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STUDIES ON THE ROLE
OF TAURINE IN THE BRAIN

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by

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Abstract

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The role of taurine in neurotransmission has been examined. Cortex slices, synaptosomes and C-6 glioma cells were employed in a study of $[^{35}\text{S}]$ - taurine uptake and its electrically-stimulated release. Both high ($K_m = 5 \times 10^{-4}\text{M}$, $V_{\text{max}} = 0.102 \mu\text{mol/g/min}$) and low ($K_m = 10.83 \times 10^{-3}\text{M}$; $V_{\text{max}} = 1.46 \mu\text{mol/g/min}$) affinity uptake systems were detected in cortex slices. Only low affinity transport was present for leucine ($K_m = 1.16 \times 10^{-3}\text{M}$, $V_{\text{max}} = 0.318 \mu\text{mol/g/min}$). After exposure to taurine at two concentrations, synaptosome preparations subsequently derived from slices contained 41% of the particle-bound taurine and 16% of the total in the tissue. The uptake of $[^{14}\text{C}]$ - GABA by C-6 glioma cells was inhibited more by β -alanine (39%) than by L-DABA (13%) whilst synaptosome preparations showed the opposite pattern, L-DABA (84%) being more effective than β -alanine (26%). $[^{35}\text{S}]$ - taurine uptake inhibition by L-DABA was small for synaptosomes (9%) and C-6 glioma (7%) whereas β -alanine showed considerable effect on C-6 glioma (41%) and slices of white matter (ependyma, 50%). Synaptosome preparations showed little effect with β -alanine (2%). When 30 minutes rather than 5 minutes incubation were employed, β -alanine depressed $[^{35}\text{S}]$ - taurine uptake to synaptosomes (28%), cortex slices (30%) and white matter slices (51%). Electrical pulses released $[^{35}\text{S}]$ - taurine from cortex slices by a calcium-dependent mechanism and subcellular fractionation indicated that the synaptosome fraction showed losses commensurate with the net taurine release when low current stimulations were used. Omission of calcium ions from the incubation medium was found to affect taurine uptake reducing it by one-third.

The passage of orally administered taurine in doses of 600 mg/Kg to the cerebral cortex and other tissues has been followed up to 72 hours. The effect of oral administration of taurine in the above doses on the limb jerk frequency of rats made epileptic by

application of powdered cobalt metal to the cerebral cortex has been studied. Optimum results occurred with prolonged frequent high dosage. Taurine may be useful against types of epilepsy with blood brain barrier defects, such as that due to brain scarring.

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Publications

Part of the work presented in this thesis is described in the following papers:

1. Wheler, G. H. T., Osborne, R.H., Bradford, H.F., & Davison, A.N., (1974).
Treatment of experimental epilepsy with taurine.
Biochem. Soc. Trans. 2, 285-286.
2. Bradford, H.F., Davison, A.N., & Wheler, G.H.T., (1976).
In Taurine. eds Huxtable R. & Barbeau, A. (Raven NY).
Taurine and Synaptic Transmission. p. 303-310.
3. Wheler, G.H.T., Bradford, H.F., & Davison, A.N., (1977).
Uptake studies of taurine in vivo and its effects on the course
of experimental focal epilepsy in rats.
Brain Research. (In press).
4. Wheler, G. H. T., Bradford, H. F., & Davison, A. N., (1977)
Uptake and release of taurine from cortex tissue slices and
their subcellular compartments. (In preparation).

CHAPTER 1

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Section 1.1. Introduction

Taurine has been known to occur in living organisms for 150 years. It was first found as a constituent of ox bile (Tiedmann & Gmelin 1827). It occurs widely throughout the phylogenetic scale, and may even have existed before the origin of life (Choughuley & Lemmon 1966). Nevertheless despite its ubiquity and antiquity little is known about its physiological and biochemical role.

The role of taurine in the body which is most definite is in bile salt metabolism (Jacobsen and Smith 1968). It has been thought that taurine in body tissues is a metabolic end product of little functional importance. Now however, the importance of taurine in several tissues including the brain is becoming established.

Taurine exists in high concentration in the mammalian brain relative to other free amino acids (Agrawal et al 1966). Topical application of taurine to the brain cortex modifies the electrical activity of the tissue as shown by the electroencephalogram (EEG) (Kaczmarek & Adey 1975). Application of taurine to individual neurons in the central nervous system exerts a strong depressant effect on their firing (Curtis & Watkins 1960). Taurine occurs in nerve endings, and its synthesising enzyme cysteinsulphinic acid decarboxylase (L - aspartate 4 - carboxylase EC 4:1:1:12) is enriched there, with half the activity occurring in the nerve ending fraction. (Agrawal et al 1971). Electrical stimulation has been shown to cause the release of taurine from brain slices and this release is sensitive to reduced calcium ion concentration in the incubating medium (Kaczmarek & Davison 1972).

There is also evidence of the involvement of taurine in cerebral pathology. Low tissue concentrations of taurine have been found in focal epilepsy in animals and human epileptics (Koyama 1972; Van Gelder et al 1972). Recently taurine has been reported to be associated with hereditary depression (Perry et al 1975). These findings together suggest that taurine may have an important function in the brain, especially in relation to excitability in the central nervous system. This study has examined further the role of taurine in the brain with special reference to its role as a potential neurotransmitter or modulator in the cerebral cortex.

1.2 Occurrence

Taurine occurs in the cerebral cortex at a concentration second to glutamate among the free amino acids, at $8 \mu\text{mol/g}$ in adult rat brain (De Belleruche & Bradford 1973). In other brain regions it has a heterogeneous distribution (Lombardini 1976). Similar levels to the cerebral cortex are found in the cerebellum, hippocampus, striatum and olfactory lobes. Higher levels are found in the neurohypophysis, pineal and retina. Lower levels are found in the brain stem and spinal cord. Taurine also occurs in human brain at a concentration $0.93 \mu\text{mol/g}$ which is similar to that for aspartate $0.97 \mu\text{mol/g}$, and greater than γ -aminobutyric acid (GABA) $0.42 \mu\text{mol/g}$ (Perry et al 1971). It occurs throughout the cerebral cortex at similar concentrations with the highest concentration in the frontal cortex $1.85 \mu\text{mol/g}$.

1.3 Chemistry

Taurine (2 - aminoethane sulphonic acid) is a colourless compound, of molecular weight 125. It is soluble in water ($10 \text{ g}/100 \text{ ml}$ at 25°C) and has a pK_a of 1.5 and pK_b of 8.74 at 25°C (Andrews & Schmidt 1927). At the pH of intracellular fluid both functional groups are ionized and it exists as a zwitterion. Taurine has been classed as a neutral amino acid (Neame 1968), like GABA, glycine and phosphoethanolamine. Steric rotation or folding of the molecule can occur but to a lesser extent than with GABA because of its bulky sulphonic acid group (Kaczmarek 1971).

1.4. Synthesis

Several pathways for taurine synthesis occur in different species (Jacobsen & Smith 1968). Of these only two pathways to taurine appear important in the brain at the present time. These are the synthesis of taurine from cysteinesulphinic acid, and from cysteamine.

Rigorous proof of a synthetic pathway demands that the appropriate precursor can be shown to be converted to taurine, with the identification of the intermediate products, and of the enzymes associated with the reaction sequence. This has been largely achieved for these two pathways.

The dietary sources of taurine precursors are methionine and cysteine (Awapara 1976). Cystine in the diet is converted to cysteine by a reaction with reduced glutathione in the tissues so that the reduced form predominates in the ratio of 16:1. Methionine sulphur is converted mole per mole into the sulphur of cysteine in the organism. In the human diet the methionine content of the diet is twice that of cysteine, hence cysteine may be regarded as the starting point for taurine synthesis. Accordingly the synthesis of cysteine from methionine is only briefly discussed.

The pathway from methionine first consists of the conversion of methionine to homocysteine. Then there is a transulphuration reaction with homocysteine being converted to cystathionine with concomitant conversion of serine to homoserine, and the formation of cysteine. The intermediates have been demonstrated, and the brain has been shown to possess all the enzymes necessary for this sequence (Mudd et al 1965).

Cysteine is then converted to cysteine sulphinic acid by cysteine oxidase (L - cysteine; oxygen oxidoreductase, E.C. 1:13:11:20). The reaction employs NAD^+ as a cofactor, and 70% of its activity is localized in the microsomal fraction (Misra & Olney 1975). Activity was 0.7 μ mole of cysteine sulphinic acid formed /h/mg of protein. This compares with the report of negligible activity reported by Ewetz & Sorbo (1966) using a homogenate prepared in 0.15 M KCl, and Yamaguchi et al (1971). The reasons for this are probably the nature of the homogenizing conditions and the fact that Yamaguchi examined the enzyme activity in the supernatant fraction rather than in whole homogenate. Appreciable activity of cysteine oxidase is present throughout the brain, with 3 times greater activity in the pons-medulla as compared with cerebral cortex.

The cysteine sulphinic acid formed then undergoes decarboxylation to yield hypotaurine in the presence of the enzyme cysteine sulphinic acid decarboxylase, L - aspartate 4 - carboxylase, EC 4:1:1:12, (this enzyme was formerly indexed as 4:1:1:29). This enzyme is soluble but partly membrane bound, and half of the activity occurs in the nerve ending fraction (Agrawal et al 1971). It uses pyridoxal phosphate as a cofactor, but is not saturated with it as is the case of its isoenzyme in the liver. The pH optimum is 7.6, and the enzyme requires free SH groups for its function.

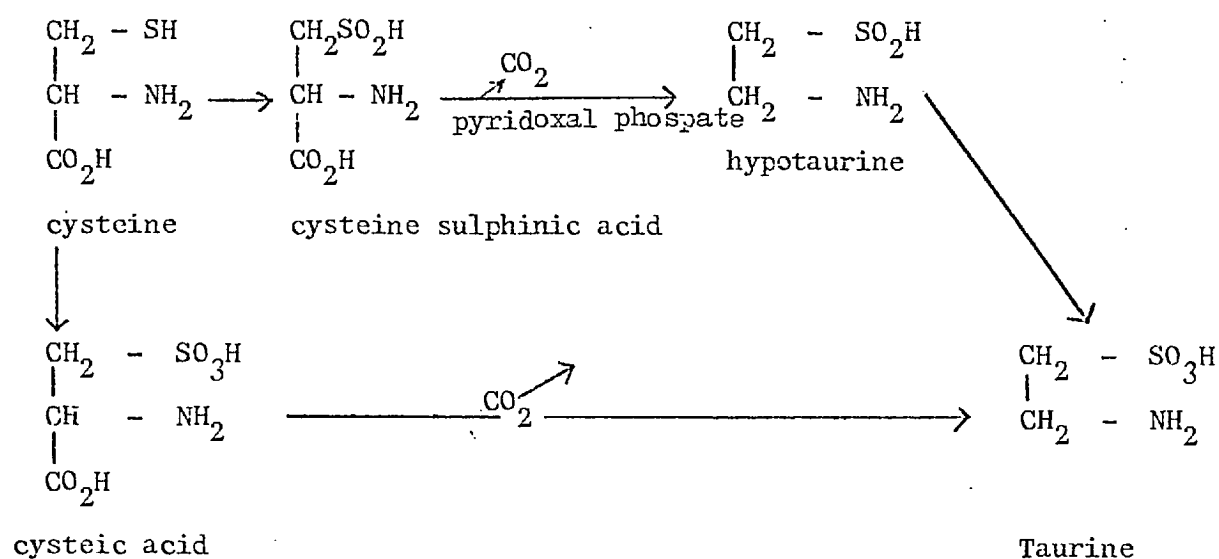
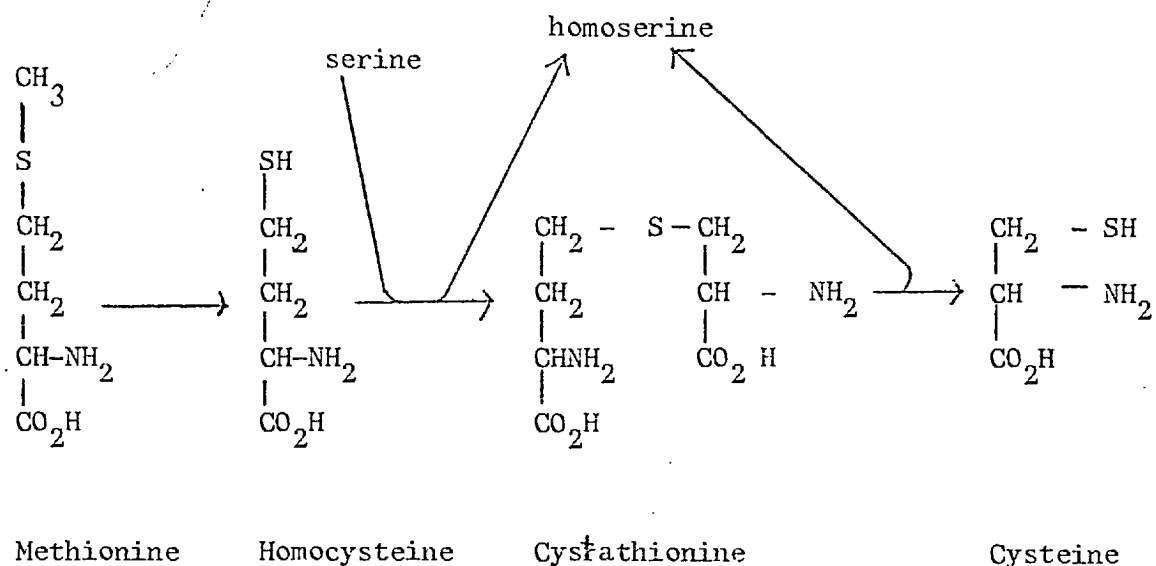
Cysteine sulphinic acid decarboxylase will also decarboxylate cysteic acid, which has been suggested as a precursor of taurine in the brain. Peck and Awapara (1966) have demonstrated the conversion of $[^{35}\text{S}]$ -methionine to cysteine, cysteinesulphinic acid, and cysteic acid in rat brain slices and homogenates. However it appears that the decarboxylation of cysteinesulphinic acid is more important

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in the brain than that of cysteic acid, as the decarboxylation of the former occurs at a rate 2 - 8 times that for the latter in all tissues where decarboxylase activity is demonstrable (Davison 1956, Jacobsen & Smith 1963, Sorbo & Heyman 1957).

Present evidence suggests that cysteine sulphinic acid and cysteic acid are decarboxylated by the same enzyme. Several observations support this view. The ratio of the decarboxylase activity to the two substrates stays the same under varying conditions including pH changes, progressive pyridoxine deficiency, thermal inactivation and partial purification of the enzyme (Jacobsen et al 1964; Blaschko & Hope 1954; Davison 1956). Both substrates inhibit each other competitively in the brain, and the K_m and K_i values for each substrate are in close agreement (Jacobsen et al 1964).

The next step in the sequence of taurine synthesis is oxidation of hypotaurine to taurine. Although the oxidation of hypotaurine to taurine has been demonstrated in vivo (Cavallini et al 1954), no enzyme has been detected so far that is able to oxidase a sulphinic group to the sulphonate level in the brain. Sumizu has reported an enzyme in the soluble fraction of rat liver that catalyses the conversion of hypotaurine to taurine with NAD as a co-factor (Sumizu 1962), but this was not confirmed by other workers (Fiori & Costa 1969). Thus the problem of the mechanism of the conversion of hypotaurine to taurine in the brain remains unsolved.

As mentioned above cysteic acid has been suggested as a precursor of taurine. Evidence against it being important included its low rate of decarboxylation to hypotaurine mentioned above, that it does not occur as such in biological materials, and that incubation



Synthesis of Taurine from Methionine

of cysteine with animal tissues has never produced relevant quantities of cysteic acid. In the liver cysteic acid is converted to taurine slowly by an NAD dependent dehydrogenase of mitochondria (Singer & Kearney 1956), and this has not been found in the brain.

Recent reports by Cavallini and coworkers have shown that the pathway to taurine from cysteamine is an important one in several tissues including the brain. The fact that taurine levels were maintained in the brain despite vitamin B₆ deficiency (Hope 1957), and the absence of cysteinesulphinic acid decarboxylase activity in tissues where taurine was present in high concentration prompted the investigation of alternative pathways. Cavallini was unable to find a vitamin B₆ independent decarboxylase for cysteine, however he did find an enzyme capable of converting cysteamine to hypotaurine (Cavallini et al 1964). This enzyme has been named pantetheinase (EC 3.5.1). A further enzyme cysteamine oxygenase (EC 1.13.11.19) converts cysteamine to hypotaurine.

The immediate source of cysteamine may be regarded as P - pantotheine. This compound is derived from cysteine which is converted to P - pantothenyl cysteine, P - pantetheine and coenzyme A (CoA) in a reaction sequence that has been well studied (Cavallini et al 1976). P - pantetheine is probably mainly derived from cysteine rather than CoA as turnover studies suggest that a relatively small quantity of CoA is hydrolysed to produce cysteamine (Novelli et al 1954).

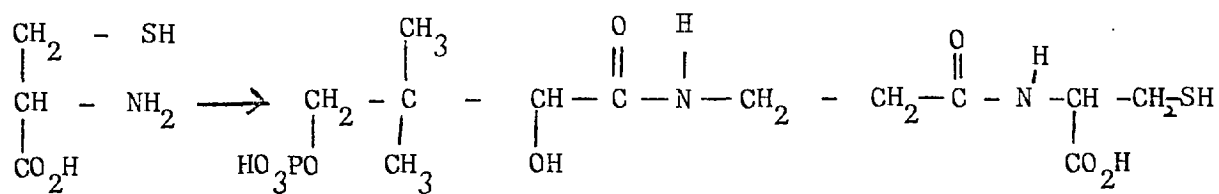
P - pantothenyl cysteine decarboxylase is the enzyme that performs the vitamin B₆ independent decarboxylation which has been studied in liver, kidney and heart (Cavallini et al 1976). It does not contain any common co-enzyme, and is strongly activated by mercaptoethanol.

Pantetheinase splits the carboamide linkage between cysteamine and pantethenic acid or P - pantothenic acid in liver, kidney, heart and muscle. No cofactor or coenzyme has been detected, and it is strongly activated by reducing agents such as mercaptoethanol or cysteine. The activity towards P - pantetheine is 10% of that for the unphosphorylated compound.

The cysteamine produced by the above reaction is oxidized by cysteamine oxygenase, which adds an oxygen molecule to the sulphydryl group of cysteamine to produce hypotaurine. It is a nonhaem iron protein, the valence of iron not being changed in the course of activity (Rotilio et al 1970). The hypotaurine that is formed by this reaction is converted to taurine in the tissues although no enzyme associated with this reaction has yet been found, as described earlier.

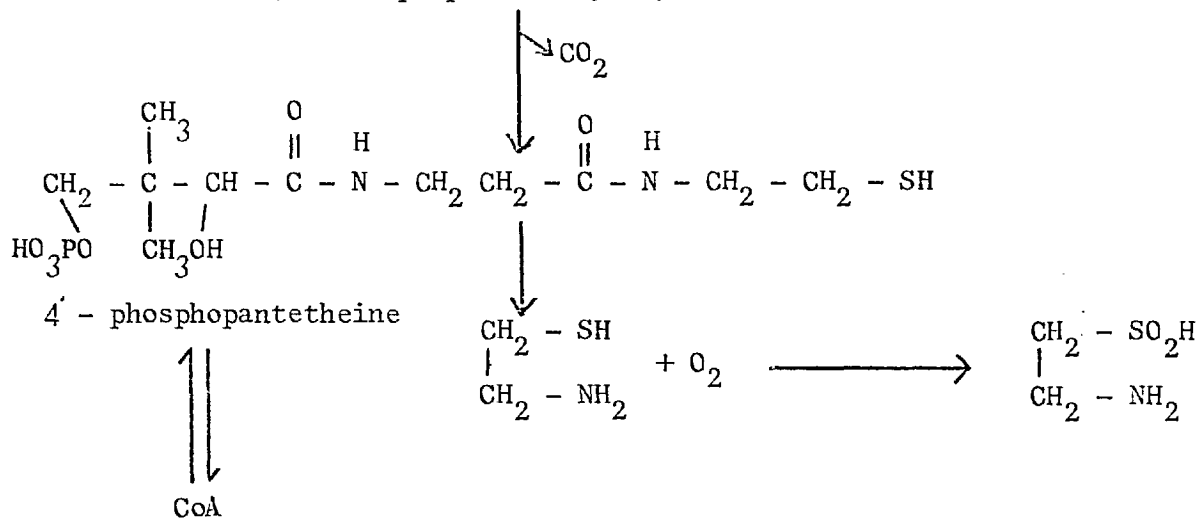
The sequences of reactions described above has not been studied in detail in the brain. Nevertheless preliminary evidence suggests that it may be quantitatively important there. When rat brain tissue in vitro was incubated with equivalent amounts of [^{14}C] labelled cysteine free or bound to P - pantothenate four times as much taurine was produced from the latter. The amounts produced were 3 n mole/h/g from cysteine and 12 n mole/h/g from P - pantothenyl cysteine (Cavallini et al 1976). These results are interesting, but before the importance of this pathway is established in the brain further work should be done to identify, purify and study the enzymes associated with this pathway in this tissue.

Two other pathways to taurine have also been proposed (Jacobsen & Smith 1968), these are the formation of taurine from



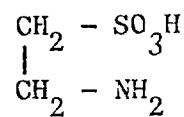
Cysteine

4'- Phosphopantothenyl cysteine



cysteamine

hypotaurine



Taurine

The Cysteamine pathway to Taurine

cystine disulphoxide, or from sulphate. The former pathway is no longer considered probable in view of the fact that cysteine disulphoxide is quite insoluble in water and extremely unstable except at acid pH (Awapara 1976). Radioactive taurine has been shown to be formed in vivo in the brains of rats injected with $[^{35}\text{SO}_4^{2-}]$, but not in vitro (Green & Robinson 1960). However since this reaction does not occur in germ - free rats (Huovinen & Gustafsson 1966), it is unlikely to be a significant pathway for taurine formation in the brain.

1.5 Metabolism

The metabolism of taurine in the brain has not been studied in great detail. Current evidence suggests that taurine is very slowly metabolized in the brain. Peck and Awapara (1966) described the formation of isethionic acid in rat brain homogenates which occurs at a very slow rate. Taurine is not converted to inorganic sulphate in homogenates of rat brain (Ikeda et al 1963), and it is likely that conversion of taurine to sulphate in vivo is due to intestinal bacteria as discussed above. Taurocyamine, the guadino derivative of taurine has been reported to occur in rat brain (Blass 1960). It belongs to a group of compounds that is converted to phosphagens which are associated with energy storage in invertebrates (Emmott & Morrison 1958). Its function in the mammalian brain remains unknown. Carbamyl taurine is excreted in the urine of rats and man in small amounts but this is not increased after administration of taurine (Schram and Crockaert 1957). The role for these compounds in mammalian brain remains to be defined.

Because of the incorporation of the other sulphur amino acids into proteins, it might be expected that taurine would also be incorporated into proteins. However taurine has not yet been shown to be a constituent of proteins. Taurine could alternatively have an indirect influence on protein synthesis. Thus taurine could influence protein synthesis by competing with other amino acids in a similar manner to which high levels of phenylalanine in plasma reduce incorporation of $[^{35}\text{S}]$ - methionine and $[^{14}\text{C}]$ - leucine into protein (Agrawal, Bone & Davison 1970), by interfering with uptake processes for these amino acids. Alternatively taurine could exert a sparing effect on other amino acids by being used for a common role, for example in osmoregulation. This is discussed further in a subsequent section regarding taurine and glutamate in epileptic tissue. Also taurine could exert an indirect effect on protein synthesis if it acts as a transmitter acting on post-synaptic

nerve membranes. For example the related amino acid GABA in the presence of Na^+ stimulates the incorporation of $[^{14}\text{C}]$ - leucine to protein in a ribosomal preparation of rat brain (Tewari & Baxter 1969).

Taurine may have a metabolic effect on brain glutamate, which is important in the brain as a probable transmitter and in cerebral metabolism. Glutamate has been reported to be synthesised from the taurine precursor cysteinesulphinic acid by brain mitochondria (Balaz, Biesold, Magyar 1963). Cysteinesulphinic acid undergoes a transamination process with oxaloacetate then deamination to finally yield glutamate, pyruvate and sulphate. The importance of this reaction is not known although it could be a mechanism which would lead to a balance in the brain between the excitatory effect of glutamate on nerve membrane and a depressant effect due to taurine.

A further way by which taurine could exert a metabolic effect on glutamate levels has been suggested by the report that glutamic acid and cysteinesulphinic acid decarboxylase are decarboxylated by the same enzyme in the brain (Davison 1956). The decarboxylation of both compounds occurs with similar localization of enzyme activity, and similar behaviour with thermal inactivation and inhibition by p - chloromercuribenzoate. However Jacobsen et al (1964) reported that glutamic acid non-competitively inhibited cysteinesulphinic acid decarboxylase in the brain.

1.6 Taurine and Cerebral Development

During foetal development and early in post-natal life taurine is present at four times the concentration in the brain compared with adults (Sturman & Gaull 1976). Taurine differs from other amino acids in this respect as although the concentrations of many amino acids are greater in foetal tissues and body fluids than in the mother, none have the four-fold difference found for taurine in the brain.

Following birth the high taurine levels in the brain fall during the first three weeks of life, and adult levels are reached about the time of weaning. For example in the rat taurine is present at $18 \mu\text{mol/g}$ in the 1-day old rat brain and by 17 days its concentration was $7 \mu\text{mol/g}$ which is close to adult levels (Agrawal et al 1971). Taurine differs from other amino acids with postulated neurotransmitter functions such as GABA and glutamate, which increase in concentration in the brain in the first three weeks after birth to reach adult levels 2 - 3 times those found in the neonate. For example GABA has been reported as $5 \mu\text{mol/g}$ at birth rising to $11 \mu\text{mol/g}$ in the adult. Aspartate shows a similar rise. Phosphoethanolamine however is similar to taurine and decreases in concentration in the brain after birth. (Agrawal et al 1968). These amino acids differ from other amino acids used for protein synthesis which do not show these variations.

In contrast to the above findings it has been reported that the synthesizing enzyme for taurine, cysteine sulphinic acid decarboxylase, increased in activity in the brain during the first four weeks of post-natal life when it reached two thirds the activity found in adult rat brain (Agrawal et al 1971). In this respect it closely paralleled the increase in activity found for the synthesizing

enzyme of GABA, glutamate decarboxylase, over the same period.

The apparent paradox between the decrease in taurine concentration and the increase in activity of its synthesizing enzyme activity was resolved by examining changes occurring in different tissue compartments separated by density gradient centrifugation (Rassin et al 1977). Rassin and co-workers found that in neonatal brain soon after birth the concentration of taurine in the supernatant fraction decreased but that in the synaptosome fraction increased. Thereafter the decrease in taurine concentration in the supernatant (60%) was greater than that in the synaptosome fraction (30%) when comparing 7-day old and adult rats. (Agrawal et al 1971). Thus taurine levels in the nerve ending fraction appears to be partly protected from the decrease in taurine concentration found in whole brain during post-natal development.

The changes in taurine concentration that occur in the early neonatal period described above are of interest in that functional connections between neurons are greatly increasing at this time. The increased activity of cysteine sulphinic acid decarboxylase over this period is consistent with an increasing role of taurine as a neurotransmitter in the brain during this period.

The cause for the high levels of taurine in the brain during development and the early post-natal period probably resides in several different factors acting together. In part it is due to high levels of taurine in the plasma both pre and post-natally, due to active transport across the placenta and high taurine levels in the

mother's milk (Sturman et al 1977). Also associated is immaturity of the blood brain barrier, which in the foetus and newborn taurine can penetrate as easily as the liver. This is the reverse of the adult liver which taurine enters much more readily than brain (Sturman et al 1977, and results presented in this thesis). Also associated may be an active uptake process, as slices of 7-day old rat brain sustained their taurine levels better than adults (Oja 1971). However Oja also reported that brain slices from young animals showed slower taurine uptake than adult rats.

The reasons for the decrease in taurine concentration during the post-natal period are not yet known. First of all it should be emphasized that although the concentration of taurine is decreasing, the total pool of taurine is increasing rapidly during this period to reach a maximum in the brain at 18 days after birth after which it slowly falls to adult levels (Sturman et al 1977). Possible explanations for this fall could be decreased taurine uptake to the brain from the blood, or decreased synthesis by enzymes other than cysteine sulphinic acid decarboxylase, which increases in activity during this period. A further possibility is increased catabolism of taurine to isethionic acid although this is slow in the adult (Peck & Awapara 1967), and has been reported to be slow also during the neonatal period by Sturman and co-workers (1977).

The significance of the high levels of taurine in foetal and neonatal brain remains open at this time. The increasing activity of cysteine sulphinate decarboxylase which is enriched in nerve endings, undoubtedly supports a possible role of taurine in neurotransmission, as it parallels the increase of glutamate decarboxylase (Agrawal et al 1971)

and occurs during a period of rapid formation of synaptic connections in the brain.

The reasons for the fall in taurine concentrations in the nerve-ending pool in brain tissue remain to be elucidated. The reasons for the high concentration of taurine in the foetal brain also await discovery. Unmyelinated nerve cells of the immature brain may be very sensitive to excitatory transmitters such as acetylcholine over almost all the cellular membrane surface (Harris & Dennis 1970). Taurine could play a part in decreasing the electrical activity of these immature cells. Further information about the role of taurine during the period of foetal development requires investigation of the change in taurine levels in defined regions and stages during development, and of the effect of altered availability of taurine during the course of development. The development of inhibitors for taurine synthesis and uptake may be useful in the further study of this process.

1.7 Approaches to the Study of Neurotransmission

The study of Neurotransmission and especially of synaptic function in the brain is difficult because of the relative inaccessibility and complexity of the central nervous system. Since thousands of synaptic contact converge on each of the millions of central neurons, and since each neuron in turn projects thousands of terminals upon other cells, neuronal interactions are very complex. This is further complicated by the possibility of different neurotransmitters occurring in a given anatomical area, where the effects of one type of transmitter may be masked by many other neurons which use other transmitters.

Despite the complexity of the central nervous system, several approaches have been useful in studying synaptic transmission in the

brain. These include the perfusion of different tissue areas in vivo, or by the study of brain tissue in vitro at different levels of complexity and function. Tissue may be studied following its isolation by slicing, by separation of different cell types, and by subfractionation. The latter includes separation of nerve ending particles called synaptosomes (Whittaker 1969).

Whole tissue may be studied by perfusing different brain areas associated with different functional roles, for example the sensory motor area and visual area of the cerebral cortex. Substances released into the CSF from these areas may be collected and measured. Possible neurotransmitters can be studied by observing their release into the perfusate during different functional states, for example during the light dark cycle for the visual cortex. Release may also be studied in conjunction with stimulation of, or lesion in, the nerve fibre tracts leading to the area being perfused.

Tissue slicing is also a useful method in the study of neurotransmission as it enables different anatomical areas to be studied isolated from the complexities of whole brain, under conditions which can be closely controlled and varied. These include different concentrations of ions, pharmacological agents, and electrical stimulation. Tissue slicing can be used in conjunction with perfusion to study the release of substances which may be quickly broken down by enzymes, for example acetylcholine, or where release is small and there is a rapid reuptake system, such as could occur if there was only a minor pool of the transmitter in the tissue. Cell separation is an additional method that may help distinguish between neuronal functions which may be associated with neurotransmission and those that are purely metabolic and could occur in neurons and glial cells or only in glia. Separated

neurons and glia can be studied in vitro in short term experiments or in tissue culture where longer acting processes, and neuronal and glial interactions can be studied.

Tissue subfractionation by homogenization and density gradient centrifugation are also useful in these studies, particularly in the isolation of synaptosomes which can be studied in vitro to yield information about transmitter function at this level.

Synaptosomes prepared by subcellular density gradient centrifugation are a good model for synaptic function, as they retain their normal morphological characteristics, metabolism and can take up and release neurotransmitters. The synaptic complex in the preparation has broken off and resealed to form morphologically identifiable units, enclosed by cell membranes which contain synaptic vesicles and mitochondria, and includes specialized membrane thickenings associated with adjacent membranes. Isolated synaptosomes like intact tissue can respire, they undergo glycolysis and oxidative phosphorylation leading to production of lactate, ATP and phosphocreatine. They can convert glucose to amino acids (De Belleruche & Bradford 1972) and incorporate amino acids into protein (Austin et al 1970). They also possess Na^+ , K^+ -ATPase and maintain normal levels of K^+ and Na^+ by a process of accumulation of K^+ (Escueta & Appel 1969), and extrusion of Na^+ (Ling & Abdel-Latif 1968). They can take up amino acids at low concentrations in the medium by a Na^+ dependent high affinity uptake process of GABA (Martin & Smith 1972), of glycine and glutamate (Logan & Snyder 1971), and taurine (Davison & Kaczmarek 1971). Synaptosomes can be stimulated to give calcium dependent transmitter release either by a high concentration of K^+ in the medium, or electrically (Bradford 1969; De Belleruche & Bradford 1972).

1.8 Electrophysiological evidence for a possible Neurotransmitter role for Taurine.

In order for a compound to be shown to be a neurotransmitter, an important criterion is that it can be shown to affect the electrical excitability of neurons in a similar way to the natural transmitter at that site. Microiontophoresis is a useful tool for studying this interaction. Employing this technique, compounds can be locally applied at low concentration close to synapses in the brain, and the resulting changes in electrical activity of single cells may be observed using an intracellular microelectrode. Much of the early work on this was done by Curtis and co-workers (1964) who facilitated investigation by developing the multibarelled microelectrode. This allowed the recording of single cell activities and the release of as many as four different suspected transmitter compounds by iontophoresis. Excitatory compounds could be identified by observing an increase in the rate of firing of single cells for both resting and spontaneously active neurones. Inhibitory compounds could be identified by their ability to decrease firing, to cause hyperpolarization of the neuronal membrane, and to increase Cl^- permeability.

Early work on the effect of taurine on excitability in the brain employed topical application of taurine to the cerebral cortex. Negative results reported for these experiments (Purpura et al 1959). The first significant results were reported by Curtis and Watkins (1960) in experiments using the method of microiontophoresis described above, to study electrical changes in the spinal cord of cats, where taurine was found to inhibit neuronal firing.

Curtis and Watkins (1960, 1965) described two classes of amino acids that affected the excitability of central neurons when applied by microiontophoresis. Firstly, there were those with a strong

excitatory action. These were dicarboxylic acids, including glutamic acid, aspartic acid. Also excitatory were cysteic acid and homocysteic acid which have a sulphonate group and a carboxyl group. Amino acids which were structurally related to these but with a single acidic group were found to be inhibitory in action. These include GABA, glycine, taurine and homotaurine. These compounds cause inhibition of cell firing, hyperpolarization and increased Cl^- permeability.

Iontophoretic studies with taurine demonstrated it had a strong inhibitory effect on the firing of spinal cord motor neurons, Renshaw cells and dorsal horn inter-neurons, (Curtis & Watkins 1960). Application of taurine caused total block of the fast spontaneous firing of spinal cord interneurons (Krnjevic & Puil 1976). The action was rapid in onset (less than one-second). It was relatively prolonged, for it outlasted the application by several seconds. It was of high potency, as there was a strong effect with moderate doses (80 nA). It caused hyperpolarization, which was sensitive to injection of Cl^- intracellularly (Hosli et al 1973).

In the cerebral cortex taurine was found to exert a weaker depressant effect than in the spinal cord (Crawford & Curtis 1964). However it depressed cortical firing quite strongly (Krnjevic & Puil 1976). In the cortex it was found to act less strongly than GABA, but more strongly than glycine (Krnjevic & Phyllis 1963, Crawford & Curtis 1964). Isethionic acid, the metabolite of taurine, was inert when applied to cortical cells (Krnjevic 1965). The depressant effect of taurine was blocked in the cerebral cortex by both strychnine and bicuculline (Curtis et al 1971), post-synaptic antagonists of the action of glycine and GABA respectively. This differed from the

spinal cord where taurine was antagonized by strychnine but not by bicuculline (Curtis et al 1971). In the medulla taurine antagonism was similar to spinal cord (Haas & Hosli 1973). The action of taurine on the cerebellum and thalamus was similar to its effects in cerebral cortex. Taurine also affected sympathetic ganglion cells where it caused depolarization, which was similar to that caused by GABA, and associated with increased Cl^- permeability (Nishi et al 1974).

1.9 Criteria for a compound having Neurotransmitter role in the brain

The effect of taurine on electrical firing in the central nervous system is important in establishing that it has the capability to act as a transmitter if it is released from nerve endings. In fact a number of criteria are associated with establishing a neurotransmitter role for a compound, of which the most significant are release from nerve endings and the ability to modify post-synaptic excitability in an identical way to the natural transmitter at that site. The criteria for establishing a compound as a neurotransmitter have been reviewed by Werman (1966) and Hebb (1970). Werman listed eight factors that would be expected to be associated with proving a transmitter role for a compound. These are (1) The presence of an enzyme for inactivating the compound after it had acted (2) The presence of the compound in the nerves from which it is released (3) Collectability of the compound following its release (4) The presence of a synthesizing enzyme to make the compound available. (5) The presence of precursors of the compound in the nerves for which it is released (6) The presence of a mechanism for the release of the transmitter (7) Identical action of the transmitter candidate and the natural transmitter (8) Pharmacological identity between the transmitter candidate and the natural transmitter.

The above criteria can be further amplified and modified to define more specifically the evidence for a transmitter role of a compound. An alternative method to enzymic inactivation of the released compound is by an uptake system to act rapidly and at low concentrations of the compound, such as would occur when it had been released from nerve terminals. This has been found to be the case for the transmitter noradrenaline (Iversen 1967). Inactivation could be by uptake

to several different tissue compartments including nerve endings, to the cell the transmitter is acting on, to glial cells, or by simple diffusion away from the site of release. It could be a combination of all these factors. The criterion of the presence of the compound in nerve can be broadened to include its presence in nerve endings and in synaptic vesicles. The criterion of collectability of the compound after its release from nervous tissue can include release in vivo, from tissue slices, and from nerve endings. Collectability should include allowance for ways by which reuptake or enzymic destruction of the compound can be overcome to make this possible. A mechanism of release should include calcium dependence of release, as calcium influx at nerve endings is associated with transmitter release (Miledi & Slater 1966). Identical action and pharmacological identity can be studied at the cellular level by intracellular microelectrodes, or when the natural transmitter produces some other recognizable response in the cells it affects.

1.10 Reasons for the present study

Several reasons prompted the further investigation of the possible role of taurine as a neurotransmitter in the brain. The evidence available pointed towards a transmitter role although there were certain factors that appeared to go against this hypothesis.

Among the factors which suggested taurine could have a transmitter role was its structural similarity to GABA, for which there is now much evidence of a transmitter role. Both molecules have an acid group and an amino group separated by two carbon atoms, although taurine has a sulphonic group and GABA a carboxyl group. The atoms are joined by single bonds so the molecule may have similar conformations, although the extra oxygen in the sulphonic group of taurine may restrict rotation or folding of this molecule.

The experiments, with microiontophoreses of taurine to central neurons, by Curtis, Krnjevic and others described earlier, suggested that taurine had a powerful inhibitory effect on central neurons, and that the electrical response of neurons to taurine was similar to that for naturally occurring inhibitory transmitters, which cause hyperpolarization, and Cl^- entry to the post-synaptic cell.

Also electrically stimulated release of taurine had been shown from spinal cord slices (Hammerstad et al 1971) and from cerebral cortex tissue slices (Davison & Kaczmarek 1971). The release of taurine from the cortex slices was sensitive to low concentration of Ca^{2+} in the medium and to raised Mg^{2+} . Moreover taurine was found to be enriched in synaptic vesicles prepared from the cerebral cortex (De Belleruche & Bradford 1972). Also the

synthesizing enzyme for taurine, cysteine sulphinic acid decarboxylase, was found to have half its activity in the nerve endings of cerebral cortex (Agrawal et al 1971).

Despite the above evidence suggestive of a neurotransmitter role there were several apparent objections. The first was that taurine does not exist exclusively in nerve endings, but has 70% of its concentration in the soluble cytoplasmic compartment of the cell (Agrawal et al 1971). In this respect taurine differed from acetylcholine but was similar to GABA, glutamate and glycine which also are found largely in the cytoplasmic fraction, but are probable neurotransmitters. However a feature which appeared to go against this was that these amino acids all undergo active metabolism in this part of the cell whereas taurine is only slowly metabolized in the brain to isethionic acid (Peck & Awapara 1967).

In addition to the above, recent reports had indicated an association of taurine with glial tissue, and the possible contamination of synaptosomes with glial components. Taurine has been shown to occur in glia, though at half the concentration it occurs in neurons (Kaczmarek 1971). Also a high-affinity uptake of taurine has been described to cultured glial cells, C-6 glioma tissue, (Schrier & Thompson 1974), and in the retina (Ehinger 1973). The possibility of glial contamination of synaptosomes was reported by Henn & co-workers (1976). They based this suggestion on the finding that particles from cultured glial cells occur in the same fraction as synaptosomes prepared from rabbit brain, the crude mitochondrial fraction (P2).

Although it is not known if glial material from whole brain behaves in a similar way, the possibility remained that glia could be the source of the released taurine. Further evidence for this possibility was the report by Bowery and Brown (1971) that GABA could be released by K^+ or electrical stimulation of superior cervical ganglion.

Further recent reports linked taurine with epilepsy, Koyama (1972) showed that taurine was decreased in concentration at the epileptic focus in cobalt induced epilepsy in animals. Van Gelder and co-workers (1972) showed that taurine was decreased in concentration in the epileptic focus of patients undergoing neurosurgery for focal epilepsy. He subsequently reported (Van Gelder 1972) that intraperitoneal administration of taurine was beneficial against epilepsy induced by cobalt in cats and mice.

The aims of this project were to look in more detail for evidence of a neurotransmitter role of taurine in the cerebral cortex, and in particular^{to}/look for evidence of specific uptake to, and release from, the nerve-ending compartment of cerebral cortex, to examine glial uptake of taurine in the cortex, and contamination of synaptosomes with glial elements "gliasomes". A further aim was to assess if oral administration of taurine was effective against cobalt induced epilepsy, and to relate taurine administration orally to its passage to the plasma and to organs where its uptake could be relevant to its action against epilepsy.

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CHAPTER 2

2.1 Preparation of cerebral cortex slices

Sprague Dawley adult female rats, weight range 200 - 250 g, were stunned by a blow on the back of the neck and the cerebral cortex was rapidly exposed and removed as described by Bradford (1972).

One cerebral cortex was placed on a surgical table for slicing, whilst the other cortex was placed in a closed petri dish on a filter paper soaked in Krebs bicarbonate medium at room temperature.

Top slices of cerebral cortex were rapidly cut using a strip of razor blade and a McIlwain slicing guide (McIlwain 1975). The slices obtained were 0.3mm in thickness, and 40 - 80 mg fresh weight as measured on a torsion balance. Each slice was then transferred to a crystallizing dish containing 50 mls of Krebs bicarbonate medium gassed with O_2/CO_2 95:5 at room temperature, while the slice from the other cerebral cortex was cut.

The slices were then gently guided between the jaws of a McIlwain Rapid Transfer holder (McIlwain 1975), drained and transferred into 25 ml beakers containing 5 mls of Krebs bicarbonate medium at 37°C and gassed with O_2/CO_2 95:5 throughout each experiment.

The Krebs bicarbonate medium used had the following composition (mM): NaCl, 124; KCl, 5; $NaHCO_3$, 26; KH_2PO_4 , 1.2; $MgSO_4$, 1.3; $CaCl_2$, 0.75; glucose, 10; with pH 7.4. This was sometimes varied, see individual experiments. It was made up with distilled deionized water. All glassware used for incubation in experiments where Ca^{2+} concentration was varied was rinsed 10 times in distilled deionized water.

2.2 Uptake Experiments

2.2 (i) Transport Studies

Top slices of adult rat cerebral cortex (40 - 80 mg weight, 0.3 mm thickness) were prepared as described. They were transferred in McIlwain Quick Transfer holders to 5 ml of Krebs bicarbonate medium in 25 ml glass beakers in a water bath at 37°C gassed with O₂/CO₂ 95:5 for 10 min. They were then drained and incubated in 12 µM [³⁵S] - taurine (specific activity 48 mCi/m mol Radiochemicals Amersham, UK.) in 5 mls of the same medium. After 5 min incubation each slice was rapidly removed in its holder, drained and rapidly rinsed in incubation medium containing non - isotopic taurine at the same concentration. The slice was rinsed twice and then transferred to 1.5 ml of 10% (w/v) TCA, homogenized, centrifuged at 1,400 g for 5 min (UV International centrifuge) and 1 ml was taken for radioactivity counting.

The above procedure was repeated for different [³⁵S] - taurine concentrations, ranging from 12 µM to 5000 µM. The experiments was repeated using [³H] - leucine instead of taurine. Also diphenylhydantion at levels found in the plasma for effective theurapeutic action in humans was added to the medium to see if this would affect the uptake of [³⁵S] - taurine.

2.2 (ii) Subcellular fractionation of slices after $[^{35}\text{S}]$ - taurine uptake

Top slices of cerebral cortex were taken as above. Individual slices were incubated in medium containing $[^{35}\text{S}]$ - taurine at 20 μM or 540 μM final concentration for 30 min. They were then drained and rapidly rinsed in 0.32 M sucrose at room temperature.

Subcellular fractionation used the method of Gray and Whittaker (1962) with modifications as described by Bradford (1969). All sucrose solutions used were made up using distilled deionized water and were prepared the day before, as it was found that bacterial growth occurred readily in this medium.

Six pooled slices were then homogenized in 9 ml of 0.32 M sucrose at 5°C in a precooled glass perspex homogenizer with teflon pestle, radial clearance 250 μ , and rotating at 900 rpm. Homogenization was made with 4 up and down strokes repeated 3 times, with a short pause between each homogenization to minimize heating of the homogenate. Subsequently all subcellular fractionation procedures were performed at 0 - 5°C.

The homogenate was centrifuged at 1000g for 10 minutes in a 50 head in Beckman L2 65B ultra centrifuge to yield supernatant (S1) and crude nuclear pellet (P1). S1 was carefully removed with a pasteur pipette and recentrifuged in the 50 head at 20,000 g for 20 min to yield the crude mitochondrial pellet (P2), and combined second supernatant and microsomes (P3 + S2). P2 was resuspended in 2 ml of 0.32 M sucrose at 5°C by gentle homogenization. 1.6 ml of the resuspended P2 was taken and layered on top of a discontinuous gradient of 1.6 ml of 0.8 M sucrose above 1.6 ml of 1.2 M sucrose. The gradients were then centrifuged in a SW 39 head for 1 hour at 76,000 g at 0 - 5°C. Subsequently three fractions were collected, a myelin fraction (P2A) at the boundary of the

0.32 M and 0.8 M sucrose, a synaptosome fraction (P2B) at the boundary of the 0.8 M and 1.2 M fraction, and the mitochondrial pellet (P2C). These were resuspended in 0.32 M sucrose and 0.8 ml of each fraction was added to 0.2 ml of 25% (w/v) TCA, and centrifuged for 10 minutes at 1,400 g in UV International centrifuge, and 1 ml of the supernatant was taken for liquid scintillation counting. Aliquots of each fraction were also taken for protein estimation (Lowry et al 1951).

2.2 (iii) Uptake studies of $[^{35}\text{S}]$ - taurine and $[^{14}\text{C}]$ - GABA in the presence of β - alanine and L - 2,4 - diaminobutyrate

The various tissue preparations were incubated with $[^{35}\text{S}]$ - taurine (20 μM) either alone, or with β - alanine or L - DABA at a final concentration of 1 mM in 5 ml of incubation medium at 37°C gassed with O_2/CO_2 95:5. Synaptosome suspensions and suspensions of C - 6 glioma tissue were incubated with $[^{14}\text{C}]$ - GABA (20 μM) as comparison, (232 mCi/m.mol Radiochemicals Amersham).

a) Cortex slices These were incubated in medium containing $[^{35}\text{S}]$ - taurine at 20 μM for 30 min. Slices were then removed, rapidly rinsed in incubation medium containing non - isotopic taurine at 20 μM . They were then homogenized in 3 ml of 0.32 M sucrose, and 0.6 ml rinsed with 0.2 ml of 20% (w/v) TCA, centrifuged as described above and then taken for liquid scintillation counting.

b) Synaptosome suspensions and synaptosome beds Synaptosome suspensions were prepared according to the method of Bradford et al (1973). Whole cerebral cortex was taken from 10 adult rats, each cortex being rapidly dissected free of white matter and placed in ice - cold 0.32 M sucrose. When 8.5 g of cortex was collected, the volume of sucrose was made up to 85 ml with ice - cold 0.32 M sucrose, and homogenized as described above in a glass perspex homogenizer to yield a 10% (w/v) homogenate. The homogenate was then centrifuged in a 30 head for 10 min at 1000 g in a Beckman L2 65B ultracentrifuge to yield SI and PI. SI was then centrifuged in the 30 head for 20 min at 20,000 g to yield P2 and the combined fraction (P2 + S2). The crude mitochondrial pellet (P2) was resuspended, & 15ml then layered onto a discontinuous sucrose density gradient which consisted of 0.8 M sucrose (20 ml), above 1.2 M sucrose (20 ml) in a centrifuge tube packed in ice. When adding sucrose of lighter density to a more dense solution it was necessary to tilt the tube containing the dense sucrose at a slight angle and to run in

slowly the lighter density sucrose down the side of the tube in order to obtain a sharp interface between the layers. The sucrose gradient was centrifuged at 76,000 g in a Beckman Spinco SW25 rotor, to yield the 3 fractions P2A, P2B and P2C. Only the synaptosome fraction (P2B) was collected, taking an equal amount of sucrose above and below it in order to assume that the final sucrose concentration was 1.0 M. Cold deionized distilled water (5°C) was then added dropwise to P2B in a chilled conical flask in an ice bucket, with continual shaking to minimize osmotic stress to the synaptosomes. Water was added till the sucrose reached a final concentration of 0.45 M, to allow for entry of some sucrose into the synaptosomes during fractionation, and so make the sucrose concentration approximately iso - osmotic with the contents of the synaptosomes. The diluted suspension of synaptosomes was then centrifuged at 54,000 g for 20 min in a 30 head in Beckman L2 65B ultra centrifuge. The resulting pellet was resuspended in Krebs bicarbonate medium, and 5 ml incubated in glass vials in a waterbath at 37°C gassed with O₂/CO₂ 95:5, with continual shaking.

Synaptosome beds were prepared according to the method of De Belleroche and Bradford (1972). The synaptosome pellet obtained by the method described above was resuspended in Krebs bicarbonate medium using 7 strokes of a teflon pestle in a glass homogenizer of loose fit (radial clearance 180 µ), and made up to 30 ml with 0.32 M sucrose. 5 ml of this suspension were placed in perspex cells with nylon gauze (aperture size 120 µ and mesh size 110 µ) at the base.

The cells were centrifuged at 550 g for 10 minutes in an IEC International centrifuge. The sucrose was then carefully poured off, the cells were then taken apart and the synaptosomes deposited

on top of the nylon gauze were removed and placed in a Quick Transfer holder, a square of the same gauze was carefully placed above the synaptosomes, and the jaws of the Quick transfer holder closed. The synaptosome beds were then incubated in 5 ml of Krebs bicarbonate medium.

Synaptosome beds were incubated for 30 min in medium containing [^{35}S] - taurine at 20 μM as described above, drained, rinsed in medium containing non - isotopic taurine (20 μM), and extracted in 5% (w/v) TCA. Synaptosome suspensions were incubated under the same conditions and samples (0.5 ml) were taken at 5, 10, 20 and 30 min and centrifuged in a bench top microcentrifuge (Ole Dish Copenhagen, Type 155) at 18,000 g for 2 min. The supernatant was removed and the synaptosome pellet was extracted with 5% (w/v) TCA, agitated on a vortex mixer and centrifuged as above for 5 min. The supernatant was taken for liquid scintillation counting. A portion of the TCA - insoluble pellet was dissolved in 4.5 N NaOH for 1 hour and assayed for protein (Lowry et al 1951).

c) C - 6 glioma cells These were suspended in incubation medium (5 ml) at a concentration of 0.9 mg protein/ml and subsequently incubated as for synaptosomes, extracted in 5% (w/v) TCA and taken for liquid scintillation counting and protein estimation as above.

d) White matter slices These were prepared by placing one cerebral hemisphere, cortex downward on a moistened filter paper on a surgical block. An incision was made in the white matter uppermost to the level of the lateral ventricle. The white matter

was then folded open and thin slices were taken from the lining of the ventricle (ependyma) with the aid of a McIlwain slicing guide. The slices obtained (30 - 40 mg fresh weight) were rapidly transferred to incubation medium at room temperature and placed between the jaws of a Quick Transfer Holder. They were then incubated and treated as for cortex slices.

2.3 Release Experiments

2.3 (i) Incubation and Stimulation of cortex slices

Top slices of cortex positioned in Quick Transfer Holders were incubated at 37°C for 30 min in incubation medium, gassed with O₂/CO₂ 95:5, and containing [³⁵S] - taurine at 20 µM final concentration. They were then drained, rinsed 3 times in medium containing non-isotopic taurine at 20 µM, and transferred to a second beaker containing 5 ml of medium in which they were electrically stimulated for 15 min.

Electrical stimulation was applied using the Quick Transfer Holders (McIlwain 1975), with an A.F. signal generator (Type HI Advance, England) and a power amplifier. An oscilloscope (Type 502A dual beam, Tektronix Guernsey Ltd) was used to measure the voltage and current passing through the apparatus. Square wave pulses were employed alternating in polarity of 0.4 M sec duration, and 50 Hz frequency. The mean current applied was 30 - 50 mA. After stimulation slices were drained and transferred consecutively to two further 5 ml aliquots of medium for 15 min periods. After rinsing three times in incubation medium, each slice was homogenized in 3 ml of 0.5M PCA. The homogenate was centrifuged at 1400 g for 10 min, and 1 ml of the supernatant was taken for liquid scintillation counting.

Some slices were incubated in calcium - free incubation medium containing 1 mM EGTA and [³⁵S] - taurine (pH 7.4). Other slices were incubated with [³⁵S] - taurine in medium of normal composition and then rapidly rinsed 3 times in calcium - free incubation medium containing 1mM EGTA before incubation in calcium - free medium for 15 min periods and stimulated as before during the second period.

Other slices were stimulated over a range of currents between 12 mA and 44 mA mean current, to see if release of [³⁵S] - taurine was current dependent.

2.3 (ii) Stimulated release from subcellular fractions of cortex slices.

Cortex slices were pre-incubated under the same general conditions as above in Krebs - bicarbonate medium containing calcium ions. They were stimulated for 20 min in two groups, one at 48 mA mean current, and the other at 36 mA mean current. At the end of the incubation period, the slices were rapidly rinsed 4 times in 0.32 M sucrose at room temperature. They were then homogenized and subfractioned as described above before extraction in 5% (w/v) TCA. Aliquots of each fraction were taken for liquid scintillation counting and protein estimation as before.

2.4 Liquid Scintillation Counting

The scintillant mixture was made up of the following components, 5 parts of Toluene P.B.D. and 4 parts of 2 - methoxyethanol. Toluene P.B.D. contained 250 g naphthalene A.R. 30 g scintillator P.B.D. (Ciba) made up to 5 litres with Toluene A.R.

Tissue extracts (1 ml) were added to 19 ml of Toluene P.B.D. mixture and counted on a LS - 200 B Beckman Liquid Scintillation counter. Quench correction was achieved using an internal standard.

2.5 Protein Assay

The method of Lowry et al (1951) was used to assay the protein content of tissue fractions and TCA or PCA precipitates.

Bovine serum Albumin (Sigma Grade V) was used as a standard. A fresh stock solution was made up for each assay. The alkaline copper reagent used was also made up freshly prior to the assay. It had the composition: Na_2CO_3 2%, CuSO_4 0.01%, Na - tartrate 0.02% and where NaOH digestion of protein precipitates had not been used it was also 0.1 M with respect to NaOH. Folin - ciocalteau reagent (BDH) was diluted 1:1 with water just prior to use.

Acid precipitates of tissue samples were resuspended in 2 volumes of 4.5 N NaOH to 1 volume of water, and stood with occasional mixing on a whirlmix for 1 hour. Standards of B.S.A. were also incubated under the same conditions.

0.1 ml aliquot of sample was added to 1.1 ml of water and 3.0 ml of alkaline copper reagent. After mixing thoroughly on a Whirlimixer (Fissons Scientific Apparatus Ltd, Loughborough, Leicester) the assay solution was allowed to stand for 10 min at room temperature. 0.3 ml of diluted Folin - Ciocalteau reagent was then added rapidly and the solution immediately mixed thoroughly on a Whirlimixer. The solution was allowed to stand for 40 min at room temperature and the optical density measured at 500 mμ in a Unicam SP 600, using a continuous flow cell. The optical density was read against a blank containing the same reagents but without the protein. The optical density for standards was found to be stable for at least a further 60 min. The standard curve covered the range 0 - 200 μg protein/tube.

2.6 Uptake studies *in vivo*

2.6 (i) Uptake of $[^{35}\text{S}]$ - taurine to Tissue

Sprague Dawley adult female rats weighing 200 - 250 g were dosed orally with $[^{35}\text{S}]$ - taurine (600 mg/Kg body weight) by stomach tube. Non - isotopic taurine and $[^{35}\text{S}]$ - taurine (specific activity 48 mCi/m mol, Radiochemicals Amersham, UK) were added to water to give a final concentration of 120 mg taurine in 1.5 ml, containing 20×10^6 cpm. The rats were lightly anaesthetized with ether, and the taurine loads delivered to the stomach via a plastic tube.

The rats were then sacrificed at times ranging between 2 hr and 3 days, and plasma and tissue samples were obtained for liquid scintillation counting. During light anaesthesia, blood was obtained by intracardiac puncture using a heparinized syringe and wide bore needle, to minimize rupture of blood platelets, which contain high concentrations of taurine. Plasma was then collected for analysis of radioactivity, taking care not to disturb the "buffy" - leucocyte coat, as leucocytes are also rich in taurine. The plasma obtained was stored at -20°C , and the radioactivity counted at the same time as the tissue samples.

Plasma was mixed with 10% (w/v) TCA to give a final concentration of 5% (w/v) TCA, and mixed with a vortex mixer. The suspension was then centrifuged at 1,400 g for 10 min and 1 ml was used for the determination of radioactivity.

Tissue samples were rapidly dissected out from cerebral cortex, liver, hind limb, skeletal and heart muscle. Approximately 0.5 g fresh weight of each tissue was homogenized in 3 ml of 10% (w/v) TCA and the homogenizer rinsed out with a further 1 ml of 10% (w/v) TCA. The homogenate was centrifuged for 10 min at 1,400 g as above.

One ml of the supernatant was taken for radioactivity counting by liquid scintillation. A quench correction was obtained using $[^{35}\text{S}]$ - taurine as an internal standard.

Correction for tissue content of blood plasma was obtained by measuring the radioactivity of plasma and tissues following a single injection of $[^3\text{H}]$ - inulin into the blood stream. The method of Kandra et al (1968) was chosen as it has been shown that inulin injected into the tail vein equilibrates with the blood volume after a short period, before significant excretion of inulin has occurred. $[^3\text{H}]$ - inulin (specific activity 300 mCi/m mol, Radiochemicals, Amersham, UK) was dissolved in water with non isotopic inulin and the pH was adjusted to 7.4. Seven microcuries of $[^3\text{H}]$ - inulin in 0.4 ml (inulin concentration 1%(w/v) solubilized by gentle heating) was injected into the tail vein of Sprague Dawley adult female rats (200 - 250 g) under light ether anaesthesia. After four minutes blood was rapidly obtained by intracardiac puncture as described above. Plasma and tissue extracts were obtained as previously described, and the content of $[^3\text{H}]$ - inulin was determined using a Beckman liquid scintillation counter LS - 200 B. A quench correction was obtained by the use of an internal standard. Results were expressed as percentage uptake per gram of tissue corrected for plasma content.

2.6 (ii) Amino Acid Analysis

Rats were dosed orally with non- isotopic taurine in the same dose and concentration as those receiving the isotope. The tissues were collected and extracted with 10% (w/v) TCA in the same manner, and analysed on an amino acid analyser as described by Bradford & Thomas (1969). Taurine was clearly separated from other sulphur containing amino acids. Split - stream analyses was used to correlate the distribution of radioactivity with the location of taurine chromatographically. For this purpose 0.5 ml fractions of the split - stream were collected and their radioactivity counted by liquid scintillation. Only one peak of radioactivity was detected and this corresponded exactly with the taurine peak.

2.7 Taurine and Cobalt - induced Epilepsy

(i) Production of Epilepsy

Adult Sprague Dawley female rats (200 - 250g) were anaesthetized using dibromo ethanol (Avertin). 0.2 ml of Avertin was added to 7.8 ml of water, and dissolved by gentle heating. Anaesthesia was induced by injection 1.5 ml intraperitoneally and 0.5 ml subcutaneously. It was maintained with further injections of the anaesthetic, 0.5 ml intraperitoneally as needed.

Anaesthetized rats were clamped in a head holder. The head swabbed with 70% alcohol, and the surface of the skull was exposed by a mild line incision, and the skin flaps were held back by clamps. A circular window (4 mm diameter) was cut in the exposed skull with a trephine. The location was 1 mm lateral to the mid - line over the right frontoparital suture. With the aid of a stereo microscope (x 30) the dura and subarachnoid membrane were removed and the area of cortex exposed was sensory motor cortex associated with the left fore limb and left hind limb as described by Dawson & Holmes (1966). Sterile cobalt metal powder was lightly sprinkled on the surface of the exposed cortex from a microspatula as approximately a monolayer. The bone was replaced, the skin sutured and the animal allowed to recover.

2.7 (ii) Study of Epilepsy and oral dosing with taurine

The rats were subsequently observed for signs of limb jerks on day 5 or 6, those animals not showing at least 30 limb jerks/10 min were discarded. Animals were placed in a glass observation cage and assessed at the same hour daily for 10 min. The limb jerks were counted independently by two observers. Discrete contralateral limb jerks were counted for the left fore limb and left hind limb during the 10 min observation period for each rat. Fore limb jerks were given a score of 1 unit and hind limb jerks counted as 2, because of the greater muscle mass to be removed. The limb jerks were counted daily until day 13, so as to follow the course of the epilepsy as it developed and subsided. Only at the end of trial were the records of the two observers compared. They were found to correspond in both pattern and limb-jerk frequency.

In addition to the control epileptic group, a group of taurine treated rats was studied. Taurine was dissolved in water, and unanaesthetized rats were dosed with 600 mg/Kg in 1.5 ml of water orally by stomach tube. Full epileptic rats (30 + limb jerks/10 min) were selected on days 5 or 6 and divided into two equal groups, one of which received taurine. Oral dosing with taurine was carried out daily from day 6 onwards through the cycle of epilepsy. The limb jerk scores were combined and plotted as in Figure 9, which also includes additional data for epileptic rats not treated with taurine and obtained on other occasions. In addition plasma for rats dosed over a 1 month period with taurine 600 mg/kg/day, was taken for liver function tests, as related amino acids such as cysteic acid may be hepatotoxic if too large amounts are ingested.

CHAPTER 3 THE UPTAKE OF TAURINE IN VITRO

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3.1 Introduction

Because the conversion of taurine to its metabolite isethionic acid takes place very slowly in the brain (Peck & Awapara 1967), a metabolic role for taurine in the brain appears unlikely. Thus it is worthwhile to look for another role of taurine such as could involve taurine fluxes across cell membranes. The study of taurine uptake is particularly relevant to the role of taurine as a possible neurotransmitter, as a means of removing synaptically released taurine in the absence of rapid enzymatic breakdown.

Both low and high-affinity uptake systems have been reported for amino acid transmitter candidates, GABA (Bond 1973) glutamate (Logan & Snyder 1971) and glycine (Balcar & Johnston 1973). Uptake studies have also been reported for taurine. Kaczmarek and Davison (1972) reported a high-affinity uptake system for taurine in cerebral cortex tissue prisms ($K_m = 5 \times 10^{-5} M$; $V_{max} = 3 \times 10^{-8}$ mole/g/min). This gave taurine an easily saturable, high-affinity uptake system similar to those for GABA, glutamate and glycine. Taurine uptake was energy dependent and relatively specific, only being competitively inhibited by hypotaurine and β -alanine, among substances closely related to taurine in structure. Other workers however (Honegger et al 1973), found only one saturable component for taurine transport in rat cortex slices. They suggested this would correspond to the un-specific low-affinity uptake system found for the other amino acids discussed. Accordingly the experiments described in this thesis were carried out for cerebral cortex tissue slices over a wide range of concentrations, to examine possible high and low-affinity uptake systems for taurine in this tissue.

Taurine is taken up to glial tissue by high affinity uptake ($K_m = 1 \times 10^{-5} M$) (Schrier & Thompson 1974) to C - 6 glioma cells, and to glial cells in the retina (Ehinger 1973). Also, recent reports have stated that synaptosomes may be contaminated with particles from glial cells, or "gliasomes" (Henn et al 1974). For these reasons the contributions of glial cells and neurons to taurine uptake in the cerebral cortex and in synaptosomes were compared.

Recent experiments have shown that β - alanine may be used as a specific marker for glial uptake. β - alanine specifically enters glial cells in the cerebral cortex and cerebellar cortex (Schon & Kelly 1975; Kelly & Dick 1976). Moreover, β - alanine competes with the uptake of taurine to cerebral cortex (Kaczmarek & Davison 1972, Lahdesmaki & Oja 1973). Also β - alanine competes with the glial uptake of GABA (Schon & Kelly 1974; Iversen & Kelly 1975), but not with the uptake of GABA to cerebral cortex slices or to synaptosomes (Iversen & Johnston 1971; Schon & Kelly 1975). Accordingly β -alanine (1 mM) was used to assess the extent of $[^{35}S]$ - taurine entry by glial cells in cerebral cortex slices, and in synaptosomes.

L2,- 4 - diaminobutyrate (L - DABA) has been shown to be specifically taken up to nerve endings in the cerebral cortex and cerebellar cortex (Dick & Kelly 1975, Iversen & Kelly 1975, Kelly et al 1975). It competes with GABA uptake in the tissue (Iversen & Johnston 1971). Hence the effect of L - DABA (1 mM) on $[^{14}C]$ - GABA uptake to synaptosomes was studied to assess the purity of this fraction. The effect of L - DABA on $[^{35}S]$ - taurine uptake to synaptosomes and samples of varying glial content was studied.

The subcellular site of uptake of taurine in cerebral cortex tissue was also studied to find out the importance of different subcellular components in taurine uptake at low and high concentrations of taurine. Intact tissue slices were incubated with $[^{35}\text{S}]$ -taurine before subcellular fractionation so as to preserve the normal anatomical relationship that would be associated with taurine uptake in vivo.

3.2 Results

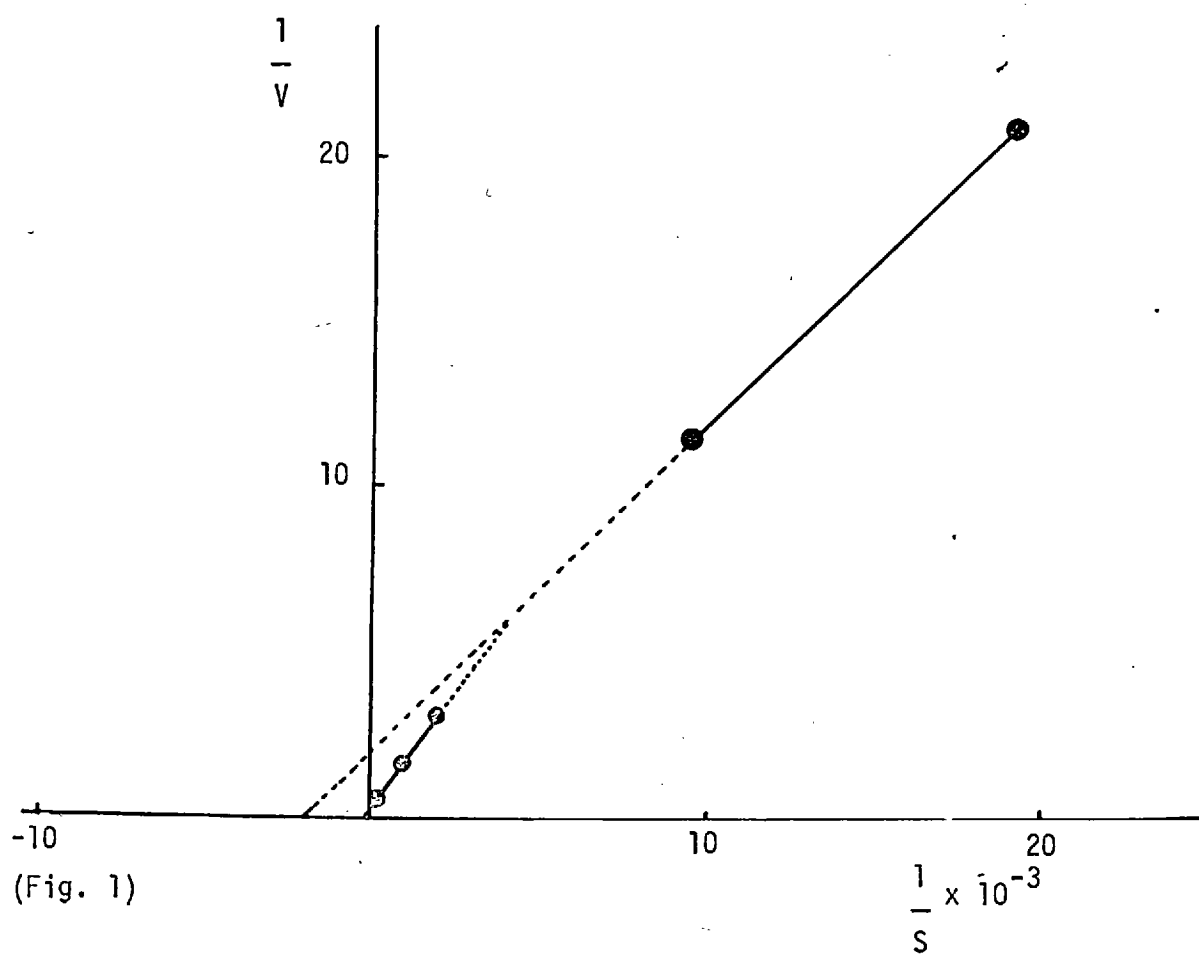
(i) Measurement of Kinetic Parameters for Uptake of taurine to cortical slices

Figure 1 shows a double reciprocal plot (Lineweaver and Burk, 1934) of velocity of $[^{35}\text{S}]$ - taurine uptake plotted against the inverse of the molar concentration S of taurine in the medium. V is taken as the initial velocity of the $[^{35}\text{S}]$ - taurine uptake and is expressed as $\mu\text{mol/g/5 min}$.

The kinetic data reveal two uptake systems for taurine, one operating at low and one at high taurine concentrations. At the low taurine concentration range ($12 - 125 \mu\text{M}$) $K_m = 5 \times 10^{-4} \text{M}$ and $V_{\text{max}} = 0.102 \mu\text{mol/g/min}$. At the higher concentration range ($200 \mu\text{M} - 5 \text{mM}$) $K_m = 10.83 \times 10^{-3} \text{M}$ and $V_{\text{max}} = 1.46 \mu\text{mol/g/min}$.

The uptake of $[^3\text{H}]$ leucine was also studied under the same conditions and was plotted in a similar way. The uptake showed a single uptake system with $K_m = 1.16 \times 10^{-3} \text{M}$ and $V_{\text{max}} = 0.318 \mu\text{mol/g/min}$. Diphenylhydantoin had no effect on $[^{35}\text{S}]$ - taurine uptake.

Fig.1. This shows a regression graph of $[^{35}\text{S}]$ - taurine uptake as a Lineweaver-Burk plot where velocity (V) is $\mu\text{mol/g/5 min}$ and concentration (S) is the molar concentration of taurine. Each line is the mean of 10 or more separate measurements. A t - test for the slopes show $p < 0.001$.



(Fig. 1)

3.2 (ii) Uptake of $[^{35}\text{S}]$ - taurine into subcellular compartments of intact cortex slices

The recovery of $[^{35}\text{S}]$ - taurine in subcellular fractions was taken as an index of entry of taurine to the subcellular compartments of the slice during incubation. The relative recoveries of $[^{35}\text{S}]$ - taurine in different subcellular fractions, following incubation in isotopic taurine at 20 μM and 540 μM are shown in Table 1. The $[^{35}\text{S}]$ - taurine content of the different fractions is given as a percentage of the total isotope present in the homogenate. The $[^{35}\text{S}]$ - taurine content varied widely among the different fractions. At 20 μM taurine most of the $[^{35}\text{S}]$ - taurine was recovered in the microsomes and soluble supernatant (P3 + S₂), and the crude mitochondrial pellet (P2) took up the next highest percentage of taurine. Within the crude mitochondrial pellet the synaptosome fraction (P2B) was most enriched. A similar pattern was obtained at 540 μM although in this case there was less $[^{35}\text{S}]$ - taurine in the synaptosomal fraction and more was recovered in the soluble supernatant fraction.

When protein content was taken into account a similar distribution of $[^{35}\text{S}]$ - taurine was observed (R.S.A.). The only difference was that the myelin fraction (P2A) showed a higher $[^{35}\text{S}]$ - taurine content than before, possibly reflecting leakage of taurine from synaptosomes. When the $[^{35}\text{S}]$ - taurine uptake was described as a percentage of the sedimentable or particle - bound $[^{35}\text{S}]$ - taurine, 41.2% was found to occur in the synaptosomal fraction at 20 μM , and 36.6% at 540 μM . Recoveries of cpm and protein were 103% and 93% respectively.

TABLE 1 UPTAKE OF $[^{35}\text{S}]$ -TAURINE TO SUBCELLULAR FRACTIONS

Fraction	P.mol/g Taurine taken up/30 minutes		Protein content mg/g
	20 μ M Taurine	540 μ M Taurine	
H	49.5 $\pm$ 8.7	879.6 $\pm$ 106.1	108.0 $\pm$ 1.6
P1	5.4 $\pm$ 0.4	91.4 $\pm$ 4.9	31.3 $\pm$ 1.2
P3 + S2	31.4 $\pm$ 5.0	578.3 $\pm$ 82.5	18.7 $\pm$ 0.8
P2	15.6 $\pm$ 3.1	246.0 $\pm$ 19.6	61.3 $\pm$ 1.5
P2A	3.7 $\pm$ 1.4	51.5 $\pm$ 12.7	3.9 $\pm$ 0.1
P2B	8.3 $\pm$ 1.9	114.5 $\pm$ 14.5	24.6 $\pm$ 0.3
P2C	2.3 $\pm$ 0.3	37.1 $\pm$ 6.7	21.5 $\pm$ 1.2
Percentage Recovery		Relative Specific Activity	Percentage of Non-Supernatant taurine
at 20 μ M Taurine			
P1	10.7 $\pm$ 0.9	0.4 $\pm$ 0.1	26.4 $\pm$ 2.1
P3 + S2	59.9 $\pm$ 0.9	3.6 $\pm$ 0.1	
P2	29.5 $\pm$ 1.2	0.5 $\pm$ 0.02	73.5 $\pm$ 2.1
P2A	6.6 $\pm$ 1.6	1.9 $\pm$ 0.4	16.5 $\pm$ 4.1
P2B	16.5 $\pm$ 0.9	0.8 $\pm$ 0.03	41.2 $\pm$ 2.0
P2C	4.5 $\pm$ 0.7	0.3 $\pm$ 0.03	11.2 $\pm$ 1.7
at 540 μ M Taurine			
P1	10.6 $\pm$ 1.1	0.4 $\pm$ 0.03	29.9 $\pm$ 2.7
P3 + S2	64.8 $\pm$ 0.6	3.9 $\pm$ 0.03	
P2	24.7 $\pm$ 0.8	0.5 $\pm$ 0.01	70.1 $\pm$ 2.7
P2A	5.6 $\pm$ 0.8	1.6 $\pm$ 0.3	15.9 $\pm$ 2.6
P2B	12.9 $\pm$ 0.9	0.6 $\pm$ 0.03	36.6 $\pm$ 2.1
P2C	4.5 $\pm$ 0.7	0.2 $\pm$ 0.03	12.6 $\pm$ 1.9

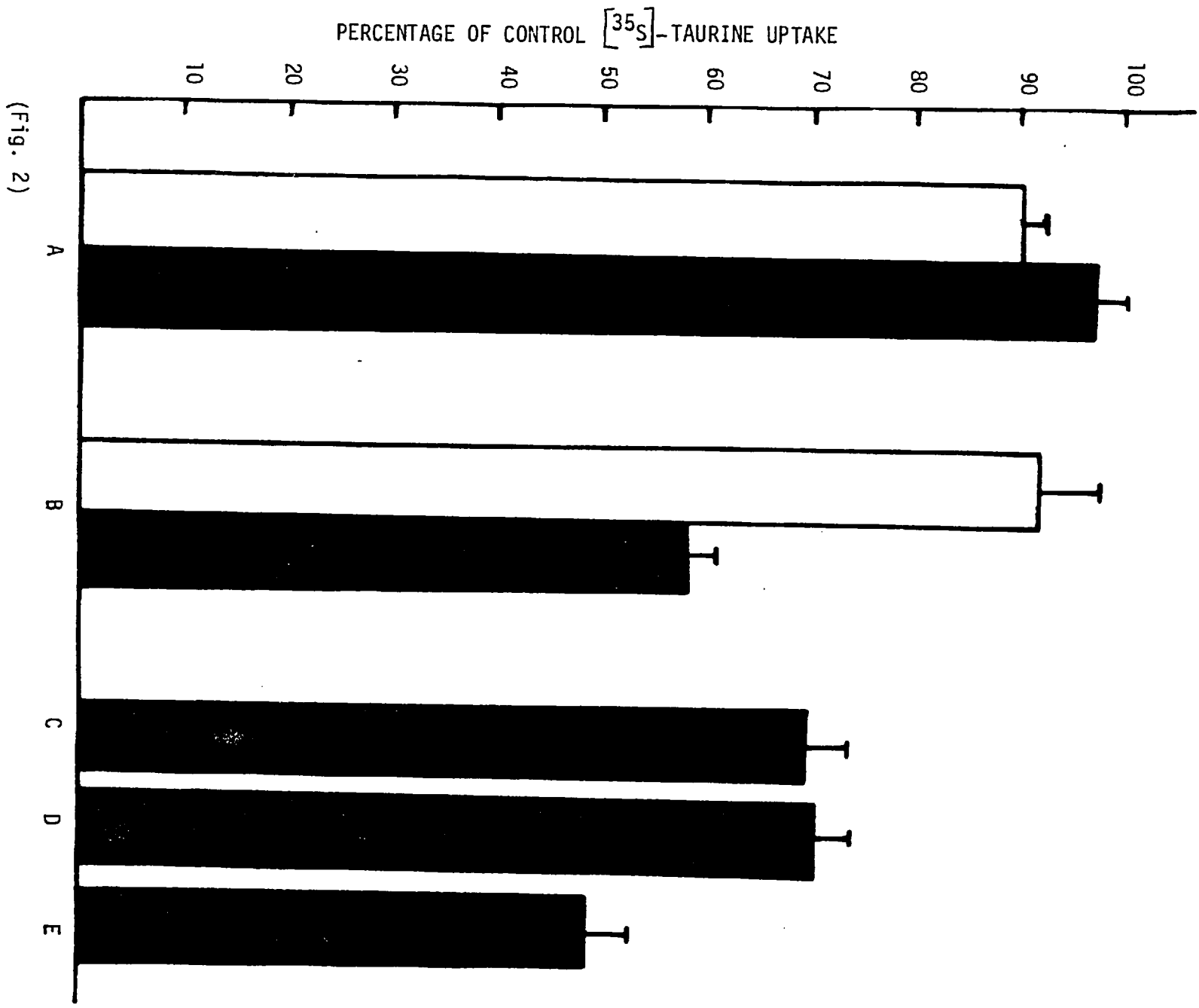
Results are $\pm$ S.E.M. Relative specific activity is Percentage of Taurine Uptake/Percentage of Protein in the fraction. Results are means of four separate estimations.

3.2 (iii) Uptake in the presence of β -alanine and L 2, 4-diaminobutyrate

(a) Uptake of $[^{35}\text{S}]$ - taurine. Figure 2 shows the effect of β - alanine (1 mM) on $[^{35}\text{S}]$ - taurine (20 μM) uptake by preparations of different neuronal and glial content, and by synaptosome preparations. Results are from incubation periods of 30 min and 5 min. After 30 min incubation it is seen that β - alanine depressed the uptake of $[^{35}\text{S}]$ - taurine by white matter slices (ependyma) by 50%. This may be compared with 30% and 28% inhibition of this uptake by cortex slices and synaptosomal beds respectively.

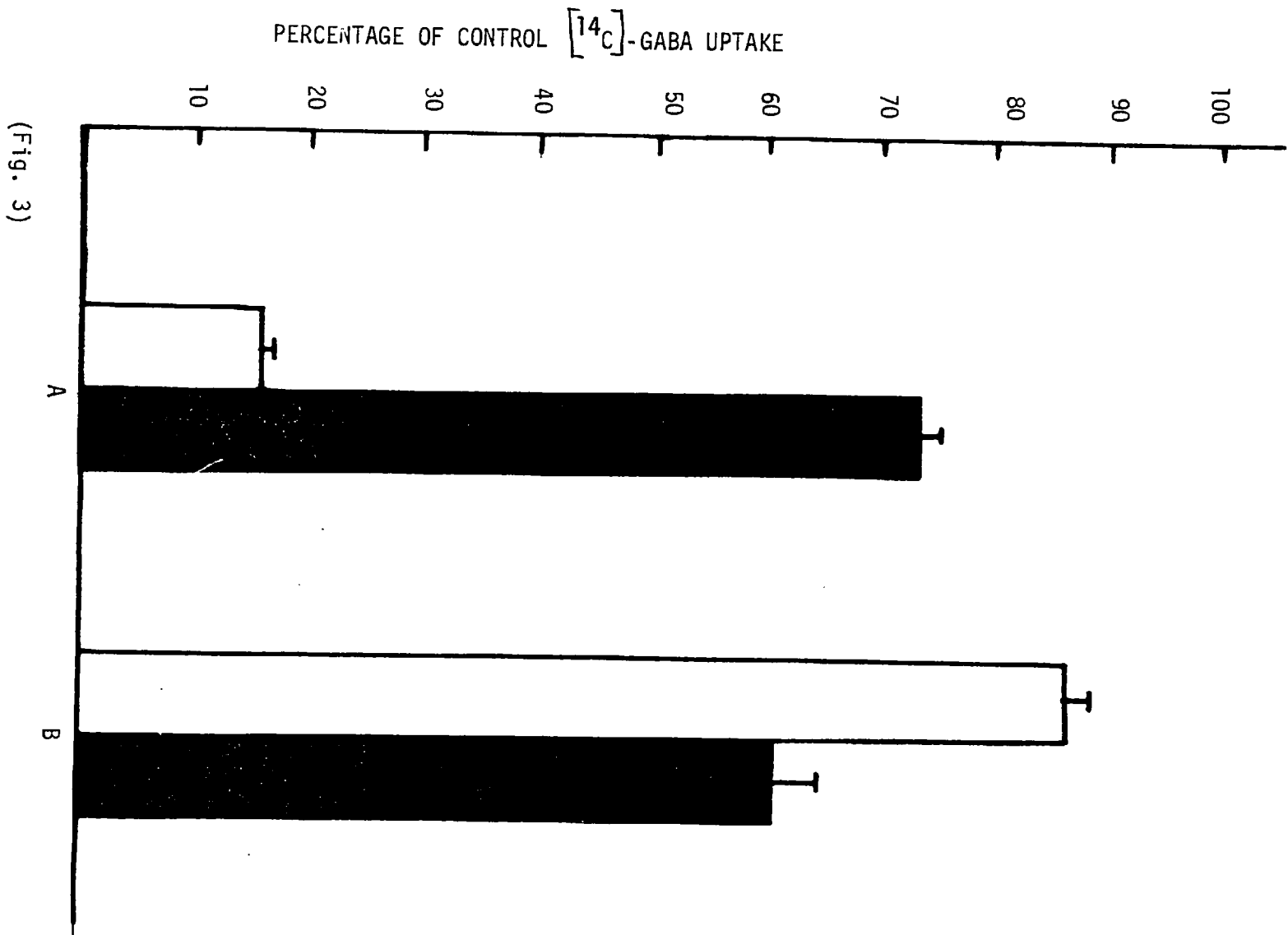
After 5 min incubation $[^{35}\text{S}]$ - taurine (20 μM) uptake was hardly depressed (2%) for synaptosome suspensions, but C - 6 glioma suspensions showed a 41% inhibition under these conditions. L - DABA (1 mM) depressed $[^{35}\text{S}]$ - taurine uptake by synaptosome suspensions by 9% and that of C - 6 glioma suspensions by 7%.

Fig. 2. This shows the uptake of $[^{35}\text{S}]$ - taurine to different tissue samples in the presence of 1 mM β - alanine or 1 mM L - DABA, as compared with control uptake. Dark areas indicate treatment with β - alanine, light areas indicate treatment with L - DABA. "A" represents synaptosome suspension; "B", C - 6 glioma suspension; "C" cortex slices, "D" Cortex beds; "E" White-matter slices. Time of incubation was 5 min for "A" and "B", and 30 min for "C", "D", "E". Four or more measurements were made for each value. Vertical bars indicate S.E.M.



(b) Uptake of $[^{14}\text{C}]$ - GABA. Figure 3 shows the amounts of $[^{14}\text{C}]$ - GABA (20 μM) taken up in 5 min in the presence of β -alanine (1 mM) or L-DABA (1 mM). For synaptosome suspensions L-DABA depressed GABA uptake by 84%. However, in C-6 glioma suspensions, uptake was only depressed by 13%. β -alanine depressed $[^{14}\text{C}]$ -GABA uptake by 26% in synaptosome suspensions, and by 39% in C-6 glioma suspensions. This β -alanine and L-DABA affected the two preparations differently, β -alanine having its main effect on the uptake by C-6 glioma suspensions, and L-DABA having its main effect on uptake by synaptosome suspensions.

Fig. 3. This compares the uptake of $[^{14}\text{C}]$ - GABA to synaptosome suspensions and C-6 glioma suspensions in the presence of 1 mM L-DABA or 1 mM β -alanine, as a percentage of control uptake. Time of incubation was 5 min in each case. Each histogram is the mean of 4 or more samples. Vertical bars indicate S.E.M. "A" represents synaptosome suspension "B", C-6 glioma suspension. Time of incubation was 5 min for "A" and "B". Dark areas indicate treatment with β -alanine, light areas indicate treatment with L-DABA.



(Fig. 3)

3.3 Discussion

The high and low-affinity uptake studies reported here compare with those established for other putative transmitters. GABA for instance shows a high-affinity uptake system of similar V_{max} ($0.115 \mu \text{ mol/g/min}$), but much lower K_m (e.g. $2 \times 10^{-5} \text{ M}$, Iversen & Neal 1968). The uptake system for taurine ($K_m = 5 \times 10^{-4} \text{ M}$) is 10 - 20 fold less sensitive than those for other transmitter amino acids ($K_m = 1 - 2 \times 10^{-5} \text{ M}$) (Iversen et al 1973), which places it in a new category of intermediate sensitivity.

Lahdesmaki & Oja (1973) have reported a high-affinity taurine uptake with a K_m of $6 \times 10^{-5} \text{ M}$, but the absolute values for K_m of these systems may vary widely with the size of the slice used (Leach et al 1976; Davies et al 1975).

The uptake of taurine was compared with that for leucine, which has a purely metabolic role in the brain. The finding that slices showed only a single, low affinity, uptake system for leucine suggests that the existence of the two transport systems for taurine is not due to artefact. Hence the higher affinity uptake system could be available for the rapid removal of the low concentrations of taurine associated with release from nerve endings. This suggestion is supported by recent evidence of a high-affinity ($K_m = 20 \mu \text{M}$) uptake of taurine to synaptosomes (Schmid et al 1975). The high-affinity uptake was sodium and energy dependent.

In experiments to study the subcellular site of $[^{35}\text{S}]$ -taurine uptake in cerebral cortex tissue slices, the slices were incubated with taurine at concentrations that would be taken up by a high and low-affinity uptake system, at $20 \mu \text{M}$ and $540 \mu \text{M}$ respectively. The subsequent localization

of $[^{35}\text{S}]$ - taurine following subcellular fractionation probably represents true uptake, as recent studies have shown that uptake of $[^{35}\text{S}]$ - taurine to cerebral cortex tissue followed by subfractionation does not undergo significant relocation during the homogenization and subfractionation process (Sieghart & Karobath 1974; Rassin et al 1977). The finding that among the particulate fractions the synaptosomes had the highest content of $[^{35}\text{S}]$ - taurine indicates that in intact cerebral cortex tissue at least part of the re-uptake of taurine could be to synaptic endings. This process would facilitate removal of taurine from its site of release and would serve to replenish taurine stores in nerve endings.

The large proportion of taurine recovered in the soluble cytoplasmic fraction could be derived largely from homogenized glial cells and neuronal cell bodies. The studies with β - alanine and L - DABA helped to assess the contribution of glial and neuronal elements to the high-affinity taurine uptake described above, because of their ability to block neuronal and glial uptake as discussed earlier. Thus it was possible to assess the contribution of glia to both synaptic uptake, as well as to uptake for cerebral cortex tissue.

Figure 2 shows that as expected β - alanine depressed $[^{35}\text{S}]$ - taurine uptake more in tissues containing glial elements than in those containing mainly neuronal elements. β - alanine however had very little effect on taurine uptake in synaptosomal suspensions. Thus virtually all the taurine entering the synaptosome suspensions is likely to be entering the synaptosomes themselves rather than any particles of glial origin "gliasomes". The fact that with longer inhibitions β - alanine depressed $[^{35}\text{S}]$ - taurine entry by 27% suggests the presence of slower uptake systems to a component of the synaptosome preparation that is of glial origin. Alternatively β - alanine may block a slower uptake system to nerve endings. Evidence for the latter possibility

is the close similarity between the depression of uptake of cortex slices and synaptosome beds during the longer incubation period of 30 min, with white matter slices showing greater depression of taurine uptake at this time, with 20% less uptake for taurine than for the cortex slices or synaptosomal beds. The fact that L - DABA had little depressant effect on taurine uptake in synaptosomes or in C - 6 glioma, suggests that it is not an effective inhibitor for the localization of taurine uptake.

The uptake of GABA in synaptosomes and C- 6 glioma was compared (Figure 3) in the presence of 1 mM β - alanine or 1 mM L - DABA in order to assess the glial contamination of synaptosomes. Since L - DABA depressed synaptosomal [14 C] GABA uptake by 84%, but had little effect on reducing C - 6 glioma GABA uptake, synaptosome preparations would appear to have little glial contamination. The inverse pattern was obtained using β - alanine. Thus this compound depressed C - glioma uptake to a greater extent (40%) than to the synaptosomal suspension (27%) when 5 min incubation periods were used. This data also supports the relative purity of the synaptosomal fraction.

L - DABA has been reported (Sellstrom & Hamberger 1975) to inhibit GABA uptake to a cortical glial preparation to the same extent, 50%, that it blocks GABA uptake to synaptosome preparations, which might be thought to conflict with the above assessment of the purity of synaptosome preparations. However this result conflicts with the autoradiographic data of uptake to cerebral cortex and sympathetic ganglia showing L - DABA uptake only in neuronal elements, mainly nerve endings (Dick & Kelly 1975). Also the I.C. 50 for L - DABA blocking GABA uptake to the neurons of sympathetic ganglia (0. 7 mM) is much higher than its counterpart for cortex slices (50 μ M) (Iversen & Kelly 1975). For these

reasons it seems likely that the glial preparation of Sellstrom and Hamberger contains synaptosomes as contaminants. It has been reported that β -alanine blocks GABA and taurine uptake to synaptosomes by up to 60% (Snodgrass et al 1973; Schmid et al 1975) but this does not correlate with the autoradiographic evidence which shows specific uptake of β -alanine to glial cells. This was also indicated by the greater effectiveness of β -alanine in blocking GABA uptake to ganglia rather than to cortex slices. The I.C. 50 values for this inhibition being 120 μ M and 21 mM respectively (Iversen & Kelly 1975). Also the fraction studied by Schmid et al was not a pure synaptosomal preparation, but a fraction (P2) which contained other elements including mitochondria.

Recently it has been suggested that in cerebral cortex tissue there is significant glial uptake of taurine (Riddall et al 1976). It was found that with increasing thickness of cerebral cortex tissue prisms, cut by a mechanical tissue slicer, the uptake of both taurine and β -alanine increased with increasing slice thickness (0.1 mm - 0.4 mm slices). It was suggested that in thicker slices the proportion of intact glial tissue increased, hence the increased uptake of taurine and β -alanine.

Thus there are several different sources of evidence for taurine uptake by glial tissue. Such glial uptake could be associated with several distinct roles for taurine. It could remove taurine liberated in the vicinity of synapses, thus taking part in rapid termination of the synaptic action of taurine. Glial uptake could also be part of a cycle for taurine between neurons and glia such as has been proposed for GABA (Sellstrom et al 1975) and glutamate (Bradford & Ward 1976). Alternatively taurine uptake to glia could be associated with release of taurine

from glia to exert a modulator action of taurine on nervous tissue, as both K^+ and electrical stimulation have been shown capable of releasing GABA from glia (Bowery & Brown 1971, 1972; Minchin & Iversen 1974), and taurine is similar in many respects to GABA (Kaczmarek 1975). At this stage it does not seem likely that glia are associated with an important metabolic role of taurine, as taurine is very slowly metabolized in the cerebral cortex (Peck & Awapara 1967). Also glial uptake is probably not the only type of uptake of taurine in the brain as high-affinity uptake of taurine ($K_m = 5 \times 10^{-5} M$) has also been shown to occur to neuroblastoma cells in culture (Borg et al 1976) and to crude synaptosome preparations ($K_m = 2 \times 10^{-5} M$) (P2) (Schmid et al 1975). Also taurine present at low concentrations (20 μM) is taken up to synaptosome suspensions and to synaptosomes prepared from intact cortical slices as reported in this thesis.

CHAPTER 4 THE RELEASE OF TAURINE FROM CORTEX
SLICES AND SUBCELLULAR COMPARTMENTS

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4.1 Introduction

Because release of amino acid transmitter compounds has been shown to occur from glial tissue (Bowery & Brown 1971, 1972; *Minchin & Iversen 1974), and from nerve trunks (Weinreich & Hammerschlag 1975), after K^+ and electrical stimulation, the calcium dependence of stimulated release has become an important criterion of synaptic release of neurotransmitters. Present evidence indicates that stimulated release from glial cells and nerve trunks does not show Ca^{2+} dependence (Bowery & Brown 1974, Sellstrom & Hamberger 1977, Weinreich & Hammerschlag 1975). On the other hand stimulated synaptic release of neurotransmitters does show calcium dependence (Sellstrom & Hamberger 1977; Osborne et al 1973). One report* has stated that K^+ stimulated release of GABA from ganglia showed some evidence of calcium dependence, although their results were contradictory. Reducing the calcium concentration in the medium decreased stimulated GABA release, however the authors noted release was not blocked by La^{3+} or by diphenylhydantion which block the entry of calcium associated with depolarization and subsequent transmitter release. Thus the effect they observed was probably due to other factors than calcium dependent release.

Clarke and Collins (1976) reported that stimulated release of endogenous taurine from visual cortex in vivo was not calcium dependent. However Collins (1977) later reported that calcium ionophores X 537A and A23187 increased taurine release. He suggested that the discrepancy with his earlier findings was due to difficulty in lowering calcium stores in vivo. Calcium dependence of taurine release has also been reported from the retina where taurine has a probable transmitter role. Pasantes-Morales and co-workers (1976) reported taurine was released from

the retina using the ionophore A23187 which is highly selective for divalent cations. Taurine release was correlated with ^{45}Ca entry into the retina.

Thus the balance of present evidence strongly indicates that stimulated release of neurotransmitters from nerve endings is calcium dependent, but from other tissue components it is not. Accordingly in experiments reported here the calcium dependence of taurine release from stimulated cerebral cortex tissue slices was examined. In addition the effect of calcium on uptake of taurine was also examined as an effect of calcium on taurine uptake could modify its stimulated release. Further reasons for examining this aspect were suggestions that taurine is associated with calcium fluxes in the brain and recent reports support this for brain, retina and heart (Welty et al 1976; Pasantes-Morales et al 1976; Read & Welty 1976).

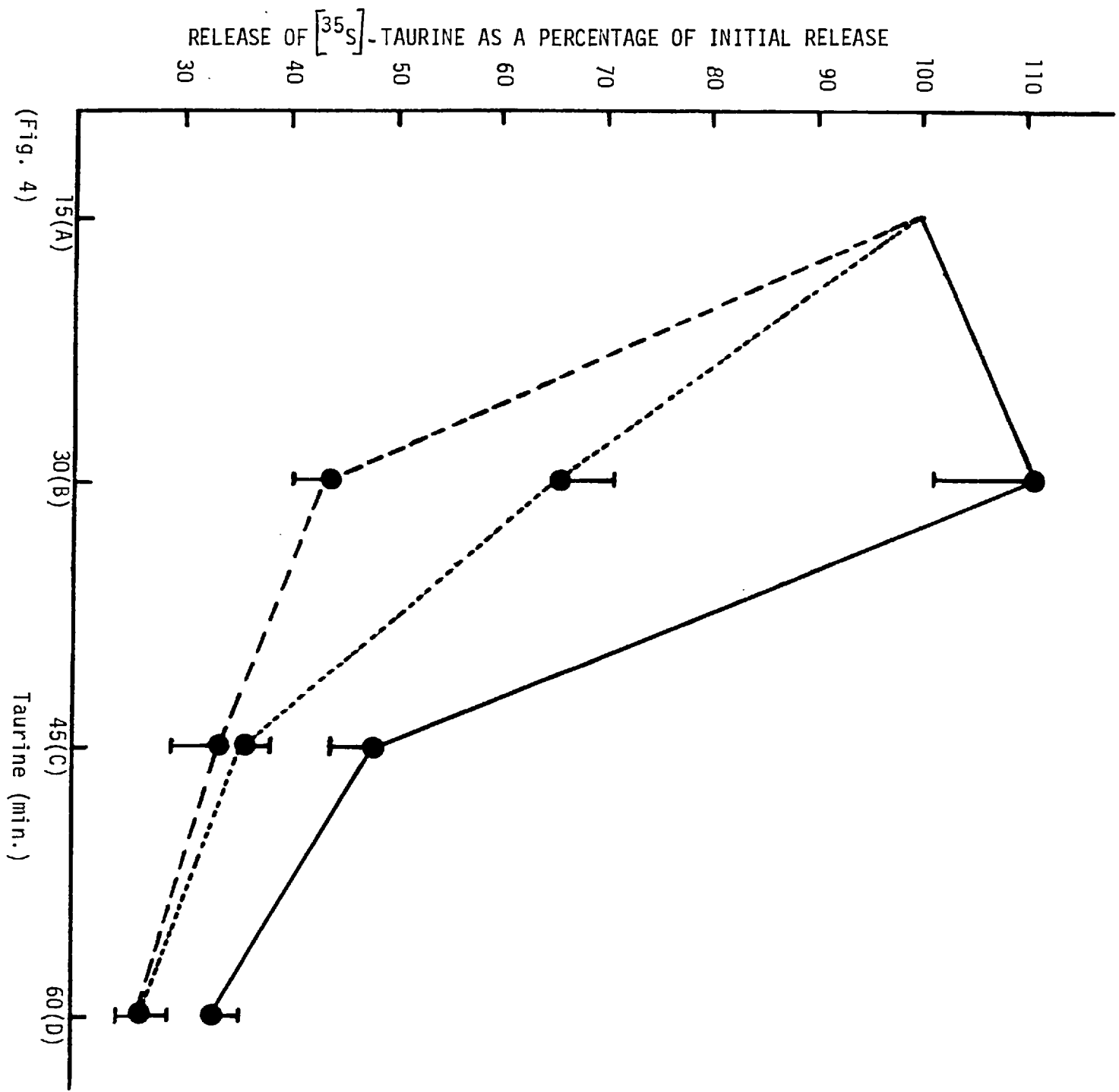
A further requirement for demonstrating a synaptic role for taurine is that the stimulated release of taurine should come from synaptic processes, rather than for other tissue pools such as neuronal cytoplasm. Thus in experiments described here cortex slices incubated in medium containing $[^{35}\text{S}]$ -taurine at $20\text{ }\mu\text{M}$ as described earlier were stimulated and then subfractionated, to compare the subcellular fractions which showed the decrease in content of $[^{35}\text{S}]$ -taurine. The advantage of using intact cortex slices is that it preserves the normal anatomical relationships of the tissue and so more closely resembles the in vivo situation than by studying an isolated tissue component such as synaptosomes. Furthermore because investigations reported here show that the electrically stimulated release of $[^{35}\text{S}]$ -taurine was current dependent, the effect of two different currents on the change in the subcellular content of $[^{35}\text{S}]$ -taurine was studied. The efflux of $[^{35}\text{S}]$ -taurine into medium was then correlated with the change in radioactivity measured for the subcellular fractions.

4.2 Results

(i) Fig. 4 shows the efflux of preloaded $[^{35}\text{S}]$ -taurine from incubated cerebral cortex slices. Points A, B, C, D represent 15 min periods of $[^{35}\text{S}]$ -taurine efflux into consecutive beakers containing taurine-free incubation medium.

When slices were stimulated electrically in the presence of Ca^{2+} there was an increase in the efflux of $[^{35}\text{S}]$ -taurine into the medium (B in Fig. 4). On the cessation of stimulation, the release of $[^{35}\text{S}]$ -taurine from the slices decreased to levels approaching those of the control, indicating that no tissue damage had occurred. Altogether about 33% of the total $[^{35}\text{S}]$ -taurine in the slices was recovered in the incubation medium during the 1-hour of incubation with stimulation. In medium free of Ca^{2+} the increase in release due to stimulation was greatly reduced (60%) during period B, compared with the control.

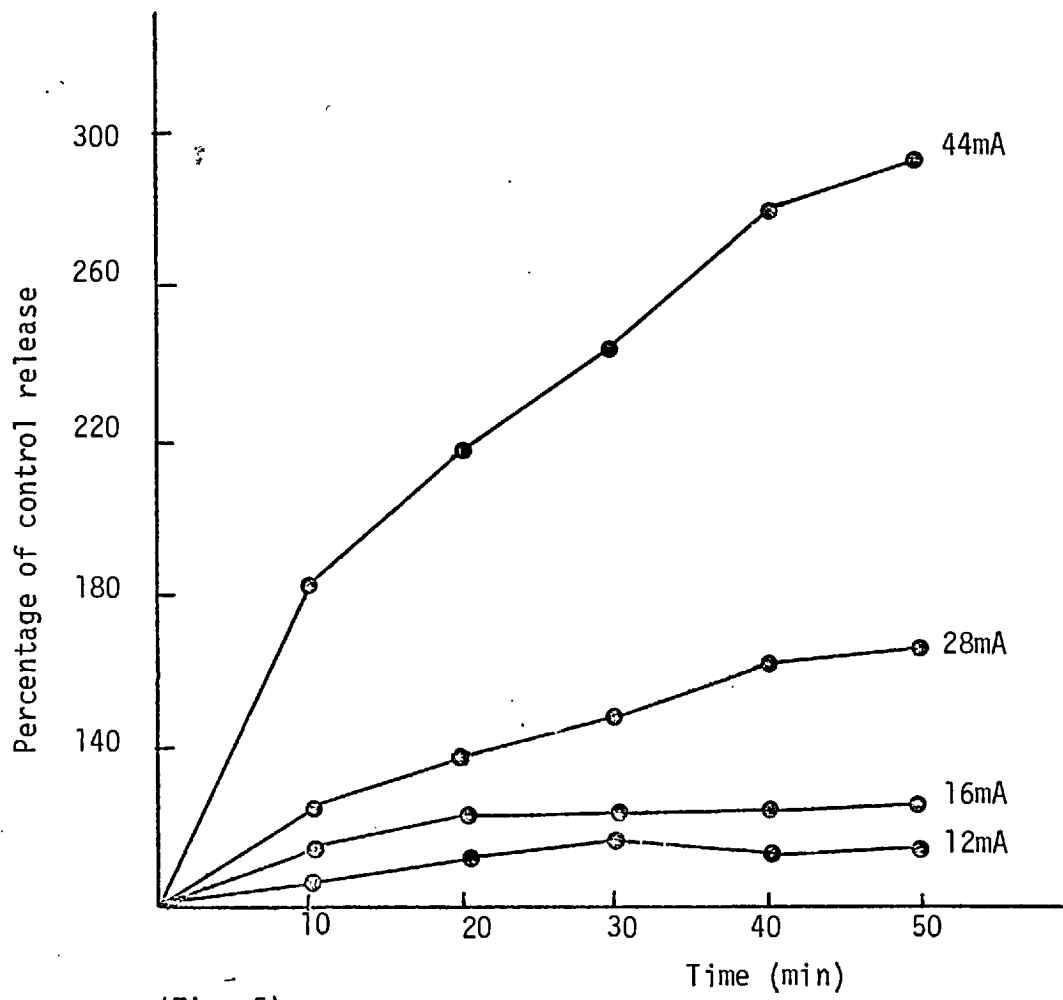
Fig. 4 This shows the effect of Ca^{2+} on electrically stimulated $[^{35}\text{S}]$ -taurine release. A, B, C, D are successive 15 minute periods of incubation in different beakers. Electrical stimulation occurred in period B. Symbols used are for electrically stimulated cortex slices in medium containing Ca^{2+} _____; for control slices; - - - - -; for slices incubated in Ca^{2+} free medium and electrically stimulated as above. Vertical lines are S.E.M. for 5 or more separate measurements. Range of cpm/g is $1 - 9 \times 10^5$ cpm/cpm g from minimum to maximum levels.



There was no significant difference between the passive (control) efflux when Ca^{2+} was present or absent from the incubating medium, though a slight increase in taurine efflux occurred in the absence of Ca^{2+} . In the case where loading of $[^{35}\text{S}]$ -taurine during preincubation occurred in Ca^{2+} -free medium, measurement showed that $[^{35}\text{S}]$ -taurine uptake was reduced by 30%. This lower content was partly the cause of the reduction in release of $[^{35}\text{S}]$ -taurine from the slices as preincubation in Ca^{2+} medium followed by stimulation in Ca^{2+} free medium caused an increase (30%) in the stimulated release of $[^{35}\text{S}]$ -taurine, but did not affect the passive release.

The proportion of $[^{35}\text{S}]$ -taurine loaded into the slices which was recovered in the medium after incubation with or without applied electrical stimulation was high (approximately 40%). This suggested that either a large proportion of the total tissue content of taurine was released, or that both uptake and release occur largely from a special compartment. Taurine release was also shown to be current dependent (Fig.5).

Fig. 5 The graph shows the current dependence of $[^{35}\text{S}]$ -taurine release from electrically stimulated cortex slices as a percentage of control release. The mean current is shown in each case on the graph, duration and frequency of stimulation were the same as in the other experiments.



(Fig. 5)

4.2. (ii) The change in $[^{35}\text{S}]$ - taurine content of subcellular fractions of slices following electrical stimulation.

Table II shows the comparison of the recoveries of $[^{35}\text{S}]$ - taurine in the subcellular fractions prepared from slices electrically stimulated at two levels of current (48 mA and 36 mA mean current).

At the higher current stimulation (48 mA), there was considerable release from all fractions (Table II). The fraction P3 + S2 showed the greatest release, and was twice that of the synaptosomal fraction. Because non-specific release due to the current used could be masking specific synaptosomal release, the lower current stimulation was employed.

Using 36 mA mean current stimulation a more specific pattern of release was obtained (Table III). The change in recovery in the synaptosome fraction was 82,000 cpm/g. This was commensurate with the increase in counts recovered in the incubation medium of 90,000 cpm/g and was significant ($p < 0.05$). The other fraction showing most change in recovery of $[^{35}\text{S}]$ - taurine was the soluble cytoplasmic fraction (P3 + S2), which showed a much smaller decrease (23,000 cpm/g) which was found to be insignificant ($p > 0.1$).

Thus, there was a striking correlation between the fall in the absolute amounts of $[^{35}\text{S}]$ - taurine recovered in the nerve endings isolated from stimulated slices, and the net amounts of $[^{35}\text{S}]$ - taurine released by these slices into the incubation medium due to electrical stimulation.

TABLE 11. CPM/G OF $[^{35}\text{S}]$ - TAURINE IN SUBFRACTIONS OF CORTEX
SLICES STIMULATED AT 48 m.A MEAN CURRENT

Fraction	Control	Stimulated	Difference
P1	120,000 $\pm$ 6,700	82,800 $\pm$ 11,500	37,200
P3 + S2	629,000 $\pm$ 25,000	325,000 $\pm$ 43,000	304,000
P2	399,000 $\pm$ 32,000	130,000 $\pm$ 19,000	269,000
P2A	89,000 $\pm$ 7,300	40,900 $\pm$ 3,500	48,100
P2B	213,600 $\pm$ 14,000	52,600 $\pm$ 10,000	161,000
P2C	189,100 $\pm$ 7,000	19,800 $\pm$ 2,800	169,300
cpm/g released into incubation medium	218,500 $\pm$ 23,000	893,500 $\pm$ 63,000	675,000

TABLE III CPM/G OF $[^{35}\text{S}]$ - TAURINE IN SUBFRACTIONS OF CORTEX
SLICES STIMULATED AT 36 m A . MEAN CURRENT

Fraction	Control	Stimulated	Difference
P1	91,200 $\pm$ 6,000	91,700 $\pm$ 7,300	500
P3 + S2	533,000 $\pm$ 15,900	509,800 $\pm$ 11,500	23,200
P2	470,000 $\pm$ 39,100	355,000 $\pm$ 37,700	115,000
P2A	75,900 $\pm$ 12,200	78,600 $\pm$ 3,800	2,700
P2B	281,700 $\pm$ 23,200	198,900 $\pm$ 25,200	82,800
P2C	57,900 $\pm$ 6,200	53,700 $\pm$ 6,700	4,200
cpm/g released into incubation medium	313,000 $\pm$ 41,600	403,000 $\pm$ 51,700	90,000

Results are $\pm$ S.E.M. Each result is the mean of 4 separate estimations.

4.3 Discussion

The release of $[^{35}\text{S}]$ - taurine into the medium due to electrical stimulation reported here was calcium dependent. Since stimulus induced release of taurine from slices was higher in calcium free medium, than for losses from control slices in the same medium containing calcium, it is possible that some endogenous calcium was still available for the process. This possibility emphasizes the importance of using a chelator such as EGTA in addition to making the medium up without calcium. Otherwise endogenous calcium stores could mask a true calcium dependent release when stimulation is carried out in calcium free medium. This may explain the conflicting results reported earlier. Alternatively the non-calcium dependent efflux of $[^{35}\text{S}]$ - taurine could be from glial cells, as the experiments discussed earlier reported calcium independence of stimulated transmitter release from glia.

The slight increase in the passive release of taurine in the absence of calcium may compare with the retina where this is a large effect (Kennedy & Voaden 1976), and it is possible this could to some extent mask a non-calcium dependent release of taurine as has been recently described for synaptosomes (Sieghart & Heckl (1976). In the latter report it was stated that decreasing the calcium concentration in the medium actually increased the potassium stimulated release of $[^{35}\text{S}]$ - taurine. This report thus is opposite to the other reports for calcium dependence of stimulated release, hence it is reasonable to look for ways to resolve the difference. Firstly it should be noted that the preparation used was a mixed one, the crude mitochondrial pellet (P2) which included mitochondria as well as synaptosomes. Also it is possible that the cortex samples used for the

preparation contained glial tissue as a contaminant. This is relevant as Henn and co-workers (1974), suggested that synaptosome preparations could contain glial elements, "gliasomes" as contaminants. Moreover it has been shown that GABA uptake to glial cells increases in calcium deficient medium whereas GABA uptake to synaptosomes or neurons stays the same or decreases (Sellstrom & Hamberger 1975). Thus if a similar mechanism operates for taurine uptake, the increased release of taurine observed by Sieghart and Heckl (1976) could be due to glial contamination.

The evidence reported in this thesis suggests the question above does not apply to experiments reported here, as experiments using β -alanine to block glial uptake, and L-DABA to block neuronal uptake of taurine and GABA, showed there was little entry of taurine to glia in the thin cortex slices used (0.3 mm thickness) or to the synaptosomes prepared from cerebral cortex.

Calcium was also found to affect the uptake of $[^{35}\text{S}]$ -taurine to cerebral cortex in experiments reported here, decreasing it by 30% when calcium was omitted from the medium, and incubating with 1 mM EGTA. This decreased uptake contributed to the decrease in release of taurine in calcium free medium, as incubating cortex slices with $[^{35}\text{S}]$ -taurine in calcium containing medium followed by stimulation in calcium free medium, showed a proportionate increase in release of taurine, compared with slices incubated and stimulated in calcium free medium. This compares with recent studies for both noradrenaline and GABA, both of whose post-synaptic action is terminated by re-uptake. Thus White (1975) reported that noradrenaline uptake to synaptosomes decreased in calcium free medium, and Sellstrom and Hamberger (1975) reported decreased calcium concentration, especially with decreased magnesium ions, decreased the synaptosomal uptake of GABA. This was not solely due to

decreased magnesium ions in the medium, as on decreasing the calcium in magnesium free medium the uptake was further decreased by one third.

The effect of calcium on taurine uptake, and on uptake of transmitters such as GABA and noradrenaline suggests a wider role for calcium in facilitating neurotransmitter re-uptake. Also since calcium affects the electrical excitability of nervous tissue, calcium dependent taurine uptake may contribute to the control of neuronal excitability in the brain.

In addition to the studies of stimulated release of taurine and its calcium dependence discussed above, the electrical stimulation of cortex slices and their subsequent subfractionation indicated the pools responsible for taurine release in intact cortical tissue. The release at the higher current (48 mA) was from several tissue fractions, hence the lower current (36 mA) was used to try and identify more specifically the site of the released taurine. At the lower current release was mainly from the synaptic pool of $[^{35}\text{S}]$ -taurine.

CHAPTER 5 STUDIES OF UPTAKE OF ORALLY ADMINISTERED
TAURINE TO THE CEREBRAL CORTEX, AND ITS
AFFECT ON COBALT INDUCED EPILEPSY.

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5.1 Introduction

A possible connection of taurine and focal epilepsy was reported in 1972 when it was found that the levels of certain amino acids were decreased at the epileptic focus. It was found that the decrease in concentration of amino acids in the focus of human focal epilepsy was similar to that for experimental cobalt-induced epilepsy in cats (Van Gelder et al 1972; Koyama 1972). Decreased taurine levels occurred at the focus in both cases as described in the introduction to this thesis.

Subsequently Van Gelder (1972) reported that intraperitoneal administration of taurine at doses of 200mg/Kg in cats and mice, produced marked improvement in the number and severity of cobalt-induced convulsions.

The above observations prompted a trial of oral administration of taurine to rats with cobalt-induced epilepsy described in this thesis. Large doses (600 mg/Kg) were used and repeated daily to rats which had been observed to convulse. The rats were observed throughout the period during which convulsions, as manifested by the fore and hind limb jerks, increased and subsided over a two-week period.

A study was also made of the uptake of orally administered [^{35}S] - taurine, to the plasma, liver, skeletal muscle and cerebral cortex, at the same dose levels. This was carried out in order to assess the effectiveness of oral dosing in relation to taurine entry to the brain, and tissues possibly relevant to its action on epilepsy.

Large repeated doses were used in this trial as previous reports had indicated that taurine did not enter the brain readily when given intravenously or intraperitoneally (Minato et al 1969; Peck & Awapara 1967). Also the report of a high-affinity uptake for taurine to cerebral cortex slices (Kaczmarek & Davison 1972) contrasted with the above reports and suggested that taurine movement across cerebral capillaries was restricted.

5.1(i) Cobalt induced epilepsy as an experimental model

Kopeloff (1960) described the epileptic effects of metallic cobalt topically applied to the sensory motor area of the cerebral cortex. Dow and co-workers (1962) subsequently investigated the development of epilepsy in this model in more detail. They observed that the epilepsy developed over several days and then gradually subsided. This made it possible to readily separate pre-convulsive, convulsive and post-convulsive events. The model showed epileptic-like electro-corticographic tracings, and peripherally there were discrete limb jerks of the forelimb or hind limb.

The cobalt model also showered biochemical similarities with human focal epilepsy. Van Gelder and co-workers (1972b) reported changes in the amino acid content of biopsies of human focal epilepsy. The epileptic focus was characterized by decreased levels of glutamic acid, GABA, and taurine, with raised glycine. A more generalized lowering of GABA and aspartate was found in other areas of the cortex. In the cobalt model of epilepsy, Koyama (1972) reported decreased levels of glutamic acid, aspartic acid, and GABA in the acute epileptic focus in cats. Levels of taurine, glycine, serine and threonine rose in the focal area during this period.

Koyama also measured pre and post-convulsive changes, and studied the efflux of amino acids into the CSF. During the preconvulsive period there was a 30% decrease in the concentration of glutamic acid, GABA, aspartic acid and taurine in the focal area. Slight decreases in aspartic acid and taurine occurred in areas distant from the focus bilaterally. Koyama also observed that during the pre convulsive period glutamic acid levels in the CSF rose to ten times its previous level. She could not find similar increases for other amino acids including aspartic acid, taurine, GABA and glycine. During this period the concentrations of urea and glutamine fluctuated markedly

but showed a reciprocal relationship to glutamic acid levels. The increased glutamic acid levels during the preconvulsive period led Koyama to suggest that the release of glutamate could play an important role in the production of focal epileptic discharges in this model. Because the levels of serine, threonine and glycine remained high in the focal area Koyama suggested these could have a purely metabolic role in this model. The concentrations of taurine fluctuated markedly, so it was not possible to make a conclusion about its relationship to the epilepsy at this time.

Van Gelder and Courtois (1972) subsequently confirmed Koyama's findings, and reported the changes in amino acid levels in the homologous area, contralateral to the epileptic region. This area was characterized by low levels of glutamic acid, GABA, and aspartic acid. They reported that the decrease was proportional to the severity of the epilepsy, and diminished with increasing distance from the focus. Since the levels of GABA, glutamic acid, and aspartic acid were decreased in constant proportion in the focus, Van Gelder suggested there was a common metabolic defect.

Subsequently Van Gelder (1974) reported enzyme changes in cobalt epileptic foci. He reported that glutamate dehydrogenase (GDH) decreased in activity during the pre-convulsive period, while other enzyme activities changed during the convulsive period. Thus glutamic acid decarboxylase (GAD) activity increased during the convulsive period, while GABA - transaminase activity decreased. Van Gelder suggested that the decrease in GDH activity could be responsible for the common changes in amino acid levels which decrease together, as it is the source of glutamate from which aspartate, and GABA are

synthesized. Van Gelder also suggested that decreases in acidic amino acid levels at the focus could be of possible importance in perpetuating the epileptic lesion by leading to a local decrease in the synthesis of acidic proteins, as the presence of these proteins is related to neuronal activity (Tiplady & Rose 1971, Yoshino & Elliott 1970), and protein synthesis is inhibited after seizure activity (Cremer 1964; Jones & McIlwain 1971; Yoshino & Elliott 1970).

Abnormal ion fluxes may also be a common defect in human epilepsy and in cobalt induced epilepsy. Slices of human epileptic tissue do not take up potassium nor extrude sodium as well as do normal brain slices (Tower 1965), and cobalt induced epilepsy has been reported to be associated with decreased concentration of K^+ and raised Na^+ , Ca^{2+} and Mg^{2+} close to the epileptic focus (Hunt and Craig 1973). These authors also reported decreased activity of Na^+ , K^+ -ATPase in this model. The significance of these changes with respect to the development of seizures in this model and in man remains to be established.

Finally abnormal folic acid metabolism may be associated with cobalt-induced epilepsy, cats were found to have five times the amount of mono-glutamate folic acid at cobalt epileptic focus, as compared with the rest of the cortex (Mayersdorf et al, 1971). Although folic acid derivatives may have a strong depolarizing effect on central neurons, its role in epileptogenesis remains unknown (Obbens 1973).

5.2 Results

(i) Entry of $[^{35}\text{S}]$ - taurine to Tissues

It was found that oral taurine was absorbed into the blood stream, where its concentration rose to a peak value within 4 hr. Some 7 hr after dosing, levels of $[^{35}\text{S}]$ - taurine in the plasma decreased significantly and by 24 hr little remained in the plasma (Fig. 6).

The brain cortex, skeletal muscle and cardiac muscle showed a similar pattern of change in $[^{35}\text{S}]$ - taurine content. Initially there was a sharp increase in entry of $[^{35}\text{S}]$ - taurine from the plasma, which coincided in time with the sharp rise in plasma levels. Then, from approximately 2 - 24 hrs there was a slight decrease in $[^{35}\text{S}]$ - taurine entry to brain and muscle, followed by a slight increase in tissue uptake over the next 3-day period. On the other hand for liver there was a sustained rise in tissue $[^{35}\text{S}]$ - taurine concentration for 24 hr after which it decreased (Fig. 7).

Fig. 6 Shows the uptake of $[^{35}\text{S}]$ - taurine in the plasma following oral administration of taurine. cpm/ml range was 30 - 0.5×10^3 cpm/ml, max to min. Vertical bars indicate S.E.M. (a)

Fig. 7 This shows the uptake of $[^{35}\text{S}]$ - taurine in cerebral cortex, (b) skeletal muscle and (c) liver after isotopic dosing as indicated in the text. The cpm/g range for the tissue was: cerebral cortex 5 - 16×10^3 cpm/g, skeletal muscle 4 - 30×10^3 cpm/g, liver 7 - 320×10^{-3} cpm/g, from minimum to maximum levels. Vertical bars indicate S.E.M.s. Values are the mean of 4 or more separate estimations.

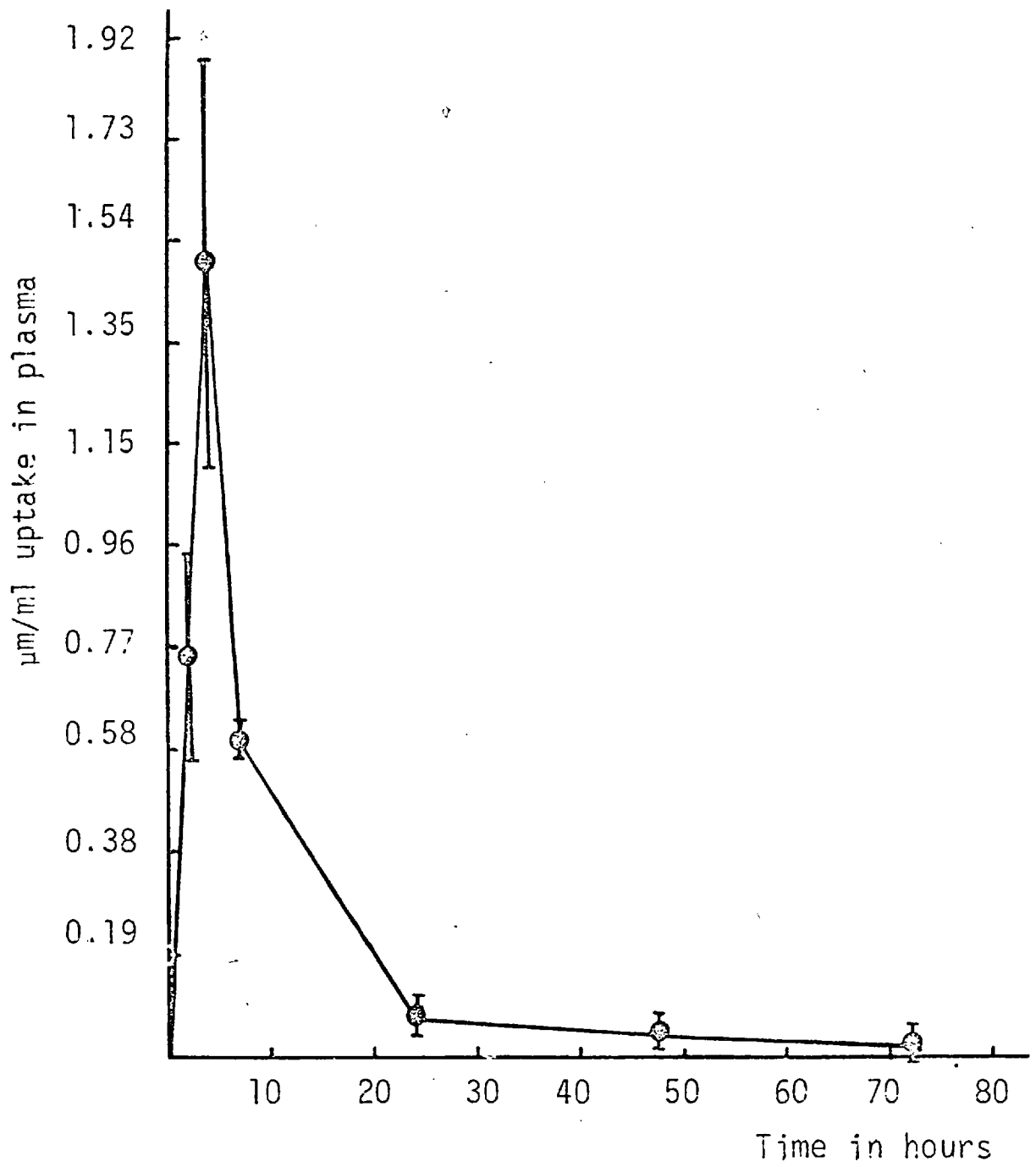


FIGURE 6

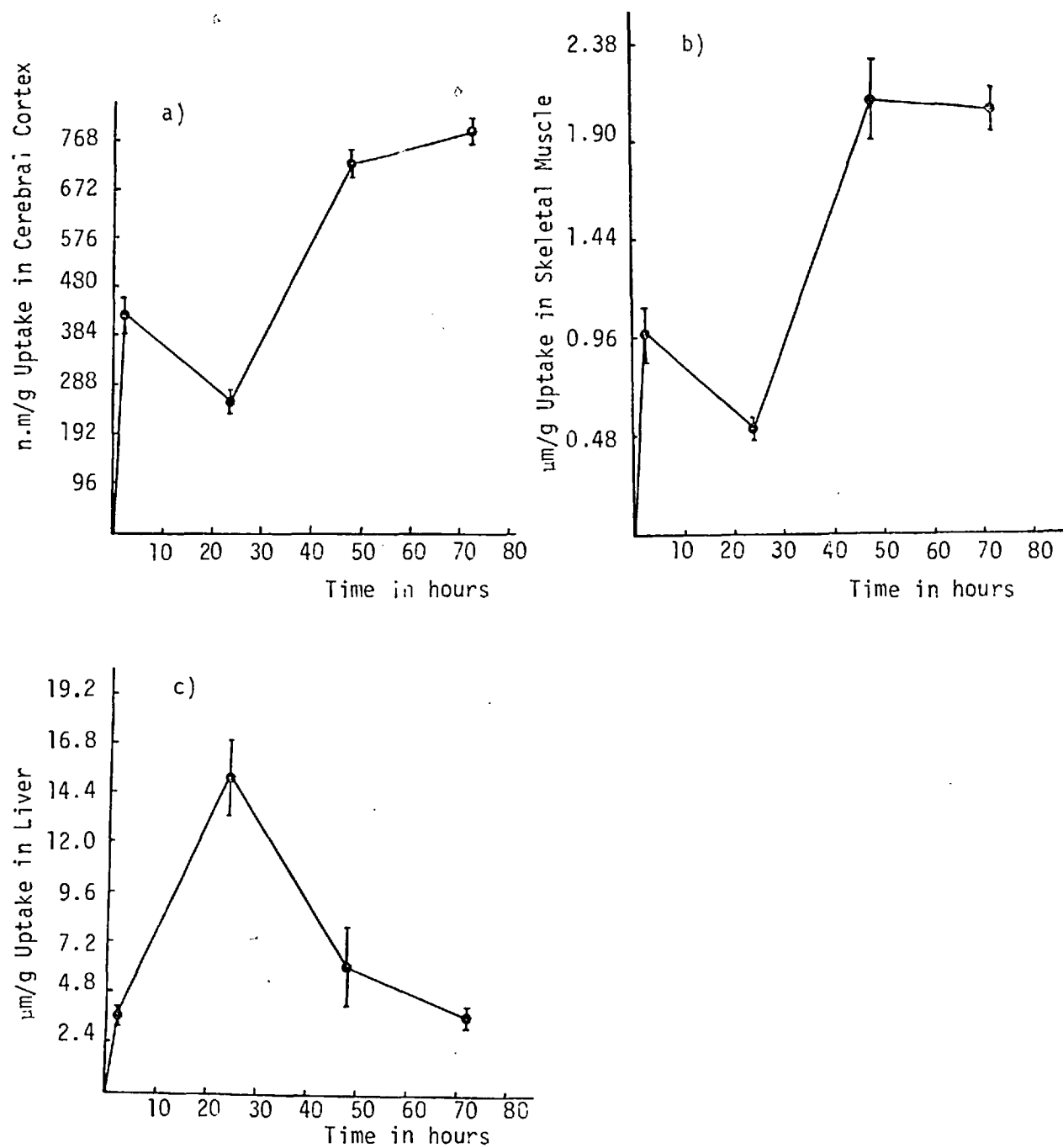


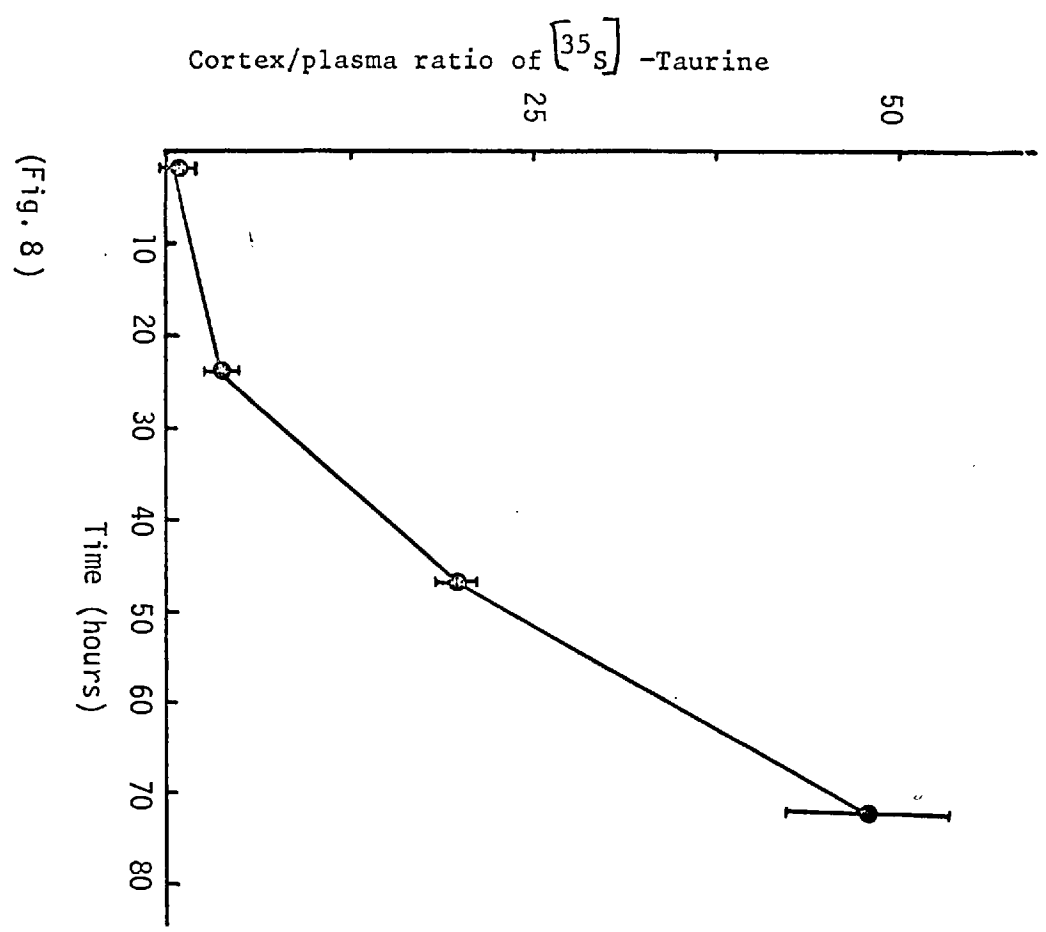
FIGURE 7

The increase in tissue concentration of $[^{35}\text{S}]$ - taurine (Fig.7) was obtained after subtracting the contribution of $[^{35}\text{S}]$ - taurine made by the plasma content of the tissue samples as estimated by the $[^3\text{H}]$ - inulin method described. The initial sharp rise in $[^{35}\text{S}]$ - taurine content of the tissues at 2 hr was reduced but not completely abolished when allowance was made for plasma content of the tissues.

Levels of non-isotopic taurine in brain and plasma was measured with an amino acid analyser before and after taurine administration and were compared with the extent of uptake of isotope. No difference in taurine levels could be detected, though the rather large variation in tissue content from sample to sample (15 - 20%) may have masked any small change which occurred. The minimal uptake of $[^{35}\text{S}]$ - taurine to the brain calculated from the radioactivity data (Fig.7), represents about 10% of the brain taurine content. The radioactivity in the extracts coincided with the single peak of taurine found with the amino acid analyser and was not detected at other points in the analysis.

The relative uptake of $[^{35}\text{S}]$ - taurine to the brain cortex and plasma is shown in (Fig.8). It may be seen that there was a progressive rise in the $[^{35}\text{S}]$ - taurine in the cortex relative to the plasma which continued when the plasma content of $[^{35}\text{S}]$ - taurine was falling and had reached low levels, up to 72 hours after dosing.

Fig.8 This shows the relative content of $[^{35}\text{S}]$ - taurine following oral dosing in cerebral cortex compared with blood plasma following oral administration. Vertical bars indicate SEM's.



5.2 (ii) Anticonvulsant action of taurine on Cobalt-induced Epilepsy

The graph limb^{of} jerk frequency plotted against time (Fig. 9) shows that in the control group of rats there was a gradual increase in limb jerks till day eight, after which there was a sharp increase in the number of limb jerks, a maximum being reached on day 10 after surgery. This was followed by a decrease in limb jerk frequency which was maintained on subsequent days. Scores collected and plotted independently by two observers showed close correlation in pattern.

The taurine treated rats did not show any significant difference from this pattern until after day eight when the large increase in limb jerks, which was a consistent feature with untreated rats was not observed. This was maintained during the full period of maximal epilepsy found in the control group on days 9 - 12 (see Figure 9). The significance was $p < 0.005$ for day 9 and $p < 0.005$ for day 10, as calculated using the student's t test.

The treated rats were observed to be behaviourally different from the control epileptic animals, being much less active, and although the limb jerks were decreased in frequency they were not abolished. Those jerks observed were markedly decreased in amplitude as compared with the limb jerks observed in the control epileptic group. No hepatotoxic effect was found with taurine administered at the above doses.

Fig. 9 This shows limb jerk score of control epileptic rats and taurine treated epileptic rats, administered 600 mg/kg of taurine daily. Numbers on the graph indicate the number of animals observed for that day. Vertical bars indicate S.E.M's.

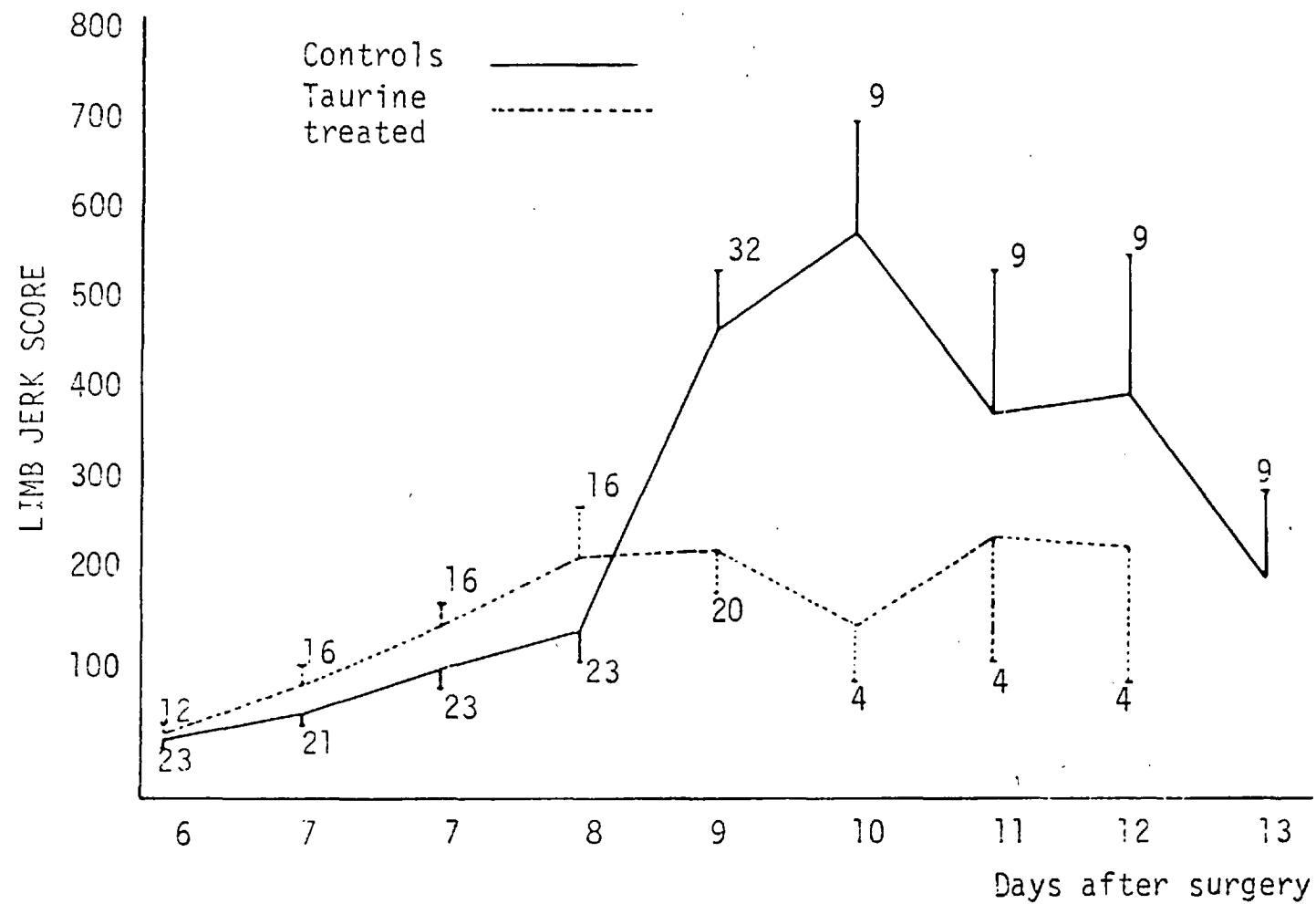


FIGURE 9

5.3 Discussion

5.3 (i) The results obtained show that even when large doses of taurine are given orally (600 mg/Kg) only a small proportion of the taurine entered the cerebral cortex. Similar results have recently been reported by other workers. Thus, Joseph & Emson (1976) found that prolonged oral dosing with 4 - 8 mg/day resulted in a 4% increase in brain taurine levels after 33 days. However, they reported that much larger repeated doses gave a 30% rise of brain taurine after 24 days.

Urquhart and co-workers (1974) reported that there was fast entry of $[^{35}\text{S}]$ - taurine to monkey whole brain after intramuscular injection. However, if this is calculated as a percentage uptake of the initial dose then the amount entering the brain is of the same low order as that reported here, i.e. 0.024% assuming 40% efficiency of counting. The low dose employed and high specific activity may have limited the availability of taurine for uptake to the brain. This would not apply to results reported in this study since the taurine dose used was relatively high. On the same of concentration these workers found ten times as much $[^{35}\text{S}]$ - taurine enters the whole brain as cerebral cortex. This suggests that there is a regional variation in the permeability of the blood brain barrier for taurine, which is in accord with other studies of the blood brain barrier (Rapoport 1976).

An interesting observation in studies reported in this thesis was that after a single large oral dose of $[^{35}\text{S}]$ - taurine the cerebral cortex content of $[^{35}\text{S}]$ - taurine rose for several days, after initially varying with plasma $[^{35}\text{S}]$ - taurine levels (Figures 6 and 7). A similar result has been reported by Sturman (1973) after intraperitoneal administration of taurine. He reported a progressive rise in whole brain taurine which was maximal after 1 week. The rise in $[^{35}\text{S}]$ - taurine content of skeletal muscle after 2-3 days reported in this thesis was also found after intraperitoneal injection of $[^{35}\text{S}]$ - taurine in rats (Awapara 1957).

An additional finding (Wheeler et al 1977) was that after a single large dose of non - isotopic taurine (600 mg/Kg), raised taurine levels in cerebral cortex could not be detected using an amino acid analyser. Moreover the taurine was not metabolized, as shown by the fact that the single peak of taurine in the amino acid analysis coincided with the distribution of administered $[^{35}\text{S}]$ - taurine. This suggested that dosing with taurine may increase the turnover or exchange of taurine in the cerebral cortex without effectively raising its concentration. Indeed Sturman (1973) has reported that the taurine content of most tissues is not increased by feeding a taurine supplemented diet for several weeks, although taurine turnover is increased. The exponential fall in brain taurine levels be observed after the levels had initially increased, suggested that there was more than one pool of taurine in the brain.

Once $[^{35}\text{S}]$ - taurine had entered the cerebral cortex it was retained despite rapidly falling plasma levels of $[^{35}\text{S}]$ - taurine which reach low levels (Figs. 6 & 7). This can be more clearly seen from the cortex/plasma ratio of $[^{35}\text{S}]$ - taurine (Fig.8) which rises progressively over a 3-day period after a single dose of $[^{35}\text{S}]$ - taurine. This supports the evidence for an active uptake system of cerebral cells for taurine, or firm retention of taurine in the brain.

Thus, it can be seen that there is a large blood brain barrier for entry of taurine to the cerebral cortex, but that administered taurine that does enter the brain is retained there for several days. This suggested that in testing the effect of taurine on experimental epilepsy larger repeated doses are important if the administered taurine is to enter the cerebral cortex.

Oral administration of taurine to rats with cobalt-induced epilepsy was studied throughout the course of the epileptic cycle. Large daily doses of taurine (600 mg/Kg) were employed as described earlier. The results (Fig.9) show that the overt manifestations of the cobalt induced epilepsy as measured by the limb jerk score were indeed improved.

The slow onset of the improvement of the epilepsy is in accord with the relative difficulty of raising the brain concentration of taurine. It is possible that more taurine was able to pass through the blood brain barrier in the region of the epileptic focus, as other studies have shown that the cobalt focus may be reactivated by systemic administration of excitatory agents, such as glutamate and pentylenetetrazole (Bradford & Dodd 1977).

Also entry of tritiated sodium folate from blood to cobalt epileptic foci has been reported to be much greater than to surrounding cortex (Obbens 1973). In addition severe convulsions can increase the permeability of the blood brain barrier to amino acids both during and after convulsions (Battistin et al 1975). It is not yet known if this is due to a breach of vascular permeability or to increased uptake activity of epileptic brain tissue.

Oral administration of large doses of taurine were found to be effective against severe epileptic limb jerks, since the maximum effects was seen during the period of maximum epileptic activity (Fig.9) when the limb jerks were decreased in amplitude and frequency although not completely abolished. The observation that the taurine treated rats moved around the cage less than untreated rats also suggested a general depressant action of taurine on the brain.

5.3 (ii) The Mode of action of taurine in Epilepsy

There is now a large amount of evidence that administered taurine can correct electrical and biochemical abnormalities in the cobalt model of epilepsy, as well as in other epileptic models.

Taurine administration has been reported to improve the EEG in cobalt epilepsy in cats and rats (Adembri et al 1974), in epilepsy due to aluminium cream (Mutani et al 1974), due to strychnine (Savoldi & Tartara 1976), and in epilepsy induced by physical means, in photic epilepsy in baboons (Wada et al 1975), and in epilepsy following osmotic stress and electrical stimulation (Thursby & Nevis 1974). Focal ictal spikes were decreased in frequency and amplitude and subsequently abolished. They were replaced by high amplitude slow waves (500 - 700 u V in baboons). Homotaurine which is closely related to taurine in structure was more effective than taurine in improving the EEG in epilepsy due to several agents, cobalt, physostigmine or strychnine (Adembri et al 1974).

It is necessary to assess the few negative reports for the action of taurine on the EEG. Thus Wada and co-workers (1975) reported that taurine did not improve the EEG in amygdaloid convulsions, although it was beneficial in photically induced epilepsy. Recently, Joseph and Emson (1976) have reported that taurine administration has no effect on focal spikes in the cobalt model in rats, despite large amounts of taurine in their drinking water (2% concentration) and despite the fact they reported that prolonged oral dosing in this way raised brain levels of taurine by up to 30%. However, it should be noted that this report conflicts with several different observations of other workers. These authors reported that intraventricular administration of taurine also had no effect on EEG, which compares with the marked improvement in the epileptic manifestations in audiogenic seizures when taurine was administered by this route

(Laird & Huxtable 1976). It also does not correspond with the marked depressant effect of taurine on central neurons when administered by iontophoresis (Curtis & Watkins 1960, 1965; Krnjevic 1976). The reason why Joseph and Emson did not find taurine affected the EEG when a number of other workers did, is not clear. Possibly it relates to their epileptic model where sticks of gelatine containing cobalt are pushed deep into the cortex causing considerable damage.

The mode of action of taurine on electrical activity at the focus is still unknown. Nevertheless the possible role of taurine as a neurotransmitter in the cortex suggests it could be released at the focus to cause inhibitory post - synaptic potentials (IPSP), which would act to limit the frequency of discharges at that point. The available evidence is consistent with this hypothesis. Taurine has a strong depressant action on central neurons and it is released from nerve endings as reported in this thesis. Raised taurine levels have been reported in the epileptic focus during convulsions (Koyama 1972), and taurine has been reported to be released into the CSF during convulsions (Kaczmarek 1976). Recently IPSP have been reported for epileptic kittens from a penicillin focus (Prince & Gutnick 1972).

Taurine may also act to decrease the spread of excitability from the epileptic focus. Fariello and co-workers (1975) studied the effect of topical application of taurine, on the spread of epileptic activity to the epileptic focus in the contralateral cerebral cortex (the mirror focus), in cats made epileptic by local application of conjugated oestrogens to the sensory motor cortex. Since the acute mirror focus was purely a propagated response without autonomous spiking, an improvement there was due to an action on conduction of electrical activity from the focus.

These workers found that taurine applied locally to the secondary focus caused a decrease in amplitude and frequency of spiking, and subsequently abolished it. Ictal events in the mirror focus were delayed and reduced in voltage. As yet there have not been other studies to support the findings of these workers. However the findings reported in this thesis that taurine administration decreased but did not completely abolish the peripheral manifestations of cobalt induced epilepsy is consistent with the above report.

Taurine administration has also been reported to improve the metabolic defects associated with cobalt induced epilepsy. Van Gelder reported that taurine administration restored glutamate levels in the cerebral cortex both in experimental animals and in humans (Van Gelder 1972; Van Gelder et al 1975). He reported that orally administered taurine decreased the raised glutamate levels found in the serum of patients with epilepsy. However Van Gelder (1974) was unable to find an improvement in the activity of enzymes associated with glutamate metabolism, including G.D.H., G.A.D. and GABA - T, which appear associated with the epilepsy in the cobalt model.

Hence taurine could be acting to correct less specific lesions. For example it could be acting as a membrane stabilizer, as suggested by Huxtable and Bressler (1973). Evidence for a possible role of taurine in this way is at yet preliminary, and is limited to the report above that taurine decreased the loss of Ca^{2+} from sarcoplasmic reticulum in response to phospholipase C. If taurine acts as a membrane stabilizer in the brain it could decrease the loss of glutamate into the CSF, thus effectively increasing the tissue levels of glutamate, and decreasing the excitatory effect of extracellular glutamate on central neurons.

The slow onset of improvement of epilepsy in the studies reported in this thesis, and by other workers in some studies (Bergamini & Mutani 1973; Bergamini et al 1974, Van Gelder 1972) suggested that taurine could exert its anti-epileptic effect through its metabolite, isethionic acid. However this is probably not the case as isethionic acid is ineffective when applied topically to the acute mirror focus in cats (Fariello et al 1975) and it is without effect when applied to central neurons by iontophoresis (Krnjević 1965). Moreover it is formed from taurine very slowly in the brain (Peck & Awapara 1967). Thus it is more likely that the slower onset of improvement when taurine is given other than directly to the brain, is due to its slow passage across the blood brain barrier. This is consistent with the prompt improvement in EEG after local cerebral application of taurine, when a marked beneficial effect was seen as early as 10 minutes after application (Fariello et al 1975) whereas other routes could take hours or days to improve the epilepsy.

Emson and Joseph (1976) suggested that taurine could have a beneficial effect on experimental epilepsy by a peripheral action on muscle tissue which contains 70% of the taurine in the body (Jacobsen & Smith 1968). Certainly orally administered taurine is readily taken up by skeletal muscle as reported in this thesis, as well as if administered by other routes (Awapara 1957, Sturman 1973). However, the fact that taurine has a strong beneficial effect on epilepsy, when applied directly to the brain, make it unlikely that a peripheral action is very important.

A further way taurine could exert its antiepileptic effect could be by influencing ion levels or ion fluxes in the brain by a mode of action similar to its action in the heart. In the latter tissue administered taurine has a beneficial effect on cardiac arrhythmias, and it promotes K^+ influx and decreases K^+ efflux (Welty & Read 1964). In the brain a similar action by taurine could decrease extracellular levels

of K^+ (Neame 1968) which might be raised after excessive firing by neurones or if there was membrane damage. Although K^+ levels may not be altered in epileptic tissue (Tower 1965), the report of raised K^+ in the CSF of monkeys undergoing convulsions (Meyer et al 1970), suggests an epileptic mechanism involving this cation may be important.

It has been suggested that glial cells may control the extracellular concentration of K^+ , preventing an extracellular build up of this ion (Henn et al 1972; Kuffler & Nicholls 1966). As taurine occurs in glial cells (Kaczmarek 1971), and there is evidence of a high-affinity uptake of taurine to glial cells (Schrier & Thompson 1974; Riddell et al 1976), taurine may have a role in glia. Since taurine is slowly metabolized in the brain (Peck & Awapara 1967), a role of taurine in glia might therefore involve aspects such as taurine fluxes or ion fluxes. As yet this possibility has not been investigated, but the role of taurine with potassium elsewhere, and the fact that slow waves which occur during convulsive activity may be from glia (Karahashi et al 1966; Castellucci & Goldring 1970), suggest taurine could modify epilepsy by an effect on K^+ fluxes in glia.

Barbeau and Donaldson (1974) suggested that taurine could act on Na^+ , K^+ - ATPase in the brain, as intraventricular administration of taurine was beneficial on epilepsy induced by ouabain, which inhibits this enzyme. They suggested taurine could chelate zinc which inhibits this enzyme and leads to convulsions. However neither these workers nor others (Akera et al 1976) found that taurine affected the activity of Na^+ , K^+ -ATPase. Hence a beneficial effect of taurine in this model is likely to be non-specific or on an event subsequent to inhibition of this enzyme.

Little work has been done on the effect of taurine on calcium levels or calcium fluxes in epileptic tissue. Raised calcium levels have been reported at the cobalt epileptic focus (Hunt and Craig 1973), but as similar changes were not found in the mirror focus, this could be part of a local inflammatory reaction to tissue damage. Recently taurine perfusion of the cerebral cortex of cats has been reported to improve the EEG changes following perfusion with medium of low calcium concentration (Kaczmarek 1976). It is not yet known if this is a specific interaction with calcium in the brain.

Taurine has now been reported to be effective against a variety of chemical and physical agents which cause epilepsy. Chemically induced convulsions where taurine is effective include cobalt (Van Gelder 1972; Adembri et al 1974), low calcium concentration (Kaczmarek 1976; Kaczmarek & Adey 1975), penicillin and strychnine (Mutani et al 1974; Savoldi & Tartara 1976); ouabain (Barbeau & Donaldson 1974); conjugated oestrogens (Fariello et al 1975), and methionic sulfoximine (Iles & Hamilton 1976). Physical models of epilepsy improved by taurine include photic epilepsy in baboons (*papio papio*) (Deroux et al 1973), epilepsy due to osmotic stress and electrical stimulation (Thursby & Nevis 1974), and in audiogenic seizures in rats (Laird & Huxtable 1976). The fact that taurine has been found to be effective against a variety of chemical and physical agents causing convulsions suggests that taurine may be acting to correct a common defect in the mechanisms producing convulsions.

CHAPTER 6 THE POSSIBLE ROLE OF TAURINE IN THE BRAIN
A GENERAL DISCUSSION

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6.1 THE ROLE OF TAURINE AS A NEUROTRANSMITTER

The evidence presented in this thesis as well as recent reports in the literature strengthen the case for taurine being a neurotransmitter in the brain. Taurine now fulfils most of the criteria for a compound being a neurotransmitter as discussed by Werman (1966) and Hebb (1970).

Taurine is present in high concentration in the brain, for example 8 μ mol/g in the cerebral cortex. It occurs in neurons (Kaczmarek 1971) at twice the concentration found in glia. Taurine also occurs in nerve endings although it is not specifically located there with respect to the rest of the cell (Agrawal et al 1971; Macaione et al 1976) with 80% of the taurine occurring in the soluble cytoplasmic compartment. In this respect taurine is similar to other amino acids including GABA which also is not exclusively located in the nerve ending fraction in the brain (Mangan & Whittaker 1966). A specific localization in nerve endings is probably not an important criterion for the role of amino acids as neurotransmitters. Free amino acids take part in many metabolic functions in the brain, so it would ^{not} be reasonable to expect a specific synaptic localization, even though selected small pools of certain amino acids might be involved in synaptic transmission.

Taurine has not yet been found to be specifically located in nerves with a defined inhibitory function in the nervous system. In this respect it differs from GABA and acetylcholine. GABA has been identified at higher concentrations in inhibitory nerves than in excitatory nerves in crustacea (Kravitz et al 1963), and in mammalian cerebellum it is concentrated in or around the purkinje cell layer (Kuriyama et al 1966). These cells have known inhibitory outputs to neurons in defined areas of the brain, where GABA is the probable transmitter, such as in the lateral vestibular nucleus close to the IVth ventricle (Dieter's nucleus). A functional distribution has also been found for acetylcholine with much higher concentration found in motor than in sensory nerves, where there is only a trace (Hebb 1963). The fact that a specific distribution of taurine has not been found in defined inhibitory nerves, may well be due to the lack of data on the taurine

content of such nerves, or to large pools in the cytoplasm obscuring specific differences in the taurine content of the nerve terminals involved. Recent evidence that goes some way towards showing a distribution of taurine in specific nerves, is the report that isotopic taurine is taken up into a specific cerebral cortex synaptosomal fraction which can be separated by centrifugation using a linear sucrose density gradient. (Sieghart & Karobath 1974). Uptake was both Na^+ and energy dependent. Taurine was found to be taken up to synaptosomes which sedimented more slowly than those containing noradrenaline, and close to those containing GABA. This was not due to non-specific adsorption during the preparation of the synaptosomes as isotopic taurine added immediately prior to homogenization did not contribute any radioactivity to the particulate fraction being studied.

Also taurine is enriched compared with other amino acids in the synaptic vesicle fraction prepared from cerebral cortex. De Belleruche and Bradford (1973) showed taurine had the highest content in synaptic vesicles of the free amino acids. Taurine was present at 34 n mol/g, glutamate being next in amount at 16 n mol/g, while other amino acids were close to or less than GABA at 3 n mol/g. The concentration for taurine was 100 times greater than that of acetylcholine levels in purified vesicles of 0.3 n mol/g (Richter & Marchbanks 1971). Taurine was partly bound to the protein of the synaptic vesicle membrane, estimated as 1% of the taurine content of synaptic vesicles for whole brain (Rassin et al 1977). Taurine was probably not a constituent of the protein of synaptic vesicles however, as it was not found in the acid hydrolysate of this fraction.

The criteria for synthesis of taurine in neurons^{are} largely satisfied.
/as described in the introduction,

the sulphur amino acids methionine and cysteine occur in the brain, and taurine is enriched in purified neurons (Kaczmarek 1971). The production of taurine from these precursors has been observed in the brain, and the enzyme responsible for the decarboxylation leading to taurine, cysteinsulphinic acid decarboxylase, has 50% of its activity in the nerve endings (Agrawal et al 1971; Macaione et al 1976). This distribution resembles that for glutamate decarboxylase of which 80% of its activity is found in the nerve ending fraction of cerebral cortex (Iversen and Neal 1969).

Taurine release also meets the criteria proposed by Werman and Hebb for a compound being a neurotransmitter. It is released from different levels of tissue preparation predominantly from a synaptosomal fraction by a Ca^{2+} dependent process as shown in this thesis. Taurine release has been observed from the perfused cerebral cortex after stimulation of the reticular formation (Jasper & Koyama 1969). Taurine is released from electrically stimulated brain-slices (Kaczmarek & Davison 1971, Bradford et al 1976). This release is decreased when calcium in the medium is lowered and magnesium is raised. The process of release is calcium dependent, although calcium also contributes to taurine uptake (Wheler et al 1977), which is similar to noradrenaline and GABA (White 1975, Sellstrom & Hamberger 1975). Stimulated release is current dependent. In addition taurine has been shown to be released from electrically stimulated synaptosomes (Lahdesmaki et al 1975).

The criterion for identical action of taurine and a naturally occurring transmitter has been partly satisfied. As discussed in the introduction, taurine can cause

hyperpolarization of neurons, and associated increased Cl^- permeability, which is similar to the action of natural inhibitory transmitters in the brain. The criterion of pharmacological identity however as yet remains unsatisfied. For this to be shown, specific pharmacological antagonists that block the action of a natural transmitter at defined inhibitory synapses will need to be developed. Such an antagonist would then need to be shown to antagonize the action of taurine and the natural transmitter in the same way, but not that of other possible transmitter compounds.

Finally there is now evidence of a means of inactivating the released taurine. Evidence has been presented for the existence of high or ^{intermediate} / sensitivity uptake systems for taurine in the brain. These systems are able to take up taurine to nerve endings, and glial tissue. Thus taurine could be removed to the nerve endings from which it was released, to glial cells, or possibly to post-synaptic cells it was acting on. Alternatively passive diffusion might lead to removal of taurine from the site of its release. Probably all these mechanisms are operating to some extent. It is unlikely that enzymic inactivation of released taurine is a significant method of removal of taurine following its release as taurine is very slowly metabolized in the brain (Peck & Awapara 1967).

From the above it is concluded that the balance of evidence is now strong for taurine being a neurotransmitter in the cerebral cortex. Further work however remains to be done to locate taurine in specific inhibitory nerves, and to develop specific pharmacological antagonists to the post-synaptic action of taurine in the brain. In areas of the brain other than cerebral cortex taurine may also be acting as a neurotransmitter. Areas which are worth investigating would be those where microiontophoresis has shown a strong

inhibitory action of taurine, these include the cerebellum, the thalamus, and midbrain, and peripherially the spinal cord.

There is also some evidence that taurine may be a neurotransmitter in the retina. Taurine occurs there is high concentration relative to other free amino acids, at 10 μ mol/g (Pasantes-Morales et al 1972). Uptake experiments with isotopic taurine have shown that it is taken up predominantly to the photoreceptor layer (Kennedy & Voaden 1974) but also to inter neurons and the synaptic cell layers, the outer and inner plexiform layers (Orr et al 1976). Uptake also occurs to glial cells in the inner layer of the retina (Ehinger 1973). Taurine also affects the electrical activity of the retina. Iontophoresis of taurine causes depression in the b-wave of the electroretinogram (Bonaventure et al 1974). Raised levels of taurine are found in the retina after periods of darkness (Pasantes-Morales 1973). Light causes release of taurine from the retina (Pasantes-Morales et al 1973) as does electrical stimulation (Pasantes-Morales et al 1974). The stimulated release may be calcium dependent as the specific calcium ionsophore A 23187 caused release of taurine from the retina which increased in a linear fashion with increasing Ca^{2+} influx (Pasantes-Morales et al 1976). This preliminary evidence suggests a transmitter role for taurine in the retina, although additional work should be done to further study the calcium dependence of taurine release and uptake, and the precise pools from which taurine is released.

Taurine may also have a role in photoreceptors in the retina, as it is taken up to this layer and recent evidence has shown that decreasing the availability of taurine in the diet may lead to degeneration of the photo-

receptor cells (Hayes et al 1975).

The reason for this is not yet known. In addition there is an apparent discrepancy between the fact that taurine is released by light, but inhibits the b - wave of the electroretinogram which is elicited by light. A possible resolution of this paradox would be if taurine is released from more than one synaptic site in the retina.

6.2 Taurine and higher functions in the brain

The large amounts of taurine in the adult brain, its probable role as a neurotransmitter in the cerebral cortex, and its higher levels in developing brain suggest its involvement in higher mental functions and the possibility that taurine abnormalities could be associated with mental retardation. In addition abnormalities of the metabolism of taurine precursors, may be shown to be associated with mental retardation. Such abnormalities of taurine precursors include hypermethioninaemia and homocystauria, cystathionuria, cystinosis/cystinuria (Jacobsen & Smith 1968). These conditions are associated with enzymic defects and an increase in the level of the immediate substrate of the enzyme. Surprisingly normal taurine levels have been reported in these cases, suggesting another source for taurine, for example from the diet.

King and co-workers (1966) reported that taurine excretion was decreased in the urine of patients with mongolism (trisomy of chromosome 21). This was unrelated to plasma levels, and was associated with increased excretion of taurine metabolites of which one was isethionic acid (Wainer et al 1966). Supplementation of the diet with pyridoxine however increased taurine excretion towards normal levels. This suggested the biochemical lesion involved pyridoxine rather than taurine as such. Also the lesion was probably localized to the kidney as plasma levels did not correspond with urinary levels.

Decreased taurine excretion has also been observed in three phenylketonuric children (Evered 1956) and in six children with unspecified mental retardation (Jacobsen & Smith 1968).

Recently Perry and co-workers (1975) have reported an hereditary mental depression associated with Parkinsonism and decreased taurine levels in the plasma, cerebrospinal fluid and brain. Symptoms commenced late in the fifth decade and led to death in 5 years. The earliest symptom was mental depression which was not responsive to tricyclic , anti-depressant drugs or electroconvulsive treatment. Parkinsonian symptoms appeared later in the disease, and were typical, with rigidity and tremor. The disease was followed through three successive generations when it was inherited as an autosomal dominant condition. The relation of taurine to the causation of this depression remains to be elucidated. It would be worth testing if taurine administration to these patients improved their clinical condition. Also of interest would be the effects of different agents affecting depression on taurine fluxes in cerebral cortex tissue. So far the interaction of drugs affecting mental function have been little tested in relation to taurine levels in the brain. Reserpine has been reported not to affect taurine levels in the brain (Mussini & Marcucci 1962). Chlorpromazine has been reported to cause an increase in brain taurine in One study (Okumura et al 1959), but Lahdesmaki and co-workers (1975) did not find it affected taurine uptake to synaptosomes. Schmid and co-workers (1975) however reported that chlorpromazine inhibited uptake of taurine to crude synaptosome preparations. Additional studies on the affect of chlorpromazine and other centrally acting drugs on taurine levels and uptake are needed to establish an association of taurine with different functional systems in the brain.

6.3 Other possible roles of Taurine in the brain

Although the evidence for taurine being a neurotransmitter in the brain is now strong, it is important not to overlook the possibility that taurine may have other roles there. As yet no role has been shown for the taurine that occurs in the cell cytoplasm although 70% of the taurine in the cell is located there (Agrawal et al 1971). Other amino acids which have been implicated as neurotransmitters, such as GABA, glycine, glutamate also are not specifically located in nerve endings, and are known to be involved in general metabolic processes apart from any neurotransmitter function they may have in the brain. Thus it is reasonable to assume a similar situation may occur for taurine. However, taurine differs from these amino acids in that it is only slowly metabolized to isethionic acid in the brain (Peck & Awapara 1967). Hence taurine could be involved in other types of function than its further metabolism such as fluxes across membranes.

Recent reports have suggested that taurine may be involved in fluxes of calcium and potassium in the brain. Welty and co-workers (1976) reported that taurine administration decreased the efflux of calcium from the brain in response to intraperitoneal injection of glucose. This effect compared with the heart where taurine decreased the efflux of calcium and potassium. Taurine has been shown to slow the rate of Ca^{2+} loss after treatment with phospholipase C which increases membrane permeability (Huxtable & Bressler 1973, 1974). It has also been shown to influence Ca^{2+} fluxes in mitochondria, though this has only been studied in liver. Taurine stimulated the ATP dependent calcium uptake by mitochondria. It was without effect on oxidative phosphorylation, but it inhibited oxygen consumption when glutamate was used as a substrate (Dolora et al 1973). This report should be treated with caution however, as the observation occurred only at the high concentration of

calcium of 10 mM, and so might not be relevant to calcium fluxes occurring during normal metabolic processes in the brain. Taurine and calcium are also linked in taurine uptake in the cerebral cortex as reported in this thesis. Since the presence of both of these substances in the extracellular fluid affects cerebral excitability, both may be linked together in the control of excitability in the brain.

The association of taurine and potassium in the brain is at present more tenuous than for taurine and calcium. Most of the evidence for the association of taurine and potassium fluxes comes from studies involving the heart. In this tissue taurine increased potassium influx and decreases K^+ efflux, such as may occur in the presence of toxic amounts of digitalis, which cause K^+ efflux associated with cardiac arrhythmias (Welty & Read 1964; Read & Welty 1965). In the brain however, as mentioned above, these workers could not find an effect of taurine to decrease K^+ efflux. Neame (1964) reported a small increase in the K^+ content of brain slices incubated with 2 mM taurine. However the difference was too small to be significant, and so cannot be taken as indicating a definite association of taurine and potassium fluxes in the brain. Further work is needed to show if this is a real effect or not, certainly the known effect of taurine on potassium fluxes elsewhere makes it worth additional investigation.

A connection between taurine and zinc in the brain has also been proposed (Barbeau & Donaldson 1974). As discussed earlier, taurine excretion in the urine is increased during zinc deficiency in the diet, and taurine is effective against convulsions caused by zinc. It is not

yet known if this is a specific effect, or a non-specific effect for example an association with membrane stabilization. So far taurine has not been shown to affect Na^+ , K^+ -ATPase in the brain (Barbeau & Donaldson 1974, Aker et al 1976). So an action of taurine is more likely to be associated with ion fluxes themselves, or binding with ions in the cell, rather than the maintenance of the membrane potential.

Taurine may well have a role in glial tissue. It occurs in glial cells, although at half the concentration that it is found in neurons (Kaczmarek 1971). There is a high-affinity uptake process of taurine to glial cells in tissue culture (Schrier and Thompson 1974), and in incubated C - 6 glioma tissue or in slices of glial tissue as reported in this thesis. For these reasons it is probable that taurine does have a role in glia.

The nature of a role of taurine in glia is not yet clear. One such probable role is that it could serve to remove synaptically released taurine. The uptake reported above is consistent with this proposal. Certainly glial uptake has been reported for other amino acid transmitters at neurons where they are the probable transmitter in some species. For example uptake studies have shown the passage to glia of GABA in lobster nerve-muscle preparation, and of glutamate at the arthropod neuromuscular junction (Orkand & Kravitz 1971; Faeder & Salpeter 1970). A second way by which glial cells could be involved with taurine in the brain is by association of taurine and K^+ . Glial cells are known to be associated with slow potential shifts in the brain (Karahashi & Goldring 1966), and K^+ may be associated with this (Somjen 1975). If taurine has an analagous role for potassium in brain as in the heart

taurine could be associated with the control of K^+ efflux in neurons and, or, glial cells, and with removal of K^+ from the extra cellular space. A third way that taurine could have a role in glial cells is by being released from them in response to changes in their membrane potential as discussed above. Evidence which suggests this possibility is the reported release of GABA by stimulating superior cervical ganglia electrically or with K^+ (Bowery & Brown 1971, 1972). Taurine released from glia could have a more general effect on cerebral excitability than the purely localized effect of release from synaptic processes. It is unlikely that glial cells have an important metabolic role associated with taurine, as taurine is present only in half the concentration in glia as is found in neurons, and its synthesizing enzyme in glial has only one eighth of the activity that occurs in neurons (Kaczmarek 1971). Thus the present evidence suggests glial cells could have a role associated with taurine fluxes and possibly associated with fluxes of potassium.

Taurine could have a role in osmoregulation in the brain, by analogy with its osmoregulatory role in other species. In the muscle, Mytilus edulis, taurine increased in concentration intracellularly with increasing salinity in the external medium (Lange 1963). The increase in taurine concentration was greater than that for other amino acids. In the flounder, Pleurentes flesus L., taurine was released from cells when the osmolarity of the medium was decreased (Lange & Fugelli 1965). Thus taurine may have a role in osmoregulation in aquatic species, although it does not vary with salinity of the extra cellular medium in all aquatic species, for example the eel, Anquilla anquilla L., (Huggins & Colley 1971). Taurine could act in osmoregulation to

maintain normal cell size, and to spare essential amino acids for other roles in the cell. Intracellular taurine levels decrease with decreasing osmolarity in the medium, but are slow to rise again when the osmolarity is raised at least in one species, Agonus Cataphractus, (Colley et al 1974). This suggests that taurine could be associated with longer term regulation of osmolarity.

So far a role of taurine as an osmoregulator has been little studied in the mammalian brain, but its role as an osmoregulator in other species suggests that it should be investigated in cortical swelling and in cerebral oedema. Recently intraperitoneal administration of taurine has been shown to decrease conditional drinking in mice (Hruska et al 1975). Since the hypothalamus regulates this activity, it is possible that taurine has a role in this tissue, possibly associated with some of the osmoregulatory phenomena described above.

Taurine could be associated with nerve conduction in the brain. At present there is little evidence for this apart from the report discussed earlier, that taurine decreased the spread of epileptic activity from the primary epileptic focus in cats to the acute secondary epileptic focus. The latter is a conduction effect as autonomous spikes have not developed in the secondary focus in the cat in acute epilepsy (Fariello et al 1975). The beneficial effect of taurine on experimental epilepsy reported in this thesis supports a role of taurine in the control of excitability in the brain, though apart from the evidence above it is not yet clear whether this is primarily associated with its role in neurons or glia.

Taurine peptides have also been reported to be found in the cerebral cortex (Reichelt & Kvamme 1973), for example N-acetyl-aspartate-taurine-taurine in mice. These peptides form in the brain in the presence of monoamines such as histamine and noradrenaline. Since N-acetyl-aspartate exists in

high concentration in the cerebral cortex, $7 \mu\text{m/g}$, (Curatalo et al 1965), and since N - acetyl - aspartate peptides are partly distributed in synaptosomes (Reichelt & Fonnum 1969), it may be that taurine containing peptides have a transmitter role in this tissue. Elucidation of this will need further experiments, to study in more detail the localization of these peptides, the factors affecting their release, and their effect on cerebral excitability.

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