0.22 a	1.27±0.08 b
6. PK	10.7±1.3	8.62±0.73 a	6.17±1.42 b,c
7. LDH(P->L)	105+15	93.00±9.4	126.00±9.40 c
8. LDH(L->P)	22±1.7	14.40 + 0.84 a	14.00±0.20 b

Legend as in Table 3.2

TABLE 3.4

DISTRIBUTION OF ALANINE AMINOTRANSFERASE ACTIVITY IN DIFFERENT REGIONS OF RAT BRAIN

	CEREBRAL CORTEX	CEREBELLUM	BRAINSTEM
A.	3437±41	2707±65	1921+60
B.	28.4±0.3	27±0.7	17.6±0.6

Activity unita: µmoles of NADH oxidised/hr. A: Activity/em wet weight; B: Activity/me protein. Number of experiments were 5. Each value is Mean + S.D.

TABLE 3.5

DISTRIBUTION OF PYRUVATE DEHYDROGENASE AND ENZYMES OF CITRIC ACID CYCLE IN DIFFERENT REGIONS OF RAT BRAIN

(ACTIVITIES. GM WET WEIGHT^{-1}. HR^{-1})

ENZYME	CEREBRAL CORTEX	CEREBELLUM	BRAIN STEM
PDH	362±42	160±15 a	160+21 b
CS	913±92	879+24	1024±62
ICDH	227±21	219+21	221+15
2-OGDH	286±42	215±21	229±42
SDH	58±4	34±3 a	60±4 c
MDH (M->0)	4158±189	2813±147 a	2874±105 b
MDH (0->M)	14450±1329	12850±1315	10995±1112

PDH: pyruvate dehydrogenase; CS: citrate synthetase; ICDH: iao-citrate dehydrogenase; 2-OGDH: 2-oxoglutarate dehydrogenase; SDH: succinate dehydrogenaae; MDH: malate dehydrogenase. Activities of PDH, ICDH, 2-OGDH and MDH(M->0) are expressed as μmoles of NAD reduced and for SDH as μmoles of succinate oxidized and for MDH(O->M) as μmoles of NADH oxidized and for CS as μmoles of DTNB reduced. Each value ia mean ± S.D. Number of experiments are five. Only those values which are significantly different ($p<0.05$) from others are indicated with a letter. a: between cerebral cortex and cerebellum; b: between cerebral cortex and brain stem; c: between cerebellum and brain stem. Neuman-Keul's multirange analysis was used for the statistical evaluation of the data.

TABLE 3.6

DISTRIBUTION OF PYRUVATE DEHYDROGENASE AND ENZYMES OF CITRIC ACID CYCLE IN DIFFERENT REGIONS OF RAT BRAIN

(ACTIVITIES. MG PROTEIN"[1]. HR^{-1})

ENZYME	CEREBRAL CORTEX	CEREBELLUM	BRAIN STEM	
PDH	3.0±0.35	1.6±0.15 a	1.5±0.2	b
CS 7.5±0.8	8.9 + 0.2	9.4 + 0.6		
ICDH	1.9±0.17	2.2± 0.2	2.0±0.14	
2-OGDH	2.4±0.3	2.2±0.2	2.1+0.38	
SDH	0.5±0.03	0.3±0.03 a	0.6±0.04	c
MDH (M->0)	34.4±1.6	28.4±1.5	26.4±0.96	b
MDH (0->M)	133±12	123±12.5	100±10	b

Legend as ln Table 3.5

TABLE 3.7

HEXOKINASE ACTIVITY IN THE HOMOGENATES OF DIFFERENT REGIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

REGION		NORMAL	SUBACUTE		ACUTE	
CEREBRAL CORTEX	A.	347+27	294+39 p<0.05	-15*	315+15 p<0.05	-9*
	B.	3.24+0.13	2.74+0.35 p<0.02	-15%	2.79+0.21 p<0.01	-14%
CEREBELLUM	A.	335+37	296+37 NS	-12%	256+26 p<0.005	-23%
	B.	3.3+0.64	2.9+0.58 NS	-12%	2.63+0.49 NS	-20%
BRAINSTEM	A.	153+21	186+23 p<0.05	+21%	203+15 p<0.005	+33*
	B.	1.49+0.24	1.8+0.15 p<0.05	+21*	1.93+0.2 p<0.02	+30*

Activity units: μmoles of NADF reduced/hr. Number of experiments are five. A: activity/gm wet wt of tissue B: activity/mg protein.

TABLE 3.8

PHOSPHOFRUCTOKINASE ACTIVITY IN HOMOGENATES OF DIFFERENT REGIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

REGION		NORMAL	SUBACUTE	ACUTE
CEREBRAL CORTEX	A.	91+9	160+8 p<0.001 +76%	185+35 p<0.001 +99*
	B.	0.85+0.08	1.5+0.5 p<0.025 +76*	1.7+0.32 p<0.001 +110*
CEREBELLUM	A.	65+10	113+6 p<0.001 +74*	120+12 p<0.001 +85%
	B.	0.64+0.14	1.11+0.12 p<0.001 +73%	1.24+0.2 p<0.001 +94*
BRAIN STEM	A.	46 + 7	59 + 6 p<0.05 +28%	74+6 p<0.001 +61*
	B.	0.44+0.07	0.61+0.07 p<0.005 +39*	0.7+0.07 p<0.001 +59*

Activity units: μmoles of NADH oxidized/hr. Number of experiments are five. A: activity/gut wet wt of tissue. B: activity/mg protein.

Hi.

TABLE 3.9

ALDOLASE ACTIVITY IN HOMOGENATES OF DIFFERENT REGIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

REGION		NORMAL	SUBACUTE	ACUTE
CEREBRAL CORTEX	A.	106 + 20	140+22 p<0.05 +J2%	178±20 p<0.001 +68%
	B.	1.0+0.16	1.31+0.19 p<0.025 +31%	1.63+0.15 p<0.001 +63%
CEREBELLUM	A.	94+9	140+13 p<0.001 +49%	190+28 p<0.001 +102%
	B.	0.92±0.03	1.36±0.07 p<0.001 +48*	1.95±0.34 p<0.001 +112%
BRAIN STEM	A.	88+5	125+12 p<0.001 +42%	180±9 p<0.001 +104%
	B.	0.85+0.03	1.23+0.18 p<0.005 +45%	1.71+0.15 p<0.001 +101%

Activity units: μmoles of NADH oxidized/hr. Number of experiments are five. A: activity/gm wet wt of tisaue B: activity/sag protein.

TABLE 3.10

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE ACTIVITY IN HOMOGENATES OF DIFFERENT REGIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

REGION		NORMAL	SUBACUTE	ACUTE
CEREBRAL CORTEX	A.	140+11	155+12 *US* +11*	179+15 p<0.005 +28%
	B.	1.31+0.11	1.45+0.14 NS +11*	1.65+0.22 p<0.02 +26%
CEREBELLUM	A.	111+7	133+13 p<0.02 +20%	144+10 p<0.001 +30%
	B.	1.09+0.12	1.28+0.07 p<0.02 +17*	1.48+0.21 p<0.01 +36%
BRAIN STEM	A.	132+9	141+12 NS +11%	160+13 p<0.05 +21%
	B.	1.28+0.15	1.42+0.25 NS +11%	1.51+0.14 p<0.05 +18%

Activity units:μmoles of NAD reduced/hr. Number of experiments are five. A: activity/gm wet wt of tissue B: activity/me protein.

TABLE 3.11

ENOLASE ACTIVITY IN THE HOMOGENATES OF DIFFERENT REGIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

REGION		NORMAL	SUBACUTE	ACUTE
CEREBRAL CORTEX	A.	209+20	236+43 NS +13*	331+43 p<0.001 +58';
	B.	1.94+0.18	2.19+0.38 NS +13*	3.03+0.24 p<0.001 +56*
CEREBELLUM	A.	117+9	174+29 p<0.005 +49*	208+25 p<0.001 +78*
	B.	1.24+0.22	1.73+0.29 p<0.05 +40%	2.12+0.05 p<0.001 +71*
BRAIN STEM	A.	132+14	178+35 p<0.05 +35*	184+18 p<0.001 +39*
	B.	1.27+0.08	1.74+0.39 p<0.05 +37*	1.75+0.23 p<0.005 +38*

Activity units: μmoles of NADH oxidized/hr. Number of experiments are five. A: activity/am wet wt of tissue B: activity/me protein.

TABLE 3.12

PYRUVATE KINASE ACTIVITY IN THE HOMOGENATES OF DIFFERENT REGIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

REGION		NORMAL	SUBACUTE		ACUTE	
CEREBRAL CORTEX	A.	1142+66	2588+150 p<0.001	+127%	2315+471 p<0.001	+103%
	B.	10.7+1.28	24.3±3.1 p<0.001	+127%	19.3+5.4 p<0.01	+81*
CEREBELLUM	A.	885+103	1291+135 p<0.001	+0.001	1900+147 p<0.001	+115*
	B.	8.6±0.73	12.7±2.6 p<0.01	+48%	19.5+2.7 p<0.001	+127*
BRAINSTEM	A.	631+107	708+83 *US*	+12%	1149+101 p<0.001	+82*
	B.	6.2+1.4	6.95+1.2 *US* "	+12'.	10.8+1.7 p<0.005	+74*

Activity units: µmoles of NADH oxidized/hr. Number of experiments are five. **A:** activity/gm wet wt of tissue, **B:** activity/tog protein.

TABLE 3.13

LACTATE DEHYDROGENASE (PYRUVATE —> LACTATE) ACTIVITY IN THE HOMOGENATES OF DIFFERENT REGIONS OF NORMAL AND HYPERAMnONEMIC RAT BRAIN

REGION		NORMAL	SUBACUTE		ACUTE	
CEREBRAL CORTEX	A.	11067+1622	10836+983 NS "	-2*	13484+1088 p<0.025	+22%
	B.	104+15	101+86 NS "	-3%	124+14.8 NS	+19%
CEREBELLUM	A.	9420+952	9720+887 NS	+3%	11984+1023 p<0.005	+27%
	B.	92.6+16	94.9+13.7 NS	+2%	123+16 p<0.02	+33%
BRAIN STEM	A.	12890+961	12782+982 NS "	+1%	14930+884 p<0.01	+16%
	B.	122+8.8	124+4.2 NS	+2%	141+12.7 p<0.05	+16%

Activity units: µmoles of NADH oxidized/hr. Number of experiments are five. A: activity/gm wet wt of tissue, B: activity/me protein.

TABLE 3.14

LACTATE DEHYDROGENASE (LACTATE --> PYRUVATE) ACTIVITY IN HOMOGENATES OF DIFFERENT REGIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

REGION		NORMAL	SUBACUTE	ACUTE
CEREBRAL CORTEX	A.	2780+222	2727+77 US -2*	1888+21 p<0.001 -32%
	B.	24+2.1	25.5+1.1 US ~ +6*	17.3+0.9 p<0.025 -28%
CEREBELLUM	A.	1509+89	2492+170 p<0.001 +65%	1775+61 p<0.005 +18*
	B.	15+1.1	24+1.8 p<0.001 +60%	18±0.8 p<0.005 +20%
BRAIN STEM	A.	1622+22	2648+212 p<0.001 +63%	1276+22 p<0.001 -21%
	B.	14.9+1.2	26+2.0 p<0.001 +74*	12.7+0.4 p<0.02 -15*

Activity units: µmoles of NAD reduced/hr. Number of experiments are five. A: activity/gm wet wt of tissue, B: activity/mg protein.

TABLE 3.15

PYRUVATE DEHYDROGENASE ACTIVITY IN HOMOGENATES OF DIFFERENT REGIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE		CEREBRAL CORTEX	CEREBELLUM	BRAIN STEM
NORMAL	A.	362±42	160±15	160±21
	B.	3.0±0.35	1.6±0.15	1.5±0.2
SUBACUTE	A.	454+60 p<0.05 +25%	307+31 p<0.001 +92%	243+28 p<0.005 +52*
	B.	4.210.29 p<0.001 +40%	3.05±0.19 p<0.001 +90*	2.38±0.21 p<0.001 +59*
ACUTE (CONVULSIVE)	A.	639+15 p<0.001 +77*	508+32 p<0.001 +218*	361+25 p<0.001 +126%
	B.	5.8±0.13 p<0.001 +93*	5.2+0.33 p<0.001 +225*	3.7+0.3 p<0.001 +147*
ACUTE (PRE-CONVULSIVE)	A	1260+49.0 p<0.001 +248*	976+12 p<0.001 +510*	1514±24 p<0.001 +846*
	B.	12.1+0.47 p<0.001 +303*	8.9+0.11 p<0.001 +456*	16.310.3 p<0.001 +986*

Units: A: pinoles of NAD reduced/ma protein/hr; B: pinoles of NAD reduced/em wet wt of tissue. Number of experiments are five. Each value is mean ± S.D.

TABLE 3.16

CITRATE SYNTHETASE ACTIVITY IN HOMOGENATES OF DIFFERENT REGIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE		CEREBRAL CORTEX	CEREBELLUM	BRAIN STEM
NORMAL	A.	913±92	879±24	1024±62
	B.	7.5±0.8	8.9±0.2	9.4±0.6
SUBACUTE	A.	982+77 NS +8%	1097+53 p<0.001 +25%	993+38 NS -5*
	B.	9.12+0.23 p<0.01 +22*	10.9+0.22 p<0.001 +22%	9.73+0.3 NS +3%
ACUTE (CONVULSIVE)	A.	1479+48 p<0.001 +62*	1241+39 p<0.001 +41*	968+44 NS -3%
	B.	13.3+0.4 p<0.001 +77%	12.8+0.4 p<0.001 +44*	9.9+0.5 NS +5*
ACUTE(PRE-CONVULSIVE)	A.	1191+41 p<0.005 +30*	958+76 NS " +9*	872+54 NS -15*
	B.	11.5+0.4 p<0.001 +53*	8.8+0.7 NS " -1*	9.4+0.6 NC

Units: A:μmoles of DTNB reduced/mg protein/hr; B: μmoles of DTNB reduced/gin wet wt of tissue/hr. Each value la mean + S.D. Number of experiments are five.

BLE 3.17

ISOCITRATE DEHYDROGENASE ACTIVITY IN HOMOGENATES OF DIFFERENT REGIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE		CEREBRAL CORTEX	CEREBELLUM	BRAIN STEM
NORMAL	A.	227±21	219±21	221±15
	B.	1.9+0.17	2.2+0.2	2.0+0.14
SUBACUTE	A.	208+33 NS -8%	224+32 NS + +2*	199+19 NS -10%
	B.	1.93+0.22 NS +2*	2.23+0.22 NS + 1%	1.95+0.21 NS -3%
ACUTE (CONVULSIVE)	A.	224+11 NS -1%	245±24 NS +12%	214+7 NS -3%
	B.	2.0+0.1 NS +5*	2.5+0.3 NS +14%	2.2+0.07 p<0.025 +10%
ACUTE (PRE-CONVULSIVE)	A.	249+12 NS +10%	238+11 NS +9%	201+11 p<0.05 -9%
	B.	2.4+0.11 p<0.001 +26%	2.2+0.1 NC	2.2±0.12 NS +10%

Units: A: pinoles of NAD reduced/ma protein/hr; B: μmoles of NAD reduced/gm wet wt of tissue/hr. Number of experiments are five. Each value is mean + S.D.

TABLE 3.18

2-OXOGLUTARATE DEHYDROGENASE ACTIVITY IN HOMOGENATES OF DIFFERENT REGIONS OF NORMAL AND *HYPERAMMONEMIC* RAT BRAIN

STATE		CEREBRAL CORTEX	CEREBELLUM	BRAIN STEM
NORMAL	A.	286±42	215+21	229±42
	B.	2.4±0.3	2.2±0.2	2.1±0.38
SUBACUTE	A.	438+41 p<0.001 +53%	422+32 p<0.001 +96*	399+36 p<0.001 +74*
	B.	4.06+0.23 p<0.001 +69%	4.2+0.22 p<0.001 +91*	3.9+0.18 p<0.001 +86*
ACUTE (CONVULSIVE)	A.	580+29 p<0.001 +103*	556+111 p<0.001 +159*	482+41 p<0.001 +110*
	B.	5.2+0.3 p<0.001 +117*	5.7+1.1 p<0.001 +159%	4.9+0.42 p<0.001 +133*
ACUTE (PRE-CONVULSIVE)	A.	595+82 p<0.001 +108*	695+42 p<0.001 +223*	782+63 p<0.001 +241%
	B.	5.7+0.8 p<0.001 +137*	6.4+0.4 p<0.001 +191%	8.4+0.68 p<0.001 +300%

Units: A: pinoles of NAD reduced/me protein/hr; B: μmoles of NAD reduced/gm wet wt of tissue/hr. Number of experiments are five. Each value is mean + S.D.

TABLE3.19

SUCCINATE DEHYDROGENASE **ACTIVITY IN HOMOGENATES OF DIFFERENT REGIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAIN**

REGION		CEREBRAL CORTEX	CEREBELLUM	BRAIN STEM
NORMAL	A.	58±4	34±3	60±4
	B.	0.5±0.03	0.3±0.03	0.6±0.4
SUBACUTE	A.	86±11 p<0.005 +48%	72±6 p<0.001 +112%	76.5±1.9 p<0.001 +28*
	B.	0.8±0.09 p<0.001 +60%	0.72±0.06 p<0.001 +140%	0.75±0.05 p<0.005 +25%
ACUTE CCONVULSIVE)	A.	135±11 p<0.001 +133*	85±5 p<0.001 +150*	98±2 p<0.001 +63*
	B.	1.2±0.1 p<0.001 +140*	0.9±0.05 p<0.001 +200*	1.0±0.02 p<0.001 +67*
ACUTE (PRE-CONVULSIVE)	A.	90±4 p<0.001 +55*	145±5 p<0.001 +326*	112±7 p<0.001 +87*
	B.	0.9±0.04 p<0.001 +80*	1.3±0.05 p<0.001 +333*	1.2±0.08 p<0.001 +100*

Units: A: µmoles of auccinate oxidized/mg protein/hr; B: µmoles of auccinate oxidized/gm wet wt of tissue/hr. Number of experiments are five. Each value la mean + S.D.

TABLE 3.20

MALATE DEHYDROGENASE (MALATE_____>OXALOACETATE) ACTIVITY IN HOMOGENATES OF DIFFERENT REGIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE		CEREBRAL CORTEX	CEREBELLUM	BRAIN STEM
NORMAL	A.	4158+189	2813±147	2874±105
	B.	34.4±1.6	28.4±1.5	26.4±0.96
SUBACUTE	A.	2794+161 p<0.001 -33%	2037+218 p<0.001 -28%	2048+264 \ p<0.001 -29
	B.	26+1.6 p<0.001 -24%	20+1.5 p<0.001 -30%	20+1.8 p<0.001 -24%
ACUTE (CONVULSIVE)	A.	1409+55 p<0.001 -66%	1514+19 p<0.001 -46%	1181+181 p<0.001 -59%
	B.	12.7+0.5 p<0.001 -63%	15.6+0.2 p<0.001 -45%	12.2+1.86 p<0.001 -54%
ACUTE (PRE-CONVULSIVE)	A.	1448+57 p<0.001 -65%	1488+153 p<0.001 -47%	2291+33 p<0.001 -20%
	B.	13.9+0.6 p<0.001 -60%	13.7+1.4 p<0.001 -52%	24.6+0.35 p<0.02 -7%

Units: A: µmoles of NAD reduced/ma protein/hr; B: pinoles of NAD reduced/gin wet wt of tissue/hr. Number of experiments are five. Each value ia mean + S.D.

TABLE 3.21

MALATE DEHYDROGENASE (OXALOACETATE--->MALATE) ACTIVITY IN HOMOGENATES OF DIFFERENT REGIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

REGION		NORMAL	SUBACUTE	ACUTE
CEREBRAL CORTEX	A.	133+12	138+25 NS +4*	151+15 NS -144
	B.	14450+1329	14769+1421 NS -2*	16130+1721 NS + 12%
CEREBELLUM	A.	123+12.5	126+10 NS + 2%	135+11 NS " +10%
	B.	12850+1315	12705+1207 NS -1*	13140+1125 NS + 2%
BRAIN STEM	A.	100 + 10	107 + 6 NS +7*	115 + 12 NS " +15%
	B.	10995+1112	11088+1131 NS +1*	12195±1220 NS +11%

Units: A: µmoles of NADH oxidized/ma protein/hr; B: µmoles of NADH oxidized/gm wet wt of tissue/hr. Number of experiments are five. Each value la mean + S.D.

TABLE 3.22

REGIONAL DISTRIBUTION OF BORST CYCLE ENZYMES IN DIFFERENT REGIONS OF RAT BRAIN

ENZYME		CEREBRAL CORTEX	CEREBELLUM	BRAINSTEM
MDH (M-->0)	A.	41581189	2813±147 tt	28741105 b
	B.	34.411.6	28.4±1.5 a	26.4±0.96 b
MDH (0-->M)	A.	14450±1329	12850±1315	1099511112 b
	B.	133±12	123+12.5	100±10 b
AAT	A.	10904±657	9511±576	8631±668 b
	B.	90.115.4	96.115.8	79.2±6.1

MDH: malate dehydrogenaae; AAT: aspartate aminotransferase. Activity was expressed for MDH(M-->0) as µmoles of NAD^+ reduced/hr and MDH(O-->M), AAT as µmoles of NADH oxidized/hr. Each value is mean ± S.D. Number of experiments are five. Only those values which are significantly different ($p<0.05$) from others are indicated with a letter (a): between cerebral cortex and cerebellum; (b): between cerebral cortex and brain stem; (c): between cerebellum and brain stem.
A: activity/gm wet weight; B: activity/mg protein.

TABLE 3.23

ASPARTATE AMINOTRANSFERASE ACTIVITY IN HOMOGENATES OF DIFFERENT REGIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

REGION		NORMAL	SUBACUTE	ACUTE
CEREBRAL CORTEX	A.	90.1+5.4	76.5+2.4 p<0.005 -15*	64.8+0.3 p<0.001 -28%
	B.	10904+657	8245+751 p<0.001 -24*	7198+32 p<0.001 -34%
CEREBELLUM	A.	96.1+5.8	82.5+2.1 p<0.005 -14%	70.5+0.3 p<0.001 -27%
	B.	9511±576	8288 + 398 p<0.01 -13*	6835 + 32 p<0.001 -28%
BRAIN STEM	A.	79.2+6.1	70+1.71 p<0.025 -12*	53+0.4 p<0.001 -33*
	B.	8631+668	7129+393 p<0.01 -17%	5141+43 p<0.001 -40%

Units: A: µmoles of NAOH oxidized/mg protein/hr; B: µmoles of NADH oxidized/gm wet wt of tiaeue/hr. Number of experiments are five. Each value la mean + S.D.

STUDIES WITH SUBCELLULAR FRACTIONS

Results presented in the earlier chapter indicated that hyperammonemic states might affect the cerebral carbohydrate metabolism. However, this conclusion la based on the studies carried out on the activities of enzymes in tissue homogenates. Activities of enzymes measured under in vitro conditions (where all the conditions are optimal) need not represent the activities under in vivo conditions where in the concentrations of cofactors and substrates and pH are not optimal. Hence, under in vivo conditions the enzyme activities might be less than that measured under in vitro conditions. Further, the measured enzyme activities need not also represent thy flux of metabolites under in vivo conditions. This is because of the fact that a given metabolite might enter more than one pathway at a given time and this would depend on the transport of the metabolites across the plasma membrane and membranes of different intracellular compartments, content and activity levels of the enzymes of these pathways, affinities of enzymes of different pathways to their substrates and cofactora and the presence or absence of ligands which regulate the enzyme activities. Due to this sharing of metabolites among different pathways, the flux of a metabolite through a given pathway is governed by several parameters. Other factors such as pool sizes and their turnover will also have an influence on the metabolic flux. The

situation becomes much more complex in experimental conditions (drug administration or altered physiological conditional where in changes in the flux of metabolites in one pathway might have an influence on other pathways. Hence, a simple measurement of enzyme activity may not give adequate information. One way of overcoming this problem is to provide the substrate to the system and to measure the levels of end products and various intermediates in the normal and altered conditions. This information along with the changes in the activities of enzymes might provide a meaningful information. Such an attempt is made in the present study where in glucose was provided to the cerebral preparations and the production of CO_2 was measured along with the levels of some of the intermediates of glycolytic pathway, citric acid cycle and of malate-aspartate shuttle. These studies have been carried out with cerebral preparations from normal and hyperammonemic rats. In these experiments, it was assumed the metabolic alterations that have occured under in vivo states, due to the administration of ammonium salts, would survive the isolation procedure. Results described below support this assumption. However, these alterations in the metabolic pathways that have occured under in vivo conditions might be due to the direct or indirect effects of the administered ammonium salts. This was investigated by studying the metabolic processes and metabolite levels in preparations obtained from

normal animals in the presence and absence of added ammonium ions (to the preparation) . Two concentrations of ammonium acetate was selected for this purpose - 1mM and 5mM - the latter concentration represents the level of ammonium ions in brain and cerebrospinal fluid in hyperammonemic states (Hawkins et al., 1973; Norenberg, 1981; Benjamin, 1962).

Homogenates, used earlier, represent a heterogenous preparation of the brain region as they contain several subcellular organelles. In addition to the ususal subcellular organelles, such as mitochondria, ribosomes, nucleus etc., the brain homogenates have an additional subcellular organelle namely "synaptosomes". This fraction represents pinched off nerve endings which contain a part of the neuronal cytoplasm (synaptoplasm) along with mitochondria (synaptic mitochondria) and is enclosed by plasma membrane. These subcellular particles also contain membrane bound vesicles, called synaptic vesicles which are involved in the storage and release of neurotransmitter. In general, the synaptosomes also contain a portion of the post synaptic membrane.

Glycolytic enzymes are supposed to be localized in the cytosol. However, some of the enzymes, especially hexokinase, are known to be reversibly associated with the mitochondria (Crane, 1953; Johnson, 1960; Beattie et al., 1963; Brunngraber et al., 1963; Craven et al., 1969; Clark and Masters,

1972,1973; Knull, 1977). The association of some of these enzymes depends upon the physiological status of the cell. It has been shown that the binding of the hexokinase to the brain mitochondria alters its activity and is dependent upon the energy charge of the cell (Krane and Sols, 1953; Knull et al., 1973, 1974; Gots and Beesman, 1974; Inui and Ishibashi, 1979; Easterby and Qadri, 1981; Linden et al., 1982). Moreover, glycolytic enzymes are also present in synaptosomes which represent the pinched off nerve terminals (Knull, 1978,1980; Szutowicz and Lysiak, 1980; Knull and Khandelwal, 1982; Ratnakumari and Murthy, 1989,1990). Hence, studies on these enzymes conducted with the homogenates might not yield appropriate information on the magnitude of activities of these enzymes in different subcellular fractions. Moreover, any changes in the relative distribution of the enzymes in any one of these fractions, especially during experimental conditions, might not be detected by the use of homogenates. Hence, it was thought that the distribution of these enzymes in different subcellular fractions not only in normal but also in hyperammonemic states might yield information on the subcellular sites of the action of ammonia. In such studies it is desirable to prepare the subcellular fractions from different brain regions and study the distribution and the changes in the activities of glycolytic enzymes during normal and experimental conditions. However, practical constraints

imposed limitations on such study. The major constraint is the amount of tissue available with the regions like cerebellum and brain stem. These regions are much smaller and yield of the subcellular fractions would not be high unless a large number of animals are sacrificed. Hence, only cerebral cortex was used in the present study to prepare a subcellular fractions. In the following description, comparisons were made between cytosol and mitochondria.

PURITY OF SUBCELLULAR FRACTIONS:

For studies involving subcellular fractions, the establishment of the purity and cross contamination is a prerequisite. The two fractions that are to be discussed currently, i.e., mitochondria and cytosol, have different buoyant densities. Hence they separate out quite early during differential centrifugation. Moreover, the crude mitochondrial pellet (P2) was resuspended in sucrose, passed through density gradients and washed with sucrose. This treatment dilutes and removes the cytosolic contamination of mitochondria. Similarly, the cytosol was centrifuged at 63,000g to remove any particulate material. Centrifugation at 1,00,000g did not improve the purity of cytosolic fraction with respect to the distribution of marker enzymes. The activity of the marker enzyme of mitochondria, i.e., succinate dehydrogenase, was determined in the cytosolic fraction (Table 4.1). It was

found to be < 2% (Table 4.2). However, In hyperammonemic states, there was an increase in the succlnate dehydrogenase activity in the cytosol. Even under these conditions, it did not exceed 8% of mitochondrial activity (Table 4.2). The activity of lactate dehydrogenase, which is considered as a. marker of cytosol, was about 20% in the mitochondria. Though it appears to be high, the changes observed in the hyperammonemic states (8% in subacute and 24% in acute states) indicated that thi3 may not be due to cytosolic contamination of mitochondria (Table 4.2). Probably some lactate dehydrogenase is tightly associated with mitochondria and this can not be removed by washing with sucrose.

METABOLIC STUDIES:

PRODUCTION OF $^{14}CO_2$ FROM [U - ^{14}C] GLUCOSE:

When the cytosol and mitochondria were incubated with [U-^{14}C] glucose, very low amounts of $^{14}CO_2$ was produced. This process was observed to be two fold higher in the mitochondrial fraction than in the cytosol (Table 4.3). This observation was surprising. Cytosolic fraction is capable of utilizing glucose as it contains glycolytic enzymes. However, these enzymes can metabolize glucose to pyruvate and they can not further oxidize pyruvate to CO, as they do not have mitochondria. Moreover, metabolism of glucose through glycolysis does not yield CO_2 unless the cytosolic fraction has some

small mitochondria which fail to sediment during centrifugation and these mitochondria might be oxidizing the pyruvate. Rupture of a small population of mitochondria during the process of homogenization followed by the release of the citric acid cycle enzymes into the cytosol could be another possibility. Under such conditions, the pyruvate formed from glucose in the cytosol may be oxidized further. This possibility will be discussed later. Another pathway for CO_2 production from glucose in the cytosol could be hexose monophosphate shunt pathway.

Production of $^{14}CO_2$ from labelled glucose in the mitochondrial fraction is also equally puzzling. Mitochondria which are usually devoid of glycolytic enzymes can not metabolize glucose unless it is converted to pyruvate. Production of $^{14}CO_2$ from glucose in this fraction suggests that glycolytic enzymes might be associated with the isolated mitochondria atleast to a small extent. This presence of glycolytic enzymes in mitochondria may not be due to contamination. It is, therefore, possible that small amounts of glycolytic enzymes might be either adsorbed or firmly associated with the mitochondria. There are several reports in the literature in support of the present observation (Brunngraber et al., 1963; Tamin et al., 1972; Knull, 1977,1978; Szutowicz and Lysiak, 1980). Further, results obtained in the present study

on the distribution of glycolytic enzymes also provide support for the above described result (See later).

In addition to the production of $^{14}CO_2$, levels of some of the intermediates of glycolytic pathway, citric acid cycle and malate-aspartate shuttle were also measured in the cytosolic and mitochondrial fractions after they were incubated with unlabelled glucose. Glycolytic intermediates such as filucose-6-phoaphate, fructose-6-phosphate, phosphoenolpyruvate, pyruvate and lactate were detected in both the fractions (Table 4.4). Excepting pyruvate and lactate the levels of other intermediates were ten times less in the mitochondrial fraction when compared with cytosol. Pyruvate content in cytosol was twice that of mitochondria while lactate content was five fold leas in mitochondria when compared with that in cytoaol. When the profiles of levels of the glycolytic intermediates were compared, in the cytosol it was lactate > glucose-6-phosphate > pyruvate > fructose-6-phosphate > phosphoenolpyruvate while in the mitochondria it was lactate > pyruvate > glucose-6-phosphate > fructose-6-phosphate > phosphoenolpyruvate (Table 4.4).

A close observation of the results indicate that CO_2 production was greater in mitochondria than cytosol though the concentrations of glycolytic intermediates wer-e lesser than those in the cytosol. This observation once again

suggests the association of small amounts of glycolytic enzymes with the mitochondria. Greater rates of CO. production with very low levels of glycolytic intermediates in the mitochondrial fraction suggests that the pool of these intermediates may be having a rapid turnover rate. Very low levels of lactate (when compared to cytosol) indicated that the preferred pathway of pyruvate utilization in mitochondria might be its oxidation while the least preferred pathway would be its conversion to lactate. This is understandable as the NADH formed in the glycolytic pathway associated with the mitochondria might be oxidized in electron transport chain. Very low amount of glucose-6-phosphate formed by this fraction could be due to the hexokinaae which is associated with the mitochondria (Crane, 1953; Johnson, 1960; Brunngraber et al., 1963; Knull, 1977).

In the cytosolic fraction, glucose-6-phosphate levels were higher than those of the mitochondria. This suggests that cytosol has adequate amounts of ATP which can be used for the phoaphorylation of glucose. Very high amounts of lactate in this fraction is understandable as the pyruvate formed during glycolysis can not be completely oxidized either due to the absence of mitochondria or due to very small number of mitochondria. Hence, pyruvate is rapidly converted to lactate in this compartment. This would favour the reoxidization of NADH to NAD for the continuation of

glycolysis.

Above said results indicate that the isolated subcellular fractions are metabolically active and can utilize glucose. The observation that isolated mitochondria can utilize glucose necessiated a study on the distribution ot the enzymes of glycolysis in this fraction as well as in the cytosol. Results of such study are described below.

In general, activities of all the glycolytic enzymes were detected both in cytosolic and mitochondrial fractions. However, the mitochondrial activity of these enzymes was several fold lesser than those in the cytosol (Table 4.5). Presence of these enzymes in the mitochondrial fraction may not be due to the contamination for the reasons mentioned earlier. There are reports in the literature to indicate the presence of glycolytic enzymes in the mitochondrial fraction (Brunngraber et al., 1963; Tamin et al., 1972; Knull, 1977,1978; Szutowicz and Lysiak, 1980). As the mitochondria in these studies have been prepared by a method different from that described in the present study it may be assumed that the presence of glycolytic enzymes observed presently may not be an experimental artifact.

The activity profile of glycolytic enzymes in cytosol was observed to be aldolase > phosphofructokinase ; glycer-

aldehyde-3-phosphate dehydrogenaae > aldolase ; enolase > glyceraldehyde-3-phosphate dehydrogenaae ; pyruvate kinase > enolase and lactate dehydrogenase > pyruvate kinase. However, the activity of hexokinase was greater than that of phosphofructokinase. This profile was the same in the mitochondria except that aldolase activity was equal to glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase to that of enolase (Table 4.5).

STUDIES ON CITRIC ACID CYCLE IN SUBCELLULAR FRACTIONS:

Studies with the subcellular fractions (mitochondria and cytosol) indicated that both these fractions are capable of producing $^{14}CO_2$ from glucose. In this connection, it was suggested that the production of $^{14}CO_2$ from glucose in the cytosolic fraction could be due to the presence of few mitochondria in the cytosol or it may be due to hexose monophosphate shunt. If the former suggestion is true then it should be possible to detect some of the intermediates of citric acid cycle in the cytosolic fraction especially after incubation with glucose. Studies were conducted along these lines and the results are presented below (Table 4.6).

Subsequent to the incubation with glucose, citrate and isocitrate levels were below the level of detection (sensitivity of the assay 5 nmoles for citrate and 8 nmoles for isocitrate) while 2-oxoglutarate and malate were detectable

in the cytosol. Malate levels were higher than the 2-oxoglutarate levels in the cytosol under these conditions. Absence of citrate and isocitrate in the cytosolic fraction rules out the possibility of a large contamination of cytosol with mitochondria. Even if the mitochondria are present, the contamination could be very little.

However, the presence of 2-oxoglutarate and malate in this fraction, at the instance, appears to contradict the above statement on contamination. It must be mentioned that the cytosol prepared from brain has many aminotransferases and amino acids such as glutamate. The glutamate in the cytosol might undergo transamination and yeild 2-oxoglutarate. Further, it is also possible that the 2-oxoglutarate that has been transported into the cytosol (due to malate-aspartate shuttle) might still be retained even after isolation. The experiments of Murthy and Hertz (1988) on pyruvate decarboxylation by astrocytes in primary cultures indicated that 2-oxoglutarate may be present in adequate quantities in the cytosol. This is supported by the fact that addition of aspartate to incubation medium (in the absence of ammonium chloride) promoted pyruvate decarboxylation in their experiments. This is possible only if aspartate is transaminated with 2-oxoglutarate in the cytosol to produce oxaloacetate which is converted to malate and transported into mito-

chondria where pyruvate is decarboxylated by pyruvate dehydrogenase complex. Results of their study is in support of the present observation. Similar explanation also holds good for the presence of malate in the cytosol. Transamination of cytosolic aspartate with 2-oxoglutarate could yeild oxaloacetate which may be converted to malate by cytoplasmic malate dehydrogenase. As the malate, thus formed, cannot be utilized fully in the cytoaol (due to the presence of few mitochondria, if any), it might accumulate in the cytosol.

Following the incubation with glucose, all the citric acid cycle intermediates could be detected in the mitochondria] fraction (Table 4.6). These might be due to the conversion of glucose to pyruvate by the glycolytic enzymes associated with the mitochondria and the subsequent utilization of the pyruvate in the citric acid cycle in this aubcellular organelle. It is also possible that these intermediates were formed by the mitochondria prior to their isolation (i.e., under in vivo state) and they were retained and survived the rigours of isolation procedure. If this were to be true, then it indicates the integrity of membranes of the isolated mitochondria. It la interesting to note that the levels of all the citric acid cycle intermediates were more or less equal in this fraction and this profile is different from that in the cytoaol. Following the incubation with glucose both ATP and ADP were detected in both the fractions.

However, the contents of these metabolites were high in mitochondria compared to cytosol (Table 4.38).

These studies on the citric acid cycle intermediates in subcellular fractions revealed the minimal or no contamination of cytosolic fraction with mitochondria. This was further verified by studying the distribution of enzymes of citric acid cycle in cytosol and mitochondria prepared from the cerebral cortex of brain from normal rats.

SUBCELLULAR DISTRIBUTION OF PTRUVATE DEHYDROGENASE **AND CITRIC ACID CYCLE ENZYMES:**

As was expected the activities of pyruvate dehydrogenase and the enzymes of citric acid cycle except malate dehydrogenase were higher in the mitochondria than in the cytosol. Though the magnitude of difference in the activity levels of these enzymes between these two fractions varied from enzyme to enzyme, in general, it was higher for citrate synthase and succinate dehydrogenase. The least difference in the activity levels were observed in the case of pyruvate dehydrogenaae. Unlike the above enzymes of citric acid cycle, malate dehydrogenase activity was more or less similar in the cytosol and mitochondria, irrespective of the direction in which the enzyme was assayed. Moreover, these results indicated that the activity of malate dehydrogenase was signifi-

cantly higher in the direction of malate formation than in the direction of oxaloacetate formation in the cytosol. However, in the mitochondria the difference between the activity of this enzyme in both the directions waa marginal but statistically significant (Table 4.7).

As was observed in the homogenates, the activity of citrate aynthaae waa higher than that of pyruvate dehydrogenase and of isocitrate dehydrogenase. Similarly, the activity of succinate dehydrogenaae waa about eight fold lesser than that of 2-oxoglutarate dehydrogenaae and by aeveral folds than that of malate dehydrogenaae. Theae results support the earlier view that auccinate dehydrogenase may be an additional point for the regulation of cerebral citric acid cycle (Table 4.7).

The profile of the diatribution of citric acid cycle enzymes excepting malate dehydrogenaae would also provide information on the purity of these two fractions. The marginal activity of the citric acid cycle enzymes except malate dehydrogenase in the cytoaol might be due to the presence of small mitochondria or mitochondrial fragments (formed during homogenization) which failed to sediment and/or nay be due to the releaae of theae enzymes due to the rupture of few mitochondria during the process of homogenization. It is alao possible that the activity of theae enzymes in cytoaol may

represent the nascently formed enzymes awaiting for their transport into mitochondria.

STUDIES ON MALATE-ASPARTATE SHUTTLE:

As was mentioned earlier, the intermediates and the enzymes of malate-aspartate shuttle are distributed between cytoaol and mitochondria. The intermediates include malate, 2-oxoglutarate, glutamate and aapartate while the enzymes involved are malate dehydrogenase and aapartate aminotransferase. Hence, the subcellular distribution of the components of malate-aspartate shuttle were analyzed and the results are presented below.

Studies on the distribution of two intermediates of malate-aspartate shuttle (following the incubation with glucose) i.e., 2-oxoglutarate and malate, are already described (Table 4.6). It may be summarized that these two intermediates are present both in cytosol and mitochondria. With regards to the amino acid components of the shuttle, it was observed that both glutamate and aspartate are present in the cytosol and mitochondria. In both these fractions glutamate levels were observed to be three fold higher than those of aspartate. Glutamate content in the cytoaol was found to be higher than that in the mitochondria while there was no difference in the aspartate levels between these two fractions (Table 4.8). Higher levels of glutamate than aapartate

in isolated fractions is understandable as aspartate is formed only by the transamination of glutamate with oxaloacetate. Since oxaloacetate concentration would be less than that of 2-oxoglutarate (see discussion above) less amounts of aspartate would be formed.

In addition to the levels of the intermediates of malate-aspartate shuttle, data is also presented on the distribution of the enzymes of malate-aspartate shuttle in the subcellular fractions. Activity levels of malate dehydrogenase, measured in both the directions, have already been described. In brief, malate dehydrogenase is present both in cytosol and mitochondria. Malate dehydrogenase activity, measured in the direction of oxaloacetate formation, was higher in the mitochondria than in the cytosol. However, activity of this enzyme when measured in the reverse direction was marginally higher in cytosol than in mitochondria. In mitochondrial fraction there were no statistically significant changes in the activity of malate dehydrogenase when measured in both the directions, while in the cytosol ita activity in the direction of malate formation was higher than the activity in the direction of oxaloacetate formation (Table 4.9). Activity levels of aspartate aminotransferase were similar in the mitochondrial and cytosolic fractions and were lesser than that of malate dehydrogenase (both directions) (Table 4.9). These results suggest that the differen-

cea in the levels of 2-oxoglutarate, malate, glutamate and aapartate may not be due to the differences in activities of the enzymes involved in the shuttle.

EFFECTS OF AMMONIA:

Results presented earlier indicated that the operational rates of the glycolytic pathway and of citric acid cycle might be stimulated in hyperammonemic states. However, this conclusion is based on the studies of enzyme activities in homogenates. As was mentioned earlier, enzyme activities are not true indicators of metabolism. Hence, levels of intermediates of glycolytic pathway and of citric acid cycle were determined in the cytosol and mitochondria prepared from the cerebral cortex of brains of rats rendered hyperammonemic by administering either subacute or acute dose of ammonium acetate. In some of the studies, ammonium acetate (1 mM and 5 mM, pH 7.4) was added to the suspensions containing subcellular fractions prepared from normal animals (in vitro addition) and were incubated with glucose. Results obtained in these studies are present below.

$^{14}CO_2$ PRODUCTION FROM [U - ^{14}C] GLUCOSE:

$^{14}CO_2$ was produced from glucose by the mitochondria and cytosolic fractions. The CO_2 produced was higher in the mitochondrial fraction than in cytosol. There were no statis-

tically significant changes in the amount of CO, evolved from glucose by the cytosol prepared from the cerebral cortex of rats injected with the subacute dose of ammonium acetate. However, under these conditions, the amount of CO_2 produced by the mitochondria was enhanced. An elevation was observed in the rate of production of CO_2 from glucose by both mitochondria and cytosol prepared from the cerebral cortex of rats injected with an acute dose of ammonium acetate. The magnitude of elevation was same in both the fractions (Table 4.10). Addition of 1mM ammonium acetate to the incubation medium resulted no significant change in the rate of $^{14}CO_2$ production in both the fractions. However, when the assay was performed in the presence of 5 mM ammonium acetate, the magnitude of change was same in both the fractions but it was statistically significant only in the mitochondrial fraction (Table 4.10).

Enhanced production of CO_2 from glucose in hyperammonemic states in both the fractions could not be due to enhanced availability of glucose as the incubation media used in these studies contained similar amounts of glucose. Moreover, there are no permeability barriers in the cytosol (as it has no membranes or membrane bound particles) to restrict the availability of glucose to the metabolic pathways. Even in the mitochondria, membrane barriers may not play a major role in determining the availability of glucose, as most of the

glycolytic enzymes might be associated either with the outer membrane or outer surface of inner membrane. Hence, the alterations in the rate of transport as a reason for enhanced CO_2 production from glucose may be ruled out atleast in the present case.

These results, thus, suggested increased glucose utilization in subcellular fractions in hyperammonemic states. If this were to be true, then the levels of metabolites of glycolytic pathway in the subcellular fractions should show changes parallel to that of CO_2 production. Moreover, it was suggested earlier that some of the CO_2 produced from glucose might be through hexose monophosphate pathway. If this were to be true, then the increased production of CO_2 by the cytosolic fraction in hyperammonemic states could also be due to increased operational rates of hexose monophosphate shunt. Under such conditions, no changes can be expected in the levels of glycolytic intermediates in the cytosolic fraction in hyperammonemic states. In order to have further information, levels of glycolytic intermediates were determined following the incubation of subcellular fractions with glucose.

Uhen the above said experiments were performed with the cortical cytosol of rats of subacute group, the following results were obtained. There was an increase in the levels of

glucose-6-phosphate, fructose-6-phosphate and phosphoenol-pyruvate. The magnitude of change in glucose-6-phosphate and phosphoenol pyruvate were similar while that of fructose-6-phosphate was lesser than that of glucose-6-phosphate or phosphoenolpyruvate. However, there were no statistically significant changes in the levels of pyruvate and lactate in the cytosol under these conditions. In the cortical cytosol obtained from rats of acute group, there was significant increase in the contents of glucose-6-phosphate, fructose-6-phosphate, phosphoenolpyruvate and pyruvate. However, under these conditions, lactate levels were decreased when compared to the control values. Further, all the changes observed in the cortical cytosol of acute group animals were higher than those in the subacute group (Tables 4.11 to 4.15).

Increased glucose-6-phosphate levels suggested that the rate of phosphorylation of glucose is enhanced in hyper-ammonemic states. This suggestion is supported by the observations of Bessman and Pal (1982) and of Jessy et al (1990). However, Hawkins et al., (1973) observed no significant increase in glucose-6-phosphate content in the extracts of supratentorial regions of brains of rats which are rendered hyperammonemic by administering an acute dose of ammonium salts and Mans et al., (1984) reported only marginal changes in the levels of this metabolite in whole brain extracts of

rats rendered hyperammonemic by portocaval shunt. It must be mentioned that glucose-6-phosphate synthesis requires ATP in the cytosol. From the above results it was apparant that ATP levels in the cytosol were more than adequate to carry out glucose phosphorylation and that the ATP levels are being maintained through out the incubation period. This is possible by substrate level phosphorylation of ADP. One such reaction is catalysed by pyruvate kinase which transfers the phosphate from phosphoenol pyruvate to pyruvate. As there would be limited amounts of ADP in the cytosol, the rate of phosphorylation of glucose under these conditions might be regulated by the production of ATP in the pyruvate kinase reaction. This also suggested that the activities of both hexokinase and pyruvate kinase might be elevated in hyperammonemic states.

Decrease in lactate levels in the cytosol both in subacute (though not statistically significant) and in acute states is surprising and is contradictory to the results obtained by several investigators. It was reported earlier that the lactate content increases in several different types of cerebral preparations (ranging from whole brain extracts to slices) under hyperammonemic states (Hawkins et al., 1973; Jessy et al., 1990; Adams et al., 1979; Raabe and Lin, 1984). It was also reported that ammonium ions in pathophysiological concentrations promotes anerobic glycolysis and thus enhances

cerebral lactate levels (Benjamin et al., 1978; Benjamin and Verjee, 1980). Though the results of the present study on lactate content indicated a decrease, it must be stated that lactate dehydrogenase activity is several folds higher than that of pyruvate kinase. Hence, the lactate dehydrogenase activity, even if it is suppressed, might still be adequate enough to convert pyruvate to lactate. Maintainance of lactate dehydrogenase activity, even at low levels, would be essential as it can regenerate NAD from NADH in the cytosol especially when malate-aspartate shuttle is interrupted as in hyperammonemic states. Suppression of lactate formation under these conditions would spare pyruvate for citric acid cycle.

Following were the changes in the levels of glycolytic intermediates when the mitochondria from the hyperammonemic animals were incubated with glucose : (1) levels of glucose-6-phosphate, fructose-6-phosphate and phosphoenolpyruvate were enhanced in both subacute and acute states, (2) there were no statistically significant differences in the pyruvate levels and (3) lactate levels were found to decrease (Tables 4.11 to 4.15). The ATP required for the phosphorylation of glucose might be directly provided to hexokinase through the membrane bound ATP/ADP translocator (Lehninger, 1984) or from phosphoenolpyruvate in the reaction mediated by pyruvate kinase associated with mitochondria. A decrease of lactate

content in this fraction would spare the pyruvate for its utilization in citric acid cycle.

The effects of ammonium ion on the levels of intermediates appear to be due to the direct effects of ammonia on the concerned metabolic pathways. This is confirmed by similar changes in these intermediates by the in vitro addition of 1mM and 5mM ammonium salts to the incubation medium. Changes observed in the levels of intermediates in the presence of 1mM ammonium acetate were similar to those occuring in subacute state while those that are taking place in the presence of 5mM ammonium acetate are similar to those ocurring in acute state (Tables 4.11 to 4.15). However, the rate of production of $^{14}CO_2$ from [U-^{14}C]glucose in the in vitro and in vivo states were not similar (Table 4.10).

The changes observed in glucose oxidation and of metabolite levels support the notion that cerebral glucose metabolism might be stimulated by ammonium ions. Studies were performed to confirm these effects by determining activities of the enzymes of glycolytic pathway in cytoaol and mitochondria isolated from the cerebral cortex of hyperammonemic rats.

STUDIES ON ENZYMES OF GLYCOLYTIC PATHWAY:

Following the administration of subacute dose of ammonium acetate, it was observed that the mitochondrial

activity of the hexokinase was enhanced while that in the cytosol was suppressed (Table 4.20). Activities of phosphofructokinase and aldolase were enhanced in both the fractions in subacute hyperammoneniic condition (Table 4.21 and 4.22). However, the change in the mitochondrial phosphofructokinase activity was statistically not significant (Table 4.21). The change in glyceraldehyde-3-phosphate dehydrogenase was similar to that of hexokianse in the sense that the mitochondrial activity was enhanced but the change in the activity in cytosol was statistically not significant (Table 4.23). Activities of enolase and pyruvate kinase, though enhanced in mitochondria in subacute condition, These changes were not statistically significant while the changes in the cytosolic activities of these enzymes were statistically significant (Tables 4.24 and 4.25). Alteration in the activity of lactate dehydrogenase, when assayed in the direction of lactate formation was surprising, as it was suppressed in both the fractions (Tables 4.26 and 4.27). This is in contrast to the results obtained with the homogenates in which there were no statistically significant alterations in the activity of lactate dehydrogenase (pyruvate to lactate) under these conditions (Table 3.13). The same was also true when the activity of this enzyme was measured in the direction of pyruvate formation. Its activity in the cytosolic fraction was enhanced by 80% while there were no significant alterations in

mitochondria (Table 4.27). However, in the homogenates the decrease in the activity of this enzyme was marginal but statistically significant (Table 3.14).

ACUTE CONDITION:

In the acute hyperammonemic states, activities of all the glycolytic enzymes were enhanced in both the cytosolic as well as in mitochondrial fractions isolated from the cerebral cortex (Tables 4.20 to 4.27). Exceptions to this are (1) changes in hexokinase activity in cytosol were statistically not significant (Table 4.20) and (2) changes in the activity of lactate dehydrogenase measured in the mitochondria, in the direction of pyruvate formation, were unaltered under these conditions (Table 4.27). As was in the subacute condition, changes observed in the activities of all the enzymes except hexokinase and lactate dehydrogenase (both the directions) were similar to those described for the homogenates (Tables 3.7 to 3.14 and 4.20 to 4.27). In the case of hexokinaae, there was a decrease in the activity of this enzyme in the homogenate (Table 3.7), while in the subcellular fractions it was unaltered in the cytosol and was enhanced in the mitochondrial fraction under these conditions (Table 4.20). In the case of lactate dehydrogenase, there was a suppression of equal magnitude in its activity in the subcellular fractions whan measured in the direction of lactate formation (Table

4.26) while in the homogenates it was elevated (Table 3.13). Similarly, when the activity of this enzyme waa measured in the opposite direction, it was enhanced in the cytosol and was unaltered in the mitochondria (Table 4.27) while in the homogenate it was suppressed (Table 3.14). As mentioned earlier, the precise reason for the anomolous behaviour of these two enzymes when measured in homogenates and aubcellular fractions is not known at present. It may be said, however, that the changes for the majority of the enzymes were in the same direction in the subcellular fractions and in the homogenates.

Majority of the changes observed in the activities of glycolytic enzymes in the aubcellular fractions of brain in hyperammonemic states resemble closely to the changes observed in the levels of their intermediates. The increase in mitochondrial hexokinase (Table 4.20) is in keeping with the enhanced glucose-6-phosphate levels observed in the mitochondrial fractions (Table 4.11) in hyperammonemic states. Though there was an elevation in the cytosolic glucose-6-phoaphate content (Table 4.11), there were no statistically significant changes in cytosolic hexokinase activity (Table 4.20). This suggests that the elevation in cytosolic glucose-6-phosphate levels could be due to the suppression of other pathways which utilize this metabolite. Increase in phosphofructo-

kinase activity in hyperammonemic states ensures the conversion of glucose-6-phosphate to fructose-1,6-diphosphate. Elevation in the activity of this enzyme could be due to the allosteric activation of the enzyme by ammonium ions (Lowry and Passonneau, 1966; Sugden and Newsholme, 1975). Enhancement in the aldolase activity in the subcellular fractions in hyperammonemic states would result in an increase in the production of triose phosphates in the cytosol and mitochondria. The observed increase in glyceraldehyde-3-phosphate dehydrogenase activity in the mitochondria could be due to increased availability of both substrates and the cofactor (NAD), as it has been reported that mitochondrial NAD/NADH ratio is elevated in hyperammonemic states (Hawkins et al., 1973). However, in the cytosol there were no statistically significant changes in the activity of this enzyme. This is understandable as NAD regenerating systems (malate-aspartate shuttle and lactate dehydrogenaae) are suppressed in hyperammonemic states and the cytosol is in a more reduced state under these conditions. Increase in the activity of enolase (Table 4.24) observed in the present study is in agreement with the increased phosphoenolpyruvate levels in both the subcellular fractions under these conditions (Table 4.13). Changes observed in pyruvate levels in the cytosol are in agreement with the activity of pyruvate kinase in this fraction. However, in the mitochondria pyruvate levels underwent

no statistically significant changes though there was an elevation in the activity of pyruvate kinase (Tables 4.14 and 4.25). This could be due to an enhancement in the transport of pyruvate into the mitochondria and in its utilization in the citric acid cycle in thia subcellular organelle. Suppression of lactate dehydrogenase activity observed in the mitochondria and cytoaol prepared from the cerebral cortex of hyperammonemic rate is in agreement with the observed fall in the lactate content in these two subcellular fractions.

These results indicated that the increased activities of the enzymes observed in the homogenates in hyperammonemic states are also reflected in the subcellular fractions. The only exception ia with lactate dehydrogenase, whose activity was enhanced in the homogenates while it was suppressed in the cytosol. The latter change is physiologically favourable as it spares the pyruvate for citric acid cycle. However, the fall in the levels of lactate and lactate dehydrogenase activity in the subcellular fractions (Tables 4.15 to 4.26) ia not in agreement with the results of several investigators who reported an enhanced lactate levels in hyperammonemic states (Hawkins et al., 1973; Adams et al., 1979; Raabe and Lin, 1984; Jessy et al., 1990). However, it must be mentioned that this anamoly between the results of the present study with that of others could be due to differences in the preparations used. Hawkins et al., (1973) reported an increase in

the lactate levels in the supratentorial regions of the brain after the administration of ammonium chloride. Adams et al., (1979) reported a similar increase in the brain lactate levels in the rats infused with ammonium acetate. Like wise Raabe and Lin (1984), O'Connor et al., (1984), Mans et al., (1984) and Jesay et al., (1990) reported a similar increase in brain lactate levels in various animal models for hyperammonemia. It must be mentioned that these levels were measured in extracts of brain by arresting post-mortem changes. However, in the present study, not only the activity of the enzyme (lactate dehydrogenase) and also the lactate levels (following incubation with glucose) were estimated in subcellular fractions. It is possible that some of the changes that occured in situ might not be reproducible faithfully under in vitro conditions. However, it must be mentioned that majority of the changes observed in these experimental conditions have been retained and they survived the rigors of the isolation procedure. In conclusion, it may be stated that the results obtained with levels of glycolytic intermediates, activities of enzymes involved in their metabolism suggest that the cerebral glycolysis might be enhanced in hyperammonemic states.

STUDIES ON CITRIC ACID CYCLE:

The energy depletion theory proposed by Bessman and

Bessman (1955) to explain the mechanism of ammonia toxicity centers around the disturbances in the operation of citric acid cycle due to the depletion of 2-oxoglutarate. This hypothesis was verified later by several investigators by measuring cerebral levels of citric acid cycle intermediates in several animal models of hyperammonemia. In the present study it was observed that the activities of citric acid cycle enzymes in homogenates were enhanced in hyperammonemic statea. Further, the presence of citric acid cycle enzymes was also observed in the cytosol, albeit lesser than that of mitochondria. Moreover, it was suggested that in hyperammonemic states the activities of glycolytic enzymes associated with mitochondria were enhanced and there might be an enhanced utilization of pyruvate in citric acid cycle under these conditions. Keeping these in view, studies were performed on the levels of citric acid cycle intermediates and the activities of citric acid cycle enzymes in the mitochondria and cytosol in present modal of hyperammonemia.

METABOLITE LEVELS:

Results obtained on $^{14}CO_2$ production indicated an enhancement in this process in both the subcellular fractions in hyperammonemic states (Table 4.10). If the $^{14}CO_2$ production from glucose la taking place due to the operation of pathways concerned with glycolysis and citric acid cycle,

then the levels of the intermediates of this pathway should be elevated in the mitochondria and cytoaol prepared from cerebral cortex of hyperammonemic rats. Results obtained on glycolytic intermediates, which were presented earlier, supported this concept. In order to obtain further evidence, levels of citric acid cycle intermediates were determined in these subcellular fractions after they are incubated with glucose.

In the cytosolic fraction prepared from the cerebral cortex of subacute group of rats, citrate and isocitrate levels could not be detected after the incubation with glucose (Tables 4.16 and 4.17). However, in the same preparation from acute group of rats, small amounts of these two intermediates of citric acid cycle were detected (Table 4.16 and 4.17). Under these conditions, there were no statistically significant changes in the mitochondrial citrate content (Table 4.16). Isocitrate content of mitochondria isolated from the subacute group of rats was unaltered while there was an elevation in the same in acute conditions (Table 4.17). The content of 2-oxoglutarate was elevated in these two subcellular fractions isolated from both the groups of hyperammonemic rata. The magnitude of elevation was higher in cytosol than in mitochondria and in acute conditions than in subacute states (Table 4.18). A similar profile of change was

observed in the malate content of these two subcellular fractions except that the increase in mitochondrial malate content in subacute conditions was not statistically significant (Table 4.19). Similar changes were noticed in the citric acid cycle intermediates in these two subcellular fractions of normoammonemic rats when incubated with glucose in the presence of ammonium ions except that some of the changes observed in the presence of 1 mM ammonium acetate were statistically not significant (Table 4.16 to 4.19).

Lack of changes in mitochondrial citrate levels in hyperammonemic states, though contradicts the suggestion of increased pyruvate utilization under these conditions, could be due to the lack of availability of oxaloacetate as malate dehydrogenase activity was suppressed (see later). The change observed in the mitochondrial citrate content in the present study was in agreement with similar reports of Hawkins et al., (1973) and Jessy et al., (1990). However, increased isocitrate levels in the mitochondria indicated an increase in the rates of citrate utilization. This was further confirmed by the enhanced 2-oxoglutarate content in the mitochondria under these conditions.

It is well known that 2-oxoglutarate is formed and utilized in the metabolism of glutamate. One such reaction of glutamate metabolism is mediated by glutamate dehydrogenase. This enzyme can bring about the formation of 2-oxoglutarate

from glutamate by oxidative deamination or utilize 2-oxoglutarate for glutamate formation by reductive amination. Studiea with [U - ^{14}C] and [1 - ^{14}C] glutamate suggested that the oxidative deamination of glutamate may be suppressed in hyperammonemic states (Yu et al., 1982, Lai et al., 1989). Though it was proposed earlier that the equilibrium of glutamate dehydrogenase reaction under physiological conditions favours glutamate formation (2-oxoglutarate utilization), this has been questioned recently aa the ammonia levels in brain in normoammonemic conditions are much below the Km value of this enzyme for ammonium ions (Cooper and Plum, 1987). However, in hyperammonemic states, the enhanced cerebral ammonia levels could drive the reaction towards glutamate formation. In fact it was proposed earlier that glutamate formation from ammonia might be the primary pathway for ammonia detoxification (Bessman and Bessman, 1955; Berl, 1971; Norenberg, 1977; Berl and Clarke, 1983; Berl, 1984) and in this process 2-oxoglutarate is drained out of citric acid cycle. If this were to be true, then the mitochondrial 2-oxoglutarate levels should register a fall in hyperammonemic states. Results obtained in the present study, however, does not support the above proposal. Moreover, glutamate dehydrogenase activity in the direction of glutamate formation was found to decrease in the mitochondria of cerebral cortex and cerebellum in hyperammonemic rate (Subbalakshmi and Murthy,

1983, 1984, 1985).

Another route of formation of 2-oxoglutarate from glutamate is by the transamination of the latter with a keto acid in a reaction mediated by aminotransferases. Though this reaction is possible with any α-keto acid, the oxaloacetate and pyruvate are the preferred keto acids. The reaction with oxaloacetate is mediated by aspartate aminotransferase and that with pyruvate by alanine aminotransferase. Though the activity of aspartate aminotransferase is higher than any other aminotransferase in brain (Benuck et al, 1971), it will be limited by the availability of oxaloacetate. It has been shown that of all the citric acid cycle intermediates, the levels of oxaloacetate are lowest in the brain (Goldberg et al., 1966; Folbergrova et al., 1974a,b; Norberg and Siesjo, 1975b, 1976; Carlsson et al., 1975). Moreover, in hyperammonemic states the levels of glutamate were found to decrease (Hindfelt, 1975; Hindfelt et al., 1977, Tyce et al., 1981; O'Connor et al., 1982) and so also the levels of oxaloacetate (due to suppression of malate dehydrogenase (Hawkins et al., 1973). In addition to this, the aspartate aminotransferase activity is also suppressed under these conditions (Subbalakshmi, 1984; Ratnakumari and tturthy, 1985,1989; and also see later). In the reaction mediated by alanlne aminotransferase, the substrates i.e., glutamate and

pyruvate are not rate limiting but the activity of this enzyme, though higher than other aminotransferases, is several fold less than that of aapartate aminotransferase. Moreover, activity of this enzyme is also suppressed in brain in hyperammonemic states(Sadasivudu and Murthy, 1973, 1976; Subbalakshmi and Murthy; 1983, 1984, 1985; Ratnakumari and Murthy, 1985, 1986, 1989, 1990). As both the routes of 2-oxoglutarate formation from glutamate (oxidative deamination and transamination) are suppressed in brain in hyperammonemic states, it appears that 2-oxoglutarate might be formed to a large extent in citric acid cycle under these conditions. However, the possibility of suppression of 2-oxoglutarate utilization as a causative factor for the enhancement in its content can not be ruled out as succinate levels were not determined in the present study. Increase in the mitochondrial malate content in hyperammonemic states could be due to the suppression of malate dehydrogenase activity in the direction of oxaloacetate formation (See later). It could also be due to an increase in the rate of synthesis of malate under these conditions.

Increase in the contents of citrate and isocitrate in the cytosol in the acute states but not in subacute states once again rises the question of contamination of cytosol either with mitochondia or with mitochondrial fragments but this can be ruled out as citrate was not detected in subacute

hyperammonemic states. Such an increase could also be due to a suppression of citrate lyase activity in the cytosol in hyperamraonemic states. However, citrate lyase activity is low in brain (Szutowicz and Lysiak, 1980) and it is not known whether the activity of this enzyme is affected by pathophysiological concentrations of ammonium ions.

Elevation in 2-oxoglutarate content in the cytoaol may be due to its synthesis in citric acid cycle in the mitochondria and its subsequent transport into cytosol. If this is true, then it should have happended prior to the isolation of the fractions and retained during isolation or it might have been formed during the incubation by the small number of mitochondria present (if any) in the cytoaol. One other reason for the rise in cytosolic 2-oxoglutarate content could be due to the suppression of cytosolic aspartate aminotransferase. Increase in cytoaolic malate content in hyperammonemic states (which was statistically significant only in acute conditions) may be due to the increased conversion of oxaloacetate to malate in this subcellular fraction or due to the failure of the carrier that transports malate from the cytosol to mitochondria. If the latter were to be true, then cytosolic 2-oxoglutarate content should have registered a fall rather than an elevation.

Whatever may be the mechanism for the reported changes

in citric acid cycle intermediates, these results indicated that there is no depletion of 2-oxoglutarate content in hyperammonemic states. Though this suggestion is not in agreement with results of Beesman and Beesman (1955), it agrees very well with the reports of several investigators (Hawkins et al., 1973; Vergara et al., 1974; O'Connor et al., 1984; Mans et al., 1984). Results of the present study also suggests that the operational rates of citric acid cycle may not be compromised in brain in hyperammonemic states. However, no statistically significant changes were noticed in the contents of ATP and ADP in mitochondria and cytoaol under both the conditions (Tables 4.39 and 4.40).

As was done in earlier experiments, the observations on the contents of citric acid cycle metabolites have to be supplimented with the results of the studies on enzyme activities. For this purpose, the mitochondria and cytosol were isolated from hyperammonemic rats and the activity levels of enzymes were determined. These results, presented below, were compared with those obtained in normal animals.

Following the administration of either subacute or acute doses of ammonium acetate, there was an elevation in the mitochondrial pyruvate dehydrogenase activity and the magnitude of elevation was much higher in acute state than in subacute state (Table 4.28). Under these conditions there

were no statistically significant changes in the citrate synthetase activity in thia subcellular fraction isolated from the cerebral cortex of either aubacute or acute hyperammonemic rats (Table 4.29). Though the isocitrate dehydrogenaae activity in the mitochondria was elevated in both the groups, the change in subacute state was statistically not significant while in the acute state the elevation in the activity was marginal but was statistically significant (Table 4.30). Activity levels of 2-oxoglutarate dehydrogenase were enhanced in the mitochondria isolated from the cerebral cortex of the rats administered with subacute dose of ammonium acetate. However, elevation in the activity of this enzyme after the administration of an acute dose of ammonium acetate was not statistically significant (Table 4.31). Succinate dehydrogenase activity was enhanced in the mitochondria in both subacute and acute states of hyperammonemia (Table 4.32). In contrast to all these changes malate dehydrogenase activity was suppressed in the direction of oxaloacetate formation in the mitochondria isolated from subacute or acute hyperammonemic rats (Table 4.33). Activity of this enzyme in the reverse direction was unaltered under these conditions (Table 4.34). An interesting observation in this atudy was the lesser magnitude of changes in the activities of all citric acid cycle enzymes in the mitochondrial fraction of the cerebral cortex of hyperammonemic rats when

compared to the changes observed under similar conditions in homogenates. Exceptions to this were the changes in the activities of succinate dehydrogenaae and malate dehydrogenase in the mitochondria in subacute hyperammonemic states.

Activities of the citric acid cycle enzymes in the cytosolic fraction prepared from the cerebral cortex of hyperammonemic rats were lesser than those of the mitochondria, a situation similar to that seen in the normal animals. However, the activities of all the citric acid cycle enzymes excepting malate dehydrogenase were enhanced to a very great extent, ranging from 1.5 to 8 fold (47% to 725';), in the cytosol. Despite the increase in the activities of these enzymes in the cytosol, it must be emphasized that the observed activity levels were much lower than those observed in the mitochondria (Tables 4.28 to 4.32). Malate dehydrogenase activity, when measured in the direction of oxaloacetate formation, was suppressed in the cytosol in both subacute and acute states of hyperammonemia. The magnitude of suppression was more or less same in both the conditions (Table 4.33). In contrast to this, activity of this enzyme when measured in the reverse direction i.e., towards malate formation, was enhanced in the cytosol under these conditions (Table 4.34). The profile of changes observed in the cytosolic activities of citric acid cycle enzymes in hyperammonemic states were

similar to those in homogenates but the magnitude of change was much higher in cytosol when compared to homogenates. An exception to this is the change in the cytosolic malate dehydrogenase activity measured in the direction of malate formation.

Increase observed in the pyruvate dehydrogenase activity in the mitochondria would facilitate the channelling of pyruvate into citric acid cycle in hyperammonemic conditions. This may be further aided by the suppression of lactate dehydrogenase and alanine aminotransferase activities as a result of which pyruvate would be spared for the citric acid cycle. This observation on pyruvate dehydrogenase activity is well in agreement with the earlier suggestion. Increased activity of pyruvate dehydrogenase in hyperammonemic states could be due to the altered states of phosphorylation of pyruvate dehydrogenase complex under these conditions. Lack of change in citrate synthetase activity in the mitochondria from the cerebral cortex of hyperaminonemic rats agreed very well with the results of mitochondrial citrate content observed under similar conditions. However, changes in the isocitrate dehydrogenase activity were not in proportion to the changes in 2-oxoglutarate content. However, stimulation of isocitrate dehydrogenaae (atleast in acute states) might drive the reaction of citrate synthetase forward and thus facilitate the conversion of citrate to isocitrate and to 2-

oxoglutarate dehydrogenase in subacute state than in acute state. This might explain the greater 2-oxoglutarate content in the acute states than in subacute states. Changes observed in 2-oxoglutarate dehydrogenase suggests that the utilization of 2-oxoglutarate in the citric acid cycle may not undergo radical changes in hyperammonemic states. As the 2-oxoglutarate dehydrogenase complex resembles pyruvate dehydrogenase complex in several aspects, the mechanism of stimulation of this enzyme in hyperammonemic states might be similar to that of pyruvate dehydrogenase. This suggestion is supported by observation on branched-chain keto acid dehydrogenase, an keto acid dehydrogenase involved in the metabolism of branched-chain keto acids, is also activated in hyperammonemic states (Jessy and Murthy 1990a,b). However, the results of the present study on 2-oxoglutarate dehydrogenase are not in agreement with the results of Lai and Cooper (1986) who reported that ammonium ions even in small concentrations would inhibit the activity of this enzyme in the mitochondria prepared from the cerebral cortex of normal rats. Though the reasons for this discrepancy are to be investigated, it could be due to differences in the assay methods and in the preparations used. In their study, Lai and Cooper (1986) have used mitochondria from the brains of normoammonemic rats while in the present study mitochondria from hyperammonemic rat brains were used. Though the reports of McKhan and Tower (1961)

indicated a suppression in the production of $^{14}CO_2$ from 2-oxoglutarate in the brain mitochondria in the presence of ammonium ions, it must be mentioned that the results of metabolic study need not necessarily be same as that of enzyme activities. Furhter, these investigators have used very high concentration (20 mM) of ammonium chloride to achieve this effect.

Increase in the activity of 2-oxoglutarate dehydrogenase would increase the production of succinate. Though succinate dehydrogenase activity was enhanced by 2 fold in the mitochondria in hyperammonemic states, still the activity of this enzyme is lesser than that of 2-oxoglutarate dehydrogenase. This might result in the accumulation of succinate under these conditions. This would also impair the production of oxaloacetate which is required for (a) condensation of acetyl CoA and (b) for transamination (with glutamate) reaction. Lack of oxaloacetate might result in accumulation of acetyl CoA which might exert feed back inhibition on pyruvate dehydrogenase. This would restrict the formation of acetyl CoA. However, it is also possible that acetyl CoA (which accumulates in hyperammonemic states) might act as a stimulator for phosphoenolpyruvate carboxykinase and enhance the production of oxaloacetate. Berl demonstrated a increase in CO_2 fixation in brain in hyperammonemic states (Berl et al.,

1962; Berl 1961) which is in supportive of the above suggestion. Moreover, association of malate dehydrogenase with other enzymes might also influence the utilization of oxaloacetate. It has been shown that malate dehydrogenase complexes with fumarase and this will bind to either citrate synthetase or aspartate aminotransferase (Backmann and Johnsson, 1976; Bryce et al., 1976; Helper and Srere, 1977; Beeckmans and Kanarek, 1981). Though the precise conditions which influence this binding are not known, it is possible that ammonia might alter this phenomenon, so that oxaloacetate is channelled into citric acid cycle.

It is interesting to observe that the activities of citric acid cycle enzymes (except malate dehydrogenase) are enhanced in the cytosol in hyperammonemic states. The magnitude of increase cannot be accounted by the simple stimulation of the activities of the preexisting enzymes. This is quite evident in the case of succinate dehydrogenase which was stimulated by 8 fold in the cytosol in acute hyperammonemic states. If this were to be true, then the enhancement in the activities of citric acid cycle enzymes could be due to any one or more of the following reasons: (1) altered buoyant densities of mitochondria (due to swelling) which prevents it in sedimenting at the centrifugal forces used in the present study (2) fragmentation of mitochondria into small vesicles which sediment at higher centrifugal forces (3) rupture of

small population ofmitochondria which are vulnerable to increased concentrations of ammonium ions. On rupturing the contents of these mitochondria including citric acid cycle enzymes are liberated into the cytosol and this change should take place in in situ condition. There are evidences for the last suggestion where in mitochondrial sensitivity to ammonium ions has been demonstrated (Drewes and Leino, 1980). The physiological role of the enzymes released into the cytosol in this fashion is difficult to assess as the co-factors required for these enzymes will be diluted in the cytosol and may not be available for the enzymes. Further, there would be alterations in the environment, in the cytosol, especially for enzymes like succinate dehydrogenase which require phospholipids and hydrophobic environment for its activity (Singer, 1972; Viveksagar, 1984).

The results obtained with the activities of the enzymes of citric acid cycle and of metabolites of this cycle suggested that there might be no comproinizat ion in the operation of citric acid cycle in hyperammonemic states. Though this is not in agreement with the hypothesis of Beesman and Beesman (1955), it concurs with the results of several investigators.

STUDIES ON MALATE-ASPARTATE SHUTTLE

Due to the equivocal results obtained upon **the verifi-**

cation of Bessman's hypothesis on the mechanism of ammonia toxicity it was suggested that the transport of reducing equivalents across the mitochondrial membranes may be affected in hyperammonemic states (Hindfelt, 1975, Duffy and Plum, 1982). It was proposed that major pathway for ammonia detoxification in brain is by way of glutamine production and this process is stimulated in hyperammonemic states due to the increased availability of ammonium ions. As glutamine synthesis occurs in cytosol, enhanced glutamine formation in hyperammonemic states was thought to deplete cytosolic glutamate and make this amino acid unavailable for malate-aspartate shuttle. As a result, the shuttle activity decreases and this keeps the cytosol in a more reduced state. This would affect glyceraldehyde-3-phosphate dehydrogenase activity as the regeneration of NAD will be adversely influenced under these conditions. This was supported by the reported elevation in the levels of malate (Hindfelt and Siesjo, 1970), increased lactate/pyruvate ratios (Hawkins et al., 1973; Adams et al., 1979; Raabe and Lin, 1984; Mans et al., 1984) and a fall in the levels of glutamate and aspartate (Hindfelt, 1975; Hindfelt et al., 1977; Tyce et al., 1981; O'Conner et al., 1982; Lin and Raabe, 1985; Lai et al., 1989) in the extracts of various brain preparations from different animal models of hyperammonemia. However, there are no reports on the cytosolic and mitochondrial levels of these

intermediates or on the enzymes of malate-aspartate shuttle in hyperammonemic states. Hence a study was conducted along these lines.

METABOLITES OF MALATE-ASPARTATE SHUTTLE:

The increase in the levels of malate and 2-oxoglutarate in the subcellular fractions (after incubation with glucose) isolated from hyperammonemic rat brain have already been described (Tables 4.18 and 4.19). In the subcellular frctions from subacute hyperammonemic states, there were no statistically signifiant differences in the contents of glutamate and aspartate (Tables 4.35 and 4.36). However, in the acute states, there was a fall in the contents of these two amino acids in the cytosol. Under these conditions, there was a significant decrease only in the content of aspartate but not glutamate in the mitochondria (Tables 4.35 and 4.36).

The results obtained for the changes in the cytosolic and mitochondrial malate and 2-oxoglutarate levels have already been discussed (Tables 4.18 and 4.19). Fall in the glutamate content in the cytosol may be due to its increased utilization for the synthesis of glutamine in hyperammonemic states or due to decreased synthesis by cytosolic aapartate aminotransferase (aspartate + 2-oxoglutarate ——> glutamate + oxaloacetate). It may also be due to the increased transport of glutamate into mitochondria under these conditions. The

last possibility may be ruled out as the cytosol has minimal contamination of mitochondria even under hyperammonemic states. Lack of changes in the mitochondrial glutamate content is understandable as this subcellular compartment is not involved in glutamine synthesis and it may also be due to the suppression of glutamate dehydrogenase activity in the direction of either glutamate formation or utilization (Yu et al., 1982; Subbalakshmi and Murthy, 1983, 1984, 19a5; Murthy et al, 1987; Lai et al., 1989). Fall in mitochondrial aspartate content may be due to reduced rates of synthesis or increased rates of utilization and transport. The latter two possibilities may be ruled out as aspartate is utilized only in very few reactions and some of them may not even present in brain or in the mitochondria (eg. urea synthesis and purine necleotide cycle respectively). Increased transport of aspartate may also be ruled out as the whole of incubation mixture (mitochondria + medium) were analyzed for aspartate content. Hence if transported, it should have been present in the medium.

According to the proposal of malate-aspartate shuttle, mitochondria are the sites of aspartate formation. Hence, if aspartate formation in the mitochondria is decreased (aa in hyperammonemic states), its transport into the cytosol (in exchange for glutamate) would also decrease. This would re-

sult in the decreased availability of aspartate for the cytosolic aspartate aminotransferase and thus oxaloacetate production in the cytoaol would be adversely affected. This would affect the conversion of oxaloacetate to malate, thus NADH to NAD, in the cytoaol. Thi3 results in the increase in reduced conditions in the cytosol. However, suppressed production of aspartate in the mitochondria is physiologically beneficial as it spares oxaloacetate required for citric acid cycle. Earlier invest1gators attributed the increase in lactate/pyruvate ratio in hyperammonemic states to the increasd NADH levels in the cytosol. However, in the present investigation, there was no such increase in lactate/pyruvate ratio and infact a decrease was noticed in lactate dehydrogenase activity and in lactate content (Tables 4.15 and 4.26). Though this is physiologically beneficial, as it sparea pyruvate for citric acid cycle, it would increase the NADH content in the cytosol (thus glycolysis). It was shown that suppression of malate-aspartate shuttle would also suppress the oxidation of glucose and also of pyruvate in brain (Fitzpatrick et al., 1983; Murthy and Hertz, 1988). Results obtained presently on $^{14}CO_2$ production from glucose in these two fractions is not in agreement with this concept. This is possible only if other shuttles for the transport of reducing equivalents (such as glycerol phosphate -dihydroxyacetone phosphate shuttle) are activated under these conditions.

As a suppliment to the metabolite levels presently analyzed activities of enzymes of malate-aspartate shuttle were also determined in the cytosol and mitochondria in hyperammonemic states. Administration of either subacute or acute dose of ammonium acetate resulted in the suppression of malate dehydrogenase activity (measured in the direction of oxaloacetate formation) both in mitochondrial and cytosolic fractions and the magnitude of this change was similar in both the experimental conditions (Table 4.33). However, when the activity of this enzyme was measured in the reverse direction, the mitochondrial activity showed no significant changes either in acute or subacute hyperammonemic states while it was elevated in the cytosolic fraction in both the experimental states (Table 4.34). Activities of aspartate aminotransferase in the cytosol and mitochondria were found to decrease upon the administration of either subacute or acute dose of ammonium acetate (Table 4.37).

Changes observed in the malate dehydrogenase activity in the cytosol and mitochondria in hyperammonemic states have already been discussed. Suppression in the mitochondrial aspartate aminotransferase actvity in both acute and subacute states agreed well with the reduced aspartate content and unaltered glutamate levels in the mitochondrial fraction under these conditions. Suppression of cytosolic aspartate

aminotransferase concured with the decreased cytosolic glutamate levels but not with the aspartate levels in this fraction. Thus, the results on malate aspartate shuttle suggests that there might be disturbances in the operation of malate-aspartate shuttle in brain in hyperammonemic states.

The results of this studies on subcellular fractions in hyperammonemic states may be summerized as follows:

(1) There is an elevation in the processsing of glucose in glycolytic pathway.

(2) The utilization of pyruvate in citric acid cycle might also be enhanced under these condition. The oxaloacetate required for citric acid cycle might be generated from residual malate dehydrogenase activity and supplimented by the anaplerotic reaction where in oxaloacetate is generated from either phosphoenolpyruvate or pyruvate by way of CO_2 fixation.

(3) The operation of malate-aspartate shuttle may be altered due to increased glutamine formation. Alternate shuttles may be stimulated under these conditions.

TABLE 4.1

ACTIVITY LEVELS OF MARKER ENZYMES IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RATS

ENZYME		MITOCHONDRIA	CYTOSOL
SDH	N	2.8±0.2	0.040±0.003
	SA	4.9±0.4 p<0.005 +76*	0.197 + 0.015 p<0.001 +393%
	A	5.56+0.71 p<0.001 +99%	0.330+0.009 p<0.001 +725%
LDH (P->L)	N	79+3	385+72
	SA	24+3 p<0.001 -70*	295+15 p<0.001 -23*
	A	34+7 p<0.001 -57%	145+29 p<0.001 -62*
LDH (L->P)	N	23+4	38+4
	SA	2 4 ±2 6 9	+ 5 p<0.001 +82*
		N.S. +4*	
	A	22+3 N.S. -4*	48±12 N.S. -26%

SDH : auccinate dehydrogenase, activity is expressed as poles of succinate oxidized/rag protein/hr.LDH : lactate dehydrogenas< pyruvate———> lactate, activity is expressed as umoles of NADH oxidized/mg protein/hr; lactate———> pyruvate, activity is expressed as µmoles of NAD reduced/mg protein/hr. Number of experiments are 5. Values are Mean ± S.D.

TABLE 4.2

RELATIVE PERCENTAGES OF MARKER ENZYMES IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RATS

ENZYME		MITOCHONDRIA	CYTOSOL
SDH	N	100±0	1.58±0.26
	SA	100+0	4.10+0.76 p<0.001
	A	100+0	6.00+1.1 p<0.001
LDH (P->L)	N	21+2.0	100+0
	SA	8.2+1.1 p<0.001	100+0
	A	23.9+4.0 N.S.	100+0
LDH (L->P)	N	63+15	100+0
	SA	35+6 p<0.005	100+0
	A	47+11 N.S.	100+0

Legned same aa in Table 4.1

TABLE 4.3

$^{14}CO_2$ PRODUCTION FROM [U-^{14}C]GLUCOSE IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX OF NORMAL RAT BRAIN

FRACTION	nMOLES OF $^{14}CO_2$ PRODUCED
MITOCHONDRIA	1.99±0.12 (5)
CYTOSOL	0.4310.05 (5)

Activity units: nmoles of CO_2 produced/mg protein/hr. Number in parenthesis indicates number of experiments. Each value is mean+S.D.

TABLE 4.4

DISTRIBUTION OF THE INTERMEDIATES OF GLYCOLYTIC PATHWAY IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF RAT CEREBRAL CORTEX

METABOLITE	MITOCHONDRIA	CYTOSOL
G-6-P	7.90±1.0	90.0±11.0
F-6-P	0.40+0.05	4.9±0.6
PEP	0.13±0.03	1.5±0.2
PYR	11.00±0.9	25.0±1.8
LACT	46.00±10.0	230.0±36.0

G-6-P: glucose-6-phosphate; F-6-P: fructose-6-phosphate; PEP: phoaphoenolpyruvate; PYR: pyruvate; LACT: lactate. Unite: nmoles/mg protein. Number of experiments are four. Each value is mean + S.D.

TABLE 4.5

DISTRIBUTION OF GLYCOLYTIC ENZYMES IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF RAT CEREBRAL CORTEX

ENZYME	MITOCHONDRIA	CYTOSOL	
HK	2.21 + 0.54	11±1	a
PFK	0.3± 0.04	2.64 + 0.32	a
ALASE	1.9+0.25	6.9±0.3	a
GLPDH	2.2+0.16	9.15±1.04	a
ENASE	3.15±0.35	19.9±1.04	a
PK	4.8±0.9	33±2	a
LDH (P-->L)	79±3	385±72	a
LDH (L-->P)	23±4	38+4	a

HK: hexokinase; PFK: phosphofructokinase; ALASE: aldolase; GLPDH: glyceraldehyde-3-phosphate dehydrogenase; ENASE: enolase; PK: pyruvate kinase; LDH: lactate dehydrogenase. Activity units for HK were µmoles of NADP* reduced/mg protein/hr; for GLPDH and LDH (L->P) µmoles of NAD reduced/ma protein/hr; for others µmoles of NADH oxidized/mg protein/hr. Each value is mean ± S.D. Number of experiments are five. Only those values which are significantly different ($p<0.05$) from mitochondria are indicated with a letter *a'.

TABLE 4.6

DISTRIBUTION OF THE INTERMEDIATES OF CITRIC ACID CYCLE IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF RAT CEREBRAL CORTEX

INTERMEDIATE	MITOCHONDRIA	CYTOSOL
CIT	35.00±2.8	ND
ISOCIT	25.00±3.0	ND
2-OG	25.00+2.0	8.0+0.7
MAL	37.00+3.0	42.0±2.5

CIT: citrate; ISOCIT: iaocitrate; 2-OG: 2-oxoglutarate; MAL: malate. Units: nmoles/mg protein. Number of experiments are four. Each value is mean + S.D.

TABLE 4.7

DISTRIBUTION OF PYRUVATE DEHYDROGENASE AND CITRIC ACID CYCLE ENZYMES IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX OF RAT BRAIN

ENZYME	MITOCHONDRIA	CYTOSOL	
PDH	9.6610.52	1.036±0.18	a
CS	65±8	0.8±0.05	a
ICDH	35.8+4	1.5+0.47	a
2-OGDH	22±3.7	3.6±0.54	a
SDH	2.8+0.43	0.04±0.002	a
MDH (M->0)	536+41	392±66	a
MDH (O->M)	472+43	576+68	a

PDH: pyruvate dehydrogenase; CS: citrate synthetase; ICDH: isocitrate dehydrogenase; 2-OGDH: 2-oxoglutarate dehydrogenase; SDH: succinate dehydrogenase; MDH: malate dehydrogenase. Activity was expressed for PDH, ICDH, 2-OGDH and MDH (M->0) as umoles of NAD reduced/mg protein/hr and for CS ae μmoles of DTNB reduced/mg protein/hr and for SDH as umoles of succinate oxidized/mg protein/hr and for MDH (0->H) as μmoles of NADH oxidized/mg protein/hr. Number of experiments are five. Each value is mean ± S.D. Only those values which are significantly different ($p<0.05$) from mitochondria are indicated with a letter 'a*.

TABLE 4.6

DISTRIBUTION OF THE INTERMEDIATES OF BORST CYCLE IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF RAT CEREBRAL CORTEX

INTERMEDIATE	MITOCHONDRIA	CYTOSOL
2-OG	25.00±2.0	8.0±0.7
MAL	37.00±3.0	42.0±2.5
ASP	65.00±7.0	72.017.0
GLU	180.0+20.0	250.0130.0

2-OG: 2-oxoglutarate; MAL: malate; ASP: aapartate; GLU: glutamate Units:nmoles/mg protein.Number of experiments are four. Each value ia mean ± S.D.

TABLE 4.9

DISTRIBUTION OF BORST CYCLE ENZYMES IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX IN RAT BRAIN

ENZYME	MITOCHONDRIA	CYTOSOL	
MDH (M->0)	536±41	392±66	a
MDH (0->M)	472±43	576±68	a
AAT	150115	129±13	

MDH: malate dehydrogenase; AAT: aapartate aminotransferase. Activity was expressed for MDH (M->0) as umoles of NAD reduced/mg protein/hr and for other enzymes as umoles of NADH oxidized/mg protein/hr. Number of experiments are five. Each value is mean ± S.D. Only those values which are significantly different ($p<0.05$) from mitochondria are indicated with a letter 'a'.

TABLE 4.10

$^{14}CO_2$ PRODUCTION FROM [U - ^{14}C] GLUCOSE IN MITOCHONbRIAL AND CYTOSOLIC FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	MITOCHONDRIA	CYTOSOL
NORMAL	1.99+0.12	0.43+0.05
SUBACUTE	2.67+0.26 p<0.005 +34%	0.49+0.045 NS +14%
ACUTE	2.70±0.19 p<0.001 +36%	0.61i0.037 p<0.001 +424
1mM	1.93+0.3 NS -3%	0.49+0.02 NS +14%
5mM	2.30+0.15 p<0.01 +164	0.50+0.03 NS +16*

Units:nmoles of CO_2 produced/mg protein/hr.
Number of experiments are 4. Each value is Mean+S.D.

TABLE 4.11

LEVELS OF GLUCOSE-6-PHOSPHATE IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX IN NORMAL AND HYPERAMMONEMIC RAT BRAINS

STATE	MITOCHONDRIA	CYTOSOL
NORMAL	7.9011.0	90.0±11.0
SUBACUTE	9.90+0.80 p<0.025 +25%	118.0±9.0 p<0.01 +31%
ACUTE	10.6+0.90 p<0.01 +34%	139.0+7.0 p<0.001 +54%
1mM	7.90+1.30 NC	102.0+10.0 NS +13%
5mM	9.70+0.70 p<0.05 +23%	116.0+8.0 p<0.01 +29%

Units: nmoles/mg protein. Number of experiments are 4. Each value is Mean+S.D.

TABLE 4.12

LEVELS OF FRUCTOSE-6-PHOSPHATE IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX IN NORMAL AND HYPERAMMONEMIC RAT BRAINS

STATE	MITOCHONDRIA	CYTOSOL
NORMAL	0.40+0.05	4.9+0.6
SUBACUTE	0.57+0.04 p<0.02 +25%	5.7+0.5 NS **+16%**
ACUTE	0.6+0.02 p<0.001 +50%	**6.5+0.7** p<0.02 +33%
1mM	0.5+0.03 p<0.02 +25%	5.6+0.5 NS **+14%**
5mM	0.57+0.04 p<0.005 +43%	**6.3+0.7** p<0.025 **+28%**

Legend same as in Table 4.11.

TABLE 4.13

LEVELS OF PHOSPHOENOLPYRUVATE IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX IN NORMAL AND HYPERAMMONEMIC RAT BRAINS

STATE	MITOCHONDRIA	CYTOSOL
NORMAL	0.13±0.03	1.5±0.2
SUBACUTE	0.17+0.017 NS +314	1.97+0.2 p<0.02 +31%
ACUTE	0.26 + 0.025 p<0-001 +100%	2.6±0.17 p<0.001 +73%
1mM	0.16+0.016 NS +2 34	1.8+0.17 NS +20%
5mM	0.23±0.02 p<0.001 +77%	2.2 + 0.27 p<0.01 +474

Legend same as in Table 4.11.

TABLE 4.14

LEVELS OF PYRUVATE IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX IN NORMAL AND HYPERAMMONEMIC RAT BRAINS

STATE	MITOCHONDRIA	CYTOSOL
NORMAL	11.0±0.9	25.0 + 1.8
SUBACUTE	10.5+0.8 NS -5%	27.5+1.8 NS +10%
ACUTE	12.2+1.2 NS +114	34.0+2.7 p<0.005 +36%
1mM	11.5+1.0 NS +44	27.0+2.0 NS +8%
5mM	12.0 + 1.0 NS +94	32.0 + 2.0 p<0.005 +28%

Legend same as in Table 4.11.

TABLE 4.15

LEVELS OF LACTATE IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX IN NORMAL AND HYPERAMMONEMIC RAT BRAINS

STATE	MITOCHONDRIA	CYTOSOL
NORMAL	46.0±10.0	230.0+36.0
SUBACUTE	28.0+3.0 p<0.02 -39%	187.5+19.0 NS -18%
ACUTE	32.0+3.0 p<0.05 -30%	150.0+25.0 p<0.02 -35%
1mM	28.5+2.0 p<0.02 -38*.	190.0+22.0 NS -17%
5mM	26.0+4.60 p<0.02 -43%	180.0+18.0 p<0.05 -22%

Legend same as in Table 4.11.

TABLE 4.16

LEVELS OF CITRATE IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX IN NORMAL AND HYPERAMMONEMIC RAT BRAINS

STATE	MITOCHONDRIA	CYTOSOL
NORMAL	35.0±2.8	ND
SUBACUTE	34.0+6.0 NS -3%	ND
ACUTE	37.1+3.2 NS +6%	2.5+0.2
1mM	36.0+4.0 NS +3%	ND
5mM	34.8+3.0 NC	2.0+0.1

Legend same aa in Table 4.11.

TABLE 4.17

LEVELS OF ISOCITRATE IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX IN NORMAL AND HYPERAMMONEMIC RAT BRAINS

STATE	MITOCHONDRIA	CYTOSOL
NORMAL	25.0±3.0	ND
SUBACUTE	27.5+2.0 NS +10%	ND
ACUTE	32.0+3.1 p<0.02 +28*	3.6+0.3
1mM	26.0+2.5 NS +4*	ND
5mM	30.0+1.5 p<0.025 +20*	3.4+0.2

Legend sane as in Table 4.11.

" **TABLE 4.18**

LEVELS OF 2-OXOGLUTARATE IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX IN NORMAL AND HYPERAMMONEMIC RAT BRAINS

STATE	MITOCHONDRIA	CYTOSOL
NORMAL	25.0±2.0	8.0±0.7
SUBACUTE	30.0+3.3 p<0.05 +20*	10.0+1.0 p<0.02 +25%
ACUTE	38.0+4.0 p<0.001 +52%	15.0+1.2 p<0.001 +88%
1mM	28.0±2.0 NS +12%	9.0±0.8 NS +13%
5mM	35.0+3.0 p<0.005 +40%	14.0+1.0 p<0.001 +75*

Legend same as in Table 4.11.

TABLE 4.19

LEVELS OF MALATE IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX IN NORMAL AND HYPERAMMONEMIC RAT BRAINS

STATE	MITOCHONDRIA	CYTOSOL
NORMAL	37.0+3.0	42.0+2.5
SUBACUTE	41.0+4.0 *US* +11%	48.0+3.0 p<0.025 +14%
ACUTE	48.0+5.0 p<0.01 +30%	58.0+6.0 p<0.005 +38%
1mM	39.0+4.0 NS +5%	45.0+4.0 NS +7%
5mM	45.0+5.0 p<0.05 +22%	52.0+6.0 p<0.025 +24%

Legend same aa in Table 4.11.

TABLE 4.20

MITOCHONDRIAL AND CYTOSOLIC HEXOKINASE ACTIVIIES IN CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	NORMAL	SUBACUTE	ACUTE
MITOCHONDRIA	2.21+0.54	3.02+0.48 p<0.05 +37*	2.98+0.37 p<0.05 +35*
CYTOSOL	11.0+1.0	9.8+1.3 NS -11*	11.9+0.9 NS +8*

Activity units: µmolea of NADP reduced/nig protein/hr. Number of experiments are five. Each value is mean + S.D.

TABLE 4.21

MITOCHONDRIAL AND CYTOSOLIC PHOSPHOFRUCTOKINASE ACTIVITIES IN CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	NORMAL	SUBACUTE	ACUTE
MITOCHONDRIA	0.3+0.03	0.34+0.04 NS +13%	0.39+0.04 P<0.005 +30*
CYTOSOL	2.64+0.32	3.94+0.4 P<0.001 +49%	4.98+0.8 P<0.001 +89*

Activity units: umoles of NADH oxidized/mg protein/hr. Number of experiments are five. Each value is mean + S.D.

TABLE 4.22

MITOCHONDRIAL AND CYTOSOLIC ALDOLASE ACTIVITIES IN CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	NORMAL	SUBACUTE	ACUTE
MITOCHONDRIA	1.9+0.25	2.7+0.37 P<0.005 +42*	2.8+0.38 P<0.005 +49*
CYTOSOL	6.92+0.3	10.6+1.1 P<0.001 +53*	14.3+0.6 P<0.001 +107*

Activity units:umoles of NADH oxidized/mg protein/hr. Number of experiments are five. Each value is mean ± S.D.

TABLE 4.23

MITOCHONDRIAL AND CYTOSOLIC GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE ACTIVITIES IN CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	NORMAL	SUBACUTE	ACUTE
MITOCHONDRIA	2.22+0.16	3.08+0.26 p<0.001 +39%	2.98+0.32 p<0.005 +34*
CYTOSOL	9.15±0.33	9.42±0.74 NS +3%	10.2±0.8 p<0.05 +11%

Activity unite: µmoles of NAD reduced/mg protein/hr. Number of experiments are five. Each value is mean ± S.D.

TABLE 4.24

MITOCHONDRIAL AND CYTOSOLIC ENOLASE ACTIVITIES IN CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	NORMAL	SUBACUTE	ACUTE
MITOCHONDRIA	3.15+0.35	3.44+0.35 NS +9*	4.29+0.35 p<0.001 +36%
CYTOSOL	19.9+1.03	26.4+1.46 p<0.001 +33%	35.9+1.06 p<0.001 +80%

Activity units: µmoles of NADH oxidized/mg protein/hr. Number of experiments are five. Each value is mean ± S.D.

TABLE 4.25

MITOCHONDRIAL AND CYTOSOLIC PYRUVATE KINASE ACTIVITIES IN CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	NORMAL	SUBACUTE	ACUTE
MITOCHONDRIA	4.8+0.9	5.5+0.8 NS +15*	6.3+0.6 p<0.02 +31%
CYTOSOL	33+2	39+2 p<0.005 +18*	46+2 p<0.001 +40*

Activity units: umoles of NADH oxidized/mg protein/hr. Number of experiments are five. Each value ia mean ± S.D.

TABLE 4.26

MITOCHONDRIAL AND CYTOSOLIC LACTATE DEHYDROGENASE (PYRUVATE -->LACTATE) ACTIVITIES IN CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	NORMAL	SUBACUTE	ACUTE
MITOCHONDRIA	79+3	24+3 p<0.001 -70%	34+7 p<0.001 -57%
CYTOSOL	385+72	295+15 p<0.05 -23%	145+29 p<0.001 -62%

Activity units: μmoles of NADH oxidized/mg protein/hr. Number of experiments are five. Each value is mean ± S.D.

TABLE 4.27

MITOCHONDRIAL AND CYTOSOLIC LACTATE DEHYDROGENASE (LACTATE-->PYRUVATE) ACTIVITIES IN CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	NORMAL	SUBACUTE	ACUTE
MITOCHONDRIA	23+4	24+1.5 NS +4%	22+3 NS -4%
CYTOSOL	38 + 4	69 + 5 p<0.001 +82%	52+6 p<0.001 +37%

Activity unite: μmoles of NAD reduced/mg protein/hr. Number of experiments are five. Each value is mean ± S.D.

TABLE 4.28

MITOCHONDRIAL AND CYTOSOLIC ACTIVITIES OF PYRUVATE DEHYDROGENASE IN CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	NORMAL	SUBACUTE	ACUTE
MITOCHONDRIA	9.66+0.52	11+1.1 p<0.05 +14%	15.2+0.37 p<0.02 +57%
CYTOSOL	1.036+0.18	3.46+0.5 p<0.001 +234%	7.2+0.52 p<0.001 +595%

Activity units: μmoles of NAD reduced/mg protein/hr. Each value is mean + S.D. Number of experiments are five.

TABLE 4.29

MITOCHONDRIAL AND CYTOSOLIC ACTIVITIES OF CITRATE SYNTHETASE IN CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	NORMAL	SUBACUTE	ACUTE
MITOCHONDRIA	65+8	67+12 NS + 3%	62+11 NS -5*
CYTOSOL	0.8+0.05	1.5+0.4 p<0.001 +88%	1.8+0.2 p<0.001 +125%

Activity units: pinoles of DTNB reduced/mg protein/hr. Number of experiments are five. Each value is mean ± S.D.

TABLE 4.30

MITOCHONDRIAL AND CYTOSOLIC ACTIVITIES OF ISOCITRATE DEHYDROGENASE IN CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	NORMAL	SUBACUTE	ACUTE
MITOCHONDRIA	35.8+4	39.5+1.3 NS +10*	43.5+ p<0.05 +20*
CYTOSOL	1.5+0.47	2.2+0.32 p<0.025 +47%	2.9+0.49 p<0.005 +93%

Activity units: umoles of NAD^+ reduced/ma protein/hr. Number of experiments are five. Each value is mean± S.D.

TABLE 4.31

MITOCHONDRIAL AND CYTOSOLIC ACTIVITIES OF 2-OXOGLUTARATE DEHYDROGENASE IN CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	NORMAL	SUBACUTE	ACUTE
MITOCHONDRIA	22+3.7	32+3.7 p<0.005 +45';	26+3.9 NS +18%
CYTOSOL	3.6+0.54	5.5+0.55 p<0.001 +54%	11.4+2.2 p<0.001 +220%

Activity units: umoles of NAD^+ reduced/mg protein/hr. Number of experiments are five. Each value is mean ± S.D.

TABLE 4.32

MITOCHONDRIAL AND CYTOSOLIC ACTIVITIES OF SUCCINATE DEHYDROGENASE IN CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	NORMAL	SUBACUTE	ACUTE
MITOCHONDRIA	2.8+0.43	4.94+0.9 p<0.005 +76*.	5.56+0.7 p<0.001 +99%
CYTOSOL	0.04+0.002	0.19+0.018 p<0.001 +393%	0.33+0.042 p<0.001 +.725%

Activity units: μmoles of succinate oxidized/rag protein/hr. Number of experiments are five. Each value ia mean ± S.D.

TABLE 4.33

MITOCHONDRIAL AND CYTOSOLIC ACTIVITIES OF MALATE DEHYDROGENASE (MALATE --> OXALOACETATE) IN CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	NORMAL	SUBACUTE	ACUTE
MITOCHONDRIA	536+41	365+15 p<0.001 -32%	341+24 p<0.001 -36%
CYTOSOL	392+66	240+22 p<0.005 -39%	228+24 p<0.001 -42%

Activity units: μmoles of NAD^+ reduced/ma protein/hr. Number of experiments are five. Each value is mean ± S.D.

TABLE 4.34

MITOCHONDRIAL AND CYTOSOLIC ACTIVITIES OF MALATE DEHYDROGENASE (OXALOACETATE --> MALATE) IN CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	NORMAL	SUBACUTE	ACUTE
MITOCHONDRIA	472+43	464+38 NS -2%	508+8 NS +8%
CYTOSOL	576+68	755+69 p<0.005 +31%	719+27 p<0.005 +25%

Activity units: μmoles of NADH oxidized/ing protein/hr. Number of experiments are five. Each value is mean + S.D.

TABLE 4.35

LEVELS OF GLUTAMATE IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX IN NORMAL AND HYPERAMMONEMIC RAT BRAINS

STATE	MITOCHONDRIA	CYTOSOL
NORMAL	180.0±20	250.000
SUBACUTE	160.0+17 NS -11*	220.0+30 NS • -12*
ACUTE	164.0+19 NS -94	200.0+20 p<0.05 -20%
lmM	170.0+15 NS -64	220.0+30 NS -12%
5mM	160.0+18 NS -11%	190.0+20 p<0.02 -244

Units: nmoles/mg protein. Number of experiments are 4. Each value is Mean+S.D.

TABLE 4.36

LEVELS OF ASPARTATE IN THE MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX IN NORMAL AND HYPERAMMONEMIC RAT BRAINS

STATE	MITOCHONDRIA	CYTOSOL
NORMAL	65.0±7.0	72.0±7.0
SUBACUTE	60.0+4.0 NS -8%	62.0+5.0 NS -144
ACUTE	51.0+5.0 p<0.02 -22%	55.0+4.0 p<0.01 -244
1mM	60.0+6.0 NS -84	68.0±5.0 NS -64
5mM	53.0+4.0 p<0.025 -184	54.0±4.0 p<0.005 -254

Units: nmoles/mg protein. Number of experiments are 4. Each value is Mean±S.D.

TABLE 4.37

MITOCHONDRIAL AND CYTOSOLIC ACTIVITIES OF ASPARTATE AMINOTRANSFERASE IN CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION	NORMAL	SUBACUTE	ACUTE
MITOCHONDRIA	150+15	99+7.6 p<0.001 -34*	82+9 p<0.001 -45%
CYTOSOL	129+13	68+7.6 p<0.001 -47%	71+6 p<0.001 -45%

Activity units: μmoles of NADH oxidized/me protein/hr. Number of experiments are five. Each value is mean + S.D.

TABLE 4.38

DISTRIBUTION OF ATP & ADP IN ttlTOCHONDRIAL AND CYTOSOLIC FRACTIONS OF RAT CEREBRAL CORTEX

INTERMEDIATE	MITOCHONDRIA	CYTOSOL
ATP	45.00+5	28.0±1.5
ADP	34.00+2.8	18.0+0.9

ATP: adenosine 5'triphosphate; ADP: adenosine 5' diphosphate. Number of experiments are four. Each value is mean ± S.D.

TABLE 4.39

LEVELS OF ATP IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX IN NORMAL AND HYPERAMMONEMIC RAT BRAINS

STATE	MITOCHONDRIA	CYTOSOL
NORMAL	45.0+5.0	28.0±1.5
SUBACUTE	47.7+4.8 NS + 6 4 NS	29.4+1.6 + 5 %
ACUTE	49.1+5.0 NS +94	30.2+2.1 NS +8%
1mM	43.7+4.0 NS -3% NS	27.4±1.4 -2 %
5mM	48.6±5.0 NS +8%	28.4+1.3 NS +34

Units: nmoles/mg protein. Number of experiments are 4. Each value is Mean±S.D.

TABLE 4.40

LEVELS OF ADP IN MITOCHONDRIAL AND CYTOSOLIC FRACTIONS OF CEREBRAL CORTEX IN NORMAL AND HYPERAMMONEMIC RAT BRAINS

STATE	MITOCHONDRIA	CYTOSOL
NORMAL	34.0±2.8	18.0+0.9
SUBACUTE	36.7+2.9 NS +8%	19.3+1.2 NS +74
ACUTE	38.0+4.0 NS +114	15.0+1.2 NS +94
1mM 28.0 + 2.0	9 . 0±0 . 8 NS -4 4	NS +34
5mM	35.0+3.0 NS +84	14.0+1.0 NS +54

Units: nmoles/mg protein. Number of experiments are 4. Each value is Mean±S.D.

STUDIES WITH CELLULAR FRACTIONS

STUDIES ON CELLULAR FRACTIONS:

Studies with homogenates and subcellular fractions revealed alterations in the cerebral carbohydrate metabolism in hyperammonemic states. The heterogeneity in the composition of the homogenates and the necessity for the studies with subcellular fractions has already been discussed.

Brain is made up of three kinds of cells viz., neurons, astrocytes and oligodendrocytes and during the process of homogenization all three kinds of cells are ruptured and their contents along with the subcellular organelles are released into the homogenizing medium. The subcellular fractions, namely mitochondria and cytosol, are thus derived from these three cell types. It must be remembered that these three cell typos perform different functions - i.e., neurons are involved in the process of reception, analysis and transmission of information; astrocytes are involved in several processes which are supportive of neuronal function while oligodendrocytes are involved in the formation of myelin sheath. Due to the heterogenity in their function, these cells differ in their structural details and in their biochemical composition. These differences in structure, function and biochemical composition evidently imply that they may be metabolically heterogenous. Thus, some of the metabolic pathways may be present only in one kind of cells,

but not in others and the same metabolites may have different metabolic fates in each cell type. For eg , glucose carbon is incorporated into acetylcholine in neuronal cells while this pathway is absent in other two cell types. Similarly, carbons of glucose are incorporated into myelin lipids in the oligodendrocytes and this process is absent in other two cell types. This metabolic heterogeneity was earlier thought to be unique to brain but recent studies indicate that this is also present in the liver cells (Haussinger, 1963; Haussinger et al., 1984). Due to this cellular heterogeneity in the cerebral metabolism, it was observed that certain toxins, pathological or altered physiological states affect only one type of cell but not other cells. Hence, it is quite possible that hyperammonemic conditions might affect only one cell type in the brain or may affect different cull types in different ways. Studies with homogenates and subcellular fractions fail to reveal the specific cell type affected in hyperammonemic states. Hence, it becomes imperative to know the action of ammonia on different cell types and on the metabolism of these cells. Prior to the description of the results of the present study, literature available on this aspect will be reviewed briefly.

Von Hosslin and Alzheimer (1912) described that hepatic diseases with encephalopathy are associated with a morbid hypertrophy of the astrocyte. This was later supported by

several investigators and Adams and Foley (1957) described the appearance of Alzheimer Type II cells as the dominant microscopic change in the brains of patients dying of hepatic coma. They observed marked increase in the number of large, pale astrocytes in the central nervoua system except in brain stem, spinal cord and cortical white matter. However, they emphasized such a change in astrocyte may not bring about neurological symptoms (delirium, seizures, coma etc.,) and the change in the astrocytea may be a mere reactive hyperplasia of protoplasmic astrocyte. Thia is understandable as the astrocytes at that time were considered as passive supporting cells of brain.

However, studied on the metabolism of glutamate family of amino acids (glutamate, glutamine, GABA, aspartate and alanine), by Waelsch and his associates (Berl, 1971; Waelsch and Lajtha, 1961; Berl et al., 1961,1962) resulted in understanding the role of astrocyte in hyperammonemic states. Using ^{14}C - glutamate as a precursor, these investigators reported that the specific radioactivity of glutamine was higher than that of glutamate and suggested that this is possible only when the metabolism of glutamate is compartmentalized in the brain. They further suggested that there are two pools of glutainate in brain - one large and the other small. Their studies revealed that these two pools of gluta-

mate do not mix with each other and that glutamine must be originating from the small but rapidly turning over pool of glutamate. Later studies of Benjamin and Quastel (1972, 1974, 1975) indicated that the small pool may be localized in the astrocytes and the large pool in the neurons. The suggestion that small pool of glutamate is associated with the synthesis of glutamine, helped in explaining the role of astrocytes in ammonia detoxification and for their reactive changes in hepatic disorders. Uith the demonstration of induction of Alzheimer Type II astrocytosis in methionine sulphoximine treated rats (Lodin et al., 1968; Phelps, 1975; Gutierrez and Norenberg, 1975, 1977) and the localization of glutamine synthetase in astrocytes (Martinez-Hernandez et al., 1977; Norenberg and Martinez-Hernandez, 1979), the implication of astrocytes in hyperammonemic states was firmly established. However, the relationship between the astroglial changes and the neurological symptoms is yet to be understood completely.

In recent years, results have appeared in the literature which contradict the theory of metabolic compartmentation. Though the original theory proposes that glutamine synthetase is exclusively localized in the astrocytes, it has been shown recently that this enzyme is present both in the neuronal perikarya and in synaptosomea (Subbalakshmi, 1984; Subbalakshmi and Murthy, 1985, 1986; Usharani, 1985). Under such conditions, ammonia might also be detoxified in the

neurons. Further, it has been reported that Na* , K*- ATPase activity is stimulated in hyperammonemi states. As this enzyme is also present in neurons and nerve terminals, its stimulation might lead to the depletion of cerebral energy stores in hyperammonemic states. Studies of Subbalakshmi (1984), Subbalakshmi and Murthy (1983), Murthy et al., (1987) revealed that ammonia at pathophysiological levels might affect the glutamate metabolism in neurons and astrocytes while the studies of Jessy (1990) and Jessy and Murthy (1990 a,b) revealed that the metabolism of branched-chain amino acida is affected in all the three cell types in hyperammonemic states. Presently, an attempt is made to study the effects of ammonium ion on the glucose metabolism of these three cell types viz., neurons, astrocytes and oligodendrocytes. Moreover, studies with the synaptosomes are also included along with the results on cell types. The rationale for this is as follows. Synaptosomes are the axonic nerve terminals thus they are a part of the neuron. However, they differ from their cell body in having mechanisms for the release of neurotransmitter. It has been reported earlier that the synaptosomal metabolism of glutamate differs from that of the neuronal perikarya (Subbalakshmi and Murthy 1985) and their response to injected ammonium acetate is also different (Subbalakshmi and Murthy 1986). Moreover, synaptosomes, though isolated as subcellular fractions, have plasma

membrane, cytoplasm and mitochondria and hence do not atrictly resemble any subcellular traction.

CELLULAR STUDIES IN NORMAL ANIMALS:

PURITY OF CELL PREPARATIONS:

In the studies involving isolated cells, establishment of cell purity and cross contamination of the isolated cells is a prerequisite. This is established by microscopic and biochemical methods. In the former both light and electron microscopic techniques are to be employed in the past. The information obtained from electron microscopic methods is usually much more extensive than that from light microscopic methods. However, the preparation of material for electron microscopic study is a time consuming process and can not be used on a day to day basis. Hence, in the present study only light microscopic studies (including phase contrast techniques) were used. These studies revealed no credible contamination of one cell type with other (Figs.1-3). However, the major drawback in these studies was the inability to identify contamination with membrane fragments and pinched off membrane vesicles (both derived from other cell types). Moreover, this technique could not reveal the contamination of synaptosomes with gliosomes (pinched off membrane vesicles of glial cells). Hence, the light microscopic observations were supplimented with the determinations of marker enzymes. In

this study, glutamine aynthetase and cholinesterases were used as a markers for aatrocytea while glutamic acid decarboxylase was used as a marker for nerve terminals (synaptosomes). These results indicated that the cross contamination was not more than 15 - 20% (Table 5.1). In other words the purity of isolated preparations were about 80 - 85%. No marker waa used for oligodendrocytes as the incubation conditions (during cell isolation) reduces the viability and survival of other cell types (Snyder et al., 1980). Other parameters such ae RNA, DNA and protein contents and the cell number were also determined (Tables 5.3 to 5.8).

There were no statistically significant differences in the purity of the cell preparations even in hyperammonemic states (Table 5.2).

PRODUCTION OP CO.:

As the protein content of the cells differs over a wide range (Table 5.7), the production of CO_2 from glucose has been expressed both per mg protein and per cell (Tables 5.9 and 5.10). However, with synaptosomes, it was expressed only per mg protein (Table 5.11). Irrespective of the mode of expression, the production of CO_2 from glucose wae higher in astrocytes than in neurons and oligodendrocytes. Among the latter two cell types, CO_2 production from glucose was higher

in neurons than in oligodendrocytes. The magnitude ot difference in this parameter was higher when the rates were expressed per cell than when it was expressed per mg protein (Table 5.9 and 5.10)). When a comparison waa made between neurons and synaptosomes, it was observed that the rate of CO_2 production was higher in the synaptosomes than in the neurons (on mg protein basis, Table 5.11). These results indicated that the isolated cells and synaptosomes are viable and can respire in the presence of glucose.

The values obtained in the present study for the production of COz from glucose in astrocytes and neurons are higher than the rates of CO_2 production in the neurons and astrocytes in primary cultures (Hertz et al., 1987). This may be due to the different treatments to which the isolated cells and the cells in primary culture are subjected. The isolated cells undergo prolonged trypsin treatment which might alter the membrane composition (acetylated trypsin used in the study does not penetrate the cell; (Farooq and Norton 1978). However, the cells grown in primary cultures undergo much gentler treatments and are allowed several weeks to recover from the trauma of dissociation procedure. Further, exposure of cells to hypertonic media during isolation procedure might also affect their membrane properties especially permeability properties. Hence, in the isolated cells the glucose permeability would naturally be higher than in the cells in the

primary cultures (Roeder et al., 1988). However, even with cells in primary cultures, Hertz et al., (1987) reported that CO_2 production with astrocytes was higher than that of neurons which ia similar to that in the present study. However, it must be mentioned that the neuronal perikarya used in the present study are usually devoid of dendritic and axonic processes and the nerve terminals. If all these components are present, as in in vivo states, it is possible that the glucose oxidation may be much higher in neurons than in astrocytes or the rates may be similar in both the cells. Infact the rate of glucose oxidation by synaptosomes was much higher than neuronal perikarya, which supports the above contention. Higher rates of glucose oxidation in synaptosomes may be due to a very low ratio of volume to surface area when compared to neurons. Very low rates of CO_2 production from glucose by oligodendrocytes indicated their metabolic inertness when compared with the other two cell types. This ia understandable as the major function of these cells is to form myelin lipids and in adult animals the rate of turnover of myelin lipids is very slow and the elaboration of myelin sheath does not take place. Moreover, as these cells are involved in lipid synthesis, part of acetyl CoA (thus glucose) might be diverted from citric acid cycle for lipid synthesis. Under such conditions, it can be expected that the rate of CO_2 production from these cells would be low. Aa the

nerve terminals (synaptosomes) outnumber the cells in brain, their contribution to cerebral glucose utilization would be much higher than the cel la. Among the cells, astrocytes outnumber the neurons by a factor of ten (Rose, 196/, 1968), hence their contribution in this respect would be greater than neurons and oligodendrocytes. One interesting observation of this study is the emergence of a new type of heterogeneity in the metabolism - i.e., functionally distinct regions of the same cell (such as neuronal perikarya and synaptosomes) might have distinct metabolic profiles.

Following is the description of levels of metabolites of glycolysis and citric acid cycle in the cells and synaptosomes after they were incubated with glucose. The contents of glucose-6-phosphate and fructose-6-phosphate were higher in astrocytes than in neurons and oligodendrocytes and this was seen irrespective of the mode of expression of the content (i.e., per cell or per mg protein; Tables 5.12 and 5.13). This suggests that the glucose uptake and utilization might be higher in astrocytes than in the other two cell types. This becomes important especialy while interpreting the results of glucose uptake in whole brain preparations such aa slices. It would be interesting to study whether astrocytic glucose uptake, phosphoryaltion and further metabolism ia affected in functional states of brain such as exitation and

depression.

The content of phosphoenolpyruvate was observed to be higher in the astrocytes than in the other two cell types when expressed per cell or per mg protein (Tables 5.12 and 5.13). This is understandable as these cells are supposed to be involved in CO_2 fixation (Berl,1971), where in phospho-enolpyruvate is converted to oxaloacetate, their requirement for phosphoenol pyruvate would be higher. Further, it roust be ascertained in future whether glycogen synthesis in these cells utilize pyruvate and phosphoenolpyruvate formed in glycolysis.

It is interesting to note that the profile for contents of pyruvate in the cells depends on the mode of expression. When expressed per cell, pyruvate was higher in astrocytes while it was same in neurons and oligodendrocytes (Table 5.12). However, when it was expressed per mg protein, it was highest in oligodendrocytes than in the other two cell types in which the contents were more or less same (Table 5.13). These two types of profiles, which depend on the mode of expression, were also observed in lactate content (Tables 5.12 and 5.13). The precise reason for this dependence of the content on the mode of expression and its physiological significance is difficult to explain at present. This may be due to the simple reason that the oligodendrocytes have less

amounts of protein (Table 5.7) hence, the number of cells per mg protein would be higher when compared to astrocytes and neurons (Table 5.5).

If this was to be the reason, then it is surprising that this difference does not appear in the case of other metabolites. Moreover, when the levels of all the other intermediates are lower in oligodendrocytes than in the other two cell types, it is difficult to envisage a higher pyruvate and lactate contents. This will only be possible (i.e., higher pyruvate and lactate contents in oligodendrocytes when all other metabolites are low) only if the utilization of pyruvate and lactate is very low in these cells or it the pyruvate/lactate pools are quite large and act as sinks. Despite higher lactate content, the contribution of oligodendrocytes to cerebral lactate pool would be low as these cells are few in number when compared to astrocytes and neurons.

When the contents of the four glycolytic intermediates of synaptosomes and neuronal perikarya are compared, there were certain striking differences (Table 5.13). The synaptosomal content of glucose-6-phosphate was lower than that of neuronal perikarya while the pyruvate and lactate contents of synaptosomes were greater than that of neuronal perikarya. The contents of fructose-6-phosphate and phosphoenolpyruvate

were similar in both the fractions. The magnitude of difference in the lactate content was very high in these two fractions when compared with the other metabolites. Obviously, the contribution of synaptosomes to cerebral lactate content would be much higher than other cells. This is surprising as the rate of CO_2 production from [U - ^{14}C]glucose was much higher in synaptosomes than the neuronal perikarya which suggests a greater oxidation of glucose in the former preparation.

As was done in earlier studies with subcellular fractions, the data on metabolites is supplimented with that on enzymes. For this purpose glycolytic enzymes were assayed in the extracts of the three differenct cell types and in synaptosomes. The activities of the glycolytic enzymes were expressed per mg protein (specific activity) and per cell (cellular activity) and the reasons for these two modes of expression of enzyme activity have already been discussed. Due to the reasons mentioned earlier, most of the discussion will be based on cellular activities unless otherwise indicated.

CELLULAR ACTIVITIES:

The cellular activity of the initial enzyme of glycolysis, i.e., hexokinase, was observed to be higher in aatrocytes than in the neurons and oligodendrocytes. Among the

later two, hexokinse activity was higher in the neurons than in oligodendrocytes (Table 5.14). Activities of the phosphofructokinase and aldolase were higher in the neurons than in the other two cell types. Among the astrocytes and oligodendrocytes, activities of these two enzymes were higher in astrocytes than in the oligodendrocytes. Glyceraldehyde-3-phosphate dehydrogenase activity was similar in all the three cell types while that of enolase was similar in only neurons and astrocytes. Enolase activity was lesser in oligodendrocytes than in other two cell types. Cellular activity of pyruvate kinase was similar in astrocytes and neurons which was greater than that of oligodendrocytes. Cellular activity of lactate dehydrogenase, measured in both the directions, was similar in astrocytes and neurons which was greater than that in oligodendrocytes. In general, cellular activities of all the glycolytic enzymes, excepting glyceraldehyde-3-phosphate dehydrogenase were lower in oligodendrocytes than in the other two cell types (Table 5.14).

The cellular activity of phosphofructokinase was greater than that of hexokinase in the neurons and oligodendrocytes while in astrocytes the activities of these two enzymes were similar. However, the cellular activities of phosphofructokinase and aldolase were equal in all the three cell types. In the oligodendrocytes, glyceraldehyde-3-phos-

phate dehydrogenase activity was greater than aldolase and enolase while in the neurons and astrocytes the activity of aldolase is equal to that of glyceraldehyde-3-phosphate dehydrogenase which was lesser than that of enolase. In all the cell types, the cellular activites of pyruvate kinase were several fold higher than those of enolase. In the oligodendrocytes, the activities of pyruvate kinase and lactate dehydrogenase (pyruvate ——> lactate) were also equal while in other two cell types pyruvate kinase activity was greater than that of lactate dehydrogenase (pyruvate --> lactate). Activity of lactate dehydrogenase, measured in the direction of pyruvate formation was lesser than that in the reverse direction in all the three cell types (Table 5.14).

SPECIFIC ACTIVITIES:

Differences were observed in the profiles obtained for the distribution of glycolytic enzymes, especially when their activities were expressed per mg protein (Table 5.15). Hexokinase activity was same in neurons and astrocytes and was higher than that of oligodendrocytes. Specific activities of phosphofructokinase and aldolaae were higher in neurons than in astrocytes and oligodendrocytes and in the latter two cell types specific activities of these two enzymes were similar. Unlike the profile of the distribution of above enzymes, specific activity of glyceraldehyde-3-phosphate dehydrogenase was higher in the oligodendrocytes than in neurons and astro-

cytes. Its activity was similar in the neurons and astrocytes. Similar activity levels were noticed for the specific activity of enolase in astrocytes and oligodendrocytes which were lower than that in neurons. Unlike the above, specific activity of pyruvate kinase was similar in neurons and astrocytes and was higher than that in oligodendrocytes. Specific activity of lactate dehydrogenase (both the directions) was higher in oligodendrocytes than in neurons and astrocytes.

As the uptake and retention of glucose by the cells are supposed to be dependent on hexokinase activity, it may be assumed that the astrocytes, with greater hexokinase activity, might take up much larger amounts of glucose and convert it to glucose-6-phosphate than neurons and oligodendrocytes. This is understandable as the astrocytes are the seats of glycogen synthesis (Phelps, 1972, 1975; Ibrahim, 1975; Cataldo and Broadwell, 1986; Cambray-Deakin et al., 1988 a, b) and the glucose-6-phosphate may also be utilized for this purpose. Moreover, it has been reported that the activity of hexose monophosphate ahunt pathway is higher in astrocytea than in other two cell types (Edmond et al., 1987) and this pathway also utilizes glucose-6-phosphate as its substrate.

Though it is said that the oligodendrocytes might also have small amount of glycogen, it was however observed to be

restricted to the early periods of brain development. Oligodendrocytes were shown to be devoid of glycogen in the mature brain (Phelps, 1972; Katzman, 1981). Lesser activities of hexokinase in these cells indicate that the glucose uptake by the oligodendrocytes might be much less compared to the neurons or astrocytes. This is supported by the observation that the oligodendrocytes had the lowest amount of glucose-6-phosphate. In the neurons and oligodendrocytes, phosphofructokinase activity was much higher than that of hexokinase while in the astrocytes both these activities were observed to be same. Phosphofructokinase is known to be a major regulatory enzyme in glycolytic pathway and controls the flux of glucose carbon through glycolytic pathway. Higher activities of this enzyme in neurons will facilitate the greater utilization of hexose phosphate in the glycolytic pathway. However, equal activities of hexokinase and phosphofructokinase in the astrocytes would allow the utilization of hexose phosphates not only in the glycolytic pathway but also for the synthesis of glycogen and in hexose monophosphate shunt. The amount of glucose carbon passing through these pathways at any given time would be determining the rate of synthesis of the intermediates of that pathway. In oligodendrocytes, which are involved in the synthesis of myelin, the glucose carbon is used not only for the energy metabolism but also for the synthesis of myelin and

also for providing the reducing equivalents required for the synthesis of lipids. The former process involves the conversion of glucose to pyruvate and then to acetyl-CoA which serves as the building blocks for myelin synthesis. The latter process, i.e., providing reducing equivalents, ia through the metabolism of glucose in hexose monophosphate shunt pathway. In present study, though the enzymes involved in the hexose monophosphate shunt were not determined, it may be assumed that their contribution to glucose metabolism will be minimal aa the animals used in this study were adult animals, in which the myelin formation takes place at very low rates. Hence, in the oligodendrocytes obtained from the adult animals, most of the glucose carbon might be diverted into the glycolytic pathway. Lesser activity levels of phosphofructokinase in the oligodendrocytes than the other two cell types is in agreement with the earlier suggestion that the rate of glucose metabolism might be low in the oligodendrocytes when compared to astrocytes and neurons.

Another regulatory point in the glycolytic pathway ia the reaction mediated by glyceraldehyde-3-phosphate dehydrogenase. In the neurons and astrocytes, activity of this enzyme was similar to that of aldolase while in the oligodendrocytes it was about three fold higher than that of aldolase. The enzyme, enolase, is involved in the formation of

phosphoenolpyruvate and its activity was observed to be higher than that of glyceraldehyde-3-phosphate dehydrogenase in the neurons and astrocytes but not in the oligodendrocytes. Such distribution would ensure greater utilization of triose phosphates for the formation of phosphoenolpyruvate in the former two cell types. Phosphoenolpyruvate participates not only in the pyruvate formation but also in the formation of oxaloacetate and thus in the anaplerotic replenishment of citric acid cycle intermediates. Studies on metabolic compartmentation revealed that the enzyme phosphoenolpyruvate carboxykinase is associated with the small compartment, which is supposed to be localized in the astrocytes (Berl, 1971). These studies suggested that phosphoenolpyruvate is involved not only in the formation of pyruvate but also of oxaloacetate in the astrocytes. Greater contents of phosphoenolpyruvate in the astrocytes than in neurons and oligodendrocytes, observed in the present study, supports such a concept. In this context, it must be mentioned that the relative activities of enolaae and pyruvate kinase are important as these two enzymes are involved in the formation utilization of phosphoenolpyruvate in the glycolytic pathway. It ia interesting to note that the pyruvate kinase activity was about ten fold higher than that of enolaae in all the three cell types. Under such circumstances, it is possible that the phosphoenolpyruvate formed in the enolase reaction may be

utilized to a greater extent for pyruvate formation. This is understandable as pyruvate is the vital intermediate of the glucose metabolism and is present at the cross road of several metabolic pathways.

The two major routes of utilization of pyruvate are the (1) formation of lactate and (2) its transport into mitochondria where pyruvate is converted to acetyl CoA. The latter intermediate may be further oxidized in the citric acid cycle or may be used for lipid synthesis. The conversion of pyruvate to lactate ia a reversible process and is mediated by the enzyme lactate dehydrogenase. Conversion of pyruvate to lactate involves the oxidation of NADH to NAD. It has been proposed that this reaction may be involved in the regeneration of NAD in the cytosol. The NAD thus regenerated is utilized in the triose-phosphate dehydrogenase reaction. Results obtained in the present study indicated that all these three cell types were capable of converting pyruvate to lactate and also the reverse reaction i.e., lactate to pyruvate. In general, the activity of the enzyme in the direction of lactate formation was observed to be much higher than in the reverse direction.

Profile of distribution of the activities of the glycolytic enzymes were more or leas same as above when their activities were expressed per mg protein. However, there are

few exceptions to this when the specific activities were considered. The activity of glyceraldehyde-3-phosphate dehydrogenase was much higher in the oligodendrocytes than the other two cell types. Similar pattern was observed with respect to lactate dehydrogenase. But for these two differences in general, the activities of the glycolytic enzymes in the oligodendrocytes were lesser than other two cell types.

As was mentioned earlier, in the present study synaptosomes have been included into the cell types. It is interesting to note that though synaptosomes are derived from perikarya, the profile of distribution of glycolytic enzymes was much different in these two neuronal preparations. The major difference was the very high activity of hexokinase and lower activity of phosphofructokinase in the synaptosomes when compared with neuronal perikarya. Similarly pyruvate kinase activity in synaptosomes was atleast half of that in the neurons and that of lactate dehydrogenase was six times higher. The differences in the activities of hexokinase indicated that the uptake of glucose would be much higher in the synaptosomes when compared to neurons. However, the measured glucose-6-phosphate levels were lower in synaptosomes than in neuronal perikarya. Such a difference in the measured content of the intermediate and the activity of the enzyme involved in its synthesis might be due to rapid utilization of the

metabolite in other reactions or due to the regulatory restrictions imposed on the activity of the enzyme under in vivo conditions. Similarly, lactate formation would also be much higher in the synaptosomes than in the neuronal perikarya. This is supported by the greater amount of lactate in the synaptosomes than in neurons. As the synaptosomes outnumber the neuronal perikarya as well as astrocytes, their contribution towards lactate formation will be much higher than any other cell type.

It la very well known that protein synthesis cannot takes place in synaptosomes as they are devoid of ribosomes. Hence, all the proteins are synthesized in the perikarya and transported through the axons into the nerve terminals. Based on the rates of transport, these proteins are devided into slow moving and fast moving proteins (Ocha, 1981). Though there is a heteroflenity in the rates of transport of these proteins, so far no information is available on the differences in the rates of transport of different enzymes of same pathway. Hence, it is quite possible that the differences in profiles of distribution of glycolytic enzymes in the synaptosomes and in the neuronal perikarya might be due to the differential rates of transport of this proteins. Another reason for such differential distribution could be due to differences in the turnover rates of these proteins in diffe-

rent compartments of the same cell. Once again no information is available on this aspect. Finally it should also be mentioned that the differences in the distribution of enzymes of glycolytic pathway in the neurons and in the nerve terminals might also be due to the differential expression of these enzymes in different subcellular fractions. This may be due to the differences in the availability of the cofactors and the minor variations in the local pH and substrate quantities. However, this may be ignored aa the assays were made under in vitro conditions, where both the preparations were permeabilized by the addition of Triton X-100 and optimal concentrations of substrates and cofactors were provided.

CITRIC ACID CYCLE INTERMEDIATES AND ENZYMES:

It was established quite early that citric acid cycle in brain is closely associated with the metabolism of amino acids of glutamate family (glutamate, glutamine, aspartate, alanine and GABA). Balazs and his associates have demonstrated that the glucose carbon enters rapidly into the carbon skeleton of these amino acids (Balazs et al., 1970). With the proposal of the compartmentalization of glutamate metabolism, it was evident that there is a bidirectional exchange of carbon skeleton between citric acid cycle and the glutamate family of amino acids. It has been proposed that there are two citric acid cycles in brain which are distinct

and do not mix with each other. One of them was proposed to be localized in neurons (associated with the large pool of glutamate) and the other in astrocytes (associated with the small pool of glutamate). It was proposed that glutamate is oxidized or in other words glutamate carbon enter the citric acid cycle in the neuronal compartment while in the astrocytes glutamate is synthesized (in a reaction mediated by glutamate dehydrogenase) which suggests the entry of carbons from citric acid cycle into glutamate (Benjamin and Quastel, 1972, 1974, 1975; Berl et al., 1978; Berl and Clarke, 1983). It was also suggested that carbon dioxide fixation occurs in the astrocytes (Berl, 1971). It was also proposed that these two pools communicate through glutamate and glutamine in order to balance the flow of carbon atoms into and out of glutamate (Benjamin and Quastel, 1972). However, recent experiments especially with cell cultures provided evidences some of which are in favour and others are not in support of the original theory of metabolic compartmentation. Of these results, the one with particular interest to the present study is the rates of $^{14}CO_2$ production from glutamate. It was shown that these rates are very high in astrocytes than in neurons (Hertz et al., 1983, 1987; 1988). This suggests that 2-oxoglutarate formed from glutamate enter citric acid cycle and this process is very high in astrocytes. In other words, **anaplerotic replenishment of citric acid cycle intermediates**

in glial cells occurs at two levels i.e., at the stage of 2-oxoglutarate (from glutamate) and also at the stage of oxaloacetate (transamination and CO_2 fixation). Another study of considerable interest is the release of citric acid cycle intermediates (2-oxoglutarate and malate) from the astrocytes and their subsequent uptake by high affinity uptake systems at the nerve terminals (Shank and Campbell, 1981, 1984 a, b). Thia atudy suggests that the citric acid cycles of neurons and aatrocytes communicates with each other not only through glutamate family of amino acid but also through their own intermediates. Despite the enormous amount of work done in thia area, very little information is available on the levels of the citric acid cycle intermediates in neurons and astrocytes and on the enzymes of citric acid cycle in these two cell types.

Moreover, in the original theory of metabolic compartmen ation the role of oligodendrocytes was completely ignored. Only recently, it has been demonstrated that theae cells can utilize not only glucose but also acetoacetate and hydroxybutyrate (Edmond et al., 4987). Results of the present investigation also demonstrated that these cells can form pyruvate and lactate from glucose and their contribution to brain lactate may be higher than the other cells.

Presently, the levels of citric acid cycle interme-

diates and the distribution of the enzymes of citric acid cycle have been studied in the cells isolated from the cerebral cortex of normal rats. Experiments were also carried out along the same lines with synaptosomes and the results were compared with those of neuronal perikarya. As was the earlier case, the contents and the activities were expressed per cell and per mg protein.

The results obtained with the production of $^{14}CO_2$ from [U - ^{14}C] glucose have already been described and discussed. In brief, these results indicated that the rate of production of $^{14}CO_2$ from glucose were higher in astrocytes than in neurons and the lowest rates were observed in oligodendrocytes (Table 5.9 and 5.10).

It was observed that the comparison of the contents of citric acid cycle intermediates in the cell types varies with mode of expression (Tables 5.12 and 5.13). The profiles were also observed to be different from those of the glycolytic intermediates. When expressed per cell it was observed that the levels of citrate, isocitrate, 2-oxoglutarate and malate were higher in the astrocytes than in the neurons. Lowest levels of these intermediates were observed in the oligodendrocytes. However, when expressed per mg protein, the contents of the measured citric acid cycle intermediates were similar in the neurons and astrocytes while their contents in

oligodendrocytes remained to be the lowest among the three cell types. In all the cell types, irrespective of the mode of expression, malate content was observed to be higher than that of other citric acid cycle intermediates.

Greater contents of citric acid cycle intermediates in astrocytes than in other cells is understandable as these cells also produced greater amounts of $^{14}CO_2$ from [U- ^{14}C] glucose and also had higher amounts of glycolytic intermediates. These results also suggest that the pool size of these intermediates (both of glycolysis and citric acid cycle) might be larger in the astrocytes than in neurons. This may be essential as these cells are supposed to export glutamine, 2-oxoglutarate and malate to the nerve endings (Shank and Campbell, 3981, 1984 a, b). Naturally this necessiates large pool size of intermediates which are required for their own use and for export purposes. Further, to maintain such large pools of citric acid cycle intermediates not only the rate of glucose utilization must be high but there must also be anaplerotic reactions at more than one stage of citric acid cycle. As was discussed earlier, the results of Yu et al., (1982, 1983); Berl et al., (1971) suggest that this may be true and the anaplerotic replenishment might occur at the stage of 2-oxoglutarate (by glutamate) and oxaloacetate (CO_2 fixation).

An interesting observation in the present study is the greater levels of malate than 2-oxoglutarate. Though this la observed in all the cell types, the magnitude of difference between these two intermediates is very high in astrocytes. If malate is formed only from 2-oxoglutarate in the citric acid cycle, its levels must be either lesser or equal to that of 2-oxoglutarate. Moreover, it must be recalled that the succinate dehydrogenase activity was much leaser than 2-oxoglutarate dehydrogenase by several fold in the mitochondria (and in the cells; see below). Under such conditions, the rate of utilization of succinate would be lower than the rate of its formation which should result in much lower levels of malate than 2-oxoglutarate. Lack of such relationship indicates that malate may be formed from other sources. Most likely source of malate is oxaloacetate which is generated from phosphoenolpyruvate (CO_2 fixation) or amino acids such as aspartate. Some of this malate might have originated in the cytosol due to the operation of malate-aspartate shuttle.

The lowest levels of citric acid cycle intermediates in the oligodendrocytes fits with the observed rates of $^{14}CO_2$ production from glucose and the levels of glycolytic intermediates in these cells.

Due to such a metabolic profile of oligodendroglial

cells, glucose would be spared for the other cells which are metabolically much more active than oligodendrocytes. Further, the cells with perpectually low requirements of energy would have low contents of ATP. In order to test thi3 tenet, ATP and ADP levels were measured in all the three cell types after they were incubated with glucose.

As in the case of citric acid cycle intermediates, the contents of ATP and ADP in the three cell types varied with the mode of expression (Tables 5.12 and 5.13). Their contents were higher in astrocytes than in neurons when expressed per cell. However, the contents were same in these two cell types, when they were expressed per mg protein. Irrespctive of the mode of expression, the oligodendroglial ATP and ADP levels were the lowest among the three cell types. However, the ATP/ADP ratio was observed to be the same in all the cell types.

Higher cellular contents of ATP in astrocytes may be the reason for their higher glucose utilization. As ATP levels are more, more of this compound would be available for hexokinase reaction which would result in trapping more glucose as glucose-6-phosphate. Conversely it may also be suggested that the higher rates of glucose utilization in astrocytes may be required to maintain the large pool size of ATP. Such high levels of ATP may be required for the astrocytes, as they are involved in the synthesis of glycogen, glutamine and

in scavenging the extracellular potassium. Lowest levels of ATP in the oligodendrocytes supports the concept that their energy requirements are lower than the other cells. Moreover, such low levels of ATP would not allow large amounts of glucose to be trapped as glucose-6-phosphate in hexokinase reaction unless the turnover rates are elevated.

A comparison of the contents of citric acid cycle intermediates between the neuronal perikarya and of aynaptosomes has also been made and the results are presented in Table (5.13). It was surprising to notice that there are no significant differences in the contents of the citric acid cycle intermediates between neuronal perikarya and synaptosomes despite of a vast difference in their rates of CO_2 production and in the levels of pyruvate and lactate. As was suggested earlier, that though the levels of the citric acid cycle intermediates are the same for neuronal perikarya and synaptosomes, it is possible that the rate of turnover of the intermediates may determine the final outcome of the metabolism.

As was done earlier, the data on metabolite levels has to be supplimented with that of enzymes. For this purpose, activity levels of citric acid cycle enzymes were determined in the neurons, astrocytes and oligodendrocytes and expressed as cellular activities and specific activities (Tables 5.17

and 5.18a).

The cellular activities of pyruvate dehydrogenase and other citric acid cycle enzymes were less in the oligodendrocytes when compared to the aatrocytes and neurons. In majority of the cases the activities were approximately ten fold lesser in these cells. Cellular activities of pyruvate, isocitrate, 2-oxoglutarate and succinate dehydrogenases were equal in both astrocytes and neurons while those of citrate synthase and malate dehydrogenase (in both the directions) were higher in astrocytes than in neurons. As was observed earlier, succinate dehydrogenase activity was several fold less when compared to that of 2-oxoglutarate dehydrogenase and malate dehydrogenase (Table 5.17).

Though the specific activities of these enzymes in the oligodendrocytes were lesser than those of neurons and astrocytes, the magnitude of difference of specific activities between oligo cells and other two cell types was lesser when compared with that of cellular activities. Specific activities of all the citric acid cycle enzymes except succinate and pyruvate dehydrogenases were similar in neurons and astrocytea. The activities of both pyruvate and succinate dehydrogenases were lesser in aatrocytes than in neurons. As was seen earlier,the specific activity of succinate dehydrogenase activity was lesser by several fold than that of 2-

oxoglutarate and malate dehydrogenasea (Table 5.18a).

The results obtained in this study indicate that on mg protein basis oligodendrocytes do contribute to a significant extent to the activities of citric acid cycle enzymes in brain. However, on the whole, their contribution would be less as the number of oligodendrocytes per unit weight of cortex will be lesser than the neurons and astrocytes. Moreover, contribution by the astrocytes would be much high when compared to the neurons, as they out number the neurons by a factor of ten (Rose, 1967, 1968).

The results obtained for the activities of pyruvate dehydrogenase and the enzymes of citric acid cycle and the levels of the intermediates of this pathway were more or less similar. The activity levels of pyruvate dehydrogenase and the levels of its substrate i.e., pyruvate do not agree with each other. The cellular content of pyruvate, as was mentioned, was higher in the astrocytes than in neurons while the cellular activity of pyruvate dehydrogenase was similar in both the cells. However, it must be stated that the activity level of the enzyme and its substrate in different cells need not match with each other especially if the substrate happens to be utilized by several other metabolic pathways. Lower activity levels of pyruvate dehydrogenase in ast: cytes than in neurons might result in sparing the pyruvate for

other pathways such as CO_2 fixation and lactate formation in the former cell type. The higher cellular levels of lactate in the astrocytes than in the neurons might be due to the same reason. It must be mentioned at this point that the measured activity of pyruvate dehydrogenase represents the total activity of the complex. As discussed earlier, pyruvate dehydrogenase activity is under the regulatory influence of several modulators in in vivo states. Of these, the phosphorylation/ dephosphorylation of the enzyme is very important. In the present study, the entire pyruvate dehydrogenase complex might have been dephosphorylated during the cell isolation procedure eventhough glucose was present and the temperature was maintained at 4°C throughout the duration. However, activity levels of pyruvate dehydrogenase determined in this study (i.e., total pyruvate dehydrogenase), though does not reflect the actual utilization of pyruvate carbon, provides information on the total capacity of the system under conditions of maximal activation. However, the studies with cell cultures indicated that the rate of decarboxylation of pyruvate, through pyruvate dehydrogenase complex, is much higher in astrocytes than in the neurons (Hertz et al., 1987; Murthy and Hertz, 1989). This supports the above contention that the total activity of this enzyme may be different from that of active form.

Higher levels of citrate in astrocytes than in neurons might be due to greater activity levels of citrate synthetase in the former cell type than the latter which promotes condensation of acetyl CoA (formed from pyruvate) with oxaloacetate. This would pull the pyruvate dehydrogenase reaction forward and prevents the accumulation of acetyl CoA which is a feed back inhibitor of pyruvate dehydrogenaae (Garland and Randle, 1964; Randle, 1981; Uieland, 1983). Moreover, higher activity of citrate synthetase in the astrocytes might also be essential for the oxidation of acetyl CoA formed from substrates like acetoacetate, B-hydroxybutyrate, fatty acida and ketogenic amino acida which are utilized at higher rates (some times exclusively; in these cells (Edmond et al., 1987; Murthy and Hertz, 1987 a, b; Hertz et al., 1987).

The levels of 2-oxoglutarate and the distribution of iaocitrate dehydrogenase resembled each other in that both were higher in astrocytes than in neurons. However, the relationship in the contents of 2-oxoglutarate and the activity of 2-oxoglutarate dehydrogenase was different. The profile of distribution of this enzyme and its substrate was similar to that of pyruvate and pyruvate dehydrogenase. As the regulatory mechanisms for pyruvate dehydrogenase and 2-oxoglutarate dehydrogenaae are similar (Randle, 1981), the interpretations for the results of pyruvate dehydrogenase are equally applicable for 2-oxoglutarate dehydrogenaae. More-

over, the results on CO_2 production from [1-^{14}C] glutamate reported by several investigators in cell cultures (Yu et al., 1982; Hertz et al., 1987; Lai et al., 1989) suggests that 2-oxoglutarate utilization in the astrocytes might be higher than in neurons. It must be mentioned here that glutamate is converted to 2-oxoglutarate in the reactions mediated by either glutamate dehydrogenase or aspartate aminotransferase and the latter compound is acted upon by 2-oxoglutarate dehydrogenase liberating CO_2. Greater activity levels of malate dehydrogenase (malate to oxaloacetate) in astrocytes than in neurons, suggested that the rate of oxaloacetate formation in the former type of cell might be higher than in the latter. This is understandable as oxaloacetate requirement of these cells would be high due to high rates of citrate formation and acetyl CoA production from several sources.

Lowest activity levels of pyruvate dehydrogenase and citric acid cycle enzymes in the oligodendrocytes might be responsible for the low levels of citric acid cycle intermediates in these cells.

The profile of distribution of pyruvate dehydrogenase and the citric acid cycle enzymes were quite different in the neuronal perikarya and synaptosomes (Table 5.18b). In the neuronAl perikarya, pyruvate dehydrogenase activity was much higher when compared to that in the synaptosomes while the

reverse was true for citrate synthase. The distribution profile for isocitrate dehydrogenase resembled that of pyruvate dehydrogenase but for the magnitude of difference. Activity levels of 2-oxoglutarate and succinate dehydrogenases were similar in these two fractions while the malate dehydrogenase activity, assayed in both the directions, waa higher in synaptosomes .than in the neurons by several folds.

A scrutiny of these results revealed a close similarity in the distribution of pyruvate dehydrogenase and of citric acid cycle enzymes in neurons and synaptosomes with that of neurons and astrocytes. Hence, the discussion made earlier for neurons and astrocytes might be equally applicable to the distributory profile of neurons and synaptosomes.

Another interesting observation made in this study was the difference in the activities of citric acid cycle enzymes in neurons and synaptosomes. It is well established that the mitochondria are assembled in the neuronal perikarya and are transported along the length of the axon into the nerve terminals. H«nce, the differences in the activities of the citric acid cycle enzymes might not due to the variation in the mitochondrial content because if the mitochondrial content were to be different between the neurons and synaptosomes then it should be reflected in the activity of all the enzymes. These studies indicate that the mitochondrial compo-

sition of neuronal perikarya might be different from that of the synaptosomes. It also suggests that these differences in the enzymic composition of mitochondria could be due to the differences in the import of different mitochondrial proteins at different rates and the assembly of a distinct population of mitochondria in neuronal perikarya to be transported into

Results obtained in this study alao indicated at the major regulatory point for the citric acid cycle might be the conversion of succinate to fumarate. The results obtained with homogenates, subcellular and cellular preparations indicated that the succinate dehydrogenase activity was much less in the brain when compared to the activities of other citric acid cycle enzymes, especially of its proceeding enzyme i.e., 2-oxoglutarate dehydrogenase. Such a profile suggests that the production of succinate might be much higher than the rate of its utilization in the citric acid cycle. Literature aurvey reveals that the auccinate levels in the brain are several fold higher than those of fumarate (Goldberg et al., 1966; Folbergrova et al., 1974 a, b; Carlsson et al., 1975; Norberg and Siesjo, 1975 b, 1976), thua supporting the above suggestion. The significance of this is difficult to assess as succinate is not utilized in pathways other than citric acid cycle. The reason for the very low activities of succi-

nate dehydrogenase in the brain when compared with the other citric acid cycle enzymes ia very difficult to suggest at this time. This la because of the fact that all the citric acid cycle enzymes including succinate dehydrogenase are located in the mitochondria, hence the difference cannot be attributed to the mitochondrial content. It ia quite possible that the amount of enzyme in the mitochondria might itself be low or the enzyme may be inhibited by some ligands. For eg., it has been reported that the succinate dehydrogenase activity is inhibited by oxaloacetate in a competitive fashion under in vitro and in vivo conditions (Uoistczak et al., 1969; Zeylemaker et al., 1969; Singer, 1972; Vinograeoe et al., 1972; Ackrell et al., 1974; Gutman, 1976).

STUDIES ON MALATE-ASPARTATE SHUTTLE:

Utilization of pyruvate (thus glucose) in the citric acid cycle in brain was shown to be dependent on the transport of reducing equivalents across the mitochondrial membranes by malate-aspartate shuttle (Fitzpatrick et al., 1983; Murthy and Hertz, 1988). Studies with subcellular fractions have demonstrated the presence of the enzymes and intermediates required for the shuttle. Very few studies have been conducted on the activitiea of the malate-aspartate shuttle in the cells and synaptosomes of brain. Results of Fitzpatrick et al., (1983), Cheeseman and Clark (1988) and

Murthy and Hertz (1988), have conclusively demonstrated the flow of carbons from glucose and pyruvate into citric acid cycle through pyruvate dehydrogenase is dependent on the operation of malate-aspartate shuttle in brain slices, synaptosomes and in primary cultures of astrocytes. In the present study, the levels of the intermediates and the enzymes involved in malate-aspartate shuttle have been estimated in the isolated neurons, astrocytes, oligodendrocytea and in synaptosomes. It must be mentioned here that these components of malate-aspartate shuttle have been studied in the whole cell preparations. Though it is ideal that such studies are conducted in the cytosol and mitochondria of the isolated cells, the paucity of the material was the major restraining factor. This is especially true with the cells such as oligodendrocytes where in the yeild is very low (2, 4 and 0.5 mg protein/gin wet weight cotex for neurons, astrocytea and oligodendrocytes respectively; Table 5.8).

LEVELS OF INTERMEDIATES OF MALATE-ASPARTATE SHUTTLE:

The contents of 2-oxoglutarate and malate in neurons, astrocytes, oligodendrocytes and in synaptosomes have already been discussed (Tables 5.12 and 5.13). In general, malate levels were higher than those of 2-oxoglutarate in all the three call types. The contents of these two intermediates were low in oligodendrocytes when compared to other two cell

types. Astrocytes had higher levels of these two intermediates than neurons.

Aspartate content when expressed per cell was same in astrocytes and neurons. However, when it was expressed in terms of mg protein, it was higher in neurons than in astrocytes. Lowest levels of aspartate were observed in the oligodendrocytes irrespective of the mode of expression of the content. Between neuronal perikarya and synaptosomes, the content of this amino acid was higher in the latter than in the former (Table 5.13).

Large amounts of aspartate in the neurons and astrocytes may be due to their higher rates of glucose utilization when compared to oligodendrocytes which have low rates of glucose utilization. ns malate-aspartate shuttle is closely associated with the utilization of glucose, it is surprising that aatrocytes with higher rates of glucose utilization than neurons, have same amount of aspartate as the neurons. Though it may be argued that some of this aspartate in the neurons may belong to the neurotransmitter pool, it must be mentioned that the neuronal perikarya, in general, do not have the machinery to release neurotransmittera. Hence, there may not be a neurotransmitter pool of aspartate in them. It is quite possible that in these cells, the pool size may be larger but its turnover rate may be slow. Moreover, the operational

rates of malate-aspartate shuttle is not the sole factor in regulating glucose metabolism. Greater contents of aspartate in synaptosomes than in neuronal perikarya (Table 5.13) is understandable as the glucose utilization fcy the synaptosomes is higher than that of neuronal perikarya and part of the aapartate in the synaptosomes may belong to the neurotransmitter pool.

The moat surprising observation of the present study is that the glutamate levels were below the level of detection in the cells (and medium) after they were incubated with glucose. Thia could not be a methodological error aa the assay system detected glutamate in the extracts of nerve terminals and in other subcellular fractions. Moreover, exogenous addition of glutamate to the extracts confirmed that the assay system is functional. The precise reason for this lack of glutamate in cell extracts is not known at present. It may be due to the rapid ratea of oxidation of glutamate or its conversion to glutamine. If this is true, it is not understandable as to how malate-aspartate shuttle functions under such conditions as exchange of aspartate across mitochondrial membranes requires the presence of glutamate. However, in synaptosomes the glutamate levels were higher than that of aspartate and part of this glutamate may belong to the neurotransmitter pool.

DISTRIBUTION OF ENZYMES OF MALATE-ASPARTATE SHUTTLE:

The distribution profile for the activity levels of malate dehydrogenase has already been discussed. In summary, the cellular activity of malate dehydrogenase (in both the directions) was higher in astrocytea than in neurons and oligodendrocytes and the latter cell type had the lowest activity of this enzyme. However, the specific activity of this enzyme in neurons and astrocytes was similar but it was higher than that of oligodendrocytes (Table 5.19a).

Both the cellular and specific activities of aspartate aminotransferase was observed to be higher in the astrocytes than in neurons (Table 5.19a). However, the magnitude of difference in the cellular activities of this enzyme in astrocytes and neurons was higher than that of the specific activities. Cellular and specific activities of aspartate aminotransferase were lower in oligodendrocytes when compared to the other two cell types. The profile of distribution of enzyme was, thus, different from that of aspartate in astrocytes and neurons. In theae two cell types aspartate levels were similar eventhough aspartate aminotransferase levels were higher in astrocytea than in neurona.

A comparision between synaptosomes and neuronal perikarya revealed that the aapartate aminotransferase activity was several fold higher in the former than in the latter

(Table 5.19b). From the present study, it is not known whether the mitochondrial or cytoaolic activity of thia enzyme is higher in these two neuronal preparations. Whatever, may be the localization, such difference represents preferential transport of this enzyme into nerve endings. Thia may be required as aspartate participates both in neurotransmission and in the operation of malate-aspartate shuttle in the nerve endings. Moreover, aspartate is also required for the purine nucleotide cycle. Though the subcellular localization of all the enzymes of purine nucleotide cycle ia not known, it is well known that electrical excitation brings about the release of several of the purine nucleotides such as AMP, IMP, inosine and hyooxanthine (Pull and McIlwain, 1972a, b; 1975; Sul et al., 1976; Hollins and Stone, 1980; Jamandas and Dumbrille, 1980; Potter and White, 1980; Jonzon and Fredholm, 1985; Hoehn and Uhite, 1989). Moreover, it was postulated that this cycle may be involved in the production of ammonia in the brain (Benjamin, 1982). It is interesting to note that the nitrogens of aspartate are also incorporated into the purine nucleotidea (Yudkoff et al., 1987).

EFFECTS OF AMMONIA:

With the proposal of metabolic compartmentation of glutamate, the importance of astrocytea in ammonia detoxification was recognized. Aa the small pool of glutamate, loca-

lized in the astrocytes, was supposed to be involved in glutamine synthesis, it was proposed that astrocytes are the seats of ammonia detoxification (Benjamin and Quastel, 1972, 1974, 1975). Reports on the exclusive localization of glutamine synthetase in the astrocytes (Martinez-Hernandez et al., 1977; Norenberg and Martinez-Hernandez, 1979) provided a strong support for this hypothesis. Moreover, the swelling, hydropic degeneration of astrocytes and formation of Alzheimer Type II astrocytes were also in agreement with the hypothesis (Zamora et al., 1973; Cavanagh, 1974; Norenberg and Lapham, 1974; Norenberg, 1976, 1977, 1981).

It was proposed earlier that ammonia detoxification takes place in a two step process: initially ammonia is incorporated into glutamate (by reductive amination of 2-oxoglutarate in the reaction mediated by glutamate dehydrogena3e) and the glutamate so formed condenses with another molecule of ammonia to form glutamine (glutamine synthetase reaction). The former process not only depletes 2-oxoglutarate from citric acid cycle but also converts NADH to NAD without the production of ATP. Moreover, glutamine synthesis requires ATP. Synergestic action of these two processes would depletes ATP and adversely affects the astrocytic metabolism and induces pathological changes.

Later investigations revealed no significant changes in

ATP and 2-oxoglutarate in the brain (Hindfelt and Siesjo, 1971). However, it was argued that the change may take place in a particular metabolic compartment and this might have been lost by dilution with the contents of these components from other compartments during the process of homogenisation. Despite such argument, several controversial results haze appeared in the literature which does not support the above contention. Use of ^{13}N and ^{15}N labelled ammonia revealed that the amount of ^{15}N incorporated into alpha amino groups of glutamate and glutamine was lesser than that into the amido group of glutamine (Yudkoff et al., 1983; Cooper et al., 1979; 1985). Moreover, a fall in the glutamate dehydrogenase activity was noticed in the astrocytes in hyperammonemic states (Subbalakshmi and Murthy, 1983; Murthy et al., 1987). These results suggested that the glutamate produced in glutamate dehydrogenase reaction may not serve as the precursor for glutamine biosynthesis. It waa demonstrated that glutamate produced by transamination of other amino acids (eft. branched-chain amino acids) might be precursor for glutamine biosynthesis (Yudkoff, 1983 a; Jessy, 1989; Jessy et al., 1989). Whatever may be pathway of production of glutamate (required for glutamine biosynthesis), it appears that 2-oxoglutarate is lost for the detoxification of ammonia. The only advantage in the transamination pathway seems to be the •paring of NADH (used in glutamate dehydrogenase reaction)

for electron transport chain. However, the loss of cytosolic pool of glutamate for glutamine biosynthesis in hyperammonemic states would result in the disruption of malate-aspartate shuttle and might affect the oxidation of pyruvate (thus glucose). All these effects are supposed to be restricted to astrocytes (Murthy and Hertz, 1988).

Though it was observed that the elevated levels of ammonium ions in hyperammonemic states might affect the membrane potentials by disturbing the ionic gradients (Lux, 1971; Hawkins et al., 1973; Llinas et al., 1974 a, b; Raabe and Gumnit, 1975; Sadasivudu et al., 1977, 1979; Benjamin et al., 1978; Subbalakshmi and Murthy, 1981; Raabe and Onstad, 1982; Alger and Nicoll, 1983; Raabe and Lin, 1983, 1984, 1985; Dabrowiecki and Albrecht, 1985; Raabe, 1986), earlier investigators have not paid much attention to the metabolic changes in neurona under these conditions. Only recently some observations have been made on the metabolic changes in neurons in the presence of pathophysiological concentrations of ammonium ions (Hertz et al., 1987; Murthy et al., 1987a, b; Murthy and Hertz, 1988; Lai et al., 1989). Like wise there are very few studies on nerve endings (synaptosomes) and on oligodendrocytes. The only report on the metabolic changes in oligodendrocytes in hyperammonemic states is that of Jessy (1989) and Jessy and Murthy, (1989,

1990 a, b) on the metabolism of branched chain amino acids.

In the present study, efforts are made to study the alterations of glucose metabolism in the three cell types (neurons, astrocytes and oligodendrocytes) and in synaptosomes isolated from the cerebral cortex of rats rendered hyperammonemic by the administration of ammonium acetate. In few cases, the effect of in vitro fortification of pH neutralized ammonium acetate was studied on the cells isolated from normoammonemic animals. As was done earlier, the results obtained in cell preparations were expressed per cell and per mg protein.

PRODUCTION OF $^{14}CO_2$ FROM [U - ^{14}C] GLUCOSE:

Neurons, astrocytea and oligodendrocytes isolated from the cerebral cortex of hyperammonemic rats were incubated with [U-^{14}C] glucose and the production of $^{14}CO_2$ was measured (Tables 5.9 and 5.10). It was observed that in the cells isolated from both subacute and acute group of rats, the rate of $^{14}CO_2$ production was enhanced significantly. This elevation was observed irrespective of the mode of expression of the rate, i.e., per cell or per mg protein. The only exception was the lack of statistical significance in the elevation in the rate of $^{14}CO_2$ production in the oligodendrocytes in subacute states when it was expressed per mg protein. The magnitude of elevation in all the three cell types

was higher in acute state than in subacute state. Among the cell types, the magnitude of elevation was higher in the ollgodendrocytes than that in astrocytes and neurons. A similar elevation in the production of $^{14}CO_2$ from glucose was observed in the synaptosomes and its magnitude was higher than that of neuronal perikarya (Table 5.11).

Studies conducted earlier revealed that the cerebral glucose metabolism is reduced in hyperammonemic states induced by portocaval anastomosis (Mans et al., 1983). Hawkins's et al., (1973) reported that the cerebral oxygen consumption was marginally elevated in rats in acute hyperammonemic states induced by the administration of ammonium chloride. However, there are very few studies carried out on the cells or synaptosomes in the presence of pathophysiological concentrations of ammonium ions. Hertz et al., (1987) reported no changes in $^{14}CO_2$ production from [U-^{14}C] glucose in primary cultures of astrocytes and of neurons when ammonia levels were elevated in the incubation medium. They have also observed no change in the production of CO_2 with pyruvate as substrate in these cells in the presence of ammonium ions. However, with this substrate, the effects of ammonium ion were shown to be dependent on the presence of glutamine/ glutamate/ aspertate in the medium (Murthy and Hertz, 1988). In absence of these amino acids, CO_2 production from pyruvate

was suppressed in the presence of pathophysiological concentration of ammonium ions. While summerizing the results, these authors concluded that elevation of ammonia concentrations seems to have no effect on the CO_2 production from glucose or pyruvate (Hertz et al., 1987). However, results of the present study, where in CO, production was elevated in the cells isolated from hyperammonemic states, were not in agreement with their results.

This elevation in glucose utilization may not be due to increased glucose availability (when compared to the cells isolated from normal animals) as the cells isolated from normal and experimental animals were incubated with same amount of glucose (5 mM). The argument that the plasma membrane of cells from normal and hyperammonemic animals might have undergone different changes during trypsinization may not be valid aa the increased rate of CO_2 production was also observed in synaptosomes (which are prepared without trypsinization) isolated from hyperammonemic animals. Moreover, the lack of effect of ammonium ions (both 1 and 5mM) under in vitro conditions (Tables 5.9 and 5.10), suggested that changes might have occured under in vivo conditions and were retained throughout the isolation procedure. Hence, it appears that there may be differences in the effects of ammonia on the metabolism of brain cells in in vivo conditions and in cultures.

The increase in glucose oxidation by cells and synaptosomes isolated from hyperammonemic animals may be a response to the increased energy demands under these conditions. It is known that hyperammonemic states stimulate Na^+, K^+ ATPase (Sadasivudu et al., 1977, 1979; Subbalakshmi and Murthy, 1983; Subbalakshmi, 1984) and influx of Na^+ into the brain cells (Benjamin et al., 1978). The latter process not only upsets the ionic gradients but also stimulates the Na^+, K^+ - ATPase. It has been established that about 1/3 of the total energy in brain is spent on the maintenance of ionic gradients and sodium pump activity even under normoammonemic states (Berl, 1971). Stimulation of the activity of this enzyme would result in increased utilization of ATP and production of ADP. The latter compound might act as a stimulant for cerebral glucose metabolism. Further, increased production of glutamine also enhances the formation of ADP. Production of ADP might also be stimulated due to an increase in CO_2 fixation in brain in hyperammonemic states (Berl, 1971). The stimulation of glucose metabolism can occur at different stages. Under such conditions, binding of hexokinase to the mitochondria might be promoted. This would increase the rate of phosphorylation of glucose and glucose uptake. It would also enhance the activity of phosphofructokinase and the conversion of inactive forms of pyruvate and

2-oxoglutarate dehydrogenases into more active forms. Moreover, changes in ADF levels would also bring about the conformational changes in mitochondria (orthodox atate to condensed state) and enhance the activity of electron transport chain (DeRobertis and DeRobertis, 1975).

The reason why such changes occur only under in vivo states but not in in vitro conditions is not known at present. It is possible that the breakdown of blood brain barrier in hyperammonemic states in in vivo conditions might allow other components of plasma which may bring about such changes. Some of these blood components could be hormones as hyperammonemic states are known to alter the ratio of insulin to glucagon in plasma.

If this change in glucose oxidation waa to be true, then corresponding changes should also occur in the levels of various intermediates and in the activities of enzymes of glucose metabolism and in the levels of ADP and ATP. Keeping this in view, the levels of intermediates and the activities of the enzymes were determined along with the levels of ATP and ADP.

Following the incubation with glucose, the cellular contents of glucose-6-phosphate were elevated in neurons, astrocytes and ollgodendrocyte3 and in synaptosomes isolated from hyperammonemic animals. However, the elevation in

glucose-6-phosphate content in the cells isolated from subacute ammonia was not statistically significant. The magnitude of elevation in the content of this metabolite was higher in oligodendrocytes than in neurons and astrocytes. Among the latter two cells, it was higher in neurons than in astrocytes. The percent increase in glucose-6-phosphate content in neurons and synaptosomes, isolated from acute hyperammonemic rats and incubated with glucose, was more or less similar. Incubation of cells isolated from normal animals with glucose in the presence of 1 and 5 mM ammonium acetate brought about changes which are similar to subacute and acute states respectively (Tables 5.20 to 5.22).

The content of fructose-6-phosphate was also elevated in the cell preparations and in synaptosomes of hyperammonemic rats after they were incubated with glucose (Tables 5.23 to 5.25). The only exception to this was seen in oligodendrocytes wherein the increase in fructose-6-phosphate when expressed per mg protein was not statistically significant. A similar change was seen in the content of phosphoenolpyruvate in the three cell types and in synaptosomes of the cerebral cortex of hyperammonemic rate (Tables 5.26 to 5.28). However, the increase in phosphoenolpyruvate content was not statistically significant in the neurone isolated from the cerebral cortex of rats administered with subacute dose of ammonium

acetate. This was also seen in oligodendrocytes when the phosphoenolpyruvate content was expressed per mg protein. Changes in pyruvate content in the cells ar synaptosomes closely followed that of phosphoenolpyruvate, though these changes in subacute states were not statistically significant in the cells and were marginal in synaptosomes. However, in the cell preparations from acute states, pyruvate content was enhanced to a great extent and all these changes were statistically significant (Tables 5.29 to 5.31). Irrespective of the mode of expression of the content and state of hyperammonemia, there were no statistically significant changes in the lactate content in the isolated neurons, astrocytes, oligodendrocytes and in synaptosomes after they were incubated with glucose (Tables 5.32 to 5.34). This is in contrast to the changes observed in the cytosol and mitochondria where in the lactate content decreased under similar conditions (Table 4.15).

The increase in glucose-6-phosphate levels in the cerebral preparations is similar to that seen in the cytosol and mitochondria and this could be due to either increased glucose supply or due to an elevation in the activity of hexokinase. The former effect could be due to changes in the rate of transport of glucose by the glucose carrier. However, as the cells are already highly permeable to glucose (indicated by high rates of $^{14}CO_2$ production from glucose when

compared with the rates in cell cultures, Yu et al., 198E.; Hertz et al., 1988; Roeder et al., 1988; Lai et al., 1989) Moreover, an elevation o£ glucose-6-phosphate content in the cytosol (in which the question of permeability does not arise) prepared from hyperammonemic rat cerebral cortex, supports the above reasoning. The increase in glucose-6-phosphate due to changes in the activity of hexokinase could be due to the enhanced binding of hexokinase to mitochondria or increased utilization of glucose-6-phosphate by which feed back inhibition on the enzyme would be relieved. Uhatever, may be the mechanism, increased glucose phosphorylation promotes the utilization of glucose in these preparations. As the uptake of glucose is supposed to be dependent on the rate of its phosphorylation, increased glucose-6-phosphate formation might even promote glucose transport. The increase in glucose-6-phosphate due to reduced rate of its utilization, an alternate possibility could be ruled out as the levels of other glycolytic intermediates and CO_2 production are also enhanced under these conditions.

Since glucose-6-phosphate and fructose-6-phosphate are equilibrated through hexose phosphate isomerase reaction, this also suggested increased glucose-6-phosphate levels and its channelling into glycolysis.

Elevation in the levels of phosphoenolpyruvate and

pyruvate also support the above idea of increased rate of glycolysis in the cells and synaptosomes in hyperammonemic states. Another possibility for an increase in the levels of some of the glycolytic intermediates could be due to gluconeogenesis which might ultimately lead to glycogen synthesis. Though there are reports that chronic hyperammonemic states increase glycogen deposition, a fall in the cerebral glycogen content was demonstrated in acute hyperammonemic states (Hawkins et al., 1973). Moreover, gluconeogenesis either does not occur or occurs at a negligible rate in brain (Scrutton and Utter, 1968; Ide et al., 1969; Phillips and Coxon, 1975; Majumdar and Eisenberg, 1977; Swanson et al., 1990). Hence, such a possibility may be ruled out. The increase in phosphoenolpyruvate due to the decarboxylation of oxaloacetate by phosphoenolpyruvate carboxykinase may be ignored as the reversal of this reaction (i.e., CO_2 fixation) was shown to be promoted in brain in hyperammonemic states. All these results suggested an increase in glycolysis in brain in hyperammonemic states.

Unaltered levels of lactate in the cells and aynaptosomes, isolated front hyperammonemic rats and incubated with glucose, was surprising. Reports available so far indicate that the lactate content is enhanced in brain in various animal models of hyperammonemia (Hawkins et al., 1973; Adams et al., 1979; Raabe and Lin, 1984; Jessy et al., 1990). It

was proposed that due to the interference with the operation of malate-aspartate shuttle in brain in hyperammonemic states, an increased conversion of pyruvate to lactate is supposed to occur in order to regenerate the cytosolic NAD from NADH. Infact, an increase in lactate/pyruvate ratio that is observed in hyperammonenic states is taken as an index for an increase in cytosolic NADH/NAD. However, the present observation fails to support this view. The precise reason for such a discrepancy is not known. It must be mentioned that in all the earlier studies where an elevation was observed in lactate content, usually extracts of whole brain were used. Hence, the possibility of increase in lactate content in regions other than cerebral cortex can not be ruled out. If this were to be true, then the increase in lactate content, in whatever region(s) occurs, must be much greater in order to nullify the lack of change observed in the cerebral cortex. In contrast to the earlier studies, the cells isolated from the brains of hyperammonemic animals were used in the present study. Uith an assumption that the changes occured under in situ conditions survive the isolation procedure. Moreover, this discrepancy of lactate content can not be ascribed to the trypsin treatment as a similar change ia noticed in synaptosomes which are not subjected to trypsinization. It is interesting to notice that Benjamin et al., (1978) have reported an increase in lactate content when

brain slices were incubated with ammonium chloride and they observed that the magnitude of increase is proportional to the ammonium chloride concentration in the medium. However, in these studies they have used slices from the cerebral cortex of normoammonemic animals. It is possible that the final out come of the brain metabolism when all the three cell types are present together could be different when a cell type is present alone. Though the slices are considered to be near physiological systems resembling the in vivo conditions, estimated levels of a component (metabolite, enzyme, uptake etc.,) represents the algebric sum of the same in neuronal perikarya, axons, dendrites, nerve terminals, astrocytes, myelin and oligodendrocytes and thus it can not be ascribed to any subcellular or cellular component.

The increase in pyruvate but not in lactate observed in the present study would be physiologicially advantageous as more pyruvate would be made available to the citric acid cycle and the deleterious effects of lactate accumulation would be avoided. However, increase in pyruvate formation through glycolysis with out any change in lactate formation would necessiate an alternate route to regenerate cytoaolic NAD^+ from NADH. This may be through the operation of an alternate shuttle such as -glycerophosphate shuttle. It la also possible that the mitochondrial fragments (formed due to the rupture of population of mitochondria which are vulne-

rable to elevation in ammonia levels, earlier might also participate in the regeneration of NAD^+.

These studies on metabolites of glycolytic pathway indicated that the glycolysis might be enhanced and there might be an increased conversion of glucose to pyruvate in the three cell types and in nerve endings in brain in hyperammonemic states. In addition to the studies on the metabolities, studies were also conducted on the activities of enzymes of glycolytic pathway in the three cell types and in synaptosomes isolated from the cerebral cortex of hyperammonemic rats. Such studies would help in understanding the changes in metabolite levels under these conditions. As was described earlier, activities of these enzymes were expressed either as cellular activity or as specific activity.

The cellular activity of hexokinase was elevated in the neurons and oligodendrocytes in the subacute state of ammonia toxicity while in acute states this response was seen in all the three cell types (Table 5.35). However, when the specific activity was taken into consideration no statistically significant changes were observed in the hexokinase activity in neurons and astrocytes while it was elevated in oligodendrocytes in both the conditions (Table 5.35). Irrespective of the mode of expression, the activity of phosphofructokinase was enhanced in all the cell types (Table 5.36). The response

of aldolaae was also similar to that of phosphofructokinase except that the increase in the specific activity of this enzyme in neurons in subacute state was of lesser magnitude than the cellular activity and was statistically not significant (Table 5.37). In the subacute states of ammonia toxicity, glyceraldehyde-3-phosphate dehydrogenase activity was not significantly different from the controls in the neuronal perikarya and astrocytes while the elevation in the activity was statistically significant in the oligodendrocytes. However, in the acute states, both the cellular and specific activities of this enzyme were enhanced in all the three cell types (Table 5.38).

The cellular and specific activities of enolase were unaltered in the neurons and in oligodendrocytes in subacute hyperammonemic while they were elevated in astrocytes under these conditions. However, in acute hyperammonemic states enolase activity was enhanced in all the three cell types and this was seen irrespective of the mode of expression (Table 5.39). The changes in the cellular and specific activities of pyruvate kinase in all the three cell types in subacute states of ammonia toxicity were not statistically significant. In acute hyperammonemic states, cellular activity of this enzyme was enhanced only in the oligodendrocytes while the changes in the neuronal and astroglial activities for

this enzyme were once again statistically not significant. However, the elevation in the specific activity of this enzyme was statistically significant in all the three cell types in acute hyperammonemic states (Table 5.40).

The cellular activity of lactate dehydrogenase (in the direction of lactate formation) was marginally enhanced only in the oligodendrocytes in both subacute and acute hyperammonemic states. The changes observed in the specific activity of this enzyme were different from the cellular activities only in the oligodendrocytes wherein the change in the specific activity was statistically not significant. The cellular and specific activities of this enzyme in astrocytes were unaltered in subacute hyperammonemic states while it was suppressed in acute hyperammonemic states (Table 5.41). Lactate dehydrogenase activity, when assayed in the direction of pyruvate formation and expressed per cell, did not show any significant change in all the three cell types in subacute hyperammonemic states. While in the acute states, a marginal elevation in the activity of this enzyme, observed in the astrocytes and oligodendrocytes, was statistically significant. Changes in its specific activity were more or less similar to that of cellular activity except in the astrocytes and neurons isolated from the acute group of hyperammonemic rats. In these two cell types the magnitude of elevation was much leaser and statistically not significant.

The activity of lactate dehydrogenase (in the direction of pyruvate formation) did not show any statistically significant change in oligodendrocytes in both acute and subacute hyperammonemic states (Table 5.42).

Though the response of the enzymes were more or less same in all the three cell types, there were subtle differences among the cell types which indicated the heterogenity of the metabolism and of the response of these cells to a given metabolic insult. The lack of change in hexokinase activity in the astrocytes in subacute hyperammonemic states could be due to the already prevailing higher activity of this enzyme in these cells when compared to the other cells. As a result of this, the astrocytic enzyme could compensate the marginal increase of glucose metabolism with out exhibiting a significant change in its activity. Increased activity of this enzyme observed in the other cell types and also in the astrocytes in acute state would result in an enhanced production of glucose-6-phosphate in all these cells. In fact, such an increase was observed in glucose-6-phosphate content not only in the isolated cells (present study), and also in the whole brain extracts. The glucose-6-phosphate, so produced, can be utilized for synthesis of glycogen or in hexose monophosphate ahunt and/or glycolytic pathway. The former pathway i.e., glycogen synthesis is present in

astrocytes (Scrutton and Utter, 1968; Ide et al., 1969; Phillips and Coxon, 1975; Majundar and Eisenberg, 1977; Swanson et al., 1990). It was also reported earlier that the glycogen content of the brain would decrease in the acute states (Hawkins et al., 1973) while in chronic states of hyperammonemia the glycogen content is enhanced (Cavanagh and Kyu, 1971; Zamora et al., 1973; Cavanagh, 1974; Norenberg and Lapham, 1974; Norenberg, 1977, 1981). As the turnover rate of glycogen la very high in the brain (Lowry et al., 1964; Watanabe and Passonneau, 1973; Karnovsky et al., 1980), it is still quite possible that some amount of glucose-6-phosphate may still be involved in glycogen synthesis in hyperammonemic states. The details about the operational rates of hexose monophosphate shunt in the cells isolated either from normal or hyperammonemic states are not available in the literature. It may be mentioned that the increased production of glucose-6-phosphate in these cells would promote the influx of glucose into the cells.

The increase in the phosphofructokinase activity in all the three cell types could be due to the direct effect of ammonia on the enzyme and this would allow the rapid conversion of fructose-6-phosphate to fructose-1,6-diphosphate. The enhanced aldolase activity would help in channelling more of fructose-1,6-diphosphate into the glycolytic pathway by forming triose phosphates. The enhancement in the activity of

glyceraldehyde-3-phosphate dehydrogenase, atleaat in acute hyperammonemic states, would channel these triose phosphates towards the later parts of glycolytic pathway. Enhancement in the enolase activity in these cells in hyperammonemic states would promote the production of phosphoenolpyruvate. The pattern of changes, observed in the enolase activity and phosphoenolpyruvate content (after incubation with glucose), were observed to be parallel in all the cell types.

Though the activity of pyruvate kinase, was enhanced in homogenates and subcellular fractions in hyperammonemic states, there was no change in the activity of this enzyme in subacute states in all the three cell types, while in acute state the elevation in the activity of this enzyme was seen only in the oligodendrocytes. However, it must be mentioned that the contribution of oligodendrocytes to the metabolism of cerebral cortex would be leaser than that of astrocytes or neurons. This is because of the fact that both the number of oligodendrocytes and the pyruvate kinase activity in theae cells were lesser than that of the other two call types (Tables 5.6 and 5.40). However, activity of pyruvate kinase, which is about 7-8 folds higher than the earlier enzymes of glycolytic pathway, might facilitate greater utilization of phosphoenolpyruvate (even if not stimulated) in the neurons and astrocytes in hyperammonemic states. Some of the phos-phoenolpyruvate, atleast in the astrocytes, would be used for

the production of oxaloacetate by way of CO, fixation. Under these conditions, reported elevation of this process in astrocytes (Berl, 1971) would support such a possibility.

The lack of changes in lactate dehydrogenase activity (pyruvate to lactate) in the cells isolated from the brains of subacute hyperammonemic animals was similar to the content of lactate. A similar profile of this enzyme and the product of its reaction were observed in neurons of acute hyperammonemic animals when they (cells) were incubated with glucose. However, under these conditions the astrocytic lactate content waa unaltered while lactate dehydrogenase activity was suppressed. In the oligodendrocytes, under these conditions, there waa a marginal but statistically significant elevation in lactate dehydrogenase activity but lactate content was unaltered. The precise reason for the discrepancy is not clear at this time.

As differences in the metabolism of the neuronal perikarya and the synaptosomes were shown earlier, responses of enzymes of glycolytic pathway in hyperammonemic states were also studied in these two neuronal preparations. While comparing the activities of the enzymes in these two preparations, only specific activities were taken into account.

In general, the changes in the activities of glycolytic enzymes in the synaptosomes and in the neuronal perikarya

were more or less similar with few exceptions. The specific activity of hexokinaae, increased in the neuronal perikarya as well as in the synaptosomes both in subacute and acute conditions. However, the changes in the hexokinase activity in neuronal perikarya were statistically not significant in both the hyperammonemic states while the change in the activity of synaptosomal hexokinase was significant only in the acute condition (Table 5.35).

The changes observed in the phosphofructokinaae activity were similar in both these preparations in acute and subacute hyperammoneniic states except that the magnitude of change was much high in synaptosomal fraction when compared to the neuronal perikarya (Table 5.36). The changes in the aldolase activity were also similar under these experimental conditions in both the preparations but for the lack of statistical significance in the elevation of the enzyme activity in neuronal perikarya in subacute states (Table 5.35). The changes in the glyceraldehyde-3-phosphate dehydrogenase activity were more or leaa same in both these preparations while those of enolaae and pyruvate kinaae were similar to that of aldolase (Tables 5.38 to 5.40). Lactate dehydrogenase activity waa suppressed to a greater extent in the synaptosomal fraction in subacute states of ammonia toxicity. In the acute states, it waa more in the neuronal perikarya, though the change was not statistically significant (Table

5.41). Activity of this enzyme when assayed in the reverse direction was enhanced only in the synaptosomal fraction in both subacute and acute states (Table 5.42).

The possible changes in the glucose metabolism due to the administration of the pathophysiological concentrations of ammonium acetate were same both in neurons and synaptosomes and these changes have already been discussed. However, any small change that is observed in synaptosomes would amplify when the total contribution of these two preparations to the cerebral glucose metabolism is taken into consideration because of the fact that the number of synaptosomes are greater than that of neuronal perikarya.

All these studies indicated that the flow of glucose carbon through the glycolytic pathway might be enhanced in all the three major cell types of brain by the presence of pathophysiological concentrations of ammonium ions, with few exceptions. This suggestion is supported by the changes observed in the intermediates of glycolytic pathway in the whole brain in acute hyperamnonemic states. However, this concept would contradict the hypothesis that the operational rates of the glycolytic pathway might be affected in the hyperammonemic states due to alterations in the transport of reducing equivalents across the mitochondrial membranes. This would also contradict the reduced glucose utilization

observed in the brain in chronic hyperammonemic states. It is quite possible that the changes induced by ammonium ions in the acute conditions could be totally different from those seen in chronic conditions. Further, operation of some compensatory mechanisms to regenerate the NAD* in the brain in hyperammonemic states might facilitate the utilization of glucose under these conditions.

The increase in CO_2 production from glucose and in the contents of glycolytic intermediates suggested that the further utilization of pyruvate might also be enhanced in hyperammonemic states. In the past, several investigators have determined the levels of citric acid cycle intermediates in the brain in various animal models of hyperammonemic states and several controversies have surfaced in these studies. Though the citric acid cycle commenses with the condensation of acetyl CoA with oxaloacetate to form citrate, there are no reports on the acetyl CoA content under these conditions. This might be due to very low levels of acetyl CoA or the labile nature of this compound. Hawkins et al., (1973) and Jessy et al., (1990) have determined citrate content in the freeze-blown brains of rats administered with ammonium acetate and urease respectively. They reported no significant changes in the levels of this metabolite in both the animal models of hyperammonemic states. There are no reports on the cerebral contents of isocitrate in hyperammo-

nemic states while the reports on the levels of 2-oxogluta-rate content in brain under these conditions are numerous. These reports may be categorized into three groups based on the direction of changes, viz.,those in which (a) an elevation (b) a decrease and (c) no change have been observed. Vergara et al., (1973) reported that the 2-oxoglutarate content in rat brain increased from +33* to +133% depending on the cerebral ammonia levels. O'Connor et al., (1984) observed a marginal elevation in 2-oxoglutarate content in the brains of mice injected with 12mM ammonium acetate. However, Shorey et al., (1967) observed a consistent fall in the cerebral 2-oxoglutarate content with time after injecting ammonium acetate to rats. Similarly, Raabe and Lin (1984) also observed a decrease in the 2-oxoglutarate content in the spinal preparations of rats injected with ammonium acetate. A fall in the cerebral 2-oxoglutarate content was also reported by Jessy et al., (1990) in the brains of rata rendered hyperammonemic by the administration of urease. However, Mans et al., (1984) reported no changes in cerebral 2-oxoglutarate content in the rats even after 7 weeks of portocaval shunt. Discrepancies in these results might to be due to variations in the species, mode and duration of hyperammoneniia.

A similar conflict is also seen in the results on the malate content in brain in hyperammonemic states. Hawkins et

al., (1973) and Hindfelt and Siesjo (1970) have reported an increase while Mans et al., (1984) reported a decrease in the cerebral malate content in the brains of Long Evans rats after portocaval shunting. However, these investigators reported an elevation of malate content in the brains of Sprague Dawly rats under similar conditions (Mans et al., 1984). Oxaloacetate being an unstable metabolite was not investigated thoroughly. However, Hawkins et al., (1973) calculated the levls of oxaloacetate from the concentrations of pyruvate, malate and lactate and the equilibrium constants of malate dehydrogenase and lactate dehydrogenase. They reported a 22% decrease in cerebral oxaloacetate content in rats administered with an acute dose of ammonium acetate. However, no studies were carrried out to localize the changes in the metabolites in aubcellular or cellular compartments or to study the abilities of the cerebral preparations of hyperammonemic rats to metabolize glucose. Such an attempt has been made in the present study by incubating the cerebral preparations of normal and also of hyperammonemic rats with glucose and estimating the levels of the citric acid cycle intermediates.

Irrespective of the mode of expression, the levels of citrate showed no significant changes in the neurons of hyperammonemic rats (both subacute and acute) when they (cells) were incubated with glucose. In the astrocytes and

oligodendrocytes, under these conditions, there were no significant changes in the cellular citrate content of subacute group of animals while the same was elevated in the cells isolated from acute group of rats. The magnitude of change in the oligodendrocytes of acute group of rats was lower than that of astrocytes and was statistically significant only when the content was expressed per cell (Tables 5.43 and 5.44). Isocitrate content was unchanged in the three cell types of brains of subacute hyperammonemic rats when they were incubated with glucose while there was an elevation in the content of this metabolite in the preparations from acute group of animals (Tables 5.46 and 5.47). Levels of 2-oxoglutarate were elevated in the neurons and oligodendrocytes of the brains of hyperammonemic (both subacute and acute) rats. However, in aatrocytea, the increase in 2-oxoglutarate waa significant only in the acute group (Tables 5.49 and 5.50). There were no statistically significant changes in the malate content in the neurons and astrocytea of subacute group of rats when they were incubated with glucose. However, in the oligodendrocytes, under the same conditions (Tables 5.52 and 5.53), there was a statistically significant increase in malate content. When incubated with glucose, the levels of this intermediate in all the three cell types of acute hyperammonemic rats were enhanced and the magnitude of elevation was more in oligodendrocytes when compared to neu-

rons.

There were no statistically significant changes in the contents of citrate and isocitrate in synaptosomes of hyperammonemic rats when they (synaptosomes) were incubated with glucose (Tables 5.45 and 5.48). However, 2-oxoglutarate content was increased in the synaptosomes under these conditions and the magnitude of increase was higher in acute than in subacute group (Table 5.51). A similar profile of changes was seen in the synaptosomal malate content except that the increase observed in the synaptosomes of subacute group of animals waa statistically not significant (Table 5.55). Thus, the changes observed in the contents of metabolites in synaptosomes and neuronal perikarya were similar except for isocitrate.

The lack of changes in the citrate content in neurons, and synaptosomes in hyperammonemic states is similar to that observed in the whole brain extracts (Hawkins et al., 1973). Though there was an elevation in the citrate content in astrocytes and oligodendrocytes of acute group of animals, the overwhelming population of nerve terminals (synaptosomes) might mask the changes in these two cells particularly astrocytes under in vivo conditions. The increase in citrate content in astrocytes could be due to ita increased rate of synthesis or decrease in the rate of its utilization. There are only two reactions that utilize citrate and they are cis-

aconitase and citrate lyase. The enzyme citrate lyase is supposed to be localized in the cytoplasm and involved in the supply of acetyl CoA for lipid synthesis (Srere, 1959; Tucek, 1967a; Smith et al, 1970; Szutowicz et al., 1974, 1976a,b, ; Szutowicz and Lyaiak, 1980). Though astrocytes synthesize lipids of their own plasma membrane, it is generally believed that this process occurs at much a slower pace when compared to citrate utilization in citric acid cycle (Lopes-Cardozo et al., 1986). Moreover, the brief duration of exposure of cells to in situ hyperammonemic state might not have brought a remarkable change in the composition of membrane lipids (which are turned over at relatively lesser rates) to warrant their synthesis. Though citrate is also used in the exchange transport of malate by the tricarboxylate carrier, this carrier is supposed to transport citrate into the mitochondria and its presence in the brain mitochondrial preparations is not well established. The other reaction which utilizes citrate is cis-aconitase. If this reaction is slowed down in ammonia exposed cells, then the isocitrate levels should have been low, which is not the case in present studies. The conversion of isocitrate to 2-oxoglutarate by NAD^+ dependent mitochondrial isocitrate dehydrogenase is a highly exergonic process and is supposed to be irreversible. Hence, this reaction pulls the aconitase reaction forward by rapidly converting isocitrate to 2-oxogluta-

rate.

The increase observed in 2-oxoglutarate levels, could also be due to increased synthesis or decreased degradation. However, the major difference in tnis metabolite and citrate is that 2-oxoglutarate is produced and utilized in many more reactions than citrate. The major non-citric acid cycle source for 2-oxoglutarate production is glutamate and the reactions involved are either transamination or oxidative deamination. Though there are several aminotransferases in the brain, the activity of aapartate aminotransferase la greater than the others (Benuck et al., 1971). Activity of this enzyme is suppressed in the brain in hyperammonemic states. Suppression of this reversible reaction, might restrain the formation and utilization of 2-oxoglutarate. Alanine aminotransferase, the activity levels of which are next to aapartate aminotransferase, was also suppressed in brain in hyperammonemic states (Ratnakumari et al., 1985, 1986; Ratnakumari and Murthy, 1989, 1990). Further, glutamate dehydrogenase, which mediates the reversible interconversion of glutamate and 2-oxoglutarate, is also suppressed (Subbalakshmi, 1984). Hence, it appears that the formation or utilization of 2-oxoglutarate from glutamate is suppressed In hyperammonemic states. Though 2-oxoglutarate is transported out of the mitochondria by the dicarboxylate carrier

(Chappell and Haarhoff, 1967; Chappell, 1968; Klingenberg, 1970; Brand and Chappell, 1974 a, b), it requires malate for exchange and the 2-oxoglutarate is once again regenerated (see malate-aspartate shuttle). All these point out to the possibility of increased 2-oxoglutarate formation in the citric acid cycle in the three cell types and nerve terminals in brain in hyperammonemic states.

Malate is the penultimate intermediate of citric acid cycle and is formed from fumarate by the action of fumarase. It is also formed from oxaloacetate by the enzyme malate dehydrogenase. The latter enzyme is also responsible for its conversion to oxaloacetate and the continuation of citric acid cycle. Though malate dehydrogenase activity is reversible, in the mitochondria it usually proceeds in the direction of oxaloacetate formation due to the high ratios of $NAD^+/NADH$ (Siesjo, 1978). Accumulation of malate in all the three cell types and in synaptosomes, could be due to increased fumarase or malate dehydrogenase (oxaloacetate to malate) or decreased conversion of malate to oxaloacetate. The latter may be due to unfavourable $NAD^+/NADH$ ratio or due to the suppression of malate dehydrogenase in the direction of oxaloacetate formation in hyperammonemic states. Moreover, it has been demonstrated that the malate generated in the cytosol (by malate-aspartate shuttle) is the primary source for the malate in the mitochondria (Brand and Chappell, 1974b; Beck et al.,

1977; Cheeseman and Clark, 1988) and any interference to either the production or transport of malate from the cytoaol to mitochondria would affect the operation of citric acid cycle by inhibiting the pyruvate dehydrogenaae activity and the production of citrate (Cheeseman and Clark, 1988; Murthy and Hertz, 1989). If so, the production of malate from fumarate appears to be of less importance. This is understandable as the conversion of succinate to fumarate (by succinate dehydrogenase) would be less due to very low activity of the enzyme. As was mentioned earlier, fumarate levels are very low in the brain when compared to succinate or malate (Goldberg et al., 1966; Folbergrova et al., 1974a, b; Carlsson et al., 1975; Norberg and Siesjo, 1975b, 1976). Hence, while considering the accumulation of malate, this should be taken into account. Aa malate ia produced in the cytosol also (cytosolic malate dehydrogenase, oxaloacetate to malate), it is possible that some of this malate might belong to cytoaolic pool. Uith the information available, it ia not possible to identify the subcellular localization of the pool of malate in which change has occured. In such a case, it will also be difficult to explain the precise or even the possible mechanism for this increase in malate content.

Another possibility for the elevation in citric acid cycle in cells and synaptosomes intermediates could be the

fall in their utilization in the citric acid cycle during hyperammonemic states. If this were to be so, some of the changes observed in the metabolites in the present study can not be explained. For example the changes in citrate (unaltered) and isocitrate levels (elevated). These two intermediates are equilibrated by the aconitase action. Under equilibrium conditions, the ratio of citrate to isocitrate is 93:7. Even if the aconitase activity is suppressed, the equilibrium concentrations should not be affected to such an extent where the profile is entirely reversed (either [citrate] = [isocitrate] or [iaocitrate] > [citrate]). Moreover, the segment of citric acid cycle between succinate and oxaloacetate ia reversible and any acumulation of these metabolites should have its influence on this segment of the citric acid cycle. Further, increased CO_2 production from glucose also rules out such a possibility unless the hexose monophosphate shunt is activated to a level as to compete with glycolysis. If such a change occurs, it would be unfavourable as the operation of hexose monophosphate shunt results in the production of NADPH which brings down the cytosolic redox state. The changes in the metabolites, as artifacts due to the trypsin treatment of cells might also be ruled out since similar changes were also seen in synaptosomes which are not exposed to trypsin.

These results suggest the possibility of an enhancement

in the operation of citric acid cycle. If this were to be true, then a parallel change should be observed in the activities of citric acid cycle enzymes. Excepting our reports on the activities of citric acid cycle enzymes in homogenates and subcellular fractions (Ratnakumari et al., 1985, 1986; Ratnakumari and Murthy, 1989, 1990), no information is available on the activities of citric acid cycle enzymes in cells. Hence, in the present study the activities of the citric acid cycle enzymes were determined in the neurons, astrocytes, oligodendrocytes and in synaptosomes isolated front the cerebral cortex of hyperammonemic rats. These activities were compared with those of normal animals.

Following the administration of pathophyslological concentration of ammonium acetate there were no statistically significant changes in pyruvate dehydrogenase activity in the neuronal perikarya in both acute and subacute conditions. However, under these conditions, cellular activity of this enzyme was enhanced to a significant extent in astrocytes and oligodendrocytes. In the astrocytes the magnitude of increase was higher in acute conditions than in subacute condition while in oligodendrocytes it was same in both the states of hyperammonemia (Table 5.61). Citrate synthetase activity was enhanced in the neuronal perikarya in acute and subacute states of hyperammonemia. Though a similar observation was made in the astrocytes for the cellular activity of this

enzyme, the magnitude o£ change was much less in these cells when compared to the neuronal perikarya. The increae in the cellular activity of citrate aynthetaae in the oligodendrocytes was marginal and statistically not significant in subacute hyperammonemic states. In acute hyperammonemic states the increase in the activity of this enzyme was statistically significant (Table 5.62).

Cellular activity of isocitrate dehydrogenase was enhanced in all the three cell types in both the hyperammonemic states. The magnitude of the increase in the cellular activity of this enzyme was higher in the acute state than that in subacute state in the neurons and astrocytes. Though a similar change was observed in the oligodendrocytes, the magnitude of difference between acute and subacute states waa not as high as in the other two cell types (Table 5.63). Following the administration of ammonium salt, there was an elevation in the cellular activity of 2-oxoglutarate dehydrogenase in all the three cell types except that the change in the oligodendrocytes in subacute atates was marginal and statistically not significant. The magnitude of increase in the activity of this enzyme was higher in the acute states than in the subacute atates and this difference was much more in the astrocytes than in neurons (Table 5.64). A similar trend was noticed in the cellular activities of succinate de-

hydrogenase. In the acute and subacute states of hyperammonemia, the magnitude in the elevation in the activity of this enzyme was much higher in the astrocytes than the neurons and oligodendrocytea under these conditions (Table 5.65).

Unlike the changes in the activities of 'these enzymes, malate dehydrogenase activity when assayed in the direction of oxaloacetate formation was suppressed to a significant extent only in the neurons in both acute and subacute hyperammonemic states. Under these conditions, the activity of astrocytic malate dehydrogenase was marginally suppressed only in the acute states, while in the oligodendrocytes there were no statistically significant changes (Table 5.66). Activity of thia enzyme when measured in the reverse direction was marginally elevated in neurons in the subacute state while there were no statistically significant changes in the activity of this enzyme in aatrocytes and oligodendrocytes (Table 5.67).

The profile of changes in the activities of the enzymes of citric acid cycle when expressed per mg protein were similar to the cellular activities with few exceptions (Tables 5.61 to 5. 67). These were (1) enhancement in the pyruvate dehydrogenase activity in the neuronal perikarya in acute hyperammonemic state (Table 5.61) (2) lack of change in the activity of citrate synthetase in the neuronal perikarya in subacute state and in the oligodendrocytes in the acute

state (Table 5.62) (3) enhanced activity of malate dehydrogenase in the astrocytes and statistically insignificant changes in the activity of the same enzyme in the oligodendrocytes in both acute and subacute states of hyperammonemia (Table 5.67).

When the specific activity of the citric acid cycle enzymes were compared between the neuronal perikarya and the synaptosomes the profile of changes were more or less same in both these fractions in hyperammonemic states (Tables 5.61 to 5.67). However, there were few exceptions which are listed below. (1) In the synaptosomes, pyruvate dehydrogenase activity was stimulated to a greater extent than in neuronal perikarya in subacute and acute hyperammonemic states. Though the activity of this enzyme was stimulated in neuronal perikarya, it was not statistically significant in subacute state and was of lesser magnitude than that of synaptosomes in the acute states (Table 5.61). (2) Citrate synthetase activity was stimulated in the neuronal perikarya in acute hyperammonemia while such a stimulation was not observed in the synaptosomes (Table 5.62). (3) There were no statistically significant changes in the activity of malate dehydrogenase, when assayed in the direction of malate formation in the neuronal perikarya in the acute states while in the synaptosomes there was a suppression in the activity of this enzyme. However, activity of this enzyme was stimulated in both these

preparations in subacute hyperammonemic states (Table 5.67).

In brief, it is evident that the activities of citric acid cycle enzymes were enhanced in the hyperammonemic states with the exception of malate dehydrogenase. Activity of this enzyme was suppressed under these conditions. Citrate synthetase activity was unaltered in the subacute states in the oligodendrocytes.

The stimulation of pyruvate dehydrogenase activity seen in the cells and synaptosomes in hyperammonemic states is similar to that seen in homogenates and in mitochondria. The possible mechanisms involved in such a stimulation have already been discussed. Such an elevation in pyruvate dehydrogenase activity would promote the channelling of pyruvate into citric acid cycle in the formation of acetyl CoA in the astrocytes, oligodendrocytes and synaptosomes in hyperammonemic states. This change, in conjunction with the suppression of lactate dehydrogenase (pyruvate to lactate) and elevated pyruvate kinase, would favour the utilization of pyruvate for citric acid cycle. However, there was no change in the activity of this enzyme in the neuronal perikarya. Enhanced activity of citrate synthetase in astrocytes and oligodendrocytes (acute states) would also favour citrate formation. This change in citrate synthetase activity is identical to the changes in citrate content in the oligodendrocytes and synaptosomes and in astrocytes in acute

states. The citrate synthetase activity was elevated while citrate content was unaltered in the neuronal perikarya in the both the hyperammonemic states. Though the elevation of citrate synthetase favours citrate formation in these cells, it would be limited by the supply of acetyl CoA (due to unaltered pyruvate dehydrogenase activity) and oxaloacetate (due to suppression of malate dehydrogenase activity).

The increase in isocitrate dehydrogenase activity in all the cerebral preparations wold promote the conversion of isocitrate to 2-oxoglutarate and this change in the enzyme activity was once again identical to that of 2-oxoglutarate content in all the cells (except in astrocytes of subacute group of animals). The stimulation of isocitrate dehydrogenase could be due to increased availability of ADP, which is an allosteric activator, for this enzyme (Lehninger, 1984). The possible reasons for the increase in ADP content in hyperammonemic states have already been discussed. Increased activities of 2-oxoglutarate dehydrogenase ensures the greater utilization of 2-oxoglutarate in citric acid cycle. This in conjunction with the suppression of glutamate dehydrogenase (2-oxoglutarate + ammonia ——> glutamate) and aspartate aminotransferase (2-oxoglutarate + aspartate ——> glutamate + oxaloacetate) suggested an enhancement in the utilization of 2-oxoglutarate and the formation of succinate in the citric acid cycle. However, thia reaction may be

limited by the availability of CoA. Enhanced activity of 2-oxoglutarate dehydrogenase in hyperammonemic states would also promote the phosphorylation of GDP to GTP and the latter may bo utilized in synthetic reactions (protein synthesis) and in phosphorylating ADP to ATP (nucleoside diphosphate kinase is present in mitochondria and cytosol, Lehninger, 1984).

The elevation of auccinate dehydrogenase activity would promote the conversion of succinate to fumarate which may be the rate limiting reaction of citric acid cycle. The activity of this enzyme is regulated by several ligands such as oxaloacetate, ADP/ATP ratio, Pi, CoQ/$CoQH_2$. It has been proposed that the binding of oxaloacetate which occurs even in in vivo conditions suppresses the activity of this enzyme (Zeylemaker et al., 1969). In hyperammonemic states the oxaloacetate binding of succinate dehydrogenase may be affected due to suppressed malate dehydrogenaae activity and the enzyme may be released from inhibition. The rest of the ligands are activators of the enzyme and the alterations in their contents would influence the activity of this enzyme.

The increase in malate content in hyperammonemic states in synaptosomes, neurons and astrocytes (acute states) could be due to (a) increased availability of fumarate (due to enhancement in succinate dehydrogenase activity) which is the precursor for malate and/or (b) suppression ot malate de-

hydrogenase (malate to oxaloacetate) activity which utilizes malate and/or (c) increased conversion of oxaloacetate to malate . However, as the malate and malate dehydrogenase are present in substantial amounts in both mitochondria and cytosol, it would be difficult to interpret these results unless the cells and synaptosomes are further fractionated into cytosol and mitochondria. If the changes observed in the malate dehydrogenase activity in the non synaptic mitochondria in hyperammonemic states holds good for the cells and synaptosomes, then the suppression of malate dehydrogenase in the mitochondria of synaptosomes (synaptic mitochondria), neurona and astrocytes (acute states) would limit the production of oxaloacatate required for citrate synthetase reaction. However, this may be overcome by the enhanced rates of CO_2 fixation resulting in the production of oxaloacetate in the astrocytes. These cells (astrocytes) may supply the oxaloacetate in the form of malate/2-oxoglutarate/glutamate/glutamine to neurons and synaptosomes (Shank and Campbell, 1984 a, b).

In summary, the results obtained on citric acid cycle indicated that the operational rates of this cycle may not be compromised in the three cell types and in aynaptosomes in the hyperammonemic states. This is further supported by the lack of changes in ATP and AOP levels in these cerebral preparations when they were incubated with glucose (Tables

5.55 and 5.60).

STUDIES ON MALATE-ASPARTATE SHUTTLE:

The results obtained with intermediates and the enzymes of glycolysis in the cells and synaptosomes indicated the possibility o£ an increase in the glycolysis in hyperammonemic study. Such an increase will be accompanied by the increased conversion of 3-phosphoglyceraldehyde to 1,3-diphosphoglycerate by the enzyme glyceraldehyde-3-phosphate dehydrogenase and during this process NAD* ia converted to NADH. With the suppression of lactate dehydrogenase activity and unaltered levels of lactate in these cerebral preparations in hyperammonemic states, it would of interesting to study the regeneraton of NAD* from NADH in the cytoplasmic compartment of theae cerebral preparations . As was mentioned earlier, malate-aspartate shuttle is known to regenerate NAD* from NADH by transferring reducing equivalents to malate. The malate ia transported into the mitochondria and the reducing equivalents are oxidized in this aubcellular compartment through electron transport system. Moreover, the operation of malate-aspartate shuttle is coupled to the oxidation of pyruvate in citric acid cycle and any interference with the shuttle would also affect the citric acid cycle in brain (Fitzpatrick et al., 1983). Aa the results of the present study also indicated that the operational ratea of citric acid cycle are not compromised in hyperammonemic atatea, it

would be interesting to study raalate-aspartate shuttle under these conditions. Hence, the metabolites and the enzymes o£ the malate-aspartate shuttle were atudied in the cells and synaptosomes isolated from the cerebral cortex of subacute and acute hyperammonemic rat brain.

The results on the contents o£ 2-oxooglutarate, malate and the activities of malate dehydrogenase (both the directions) in the cells and in synaptosomes have already been described and discussed (Tables 5.49 to 5.54, 5.66 and 5.67). Upon incubation with glucose, there was a fall in the aspartate content of astrocytes and oligodendrocytes isolated from the hyperammonemic rats and the magnitude of decrease was greater in acute states than in subacute states (Table 5.69). In the neuronal perikarya and synaptosomes, though there was a similar decrease in aspartate content under these conditions, the change observed was not statistically significant in these cerebral preparations from subacute hyperammonemic rats (Table 5.71). Similar changes were noticed when the aspartate content was expressed per mg protein (Table 5.70). Glutamate content was below the level of detection in the neurons, astrocytes and oligodendrocytes of hyperammonemic animals when they were incubated with glucose. In the synaptosomes of hyperammonemic rata, following the incubation with glucose, there were no statistically significant changes in the glutamate content (Table 5.72).

Changes observed in aapartate aminotransferase activity in the three cell types and synaptosomes of hyperammonemic rats closely resembled the changes in the aspartate levels. There was a fall in the aspartate aminotransferase activity in the synaptosomes, astrocytes and oligodendrocytes isolated from hyperammonemic rats (both subacute and acute states). In the neuronal perikarya, the fall in the aspartate amino-transferase activity was not statistically significant in subacute states, while the same was significant in the acute hyperammonemic states (Table 5.68).

The observed increase in 2-oxoglutarate and malate, which are the components of both citric acid cycle and malate-aspartate shuttle, has already been discussed. These results suggested that these might not be the rate limiting components for the operation of malate-aspartate shuttle in hyperammonemic states. Such a conclusion may also be drawn from the results of Murthy and Hertz (1988). These investi-gators have shown that addition of aspartate relieved the inhibition of pyruvate decarboxylation by high concentrations of ammonium ions in primary cultures of astrocytes. The aspartate taken up by the cell undergoes transamination with 2-oxoglutarate in the cytosol and provides the glutamate required for the operation of malate-aspartate shuttle and glutamine synthesis. If 2-oxoglutarate were to be rate limi-ting, then this effect of aspartate might not have been seen.

Moreover, in the absence of ammonium ions, aspartate enhanced the pyruvate decarboxylation, which is in support of such a concept. In the present study, total 2-oxoglutarate levels were measured instead of cytosolic and mitochondrial contents of this compound. Hence, the observed increase in 2-oxoglutarate could nave occured in any one of the subcellular compartments. However, the results on subcellular fractions indicated that 2-oxoglutarate content was elevated in both cytosol and mitochondria and probably such a change might have occured in these cells also. Moreover, 2-oxoglutarate, eventhough synthesized in the mitochondria, is transported into cytosol. Hence, it is possible that the increase in mitochondrial 2-oxoglutarate might also elevate cytosolic 2-oxoglutarate contents due to its transport. This suggetion is equally applicable for malate. Aa was mentioned earlier, the increase in 2-oxoglutarate in hyperammonemic states could be due to the suppression of aspartate aminotransferase and glutamate dehydrogenase and increased activities of isocitrate dehydrogenase. Similarly, the increase in malate content could be due to aupressed malate dehydrogenase (malate to oxaloacetate) activity and an increase in its synthesis in citric acid cycle under these conditions.

The fall in the aspartate levels in the cells and synaptosomes observed in the present study in hyperammonemic states is supported by similar observations made earlier in

the whole brain preparation and in primary cultures of neurons and of astrocytes. These results suggested that aapartate might become the rate limiting factor for the operation of malate-aspartate shuttle in hyperammonemic states. It must be mentioned that according to the scheme of malate-aspartate shuttle, aspartate is synthesized in the mitochondria and is transported into the cytosol. Fall in the aspartate content could be due to the decreased rate of its synthesis in hyperammonemic staes. Such an effect is possible due to suppression of aspartate aminotransferase in the cells and synaptosomes. This would spare the oxaloacetate for citric acid cycle in the mitochondria. Though the decrease in aspartate levels could be attributed to increased rate of its utilization, it must be mentioned that the major reaction that utilizes aspartate is that of aspartate aminotransferase. As per the scheme of malate-aspartate shuttle, this would occur in the cytosol. The studies on subcellular fractions indicated that the cytosolic activity of aspartate aminotransferase was suppressed in hyperammonemic states. Hence, the increased utilization through aspartate aminotransferase mediated reaction might be ruled out under these conditions. However, aspartate ia also utilized in the purine nucleotide cycle and in arginine biosynthesis. The former metabolic cycle is primarily involved in the production of ammonia in brain (Lowenstein, 1972; Benjamin, 1982) and any

increase in the operational rates of this cycle would be a physiological disadvantage especially in hyperammonemic states when endogenous ammonia levels are already high. Though, the incorporation of aspartate nitrogen into arginine was shown, no such incorporation was seen in urea (Yudkoff et al., 1987). Hence, urea synthesis may not be a major pathway for the disposal of aspartate nitrogens in brain. Hence, the observed decrease in aspartate levels due to increased utilization seems to be a remote possibility.

The fall in aspartate levels and the suppression of aspartate aminotransferase activity especially in cytosol would adversely affect the production of oxaloacetate and thus malate in this subcellular compartment in hyperammonemic states. This would also restrict the conversion o£ NADH to NAD* in the cytosol under these conditions. It was suggested that this would stimulate the conversion of pyruvate and lactate so that NAD* is regenerated in the cytoaol (Cooper and Plum, 1987). The increase in lactate levels in brain in hyperammonemic states was taken as an evidence for this concept (Hawkins et al., 1973; Adams et al., 1979; Raabe and Lin, 1984; Jessy et al., 1990). However, in the present study, when the cells and synaptosomes isolated from hyperammonemic rat brain were incubated with glucose, either the lactate content decreased or did not change and in several instances lactate dehydrogenase activity (pyruvate to

lactate) was suppressed in hyperammonemic states. Though the precise reason for this discrepancy is not known at present, it is possible that the residual activity of lactate dehydrogenase may be adequate enough to regenerate NAD^+ in the cytosol or some other shuttle mechanism or NAD^+ regenerating reaction may be operative under these conditions. Future studies might clear this discrepancy.

The results obtained with cellular fractions in hyperammonemic states are summerized as follows.

(1) In hyperammonemic states, utilization of glucose might be enhanced in the different cell types and in nerve terminals due to the stimulation of glycolysis and citric acid cycle.

(2) However, the production of oxaloacetate in the mitochondria might be suppressed in the hyperammonemic states. The oxaloacetate required for the operation of citric acid cycle might be anaplerotically replenished by CO_2 fixation in astrocytes and by the supply of citric acid cycle intermediates from astrocytes to neurons and synaptosomes.

(3) There is a possibility of the alterations in the operation of malate-aspartate shuttle in hyperammonemic states due to decreased synthesis of aspartate.

(4) The results indicated the possibility of the operation of an alternate mechanism for the regeneration of NAD* from NADH in the cytosol.

TABLE 5.1

DISTRIBUTION OF MARKER ENZYMES IN SYNAPTOSOMES, NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF CEREBRAL CORTEX OF NORMAL RAT BRAIN

ENZYME	SYNAPTOSOMES	NEURONS	ASTROCYTES	OLIGOCELLS
GS	5.1+0.3 34%	4.3+0.8 29%	15.0+2.6 100%	3.7+0.4 25%
GAD	702+172 100%	84+12 12%	21 + 7 " 3%	8 + 1 1%
AChE	0.18+0.01 9%	0.36+0.02 18%	1.98+0.11 100%	0.58+0.03 29%
PChE	0.04+0.003 6%	0.18+0.01 26%	0.68+0.05 100%	0.09+0.007 14%

GS: glutamine synthetase; GAD: glutamic acid decarboxylase, AChE: acetylcholinesterase; PChE: pseudocholinesterase. Activity units:
GS: μmoles of γ-glutamyl hydroxamate formed/mg protein/hr.
GAD: μmoles of GABA formed/mg protein/hr.
AChE and PChE: umoles of DTNB reduced/mg protein/hr.
Number of experiments are five. Each value is mean + S.D.

TABLE 5.2

DISTRIBUTION OF MARKER ENZYMES IN SYNAPTOSOMES, NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF CEREBRAL CORTEX OF ACUTE HYPERAMMONEMIC RAT BRAIN

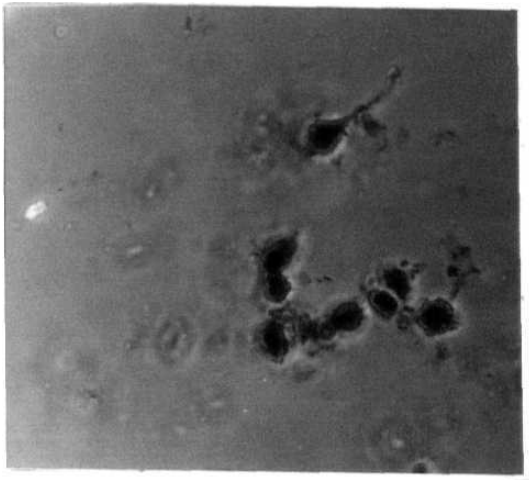
ENZYME	SYNAPTOSOMES	NEURONS	ASTROCYTES	OLIGOCELLS
GS	5.3+0.49 20%	6.2+0.57 24%	25.7+1.98 100%	6.65+0.35 26%
GAD	204+25 100%	29+3.5 14%	9.3+1.1 5%	4+0.6 2%
AChE	0.19+0.02 9%	0.33+0.01 16%	2.11+0.21 100%	0.55+0.03 26%
PChE	0.04+0.003 6%	0.20+0.02 28%	0.72 + 0.06 100%	0.10 + 0.008 14%

Legend as in Table 5.1.

1. Light microscope photomicrographs of: neurons isolated from rat cerebral cortex.

2. Light microscope photomicrographs of astrocytes isolated from rat cerebral cortex.

3. Light microscope photomicrographs of oligo ceils isolated from rat cerebral cortex.



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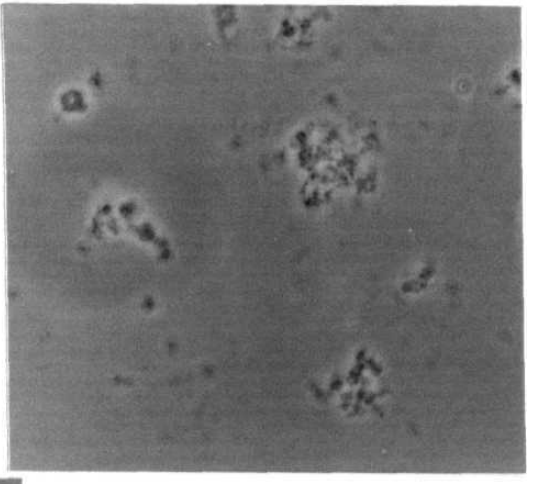
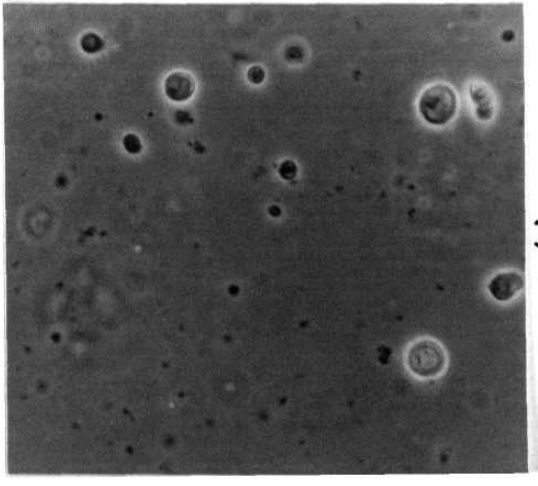


2



3

TABLE 5.3

RNA CONTENT OF BULK ISOLATED CELLS FROM THE CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RATS

FRACTION	NORMAL	ACUTE		
NEURONS	23.0±6.0(6)	32.0±6.0(6)	+39*	p<0.05
ASTROCYTES	6.9511.07(4)	9.3±1.6(5)	+34*	p<0.05
OLIGO CELLS	10.0±2.0(5)	12.0+1.4(5)	+20%	NS

UNITS: pgms RNA/cell. For other details see Table 5.1.

TABLE 5.4

DNA CONTENT OF BULK ISOLATED CELLS FROM THE CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RATS

FRACTION	NORMAL	ACUTE		
NEURONS	7.9±1.6(6)	8.2±1.0(5)	+4%	NS
ASTROCYTES	7.9611.9(5)	8.5+1.9(5)	+6%	NS
OLIGO CELLS	6.9±1.5(5)	6.611.4(5)	-4%	NS

UNITS: pgms DNA/cell. Each value is Mean + S.D. Number in parenthesis indicates number of experiments.

TABLE 5.5

CELL NUMBER (/MG OF CELLULAR PROTEIN) IN NORMAL AND HYPERAMMONEMIC RATS

FRACTION	NORMAL	SUBACUTE	ACUTE
NEURONS	2.3+0.4	2.4+0.5 -2.5% NS	2.2+0.7 -6% NS
ASTROCYTES	1.5+0.1	1.6+0.2 +3% NS	1.4+0.5 -5% NS
OLIGO CELLS	6.2+0.3	6.0+0.8 -3% NS	5.9+1.0 -5% NS

CELL NUMBER X 10^6. Each value is Mean $\pm$ S.D. Number of experiments are 5. For each experiment three animals were used.

TABLE 5.6

CELL NUMBER (/GRAM WET WEIGHT OF CEREBRAL CORTEX) IN NORMAL AND IN HYPERAMMONEMIC RAT BRAIN

FRACTION	NORMAL	SUBACUTE	ACUTE
NEURONS	4.4$\pm$0.6(5)	4.3$\pm$0.7(5) -2*	4.3$\pm$0.6(5) -2*
ASTROCYTES	6.4 + 0.7(5)	6.5 + 0.8(5) +2%	6.5$\pm$1.0(5) +24
OLIGO CELLS	2.9+0.2(5)	2.8+0.5(5) -2%	2.8+0.3(5) -2%

Cell number X 10 . Numbers in parenthesis indicates the number of experiments. Each value is Mean+SD.

TABLE 5.7

PROTEIN CONTENT OF BULK ISOLATED CELLS FROM THE CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RATS

FRACTION	NORMAL	SUBACUTE	ACUTE
NEURONS	439+70	458+23 +4* NS	464+36 +6% NS
ASTROCYTES	668+59	673+21 + 1* NS	685+70 +3* NS
OLIGO CELLS	160+5"'	165+30 +3% NS	155+23 -3% NS

Protein content ie expressed as picograms/cell. Number of experiments are 7. For each experiment three animals were used. Each value is Mean ± S.D.

TABLE 5.8

PROTEIN CONTENT (/GM WET WEIGHT) OF SYNAPTOSOMES AND BULK ISOLATED CELLS FROM THE CEREBRAL CORTEX OF NORMAL AND HYPERAMMONEMIC RATS

FRACTION	NORMAL	SUBACUTE	ACUTE
NEURONS	2.0+0.3	2.0+0.03 + 2% NS	2.0+0.3 +2* NS
ASTROCYTES	4.3±0.5	4.4+0.-05 +2.8V NS	4.4+0.7 +2.8% NS
OLIGO CELLS	0.46+0.01	0.47+0.07 +1% NS	0.43+0.05 -7* NS
SYNAPTOSOMES	9.00+2.8	8.40+2.0	7.30±1.2

protein content is expressed as mg cellular protein/em wet wt of cerebral cortex. Number of experiments are 7. For each experiment 3 animals were used. Each value is mean + S.D.

TABLE 5.9

$^{14}CO_2$ PRODUCTION FROM [U-^{14}C]GLUCOSE FROM NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAINS
(fmoles of CO_2 produced/cell/hr)

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	**2.48±0.27**	**13.7±1.34**	**0.53±0.06**
SUBACUTE	2.98+0.17 p<0.01 +21%	15.5+0.87 p<0.05 +13%	0.60+0.02 p<0.02 +13%
ACUTE	3.10+0.14 p<0.005 +26%	17.9±0.71 p<0.001 +31*	0.85±0.03 p<0.001 +60%
1mM	2.45+0.11 NS -1*	13.1+1.4 NS -4%	0.52+0.05 NS -2%
5mM	2.70+0.15 NS +10%	15.1+1.0 NS +11%	0.66+0.08 p<0.02 +25%

Number of experiments are five. Each value is mean+S.D.

TABLE 5.10

$^{14}CO_2$ PRODUCTION FROM [U-^{14}C]GLUCOSE FROM NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAINS
(nmoles of CO_2 produced/mg protein/hr.)

FRACTION	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	**5.65±0.63**	**20.5±2.0**	3.3+0.4
SUBACUTE	**6.50+0.37** p<0.05 +15%	23.0+1.3 p<0.05 +12%	3.6+0.13 NS +9%
ACUTE	6.96+0.3 p<0.005 +23%	25.0+1.0 p<0.005 +22%	5.0+0.18 p<0.001 +52%
1mM	5.63+0.25 NS -1%	19.7+2.1 NS -4%	3.2+0.3 NS -3%
5mM	6.20+0.35 NS +10%	22.7+1.5 NS +11%	4.1+0.15 p<0.02 **+24%**

Number of experiments are five. Each value is mean+S.D.

TABLE 5.11

$^{14}CO_2$ PRODUCTION FROM [U-^{14}C]GLUCOSE IN NEURONAL AND SYNAPTOSOMAL FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

STATE	NEURONS	SYNAPTOSOMES
NORMAL	5.65+0.63	14.0±1.0
SUBACUTE	6.50+0.37 p<0.05 +15%	17.8+1:48 p<0.005 +27%
ACUTE	6.96±0.3 p<0.005 +23%	19.4+1.1 p<0.001 +39%
1mM	5.63+0.25 NS -1%	14.4+1.25 NS +3%
5mM	6.20+0.35 NS +10%	16.0+2.0 NS +14%

Activity units: nmoles of CO_2 produced/mg protein/hr.
Number of experiments are 4. Each value is Mean±S.D.

TABLE 5.12

METABOLITE LEVELS IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES FOLLOWING THE INCUBATION WITH GLUCOSE IN CEREBRAL CORTEX OF NORMAL RAT BRAIN
(fmoles/cell)

METABOLITE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
G-6-P	33.90±7.8	85.0±12 a	2.60±0.5 b,c
F-6-P	1.26±0.13	4.010.3 a	0.1410.1 b,c
PEP	0.4310.07	1.310.12 a	0.0410.003 b,c
PYR	3.5010.3	6.0+0.4 a	2.4010.13 b,c
LACT	8.0011.3	13.011.33 a	6.2010.5 b,c
CIT	5.2010.4	7.310.6 a	0.9710.08 b,c
ISOCIT	4.3010.39	8.0±0.67 a	0.80±0.06 b,c
2-OG	5.20+0.48-'.	7.310.67 a	0.9710.08 b,c
MAL	6.5010.3	12.011.3 a	1.30±0.11 b,c
ASP	12.20±1.3	12.711.3	1.4510.13 b.c
ATP	13.5011.1	23.0±2.53 a	2.9010.3 b,c
ADP	9.60+0.65	16.011.2 a	1.610.13 b,c

G-6-P: glucose-6-phosphate; F-6-P: fructose-6-phosphate; PEP: phosphoenolpyruvate; PYR: pyruvate; LACT: lactate. CIT: citrate; ISOCIT: iaocitrate; 2-OG: 2-oxoglutarate; MAL: malate; ASP: aspartate; ATP: adenoaine 5'tri phosphate; ADP: adenosine 5'di-phoaphate. Number of experiments are five. Each value *La* mean ± S.D. Values which are significantly different ($p<0.05$) from other fractions were indicated by letters. a: between neurons and astrocytes; b: between neurona and oligodendrocytes; c: between astrocytes and oligodendrocytes.

TABLE 13

METABOLITE LEVELS IN SYNAPTOSOMES, NEURONS, ASTROCYTES AND OLIGODENDROCYTES FOLLOUING THE INCUBATION WITH GLUCOSE IN CEREBRAL CORTEX OF NORMAL RAT BRAIN
(nmoles/mg protein)

METABOLITE	SYNAPTOSOMES	NEURONS	ASTROCYTES	OLIGODENDRO-CYTES	
G-6-P	52.0±5.4	70.7011.5 d	127.0±18 a	27.9±2.4	b,c
F-6-P	2.5+0.3	2.90+0.3	6.0±0.4 a	0.85±0.06	b,c
PEP	0.8±0.1	0.98±0.16	2.0±0.18 a	0.26+0.017	b,c
LACT	74.0±10	18.50±3.0 d	19.5±2.0	38.50±3.0	b,c
PYR	12.5+0.8	8.00+0.6 d	9.0+0.6	15.00+0.8	b,c
CIT	18.0±1.2	12.00±1.0 d	11.0±0.9	6.00±0.8	b,c
ISOCIT	18.0±2.0	10.00±0.9 d	12.0±1.0	5.00±0.4	b,c
2-OG	19.0i2.0	12.00 + 1.1 d	11.0±1.0	6.00 + 0.5	b,c
MAL	19.0±2.0	15.00+0.6 d	18.0±2.0	8.00±0.7	b,c
ASP	36.0±4.0	28.00±3.0 d	19.0±2.0 a	9.0010.8	b,c
GLU	17.0+2.0	ND V.	ND	ND	
ATP	32.014.0	31.0012.5	35.013.8	18.0012.0	b,c
ADP	14.011.0	22.0011.5 d	24.0±1 8	9.8±0.8	b,c

Legend same as in Table 5.12. d: between synaptosomes and neurons.

TABLE 5.14

DISTRIBUTION OF GLYCOLYTIC ENZYMES IN CELLULAR FRACTIONS OF CEREBRAL CORTEX OF RAT BRAIN
(Picomoles/cell/hr)

ENZYME	NEURONS	ASTROCYTES	OLIGOCELLS
HK	0.48±0.09	1.0310.27 a	0.13±0.006 b,c
PFK	3.29+0.59	1.55±0.35 a	0.31+0.04 b,c
ALASE	2.2+0.52	1.4910.18 a	0.31±0.03 b,c
GLPDH	1.5±0.2	1.9±0.25	1.38±0.25
ENASE	5.1411.01	3.25±0.54	0.64±0.013 b,c
PK	29.2±3.5	37.5616.3	5.2711.0 b,c
LDH (P->L)	14.312.5	15.8±0.88	5.2610.58 b.c
LDH (L->P)	4.410.2	4.4±0.3	2.1±0.2 b,c

HK: hexokinase; PFK: phosphofructokinase; ALASE: aldolase; GLPDH: glyceraldehyde-3-phosphate dehydrogenase; ENASE: enolase; PK: pyruvate kinase; LDH: lactate dehydrogenase. Activity was expressed for Hk aa NADP reduced; for GLPDH and LDH (L->P) as NAD^+ reduced; for others as NADH oxidized. Number of experiments are five. Each value ia mean ± S.D. Values which are significantly different ($p<0.05$) from other fractions are indicated with a letter *a':between neurons and astrocytea; `b':between neurons and oligodendrocytes; `c':between oligodendrocytes and astrocytes.

TABLE 5.15

DISTRIBUTION OF GLYCOLYTIC ENZYMES IN THE CELLULAR FRACTIONS OF CEREBRAL CORTEX OF RAT BRAIN
(μmoles/mg protein/hr)

ENZYME	NEURONS	ASTROCYTES	OLIGOCELLS
HK	1.15 + 0.29	1.52±0.4	0.81+0.06 b,c
PFK	7.60±1.7	2.30±0.6 a	1.90+0.2 b
ALASE	5.0±0.9	2.25i0.35 a	1.96±0.21 b
GLPDH	3.4±0.6	3.0±0.2	8.4±0.5 b,c
ENASE	10.66±1.85	4.86±0.57 a	3.98±0.11 b
PK	53.3±9.11	56.76±11.4	33.0±4.5 b,c
LDH (1)	21.8+6.1	23.8±2.0	33.0+4.5
LDH (2)	8.4±0.9	7.1±0.5	13±1.4 b,c

For details see Table 5.14.

TABLE 5.16

DISTRIBUTION OF GLYCOLYTIC ENZYMES IN NEURONAL AND SYNAPTOSOMAL FRACTIONS OF CEREBRAL CORTEX OF RAT BRAIN

ENZYME	NEURONS		SYNAPTOSOMES	
HK	1.15±0.29	"	5.64+0.73	a
PFK	7.6+1.7		1.78+0.19	a
ALD	5.0±0.9		4.4+0.64	
GLPDH	3.4+0.6		3.79±0.35	
ENOLASE	10.7±1.85		4.8±0.78	a
PK	53.3+9.1		20±2	a
LDH (P->L)	21.8±6.1		121±9	a
LDH (L->P)	8.4±0.9		23+3	a

HK: hexokinase; PFK: phosphofructokinase; ALASE: aldolaae; GLPDH: glyceraldehyde-3-phosphate dehydrogenase; ENASE: enolase; PK: pyruvate kinase; LDH: lactate dehydrogenase. Activity unite for HK were μmoles of $NADP^+$ reduced/mg protein/hr; for GLPDH and LDH (L->P) μmoles of NAD reduced/mg protein/hr; for others μmoles of NADH oxidized/me protein/hr. Each value ia mean ± S.D. Number of experiments are five. Only those values which are significantly different ($p<0.05$) from neurons are indicated with a letter 'a'.

TABLE 5.17

DISTRIBUTION OF PYRUVATE DEHYDROGENASE AND CITRIC ACID CYCLE ENZYMES IN CELLULAR FRACTIONS OF RAT CEREBRAL CORTEX (picomoles/cell/hr)

ENZYME	NEURONS	ASTROCYTES	OLIGO CELLS	
PDH	10.911.5	13.3511.6	1.92±0.3	b,c
CS	7.8+0.9	11.3+1.2 a	1.4410.18	b.c
ICDH	7.210.6	9.6±1.27	1.9810.13	b.c
2-OGDH	9.3±0.9	11.67±1.9	2.4±0.5	b,c
SDH	0.28+0.03	0.22±0.024	0.028+0.001	b,c
MDH (M->O)	29.3±1.3	104±8.0 a	6.9010.9	b,c
MDH (O->M)	35.2±0.68	42.014.2	5.9010.5	b,c

PDH:pyruvate dehydrogenase; CS:citrate synthetase; ICDH: isocitrate dehydrogenase; 2-OGDH:2-oxoglutarate dehydrogenase; SDH: succinate dehydrogenaae; MDH: malate dehydrogenaae. Activity was expressed for PDH, ICDH, 2-OGDH and MDH (M-->0) as pmoles of NAD^+ reduced and for CS &a pinoles of DTNB reduced and for SDH as pmoles of succinate oxidized and for MDH (0->M) as pinoles of NADH oxidized. Only those values which are significantly different ($p<0.05$) from other are indicated with a letter a: between neurons and astrocytes; b : between neurons and oligodendrocytes; c: between astrocytes and oligodendrocytes. Number of experiments are five. Each value is mean + S.D.

BLE 5.18a

DISTRIBUTION OF PYRUVATE DEHYDROGENASE AND CITRIC ACID CYCLE ENZYMES OF RAT CEREBRAL CORTEX
(µmoles/mg protein/hr)

ENZYME	NEURONS	ASTROCYTES	OLIGO CELLS
PDH	24.9±1.7	20.0±1.8	12.0+2.1 b,c
CS	20.9+6.0	16.0±2.6	9.0±1.3 b,c
ICDH	16.7±1.4	14.4±1.9	12.2± 0.8
2-OGDH	21.6+3.7	17.5±2.6	15.2+2.6
SDH	0.51+0.17	0.30±0.06 a	0.17±0.02 b,c
MDH (M->O)	55.0±6.8	56.0±6.4	43.0±6.4
MDH (O->M)	69.5±4.9	60.0±9.4	37.0±4.2 b,c

Legend same as in Table 5.17.

TABLE 5.18b

DISTRIBUTION OF PYRUVATE DEHYDROGENASE & CITRIC ACID CYCLE ENZYMES IN NEURONS AND SYNAPTOSOMES

ENZYME	NEURONS	SYNAPTOSOMES
PDH	24.9+1.7	3.42±0.33 a
CS	20.9±6.0	32±4 a
ICDH	16.7±1.4	11.4±1.72 a
2-OGDH	21.6+3.7	19.5il.99
SDH	0.51±0.17	0.53±0.06
MDH (M->O)	55.0±6.8	388+33 a
MDH (O->M)	69.5+4.9	462+35 a

Legend same as in Table 5.17. a: Statistically significant diffenrence between neurons and synaptosomes.

TABLE 5.19a

DISTRIBUTION OF BORST CYCLE ENZYMES IN CELLULAR FRACTIONS OF RAT CEREBRAL CORTEX

ENZYME		NEURONS	ASTROCYTES		OLIGO CELLS	
MDH (M->O)	A.	29.3±1.3	104±8.0	a	6.90±0.9	b,c
	B.	55.0±6.8	56.0±6.4		43.0±6.4	b,c
MDH (O->M)	A.	35.2i0.68	42.0i4.2	a	5.90±0.5	b,c
	B.	69.5±4.9	60.0±9.4		37.0±4.2	b,c
AAT	A.	13.4+2.5	23.5±3.2	a	1.92±0.2	b,c
	B.	27.0 + 4.9	35.0±5.0		12.0±1.2	b.c

MDH: malate dehydrogenase; AAT: aspartate aminotransferase. A: µmoles of activity/cell/hr; B: picomoles of activity/mg protein/hr. Activity is expressed for MDH (M->0) as NAD^+ reduced and for others as NADH oxidized. Number of experiments are five. Each value is mean + S.D. For other details see Table 5.17.

TABLE 5.19b

DISTRIBUTION OF BORST CYCLE ENZYMES IN NEURONS AND SYNAPTOSOMES OF RAT CEREBRAL CORTEX

ENZYME	NEURONS	SYNAPTOSOMES	
MDH (M->O)	55.0±6.8	388±33	a
MDH (O->M)	69.5±4.9	462±35	a
AAT	27.0±4.9	192±8.3	a

Legend same as for Table 5.19a. a: Statistically significant difference exists between neurons and synaptosomes.

TABLE 5.20

LEVELS OF GLUCOSE-6-PHOSPHATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	33.9±7.8	85.0±12.0	2.79+0.24
SUBACUTE	39.8+2.5 NS +17%	88.0+9.0 NS +4%	3.17+0.37 NS +14%
ACUTE	54.0+7.2 p<0.01 +59%	109.0+4.9 p<0.02 +28%	5.10+0.5 p<0.001 +83%
1mM	41.0+3.0 NS +21%	93.0+9.0 NS +9%	3.10+0.4 NS +11%
5mM	48.0±6.0 p<0.05 +42%	105.0+7.3 p<0.05 +24%	4.70+0.4 p<0.001 +68%

Units: fmoles/cell. Each value is mean+S.D. Number of experiments are five.

TABLE 5.21

LEVELS OF GLUCOSE-6-PHOSPHATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	70.7±15.3	127.0+18.0	16.0+3.0
SUBACUTE	95.5+6.0 p<0.05 +35%	137.0+14.0 NS +8%	19.0+2.2 NS +19%
ACUTE	119.0+16.0 p<0.001 +68%	156.0+7.0 p<0.025 +23%	30.0+3.0 p<0.001 +83%
1mM	95.0+7.0 p<0.05 +34%	140.0+13.5 NS +9%	19.0+2.5 NS +19%
5mM	110.0±14.0 p<0.05 +56%	158.0+11.0 p<0.05 +24%	29.0+2.5 p<0.001 +81%

Units: nmoles/mg protein. Each value is mean±S.D. Number of experiments are five.

TABLE 5.22

LEVELS OF GLUCOSE-6-PHOSPHATE IN NEURONS AND SYNAPTOSOMES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	SYNAPTOSOMES
NORMAL	70.7±15.3	52.0+0.54
SUBACUTE	95.5+6.0 p<0.05 +35*	65.0+6.0 p<0.02 +25*
ACUTE	119.0+16.0 p<0.001 +68*	85.0+7.0 p<0.001 +63%
1mM	95.0+7.0 p<0.05 +34*	51.0+9.0 NS -2%
5mM	110.0±14.0 63.0	+ 5.0 p<0.05 +56* p<0.025 +21*

Units: nmoles/mg protein. Each value is mean±S.D. Number of experiments are five.

TABLE 5.23

LEVELS OF FRUCTOSE-6-PHOSPHATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	1.26±0.13	4.0±0.3	0.14±0.01
SUBACUTE	1.54+0.1 p<0.02 +22*	4.8+0.3 p<0.01 +20*	0.16+0.01 p<0.05 +14%
ACUTE	1.9+0.14 p<0.001 +51%	5.8+0.3 p<0.001 +45*	0.18+0.01 p<0.005 +26*
1mM	1.65+0.1 p<0.005 +31*	4.9+0.3 p<0.01 +23*	0.15+0.008 NS +7*
5mM	1.78+0.09 p<0.001 +41*	5.3+0.3 p<0.001 +33*	0.16+0.01 p<0.025 +14*

Units: f moles/cell. Each value is mean±S.D. Number of experiments are five.

TABLE 5.24

LEVELS OF FRUCTOSE-6-PHOSPHATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	2.90 + 0.3	6.0±0.4	0.85 + 0.06
SUBACUTE	3.70±0.3 p<0.01 +284	7.4 + 0.5 p<0.005 + 234	0.94 + 0.07 NS + 114
ACUTE	4.2 + 0.3 p<0.001 +45%	8.3±0.4 p<0.001 +38%	1.04±0.06 p<0.005 +224
1mM	3.80+0.3 p<0.01 +314	7.3+0.4 p<0.005 +224	0.96+0.05 p<0.05 +134
5mM	4.10+0.2 p<0.001 +41%	8.0+0.4 p<0.001 +334	1.00+0.05 p<0.01 +184

Units: nmoles/mg protein. Each value is mean±S.D. Number of experiments are five.

TABLE 5.25

LEVELS OF FRUCTOSE-6-PHOSPHATE IN NEURONS AND SYNAPTOSOMES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	SYNAPTOSOMES
NORMAL	2.90±0.3	2.5 + 0.3
SUBACUTE	3.70+0.3 p<0.01 +284	3.3+0.36 p<0.02 +324
ACUTE	4.2+0.3 p<0.001 +454	4.1+0.3 p<0.001 +644
1mM	3.80+0.3 p<0.01 +314	3.2+0.3 p<0.02 +284
5mM	4.10+0.2 p<0.001 +414	3.9+0.3 p<0.001 +564

Units: nmoles/mg protein. Each value is mean±S.D. Number of experiments are five.

TABLE 5.26

LEVELS OF PHOSPHOENOLPYRUVATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	0.43±0.07	1.3±0.12	0.04±0.003
SUBACUTE	0.46±0.04 NS +7%	1.61±0.11 p<0.01 +24%	0.048±0.003 p<0.01 +20%
ACUTE	0.6+0.02 p<0.005 +40%	2.3+0.14 p<0.001 +77%	0.064+0.003 p<0.001 +60%
1mM	0.48+0.09 NS +12%	1.63+0.13 p<0.01 +25%	0.045+0.003 NS +13%
5mM	0.55+0.02 p<0.02 +28%	1.87+0.2 p<0.005 +44%	0.055+0.003 p<0.001 +38%

Units: fmoles/cell. Each value is mean±S.D. Number of experiments are five.

TABLE 5.27

LEVELS OF PHOSPHOENOLPYRUVATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	0.98±0.16	2.0±0.18	0.26±0.017
SUBACUTE	1.10+0.10 NS +12%	2.50+0.17 p<0.01 +25%	0.29±0.02 NS +11%
ACUTE	1.33+0.04 p<0.01 +36%	3.3+0.20 p<0.001 +65%	0.38+0.02 p<0.001 +46%
1mM	1.10+0.20 NS +12%	2.45+0.20 p<0.02 +22%	0.28+0.02 NS +8%
5mM	1.26+0.04 p<0.02 +28%	2.80+0.30 p<0.005 +40%	0.34+0.02 p<0.001 +31%

Units: nmoles/mg protein. Each value Is mean+S.D. Number of experiments are five.

TABLE S.28

LEVELS OF PHOSPHOENOLPYRUVATE IN NEURONS AND SYNAPTOSOMES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	SYNAPTOSOMES
NORMAL	0.98±0.16	0.80±0.1
SUBACUTE	1.10+0.10 NS +12*	0.99+0.03 p<0.02 +24%
ACUTE	1.33+0.04 p<0.01 +36*	1.17+0.04 p<0.001 +46*
1mM	1.10+0.20 NS +12*	0.95+0.09 NS +19*
5mM	1.26+0.04 p<0.02 +28%	1.12+0.05 p<0.001 +40%

Units: nmoles/mg protein. Each value *La* mean+S.D. Number of experiments are five.

TABLE 5.29

LEVELS OF PYRUVATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	3.5±0.3	6.0+0.4	2.40±0.13
SUBACUTE	3.7±0.3 NS +6*	6.4+0.6 NS +7*	2.65+0.15 p<0.05 +10%
ACUTE	6.3±0.5 p<0.001 +80*	11.3+1.2 p<0.001 +88%	4.10+0.37 p<0.001 +71*
1mM	3.7±0.3 NS +6*	7.0±0.8 NS +17*	2.70±0.22 NS +13*
5mM	5.2±0.4 p<0.001 +49*	10.0±0.9 p<0.001 +67*	3.50+0.3 p<0.001 +46*

Units: fmoles/cell. Each value i3 mean+S.D. Number of experiments are five.

TABLE 5.30

LEVELS OF PYRUVATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND KYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	8.0+0.6	9.0±0.6	15.0±0.8
SUBACUTE	8.9+0.8 NS +11%	9.9±1.0 NS +10%	15.9+0.9 NS + 6%
ACUTE	14.0±1.2 p<0.001 +75%	16.2+1.7 p<0.001 +80%	24.0+2.2 p<0.001 +60%
1mM	8.5+0.7 NS +6%	10.5+1.2 NS +17%	16.5+1.4 NS +10%
5mM	12.0+0.9 p<0.001 +50%	15.0+1.4 p<0.001 +67%	22.0+2.1 p<0.001 +47%

Units: nmoles/mg protein. Each value is mean+S.D. Number of experiments are five.

TABLE 5.31

LEVELS OF PYRUVATE IN NEURONS AND SYNAPTOSOMES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	SYNAPTOSOMES
NORMAL	8.0±0.6	12.5±0.8
SUBACUTE	8.9+0.8 NS +11%	13.9+0.7 p<0.05 +11%
ACUTE	14.0+1.2 p<0.001 +75%	19.7+2.0 p<0.001 +58%
lmM	8.5+0.7 NS +6*	14.0+1.0 NS " +12%
5mM	12.0+0.9 p<0.001 +50%	16.0+1.8 p<0.01 +33%

Units: nmoles/mg protein. Each value is mean+S.D. Number of experiments are five.

TABLE 5.32

LEVELS OF LACTATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	8.0+1.3	13.0±1.33	6.2±0.5
SUBACUTE	7.9+0.4 NS -14	12.3+1.3 NS -54	6.8+0.5 NS +104
ACUTE	8.8+1.4 NS +104	12.2+2.1 NS -64	6.4+0.6 NS +34
1mM	6.96+0.7 NS -134	13.0+2.0 NC	6.5+0.4 NS +54
5 mM	9.57 + 0.9 NS +204	14.0 + 1.3 NS +84	7.0 + 0.5 NS +134

Units: fmoles/cell. Each value is mean±S.D. Number of experiments are five.

LEVELS OF LACTATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	18.5±3.0	19.5+2.0	38.5±3.0
SUBACUTE	19.0+1.0 NS +34	19.0+2.0 NS -34	41.0+3.0 NS +64
ACUTE	19.5+3.0 NS +54	17.5+3.0 NS -104	38.0+3.6 NS -14
1mM	16.0+1.6 NS -134	19.7+3.0 NS +14	40.0+2.6 NS +44
5mM	22.0+2.0 NS +19%	21.5+2.0 NS +104	43.5+3.0 NS 13%

Units: nmoles/mg protein. Each value *La* mean+S.D. Number of experiments are five.

TABLE 5.34

LEVELS OF LACTATE IN NEURONS AND SYNAPTOSOMES
OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	SYNAPTOSOMES
NORMAL	18.5±3.0	74.0±10.0
SUBACUTE	19.0+1.0 NS +3*	69.0+10.0 NS -74
ACUTE	19.5+3.0 NS +5*	82.0+6.0 NS -11%
1mM	16.0+1.6 NS -134	68.0+10.0 NS -8%
5mM	22.0+2.0 NS +19%	66.0+3.6 NS -11%

Units: nmoles/mg protein. Each value is mean+S.D. Number of experiments are five.

TABLE 5.35

HEXOKINASE ACTIVITY IN SYNAPTOSOMES AND CELLULAR FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

FRACTION		NORMAL	SUBACUTE	ACUTE
NEURONS	A.	0.48±0.09	0.65+0.11 p<0.05 +35%	0.74+0.15 p<0.02 +54%
	B.	1.29±0.4	1.34+0.34 NS +4%	1.54+0.34 NS +19%
ASTROCYTES	A.	1.03±0.27	1.02±0.23 NS -1%	1.43±0.49 p<0.05 +56%
	B.	1.36+0.4	1.54+0.25 NS +13%	1.62+0.39 NS +19%
OLIGO CELLS	A.	0.13+0.006	0.17+0.003 p<0.001 +29%	0.18+0.04 p<0.025 +38%
	B.	0.81+0.06	1.01+0.15 p<0.025 +25%	1.01+0.17 p<0.05 +25%
SYNAPTOSOMES	B.	5.64±0.73	6.34±0.59 NS +12%	6.98+0.55 p<0.02 +24%

Units: A: pmoles of NADP reduced/cell/hr. B: μmoles of NADP reduced/mg protein/hr. Each value is Mean+S.D.
No. of experiments are 5.

TABLE 5.36

PHOSPHOFRUCTOKINASE ACTIVITY IN SYNAPTOSOMES AND CELLULAR FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION		NORMAL	SUBACUTE	ACUTE
NEURONS	A.	3.29+0.59	4.52+0.97 p<0.05 +37%	7.03+1.6 p<0.005 +114%
	B.	7.6+1.7	10.5+1.5 p<0.025 +38%	15.4+3.4 p<0.005 +103%
ASTROCYTES	A.	1.55+0.35	2.8±0.57 p<0.005 +81%	6.9±2.4 p<0.005 +345%
	B.	2.35+0.57	4.24+0.62 p<0.005 +80%	8.24+1.26 p<0.005 +251%
OLIGO CELLS	A.	0.31+0.04	0.52+0.07 p<0.005 +67%	0.89+0.22 p<0.001 +188%
	B.	1.94 + 0.21	3.1 + 0.41 5. p<0.001 +60%	13 + 0.86 p<0.001 +164%
SYNAPTOSOMES	B.	1.78+0.19	3.18+0.22 p<0.001 +79%	4.52+0.55 p<0.001 +154%

Units: A: picomoles of NADH oxidized/cell/hr; B: μmoles of NADH oxidized/me protein/hr. Each value la Mean+S.D.
No. of experiments are 5.

TABLE 5.37

ALDOLASE ACTIVITY IN SYNAPTOSOMES AND CELLULAR FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION		NORMAL	SUBACUTE	ACUTE
NEURONS	A.	2.2+0.52	3.0+0.54 p<0.05 +36*	3.69+0.29 p<0.001 +68%
	B.	5+0.9	6+1 NS +20%	10+2 p<0.001 +100%
ASTROCYTES	A.	1.49+0.18	2.69+0.63 p<0.005 +81%	4.2+0.61 p<0.001 +182%
	B.	2.25+0.35	4+0.41 p<0.001 +78%	7.98+1.55 p<0.001 +255%
OLIGO CELLS	A.	0.31±0.03	0.5±0.13 p<0.02 +58%	0.68±0.06 p<0.001 +117%
	B.	1.96±0.21	2.76±0.69 p<0.05 +41%	3.88+0.26 p<0.001 +98%
SYNAPTOSOMES	B.	4.4+0.64	7.4+0.58 p<0.001 +68%	10.1+1.1 p<0.001 +130%

Units: A: picomoles of NADH oxidized/cell/hr; B: μmoles of NADH oxidized/mg protein/hr. No. of experiments are 5. Each value is mean + S.D.

TABLE 5.38

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE ACTIVITY IN SYNAPTOSOMES AND CELLULAR FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION		NORMAL	SUBACUTE	ACUTE
NEURONS	A.	1 5+0.2	1.48+0.22 NS -1%	2.05+0.3 p<0.02 +37%
	B.	3.36+0.62	3.4+0.3 NS +1%	4.33+0.39 p<0.02 +29%
ASTROCYTES	A.	1.9+0.25	2.1+0.3 NS +10%	2.7+0.46 p<0.01 +42%
	B.	2.95+0.2	3.3+0.39 NS +12%	3.51+0.19 p<0.005 +19%
OLIGO CELLS	A.	1.38+0.25	1.75+0.14 p<0.025 +27%	1.77+0.23 p<0.05 +28%
	B.	8.42+0.5	10.53+0.9 p<0.005 +25%	10.44+1.27 p<0.02 +24%
SYNAPTOSOMES	B.	3.79+0.35	4.13+0.19 NS +9%	4.1+0.35 NS +8%

Units: A: pinoles of NAD reduced/cell/hr; B: μmoles of NAD reduced/nig protein/hr. Each value ia Mean+S.D.
No. of experiments are 5.

TABLE 5.39

ENOLASE ACTIVITY IN SYNAPTOSOMES AND CELLULAR FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION		NORMAL	SUBACUTE	ACUTE
NEURONS	A.	3.83+0.81	4.89+0.58 NS -5*	7.39+1.5 p<0.005 +44*
	B	10.66+1.85	11.52+1.87 NS +8*	16.02+1.63 p<0.005 +50*
ASTROCYTES	A.	3.25+0.54	4.5+0.89 p<0.05 +38%	6.37+0.9 p<0.001 +96*
	B	4.86+0.57	6.16+0.97 p<0.05 +27*	9.7+1.92 p<0.001 +100*
OLIGO CELLS	A.	0.64+0.01	0.66+0.02 NS +3*	1.13+0.38 p<0.02 +77%
	B.	3.98+0.19	4+0.34 NS . +1*	5.12+0.89 p<0.025 +29*
SYNAPTOSOMES	B.	4.8+0.78	7.66+0.76 p<0.001 +60%	9.9+1.1 p<0.001 +106*

Units: A: pinoles of NADH oxidized/cdl/hr; B: poles of NADH oxidized/me protein/hr. Each value *la* Mean+S.D.
No. of experiments are 5.

TABLE 5.40

PYRUVATE KINASE ACTIVITY IN SYNAPTOSOMES AND CELLULAR FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION		NORMAL	SUBACUTE	ACUTE
NEURONS	A.	29.2±3.5	30.2 + 5.7 NS +3*	32.3+2.9 NS +11%
	B.	53.35±9.1	61.8+10.5 NS +16%	71.2+9.2 p<0.02 +33%
ASTROCYTES	A.	37.56+6.3	36.3+3.6 NS -3%	44.9+8.8 NS +19%
	B.	56.76+11.4	68.4+13.2 NS +7%	80+9.1 p<0.01 +41%
OLIGO CELLS	A.	5.27+1	5.77+1.2 NS +9%	7.29+1.1 p<0.02 +38%
	B.	33+7.1	34.4+5.4 NS +4%	42.2+4.6 p<0.05 +28%
SYNAPTOSOMES	B.	20+2	24+3 p<0.05 +20%	31+2 p<0.001 +55%

Units: A:pmoles of NADH oxidized/cell/hr; B: µmoles of NADH oxidized/me protein/hr. Each value is Mean+S.D.
No. of experiments are 5.

TABLE **5.41**

LACTATE DEHYDROGENASE (PYRUVATE --> LACTATE) ACTIVITY IN SYNAPTOSOMES AND CELLULAR FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION		NORMAL	SUBACUTE	ACUTE
NEURONS	A.	14.3+2.5	11.8+1.56 NS -17%	11.4+2.6 NS -20%
	B.	29.3+6.1	27.8+1.1 NS -7%	24.6+3.9 NS -17%
ASTROCYTES	A.	15.8+0.88	16.9+1.7 NS +7%	10+1.6 p<0.001 -37%
	B.	23.8±2	20.7±2.8 NS -13%	19.2+3.7 p<0.05 -19%
OLIGO CELLS	A.	5.26+0.58	6.2+0.55 p<0.05 +18%	6.1+0.52 p<0.05 +16%
	B.	33+4.5	37.2+3.3 NS +13%	35.2+3.3 NS +7%
SYNAPTOSOMES	B.	121+9	95+8 p<0.005 -21%	108+4 p<0.02 -11%

Units: A: pmoles of NADH oxidized/cell/hr; B: μmoles of NADH oxidized/mg protein/hr. Each value ia Mean+S.D.
No. of experiments are 5.

TABLE 5.42

LACTATE DEHYDROGENASE (LACTATE-->PYRUVATE) ACTIVITY IN SYNAPTOSOMES AND CELLULAR FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION		NORMAL	SUBACUTE	ACUTE
NEURONS	A.	4.4+0.2	3.8+0.48 p<0.05 -14%	4.1+0.6 NS -7%
	B.	8.4+0.9	8.8+0.9 NS + 5%	9.4+1.2 NS +12%
ASTROCYTES	A.	4.7+0.3	4.9+0.5 NS +4%	5.7+0.6 p<0.001 -21%
	B.	7.1+0.5	7.8+1.1 NS +10%	7.9+1.0 NS +11%
OLIGO CELLS	A.	2.10+0.2	2.3+0.14 NS +8%	2.4+0.13 p<0.05 +14%
	B.	13+1.4	13.4+1.4 NS +3%	14.1+1.3 NS +8%
SYNAPTOSOMES	B.	23+3	34±4 p<0.005 +48%	47+8 p<0.001 +104%

Units: A: pmoles of NAD reduced; B: μmoles of NAD reduced/Dig protein/hr. Each value is Mean+S.D. No. of experiments are 5.

TABLE 5.43

LEVELS OF CITRATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	5.2±0.4	7.3±0.6	0.97±0.08
SUBACUTE	4.9+0.3 S -6%	6.6+0.4 NS -10*	0.90+0.08 NS -7%
ACUTE	5.6+0.45 NS +8%	10.3+0.8 p<0.001 +41%	1.15+0.1 p<0.05 +19%
1mM	5.3+0.4 NS +2%	7.3+0.67 NC	1.00+0.06 NS +3%
5mM	5.4+0.4 NS +4%	9.0+0.53 p<0.01 +23%	1.05+0.11 NS +8%

Units: fmoles/cell. Each value is mean±S.D. Number of experiments are five.

TABLE 5.44

LEVELS OF CITRATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	12.0+1.0	11.(HO.9	6.00±0.8
SUBACUTE	11.8+0.8 NS -2%	10.2+0.6 NS -7*	5.50+0.5 NS -8%
ACUTE	12.4±1.0 NS +3%	14.7+1.2 p<0.005 +35%	6.80+0.7 NS +13%
1mM	12.2+1.0 NS +2%	11.0+1.0 NC	6.20+0.4 NS +3%
5mM	12.5+0.9 NS +4%	13.5+0.8 p<0.01 +23%	6.50+0.7 NS +8%

Units: nmoles/mg protein. Each value is mean+S.D. Number of experiments are five.

TABLE 5.45

LEVELS OF CITRATE IN NEURONS SYNAPTOSOMES
OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	SYNAPTOSOMES
NORMAL	12.0±1.0	18.0±1.2
SUBACUTE	11.8±0.8 NS -2%	17.2+0.9 NS -4%
ACUTE	12.4+1.0 NS +3%	19.2+1.8 NS +7%
1mM	12.2+1.0 NS +2%	18.2+2.0 NS +1%
5mM	12.5+0.9 NS +4%	19.0+1.7 NS +6%

Units: nmoles/mg protein. Each value ia mean±S.D. Number of experiments are five.

LEVELS OF ISOCITRATE IN NEURONS, ASTROCYTES AND
OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	4.3±0.39	8.0±0.67	0.80±0.06
SUBACUTE	4.8+0.4 NS +12%	8.4+1.3 NS + 5%	0.80+0.08 NC
ACUTE	6.8+0.6 p<0.001 +58%	14.0+1.4 p<0.001 +75%	1.10+0.08 p<0.001 +38%
1mM	5.2+0.4 p<0.02 +21%	8.7+0.8 NS +9%	0.81+0.05 NS +1%
5mM	6.5+0.5 p<0.001 +51%	12.0+1.0 p<0.001 +50%	1.00+0.08 p<0.01 +25%

Units: fmoles/cell. Each value is mean±S.D. Number of experiments are five.

TABLE 5.47

LEVELS OF ISOCITRATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	10.0±0.9	12.0±1.0	5.0+0.4
SUBACUTE	11.5+0.9 NS +15%	13.0+2.0 NS +8*	4.8+0.5 NS -4%
ACUTE	15.0+1.4 p<0.001 +50%	20.0+2.0 p<0.001 +67%	6.5+0.5 p<0.005 +30%
1mM	12.0+1.0 p<0.025 +20%	1.3.0+1.2 NS +8%	5.0+0.3 NC
5mM	15.0+1.2 p<0.001 +50%	18.0+1.5 p<0.001 +50%	6.1+0.5 p<0.02 +22%

Units: nmoles/mg protein. Each value is mean±S.D. Number of experiments are five.

TABLE 5.48

LEVELS OF ISOCITRATE IN NEURONS AND SYNAPTOSOMES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	SYNAPTOSOMES
NORMAL	10.0+0.9	18.0±2.0
SUBACUTE	11.5+0.9 NS +15%	17.5+1.6 NS -3%
ACUTE	15.0+1.4 p<0.001 +50%	21.0+2.2 NS +17%
1mM 12.0 + 1.0	18.5 + 2.0 p<0.025 +20%	NS +3%
5mM	15.0+1.2 p<0.001 +50%	19.5+1.8 NS +8%

Units: nmoles/mg protein. Each value Is roean+^S.D. Number of experiments are five.

TABLE 5.49

LEVELS OF 2-OXOGLUTARATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	5.2±0.48	7.3±0.67	0.97±0.08
SUBACUTE	6.7+0.5 p<0.005 +30%	8.4+0.9 NS +15%	1.25+0.135 p<0.02 +29%
ACUTE	9.0+1.0 p<0.001 +73%	12.9+1.4 p<0.001 +77%	1.37+0.13 p<0.005 +41%
1mM	6.5+0.7 p<0.025 +25%	8.3+0.67 NS +14%	1.00+0.1 NS +3%
5mM	7.8+0.4 p<0.001 +50%	10.7+1.2 p<0.00~5 +47%	1.30+0.13 p<0.005 +34%

Unita: fmoles/cell. Each value is mean±S.D. Number of experiments are five.

TABLE 5.50

LEVELS OF 2-OXOGLUTARATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	12.0±1.1	11.0±1.0	6.0+0.5
SUBACUTE	16.2+1.2 p<0.005 +35%	13.0+1.4 NS +18%	7.5+0.81 p<0.02 +25%
ACUTE	20.0+2.3 p<0.001 +67*	18.5+2.0 p<0.001 +68%	8.1+0.8 p<0.005 +35%
1mM	15.0+1.6 p<0.025 +25*	12.5+1.0 NS +14%	6.5+0.6 NS +8*
5mM	18.0+1.0 p<0.001 +50*	16.0+1.8 p<0.005 +45*	8.0+0.8 p<0.01 +33*

Units: nmoles/mg protein. Each value la mean±S.D. Number of experiments are five.

TABLE 5.51

LEVELS OF 2-OXOGLUTARATE IN NEURONS AND SYNAPTOSOMES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	SYNAPTOSOMES
NORMAL	12.0±1.1	19.0±2.0
SUBACUTE	16.2+1.2 p<0.005 +35%	24.0+3.0 p<0.05 +26%
ACUTE	20.0±2.3 p<0.001 +67%	30.0+4.0 p<0.005 +58%
1mM	15.0±1.6 p<0.025 +25%	22.0±1.0 p<0.05 +16%
5mM	18.0±1.0 p<0.001 +50%	26.0+3.0 p<0.01 +37%

Units: nmoles/mg protein. Each value is mean±S.D. Number of experiments are five.

TABLE 5.52

LEVELS OF MALATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	6.5±0.3	12.0±1.3	1.30±0.11
SUBACUTE	6.7+0.4 NS +3%	13.5+1.3 NS +13%	1.80+0.1 p<0.001 +38%
ACUTE	9.0±0.86 p<0.005 +38*	17.5+0.8 p<0.001 +46%	2.00+0.17 p<0.001 +59%
1mM	6.8+0.3 NS +5*	13.3+1.3 NS +11*	1.45+0.13 NS +12*
5mM	8.3+0.87 p<0.01 +28*	15.3+2.0 p<0.05 +28*	1.77+0.1 p<0.001 +36*

Units: fmoles/cell. Each value is mean+S.D. Number of experiments are five.

TABLE 5.53

LEVELS OF MALATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	15.0±0.6	18.0±2.0	8.0±0.7
SUBACUTE	16.0±0.9 NS +7%	21.0+2.0 NS +17%	11.0+0.8 p<0.001 +38*
ACUTE	20.0±1.9 p<0.005 +33%	25.0+1.2 p<0.001 +39%	12.0±1.0 p<0.001 +50%
1mM	15.7±0.7 NS +5*	20.0+2.0 NS + 11*	9.0+0.8 NS + 12%
5mM	19.0+2.0 p<0.01 +27%	23.0+3.0 p<0.05 +28%	11.0+0.9 p<0.005 +38%

Units: nmoles/mg protein. Each value is mean±S.D. Number of experiments are five.

TABLE 5.54

LEVELS OF MALATE IN NEURONS AND SYNAPTOSOMES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	SYNAPTOSOMES
NORMAL	15.0+0.6	19.0±2.0
SUBACUTE	16.0+0.9 NS +7%	22.0+2.0 NS +16%
ACUTE	20.0+1.9 p<0.005 +33*	26.0+3.0 p<0.01 +37%
1mM	15.7+0.7 NS +5*	21.0+1.0 NS +10*
5mM	19.0+2.0 p<0.01 +27*	25.0+2.0 p<0.01 +32*

Units: nmoles/mg protein. Each value la mean±S.D. Number of experiments are five.

TABLE 5.55

LEVELS OF ATP IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	13.5±1.1	23.0±2.53	2.90±0.3
SU3ACUTE	12.2+0.9 NS -10%	23.7 + 2.4 NS + 3%	3.30 + 0.3 NS +145
ACUTE	15.0+1.4 NS +11%	22.8+2.4 NS -1*	3.30+0.32 NS +14%
1mM	13.6+1.6 NS +1%	22.7+2.4 N.S -1\	2.85+0.26 NS -2%
5mM 15.0 + 1.4	23.0 + 2.1 NS +11%	2.98 + 0.3 NC	NS +3%

Units: fmoles/cell. Each value is mean±S.D. Number of experiments are five.

TABLE 5.56

LEVELS OF ATP IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	**NEURONS**	**ASTROCYTES**	**OLIGODENDROCYTES**
NORMAL	31.0±2.5	35.0±3.8	18.0±2.0
SUBACUTE	29.2+2.2 NS -6%	36.8+3.7 NS +5%	20.0+2.0 NS +7%
ACUTE	33.2+3.2 NS +7%	32.6+3.4 NS -7%	19.5+1.9 NS -5%
1mM	31.3±2.4 NS -1%	34.0+3.6 NS -2%	17.7+1.6 NS -1%
5mM	32.6+2.8 NS +5%	34.5+3.2 NS -1%	18.5+1.9 NS +3%

Units: nmoles/mg protein. Each value is mean+S.D. Number of experiments are five.

TABLE 5.57

LEVELS OF **ATP** IN NEURONS AND SYNAPTOSOMES
OF NORMAL AND **HYPERAMMONEMIC** RAT BRAIN

STATE	NEURONS	SYNAPTOSOMES
NORMAL	31.0±2.5	32.0±4.0
SUBACUTE	29.2±2.2 NS -6*	34.0+2.7 NS +6%
ACUTE	33.2+3.2 NS +7%	35.5+3.8 NS +11%
1mM	31.3+2.4 NS -1%	31.0+3.0 NS -3*
5mM	32.6±2.8 NS +5*	33.3+3.1 NS +4%

Units: nmoles/mg protein. Each value is mean±S.D. Number of experiments are five.

TABLE 5.58

LEVELS OF ADP IN NEURONS, ASTROCYTES
OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	9.6±0.65	16.0±1.2	1.60±0.13
SUBACUTE	8.7+0.8 NS -9%	16.2+1.2 NS +1%	1.70+0.13 NS + 6*
ACUTE	10.2±0.9 NS +6%	17.8+1.4 NS +11*	1.83±0.15 NS +14%
1mM	9.4+0.6 S -2%	15.5+1.1 NS -3%	1.66+0.13 NS +4%
5mM	10.2+0.7 NS +6%	17.1+1.3 NS +7%	1.70+0.14 NS +6%

Units: fmoles/cell. Each value is mean±S.D. Number of experiments are five.

TABLE 5.59

LEVELS OF **ADP** IN NEURONS, ASTROCYTES
OLIGODENDROCYTES OF NORMAL AND **HYPERAMMONEMIC** RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	22.0+1.5	24.0+1.8	9.8±0.8
SUBACUTE	20.9+2.0 NS -54	25.1+1.8 NS + 54	10.1+0.9 NS +4%
ACUTE	22.5+1.9 NS +2 %	25.4+2.0 NS + 64	10.8+0.9 NS +94
1mM	21.7+1.4 NS -24	23.3+1.7 NS -34	10.3+0.8 NS +54
5mM	23.5+1.7 NS +74	25.7+2.0 NS +74	10.6+0.9 NS +84

Units: nmoles/mg protein. Each value is mean±S.D. Number of experiments are five.

TABLE 5.60

LEVELS OF ADP IN NEURONS AND SYNAPTOSOMES
OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	SYNAPTOSOMES
NORMAL	22.0+1.5	14.0+1.0
SUBACUTE	20.9+2.0 NS -54	14.6+1.1 NS +4 4
ACUTE	22.5+1.9 NS +24	15.5+0.9 NS +114
1mM	21.7+1.4 NS -24	13.7+0.8 NS -24
5mM	23.5+1.7 NS +74	14.7+1.0 NS +54

Units: nmoles/mg protein. Each value is mean±S.D. Number of experiments are five.

BLE 5.61

PYRUVATE DEHYDROGENASE ACTIVITY IN SYNAPTOSOMES AND CELLULAR FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION		NORMAL	SUBACUTE	ACUTE
NEURONS	A.	10.9+1.5.	12.3+1.3 NS +13%	11.2+1.97 NS +3%
	B.	25 + 1.7 29 + 4.4	31 . 6 + 4 . 4 NS +17*	p<0.02 +27%
ASTROCYTES	A.	13.3+1.6	21+1.5 p<0.001 +58*	22.8+4.3 p<0.005 **+71***
	B.	20 + 1.8	25+3.9 p<0.05 +25%	27 + 3.6 p<0.01 +34%
OLIGO CELLS	A.	1.92+0.3	2.6+0.54 p<0.05 +35%	2.64+0.2 p<0.005 +38*
	B.	12+2	15.4+2.5 p<0.05 +28*	15.4+1.7 p<0.025 +28*
SYNAPTOSOMES	B.	3.42+0.33	8.7+0.77 p<0.001 +154*	13.2+2.6 p<0.001 **+285%**

Units: A: pmoles of NAD reduced/cell/hr; B: µmoles of NAD reduced/mg protein/hr. **Number of experiments are five. Each value is mean +** S.D.

TABLE 5.62

CITRATE SYNTHETASE ACTIVITY IN SYNAPTOSOMES AND CELLULAR FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION		NORMAL	SUBACUTE	ACUTE
NEURONS	A.	7.82+0.9	.12.8+1.1 p<0.001 +64%	13.4+3.2 p<0.01 +71%
	B.	20 . 9 + 6 . 1	23+4 . 3 29 + 4 . 5 NS +12%	p<0.05 +39%
ASTROCYTES	A.	11.3+1.2	14.6+2.2 p<0.02 +29%	12.9+0.7 p<0.05 +15%
	B.	16+2.6	20.2+3.7 p<0.05 +26%	24.4+5.4 p<0.02 +52%
OLIGO CELLS	A.	1.44+0.18	1.6+0.33 NS +11%	1.91+0.18 p<0.02 +33%
	B.	9+1.2	9.3+1.5 NS . +3%	10.8+1.5 NS +20%
SYNAPTOSOMES	B.	32+4	37+8 NS +16%	32+2 NC

Unite: A: pinoles of DTNB reduced/cell/hr; B: µmoles of DTNB reduced/mg protein/hr. Number of experiments are five. Each value is mean + S.D.

TABLE 5.63

ISOCITRATE DEHYDROGENASE ACTIVITY IN SYNAPTOSOMES AND CELLULAR FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION		NORMAL	SUBACUTE	ACUTE
NEURONS	A.	7.2+0.6	8.75+1.0 p<0.02 +22%	11.8+1 p<0.001 +64%
	B.	16.7+1.1	21+2.4 p<0.05 +27%	26+2 p<0.0Ql +56%
ASTROCYTES	A.	9.6+1.27	12.2+1.4 p<0.05 +27%	15.7+1.3 p<0.001 +64%
	B.	14.4 + 1.9	19 + 2.2 p<0.025 +32%	22 + 1.8 p<0.005 +53%
OLIGO CELLS	A.	1.98+0.13	2.82+0.3 p<0.005 +42%	3.1+0.27 p<0.001 +57%
	B.	12.2+0.8	16.9+1.8 p<0.005 +39%	18+1.6 p<0.001 +48%
SYNAPTOSOMES	B.	11.4+1.72	15.7+0.99 p<0.005 +32%	16.2+3.3 p<0.025 +42%

Units: A: pmoles of NAD reduced/cell/hr; B: μmoles of NAD reduced/mg protein/hr. Number of experiments are five. Each value is mean + S.D.

TABLE 5.64

2-OXOGLUTARATE DEHYDROGENASE ACTIVITY IN SYNAPTOSOMES AND CELLULAR FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION		NORMAL	SUBACUTE	ACUTE
NEURONS	A.	9.28+0.9	11.64+2.1 p<0.05 +25%	14.9+3.5 p<0.01 +61%
	B.	21.6+3.7	27+3.3 p<0.05 +26%	32.2+4 p<0.005 +50%
ASTROCYTES	A.	11.7+1.9	18.8+2.7 p<0.005 +61%	31.9+2.9 p<0.001 +173%
	B.	17.5+2.6	26.8+3.7 p<0.005 +53%	34.2+3.3 p<0.001 +95%
OLIGO CELLS	A.	2.4+0.5	2.8+0.5 NS +15%	4.28+1.3 p<0.025 +76%
	B.	15.2+2.6	18.2+3.7 NS . +20%	20.5+3.3 p<0.025 +35%
SYNAPTOSOMES	B.	19.5+1.99	25+1.62 p<0.005 +28%	24+3.3 p<0.05 +23%

Units: A: pinoles of NAD reduced/cell/hr; B: μmoles of NAD^+ reduced/me protein/hr. Number of experiments are five. Each value is mean + S.D.

TABLE. 5.65

SUCCINATE DEHYDROGENASE ACTIVITY IN SYNAPTOSOMES AND CELLULAR FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION		NORMAL	SUBACUTE	ACUTE
NEURONS	A.	0.28±0.034	0.45+0.11 p<0.02 +61%	0.61+0.07 p<0.001 +118%
	B.	0.51±0.17	0.96±0.19 p<0.005 + 88%	1.23+0.22 p<0.001 +141%
ASTROCYTES	A.	0.22±0.024	0.51±0.097 p<0.001 +137%	0.59+0.087 p<0.001 +174%
	B.	0.3+0.06	0.69+0.08 p<0.001 +130%	0.9+0.17 p<0.001 +207%
OLIGO CELLS	A.	0.03+0.001	0.03+0.004 NS +7%	0.04+0.001 p<0.001 +43%
	B.	0.17+0.02	0.18+0.03 NS +6%	0.23+0.04 p<0.02 +35%
SYNAPTOSOMES	B.	0.53+0.06	0.73+0.016 p<0.001 +38%	0.92+0.15 p<0.005 +74%

Units: A: pinoles of succinate oxidized/cell/hr; B: µmoles of succinate oxidized/mg protein/hr. Number of experiments are five. Each value is mean + S.O.

TABLE 5.66

MALATE DEHYDROGENASE (MALATE —> OXALOACETATE) ACTIVITY IN SYNAPTOSOMES AND CELLULAR FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION		NORMAL	SUBACUTE	ACUTE
NEURONS	A.	29+1.3	20.6+2.6 p<0.001 -30%	18.9+2.75 p<0.001 -35%
	B.	55+6.8	45+5.6 p<0.05 -18%	41+5 p<0.01 -25%
ASTROCYTES	A.	104+8.7	101+18.6 NS -3%	84+10 p<0.01 -19%
	B.	156+6.4	141+11.1 NS -9%	116+14.5 p<0.001 -25%
OLIGO CELLS	A.	6.86+0.9	6.5±0.6 NS -5%	7.3+1.1 NS +6%
	B.	43+6.4	39.4±5.3 NS -8%	41+5.6 NS -4%
SYNAPTOSOMES	B.	388+33	285+24 P<0.005 -27%	251±21 P<0.005 -35%

Units: A: pr, lea of NAD reduced/cell/hr; B: µmoles of NAD reduced/me protein/hr. Number of experiments are five. Each value is mean + S.D.

TABLE 5.67

MALATE DEHYDROGENASE (OXALOACETATE --> MALATE) ACTIVITY IN SYNAPTOSOMES AND CELLULAR FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION		NORMAL	SUBACUTE	ACUTE
NEURONS	A.	35±0.7	42.3+1.8 p<0.001 +20%	34+6.8 NS -3%
	B.	70+5	93+13.6 p<0.01 +34%	75+8 NS +8%
ASTROCYTES	A.	42+4.2	42.6+2.2 NS +2*	41.7+7 NS -1*
	B.	63±11	78±7.0 p<0.025 +30%	71.6±8 NS +19%
OLIGO CELLS	A.	5.9+0.5	6.6+0.4 NS +12%	6.9+1 NS +17%
	B.	37+4.2	42.4+4.7 NS +15%	41+5.4 NS +12%
SYNAPTOSOMES	B.	462+35	556+28 p<0.005 +20%	383+25 p<0.005 -17%

Units: A: pmoles of NADH oxidized/cell/hr; B: µmoles of NADH oxidized/mg protein/hr. Number of experiments are five. Each value *La* mean + S.D.

TABLE 5.68

ASPARTATE AMINOTRANSFERASE ACTIVITY IN SYNAPTOSOMES AND CELLULAR FRACTIONS OF NORMAL AND HYPERAMMONEMIC RAT BRAINS

FRACTION		NORMAL	SUBACUTE	ACUTE
NEURONS	A.	13.4+2.5	13.5+1.6 *US*	11.9+2.5 *NS* -11%
	B.	27+5	32+3.7 NS + 17%	27.8+4.6 NS + 2%
ASTROCYTES	A.	23.5+3.2	14.4+4.9 p<0.02 -39V	16+3.2 p<0.01 -32%
	B.	35+5	24+4.5 p<0.025 -31%	21.8+2.8 p<0.001 -38%
OLIGO CELLS	A.	1 . 9±0 . 2	1.45+0.3 p<0.02 -24%	1.3+0.3 p<0.005 -32%
	B.	12+1.2	8.7+1.6 p<0.01 -28%	8.2+1.6 p<0.005 -32%
SYNAPTOSOMES	B.	192+8.3	108+13 P<0.001 -44%	84+12 P<0.001 -56%

Units: A: proles of NADH oxidized/cell/hr; B: umoles of NADH oxidized/mg protein/hr. Number of experiments are five. Each value is mean + S.D.

TABLE 5.69

LEVELS OF ASPARTATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	12.2 + 1.3	12.7±1.3	1.45+0.13
SUBACUTE	10.0±1.25 NS -18%	9.7+0.8 p<0.01 -244	1.17+0.12 p<0.02 -194
ACUTE	9.0±0.45 p<0.005 -264	7.0+0.63 p<0.001 -454	1.00+0.08 p<0.001 -31%
1mM	11.3+0.9 NS -74	11.3+1.0 NS -11%	1.32+0.11 NS -9%
5mM	10.4±1.3 NS -15%	8.0+0.7 p<0.001 -374	1.13+0.09 p<0.01 -224

Units: f moles/cel l. Each value la mean±S.D. Number of experiments are five.

TABLE 5.70

LEVELS OF ASPARTATE IN NEURONS, ASTROCYTES AND OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	ASTROCYTES	OLIGODENDROCYTES
NORMAL	28.0±3.0	19.0+2.0	9.0±0.8
SUBACUTE	24.0+3.0 NS -144	12.0+1.2 p<0.001 -374	7.0+0 7 p<0.01 -224
ACUTE	20.0+1.0 p<0.005 -28%	10.0+0.9 p<0.001 -474	6.0+0.5 p<0.001 -334
1mM	26.0+2.0 NS -74	17.0.1.5 NS -114	8.2+0.7 NS -94
5mM	24.0+3.0 NS -144	12.0+1.2 p<0.001 -374	7.0+0.6 p<0.01 -224

Units: nmoles/mg protein. Each value is mean+S.D. Number of experiments are five.

TABLE 5.71

LEVELS OF ASPARTATE IN NEURONS AND SYNAPTOSOMES OLIGODENDROCYTES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	SYNAPTOSOMES
NORMAL	28.0±3.0	36.0±4.0
SUBACUTE	24.0+3.0 NS -14%	30.0+3.0 NS -17*
ACUTE	20.0+1.0 p<0.005 -28%	28.0+2.0 p<0.02 -22%
1mM	26.0+2.0 NS -7%	31.0±4.0 NS -14%
5mM	24.0+3.0 NS -14%	28.0+3.0 p<0.02 -22%

Units: nmoles/mg protein. Each value is mean+S.D. Number of experiments are five.

TABLE 5.72

LEVELS OF GLUTAMATE IN NEURONS AND SYNAPTOSOMES OF NORMAL AND HYPERAMMONEMIC RAT BRAIN

STATE	NEURONS	SYNAPTOSOMES
NORMAL	ND	170±20
SUBACUTE	ND	160+20 NS -6%
ACUTE	ND	150+20 NS -12 %
1mM	ND	180+30 NS +6%
5mM	ND	150+30 NS -12%

Units: nmoles/mg protein. Each value is mean+S.D. Number of experiments are five.

SUMMARY

SUMMARY

(1) Metabolism of glucose and the activities of enzymes of enzymes involved in glucose metabolism were studied in the homogenates, subcellular fractions and three different cell types (neurons, astrocytes and oligodendrocytes) were studied in the brains of normal and hyperammmonemic rats.

(2) Hyperammonemia waa induced by an intraperitoneal administration of either a subacute dose (0.35 mmoles/100 gm body-weight) or an acute dose (2.5 mmoles/100 gm body weight) of ammonium acetate. Brains were isolated 25 min after the administration of ammonium acetate. Homogenates were prepared from three different brain regions (cerebral cortex, cerebellum and brain stem) while subcellular fractions and different cell types were isolated from cerebral cortex only. Similar preparations were also obtained from the brains of normoammonemic rats.

(3) In general, administration of ammonium acetate resulted in an elevation in the activities of all the glycolytic enzymes in the three regions of the brain with very few exceptions. This change was seen in both subacute and acute group of rats. Similarly, the activities of pyruvate dehydrogenase and other enzymes of citric acid cycle were elevated under these conditions. The only exception to this was the

decrease in malate dehydrogenase activity (malate ⟶ oxaloacetate) in all the three regions of brain in hyperammonemic states. Aapartate aminotransferase activity was also suppressed under these conditions. From these results, it was suggested that the operational rates of glycolytic pathway and citric acid cycle may not be compromised in brain in hyperammonemic states. How ever, the operation of malate-aspartate shuttle may be affected under these conditions.

(4) Cytosol and mitochondria were prepared from the cerebral cortex of normal and hyperammonemic rat brain by differential and density gradient (Ficoll) centrifugations. These were incubated with [U-^{14}C]glucose and the production of $^{14}CO_2$ was measured. In fractions isolated from the brains of hyperammonemic rats, there was an increase in the $^{14}CO_2$ production. Addition of 5 mM ammonium acetate to the subcellular fractions of normal rat Drain also enhanced the production of $^{14}CO_2$ but it was statistically significant at 5 mM ammonium acetate.

(5) These subcellular fractions were incubated with glucose for 30 min and the levels of glucose-6-phosphate, fructose-6-phosphate, phosphoenolpyruvate, pyruvate, lactate, citrate, isocitrate, 2-oxoglutarate, malate, glutamate, aapartate, ATP and ADP were measured. In general, there was an increase in the contents of all these metabolites except lactate, ATP,

ADP, glutamate and aspartate. More or leas similar results were obtained when the subcellular fractions of cerebral cortex of normal rata were incubated with 1 and 5 rafl ammonium acetate and glucose. In all these conditions, a fall in the contents of lactate, aspartate and glutamate was observed. These observations supported the above suggestion (see above -3).

(6) Activities of enzymes of glycolytic pathway, pyruvate dehydrogenase and of citric acid cycle were also determined in the cortical cytosol and mitochondria of normal and hyperammonemic rats. The activities of all the glycolytic enzymes (except lactate dehydrogenase), pyruvate dehydrogenase and of citric acid cycle (except malate dehydrogenase, malate ——> oxaloacetate) were observed to be elevated.

(7) Lactate dehydrogenase activity (pyruvate ——> lactate) and malate dehydrogenase (malate ——> oxaloacetate) were found to be suppressed under these conditions in these subcellular fractions. Similarly, the activities of aspartate aminotransferase and alanine aminotransferase were also suppressed.

(8) An interesting observation of this study was an elevation in the activities of pyruvate dehydrogenase and citric acid cycle in the cytosol in hyperammonemic states. This result was interpreted to be due to the presence of a small popu-

lation of mitochondria which are vulnerable to elevated levels of ammonia in brain. These mitochondria might rupture and release their contents into the cytosol in hyperammonemic states.

(9) Neuronal perikarya, aatrocytes, oligodendrocytes and synaptosomes were also prepared from the cerebral cortex of normal and hyperammonemic rate. Theae preparations were characterized by light microscopy (except eynaptosomes) and by using marker enzymes.

(10) Theae cell preparations and synaptosomes were incubated with [U-^{14}C]glucose and production of $^{14}CO_2$ was determined. There was an increase in the production of $^{14}CO_2$ from glucose in the preparations from hyperammonemic rats.

(11) Theae preparations were incubated with glucose and the metabolites (aee above -5) were determined. Excepting glutamate, all the above mentioned metabolites were detectable in these preparations and the glutamate content was below the level of detection under these experimental conditions.

(12) The levels of all the metabolites (except citrate, aspartate, ATP and ADP) were elevated in all the preparations (when they were incubated with glucose) from the hyperammonemic rats. This effect was mimicked to a large extent by the in vitro addition of 1 and 5 mM ammonium acetate to the

preparations from normal animals. Aspartate levels were observed to decrease under these conditions and those of ATP and ADP were unaltered.

(13) Activities of enzymes of glycolytic pathway, of pyruvate dehydrogenase and of citric acid cycle were also determined in the above preparations from normal and hyperammonemic rats. There was a generalized increase in the activities of all these enzymes except malate dehydrogenase (malate ⟶ oxaloacetate) and aspartate aminotransferase.

(14) From these results it is suggested that the operational rates of glycolytic pathway and of citric acid cycle, and thus glucose utilization, may not be compromized atleast in acute hyperammonemic states but the transport of reducing equivalents across mitochondrial membranes may be affected under these conditions.

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Cerebral Citric Acid Cycle Enzymes in Methionine Sulfoximine Toxicity

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The activity levels of pyruvate dehydrogenase. enzymes of the citric acid cycle, a.spartate and alanine aminotransferases, and $NADP^+$-isocitrate dehydrogenase were determined in the cerebral cortex, cerebellum, brain stem, corpus striatum, hippocampus, and midbrain regions of normal rats and rats injected with acute and subacute doses of methionine sulfoximine (MSI). In both conditions there was an elevation in the activities of pyruvate dehydrogenase and all the enzymes of the citric acid cycle except malate dehydrogenase. whereas the activities of aminotransfera.ses and $NADP^+$-isocitrate dehydrogenase were suppressed in all the cerebral regions. It is suggested that the operational rates of the citric acid cycle would be enhanced in MSI-induced hyperammonemia and that there might be a derangement in the transport of reducing equivalents across mitochondrial membranes. It has been suggested that the convulsant action of the drug is due to its effects on ionic gradients and may not be due to depletion of α-ketoglutarate from the citric acid cycle.

Key words: methionine sulfoximine. **hyperammonemia, brain,** citric acid cycle

INTRODUCTION

Methionine sulfoximine (MSI) is a potent convulsant used to study experimental epilepsy, and the symptoms observed in experimental animals have been very close to those encountered clinically in humans. The principle mechanism of action of MSI has been shown to be irreversible inhibition of glutamine synthetase. an enzyme involved in detoxifying ammonia in extrahepatic tissues such as brain [Sellinger and Weiler. 1963; Lamar and Sellinger, 1965; Lamar, 1968; Ronzio et al, 1969], thus leading to a hyperammonemic state (Tews and Stone, 1964; Folbergrova et al, 1969; Subbalakshmi and Murthy. 1984).

Elevated ammonia levels in brain may interfere with the energy production in brain by draining α-ketoglutarate, from the citric acid cycle, into glutamate formation

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by reductive amination mediated by glutamate dehydrogenase [Bessman and Bessman, 1955]. However, the evidence for alteration in the levels of α-ketoglutarate and ATP in brain in **hyperammonemic** states is contradictory [Schenker et al. 1967; Hindfelt and Seisjo, 1970; Hawkins et al. 1973; Vergara et al. 1974; McCandless and Schenker, 1981]. Though much **information** is available **on** the content **of the** citric acid cycle intermediates in hyperammonemic states, less attention has been focused on MSI toxicity.

We report an elevation in the activities of pyruvate dehydrogenase and key **enzymes** of the citric acid cycle except malate dehydrogenase in MSI toxicity. furthermore, we observed a suppression in the activities of **aspartate** and alanine aminotransferases and $NADP^+$-dependent isocitrate dehydrogenase. These **results** indicated an increase, rather than a decrease, in the operational rates of cerebral citric acid cycle in MSI toxicity. It is also suggested that the transport of reducing equivalents across (he mitochondrial **membranes** might be impaired **in** MSI **toxicity.**

MATERIALS AND METHODS

Coenzyme A, acetyl coenzyme A, thiamine **pyrophosphate,** sodium **pyruvate,** α-ketoglutarate, DL-dithiothreitol, L-malic acid, **DL-isocitratc,** succinate, **oxaloacetate,** ADP, L-aspartatc, L alanine; lactate and malate dehydrogenases; dithiobis nitrobenzoic acid, phenazine methosulfate, NAD^+, NADH, and $NADP^+$ were purchased from Sigma Chemical Company, St. Louis. Missouri. 2-(4-Iodophenyl)-3-(4-nilrophenyl)-5-phenyl tetrazolium chloride (INT) was from **Loba** Chemie, India. Dichlorophenol indophenol was purchased from V.P. Chest **Institute,** India and **Triton** X-l(X) was from Koch-Light Laboratories. UK. The rest of the chemicals were **of** AnalaR or GR grade and were purchased locally. The commercial **enzymes** were **dialyzed to** remove ammonium sulfate **and** were **reconstituted** into 50% (v/v) glycerol **to** give a protein value of 0.5 ing/ml.

Adult Wistar rats **of 250-300** g body weight and of either sex from an inbred colony **of the** vivarium were used. **Food** (balanced pellet diet from **Pragathi** Animal **Feeds,** India) and water was given ad libitum.

Drug Treatment

Methionine sulfoximine was administered intraperitoneally with saline as a carrier. Tor acute experiments a dose of **300** mg/kg body weight was used, **for** the subacute group a dose of 150mg/kg body weight was used. Control animals received none. The animals in the acute group were decapitated at the end of 3.5 hr and those of the subacutc group at the end of 17.5 hr. Brains were quickly removed and washed with ice-cold saline. Cerebral cortex, cerebellum, brain stem, hippocampus, and corpus striatum were separated at 4°C; the rest of the brain designated as midbrain includes thalamus, **hypothalamus,** and related structures. **Finally, 10%** homogenates (w/v) were prepared in 0.32 M sucrose containing 0.2% (v/v) Triton X-100.

In Vitro Experiments

To the homogenates of **different** cerebral regions that were prepared from normal animals, methionine sulfoximinc was added to a final concentration of 1 μM/ml. and the enzyme assays were **performed** with this preparation.

Enzyme Assays

In the assays of dehydrogenases (except succinate dehydrogenase) phenazine methosuitate was used as intermediary electron acceptor and INT as the final electron acceptor. The reduction of INT was followed in a spectrophotometer at 500 nm. In the assay of succinate dehydrogenase the final electron acceptor was dicholorophenol indophenol. All assays were carried out at 37°C in a Gilford spectrophotometer with a thermoprogrammer.

Pyruvate dehydrogenase was assayed by the method of Hinman and Blass [1981]: citrate synthase by the method of Shepherd and Garland [1969]; NAD^+-isocitrate dehydrogenase by the method of Plaut [1969]; α-ketoglutarate dehydrogenase by the method of Reed and Mukherjee [1969]; succinate dehydrogenase by the method of Veeger et al [1969]; malate dehydrogenase by the method of Yoshida [1969]; aspartate, alanine aminotransferases, and $NADP^+$-isocitrate dehydrogenase by the method of Bergmeyer and Bernt [1974a,b,c]. In all the assays the final volume was 250 μl, and 10 μl of 10% (w/v) homogenate was used except for aminotransferases. where only 1 μl was used. Protein content was determined by the biuret method as described by Varley [1969].

INT was converted to formazan by both enzymatic and chemical methods. In the former, purified malate dehydrogenase was used and NAD^+ concentrations were varied from 0.01 to 0.1 μm. In the chemical method INT concentration was varied from 20 to 100 nm, and reduction was carried by the addition of 10 μl of 1% ascorbic acid and 10 μl of 1 N NaOH in sodium phosphate buffer (12.5 μm; pH 7.8). A standard curve was prepared for NAD^+ and INT by correlating the optical density values obtained by these methods.

Statistical analysis of the method was carried out by the Student t test.

RESULTS

Behavioral Changes

The behavioral changes in rats following the administration of MSI observed in the present investigation were similar to those reported earlier [Subbalakshmi and Murthy, 1981, 1983, 1984; Subbalakshmi, 1981; 1984]. The preconvulsive phase included lethargy, abnormalities in gait and posture, and loss of righting reflexes. The onset of convulsions was noticed at the end of 3.5 hr after the administration of MSI in the acute group; in the subacute group it was at the end of 17.5 hr. Following this, the animals exhibited uncontrolled rolling along their body axis, and mortality after this period was high. Hence, the animals were sacrificed during convulsions.

Changes in Pyruvate Dehydrogenase and the Enzymes of the Citric Acid Cycle

In the normal animals, the activity of malate dehydrogenase was the highest and that of succinate dehydrogenase was the lowest. Activities of pyruvate. a-ketoglutarate, and malate dehydrogenases were higher in cerebral cortex, whereas brain stem, midbrain, and hippocampus had the highest levels of citrate synthase. NAD^+-isocitrate dehydrogenase, and succinate dehydrogenase, respectively (Tables I and II).

In animals injected with an acute dose of MSI, an increase in the activities of pyruvate dehydrogenase was noticed along with the enzymes of the citric acid cycle

TABLE I. Distribution and Levels of Pyruvate Dehydrogenase (PDH), Citrate Synthase (CS), and NAD$^+$-Isocitrate Dehydrogenase (ICDH) in Different Regions of Brain in Normal and Methionine Sulfoximine-Injected Rats

Enzyme	State	CC	CB	BS	CoSt	HC	MB
PDH	N	3.0 ± 0.35	1.6 ± 0.15	1.5 ± 0.2	1.3 ± 0.1	1.7 ± 0.3	1.7 ± 0.2
	A	23 ± 0.33*	22 ± 0.4*	17 ± 0.2*	22 ± 0.3*	21 ± 0.3*	22 ± 0.3*
	S	8.4 ± 0.7*	6.7 ± 0.2*	3.9 ± 0.1*	5.6 ± 0.2*	7 ± 0.3*	5.5 ± 0.4*
	I	4.5 ± 0.04*	4.0 ± 0.05*	3 ± 0.04*	2.6 ± 0.1*	4 ± 0.02*	5.4 ± 0.1*
CS	N	7.5 ± 0.8	8.9 ± 0.2	9.4 ± 0.6	7.8 ± 0.5	10.9 ± 0.2	8.2 ± 0.3
	A	9.1 ± 0.4**	10.3 ± 1.2***	9.5 ± 1.3	9.3 ± 0.9***	10 ± 0.4**	9 ± 0.8
	S	6.7 ± 0.4	7.3 ± 0.9***	5.3 ± 0.7*	6.4 ± 0.6***	9.7 ± 0.4**	7 ± 0.7***
	I	1.1 ± 0.2*	1.1 ± 0.08*	1.1 ± 0.1*	0.4 ± 0.05*	1.5 ± 0.2*	1.6 ± 0.1*
ICDH (NAD$^+$)	N	1.9 ± 0.17	2.2 ± 0.2	2.0 ± 0.14	1.8 ± 0.1	2.9 ± 0.28	2.4 ± 0.08
	A	9.2 ± 0.6*	9.0 ± 0.3*	8.1 ± 0.4*	8.8 ± 0.2*	7.7 ± 0.2*	8.8 ± 0.35*
	S	2.5 ± 0.1*	3.1 ± 0.05*	2.4 ± 0.1**	2.4 ± 0.1*	4.0 ± 0.07*	2.2 ± 0.05***
	I	2.7 ± 0.1*	3.8 ± 0.07*	2.9 ± 0.05*	3.5 ± 0.05*	4.4 ± 0.15*	2.9 ± 0.15*

N = Normal rats, A = Acute, S = Subacute group of rats; I = In Vitro (1 μmole of MSI was added to 1 ml homogenates of normal rats). CC = Cerebral cortex; CB = Cerebellum; BS = Brain stem; CoSt = Corpus striatum; HC = Hippocampus; MB = Midbrain. The number of animals in each group varied from three to six. Values are mean ± SD. Activities of PDH and ICDH (NAD$^+$) are expressed as μmoles of NAD$^+$ reduced per milligram protein per hour and that of CS as μmoles of citrate formed per milligram protein per hour. *= $P < 0.0005$; **= $P < 0.0025$; ***= $P < 0.01$ versus control value.

TABLE II. Distribution and Levels of α-Ketoglutarate Dehydrogenase (α-KGDH), Succinate Dehydrogenase (SDH), and Malate Dehydrogenase (MDH) in Different Regions of Brain in Normal and Methionine Sulfoximine-Injected Rats

Enzyme	State	CC	CB	BS	CoSt	HC	MB
α-KGDH	N	2.4 ± 0.3	2.2 ± 0.2	2.1 ± 0.38	2 ± 0.2	2.75 ± 0.2	2 ± 0.2
	A	9.5 ± 1.0*	10.3 ± 0.4*	10 ± 0.2*	11 ± 0.3*	11.6 ± 0.5*	12.7 ± 0.9*
	S	6.5 ± 0.1*	7.2 ± 0.4*	3.5 ± 0.3*	7 ± 0.3*	6.8 ± 0.07*	8 ± 0.1*
	I	5.3 ± 0.4*	5.3 ± 0.4*	3.4 ± 0.3*	3 ± 0.3*	7.1 ± 1.0*	8.4 ± 0.2*
SDH	N	0.5 ± 0.03	0.3 ± 0.03	0.6 ± 0.04	0.6 ± 0.03	1.0 ± 0.05	0.6 ± 0.02
	A	2.1 ± 0.12*	2.0 ± 0.2*	1.8 ± 0.06*	2.2 ± 0.13*	2.1 ± 0.06*	2.7 ± 0.12*
	S	1.4 ± 0.24*	1.1 ± 0.07*	1.1 ± 0.09*	1.2 ± 0.11*	1.6 ± 0.13*	1.2 ± 0.09*
	I	2.2 ± 0.1*	2.9 ± 0.18*	1.8 ± 0.17*	1.5 ± 0.09*	2.8 ± 0.1*	3.2 ± 0.1*
MDH	N	34.4 ± 1.6	28 ± 1.5	26.4 ± 0.96	30.5 ± 1.8	44.7 ± 1.4	28 ± 1.2
	A	23 ± 0.7*	24 ± 0.4*	16 ± 0.3*	25 ± 0.5*	20.3 ± 0.3*	22 ± 0.8*
	S	17 ± 0.2*	13 ± 0.4*	10.7 ± 0.3*	14 ± 0.1*	19 ± 0.4*	19 ± 0.4*
	I	12 ± 0.08*	11 ± 0.2*	9.3 ± 0.02*	7 ± 0.1*	16 ± 0.1*	14 ± 0.1*

Activities of α-KGDH and MDH are expressed as μmoles of NAD^+ reduced per milligram/protein per hour and that of SDH as μmoles of succinate oxidized per milligram protein per hour. For other details see footnotes to Table I.

except malate dehydrogenase and citrate synthase. The magnitude of increase observed in the activity of pyruvate dehydrogenase was 17-fold in the corpus striatum, whereas in other regions, it was 12-fold to 13-fold except in cerebral cortex, where the change was only sevenfold (Table I). A statistically significant increase in the activity of citrate synthase was noticed in cerebral cortex, cerebellum, and corpus striatum, but in other regions the changes were not statistically significant (Table I). A fourfold elevation in the activity of NAD^+-isocitrate dehydrogenase was observed in all regions excepting in hippocampus (Table I). Elevation in the activity of α-ketoglutarate dehydrogenase was highest (sixfold) in the midbrain, whereas in other regions it was threefold to fivefold over the controls (Table II). The magnitude of increase in the activity of succinate dehydrogenase was highest in the cerebellum and least in the midbrain (Table II). In contrast to the elevation observed in the above enzymes of the citric acid cycle, the activity of malate dehydrogenase was suppressed by the administration of MSI. This effect was maximal in hippocampus and least in corpus striatum (Table II).

The overall pattern of changes observed in the activities of the enzymes of the citric acid cycle in the subacute group of animals was more or less the same as in the acute group (Table I and II). However, the magnitudes of elevation in the activity of pyruvate and isocitrate (NAD^+) dehydrogenases were less than in the acute group (Table I). The activity of citrate synthase was suppressed in the brain of the subacute group of animals following the administration of MSI (Table I). Maximal increase in the activity of NAD^+-isocitrate dehydrogenase occurred in hippocampus and cerebellum and the least in midbrain (Table I). There was a twofold to fourfold stimulation in the activities of α-ketoglutarate dehydrogenase and succinate dehydrogenase following the administration of a subacute dose of MSI (Table II). Malate dehydrogenase was suppressed in all cerebral regions of the subacute group of animals (Table II).

Addition of MSI (in vitro) to the assay mixtures also resulted in similar changes in the activities of these enzymes except for citrate synthase, where an 80–90% inhibition was observed. The magnitude of activation was, however, less for pyruvate and α-ketoglutarate dehydrogenases (Tables I and II).

Changes in Aminotransferases and $NADP^+$-Isocitrate Dehydrogenase

The activities of both the aminotransferases were higher in cerebral cortex, cerebellum, hippocampus, and midbrain than the other two regions in the normal animals (Table III). In both normal and experimental animals the activity of aspartate aminotransferase was higher than that of alanine aminotransferase. The activities of both the aminotransferases were inhibited in all the cerebral regions following the administration of MSI. The enzyme activity was suppressed by a factor of about 3-4 in the acute group of animals and about 1-2 in the subacute group. However, in vitro addition of MSI to the assay mixture had only marginal effects on the activity of these enzymes (Table III).

The activity level of $NADP^+$-isocitrate dehydrogenase was more or less the same in all regions of the brain except in hippocampus and midbrain, where it was greater (Table III). Administration of MSI. both in acute and subacute doses, suppressed the activity of this enzyme in all cerebral regions studied. However, the drug had no effect on this enzyme when added in vitro except in cerebellum, where it was stimulated (Table III).

TABLE III. Distribution and Levels of Aspartate Aminotransferase (AAT), Alanine Aminotransferase (AlAT) and NADP$^+$-Isocitrate Dehydrogenase (ICDH) in Different Regions of Brain in Normal and Methionine Sulfoximine Injected Rats

Enzyme	State	CC	CB	BS	CoSt	HC	MB
AAT	N	90.1 ± 5.4	96 ± 5.8	79.2 ± 6.1	84 ± 0.97	139 ± 2.6	100 ± 14
	A	29.8 ± 0.9*	26 ± 0.6*	26.6 ± 0.7*	32 ± 0.69*	27 ± 0.6*	27 ± 1.8**
	S	37.1 ± 0.4*	49 ± 0.57*	35.2 ± 0.7**	42 ± 1.28*	78 ± 5.8*	52 ± 0.8**
	I	86.7 ± 8.2	86 ± 1.2**	85.9 ± 1.7***	74 ± 5.7**	123 ± 3.6*	87 ± 1.5***
AlAT	N	28.4 ± 0.3	27 ± 0.7	17.6 ± 0.6	14.2 ± 0.3	37.4 ± 0.5	27.5 ± 0.2
	A	6.9 ± 0.49*	7.9 ± 0.4*	5.7 ± 0.4*	5.2 ± 0.4*	6.7 ± 0.3*	7 ± 0.2*
	S	13.1 ± 0.48*	9.3 ± 0.4*	4.9 ± 0.4*	10.5 ± 0.2**	21 ± 0.5*	11 ± 1.1*
	I	30 ± 1.38***	29 ± 1.3***	20.6 ± 0.9**	21.1 ± 0.6*	41.7 ± 0.6*	31.9 ± 0.7*
ICDH (NADP$^+$)	N	3.6 ± 0.2	2.6 ± 0.1	2.8 ± 0.07	2.6 ± 0.2	4.3 ± 0.17	4.2 ± 0.18
	A	1.6 ± 0.3*	1.2 ± 0.1*	1.3 ± 0.12*	1.7 ± 0.04*	1.8 ± 0.25*	1.4 ± 0.14
	S	1.4 ± 0.03*	1 ± 0.1*	0.8 ± 0.02*	1.3 ± 0.08*	2.7 ± 0.17**	1.7 ± 0.16
	I	3.2 ± 0.09**	4 ± 0.1*	2.6 ± 0.06**	2.7 ± 0.3	4.7 ± 0.17**	4.2 ± 0.27

Activities of AAT and AlAT are expressed as μmoles of NADH oxidized per milligram/protein per hour and that of ICDH (NADP$^+$) as μmoles of NADP$^+$ reduced per milligram protein per hour. For other details see footnotes to Table I.

Changes in Total Protein Content

In normal rats cerebral cortex had the highest protein content and hippocampus had the lowest (Table IV). Following the administration of an acute dose of MSI. a small but significant decrease in the protein content was observed in cerebral cortex and corpus striatum. However, in brain stem and hippocampus an elevation in the protein content was observed, whereas in cerebellum and midbrain there was no change. The rise observed in hippocampus was greater than the changes observed in any other region (Table IV).

In rats administered a subacute close of MSI, protein content was elevated in all cerebral regions. The observed increase was maximal in brain stem and least in the cerebral cortex (Table IV).

DISCUSSION

Cerebral dependence on glucose for the sustenance of vital processes has been repeatedly documented in the past. A major portion of this energy (about one-third) is known to be diverted, through the enzyme Na^+, K^+-ATPase [Siesjo, 1978], for the maintenance of ionic gradients, which are vital for cerebral functioning. Earlier, we reported an elevation in the Na^+, K^+-ATPase activity in homogenates, neuronal perikarya, glial cells, and synaptosomes following the administration of MSI [Subbalakshmi and Murthy. 1981, 1983, 1984; Subbalakshmi, 1981, 1984]. Elevation in the activity of this enzyme would not only affect the ionic gradients but also enhance the production of ADP. which is supposed to act as a positive modulator for glucose utilization IMcIlwain and Bachelard, 1971; Siesjo, 1978]. However, this hypothesis contradicts the role of ammonia (accumulated owing to the inhibition of glutamine synthetase by MSI) in cerebral dysfunction, as proposed by Bessman and Bessman [1955]. As mentioned earlier, reductive amination of α-ketoglutarate to form glutamate during ammonia toxicity was supposed to interfere with the operation of the citric acid cycle and energy production. As not much work has been done in the past with regard to the applicability of the Bessman hypothesis to MSI toxicity. an attempt has been made in the present investigation to study the changes in the activities of the citric acid cycle enzymes in MSI toxicity.

The results indicated, in brief, an overall increase in the activities of the enzymes of the citric acid cycle in brain following the administration of MSI, which suggested an increase in the oxidation of pyruvate in MSI toxicity. The increase observed in pyruvate dehydrogenase activity suggested the channeling of more pyru-

TABLE IV. Protein Levels in Different Regions of Brain in Normal and Methionine Sulfoximine-Injected Rats

Region	Normal	Acute	Subacute
CC	121 ± 10 (5)	105 ± 6 (5)***	142 ± 13 (4)***
CB	99 ± 16 (5)	104 ± 6 (5)	139 ± 7 (4)**
BS	109 ± 17 (5)	125 ± 7 (5)***	203 ± 23 (4)*
CoSt	116 ± 9 (5)	103 ± 9 (5)***	147 ± 10 (4)**
HC	75 ± 8 (5)	104 ± 11 (5)**	104 ± 5 (4)*
MB	102 ± 5 (5)	103 ± 4 (5)	140 ± 2 (4)*

Protein levels in milligrams per pram wet weight of tissue. For oiher details sec footnotes to Table I. Figures in parentheses indicate number of animals,

vate into the citric acid cycle and enhanced production of acetyl CoA. Owing to the lack of change in the activity of citrate synthase in many cerebral regions. it can be assumed that either this committed step of the citric acid cycle would be rate-limiting or the citrate formation might be proceeding at normal rates because of an enhanced availability of acetyl CoA. Increased activity of isocitrate dehydrogenase (NAD^+) would favor citrate formation by citrate synthase and simultaneously enhance the production of a-ketoglutarate.

The availability of a-ketoglutarate for the citric acid cycle depends on the rate of its utilization by aminotransferases and glutamate dehydrogenase. It was shown earlier that the equilibration of glucose carbon with that of glutamate and aspartate would be through transamination rather than by reductive amination [Machiyama et al. 1970]. Besides the present report on the suppression of aminotransferases in the cerebral homogenates prepared from MSI-treated animals. we earlier demonstrated the suppression of aminotransferases and glutamate dehydrogenase in the synaptosomes. which outnumber both neuronal and glial perikarya. in the MSI-intoxicated animals [Subbalakshmi and Murthy, 1984]. Thus the suppression of aminotransferases would spare both a-ketoglutarate and oxaloacetate for the citric acid cycle. This suggestion agrees well with the reported fall in the content of both glutamate and aspartate in MSI toxicity [Tews and Stone, 1964]. Furthermore, the elevation observed in the activity of a-ketoglutarate dehydrogenase would promote the utilization of a-ketoglutarate for the operation of the citric acid cycle rather than for the transamination pathway. A similar increase in the activity of this enzyme was reported in both acute and chronic ammonia toxicity by Sadasivudu and Rangavalli [1981]. Increased activity of succinate dehydrogenase would favor enhanced production of fumarate and subsequently of malate.

The suppression of malate dehydrogenase observed in the present study was surprising. as it would hinder the operation of the citric acid cycle by limiting oxaloacetate production. However, such a condition would be prevented by anaplerotic formation of oxaloacetate, especially from pyruvate by carbon dioxide fixation (mediated by pyruvate carboxylase), which was reported to be stimulated in hyperammonemic states [Berl. 1971]. Furthermore. it appears that there might be a relationship between this process and the stimulation of Na^+, K^+-ATPase, as both the processes have been shown to be inhibited by ouabain [Cheng, 1971].

In addition to ADP, the rate of glucose utilization would also depend on the redox state of the cell. which is governed by the ratio of NAD(P)H to $NAD(P)^+$. Mitochondrial NAD(P)H would be reoxidized by the electron transport system. However, owing to the absence of such a system in cytosol, and the relative impermeability of these compounds across mitochondrial membranes. the rate of reoxidation of cytosolic NAD(P)H depends on other transport systems. Thus, in brain the reducing equivalents are transported by the operation of the malate-aspartate shuttle, $NADP^+$-dependent isocitrate dehydrogenase, the pyruvate-alanine shuttle, and a-glycerophosphate dehydrogenase [Dennis and Clark, 1978; Siesjo, 1978]. Since the activity of the last enzyme was shown to be negligible [Siesjo, 1978], the activities of the enzymes of the other two systems have been studied in the present work. The malate-aspartate shuttle is made up of cytosolic and mitochondrial isozymes of malate dehydrogenase and aspartate aminotransferase. The results obtained indicated a suppression in the activities of malate dehydrogenase. aspartate and alanine aminotransferases, and $NADP^+$-isocitrate dehydrogenase both in acute and subacute MSI

toxicity. These results suggested an impairment in the transport of reducing equivalents across mitochondrial membranes in MSI toxicity. Probably under these conditions the cytosol is in a more reduced state than normal.

Although the mechanism of action of MSI on the enzymes of citric acid cycle was not elucidated in detail, the results obtained by in vitro fortification of the homogcnates of normal rat brain wilh MSI indicated the direct action of the drug on these enzymes at least in the acute stale. In the subacute state, however, besides the above mechanism, increased synthesis of mitochondrial proteins might also be involved. This suggestion would be in agreement with the increase observed in the protein content in the subacute state in this study and the ullrastructural evidence presented by Gutierrez and Norenberg [1975, 1977].

Our results thus indicate that in MSI induced hyperammonemia the operational rates of the citric acid cycle might be enhanced by the elevated activity levels of the enzyme and that the ADP generated in the maintenance of ionic gradients (owing to elevated Na^+, K^+-ATPase activity) might be acting as a positive modulator. The oxaloacetate required for this process might be generated by anaplerotic reactions. Furthermore, these results also indicate a possibility of derangement in the transport of reducing equivalents across mitochondrial membranes. The convulsant action of the drug may be due to its effects on glutamate metabolism and on ionic gradients [Subbalakshmi and Murthy, 1984] and may not be due to the depletion of α-ketoglutarate from the citric acid cycle and interference with energy production by the accumulated ammonia.

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ACUTE EFFECTS OF AMMONIA ON THE ENZYMES OF CITRIC ACID CYCLE IN RAT BRAIN

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Abstract—Activities of the enzymes of citric acid cycle were determined along with aspartate and alanine aminotransferases and $NADP^+$-isocitrate dehydrogenase in the brains of rats treated with an acute dose of ammonium acetate and compared with those of normal animals. Elevation in the activities of pyruvate. α-ketoglutarate and succinate dehydrogenases and citrate synthase was observed in hyperammonemic animals. The activities of malate. $NADP^+$-isocitrate dehydrogenases and aminotransferases decreased under these conditions. The results suggest that ammonia toxicity might not be due to the depletion of α-ketoglutarate from citric acid cycle.

Elevated concentration of ammonia either in blood or in brain is known to be neurotoxic and produces convulsions or coma. It was suggested that ammonia interferes with cerebral energy metabolism by more than one mechanism. The diversion of α-ketoglutarate for the detoxification of ammonia resulting in the formation of glutamate (through glutamate dehydrogenase reaction) was supposed to deplete the citric acid cycle intermediates, thus affecting ATP production. This would be further aggravated by the loss of ATP during glutamine formation [through glutamine synthetase reaction] (Bessman and Bessman, 1955). This hypothesis received support from the observed reduction in cerebral oxygen consumption *in vivo;* inhibition of the oxidation of keto acids such as pyruvate and α-ketoglutarate (McKhann and Tower, 1967); decrease in cerebral α-ketoglutarate content (Bcssman and Bessman, 1955; Clark and Eiseman, 1958) and a fall in the levels of high energy phosphates in the basal parts of brain and in reticular activating system in hyperammonemic states (Schenker *et at.*. 1967; Bessman and Pal, 1976; McCandless and Schenker. 1981). However, these evidences were not unequivocal as in later works no changes were noticed in the oxygen consumption of cerebral slices in the presence of ammonium salts or in the rate of decarboxylation of either pyruvale or α-ketoglutarate. Similarly, ammonium ion induced depletion of α-ketoglutarate (Clark and Eiseman, 1958) or ATP was not observed (Shorey *et* a/., 1967; Schenker and Mendelson, 1964; Hindfelt and Siesjo, 1970, 1971; Hawkins *et al.*, 1973; Hindfelt *et al.*, 1977). in fact, an elevation in the α-ketoglutarate content of brain was reported (Hindfelt and Siesjo, 1970). Despite the voluminous information on the levels of various Krebs cycle intermediates, little information is available on the enzymes of citric acid cycle in hyperammonemic slates. Presently, we report an elevation in the activities of pyruvate dehydrogenase. citrate synthase. α-ketoglutarate dehydrogenase and succinate dehydrogenase in brain in acute hyperammonemic slates. Under these conditions, activities of malate dehydrogenase, aspartate and alanine aminotransferases and $NADP^+$-isocitrate dehydrogenase were suppressed. These results were discussed in relation to the operation of citric acid cycle and the transport of reducing equivalents in brain under hyperammonemic states.

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EXPERIMENTAL PROCEDURES

Materials

Coenzyme A. acetyl coenzyme A. thiamine pyrophosphate. sodium pyruvate. α-ketoglutarate, DL-dithiothrcilol. L-malic acid. DL-isocitric acid. disodium succinate. oxaloacetic acid, L-aspartic acid. 5.5 -dithiobis 2-nitrobenzoic acid. ADP. phenazine methosulfate. NAD^+, NADH. $NADP^+$, and lactate and malate dchydrogenases were purchased from Sigma Chemical Co. 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride (INT) was from Loba Chemie. India. 2,6-Dichlorophenol indophenol was purchased from V.P. Chest Institute, India. Triton X-100 was procured from Koch–Light Laboratories Ltd, U.K. The rest of the chemicals were of analytical grade and were purchased locally. The commercial enzymes were dialyzed to remove ammonium sulfate and were

reconstituted into **50%** (v/v) **glycerol** to a final protein **concentration** of 0.5 nig/ml.

Adult albino rats of Wistar strain (of either sex) **weighing** 250 300 g body weight from the inbred colony of the vivarium were used **in this** study. Pood (balanced **pellet** diet from Pragati Animal feeds, India) and water were provided ***ad libitum***

Induction of hyperammonemia and preparation of homogenates (in vivo)

Experimental animals were injected intraperitoneally with 25 mmol/kg body weight **of ammonium acetate** using saline as a carrier while the control animals received none. The animals entered into convulsions at the end of 35 nun. They were decapitated at the end of 20 min (preconvulsive suite) or during convulsions. Brains were quickly removed and washed with ice-cold saline. Cerebral cortex, **cerebellum,** brain stem, hippocampus, corpus striatum were dissected out at 2 C and rest of the brain was designated as mid brain. Ten percent (w/v) homogenates were prepared in ice-cold 0.32 M sucrose. **Triton** X-100 was added to a final concentration of **0.2%** (v/v).

In vitro ***experiments***

To the homogenates of different cerebral regions which were prepared from normal animals, ammonium acetate was **added** to a final concentration of 10 μmol and the enzyme assays were performed with this preparation

Enzyme assays

The dehydrogenases of citric acid cycle were assayed by dye reduction method using phenazine methosulfate as an intermediary **electron acceptor** and INT as final **electron** acceptor except in the case of succinate dehydrogenase where dichlorophenol indophenol was **used** as final electron acceptor. The formation of formazan was followed at 500 nm and all the enzymes were assayed at 37 C in Gilford spectrophotometer using a thermoprogrammer. The final volume of the assay mixture was always 250/;l and appropriate blanks (without substrate) were run simultaneously

Pyruvate dehydrogenase was assayed by the method of Hinman and Blass (1981) and citrate synthase by the method of Shepherd and Garland (1969). Assay of NAD"-isocitrate dehydrogenase was carried out as described by Plant (1969); α-ketoglutarate dehydrogenase by the method of Reed and Mukherjee (1969) and malate dehydrogenase by the method of Yoshida (1969) except that phenazine methosulfate and INT were used as electron acceptors. Succinatc dehydrogenase was assayed by the method of Veeger *et at.*, (1969), aspartate and alanine aminotransferases by the method of Bergmeyer and Bernt (1974). NADP'-Isocitrate dehydrogenase activity was determined by the method of Bergmeyer and Bernt (1974) using the aforesaid electron acceptors, in all the assays 10 μl of 10% homogenate was used except for aspartate and alaninc aminotransferases where 10 μl of 1% homogenate was used Protein content was determined by the biuret method (Varley, 1969).

Preparation of formazan

Forma7an was prepared from INT by chemical and enzymatic methods. In the former, varying concentrations of INT (20-100 nmol) was reduced with 10 μl of 1% ascorbic acid and 10 μl of 0.1 N NaOH in a final volume of 250 μl. In the enzymatic reduction, **purified** malate dchydrogenase (Sigma), malate and varying concentrations of NAD' (10 100 nmol), in a final volume of 250 μl, were incubated along with INT and phena/ine mcthosulfate at 37 C till the optical density remained constant. **The** values obtained in these experiments were used to prepare a **Standard** curve for **the** estimation of NADH formed in the enzyme assays described above.

Statistical analysis of the results were carried out by Student's (-test.

RESULTS

Following the administration of ammonium salts, rats entered into prcconvulsivc state in about 15-20 min. In this state, the animals were lethargic and were less responsive. These animals entered **into** convulsive slate by about 30 35 min and exhibited both clonic and tonic seizures. The ***in tiro*** dose of ammonium **acetate** used presently was higher than that used by Bessman and Pal (1976) as it was observed that the dose recommended by them failed to elicit any behavioural response in our animals.

Pyruvate dehydrogenase activity (*Table I*)

The activity levels of **pynivate dehydrogenase** was more or less the same in all the regions of brain studied presently except in cerebral cortex where it was twice to that of the other regions. Administration of ammonium **acetate** resulted in an elevation in the activity of this enzyme in all the regions both in prcconvulsivc and convulsive states. The magnitude of elevation was. however. greater in the preconvulsive state than in convulsions. Of all the regions, maximal elevation was observed in corpus **striatum** and hippocampus in preconvulsive and convulsive states and minimal in cerebral cortex. ***In vitro,*** addition of ammonium acetate also elevated the activity of this enzyme in all the regions of brain

Enzymes of citric acid cycle (*Tables 1 and 2*)

Of the enzymes of citric acid cycle studied, the activity of malate dehydrogenase was highest and that of succinatc dehydrogenase was lowest in all the regions of brain. There was not much of regional variation in the distribution of the enzymes of citric acid cycle except succinatc dehydrogenase (lesser in cerebellum than in other regions) and malatc dehydrogenase (highest activity in hippocampus when compared to other regions).

Following the administration of ammonium acetate, an elevation in the activity of citrate synthase was observed in cerebral cortex, corpus striatum and mid brain regions while the change in cerebellum, hippocampus and brain stem was not statistically significant in the prcconvulsivc state. In the convulsive state, the activity of this enzyme was elevated

Table 1. Distribution and levels of pyruvate dehydrogenase (PDH), citrate synthase (CS) and NAD^+-isocitrate dehydrogenase (ICDH) in different regions of brain in normal and ammonium acetate injected rats

Enzyme	State	CC	CB	BS	CoSt	HC	MB
PDH	N	3.0 ± 0.35	1.6 ± 0.15	1.5 ± 0.2	1.3 ± 0.1	1.7 ± 0.3	1.7 ± 0.2
	P	12.1 ± 0.47*	8.9 ± 0.11*	16.3 ± 0.3*	24.0 ± 0.3*	11.3 ± 0.3*	13.9 ± 0.7*
	C	5.8 ± 0.13*	5.2 ± 0.33*	3.7 ± 0.3*	3.4 ± 0.9*	4.9 ± 0.7*	3.5 ± 0.9**
	I	6.3 ± 0.17*	5.7 ± 0.44*	4.2 ± 0.06*	4.4 ± 0.2*	6.9 ± 0.3*	4.7 ± 0.3*
CS	N	7.5 ± 0.8	8.9 ± 0.2	9.4 ± 0.6	7.8 ± 0.5	10.9 ± 0.2	8.2 ± 0.3
	P	11.5 ± 0.4*	8.8 ± 0.7	9.4 ± 0.6	12.6 ± 0.5*	10.6 ± 0.2†	12.1 ± 0.1*
	C	13.3 ± 0.4*	12.8 ± 0.4*	9.9 ± 0.5	10.5 ± 0.3*	8.6 ± 0.2*	12.2 ± 0.5*
	I	1.2 ± 0.2*	1.2 ± 0.2*	1.2 ± 0.1*	0.84 ± 0.2*	1.4 ± 0.2*	1.2 ± 0.2*
ICDH (NAD^+)	N	1.9 ± 0.17	2.2 ± 0.2	2.0 ± 0.14	1.8 ± 0.1	2.9 ± 0.28	2.4 ± 0.08
	P	2.4 ± 0.11*	2.2 ± 0.1	2.2 ± 0.12	2.9 ± 0.1*	1.7 ± 0.09	2.4 ± 0.1
	C	2.0 ± 0.10	2.5 ± 0.3†	2.2 ± 0.07†	2.8 ± 0.4*	2.3 ± 0.09*	2.6 ± 0.1†
	I	4.4 ± 0.07*	4.1 ± 0.3*	4.9 ± 0.3*	5.8 ± 0.2*	7.1 ± 0.2*	6.3 ± 0.4*

N = Normal rats; P = Preconvulsive state; C = Convulsive state; I = Homogenates of normal animals fortified with ammonium acetate to a final concentration of 10 μmol (*in vitro*). CC = Cerebral cortex; CB = Cerebellum; BS = Brainstem; CoSt = Corpus striatum; HC = Hippocampus; MB = Mid brain. The number of animals in each group varied from three to six. The values are mean ± SD. Activities of PDH and ICDH (NAD^+) are expressed as μmol of NAD^+ reduced/mg protein/h and that of CS as μmol of citrate formed/mg protein/h. Statistical significance was calculated by Student's *t*-test. $*P < 0.0005$, $**P < 0.0025$; $+P < 0.01$ versus control value.

in all the regions except in brain stem and hippocampus. NAD^+-isocitrate dehydrogenase activity was elevated only in corpus striatum in both preconvulsive and convulsive states. In all the other regions of brain the changes were statistically not significant except in hippocampus. An elevation in the activity of α-ketoglutarate dehydrogenase was observed in all the cerebral regions both in preconvulsive and con-

Table 2. Distribution and levels of α-ketoglutarate dehydrogenase (α-KGDH); succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) in different regions of brain in normal and ammonium acetate injected rats

Enzyme	State	CC	CB	BS	CoSt	HC	MB
α-KGDH	N	2.4 ± 0.3	2.2 ± 0.2	2.1 ± 0.38	2.1 ± 0.2	2.7 ± 0.2	2.0 ± 0.2
	P	5.7 ± 0.8*	6.4 ± 0.4*	8.4 ± 0.68*	17.4 ± 0.1*	6.1 ± 0.2*	6.8 ± 0.1*
	C	5.2 ± 0.3*	5.7 ± 1.1*	4.9 ± 0.42*	4.6 ± 0.4*	5.9 ± 0.6*	5.3 ± 1.01*
	I	5.3 ± 0.1*	5.4 ± 0.2*	4.2 ± 0.19*	4.1 ± 0.1*	5.5 ± 0.3*	4.3 ± 0.1*
SDH	N	0.5 ± 0.03	0.3 ± 0.03	0.6 ± 0.04	0.6 ± 0.03	1.0 ± 0.05	0.6 ± 0.02
	P	0.9 ± 0.04*	1.3 ± 0.05*	1.2 ± 0.08*	1.3 ± 0.09*	0.9 ± 0.04†	1.2 ± 0.12*
	C	1.2 ± 0.1*	0.9 ± 0.05*	1.0 ± 0.02*	1.5 ± 0.07*	1.2 ± 0.06*	1.7 ± 0.02*
	I	2.7 ± 0.3*	4.2 ± 0.17*	1.8 ± 0.07*	2.3 ± 0.22*	5.9 ± 0.3*	3.6 ± 0.21*
MDH	N	34.4 ± 1.6	28.4 ± 1.5	26.4 ± 0.96	30.5 ± 1.8	44.7 ± 1.4	28.3 ± 1.2
	P	13.9 ± 0.6*	13.7 ± 1.4*	24.6 ± 0.35†	12.9 ± 0.4*	10.5 ± 0.3*	11.5 ± 0.2*
	C	12.7 ± 0.5*	15.6 ± 0.2*	12.2 ± 1.86*	21.2 ± 3.2*	25.9 ± 0.8*	19.6 ± 0.2*
	I	24.3 ± 0.3*	29.6 ± 0.9	25.6 ± 0.11	23.7 ± 0.1*	39.1 ± 0.6*	30.2 ± 1.3†

Activities of α-KGDH and MDH are expressed as μmol of NAD reduced/mg protein/h and that of SDH as μmol of succinate oxidized/mg protein/h. For other details see Table 1. $*P < 0.0005$; $**P < 0.0025$; $†P < 0.01$ versus control value.

Table 3. Distribution and levels of aspartate aminotransferase (AAT), alanine aminotransferase (AlAT) and $NADP^+$-isocitrate dehydrogenase (ICDH) in different regions of brain in normal and ammonium acetate injected rats

Enzyme	State	CC	CB	BS	CoSt	HC	MB
AAT	N	90.1 ± 5.4	96.1 ± 5.8	79.2 ± 6.1	84 ± 0.97	139 ± 2.6	100 ± 14
	P	76.8 ± 0.3*	73.2 ± 0.3*	54.8 ± 1.5*	50 ± 1.0*	60 ± 0.6*	78 ± 1**
	C	64.8 ± 0.3*	70.5 ± 0.3*	53.0 ± 0.4*	36 ± 0.34*	37 ± 0.3*	38 ± 0.3*
	I	82.3 ± 3.1†	88.0 ± 4.5†	85.7 ± 5.8	78 ± 5.82†	126 ± 1.7†	84 ± 7
AlAT	N	28.4 ± 0.3	27 ± 0.7	17.6 ± 0.6	14.2 ± 0.3	37.4 ± 0.5	27.5 ± 0.2
	P	6.7 ± 0.5*	6.7 ± 0.6*	10.3 ± 0.3*	10.1 ± 0.3*	6.1 ± 0.4*	7.9 ± 0.3*
	C	7.9 ± 0.3*	9 ± 0.3*	7.1 ± 0.3*	6.7 ± 0.2*	9.1 ± 0.2*	9.3 ± 0.2*
	I	27.3 ± 1.0†	30 ± 0.9**	17.9 ± 0.3	15.8 ± 0.5**	38.8 ± 1.2†	29.6 ± 0.5*
ICDH ($NADP^+$)	N	3.6 ± 0.2	2.6 ± 0.1	2.8 ± 0.07	2.6 ± 0.2	4.3 ± 0.17	4.2 ± 0.18
	P	1.9 ± 0.2*	1.7 ± 0.1*	3.1 ± 0.2†	2.8 ± 0.09†	3.4 ± 0.05*	3.1 ± 0.15*
	C	3.1 ± 0.07**	3.5 ± 0.2*	2.9 ± 0.2	2.6 ± 0.3	2.8 ± 0.19*	2.6 ± 0.07*
	I	2.9 ± 0.07**	2.9 ± 0.2†	2.5 ± 0.1**	2.1 ± 0.1†	4.5 ± 0.31	4.7 ± 0.34†

Activities of AAT and AlAT are expressed as μmol of NADH oxidized/mg protein/h and that of ICDH ($NADP^+$) as μmol $NADP^+$ reduced/mg protein/h. For other details see Table 1. $*P < 0.0005$; $**P < 0.0025$; $†P < 0.01$ versus control value.

Table 4. Protein levels in different regions of brain in normal and ammonium acetate injected rats

Region	Normal	Preconvulsive state	Convulsive state
CC	121 ± 10 (5)	104 ± 6 (4)†	111 ± 10 (5)
CB	99 ± 16 (5)	109 ± 5 (4)	97 ± 8 (5)
BS	109 ± 17 (5)	93 ± 13 (4)	97 ± 14 (5)
CoSt	116 ± 9 (5)	94 ± 4 (4)**	105 ± 5 (5)†
HC	75 ± 8 (5)	113 ± 9 (4)*	102 ± 12 (5)**
MB	102 ± 5 (5)	100 ± 3 (4)	99 ± 3 (5)

Protein: mg/g wet wt of tissue.
Figures in parentheses indicate number of animals.
For other details see Table 1.

vulsive states following (he administration of ammonium acetate. As in the case of pyruvate dehydrogenase, the magnitude of change in the activity of α-ketoglutarate dehydrogenase declined following the onset of convulsions. Succinate dehydrogenase activity was elevated under these conditions, the change being greater in the convulsive state than in preconvulsive state in all the regions. However, malate dehydrogenase activity was suppressed by at least 50% in all the cerebral regions in both the slates. Enrichment of homogenates by *in vitro* addition of ammonium acetate resulted in changes similar to those described above except that the activity of citrate synthase which was suppressed in all the regions.

Aminotransferases ami NADP⁺-isocitratedehydrogenase (Table .')

Both aspartate and alanine aminotransferases were suppressed in all the cerebral regions in preconvulsive and convulsive stales, while the magnitude of decrease was greater for alanine aminotransferase than for aspartatc aminotransferase. A fall in the activity of NADP⁺-isocitrate dehydrogenase was observed in all the regions except in brain stem and corpus striatum in the preconvulsive state while in convulsive state significant fall was observed in cerebral cortex, hippocampus and mid brain. Under these conditions, the activity of NADP⁺-isocitrate dchydrogenasc was elevated in cerebellum. Unlike the enzymes of citric acid cycle, *in vitro* addition of ammonium acetate had marginal effects on the aminotransferases and NADP⁺-isocitrate dchydrogenasc

DISCUSSION

In extrahepatic tissues, such as brain, ammonia was shown to be detoxified chiefly by the formation of glutamate (α-ketoglutarate + ammonia ⇌ glutamate) and glutamine (glutamate + ammonia + ATP → glutamine) mediated by the enzymes glutamale dchydrogenasc and glutamine synthetasc respectively (Benjamin, 1982; Kvamme, 1983). It was postulated that in hyperammonemic states there would be an increased utilization of α-ketoglutarate which would affect the operation of citric acid cycle (thereby interfere with energy production) and deplete cellular energy stores (Bessman and Bessman, 1955). Studies on metabolic compartmentation and histological changes under these conditions indicated that astroglia might be the sites of ammonia detoxification (Berl, 1971; Zamora *et* a/., 1973; Norenberg, 1977).

Results obtained presently indicated that elevated ammonia levels might be enhancing the operational rates of cerebral citric acid cycle. Increased activity of pyruvale dehydrogenase would promote channelling of pyruvate into the citric acid cycle in the form of acetyl CoA. This observation was, however, in contradiction to the suggested inhibition of lhc oxidation of α-keto acid in hypcrammonemic states (McKhann and Tower, 1967). Elevated activity of citrate synthasc, under these conditions, would promote the utilization of acetyl CoA and citrate formation in cerebral cortex, corpus striatum and mid brain regions. In cerebellum and brain stem, where the activity of this enzyme was unchanged, citrate formation might be rate limiting and proceed at normal rates. The increased utilization of α-ketoglutarate, due to elevated α-ketoglutarate dehydrogenasc, would promote its formation from isocitrate although the activity of NAD⁺-isocitrate dchydrogenasc was unchanged under these conditions. However, the availability of α-ketoglutarate to citric acid cycle depends on the activities of aminotransferases and glutamatc dchydrogenasc, which use this metabolite.

Among the aminotransferases, the activities of aspartate and alaninc aminotransfcrascs were reported to be highest and that a major portion of glucose carbon enters the carbon skeleton of glutamatc through the reaction mediated by these enzymes (Benuk *et at,,* 1971; Machiyama *et* a/., 1970). Decreased activity levels of these enzymes, observed presently, would spare α-ketoglutarate, oxaloacetate

and pyruvate for citric acid cycle. The reported inhibition of glutamate dehydrogenase in the astroglia and synaptosomes (which together outnumber neuronal perikarya) in acute hyperammonemia would also spare α-ketoglutarate for citric acid cycle (Subbalakshmi and Murthy. 1983, 1984). Thus, it appeared that in hyperammonemic states α-ketoglutarate would be channelled more into citric acid cycle than for the formation of aspartate or glutamate. A similar increase in the activity of cerebral α-ketoglutarate dehydrogenase was reported earlier in acute ammonia toxicity (Sadasivudu and Rangavalli, 1981). Increased activity of succinate dehydrogenase would favour utilization of succinate and subsequently fumarate.

Suppression of malate dehydrogenase in hyperammonemic states would result in the accumulation of malate and limit the formation of oxaloacetate which might interfere with the operation of citric acid cycle. Increased malate levels and a fall in the content of oxaloacetate, reported earlier by Hawkins *et al.*, (1973) in acute hyperammonemic states, were in concurrence with the present observation. However, this situation might be averted by anaplerotic replenishment of oxaloacctate by carbon dioxide fixation, a process stimulated in hyperammonemic states (Berl, 1971).

Besides its participation in citric acid cycle, malate dehydrogenase along with aspartate amino transferase is also involved in the transport of reducing equivalents across the mitochondrial membrane (Shank and Campbell. 1983). The other two systems involved in this process arc alanine aminotransferase (alanine–pyruvate shuttle) and $NADP^+$-isocitrate dehydrogena.se (Sicsjo. 1978). Following the administration of ammonium acetate. activities of these two enzymes were found to be decreased suggesting an impairment in the transport of reducing equivalents across mitochondria. Under these conditions the cytosol may bc in a more reduced slate than mitochondria (where electron transport chain reoxidizes NAD(P)H generated). A similar observation was made by Hindfelt and Siesjo (1970) and Hawkins *et al.* (1973).

Thus, the results, obtained presently, were suggestive of enhanced oxidation of α-keto acids (pyruvate and α-ketoglutarate) due to the stimulation of the enzymes of citric acid cycle. The ADP generated due to an increase in glutamine synthesis (Subbalakshmi and Murthy. 1983; Benjamin. 1982; Kvamme. 1983) and Na^+, K^+-ATPase (Sadasivudu *et al.*, 1977; Subbalakshmi and Murthy. 1981) might be acting as a positive modulator. Oxaloacetate required for the continuation of citric acid cycle would be generated by carbon dioxide fixation. The results also indicated an impairment in the transport of reducing equivalents from cytosol to mitochondria. Hence ammonia might not be depleting α-ketoglutarate from citric acid cycle as suggested earlier (Bessman and Bessman. 1955; Schenker *et al.*, 1967; Bessman and Pal. 1976).

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Activities of Pyruvate Dehydrogenase, Enzymes of Citric Acid Cycle, and Aminotransferases in the Subcellular Fractions of Cerebral Cortex in Normal and Hyperammonemic Rats

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Activity levels of pyruvate dehydrogenase. enzymes of citric acid cycle, aspartate and alanine aminotransferases were estimated in mitochondria, synaptosomes and cytosol isolated from brains of normal rats and those injected with acute and subacute doses of ammonium acetate. In mitochondria isolated from animals treated with acute dose of ammonium acetate, there was an elevation in the activities of pyruvate, isocitratc and succinate dehydrogenases while the activities of malate dehydrogenase (malate→oxaloacetate), aspartate and alanine aminotransferases were suppressed. In subacute conditions a similar profile of change was noticed excepting that there was an elevation in the activity of α-ketoglutarate dehydrogenase in mitochondria. In the synaptosomes isolated from animals administered with acute dose of ammonium acetate, there was an increase in the activities of pyruvate, isocitrate, α-ketoglutarate and succinate dehydrogenases while the changes in the activities of malate dehydrogenase, aspartate and alanine amino transferases were suppressed. In the subacute toxicity similar changes were observed in this fraction except that the activity of malate dehydrogenase (oxaloacetate→malate) was enhanced. In the cytosol, pyruvate dehydrogenase and other enzymes of citric acid cycle except malate dehydrogenase were enhanced in both acute and subacute ammonia toxicity though their activities are lesser than that of mitochondria. In this fraction malate dehydrogenase (oxaloacetate→malate) was enhanced while activities of malate dehydrogenase (malate→oxaloacetate), aspartate and alanine aminotransferases were suppressed in both the conditions. Based on these results it is concluded that the decreased activities of malate dehydrogenase (malate→oxaloacetate) in mitochondria and of aspartate aminotransferase in mitochondria and cytosol may be responsible for the disruption of malate-aspartate shuttle in hyperammonemic state. Possible existence of a small vulnerable population of mitochondria in brain which might degenerate and liberate their contents into cytosol in hyperammonemic states is also suggested.

KEY WORDS: Citric acid cycle enzymes: hyperammonemia; cytosol; mitochondria; synaptosomes.

INTRODUCTION

Derangement in the cerebral energy metabolism was proposed to be one of the many mechanisms by which ammonia exerts toxic effects on the central nervous system. It was hypothesized that removal of a-ketoglutarate

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from citric acid cycle for the purpose of ammonia detoxification (in the glutamate dehydrogenase reaction), results in the formation of glutamate and the conversion of the latter to glutamine, would adversely affect the cerebral energy' stores (1-3). Moreover, by removing cytosolic pool of glutamate for the synthesis of glutamine, ammonia was postulated to interfere with the operation of malate-aspartate shuttle and thereby the transport of reducing equivalents from cytosol to mitochondria (4, 5). Several conflicting reports were made in the past with respect to the depiction of α-ketoglutarate stores while evidences have accumulated which strongly favour the latter concept (6-9). It was observed that not many studies were made in the past on the subcellular distribution and changes in the activities of the enzymes involved in carbohydrate metabolism in hyperammonemia.

Earlier, we reported an elevation in the activities of pyruvate dehydrogenase and enzymes of citric acid cycle, except malate dehydrogenase, in the homogenates prepared from different regions of brains of hyperammonemic rats (10). We have also reported a fall in the activities of aspartate and alanine aminotransferases, malate dehydrogenase and NADP-dependent isocitrate dehydrogenase in these preparations and suggested that transport of reducing equivalents across mitochondria might be affected in hyperammonemic states. As brain has two types of milochondrial populations i.e., synaptic and non-synaptic and both these have citric acid cycle enzymes, a study with homogenates will not reveal whether the changes in the activities of these enzymes are occurring in the synaptic or non-synpatic mitochondria. Moreover, enzymes involved in the transport of reducing equivalents are present in both cytosol and mitochondria. Hence it becomes essential to localize the changes (suppression) in the activities of the enzymes of malate-aspartatc shuttle in the brains of hyperammonemic rats and such an attempt has been made in the present study.

Based on the results obtained in the present study, we suggest that (i) suppression of malate-aspartate shuttle may be due to decreased malate dehydrogenase activity in the direction of malate formation in the mitochondria (ii) there is a small population of mitochondria which are vulnerable to patho-physiological ammonia concentrations which degenerate/rupture and liberate their contents into the cytosol and (iii) the apparent increase in citric acid cycle enzymes observed in homogenales may be due to release of these enzymes into cytosol and loss of regulatory control over these enzymes in an altered subcellular environment.

EXPERIMENTAL PROCEDURE

Adult albino rats of Wistar strain of 250–300 gms. body weight were maintained in groups of 6–8 per cage under natural light-dark cycles at a constant temperature. Food and water were provided ad libitum. These animals were divided into three groups with ten animals each and two animals were used for each experiment. Animals in group I were administered intraperitoneally with 0.35 mmol of ammonium acetate/100 g body weiphts (subacute experiments) and the animals in group II received 2.5 mmol of ammonium acetate per 100 gms. body weight (acute experiments) while group III animals received none and served as controls. Animals in group I and II were sacrificed 25–30 min after the administration of ammonium acetate. Mitochondria, synaptosomes and cylosol were prepared by the method of Cotman (11) as described by Subbalakshmi and Murthy (12). These fractions were frozen overnight and Triton X-100 was added to a final concentration of 0.1% v/v after thawing the preparations.

Protein, present in 20 µl aliquot of subcellular fractions, was determined by the method of Lowry et al. (13). Ammonia content in brains frozen in liquid nitrogen and in the scrum was determined as described earlier (12).

Enzyme Assays. Activities of pyruvate dehydrogenase. citrate synthase, isocilrate dehydrogenase. α-ketoglutarate dehydrogenase, malale dehydrogenase (in the direction of oxaloacetate formation), aspartate and alanine aminotransferases were assayed as described earlier (10). Malale dehydrogenase activity, (in the direction of malate formation) was assayed as suggested by Yoshida (14). Lactate and succinate dehydrogenases were assayed by the methods of Bergmeyer and Bernt and Nandakumar et al. (15, 16) respectively. After determining the optimal concentrations of enzyme protein, substrate and cofactors, suitable alterations were made (or each enzyme (Table II. Statistical analysis of data was performed by Student's *t* test.

RESULTS

Rats administered with subacute dose of ammonium acetate (group I) showed no convulsions even upto 10 hours. However, they were sacrificed 25-30 min after the administration of ammonium acetate. This time period was chosen as the animals injected with acute dose entered into convulsions at this time period. Rats injected with acute dose of ammonium acetate (group 11) exhibited convulsions in about 25-30 min which was usually the terminal phase. In this group all the animals irrespective of their sex, succumbed to the toxic effects of ammonia al about 45 min after the administration of ammonium acetate. Hence, they were sacrificed during convulsions and used for experimentation. After the administration of ammonium acetate there was an increase in blood and brain ammonia levels (Table II).

In normal animals, activities of pyruvate, isocitrate(NAD^+), α-ketoglutarate and succinate dehydrogenases were higher in mitochondrial fraction than in cylosol or synaptosomes. Activities of these enzymes were found

Table I. Methods for the Assay of Pyruvate Dehydrogenase, Lactate Dehydrogenase and Enzymes of TCA Cycle and Borst Cycle With Subcellular Fractions

Enzyme	Method
Pyruvate dehydrogenase (E.C.1.2.4.1)	K-PO_4 buffer (pH 7.8) 50 mM, NAD 2.5 mM, TPP 0.2 mM, CoA 100 μM, DTT 300 μM, Pyruvate 5 mM, $MgCl_2$ 1 mM, PMS 6.50 μM, INT 300 μM, O.D change at 500 nm. (10)
Citrate synthase (E.C.4.1.3.7)	Tris-HCl buffer (pH 8.0) 96 mM, DTNB 97 nM, OAA 240 μM, acetyl CoA 48 μM. O.D. change at 412 nm. (10)
Isocitrate dehydrogenase (E.C.1.1.1.41)	Tris acetate buffer (pH 7.2) 33mM, NAD 333 μM, ADP 6.7 mM, isocitrate 5.3 mM, $MgCl_2$ 1 mM, PMS 6.50 μM, INT 300 μM. O.D change at 500 nm. (10)
α-Ketoglutarate dehydrogenase (E.C.1.2.4.2)	K-PO_4 buffer (pH 8.0) 50 mM, NAD 2.0 mM, TPP 200 μM, CoA 60 μM, α-ketoglutarate 1.0 mM, $MgCl_2$ 1 mM, PMS 6.50 μM, INT 300 μM. O.D change at 500 nm. (10)
Succinate dehydrogenase (E.C.1.3.99.1)	Tris-PO_4 buffer (pH 7.6) 100 mM, succinate 40 mM, PMS 6.50 μM, INT 4 mM, O.D at 500 nm. (16)
Malate dehydrogenase (E.C.1.1.1.37)	Tris-HCl buffer (pH 8.8) 83 mM, NAD 3.3 mM, malate 3.2 mM, INT 300 μM, PMS 6.50 μM. O.D change at 500 nm (10)
Malate dehydrogenase (E.C.1.1.1.37)	Tris-HCl buffer (pH 8.8) 83 mM, NADH 160 μM, oxaloacetate 3.4 mM. O.D change at 340 nm. (14)
Aspartate aminotransferase (E.C.2.6.1.1)	K-PO_4 buffer (pH 7.4) 80 mM, NADH 520 μM, aspartate 20 mM, α-ketoglutarate 18 mM, MDH 10 μg. O.D. change at 340 nm. (15)
Alanine aminotransferase (E.C.2.6.1.2)	K-PO_4 buffer (pH 7.4) 80 mM, NADH 520 μM, alanine 40 mM, α-ketoglutarate 18 mM, LDH 4 μg. O.D. change at 340 nm. (15)
Lactate dehydrogenase (E.C.1.1.1.27)	K-PO_4 buffer (pH 7.5) 48 mM, pyruvate 0.6 mM, NADH 180 μM. O.D. change at 340 nm. (15)
Lactate dehydrogenase (E.C.1.1.1.27)	K-PO_4 buffer (pH 7.5) 48 mM, lactate 1.2 mM, NAD 333 μM, PMS 6.5 μM, INT 300 μm. O.D. change at 500 nm. (15).

In all the above assays, except succinate dehydrogenase, final volume was 250 μl. In the assay for succinate dehydrogenase, the final volume was 1.0 ml and the assay was colorimetric. The assay mixture for SDH was incubated for 15 min and the reaction was arrested with 2 ml of glacial acetic acid. Colour produced due to the formation of formazan was extracted into 5 ml of toluene. In all the assays, the incubation temperature was 37°C. Corrections were made for non specific activity with suitable blanks. In the spectrophotometric assays changes in absorbance were recorded at 15 sec intervals for 10 minutes and the values in linear kinetic zone were used for calculating enzyme activity. Relationship between formazan formed and NAD* reduced was established as earlier (10). Abbreviations: TPP: thiamine pyrophosphate; DTT: dithiothreitol; PMS: phenazine methosulphate; INT: 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride; DTNB: 5-5'dithiobis(2-nitrobenzoic acid); CoA: coenzyme A; MDH: malate dehydrogenase; LDH: lactate dehydrogenase; ADP: adenosine diphosphate; NAD: nicotinamide adenine dinucleotide (oxidized); NADH: nicotinamide adenine dinucleotide (reduced). ( ) indicate the reference to the method.

Table II. Ammonia Levels in Blood and Brains of Normal and Hyperammonemic Rats

Condition	Brain	Blood
Normal	0.45 ± 0.12(3)	0.073 ± 0.01(3)
Subacute	1.40 ± 0.35(3)	1.110 ± 0.16(5)
	$P<0.005$ + 211%	$P<0.001$ + 1420%
Acute	2.64 ± 0.50(3)	1.790 ± 0.08(4)
	$P<0.001$ + 487%	$P<0.001$ + 2352%

Values are expressed in brain as μmol of ammonia/gm·wet wt. and in blood as μmol of ammonia/ml. Number in parenthesis indicates number of experiments.

to be higher in synaptosomes than in cytosol. However, in the case of malate dehydrogenase, the magnitude of difference between different fractions was not as high as with other enzymes. Activity levels of aspartate aminotransferase were observed to be higher in synaptosomal and cytosolic fractions than in mitochondria. An opposite trend was observed in the distribution of alanine aminotransferase (Tables IV - VI).

Effects of Ammonia

Mitochondria (Table IV). Administration of subacute dose of ammonium salts (group I) resulted in a marginal elevation in the activity of pyruvate dehydrogenase without altering those of citrate synthase and isocitrate dehydrogenase. Activities of α-ketoglutarate and succinate dehydrogenases were enhanced under these conditions. In contrast to the above said enzymes, malate dehydrogenase activity, when measured in the direction of oxaloacetate formation, was suppressed in subacute ammonia toxicity. However, in the reverse direction, i.e., in the direction of malate formation, it was unaltered. Activities of aspartate and alanine aminotransferases decreased under these conditions.

Administration of an acute dose of ammonium acetate (group II) resulted in an elevation in the activities of pyruvate, isocitrate and succinate dehydrogenases in the mitochondrial fraction. Under these conditions, activities of cirate synthase and a-ketoglutarate dehydrogenase were unaffected. Changes observed in the activities

of malate dehydrogenase and aspartate and alanine aminotransferases were similar to those observed in subacute toxicity. It is interesting to note that there is a small but statistically significant increase in the mitochondrial protein under these conditions (Table III).

Synaptosomes (Table V). Following the administration of a subacute dose of ammonium acetate, there was an elevation in the activities of pyruvate, isocitrate, α-ketoglutarate and succinate dehydrogenases while that of citrate synthase was unchanged in the synaptosomes. Malate dehydrogenase activity, in the direction of oxaloacetate formation, was suppressed in synaptosomes in subacute ammonia toxicity. However, in the reverse direction, activity of this enzyme was enhanced under these conditions. Activities of both the aminotransferases decreased in the synaptosomes in subacute ammonia toxicity.

Pattern of changes in the activities of pyruvate dehydrogenase, citric acid cycle enzymes and the aspartate and alanine aminotransferases in synaplosomes in acute ammonia toxicity were similar lo those observed in the subacute toxicity. Exceptions to this were seen in the activity of malate dehydrogenase in the direction of malate formation which was suppressed in acute ammonia toxicity. Though the activity of alanine aminotransferase was suppressed under these conditions, the change was statistically not significant. Protein content of cortical synaptosomes decreased in acute ammonia toxicity while in subacute toxicity this change was statistically not significant (Table III).

Cytosol (Table VI). Administration of a subacute dose of ammonium acetate resulted in an elevation in the activities of pyruvate dehydrogenase, citrate synthase, isocitrate, α-ketoglutarate and succinate dehydrogenases in the cytosol. Eventhough the magnitude of their increase in cytosol was much higher than that in the other two fractions, activities of these enzymes in the cytosol were lower than that of mitochondria and

Table III. Protein Levels in Subcellular Fractions of Cerebral Cortex in Normal and Ammonium Acetate Injected Rats

Fraction	Normal	Acute	Subacute
Mitochondria	7.0 ± 1.0(21)	9.3 ± 2.7(15) $P<0.005$ +33%	8.0 ± 2.0(9) NS +14%
Synaptosomes	9.0 ± 2.8(16)	7.3 ± 1.2(15) $P<0.05$ −19%	8.4 ± 2.0(9) NS −7%
Cytosol	28.0 ± 4.0(24)	29.9 ± 3.0(20) NS +7%	27.0 ± 4.0(9) NS −4%

Protein: mg/gm-wet wt of tissue. Numbers in parenthesis indicates the number of experiments. Each value is mean ± SD.

Table IV. Effect of Ammonium Acetate on Pyruvate Dehydrogenase and Enzymes of Citric Acid Cycle and of Borst Cycle in the Mitochondria of Rat Cerebral Cortex

Enzyme	Normal	Acute	Subacute
1. PDH	9.66 ± 0.52	15.2 ± 0.37 $P<0.02$ +57%	11.0 ± 1.1 $P<0.05$ +14%
2. CS	65 ± 8	62 ± 11 NS −5%	67 ± 12 NS +3%
3. ICDH (NAD)	35.8 ± 4	43 ± 5 $P<0.05$ +20%	39.5 ± 1.3 NS +10%
4. α−KGDH	22 ± 3.7	26 ± 3.9 NS +18%	32 ± 3.7 $P<0.005$ +45%
5. SDH	2.8 ± 0.43	5.56 ± 0.7 $P<0.001$ +99%	4.94 ± 0.9 $P<0.005$ +76%
6. MDH (MAL→OAA)	536 ± 41	341 ± 24 $P<0.001$ −36%	365 ± 15 $P<0.001$ −32%
7. MDH (OAA→MAL)	472 ± 43	508 ± 8 NS +8%	464 ± 38 NS −2%
8. AAT	150 ± 15	82 ± 9 $P<0.001$ −45%	99 ± 7.6 $P<0.001$ −34%
9. AlAT	23 ± 3.7	2.7 ± 0.23 $P<0.001$ −88%	8.7 ± 1.8 $P<0.001$ −62%

Activity is expressed as **Mean** ± SD.
PDH: pyruvate dehydrogenase; CS: citrale synthetase; ICDH: isocitrate dehydrogenase; α-KGDH: α-ketoglutarate dehydrogenase; SDH: succinate dehydrogenase; MDH: malate dehydrogenase; AAT: aspartate aminotransferase; A1AT: alanine aminotransferase.
Activity units for PDH, ICDH, «-KGDH, MDH(NAD) are μmol of NAD reduced/mg protein/hr and for CS is μmol of citrate formed/mg protein/hr. SDH is μmol of succinate oxidized/mg protein/hr and for MDH(NADH), AAT and A1AT is μmol of NADH oxidized/mg protein/hr No. of experiments are $. For each experiment two animals were used.

Table V. Effect of Ammonium Acetate on Pyruvate Dehydrogenase and Enzymes of Citric Acid Cycle and of Borst Cycle in the Synaptosomes of Rat Cerebral Cortex

Enzyme	Normal	Acute	Subacute
1. PDH	3.42 ± 0.33	13.2 ± 2.6 $P<0.001$ +285%	8.7 ± 0.77 $P<0.001$ +154%
2. CS	32 ± 4	32 ± 2 NS	37 ± 8 NS +16%
3. ICDH (NAD)	11.4 ± 1.72	16.2 ± 3.3 $P<0.025$ +42%	15.7 ± 0.99 $P<0.005$ +32%
4. α-KGDH	19.5 ± 1.99	24 ± 3.3 $P<0.05$ +23%	25 ± 1.62 $P<0.005$ +28%
5. SDH	0.53 ± 0.06	0.92 ± 0.15 $P<0.005$ +74%	0.73 ± 0.016 $P<0.001$ +38%
6. MDH (MAL→OAA)	388 ± 33	251 ± 21 $P<0.005$ −35%	285 ± 24 $P<0.005$ −27%
7. MDH (OAA→MAL)	462 ± 35	383 ± 25 $P<0.005$ −17%	556 ± 28 $P<0.005$ +20%
8. AAT	192 ± 8.3	84 ± 12 $P<0.001$ −56%	108 ± 13 $P<0.001$ −44%
9. AlAT	5.2 ± 0.7	4.3 ± 0.53 NS −18%	3.8 ± 0.22 $P<0.005$ −27%

Legend as in Table IV.

synaptosomes. As observed in the other two fractions, malate dehydrogenase activity in the direction of malate to oxaloacetate was suppressed. However, activity of this enzyme in reverse direction was elevated. Activities of both the aminotransferases were suppressed in this subcellular fraction following the administration of subacute dose of ammonium acetate.

Administration of acute dose of ammonium acetate brought about changes in the activities of these enzymes which are similar to those described above. In the case of pyruvate dehydrogenase, citrate synthase, isocitrate, α-ketoglutarate and succinate dehydrogenases and alanine aminotransferase, the magnitude of change under these conditions was higher than that of subacute toxicity.

DISCUSSION

In studies dealing with subcellular fractions, it is customary to establish the purity of the fractions by determining the marker enzymes. However, caution must be exerted in situations where the activities of marker enzymes are also altered in the experimental condition (17). In the present case, activities of the markers (lactate dehydrogenase and succinate dehydrogenase for cytosol and mitochondria respectively) were also altered substantially in the hyperammonemic state (Table VII). Hence, the relative activities of the marker enzymes were taken into consideration. Changes in the ratio of succinate dehydrogenase activity between mitochondria and synaptosomes were statistically not significant indicating that there were no alterations in the purity of these preparations. However, the cytosol/mitochondria ratio of lactate dehydrogenase was altered only in the subacute condition. A statistically significant increase in the ratio for succinate dehydrogenase in these two fractions was observed in hyperammonemic states. Though these results indicated a contamination of mitochondria with cytosol, this is difficult to comprehend as the buoyant densities of these two fractions are different and they are separated at an early stage of preparation. Hence, the changes in these ratios are due to drug induced changes in the activities of lactate and succinate dehydrogenases rather than contamination of fractions.

Increased activity of pyruvate dehydrogenase observed presently in the conical mitochondria isolated from the brains of hyperammonemic rats is in agreement with our earlier reports in homogenates (10). Such an increase in the activity of this enzyme might permit channelling of more pyruvate into citric acid cycle. However, lack of change in the activities of citrate synthase and isocitrate dehydrogenase in subacute conditions and citrate synthase and a-ketoglutarate dehydrogenase in acute states might limit the flow of carbons through this cycle. Suppression of malate dehydrogenase activity in the direction of oxaloacetate production lowers the formation of oxaloacetate and results in the accumulation of malate. A fall in the production of oxaloacetate would affect the rate of synthesis of citrate. Moreover, this would

Table VI. Effect of Ammonium Acetate on Pyruvate Dehydrogenase and Enzymes of Citric Acid Cycle and of Borst Cycle in the Cytosol of Rat Cerebral Cortex

Enzyme	Normal	Acute	Subacute
1. PDH	1.036 ± 0.18	7.2 ± 0.52 $P<0.001$ +595%	3.46 ± 0.5 $P<0.001$ +234%
2. CS	0.8 ± 0.05	1.8 ± 0.2 $P<0.001$ +125%	1.5 ± 0.4 $P<0.001$ +88%
3. ICDH (NAD)	1.5 ± 0.47	2.9 ± 0.49 $P<0.005$ +93%	2.2 ± 0.32 $P<0.025$ +47%
4. α-KGDH	3.6 ± 0.54	11.4 ± 2.2 $P<0.001$ +220%	5.5 ± 0.55 $P<0.001$ +54%
5. SDH	0.04 ± 0.002	0.33 ± 0.042 $P<0.001$ +725%	0.197 ± 0.018 $P<0.001$ +393%
6. MDH (MAL→OAA)	392 ± 66	228 ± 24 $P<0.001$ −42%	240 ± 22 $P<0.005$ −39%
7. MDH (OAA→MAL)	576 ± 68	719 ± 27 $P<0.005$ +25%	755 ± 69 $P<0.005$ +31%
8. AAT	129 ± 13	71 ± 6 $P<0.001$ −45%	68 ± 7.6 $P<0.001$ −47%
9. AIAT	12 ± 1.8	7.8 ± 0.76 $P<0.001$ −36%	5.98 ± 0.78 $P<0.001$ −52%

Legend as in Table IV.

also limit the amount of oxaloacetate available for transamination of glutamate, which together with the suppression of aspartate aminotransferase, would lower the production of aspartate in mitochondria. As mitochondrial aspartate is exchanged for cytosolic glutamate during the operation of malate-aspartate shuttle, reduction in aspartate levels would affect the operation of this shuttle. The reported fall in aspartate and increase in malate levels in brain in hyperammonemic states are in agreement with this suggestion (9, 18). Though malate dehydrogenase activity in the direction of malate formation is unaffected, it would be of little consequence as malate is not the substrate for citrate synthase and aspartate aminotransferase.

It is quite possible that more than one mechanism may be involved in bringing about the observed changes in the activities of different enzymes in hyperammonemic states. These may be an increase in mitochondrial content (Table III); changes in the phosphorylation-dephosphorylation of enzymes such as pyruvate, isocitrate and α-ketoglutarate dehydrogenases (19, 20) and changes in the membrane fluidity (21). Though an increase in the mitochondrial protein content was observed in the present investigation, further studies are required in this direction.

Changes in the activities of malate dehydrogenase (in the direction of OAA formation) and aspartate aminotransferase in cytosol in hyperammonemic states are similar to those of mitochondria. However, in cytosol malate dehydrogenase is supposed to be involved in the synthesis of malate and it is interesting to note that the enzyme activity in the direction is enhanced in hyperammonemic states. Despite this, production of malate in this compartment would be limited due to the **reduction** in the amount of aspartate available (c.f. above) and fall in the activity of aspartate aminotransferase in this compartment. Thus, it appears that reduction of malate dehydrogenase activity in mitochondria and of aspartate aminotransferase in mitochondria and cytosol might be the reasons for the failure of malate-aspartate shuttle in hyperammonemic states. Moreover, reduced **aspartate** aminotransferase activity would affect the production of glutamate which is required for the exchange with mitochondrial aspartate and also for glutamine synthesis. It is interesting to note that addition of either glutamate or aspartate normalized the malate-aspartate shuttle in the primary cultures of astrocytes in the presence of pathophysiological concentrations of ammonium chloride (22). Under in vivo conditions, such a situation is averted by the augmented production of glutamate from the transamination of branched chain amino acid and of oxaloacetate by carbon dioxide fixation (23, 24).

Changes observed in the cytosolic activities of pyruvate dehydrogenase and citric acid cycle enzymes in hyperammonemic states are surprising as this fraction is supposed to be devoid of them. Activities of these enzymes, though less in this fraction when compared to the milochondria, were enhanced in hyperammonemic state. Such an increase is difficult to explain unless it is assumed that at least some mitochondria have altered

Table M1. Activity Levels and Relative Percentages of Marker Enzymes in Subcellubr Fractions of Normal and Hyperammonemic Rats

Enzyme		Cytosol	Mitochondria	Synaptosomes
SDH	N	0.040 ± 0.003	2.8 ± 0.2	0.53 ± 0.04
	SA	0.197 ± 0.015	4.9 ± 0.4	0.73 ± 0.01
		$P<0.001$ +393%	$P<0.005$ +76%	$P<0.001$ +38%
	A	0.330 ± 0.009	5.56 ± 0.71	0.90 ± 0.10
		$P<0.001$ +725%	$P<0.001$ +99%	$P<0.05$ +74%
LDH(1)	N	385 ± 72	79 ± 3	121 ± 9
	SA	295 ± 15	24 ± 3	95 ± 8
		$P<0.001$ −23%	$P<0.001$ −70%	$P<0.005$ −21%
	A	145 ± 29	34 ± 7	108 ± 4
		$P<0.001$ −62%	$P<0.001$ −57%	$P<0.01$ −11%
LDH(2)	N	38 ± 4	23 ± 4	23 ± 3
	SA	69 ± 5	24 ± 2	34 ± 4
		$P<0.001$ +82%	N.S. +4%	$P<0.005$ +48%
	A	48 ± 12	22 ± 3	47 ± 8
		N.S. +26%	N.S. −4%	$P<0.001$ +104%
			Relative Percentages	
SDH	N	1.58 ± 0.26	100 ± 0	19.3 ± 3.5
	SA	4.10 ± 0.76	100 ± 0	15.2 ± 2.6
		$P<0.001$		N.S.
	A	6.00 ± 1.1	100 ± 0	15.7 ± 4.8
		$P<0.001$		N.S.
LDH(1)	N	100 ± 0	21 ± 2.0	31.6 ± 3.7
	SA	100 ± 0	8.2 ± 1.1	32.0 ± 3.4
			$P<0.001$	N.S.
	A	100 ± 0	23.9 ± 4.0	75.6 ± 12.0
			N.S.	$P<0.001$
LDH(2)	N	100 ± 0	63 ± 15	63 ± 14
	SA	100 ± 0	35 ± 6	50 ± 7
			$P<0.005$	N.S.
	A	100 ± 0	47 ± 11	101 ± 23
			N.S.	$P<0.02$

LDH : Lactate dehydrogenase (1) pyruvate → lactate, activity is expressed as μmoles of NADH oxidized/mg protein/hr; (2) lactate → pyruvate. activity is expressed as μmoles of NAD reduced/mg protein/hr. Rest of the legend same as in Table IV.

their buoyant density due to swelling and sediment at higher centrifugal forces or a population of mitochondria degenerate and release their contents in to cytosol in hyperammonemic states. It is interesting to note that such changes have been reported in the mitochondria in hyperammonemic states (25). Despite these changes, availability of substrates and NAD^+ required for these enzymes in cytosol might be inadequate and rate limiting. Hence, such changes may not influence cellular energy metabolism under these conditions.

As synaptosomes used in the present study have both mitochondria and cytosol, changes observed in the activities of pyruvate dehydrogenase and citric acid cycle enzymes might be similar to those described above. A small population of synaptic mitochondria might have also degenerated under these conditions and liberated their contents into synaptoplasm.

One pertinent point to be discussed at this juncture is whether the activities of enzymes measured in vitro especially under optimal conditions serve as representatives of in vivo changes. These enzymes from normal and hyperammonemic rats were measured under identical assay conditions where optimal concentrations of substrates and cofactors and pH are provided. It is apparent that the observed changes in the activities of these enzymes are not experimental artifacts, but have occurred *in situ* and have survived the isolation procedure and hence they may represent changes that have taken place in vivo.

Present study, thus, suggests a derangement in the operation of malate-aspartate shuttle in the hyperammonemic states might be due to the suppression of malate dehydrogenase in mitochondria and of aspartate aminotransferase in mitochondria and cytosol and the

possibility of exislcnce of a small population of mitochondria which arc highly vulnerable to ammonia. Further studies are being conducted to localise these changes in the specific cellular compartments of brain.

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Effect of methionine sulfoximine on pyruvate dehydrogenase, citric acid cycle enzymes and aminotransferases in the subcellular fractions isolated from rat cerebral cortex

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The effect of acute and subacute doses of L-methionine-DL-sulfoximine (MSI) were studied on the **activities of** pyruvate dehydrogenase, **enzymes of citric** acid cycle and aspartate and alanine aminotransferases in t he mitochondria, synaptosomes and **cytosol** of rat brain. In general, the activities **of pyruvate** dehydrogenase and **of** the citric acid **cycle** enzymes, except malate dehydrogenase (malate→oxaloacetate), were elevated in all 3 subcellular fractions. **Malate** dehydrogenase activity (malate→oxaloacetate) was suppressed in the mitochondria while the activity of this **enzyme** in the reverse direction was enhanced in the **cytosol.** Activities of aspartate and alanine aminotransferases were suppressed under these conditions. As the **effects** of MSI on these **enzymes** were similar to those **observed** upon the administration of ammonium salts, it is suggested that the hyperammonemie stale induced by MSI might derange the operation of the malate-aspartate shuttle Increased activities of citric acid cycle enzymes in the cytosol suggested the existence of a small population ol mitochondria which was highly **vulnerable** either to ammonia or to **MSI.**

Methionine sulphoximine is a potent convulsant with prolonged latency period and the onset of **convulsions** varies with the dosage of the drug administered and the age of the animal [5. 6]. Though it has been suggested earlier that the toxic effects of this drug are primarily due to the inhibition of glutamine synthetase (GS). recent evidence is not supportive of such a suggestion [5]. However, the induction of hyperammonemic state by methionine sulphoximine (due to the inhibition of GS) has been confirmed by several investigators [5. 6, 10].

Pathophysiological concentrations of ammonia arc known to affect the cerebral energy **metabolism** either by draining away the intermediates of citric acid cycle or by **depleting** cerebral energy reserves [1]. Though there are numerous reports on the levels of these intermediates in hyperammonemic states **[2],** very little information is

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available on the activities of enzymes and the specific mechanisms involved in the alteration of the levels of these intermediates in MSI induced hyperammonemic states. In the present study, we report the changes in the activity levels of pyruvate dehydrogenase and citric acid cycle enzymes along with those of aminotransferases in mitochondria, synaptosomes and cytosol isolated from the brains of rats treated with methionine sulphoximine and compared them with those of normal rats. Results of the present study indicated a generalized increase in the activities of pyruvate dehydrogenase and of citric acid cycle enzymes except that of malate dehydrogenase in the mitochondria and synaptosomes isolated from the brains of methionine sulphoximine treated animals. Further, results of the present study also indicated suppression of the activities of malate dehydrogenase (in the direction of malate→oxaloacetate) and of the aminotransferases which is suggestive of a derangement in the transport of reducing equivalents across the mitochondnal membranes through the malate-aspartate shuttle. It was also observed that the activities of these enzymes were enhanced in the cytosol which is suggestive of the existence of a small population of mitochondria which degenerates and liberates their contents into the cytosol under these conditions.

L-Methionine-DL-sulphoximine was dissolved in saline (pH 7.0) and was administered intraperitoneally to 6-month-old Wistar rats. The subacute group received 150 mg of the drug kg b.wt. while the acute group received 300 mg of the drug, kg b.wt. The subacute and acute groups of animals were decapitated at the end of 17.5 and 3.5 h, respectively. Methods adopted for the preparation of the subcellular fractions (mitochondria, synaptosomes and cytosol) from the cerebral cortex and for the enzyme assays have been described earlier [8]. Ammonia content was determined as described earlier [10] in the extracts of frozen (liquid nitrogen) brains. Protein content was determined by the method of Lowry et al. [4]. Statistical analysis of the data was by Newman Keul's multiple-range analysis.

Behavioural changes observed prior to the onset of convulsions in the rats administered with MSI have been described earlier [5, 6, 9, 10]. Acute group of animals entered into convulsions between 3.5 and 4 h while the onset of convulsions was

TABLE I

AMMONIA LEVELS IN BLOOD AND BRAINS OF NORMAL AND METHIONINE SULFOXIMINE TREATED RATS

Values are expressed in brain as μmol of ammonia g wet wt. and in blood as μmol of ammonia/ml. Number in parentheses indicates number of experiments.

Condition	Brain	Blood
Normal	0.45 ± 0.12(3)	0.073 ± 0.01(3)
Subacute	2.10 ± 0.20(5) $P<0.001$, +367%	0.700 ± 0.10(5) $P<0.001$, +859%
Acute	2.70 ± 0.50(5) $P<0.001$, +500%	0.800 ± 0.10(5) $P<0.001$, +996%

observed to be at 17.5 h in the subacute group. The mortality rale was very high in both the groups alter the convulsions, hence the animals were sacrificed during the convulsive phase. Blood and brain ammonia levels in the normal animals (Table I) were within the range of reported values [2]. Following the administration of MSI. the ammonia levels in the brain and blood were enhanced. The magnitude of elevation in the ammonia levels in both the groups were observed to be higher in the blood than in the brain. As was expected, the increase in the blood and brain ammonia levels were higher in acute group of animals than in subacute group of animals.

TABLE II

EFFECT OF METHIONINE SULFOXIMINE ON PYRUVATE **DFHYDROGENASE** AND **ENZYMES OF CITRIC ACID CYCLE AND BORST CYCLE IN THE MITOCHONDRIA OF RAT CEREBRAL CORTEX**

Activity is expressed as mean ±S.D.
PDH, pyruvate dehydrogenase; CS. citrate synthetase; ICDH, isocitrate dehydrogenase; α-KGDH, α-ketoglutarate **dehydrogenase:** SDH. succinate **dehydrogenaie; MDH,** malate **dehydrogenase;** AAT, aspartate aminotransferase; AI\T. **alanine** aminotransferase. Activity units for PDH, ICDH. α-KGDH, MDH(NAD') are μmol of NAD' reduced, mg proleinh and for CS μmol of **citrate** formed mg protein h. SDH is μmol of succinate oxidized mg protein h and Tor MDH(NADH), AAT and AIAT is μmol of NADU **oxidized** mg **protein h.** No. ol experiments is 5. For each experiment two animals were used

Enzyme	Normal	Acute	Subacute
PDH	9.66 ± 0.52	15.0 ± 3.0 $P<0.001$, + 56%	11.0 ± 0.8 $P<0.01$, + 12%
CS	65 ± 8	69 ± 0.4 N.S., + 6%	64 ± 7 N.S., − 1.5%
ICDH (NAD')	35.8 ± 4	45 ± 2.5 $P<0.001$, + 25%	39.0 ± 3.1 $P<0.2$, + 8%
α-KGDH	22 ± 3.7	44 ± 3.0 $P<0.001$, + 100%	36 ± 4.0 $P<0.001$, + 64%
SDH	2.8 ± 0.43	12.2 ± 1.3 $P<0.001$, + 336%	8.40 ± 0.65 $P<0.001$, + 200%
MDH (MAL→OAA)	536 ± 41	270 ± 33 $P<0.001$, − 50%	395 ± 45 $P<0.001$, − 26%
MDH (OAA→MAL)	472 ± 43	477 ± 40 N.S., + 1%	495 ± 52 N.S., + 5%
AAT	150 ± 15	112 ± 13 $P<0.001$, − 19%	117 ± 14 $P<0.001$, − 22%
AIAT	23 ± 3.7	10 ± 1.1 $P<0.001$, − 56%	14 ± 2.5 $P<0.005$, − 39%

TABLE III

EFFECT OF METHIONINE SULFOXIMINE ON PYRUVATE DEHYDROGESASE AND ENZYMES OF CITRIC ACID CYCLE AND BORST CYCLE IN THE SYNAPTOSOMES OF RAT CEREBRAL CORTEX

Legend as in Table II.

Enzyme	Normal	Acute	Subacute
PDH	3.42 ± 0.33	10.0 ± 1.2 $P < 0.001$, + 194%	9.0 ± 0.5 $P < 0.001$, + 165%
CS	32 ± 4	40 ± 0.4 $P < 0.001$, + 25%	38 ± 4 $P < 0.025$, + 19%
ICDH (NAD^+)	11.4 ± 1.72	20.0 ± 1.4 $P < 0.001$, + 82%	16.0 ± 1.4 $P < 0.005$, + 45%
α-KGDH	19.5 ± 1.99	30 ± 2.7 $P < 0.001$, + 58%	25 ± 2.8 $P < 0.02$, + 32%
SDH	0.53 ± 0.06	0.80 ± 0.1 $P < 0.001$, + 60%	0.70 ± 0.04 $P < 0.001$, + 40%
MDH (NAD^+) (MAL→OAA)	388 ± 33	215 ± 25 $P < 0.001$, −44%	281 ± 29 $P < 0.001$, −27%
MDH (NADH) (OAA→MAL)	462 ± 35	472 ± 45 N.S., + 2%	455 ± 47 N.S., −1.5%
AAT	192 ± 8.3	95 ± 9 $P < 0.001$, −50%	141 ± 11 $P < 0.001$, −26%
AlAT	5.2 ± 0.7	2.4 ± 0.4 $P < 0.001$, −52%	3.9 ± 0.4 $P < 0.02$, −22%

Purity of the isolated subcellular fractions and the activity levels of the enzymes of citric acid cycle and of aminotransferases in the mitochondrial. synaptosomal and cytosolic fractions prepared from the normal animals have been described earlier [8]. Activities of pyruvate, α-ketoglutarate and succinate dehydrogenases were enhanced in the mitochondrial fraction isolated from the brains of subacute group of animals when compared to the controls. Activity levels of citrate synthase. isocitrate dehydrogenase and malate dehydrogenase (oxaloacetate→malate) were unaltered under these conditions. However, activities of malate dehydrogenase (malate→oxaloacetate) and of aminotransferases were suppressed in the mitochondrial fraction isolated from animals injected with subacute dose of MSI.

In the synaptosomal fraction, activity levels of pyruvate. isocitrate. α-ketoglutarate and succinate dehydrogenases were enhanced following the administration of a subacute dose of MSI. Citrate synthase activity was elevated marginally but not to a significant extent. Malate dehydrogenase activity measured in the direction of malate

to oxaloacetate was suppressed while the activity of same in the reverse direction was unaltered. Activities of both the aminotransferases were suppressed in the synaptosomal fractions prepared from the subacute group ol animals.

In the cytosol more or less a similar profile of changes were observed and the magnitude of changes in the activities of pyruvate dehydrogenasc and succinate dehydrogenase were much higher than those in the mitochondria. In contrast to the observations made in the synaptosomal fractions, activity of malate dehydrogenasc (oxaloacetate → malate) was enhanced in cytosol. The magnitudes of changes in (he activities of aminotransfcrases in the cytosol were similar to those described above.

In all the 3 subcellular fractions isolated from the brains of rats administered with an acute dose of MSI, changes in the enzyme activities were more or less similar to those observed in the subacutc state with few exceptions. In general, the magnitude

TABLE IV

EFFECT OF METHIONINE SULFOXIMINE ON PYRUVATE DEHYDROOF-NASE AND ENZYMES OF CITRIC ACID *i* YC'LE AND BORST CYCLE IN THI CYTOSOL OF RAT CERI BRAL CORTEX

Legend as in Table II

Enzyme	Normal	Acute	Subacute
PDH	1.036 ± 0.18	4.6 ± 0.2 $P<0.001$, + 360%	2.50 ± 0.3 $P<0.001$, + 150%
CS	0.8 ± 0.05	1.6 ± 0.2 $P<0.001$, + 100%	1.1 ± 0.1 $P<0.001$, + 38%
ICDH (NAD^+)	1.5 ± 0.47	2.7 ± 0.3 $P<0.005$, + 80%	2.0 ± 0.2 N.S., + 33%
α-KGDH	3.6 ± 0.54	6.2 ± 0.6 $P<0.001$, + 72%	4.5 ± 0.5 $P<0.001$, + 25%
SDH	0.04 ± 0.002	0.36 ± 0.04 $P<0.001$, + 800%	0.180 ± 0.02 $P<0.001$, + 350%
MDH (NAD^+) (MAL→OAA)	392 ± 66	171 ± 52 $P<0.001$, − 56%	301 ± 34 $P<0.02$, − 23%
MDH (NADH) (OAA→MAL)	576 ± 68	764 ± 74 $P<0.001$, + 33%	682 ± 68 $P<0.01$, + 18%
AAT	129 ± 13	67 ± 16 $P<0.001$, − 48%	102 ± 9 $P<0.001$, − 21%
AIAT	12 ± 1.8	3.2 ± 0.3 $P<0.001$, − 73%	8.20 ± 0.9 $P<0.001$, − 32%

TABLE III

EFFECT OF METHIONINE SULFOXIMINE ON PYRUVATE DEHYDROGENASE AND ENZYMES OF CITRIC ACID CYCLE AND BORST CYCLE IN THE SYNAPTOSOMES OF RAT CEREBRAL CORTEX

Legend as in Table II.

Enzyme	Normal	Acute	Subacute
PDH	3.42 ± 0.33	10.0 ± 1.2 $P < 0.001$, + 194%	9.0 ± 0.5 $P < 0.001$, + 165%
CS	32 ± 4	40 ± 0.4 $P < 0.001$, + 25%	38 ± 4 $P < 0.025$, + 19%
ICDH (NAD^+)	11.4 ± 1.72	20.0 ± 1.4 $P < 0.001$, + 82%	16.0 ± 1.4 $P < 0.005$, + 45%
α-KGDH	19.5 ± 1.99	30 ± 2.7 $P < 0.001$, + 58%	25 ± 2.8 $P < 0.02$, + 32%
SDH	0.53 ± 0.06	0.80 ± 0.1 $P < 0.001$, + 60%	0.70 ± 0.04 $P < 0.001$, + 40%
MDH (NAD^+) (MAL→OAA)	388 ± 33	215 ± 25 $P < 0.001$, −44%	281 ± 29 $P < 0.001$, −27%
MDH (NADH) (OAA→MAL)	462 ± 35	472 ± 45 N.S., + 2%	455 ± 47 N.S., − 1.5%
AAT	192 ± 8.3	95 ± 9 $P < 0.001$, −50%	141 ± 11 $P < 0.001$, −26%
AlAT	5.2 ± 0.7	2.4 ± 0.4 $P < 0.001$, −52%	3.9 ± 0.4 $P < 0.02$, −22%

Purity of the isolated subcellular fractions and the activity levels of the enzymes of citric acid cycle and of aminotransferases in the mitochondrial. synaptosomal and cytosolic fractions prepared from the normal animals have been described earlier [8]. Activities of pyruvate. α-ketoglutarate and succinate dehydrogenases were enhanced in the mitochondrial fraction isolated from the brains of subacute group of animals when compared to the controls. Activity levels of citrate synthase. isocitrate dehydrogenase and malate dehydrogenase (oxaloacetate→malate) were unaltered under these conditions. However, activities of malate dehydrogenase (malate→oxaloacetate) and of aminotransferases were suppressed in the mitochondrial fraction isolated from animals injected with subacute dose of MSI.

In the synaptosomal fraction, activity levels of pyruvate, isocitrate, α-ketoglutarate and succinate dehydrogenases were enhanced following the administration of a subacute dose of MSI. Citrate synthase activity was elevated marginally but not to a significant extent. Malate dehydrogenase activity measured in the direction of malate

to oxaloacetatc was suppressed while the activity of same in the reverse direction was unaltered. Activities of both the aminotransferases were suppressed in the synaptosomal fractions prepared from the subacute group of animals.

In the **cytosol** more or less a similar profile of changes were observed and the magnitude of changes in the activities of pyruvate dehydrogenase and succinate dehydrogenase were much higher than those in the mitochondria. In contrast to the observations made in the synaptosomal fractions, activity of nialate dehydrogenase (oxaloacetate→malate) was enhanced in cytosol. The magnitudes of changes in the activities of aminotransferases in the cytosol were similar to those described above.

In all the 3 subcellular fractions isolated from the brains of rats administered with an acute dose of MSI. changes in the enzyme activities were more or less similar lo those observed in the subacute state with few exceptions. In general, the magnitude

TABLE IV

EFFECT OF METHIONINE SULFOXIMINE ON PYRUVATE **DEHYDROGENASE** AND **ENZYMES** OF CITRIC **ACID** CYCLE AND BORST **CYCLE** IN TUT **CYTOSOL OF** RAT **CEREBRAL CORTEX**

legend as in **Table** II.

Enzyme	Normal	Acute	Subacute
PDH	1.036 ± 0.18	4.6 ± 0.2 $P < 0.001$, + 360%	2.50 ± 0.3 $P < 0.001$, + 150%
CS	0.8 ± 0.05	1.6 ± 0.2 $P < 0.001$, + 100%	1.1 ± 0.1 $P < 0.001$, + 38%
ICDH (NAD⁺)	1.5 ± 0.47	2.7 ± 0.3 $P < 0.005$, + 80%	2.0 ± 0.2 N.S., + 33%
α-KGDH	3.6 ± 0.54	6.2 ± 0.6 $P < 0.001$, + 72%	4.5 ± 0.5 $P < 0.001$, + 25%
SDH	0.04 ± 0.002	0.36 ± 0.04 $P < 0.001$, + 800%	0.180 ± 0.02 $P < 0.001$, + 350%
MDH (NAD⁺) (MAL→OAA)	392 ± 66	171 ± 52 $P < 0.001$, − 56%	301 ± 34 $P < 0.02$, − 23%
MDH (NADH) (OAA→MAL)	576 ± 68	764 ± 74 $P < 0.001$, + 33%	682 ± 68 $P < 0.01$, + 18%
AAT	129 ± 13	67 ± 16 $P < 0.001$, − 48%	102 ± 9 $P < 0.001$, − 21%
AlAT	12 ± 1.8	3.2 ± 0.3 $P < 0.001$, − 73%	8.20 ± 0.9 $P < 0.001$, − 32%

of change was much higher in the acute group of animals than in the subacute. The activity of isocitrate dehydrogenase in the mitochondrial fraction isolated from acute group was enhanced significantly.

Results obtained in the present study on the enzymes of citric acid cycle and aminotransferases in the subcellular fractions were similar to those reported in the cerebral homogenates prepared from rats treated either with MSI or ammonium acetate and to those in the subcellular fractions prepared from brains of rats treated with ammonium acetate [6-8]. Increased activity of pyruvate dehydrogenase in the mitochondria might favour the channeling of pyruvate into the citric acid cycle. Similarly, increased activities of isocitrate, α-ketoglutarate and succinate dehydrogenases might enhance carbon flux through the citric acid cycle in cerebral mitochondria in MSI toxicity. Though citrate synthetase activity in the mitochondria was not altered under these conditions, increased activities of other enzymes might pull the reaction forward. However, reduction in malate dehydrogenase activity in the direction of oxaloacetate formation might act as a constraint and result in the accumulation of malate. Though malate dehydrogenase activity in the direction of malate formation was unaltered, it will be of little importance as malate is not the substrate for citrate synthetase. Similar changes might also occur in the synaptosomal fraction as the synaptosomes are known to contain mitochondria. The decrease in the activity of malate dehydrogenase (malate→oxaloacetate) would not only limit the oxaloacetate formation but also results in accumulation of malate. Suppression in the aspartate aminotransferase activity together with that of malate dehydrogenase might lower the production of aspartate in mitochondria. Hence, it appears that suppression of malate dehydrogenase activity in mitochondria and aspartate aminotransferase both in mitochondria and cytosol might be the reasons for the failure of the malate-aspartate shuttle in hyperammonemic states. The decreased activity of aspartate aminotransferase would limit the formation of glutamate in cytosol which is required for the exchange with aspartate from mitochondria.

Enhancement in the activities of pyruvate dehydrogenase and of citric acid cycle enzymes in the cytosol were surprising as this subcellular fraction is supposed to be free of mitochondria. However, it must be mentioned that even under these conditions, activities of these enzymes were much lesser in the cytosol compared to the mitochondria. Such an increase in the activities of these enzymes in the cytosol may be due to altered buoyant density and/or fragmentation of mitochondria as a result of which they do not sediment along with larger mitochondria. It might also be due to the degeneration of the mitochondria and subsequent release of the enzymes into the cytosol. It has been reported that in MSI toxicity mitochondrial number increases and subsequently the mitochondria undergo degeneration [3]. It is quite possible that such degenerating mitochondria might be releasing their contents into the cytosol. In an earlier study, we have reported similar changes in the activities of these enzymes in animals injected with an acute dose of ammonium acetate. Thus the observed changes in the activities of enzymes of citric acid cycle and of aminotransferases might be due to the enhancement of cerebral ammonia levels in MSI toxicity.

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