AMINO ACID COMPOSITION OF BODY FLUIDS AND NERVOUS TISSUE OF NORMAL CALVES AND THOSE AFFECTED WITH NEURAXIAL OEDEMA.

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This thesis contains no material which has been accepted for the award of any other degree or diploma, and to the best of the authors knowledge, contains no published or written material from another person, except where due reference has been made in the text. Parts of this thesis have been published, and copies of the papers are included.

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SUMMARY.

High performance liquid chromatography was used in amino acid analyses of body fluids and nervous tissues from newborn Poll Hereford calves. After separation on a lithium cation exchange column, primary amino acids were derivatised with ortho-phthalaldehyde and detected fluorimetrically. Normal values are presented for plasma, cerebrospinal fluid, dorsal spinal cord, ventral spinal cord, medulla oblongata, pons, white matter of the cerebellum, grey matter of the cerebellum, frontal cortex, temporal cortex, occipital cortex, amygdala, thalamus, putamen, caudate nucleus and substantia nigra. Normal ratios of branched chain amino acids to alanine are presented for urine, formalin-fixed cerebrum and fixative.

Amino acid analyses of tissues and body fluids from calves affected with neuraxial oedema has shown that this "disease" consists of two separate neurological conditions :- Inherited Congenital Myoclonus and Maple Syrup Urine Disease. Both conditions are inherited in an autosomal recessive manner.

Calves affected with Inherited Congenital Myoclonus are bright and alert, but unable to stand from birth. If fed, they are viable for an indefinite period. The clinical signs are stimulus-responsive and spontaneous myoclonic spasms. There are no lesions in the central nervous system, but most Inherited Congenital Myoclonus calves have traumatic hip joint lesions, the result of myoclonic spasms in utero. Concentrations of free amino acids, including known neurotransmitters, in tissues and fluids were not consistently different from normal calves. Normal levels of minerals and enzymes were also found in plasma and/or cerebrospinal fluid. The biochemical lesion in Inherited Congenital Myoclonus has been shown to be a glycine receptor site abnormality in the spinal cord, with no effect on the free glycine pool.

Calves affected with Maple Syrup Urine Disease are apparently normal at birth, but develop clinical signs of dullness and opisthotonus 1-3 days after birth. Death occurs within the first week. Vacuolation of the white matter of the central nervous system gives the oedematous appearance which originally initiated the term, "neuraxial oedema". Elevated levels of the three branched chain amino acids valine, isoleucine and leucine are evident in all samples analysed. The branched chain amino acids have also been shown

to be elevated at birth. Ratios of valine, isoleucine and leucine to alanine are elevated in urine and fixed nervous tissue. A deficiency in the activity of the enzyme, branched chain keto acid decarboxylase has been demonstrated in fibroblast cultures from these calves. As a result of this block, the corresponding branched chain keto acids, ketoisovaleric, ketomethylvaleric and ketoisocaproic, are also elevated. This is the first reported aminoacidopathy in ruminants and the only known model of Maple Syrup Urine Disease in man.

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LIST OF ABBREVIATIONS.

ALA	Alanine
AP	Alkaline phosphatase
ARG	Arginine
ASN	Asparagine
ASP	Aspartate
BCAA	Branched chain amino acid
BCKA	Branched chain keto acid
BCKAD	Branched chain keto acid decarboxylase
CIT	Citrulline
CNS	Central nervous system
CPK	Creatine kinase
CSF	Cerebrospinal fluid
CV	Coefficient of variation
DABSYL-Cl	4-dimethylaminoazobenzene 4-sulphonyl chloride
DABTH	Dimethylaminobenzenethiohydantoin
DANSYL-Cl	Dimethylaminonapthalene
DHBA	Dihydroxybenzylamine
DNPH	2,4 dinitrophenylhydrazine
DOPA	DL-beta-3,4-dihydroxyphenylalanine
DOPAMINE	3 hydroxytryptamine
EDTA	Ethylenediaminetetraacetic acid
FMOC-Cl	9-fluorenylmethylchloroformate
GABA	Gamma aminobutyric acid
GAD	Glutamic acid decarboxylase
GGT	Gamma glutamyl transferase
GLC	Gas-liquid chromatography
GLU	Glutamate
GLN	Glutamine
GLY	Glycine
GOT	Aspartate amino transferase
HIS	Histidine
HPLC	High pressure liquid chromatography
ICM	Inherited Congenital Myoclonus
ILE	Isoleucine

LIST OF ABBREVIATIONS.

KICA	Ketoisocaproic acid
KIVA	Ketoisovaleric acid
KMVA	Ketomethylvaleric acid
LEU	Leucine
LOG	Logarithm
LYS	Lysine
MET	Methionine
MOET	Multiple ovulation-embryo transfer
MSUD	Maple Syrup Urine Disease
NBD-Cl	7-chloro,4-nitrobenzene,2-oxa,1,3-diazole chloride
NLE	Norleucine
OCT	Ornithine carbamyl transferase
OPA	Orthophthalaldehyde
PCA	Perchloric acid
PEA	Phosphoethanolamine
PHE	Phenylalanine
PSE	Phosphoserine
PTH	Phenylthiohydantoin
SD	Standard deviation
SER	Serine
SSA	Sulphosalicylic acid
TAU	Taurine
THR	Threonine
TRY	Tryptophan
TYR	Tyrosine
VAL	Valine
WISP	Waters Intelligent Sample Processor

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Table 41: Distinguishing features of the ICM and MSUD genotypes.

INTRODUCTION

(a) Neuraxial Oedema : Literature Review.

Neuraxial Oedema, a lethal neurological disease of newborn Poll Hereford and Poll Hereford cross calves, was first described in the United States of America by Cordy et al (1969). The condition was characterised by severe extensor spasms upon tactile or auditory stimuli, resulting in an inability to stand. Three of the twelve cases examined by Cordy had severe vacuolation of the white matter of the cerebellum and brain stem, while peripheral nerves appeared to be unaffected. One calf with no obvious lesions in the central nervous system (CNS) was found to have fractures of both femoral heads. Widespread vacuolation suggested a generalised oedema of the CNS, and the disease was named Neuraxial Oedema. Examination of breeding records suggested the condition was inherited in an autosomal recessive manner.

The disease was reported in Victoria by Blood and Gay (1971), who described a similar clinical presentation but failed to find any significant lesions in the CNS. One of the nine cases examined showed degeneration of both femoral heads and acetabulae.

Weaver (1974) described cases in Britain, and a year later Davis et al (1975) reported cases in New Zealand from matings of Poll Hereford cross Friesian cows to a Poll Hereford cross Friesian bull. One of the two calves examined had fractures in both femoral heads, but neither had lesions in the CNS. Hartsough (1978) described a 19 day old Poll Hereford calf in the United States with "striking destructive changes of both femoral necks" that was unable to stand from birth. CNS changes were not observed.

An 8% prevalance of the disease was reported on a property in northern New South Wales (Chick et al, 1980). Following analysis of the breeding records, it was concluded the condition was inherited in an autosomal recessive manner. The possibility of incomplete penetrance of the gene was considered when it was observed that affected calves were born earlier, on average, than normal calves. It was postulated that environmental factors, such as increased temperature, may influence penetrance of the gene. CNS or hip joint lesions in the calves were not reported.

Donaldson and Mason (1984) also observed that affected animals were born earlier, but discounted any environmental role since the temperature trend in Tasmania was exactly the opposite to that during the calvings reported by Chick et al (1980). Five of the thirteen calves had hip joint abnormalities. Two calves that did not develop clinical signs until 2 days of age had histological lesions in the CNS similar to those described by Cordy et al, (1969). Again, analysis of breeding records supported an autosomal recessive mode of inheritance.

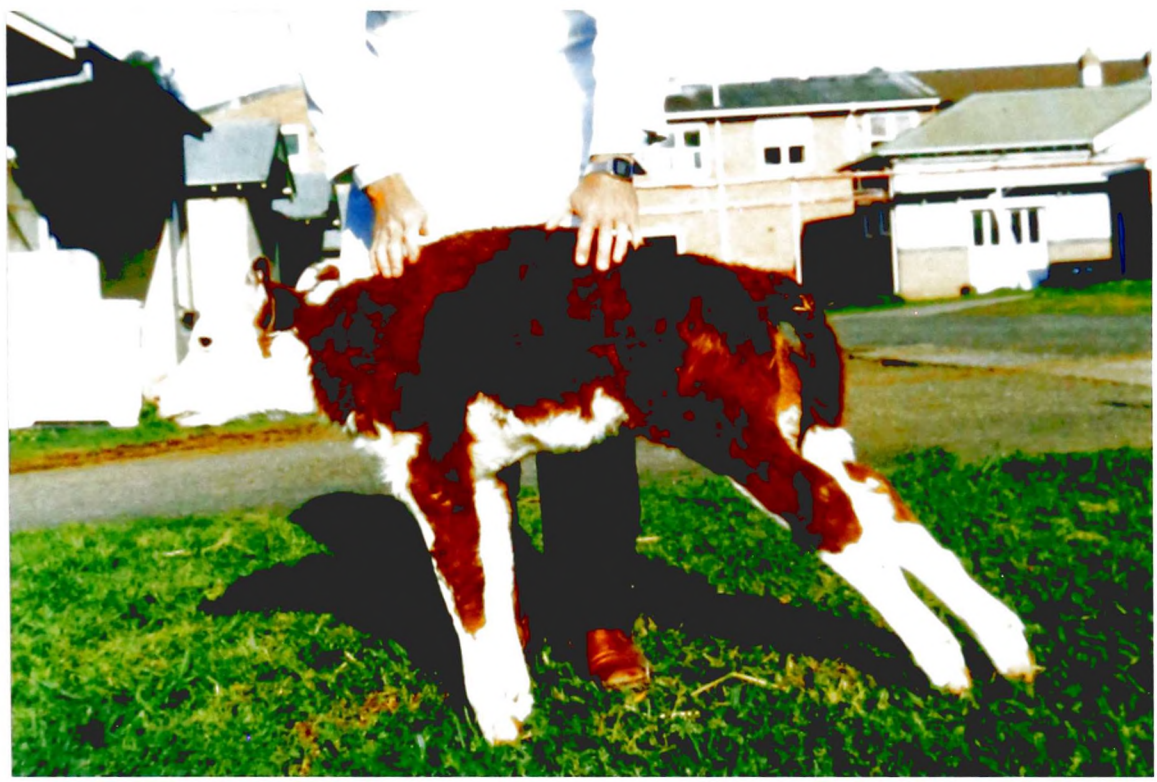
Healy et al (1985) confirmed the mode of inheritance after three years of breeding experiments involving obligate heterozygote cows (Poll Hereford and Poll Hereford crosses) mated with heterozygote Poll Hereford bulls. Of 55 calves born, 42 were normal, while 13 presented with characteristic clinical signs: the calves were bright and alert, but found in lateral recumbency with hind legs crossed, unable to progress beyond sternal recumbency as reported by Blood and Gay (1971), but managing free head movement (Fig. 1). Tactile, auditory and visual stimuli elicited extensor spasms, with responses lessening on repeated stimulation. Spontaneous extensor spasms were also reported. One calf was observed to spasm during delivery. Attempts to stand the calves resulted in extensor rigidity of limb musculature (Fig. 2). The mean gestation period for affected calves was significantly shorter than for normal calves (273 ± 7.4 vs 283 ± 4.5; $p<0.001$). This finding accounted for the observations by Chick et al (1980) that affected calves were generally born earlier in the calving season. The shorter gestation period reflects an abnormality in the foetal/maternal relationship, suggesting prenatal occurrence of the condition. One calf was delivered dead but had hip joint lesions that characterise the disease. All of the 8 cases born in 1983 showed hip joint lesions. The hip lesions predated parturition, proving expression of the disease in utero. No significant lesions were observed in the CNS of any of the affected calves, and water content of the cerebellum was normal. As oedema of the CNS was not apparent in this disease, and myoclonic spasms were the most consistent clinical feature, it was proposed to describe the condition as Inherited Congenital Myoclonus (ICM) (Harper et al, 1986a).

A similar condition has been reported in mice (Chai, 1961), where affected animals were classified as "spastic". Clinical signs were not apparent until 2-6 weeks of age. The condition was characterised by spasms that lasted one to a few minutes following handling or sudden disturbance.

FIGURE 1.

Poll Hereford cross Shorthorn calf affected with ICM. The calf is bright and alert, but unable to rise.

FIGURE 2.



Poll Hereford calf affected with ICM lifted to a standing position. Note extension of all the limbs and whole body rigidity.

In severe cases, the backs were arched and bodies and legs quivered on stimulation. In some affected mice the hindlegs were crossed. As with ICM in calves, no significant histological lesions were observed in the CNS. Breeding experiments confirmed an autosomal recessive mode of inheritance.

Inconsistencies in reports of neuraxial oedema concerning time of onset of clinical signs and presence of CNS lesions remained until the finding of a different neurological condition. Healy et al (1986) reported CNS lesions in calves that were normal at birth, though affected calves consistently had an "unclean" appearance about the white parts of their faces (Fig. 3). Neurological signs of recumbency, dullness and opisthotonus developed at 1 to 3 days of age (Fig. 4). This contrasted with ICM calves that were affected from birth and did not have histological lesions in the CNS. Urine available from 2 of the calves with CNS lesions gave a positive result in a 2,4-dinitrophenylhydrazine test (Dancis and Levitz, 1978). Amino acid analyses of formalin-fixed brain tissue from 5 of the 12 neonatal Poll Hereford calves examined, revealed elevated ratios of branched to straight chain amino acids. Concentrations of the branched chain amino acids valine, isoleucine and leucine in plasma and/or serum, cerebrospinal fluid (CSF) and urine were significantly higher in calves with CNS status spongiosus compared with normal calves (Harper et al, 1986b). A deficiency in the activity of the enzyme, branched chain ketoacid decarboxylase (BCKAD), was demonstrated in fibroblast cultures from a calf affected with CNS lesions (G. Brown, unpublished data). These findings are consistent with a diagnosis of Maple Syrup Urine Disease (MSUD). MSUD is inherited in autosomal recessive manner in man. Four phenotypic forms of MSUD have been classified in man (Tanaka and Rosenberg, 1985); 1. Classic; development of severe ketoacidosis soon after birth, resulting in seizures, coma and death if left untreated. 2. Intermittent; episodes of ketoacidosis triggered by infection or protein imbalance - rarely fatal. 3. Intermediate; no obvious ketoacidosis, but mental retardation. 4. Thiamine responsive; lethargy accompanied by ketoacidosis. All MSUD calves examined to date have presented with a disorder resembling the classic form of MSUD in infants.

Two breeding experiments were conducted to preclude a possible relationship between ICM and MSUD. Firstly, an obligate heterozygote bull for MSUD was mated with 31 cows that were obligate heterozygotes for ICM.

FIGURE 3.

A clinically normal Poll Hereford calf (left) and a half sibling affected with MSUD (right). Both calves are less than 24 hours old. Note the "unclean" appearance of the facial hair on the MSUD-affected calf.

FIGURE 4.

Poll Hereford calf in the terminal stages of MSUD. Note the hyperextension of neck and head (opisthotonus).

Secondly, an obligate heterozygote bull for ICM was mated with 12 cows that obligate heterozygotes for MSUD. All of the resultant 43 calves were clinically normal, confirming ICM and MSUD are two distinct diseases (Healy et al, 1986; Healy, unpublished observations).

(b) Aims of this Study.

The aim of this work was to provide reference values for amino acid concentrations in the body fluids and nervous tissues of newborn Poll Hereford calves. The data generated was then to be compared with values in calves affected with "neuraxial oedema", in an effort to determine a possible biochemical lesion. Preliminary work indicated few differences in body fluids, so more subtle differences in nervous tissues were considered. Further work revealed that "neuraxial oedema" consisted of two distinct disease entities, ICM and MSUD. The study then developed into a detailed description of amino acid distributions in plasma, CSF and nervous tissues of normal calves, ICM-affected calves and MSUD-affected calves.

(c) Methods of Amino Acid Detection : Literature Review.

Amino acid separations involving chromatographic techniques based on either charge or hydrophobicity have become an essential tool in biological sciences, particularly in the screening of inherited disorders. Advances made in the field of amino acid detection and quantitation has paralleled the rapid developments in chromatographic techniques that has occurred since 1940. Traditionally, amino acids have been separated and quantitated using amino acid analysers, which employ ion exchange columns. The need for amino acid separations led to the rediscovery of column liquid chromatography by Martin and Synge in 1941. Turba (1941) was the first to show that basic amino acids adsorb on acidic earth, while Wachtel and Cassidy (1942) used adsorption on charcoal to separate a mixture of glycine, leucine, phenylalanine and tyrosine. Acid alumina was used by Wieland (1942) to separate neutral amino acids, exploiting the negative charge of these components in 90% ethanol. Charcoal was used by Schramm and Primosigh (1943) for the separation of neutral amino acids into an aliphatic and aromatic group, the aromatics being retained on the column until elution with a phenol/acetic acid solution.

Consden et al (1944) improved the separation by the introduction of paper chromatography which enabled partial quantitation of a mixture of amino acids. The principle of partition chromatography applied: when a solute distributes itself between two immiscible liquids, the ratio of solute between the two phases at equilibrium is its partition coefficient. This coefficient is specific for each amino acid. When the hydrophobic cellulose of filter paper is hydrated, the water molecules in between the cellulose molecules provide a tightly bound aqueous stationary phase. When a solvent containing amino acids rises up the filter paper by capillary action, the amino acids separate according to their partition coefficients between the solvent/water phases. The filter paper is then sprayed with ninhydrin so the spots can be detected.

Dent (1947) applied paper chromatography to the analysis of free amino acids in blood and urine. In a two dimensional filter paper method termed a "map of the spots" (Dent, 1948), over 60 amino acids were separated using 2 solvents, phenol and a collidine mixture. The method enabled the presence of gamma aminobutyric acid (GABA) in biological fluids to be confirmed. Polson et al (1947) described a semi-quantitative method that involved comparison of the intensity of the ninhydrin spots with those given by a series of known dilutions of amino acids. Fisher et al (1948) also used a series of known dilutions to show that the area of the spots was proportional to the log of the concentration of components. The accuracy of quantitation with paper chromatography approximated 10%, but results were variable.

Two amberlite resins, IR-100 and IR-4 were examined by Cleaver et al (1945) to determine the conditions under which amino acids could be adsorbed. The cation exchange resins only slightly retained the neutral amino acids, while the anion exchange resins adsorbed the dicarboxylic acids. Dicarboxylic acids, histidine and aromatic amino acids could also be isolated by adsorption onto a silver sulphide precipitate (Hamoir, 1945). However cystine and cysteine were irreversibly adsorbed. Neutral amino acids were further examined by Cleaver and Cassidy (1950) and it was found their adsorption by ion exchange resins increased with increasing chain length, phenylalanine being the most strongly retained.

Mixed success was experienced with alumina columns. Thompson et al (1953) were able to separate most of the alpha amino acids from similar

compounds using a mixture of columns of copper carbonate and alumina, which adsorbs the alpha amino acids as their copper complexes. O'Connor and Bryant (1952) studied the adsorption of amino acids on sparingly soluble inorganic salts such as zinc sulphite, lead sulphite and barium sulphate, while Robson and Selim (1953) were able to isolate basic amino acids in protein hydrolysates on a charcoal column. The amino acid solution was passed through charcoal to remove the aromatic amino acids, then the basic amino acids in the filtrate were transformed into their picrates. These in turn, quantitatively adsorbed onto charcoal, while the free monoamino acids and acidic diamino acids passed out in the eluate.

Until 1950, more than three or four amino acids could not be analysed in a single run because no one method could encompass the varying acid-base properties of the physiological amino acids. Following previous work on the separation of amino acids on columns of starch, Moore and Stein (1951) described an amino acid separation on a sulphonated polystyrene resin (Dowex 50). Eluting buffers of progressively increasing pH resulted in quantitative recoveries of all except the basic amino acids. These were determined with the aid of a second column using buffers of a higher pH range.

Further work by Moore and Stein (1954a) led to the development of an amino acid analyser. The use of a longer column, a resin with 4% cross linkage, and an eluant of continuously changing pH and ionic strength, resulted in the separation of fifty synthetic components from a mixture, including basic amino acids without the aid of a second column. Recoveries were within 3% of the theoretical values, though the analysis took one week to complete. Amino acid concentrations were estimated photometrically after derivatisation with ninhydrin. Increasing the strength of the ninhydrin buffer five-fold eliminated the need to adjust the pH of the effluent fractions prior the analysis (Moore and Stein, 1954b).

The first fully automated amino acid analyser was constructed by Spackman, Stein and Moore in 1958. After separation through a column of sulphonated polystyrene resin, individual amino acids were met by a capillary stream of ninhydrin reagent. The reaction was facilitated by the spiral of capillary tubing being immersed in a boiling water bath. The amino acid-ninhydrin complex was monitored continuously at 570nm, and 440nm for proline.

The recorded peak heights were integrated by multiplying the height by the width at half the height. For loads of 0.1-3.0 umoles, precision of integration for each amino acid was 100±3%. This automated procedure reduced amino acid analyses of plasma, urine and mammalian tissues from 1 week to 2 days.

Gas-liquid chromatography (GLC) is a more recent development in the separation and quantitation of amino acids. The procedure, developed by James and Martin (1952), differs from liquid/liquid chromatography in that the mobile phase is an inert carrier gas, for example, nitrogen. Since gas is compressible, a gradient of gas velocity down the column is produced. Components in the sample are converted to a volatile form, either by employing high temperatures or prior derivatisation. The components partition between the moving and stationary phases according to their individual gas/liquid partition coefficients. The dissolved molecules may be held by the stationary phase either with Van der Waals forces or by hydrogen bonding. The separated components in the gas phase leaving the column may be detected by flame ionisation. The carrier gas stream is mixed with hydrogen and air, and burnt in a high voltage electric field. The current generated by the flow of ionised fragments in the flame is integrated.

Although GLC is commonly used for analysis of complex fatty acid mixtures, Gehrke et al (1968) quantitated amino acids in proteins and biological substances. This technique was utilised by Gabrys and Konecki (1981) to obtain a chromatogram of free amino acids in the cytosol of the hypophysis, pineal gland, thyroid, spinal cord, thymus and lymph nodes of cattle. Despite coelution of some amino acids, the use of two columns enabled the quantitation of sixteen physiological amino acids within one hour. Repeatability was good (variation<10%). The most comprehensive evaluation of GLC to date was reported by Labadarios et al, (1986). An improved sample clean-up technique was developed involving the removal of glucose, which resulted in one of the most reproducible methods published (variation<3%). Plasma amino acid levels were determined simultaneously by GLC and ion-exchange techniques. No significant differences between corresponding values for 11 of the 14 amino acids identified were found. The high resolution, reproducibility and short runtime (<60 mins) suggest GLC would be an attractive alternative to amino acid analysers. However, its use is limited to analysis of compounds that can be made volatile. Only 25% of known organic compounds can be analysed by GLC, as opposed to 75% by liquid

chromatography (LC). The operation of a GLC instrument is also more involved, and the sample is usually destroyed during detection.

Thin layer chromatography using silica gel has been used to quantitate isomers of hydroxyproline and proline, essential components of certain types of collagen (Bellon et al, 1983). Prior to application to the gel, the sample was reacted with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl), producing fluorophors specific for secondary amines. Preliminary ion exchange chromatography removed inorganic ions that inhibit fluorescence of the amino acid/NBD-Cl complex.

(d) High Performance Liquid Chromatography.

The principle of amino acid analysers paved the way for a similar system operated under high pressures to further reduce the analysis time and enhance resolution:- high pressure/performance liquid chromatography (HPLC). Although amino acid analysers have improved dramatically since 1950, their major disadvantage is that they are a dedicated system. HPLC on the other hand, can be adopted to achieve separation of a wide variety of compounds by simply changing the column, mobile phase and/or detector.

Separation of amino acids by HPLC has involved the use of either ion exchange or reverse phase packings. The separations are highly reproducible and, with most detectors, the samples are not destroyed. Since many compounds can be determined in one assay, metabolic profiles of naturally occurring constituents in physiological fluids can be obtained and used as biochemical markers of disease states or nutritional status.

Reverse phase HPLC has been used in biochemical and biomedical research since early 1970, but has only recently made its way into the clinical laboratory. Reverse phase columns utilise silica gel reacted with silanes (SiOH) to form very robust silyl-ether linkages. Onto this linkage can be attached a carbon chain of usually eight (C8 column) or eighteen (C18 column) carbon length, resulting in a highly non-polar surface. The mode of separation can be considered as liquid/liquid partition, and is achieved by the use of a high polarity solvent which results in the most polar sample components eluting first. Decreasing the polarity of the solvent speeds elution. Consider each component in the sample partitioning itself between the non-polar stationary phase and the polar mobile phase - two immiscible

liquids. Each component has a partition coefficient determined by its own polarity. The more polar components will partition more readily into the polar phase and elute first. To use reverse phase on highly polar samples, either ion-pairing or reaction with a derivatising reagent needs to be effected to render the molecule relatively non-polar, thereby slowing progress through the column so separation can occur.

The separation of underivatised amino acids using ion-pair reverse phase techniques is a relatively new procedure. Walker and Pietrzyk (1985) utilised sodium lauryl sulphate as the ion-pairing agent coupled with OPA post column derivatisation. The procedure was applied to the analysis of blood stains on filter paper, with specific reference to MSUD and phenylketonuria. A disadvantage of this method is the requirement of different ion-pairing reagents to achieve separation of some mixtures of amino acids. Most reverse phase separations of amino acids however utilise precolumn derivatisation coupled with isocratic elution, avoiding possible artifacts associated with gradient systems.

Despite the risk of drifting baselines and "ghost" peaks, ion-exchange HPLC which utilises gradient elutions, has proven superior to reverse phase HPLC in the separation of physiological amino acids. As with amino acid analysers, the columns consist of a styrene resin with many negatively charged sulphonate functional groups covalently attached. These negatively charged groups readily associate with any positively charged cations in solution. If the column is equilibrated with a low pH buffer containing a high concentration of the cation of choice, for example lithium, this low molecular weight cation will ionically bind to the functional groups. A mixture of amino acids at this low pH ensures complete protonation. In competition for sites, the large, positively charged amino acids easily displace the smaller lithium cations from the resin, and are subsequently ionically bound to the column. A gradual increase in pH of the mobile phase results in individual amino acids becoming less positively charged, according to their pK values. As a molecule approaches its neutral form, it elutes from the column and separation is effected.

Since the physiological amino acids exhibit only minimal native fluorescence, do not absorb light in the visible range, and only three (tyrosine, tryptophan and phenylalanine) absorb significant light in the ultra-violet range, detection of these compounds at appropriate sensitivities

is not possible without derivatisation. In this procedure, an amino acid is reacted with a compound forming a complex that can be detected either fluorimetrically, spectrophotometrically or electrochemically, depending on the derivatising reagent used. The most common derivatising reagent for ultra-violet detection of amino acids is phenylthiohydantoin (PTH), which reacts with both primary and secondary amines. By nature of the reaction, this procedure is used mainly for protein sequence studies. Derivatising reagents for visible detection include ninhydrin and 4-dimethylaminoazobenzene 4-sulphonyl chloride (DABSYL-Cl), both of which react with primary and secondary amines; 7-Chloro,4-nitrobenzene,2-oxa,1,3-diazole chloride (NBD-Cl) which reacts with only secondary amines, and dimethylaminobenzenethiohydantoin (DABTH) which reacts with only primary amines. The dabsyl procedure is still occasionally used for amino acid profiling utilising a C18 reverse phase column, despite the fact that sensitivity is about one fifth that of the fluorometric dansyl chloride procedure (Deyl et al, 1986).

Higher sensitivity is an advantage of fluorimetry over other forms of detection. Derivatising reagents that render amino acids fluorescent include ortho-phthaladehyde (OPA) which reacts with primary amines, and dimethylaminonapthalene (DANSYL-Cl) and 9-fluorenylmethylchloroformate (FMOC-Cl) which react with both primary and secondary amines.

OPA in the presence of 2-mercaptoethanol, a reducing agent, reacts with primary amines to produce highly fluorescent thio-substituted isoindole derivatives. (1 alkyl thio, 2 alkyl isoindoles). Although OPA is unable to form fluorescent derivatives with secondary amines unless the reaction is carried out in the presence of oxidative reagents such as hypochlorite (Bohlen and Mellet, 1979), it is favoured because it lacks intrinsic fluorescence and is thus well suited to the analysis of complex biological mixtures. The derivatisation procedure, whether pre (C8 or C18) or post (ion-exchange) column, is simple; multiple derivatives of several amino acids that are formed with the dansylation procedure are absent with OPA derivatisation; and OPA is stable in water, the basis of mobile phases used in ion-exchange chromatography (Roth, 1971; Benson and Hare, 1975). Furthermore, the isoindole complexes of the OPA derivatives are electrochemically active and coulometric detectors in series provide a means of verification of the identity of peaks (Joseph and Davies, 1983).

To obtain as complete an amino acid profile as possible from biological fluids and tissues, ion-exchange would be favoured over reverse phase. Post column derivatisation must then be used, the choice of which would be OPA or ninhydrin. Although the latter reacts with both primary and secondary amines, the sensitivity of the fluorescent OPA system is a deciding factor in the analysis of physiological amino acids. This was the system of choice in this study.

(e) Amino Acids : Literature Review.

Data available on amino acid concentrations in tissue and/or body fluids in cattle is limited, but encompasses a range of chromatographic techniques. Paper chromatography was used to isolate free amino acids in pineal gland extracts from bulls (El-Mallah, 1976). Cystine, glutamic acid, aspartic acid and alanine formed 65% of the 14 amines detected. Lahdesmaki et al (1977) identified and quantitated 10 amino acids in synaptic vesicles of calf cortex using two dimensional thin layer chromatography coupled with an amino acid analyser. The most abundant amino acids were taurine and glutamate. In a collaborative study involving 4 laboratories, free amino acids of plasma from a "preruminant" calf (whole milk diet) and a "ruminant" calf (calf rearing mixture and hay diet) were determined with amino acid analysers (Williams et al, 1980; Appendix 1). Interesting findings to emerge from this study were that different procedures of deproteinisation had little effect on average amino acid concentrations, and the concentrations were higher in the preruminant plasma for 18 of the 21 amino acids measured. As variation between laboratories was quite high for many amino acids such as lysine and glutamic acid, improvement in precision was considered necessary in analysis of physiological fluids when assessing dietary amino acid intakes. The system was considered satisfactory in measuring abnormally high or low concentrations associated with metabolic disorders. Gabrys and Konecki (1981) employed gas/liquid chromatography to analyse amino acid concentrations in the cell cytoplasm of various tissues of the cow, including the pineal gland and spinal cord. Glutamate, the most abundant amino acid in the glandular tissues analysed, comprised over 50% of the total amino acids detected in the pineal gland, while both glutamate and aspartate contributed over 60% in the spinal cord.

Interest in metabolic diseases in man has led to much literature on amino acid concentrations in nervous tissue, plasma, urine and CSF. Perry et

al (1971a) has recorded perhaps the most detailed study of amino acid distributions in various regions of the CNS. Concentrations of 35 free amino acids were determined in frontal, temporal and occipital cortex, cerebellum, thalamus, putamen-globus pallidus, caudate, amygdala, substantia nigra, red nucleus, corpus callosum and hypothalamus mamillary bodies in the human brain. The amino acids were detected as their ninhydrin derivatives on an amino acid analyser. The concentration of GABA was found to be highest in the putamen-globus pallidus and substantia nigra. Glutamate was high in all brain regions except the corpus callosum, hypothalamus mamillary bodies, substantia nigra and red nucleus (half the concentration). Taurine was twice as high in the cerebellum compared to the other 11 regions; phosphoethanolamine was highest in the temporal cortex and amygdala; aspartate was highest in the substantia nigra, occipital cortex, thalamus and cerebellum. The consistent differences in each of the 5 brains examined supported the conclusion that the results reflected true regional variations. It was postulated that the relatively high concentrations of GABA in the putamen-globus pallidus and substantia nigra, and taurine in the cerebellum, suggested a neurotransmitter inhibitory function in the synapses of these regions.

Values for amino acid concentrations in CSF of man are available prior to 1970, but the sample sizes are small (Perry and Jones, 1961; Dickinson et al, 1965). The first comprehensive report (van Sande et al, 1970) involved 78 subjects, 13 of which were considered controls. Separation was with an ion-exchange column on an amino acid analyser, with subsequent detection of ninhydrin-positive substances. Although a range of aminoacidurias was tabulated, MSUD was not included. In most cases of metabolic diseases studied, proline, isoleucine, tyrosine, phenylalanine and histidine were elevated in CSF, but not necessarily in the serum. It was then postulated that CSF does not derive its amino acids by passive diffusion. To add further to the base data for human CSF, 31 primary amino acids from 15 subjects were separated by a triple column, isocratic ion-exchange HPLC (Ferraro and Hare, 1984). The resulting values were considered more reliable reference levels than previous reports because artifacts from gradient elution were eliminated and CSF samples were treated immediately on collection. In vitro studies of plasma have shown that asparagine and glutamine are enzymatically deaminated to yield the corresponding free acids, aspartate and glutamate, and ammonia (Perry and Hansen, 1969). Previous references often failed to detect glutamine, and only very low levels of

asparagine (van Sande et al, 1970). Ferraro and Hare (1984) considered that GABA, aspartate and glutamate concentrations had been overestimated in the past because these neurotransmitters are compromised in less resolving systems by closely eluting compounds, and concentrations are affected by different sample treatments. GABA concentrations have been found to rise in CSF maintained for short intervals at room temperature (Grossman et al, 1980). The in vitro increase of GABA has been documented to be enzyme mediated (Hare, 1981).

Correlation between contamination by blood, and CSF amino acid levels has been reported (Kornhuber et al, 1986). Samples of CSF that appeared macroscopically free of erythrocytes had contamination greater than 5 erythrocytes/ul when tested with a Fuchs-Rosenthal chamber. This resulted in a significant positive correlation between red cell counts and CSF concentrations of aspartate, alanine, asparagine, citrulline, glutamate, phenylalanine and taurine. Blood contamination did not influence glutamine, histidine, isoleucine, leucine, valine, lysine, methionine, serine, threonine, tyrosine or ornithine concentrations.

Stein and Moore (1954) analysed plasma from 5 normal adult males on a Dowex 50-X4 column. Twenty-eight ninhydrin-positive substances were identified, and with the exception of glutamine, all were quantitated. Analysis of whole blood revealed higher concentrations of taurine, aspartate, glutamate, glycine and ornithine compared with plasma. Amino acid analysis of foetal plasma from 22-33 weeks showed no correlation between concentration and age of gestation (Kamoun et al, 1985). Comparison with maternal plasma revealed significantly lower concentrations in foetal plasma for alanine, arginine, citrulline, cystine, glutamate/glutamine, glycine, isoleucine and leucine. However, lysine, methionine, ornithine, serine and threonine were significantly higher. Data gathered from amino acid analyses of large numbers of plasma samples have been reported for children (Armstrong and Stave, 1973; Applegarth et al, 1979) and for adults (Schreiber, 1986). Comparison between levels in 52 children and 80 adolescents has also been reported (Gregory et al, 1986), and results compare well with earlier data, forming a sound basis for normal values in human plasma (Appendix 1).

Frampton, Yardley and MacMahon (1986) have provided the only report of amino acid concentrations in the newborn chick. Plasma samples were deproteinised with picric acid and amino acids separated by reversed phase

HPLC after derivatisation of the samples with OPA. With the exceptions of taurine, aspartate, threonine and serine, primary amino acid concentrations are similar to those found in the plasma from children (Appendix 1).

Concentrations of 27 amino acids in whole rabbit brain have been determined using an amino acid analyser (Agrawal et al, 1966). Postnatal increases were found with glutamate, glutamine, GABA and aspartate. Postnatal decreases were found with phosphoethanolamine, taurine and glycine. The remaining 20 amino acids did not differ significantly during the first 30 days, by which time maturation of the developing rabbit brain is considered complete. Reference values have been reported for plasma amino acid concentrations in 145 male rabbits (Bauer et al, 1986). Sixteen amino acids were separated by GLC equipped with a flame ionisation detector (Appendix 2). The method failed to separate glutamine from glutamic acid, and asparagine from aspartic acid.

Prior to the development of automated procedures for amino acid analyses, Tallan, Moore and Stein (1954) quantitated over 30 free amino acids in liver, brain, pancreas, gastrocnemius muscle, bladder, kidney, urine and plasma of the cat. Amines were separated on a sulphonated polystyrene resin and the ninhydrin-positive components plotted to yield a detailed eluent profile. Taurine and glutamate concentrations were consistently the highest in all tissues. GABA concentrations were highest in the brain. Glutamate, aspartate, glutamine and GABA have been quantitated in the cerebral cortex of newborn kittens (Berl and Purpura, 1963) and compared with values found in the mature cat. Glutamate, aspartate and GABA were found to occur in lower concentrations in the newborn kitten, though glutamine concentration remained constant. It was suggested that since glutamate formation is the main pathway for removal of ammonia from the brain, maintenance of low brain ammonia levels are important in the neonatal animal.

Free amino acid concentrations in the cortex of the dog have been determined by ion exchange chromatography of ninhydrin derivatives (Tews et al, 1963). The most abundant primary amino acids were glutamate and aspartate.

Normal values for 19 primary amino acids in the cortex, hippocampus, striatum, diencephalon, brainstem and cerebellum of the rat brain have been determined by reversed phase HPLC analysis of OPA derivatives (Erecinska et

al, 1984). Rat striatum has also been analysed by reversed phase HPLC analysis of dansyl derivatives (Zecca and Ferrario, 1985). In a study relating physiological responses to graded intakes of protein in rats, a relationship between serum and brain amino acid levels has been established (Gustafson et al, 1986; Appendix 1). Serum was collected under anaesthetic immediately prior to removal of the whole brain, which was snap frozen in liquid nitrogen and stored at $-70^{o}C$ until analysed. It was found that amino acid concentrations in the brain as a whole are dependent on the respective concentrations in the serum. High correlations (>0.9) were found between brain and serum for threonine, serine, glycine, valine, methionine, isoleucine, leucine, tyrosine, lysine, histidine and tryptophan. The slopes of most of the linear regressions were less than one, suggesting amino acid concentrations in brain fluctuate much less than in serum. Only histidine, serine, threonine and glutamine had slopes greater than one, indicating more rapid changes in the levels of these four amino acids in brain than in serum. The acidic amino acids, taurine, aspartate and glutamate showed very poor correlations between brain and serum levels (<0.3), thought to reflect a high degree of synthesis in the brain. Poor correlations were also noted with glutamine and alanine concentrations in CNS and serum.

During an investigation on protein synthesis, Krishnamurti, Heindz and Galzy (1984) determined amino acid concentrations in plasma of the ewe and foetus by separating OPA derivatives on a C-18 column (Appendix 2). Maternal and foetal blood were collected at 120 days gestation (average gestation:151 days). Of the 15 primary amino acids separated, no differences were observed between maternal (n=11) and foetal (n=15) plasma for aspartate, histidine, threonine, glycine, lysine, valine, methionine or phenylalanine. On the basis of a difference of 1 standard deviation or more, glutamate, isoleucine and leucine were lower in foetal blood, while serine, tyrosine, alanine and arginine were higher. For only glutamate, isoleucine, leucine and serine were there common foetal/maternal differences in humans (Kamoun et al, 1985) and sheep.

MATERIALS AND METHODS

a) Animals.

Where possible, sires and dams of calves affected with either ICM or MSUD were purchased by the Department of Agriculture to form the experimental breeding herd. Nervous tissue and body fluids were collected from calves either born in this herd, or referred to the laboratory by field veterinarians. With all samples, comparisons were made between three genotypes: clinically normal, ICM and MSUD. Classification of genotype was made on the basis of clinical signs and pathology (Table 1), and/or amino acid concentrations.

An obligate heterozygote cow for MSUD in the Departments herd was treated with follicle stimulating hormone on two occasions to induce multiple ovulations. One week after insemination with semen from an obligate heterozygote, embryos were recovered nonsurgically. Embryos were transferred nonsurgically to recipients whose oestrus cycles were synchronised with those of the donor. After the programs, the donor and three other obligate heterozygotes were mated with the same bull. One male and three females of the resultant ten calves were affected with MSUD, providing 4 of the 6 cases for brain analyses. (For a more detailed description of multple ovulation-embryo transfer (MOET) procedures, refer to the reprint by Healy, et al (1987) at the end of this thesis).

b) Samples.

Nervous tissue was collected from 7 normal, 6 ICM and 6 MSUD affected calves. All calves were destroyed at 1-4 days of age by either decapitation (5 normals and 4 ICM) or an overdose of phenobarbitone and subsequent exsanguination (2 normals, 2 ICM and 6 MSUD). The brains from the decapitated calves were snap frozen whole in liquid nitrogen within 10 minutes of death and kept at -20°C for 2 years. Brains from calves injected with phenobarbitone were either stored whole at -20°C for 6 months (2 normals, 1 ICM and 1 MSUD) or dissected immediately into the appropriate regions, homogenised in 6% perchloric acid and stored at -20°C until further processing (1 ICM and 5 MSUD).

Only one side of the brain was available for biochemistry from the 6 MSUD cases; the other half taken for histopathology. For one of the MSUD cases, the thalamus and the white and grey matter of the cerebellum were not available for amino acid analysis. With the exceptions of one ICM and one MSUD case, all calves that provided brain tissue were born in the experimental herd.

The 13 physio-anatomically distinct regions of the brain used for analysis were medulla oblongata, pons, grey and white matter of the cerebellum, frontal, temporal and occipital cortex, amygdala, thalamus, putamen, caudate nucleus, substantia nigra and red nucleus. After dissection, the tissues were weighed and homogenised (Dounce homogeniser) in 1-10 mls of 6% perchloric acid (PCA) containing 0.16mmoles/l norleucine as the internal standard (10ml PCA/g tissue). The homogenate was centrifuged at 1500g for 15mins and the pH of the supernatent adjusted to 4.0 with 2N potassium hydroxide. After placing the tube at -20°C for 30mins or 4°C overnight, the potassium perchlorate, which precipitates in the cold, was removed by centrifuging at 1500g for 15mins at 0°C (Saifer, 1971). The supernatent was rotary evaporated almost to dryness under reduced pressure, and the residue dissolved in mobile phase. This mixture containing free amino acids was centrifuged at 47,000g for 30mins at 0°C, and an aliquot injected onto the column. Samples awaiting analysis were stored at -20°C prior to the final centrifuge.

Formalin-fixed cerebrum from 4 normal calves, 6 ICM calves and 10 MSUD calves was extracted by the same procedure used for fresh nervous tissue. Where possible, samples were taken from the temporal cortex.

Spinal cords collected at postmortem from 5 normal calves and 5 ICM-affected calves, were snap frozen in liquid nitrogen and stored at -20°C for 12 months. The thoracic region of the cord was then dissected into dorsal and ventral portions after removal of the dura mater, arachnoid and pia mater. Each portion was homogenised in 6% PCA (1g/5ml) containing 0.16mmoles/l norleucine (Saifer, 1971). The homogenate was stored at -20°C until further processing.

CSF was collected from the lumbar cistern (enlarged area in the subarachnoid cavity) from 10 normal calves, 10 ICM calves and 5 MSUD calves. Only macroscopically clear CSF was retained for analysis. One millilitre was

transferred to a Centrifree Micropartition System (Amicon Scientific Australia) which has a molecular weight cut-off of 25,000, and centrifuged at 4°C on a fixed head rotor for 60mins at 2,000g. The protein-free filtrate was stored at 4°C until injection on the amino acid column.

CSF from 4 calves affected with ICM and 4 clinically normal calves was collected from the lumbar cistern and acidified immediately with hydrochloric acid for the determination of catecholamine levels. Samples were stored at -70°C within 30mins of collection and analysed within 2 weeks.

Serum or heparin plasma was obtained from 10 normal calves, 10 ICM calves and 10 MSUD calves. As with the CSF, all plasma/serum samples were deproteinised using the Centrifree filtration technique. This method was preferred to the conventional protein precipitates such as sulphosalicylic acid (SSA), to avoid the large SSA peak that elutes with the void, and often interferes with the quantitation of taurine. Serum and heparin plasma were collected from 7 normal calves between 1-2 days of age and both samples deproteinised by filtration. The 4 MSUD calves from the MOET program were bled at regular intervals for 3-4 days, ranging from presuckle samples to samples taken just prior to postmortem. Plasma from 3 normal calves was collected over the same period.

Plasma branched chain keto acid concentrations were determined on the final bleeds of 6 MSUD cases and 7 control calves at similar ages. One MSUD case from the MOET program was monitored at each 4 hour bleed until it was recumbent at 3 days. Two calves not affected with MSUD were bled daily for 3 days to determine control keto acid levels over this period.

Samples for organic acid analyses were prepared using the procedure developed by Daish and Leonard (1985), using Bond Elut SAX (quaternary amine) 2.8ml disposable anion exchange columns (Analytichem International). After conditioning the column with 2.8ml methanol, then 2.8ml water, 1ml plasma was drawn through under negative pressure (organic acids retained on resin). The column was then washed twice with 2.8ml water to remove unbound plasma components, and centrifuged at 100g for 5mins to remove excess water. Organic acids were eluted with two 0.5ml washes of 0.5M sulphuric acid. The column was centrifuged at 100g for 5mins after both acid washes. The aliquots were pooled and injected onto the analytical column on the same day.

Urine collected from MSUD calves was mixed with 4 volumes of 0.2% 2,4-dinitrophenylhydrazine (DNPH) in a qualitative test for keto acids (Dancis and Levitz, 1978).

c) Chromatography of Amino Acids.

Water used for buffers and/or sample preparation was glass distilled and purified through a Milli-Q water system (Millipore/Waters Assoc.). Water passes through an activated carbon cartridge to remove dissolved organic contaminants and residual chlorine, two mixed bed deionization cartridges to remove ionized inorganics, and an ion exchange cartridge to remove dissolved inorganic electrolytes. The final processing element in the system is a Millistak-GS filter unit that retains microorganisms greater than 0.22um.

The HPLC system consisted of two Waters Model 6000A solvent delivery pumps for gradient elutions; a Model 710B Waters Intelligent Sample Processor (WISP) for automatic injections, equipped with a 2000ul sample loop; an Eldex single piston auxillary pump for post column derivatisation, delivering OPA to a reaction coil at 0.4 ml/min; a Waters 420 fluorescence detector comprising an 8ul flow cell, 4 watt fluorescent lamp, with excitation via 338nm interference and emission via a 425nm long pass filter; a Waters M730 Data Module, a microprocessor based printer/plotter/integrator for integration of peak areas or heights; and an M720 System Controller that integrates all components and controls the two pumps to produce a continuous gradient.

Boric acid, citric acid, nitric acid, sulphuric acid, perchloric acid, sulphosalicylic acid and sodium-free ethylenediaminetetraacetic acid (EDTA) were obtained from Ajax; HPLC grade isopropanol and methanol from Waters; Brij 35, B-mercaptoethanol and OPA from Sigma, and lithium hydroxide from Pierce. All reagents were analytical reagent grade.

Norleucine, taurine and GABA were obtained from Calbiochem; DL-citrulline from British Drug Houses, and L-aspartate, L-threonine, L-serine, L-asparagine, L-glutamate, L-glutamine, glycine, L-alanine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-tryptophan, L-lysine monohydrochloride and L-arginine from Pierce. Three stock solutions of the (a) acidic (b) basic and (c) neutral amino acids were prepared in 0.1M hydrochloric acid and stored in aliquots at

-20°C. Mixing the three groups in equal quantities prior to analysis provided an external standard of 21 physiological amino acids at individual concentrations of 0.3mmoles/l.

Separations were achieved with a lithium cation exchange polystyrene resin (120 x 4.6mm, particle size of 10um), maintained at 43°C by an electric column heating block. Amino acids were separated according to individual pK values by using a pH gradient elution system involving two buffers, "A" and "B". Stock solutions of 2.0M citric acid, 0.5M boric acid and 2M nitric acid were prepared and passed through a 3cmx75cm cation exchange column. The column was packed with standard grade "Amberlite" IR-120 resin in the sodium form. Conversion to the hydrogen form, required for a lithium HPLC system, was achieved by successive washes with 1 litre 0.5M sulphuric acid, 3 litres Milli-Q water, 1 litre 2M lithium hydroxide, followed by another 3 litres water. The 3 stock acid solutions were then passed through the column to remove contaminating amines. The filtrates were stored in acid-washed amber bottles, placed under a blanket of ultra-pure grade nitrogen, and either stored at 0°C (citric acid), 4°C (boric acid) or room temperature (nitric acid) until use.

Buffer A consisted of 1 litre 0.151M citric acid, 1 litre 0.454M lithium hydroxide, 117ml methanol and 225ml 2M nitric acid, resulting in a pH of 2.50. Buffer B comprised 900ml 0.05M boric acid, 800ml 1.056M lithium hydroxide, 270ml 2N nitric acid and 2ml 10mg/ml EDTA (preventing accumulation of metal ions on the column), giving a pH of 10.40. Fermentation traps were placed over the inlets of both buffer bottles. The trap over Buffer A contained 2N nitric acid. Air that passed into the bottle to replace spent liquid was stripped of ammonia and amines before reaching the buffer surface. Similarly, the trap over buffer B, which contained 2M lithium hydroxide, served to remove carbon dioxide from air entering the bottle. This eliminated the decrease in pH observed when carbon dioxide dissolves in basic solutions.

A stock borate solution of 0.5M boric acid and 0.5M potassium hydroxide, pH 10.4, was the base for the OPA derivatising reagent. To 1 litre of this buffer was added 1ml 30% Brij 35, 700mg OPA dissolved in 10ml methanol, and 2ml mercaptoethanol. This solution was prepared fresh every 2-3 days. All buffers were filtered through a 0.45um filter unit, and degassed prior to use.

Prior to injection, the column was equilibrated with buffer A for 30 mins. Upon injection of a sample, buffer A gave way to buffer B in a linear gradient that resulted in 25% "A" and 75% "B" at 60 mins. The following 15 mins led to 100% "B" via a concave gradient (curve 9). Buffer B was maintained until 100 mins, when 100% "A" was introduced to equilibrate the system in preparation for the next injection. A flow rate of 0.4ml/min was maintained throughout the run. The 22 primary amino acids were eluted within 110mins. A profile of an external standard after buffer clean-up on the amberlite resin is illustrated in Figure 1.

2) Chromatography of Organic Acids.

Alpha ketoisocaproic acid (KICA), alpha ketoisovaleric acid (KIVA) and DL-alpha keto-beta methylvaleric acid (KMVA) were obtained from Sigma; 2,4 dinitrophenlyhydrazine was laboratory grade reagent obtained from British Drug Houses. Stock solutions of 10mM KICA, 10mM KIVA and 10mM KMVA were prepared in 5mM sulphuric acid and stored in aliquots at -20°C. A solution of 2% DNPH was prepared in 2N hydrochloric acid and stored on the bench in a brown glass bottle.

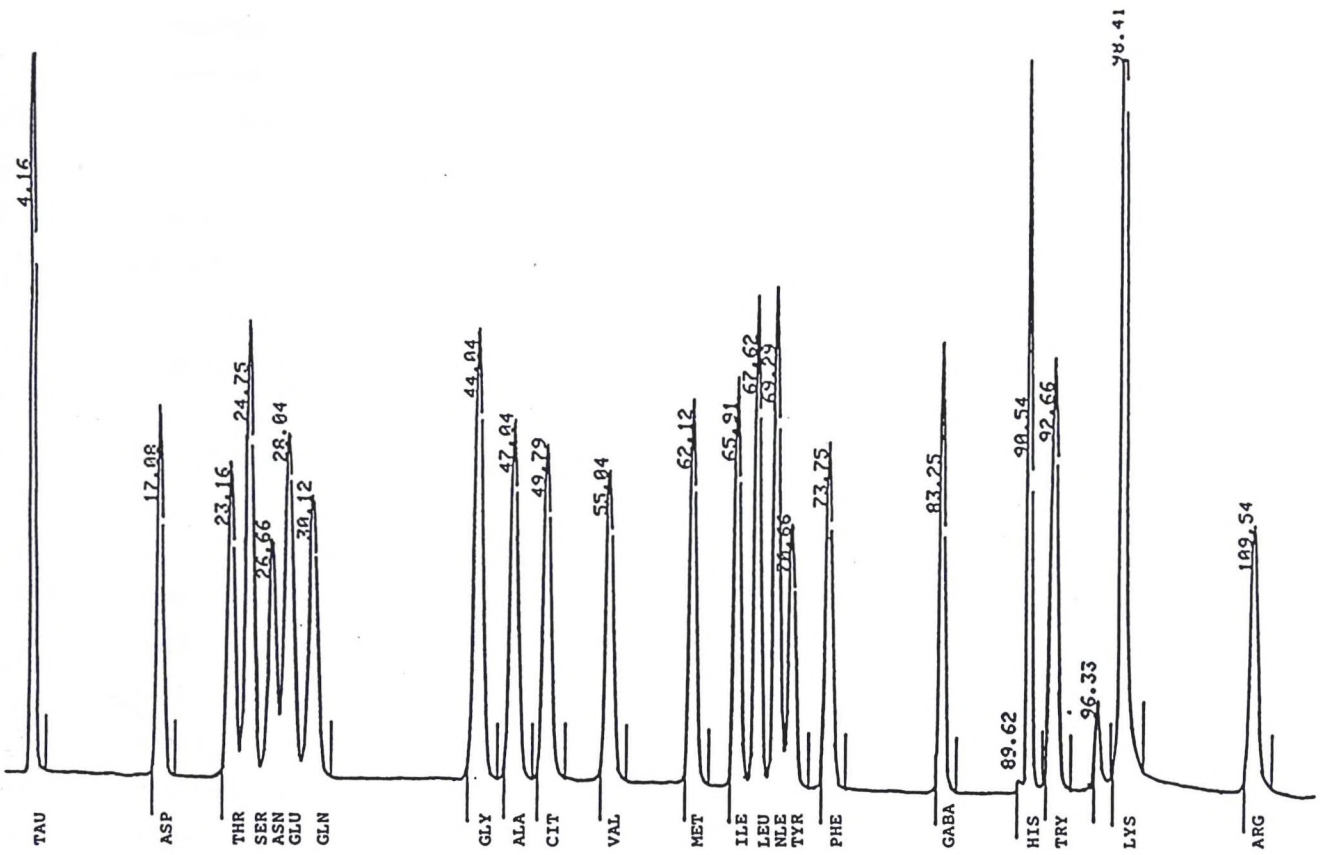
The HPLC system for organic acid analysis utilised only one solvent delivery pump; a Waters U6K manual injector with 2000ul sample loop; and a Waters 441 absorbance detector, fitted with a zinc lamp and 214nm filter and aperture.

Separations were achieved with a hydrogen cation exchange resin (300 x 7.8mm, particle size of 9um: Biorad Aminex HPX-87H column). A guard column containing the same cation exchange resin was installed immediately before the analytical column (40 x 4.6mm, Biorad Aminex HPX-85H). Organic acids were analysed with an isocratic elution system using a mobile phase of 6mM sulphuric acid and flow-rate of 0.5ml/min. The mobile phase was filtered and degassed for 30mins before use. The 3 branched chain keto acids were eluted within 17mins.

3) Chromatography of Catecholamines.

DL-beta-3,4-dihydroxyphenylalanine (dopa) and 3,4-dihydroxybenzylamine (DHBA) were obtained from Sigma. L-adrenaline, 3-hydroxytyramine (dopamine), arterenol bitartrate (noradrenalin) and serotonin

FIGURE 5.



MILLIPORE Waters Chromatography Division Part No. 74701

ELUTION PROFILE OF AMINO ACID EXTERNAL STANDARD.

Each peak represents a concentration of 4.5 nmoles. Retention times are indicated at the top of each peak.

creatine sulfate complex (serotonin) were obtained from Calbiochem. An external standard consisting of 0.4umoles noradrenalin, 1.0umole adrenalin, 2.0umoles dopamine, 1.0umoles DHBA and 1.0umole serotonin was dissolved in 1 litre dilute HCL to a pH of 2.0. The standard was aliquoted and stored at -70°C until use.

The HPLC system was similar to that used for organic acid separations, with the exception of an electrochemical detector replacing the fluorescence detector, to discriminate catecholamines from amino acids. Separations were achieved on a Waters uBondapak C18 reverse phase stainless steel column (300 x 3.9mm, particle size of 10um). The mobile phase consisted of 6.8g sodium acetate, 100mg EDTA, one bottle of Pic B-8 (1-octane sulphonic acid; Millipore/Waters Associates; catalogue number 85142); the pH adjusted to 4.8 with either sodium acetate or acetic acid; made to 1 litre, then 70ml acetonitrile added. The flow-rate was 1.5ml/min. Each of the components of the external standard eluted within 20mins.

f) Clinical Chemistry.

Plasma collected from 6 clinically normal calves and 6 ICM-affected calves was analysed to determine the concentrations of calcium (Sarker and Chauhan, 1967); magnesium (Gindler and Heth, 1971); phosphorus (Kallner, 1975); protein (Gornell et al, 1949); albumin (Bartholomew and Delany, 1966); glucose (Boehringer Mannheim kit:608459) and urea (Boehringer Mannheim kit:124770). The following enzyme activities were also determined : alkaline phosphatase (Healy, 1971); gamma glutamyltransferase (Boehringer Mannheim monotest kit:235075); ornithine carbamoyltransferase (Ohshita et al, 1976); creatine kinase (Boehringer Mannheim automated kit:475734); and aspartate aminotransferase (Boehringer Mannheim automated kit:487350).

CSF collected from 14 clinically normal calves and 14 ICM-affected calves was analysed for calcium, magnesium, phosphorus, glucose, protein and urea concentrations. Aspartate aminotransferase and creatine kinase activities were also determined.

g) Statistics.

With the exception of serum and plasma comparisons, a 5% significance level has been adopted for testing differences between mean

values for body fluids and spinal cord data. For the brain data, differences between the means that are significant at levels of 5%, 1% and 0.1% have been identified.

The t statistic for paired observations was calculated on the concentrations of twenty amino acids in serum and plasma samples from newborn calves (Table 3). Significant differences at 5%, 1% and 0.1% have been identified.

Amino acid concentrations in plasma and CSF from ICM- and MSUD-affected calves were compared to the values found in normal calves (Tables 4 and 5) by transforming the data to the logarithm (log) of base 10 and calculating the t statistic for unpaired observations, utilising a pooled estimate of variance. The t statistic was calculated in the same manner for BCKA concentrations in plasma from normal and MSUD-affected calves (Table 11).

The t statistic for unpaired observations was calculated on the original data to test for differences in catecholamine concentrations in CSF from normal and ICM-affected calves (Table 7); plasma and CSF clinical chemistry results between normal and ICM-affected calves (Tables 9 and 10); and ratios of BCAA's to alanine in fixed cerebrum from normal, ICM- and MSUD-affected calves (Table 13).

Spinal Cords.

To test for homogeneity of variance in amino acid concentrations of dorsal and ventral regions of the spinal cord in 5 normal and 5 ICM calves, the chi-square statistic was computed. As the variance was low for both regions and both genotypes, concentrations were analysed without a log transformation. Amino acid concentrations in the ventral spinal cord of each calf were subtracted from the corresponding amino acids in the dorsal region for both normal and ICM calves. The t statistic for the difference between the two genotypes was determined for each amino acid.

As no genotype effects on site differences were detected, the two genotypes were pooled, and the t statistic for the differences between the two sites determined for each amino acid.

Brain sites and regions.

Comparisons of amino acid concentrations in individual brain sites between 7 normal and 6 ICM, and between 7 normal plus 6 ICM and 6 (5 for 3 sites) MSUD calves were made on log-transformed data, using the t statistic for unpaired observations.

Brain sites were grouped into regions on anatomical and/or physiological criteria, and tests of differences conducted within and between sites and/or regions. Thus, the medulla oblongata was compared to the pons, both being anatomically distinct from the other brain sites; and the white matter of the cerebellum (axons) compared to the grey matter of the cerebellum (cell bodies), with all 4 sites forming part of the hindbrain and the latter two sites forming the total cerebellum. The frontal cortex was compared to the temporal cortex, then pooled for comparison with the occipital cortex, again anatomically distinct sites and representing the whole cortex; the putamen was compared to the caudate nucleus, then pooled for comparison with the amygdala, the three sites forming part of the basal ganglia. The amount of tissue available from the red nucleus was small (1-10mg) in comparison to the other sites (500-1000mg). Data generated from the red nucleus was omitted from the analyses as a strong correlation was found between most amino acid concentrations and tissue weights. The groupings involving the remaining 12 sites formed the basis of comparisons within and between regions in an effort to make useful comparisons among sites/regions, both within and between genotypes. The following combinations were chosen.

A1 : Hindbrain.
A2 : Cortex and basal ganglia.
A3 : Thalamus and substantia nigra.
A4 : Cortex, basal ganglia, thalamus and substantia nigra.
A5 : Hindbrain, cortex, basal ganglia, thalamus and substantia nigra.

B1 : Medulla oblongata, pons and thalamus.
B2 : Cerebellum and cortex.
B3 : Basal ganglia and substantia nigra.
B4 : Cerebellum, cortex, basal ganglia and substantia nigra.
B5 : Medulla oblongata, pons, thalamus, cerebellum, cortex, basal ganglia and substantia nigra.

C1 : Medulla oblongata, pons and substantia nigra.

C3 : Basal ganglia and thalamus.

C4 : Cerebellum, cortex, basal ganglia and thalamus.

C5 : Medulla oblongata, pons, substantia nigra, cerebellum, cortex, basal ganglia and thalamus.

To assess the similarities or otherwise of amino acid concentrations in 12 brain sites and various regions between the 7 normal calves and 6 ICM calves, ie to determine interactions of genotype by site/region, analyses of variance were computed on the log-transformed data. The interaction of genotype by site was also evaluated using analysis of variance for a comparison of the MSUD genotype with the combined normal and ICM genotypes.

Amino acid concentrations in normal and ICM-affected calves were treated as one group and tested for site/region differences. For each of the 13 animals, the log of the concentration at one site/region was subtracted from the log of the concentration at the other site/region, and the t statistic computed to test the hypothesis of a mean difference of zero. Where differences between groups of sites/regions were compared (A1-C5), the means for one group of sites/regions was subtracted from the mean of the other group, and the t statistic calculated on the average difference, again testing the hypothesis of a mean difference of zero. The same procedure was applied to test for site/region differences within the MSUD genotype, the sample size in this case being 5 or 6.

RESULTS.

The coefficients of variation (CV) of retention times for a standard of 22 amino acids injected daily for 5 days (Table 2), were less than 5.5%. With the exception of tryptophan (8.6%), the CV's of response factors were less than 6.5%. The CV's of peak heights were less than 6.8% - again with the exception of tryptophan (9.5%).

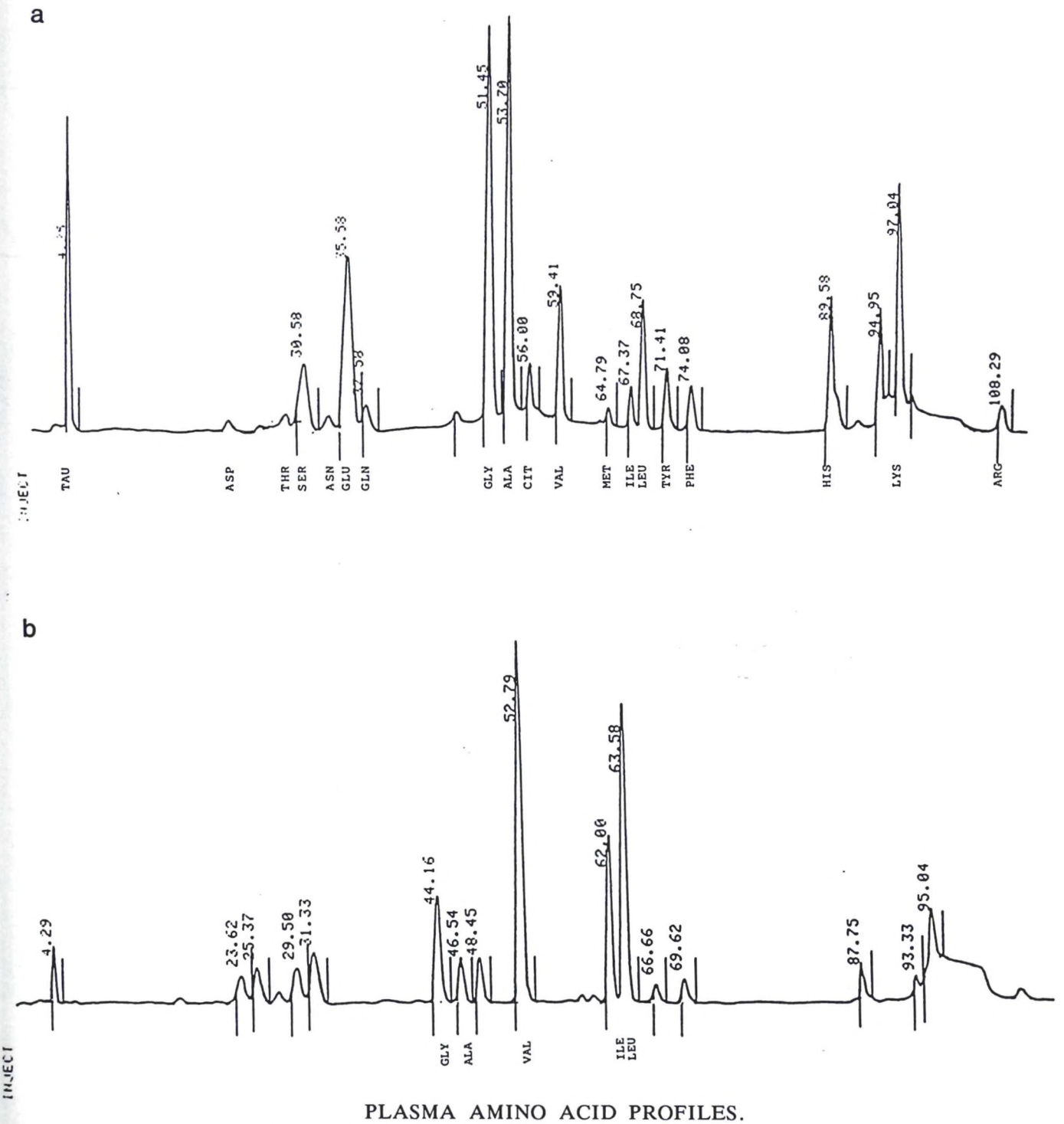
Serine, glutamate, taurine and arginine were significantly higher in serum of newborn calves than plasma (Table 3). The remaining 16 amino acids did not differ significantly.

Means and standard deviations of plasma amino acid concentrations for 10 normal, 10 ICM and 10 MSUD calves are presented in Table 4. As the distribution was skewed the data was log-transformed before determining the t-statistic. Citrulline, valine, isoleucine, leucine, phenylalanine and tyrosine were lower in ICM calves. In plasma from MSUD calves, alanine was depressed, while citrulline, valine, isoleucine, leucine, phenylalanine and arginine were elevated.

Table 5 presents means and standard deviations of amino acid concentrations in the CSF from 10 normal, 10 ICM and 5 MSUD calves. Analysis of the log-transformed data revealed no differences in amino acid concentrations between normal and ICM calves. In CSF from MSUD-affected calves, alanine was depressed while threonine, serine, asparagine, glutamine, glycine, citrulline, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine and arginine were elevated. In the CSF from one MSUD calf, a peak corresponding to the position of GABA on the chromatogram was detected.

An amino acid profile of presuckle plasma from a calf affected with MSUD is presented in Figure 6, with a profile from a normal calf for comparison. The rise in BCAA's after 2 days is obvious from the profile in Figure 7. The chromatograms in Figure 8 show a transient hyperglycemia in an MSUD-affected calf. Glycine concentration was 6.58mM at 8 hours (a), and 0.93mM at 2 hours (b) prior to death.

FIGURE 6.

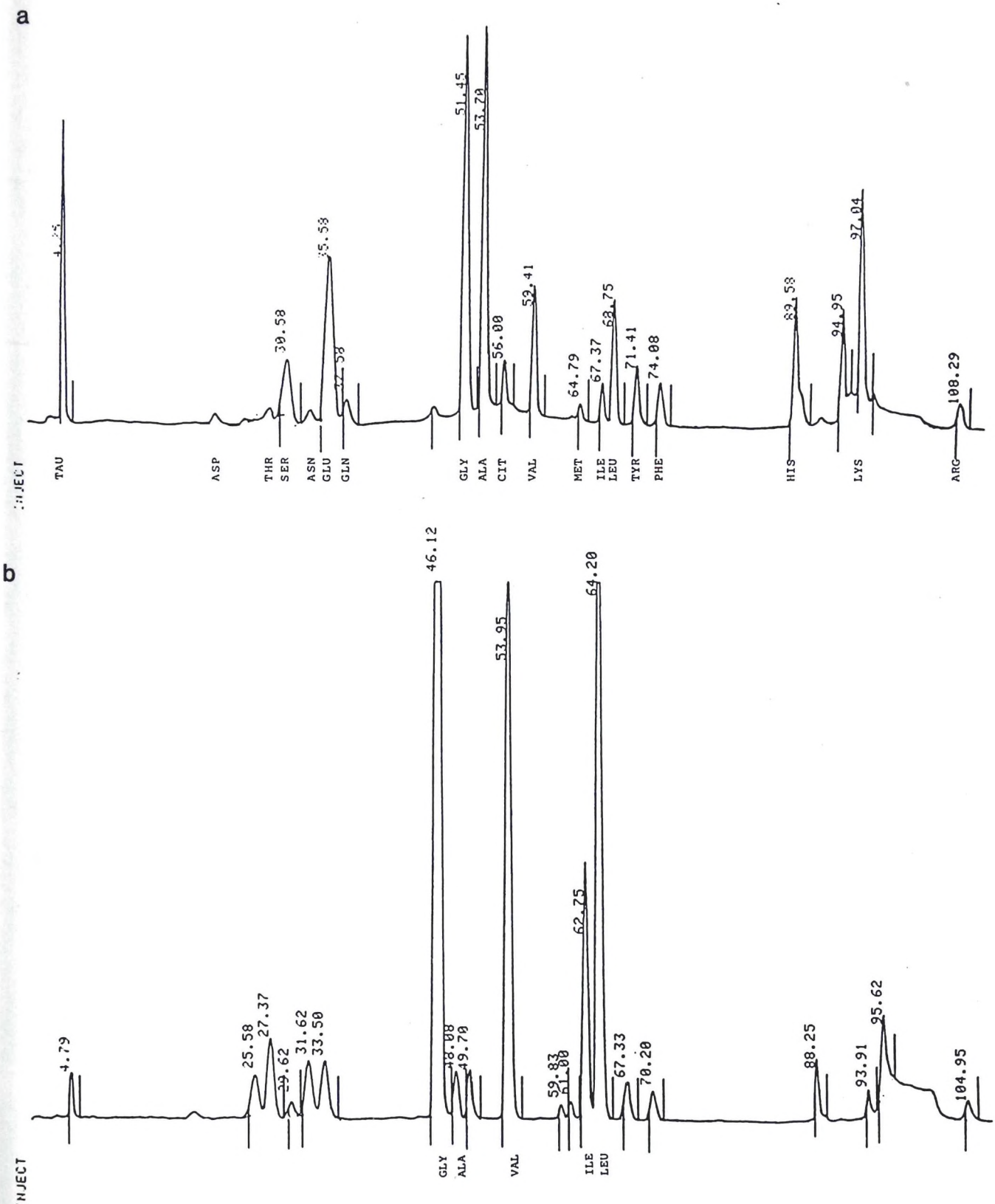


PLASMA AMINO ACID PROFILES.

a) Normal Hereford calf at birth, and b) MSUD calf at birth.

Note the elevated levels of valine, isoleucine and leucine and depressed glycine and alanine levels.

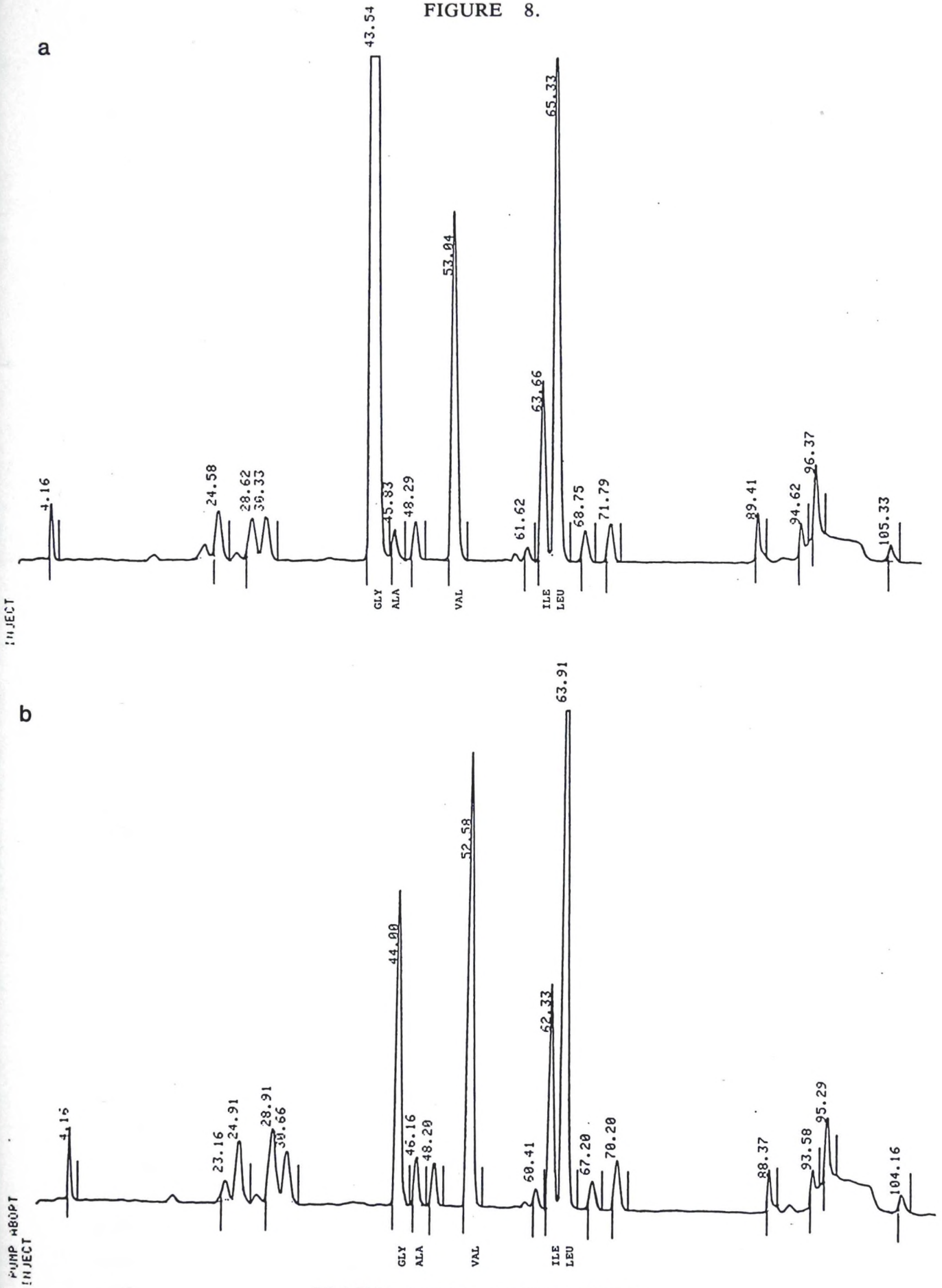
FIGURE 7.



PLASMA AMINO ACID PROFILES.

a) Normal Hereford calf, and b) MSUD calf 32 hours after birth. Concentrations of valine, isoleucine, leucine and glycine have increased after feeding, while alanine remains depressed.

FIGURE 8.



PLASMA AMINO ACID PROFILES.

a) MSUD calf at 24 hours after birth, with severe hyperglycinaemia, and

b) the same calf 30 hours after birth.

The range of amino acid concentrations obtained from analysis of heparin plasma taken at 4 hourly intervals over 3 days from 3 normal calves and 4 MSUD calves is presented in Table 6. With the exceptions of valine, isoleucine, leucine and arginine in the MSUD group, whose concentrations increased over the 3 days, no consistent changes were evident. The changes in BCAA concentrations with time in plasma from a normal and an MSUD-affected calf are illustrated in Figure 9.

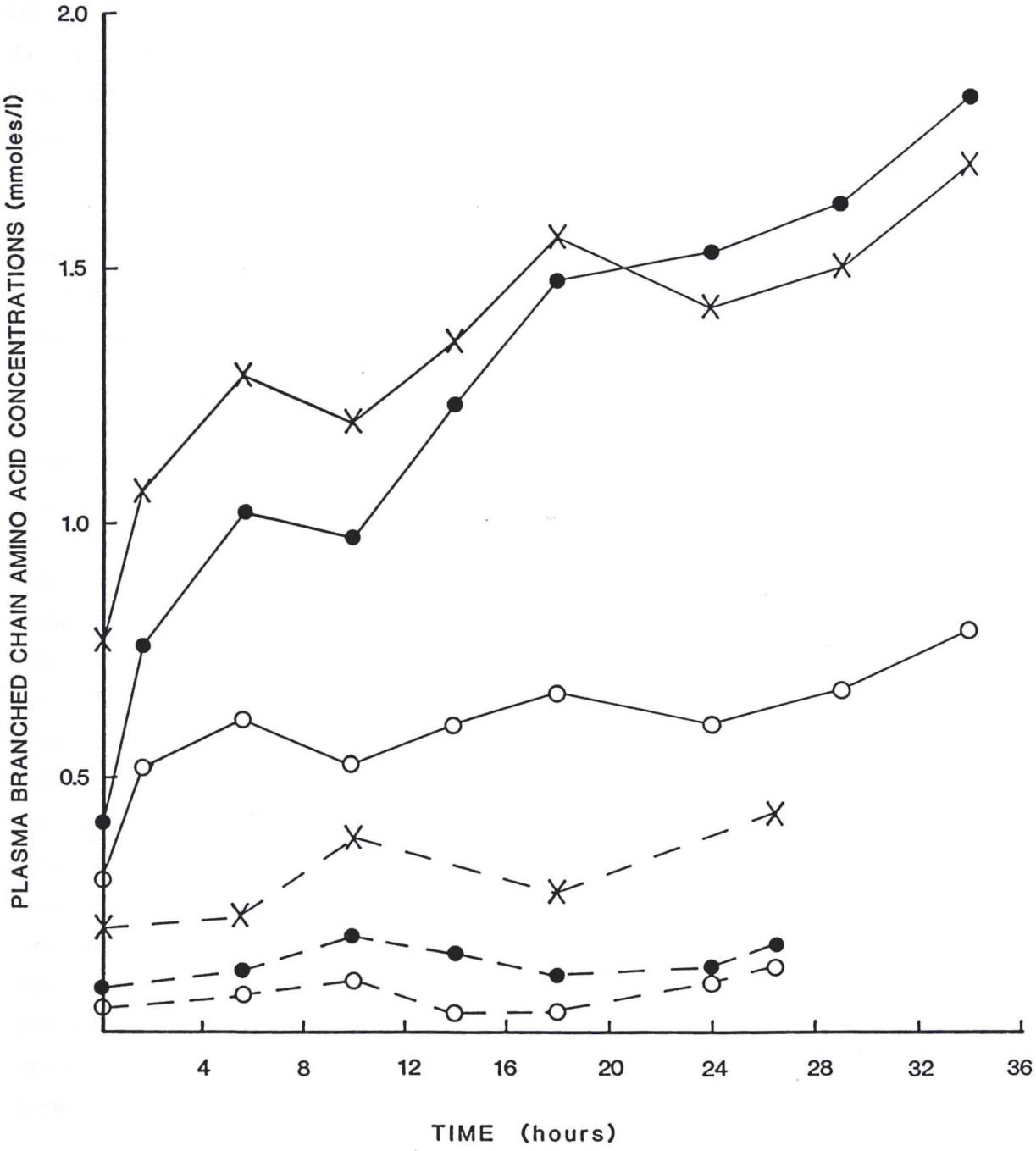
The concentration of noradrenalin in the CSF of 4 ICM-affected calves did not differ significantly from the concentration in 4 normal calves (Table 7). Adrenalin, dopa, dopamine and serotonin could not be detected in any samples. Three peaks that were consistently detected in all samples but not identified, showed no differences in height between the 2 genotypes.

Ratios of the three BCAA's to alanine in plasma, CSF, urine, formalin-fixed cerebrum and the associated formalin from a calf affected with MSUD are presented in Table 8. With the exception of the formalin fixative, the ratios of BCAA's to alanine clearly discriminate between the genotypes.

Plasma concentrations of calcium, magnesium, phosphate, protein, albumin, glucose and urea in ICM-affected calves were not different from concentrations found in normal calves (Table 9). Plasma activities of alkaline phosphatase, creatine kinase, glutamyl transferase, aspartate aminotransferase and ornithine carbamoyltransferase were not different between the 2 genotypes (Table 9). No differences were observed between normal and ICM calves in CSF concentrations of calcium, magnesium, phosphate, protein, glucose and urea (Table 10). Creatine kinase and aspartate aminotransferase activities in CSF samples did not differ between the 2 genotypes (Table 10).

Concentrations of the 3 BCKA's in plasma from 6 MSUD calves were found to be 300-400 times higher than the concentrations in normal calves (Table 11). Analysis of the log-transformed data confirmed the differences as significant. KMVA could not be quantitated at a concentration of less than 5 umoles/l by the method of Daisch and Leonard (1985) because of partial coelution with 3-hydroxybutyrate. Using default values of 5 for KMVA in the normal group, which would have overestimated the true value, comparison with KMVA values from the MSUD group still resulted in highly significant differences. Table 12 shows elevated levels of KIVA, KMVA and KICA at birth

FIGURE 9.



Valine (cross), isoleucine (open circle) and leucine (closed circle) concentrations in plasma samples collected at regular intervals from a calf affected with MSUD (unbroken lines) and a clinically normal calf (broken lines).

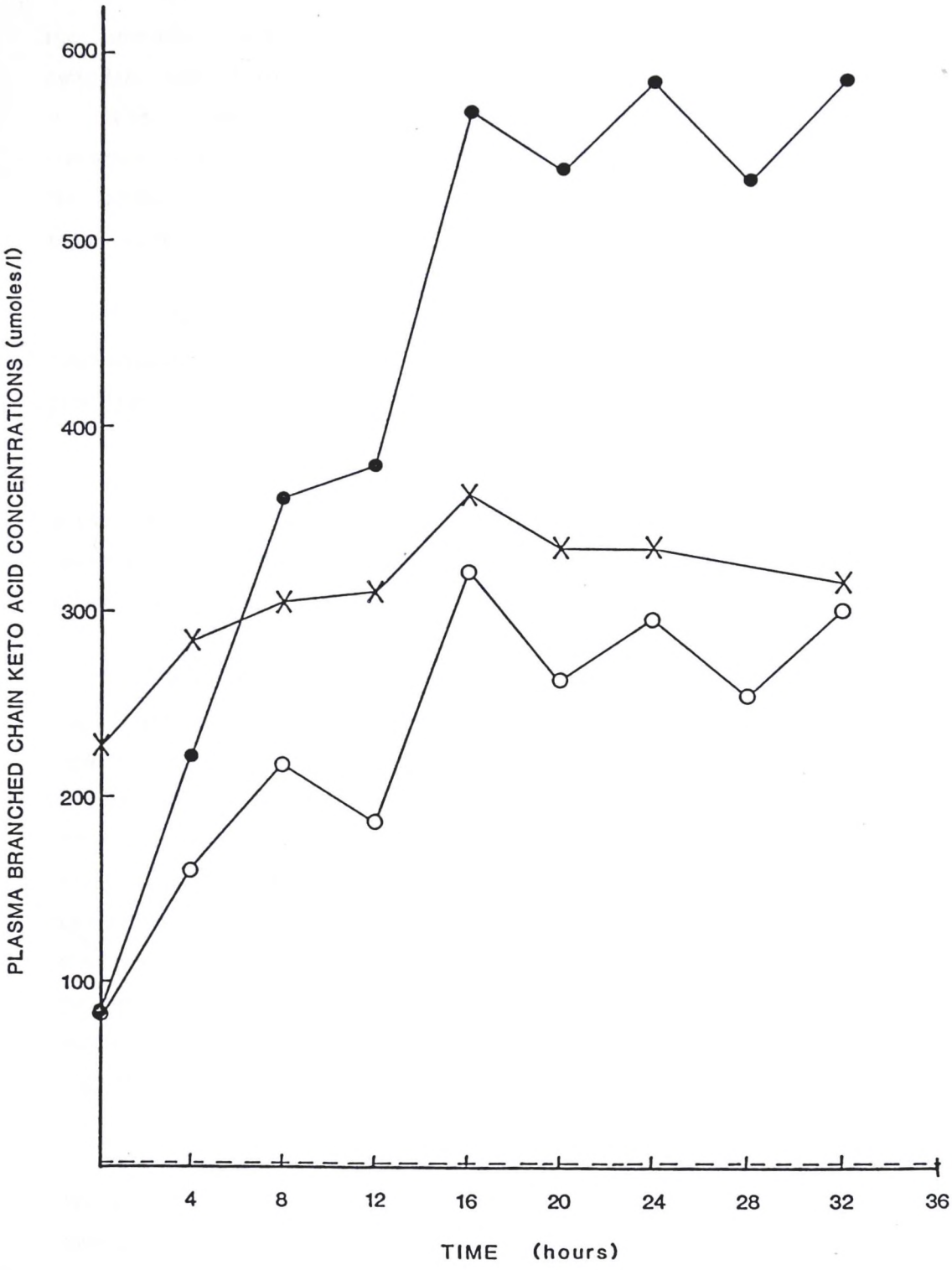
in one MSUD-affected calf (82, 171, 95 respectively). Concentrations increased to 16 hours when they were 321, 272 and 678 umoles/l respectively. Between 16 and 32 hours levels of the 3 acids tended to plateau, ranging in concentration between 321-301, 272-238 and 678-697 umoles/l for KIVA, KMVA and KICA respectively. KICA showed the most remarkable increase throughout the 32 hours (95-697 umoles/l) Concentrations of BCKA's in normal calves from birth to 32 hours showed little change. The contrast is illustrated in Figure 10.

There were no consistent differences in concentrations of BCAA's in formalin fixed tissue between the genotypes. On the other hand, Tables 13 and 14 shows that examination of the ratios of BCAA's to alanine discriminates between MSUD and the 2 other genotypes.

Tables 15 and 16 list the average amino acid concentrations in dorsal and ventral regions of the spinal cord in normal and ICM-affected calves. The differences in concentrations between dorsal and ventral sites for each calf were calculated. Estimation of the t statistic on these differences revealed no genotype effect on site differences. This finding allowed genotypes to be pooled for t tests of site differences for each amino acid. The null hypothesis assumed that the differences between the site means did not differ significantly from zero. The correlations of site differences among amino acids were also determined. Aspartate, glutamine, alanine and GABA were found to have significantly higher concentrations in the dorsal cord than the ventral cord, while glycine, methionine and phosphoethanolamine dorsal concentrations were significantly less than ventral. Differences in concentration between the two sites were positively correlated within these groups of amino acids and negatively correlated between groups. Means of the two regions were calculated to test for differences in whole cord amino acid concentrations between normal and ICM calves. Only threonine was found to differ between the two genotypes, being lower in ICM-affected calves ($p<0.05$).

This finding was not reflected in amino acid concentrations of brain sites from ICM calves. Tables 17-28 summarise the concentrations of amino acids found at 12 different sites of the brains from normal, ICM and MSUD calves. Comparison with normal calves showed taurine to be significantly higher (5%) for ICM calves in the medulla, pons, white matter of the cerebellum and the substantia nigra. Phosphoserine was found to be higher in

FIGURE 10.



Ketoisovaleric acid (cross), ketomethylvaleric acid (open circle) and ketoisocaproic acid (closed circle) concentrations in plasma collected at regular intervals from a calf affected with MSUD (unbroken lines). Normal levels are too low (0.1–5.0 umoles/l) to be plotted accurately on this scale (broken line).

For corrected page, see pocket in back

the medulla, grey and white matter of the cerebellum, frontal cortex, amygdala and thalamus. The other amino acids to have higher concentrations in ICM brains were valine, isoleucine and alanine (caudate nucleus). Glutamate concentration was greater in the caudate of ICM calves but lower in the medulla. Aspartate was the only other amino acid to be lower in ICM brains (pons).

On an average of all the brain sites, only taurine and phosphoserine concentrations were significantly different ($p<0.05$) between the two genotypes, both being higher in ICM calves.

Differences between brain sites/regions were compared between the normal and ICM genotypes (Tables 29, 30 and 31). No interactions were observed between any combination of sites for serine, glutamine, citrulline, valine, methionine, leucine, tyrosine, GABA, histidine, lysine or arginine.

While the concentration of taurine was higher in the white matter of the cerebellum than the grey for both normal and ICM calves, and taurine concentration in the grey matter was similar for both genotypes, the concentration of taurine in the white matter of the cerebellum of ICM calves (70 umoles/g) was much higher than for normal calves (42 umoles/g). Comparison of the medulla and pons with the cerebellum showed a significant interaction that suggested the concentration of taurine in the grey matter of the cerebellum of ICM calves was lower than would be expected given the pattern at the other 3 sites for both genotypes. One other significant interaction of site by genotype for taurine involved all the brain regions, suggesting a reflection of the previous 2 interactions.

The only interaction of site by genotype for phosphoserine was between the white and grey matter of the cerebellum. As was the finding with taurine, analysis of patterns within the matrix showed the level of phosphoserine of ICM calves to be much higher than normal in the white matter of the cerebellum. The remaining 13 significant interactions involved amino acids that were not different on average over all the brain sites. Having established few concentration differences within brain sites between normal and ICM calves and few interactions of sites/regions with these genotypes, data for the normal and ICM calves were pooled to assess differences in amino acid concentrations between sites/regions in the brain.

The summary on site differences provides considerable information on amino acid distributions in the "normal" calf brain (Tables 32, 33 and 34). If a 0.1% significance level is adopted to reduce the possibility of spurious results, the tables may be summarised as follows. Similar amino acid concentrations were observed in the medulla oblongata and pons; the frontal, temporal and occipital cortex; and the putamen and caudate nucleus, with the exception of arginine which was higher in the putamen. Taurine, GABA, arginine and phosphoserine were higher, and alanine lower in the white matter of the cerebellum of normal calves than the grey. Leucine concentrations were lower in the amygdala than the putamen and caudate nucleus. Threonine, glutamate, valine and phosphoethanolamine were lower in the medulla and pons compared to the cerebellum, while arginine was higher. Glycine, methionine, leucine, phenylalanine and histidine levels were lower in the cortex than the basal ganglia. Citrulline and leucine were lower in the substantia nigra than the thalamus. The cortex and basal ganglia had lower concentrations of taurine, glycine, GABA, histidine and arginine compared to the thalamus and substantia nigra. Lower concentrations of threonine, serine, glutamate, leucine and phosphoethanolamine were observed in the hindbrain compared to the rest of the brain, while concentrations of taurine, alanine, arginine and phosphoserine were higher. Compared to the thalamus, the medulla and pons had lower concentrations of threonine, serine, glutamate, leucine, tyrosine, phenylalanine, histidine and phosphoethanolamine. The cerebellum had higher concentrations of taurine, phenylalanine and arginine than the cortex, but a lower level of aspartate. The basal ganglia had lower levels of GABA and arginine than the substantia nigra. Glycine, leucine, phenylalanine and histidine were lower in the combined cerebellum and cortex than the basal ganglia and substantia nigra. The combined cerebellum, cortex, basal ganglia and substantia nigra areas had higher levels of glutamate and phosphoethanolamine than the medulla, pons and thalamus, but lower levels of taurine, arginine and phosphoserine. The concentrations of leucine, phenylalanine, GABA and histidine were higher in the substantia nigra compared to the medulla and pons. Less arginine was observed in the basal ganglia than the thalamus. The cerebellum and cortex had lower concentrations of threonine, serine, glutamate, glycine, leucine, phenylalanine and histidine than the basal ganglia and thalamus. Higher levels of taurine and arginine, and lower levels of threonine, glutamate, valine and phosphoethanolamine were observed in the medulla, pons and substantia nigra compared to the cerebellum, cortex, basal ganglia and thalamus.

Tables 17–28 show the amino acid concentrations found in the 12 brain sites from 6 (or 5) MSUD calves, and indicates where mean concentrations for the MSUD genotype differed significantly from the pooled normal and ICM genotypes. The concentrations of valine, isoleucine, leucine and phosphoethanolamine were elevated in all brain sites of MSUD calves. The other amino acid concentrations that were elevated in the MSUD group were glutamine (all regions except the amygdala and frontal cortex), taurine (grey matter of cerebellum, temporal and occipital cortex) and citrulline (medulla, grey matter of cerebellum, occipital cortex, caudate nucleus and substantia nigra). Aspartate and glutamate were depressed in all sites. Alanine was depressed in all sites except the putamen and substantia nigra. Glycine was depressed in the grey and white matter of the cerebellum, thalamus, putamen, caudate nucleus and substantia nigra. Arginine was lower in all sites except the amygdala; GABA lower in all sites except the amygdala, putamen and caudate nucleus; threonine lower in all sites except the medulla and substantia nigra; and serine lower in all sites except the medulla, pons, grey and white matter of the cerebellum and the substantia nigra. Histidine was depressed in only the grey matter of the cerebellum, thalamus, putamen and caudate nucleus; and methionine depressed in the amygdala, thalamus, putamen, caudate nucleus and substantia nigra. Normal levels of phenylalanine and tyrosine were observed in all brain sites from MSUD calves. Levels of phosphoserine in MSUD brain sites were generally closer to ICM levels, which were different mostly from the normal levels.

On an average of all the brain sites, valine, isoleucine, leucine, citrulline, glutamate and phosphoethanolamine were elevated in the MSUD group, while glycine, alanine, arginine, GABA, aspartate, threonine and glutamate were depressed. Lysine was also depressed over all sites, but the possibility of another peak coeluting in the normal and ICM profiles raised doubt as to the significance of this result.

Tables 35, 36 and 37 summarise brain site/region differences observed within the MSUD genotype. At first glance, a comparison of these results with the site differences found in normal calves (Tables 32–34) suggests the MSUD brain is more homogeneous with respect to amino acid distributions. This interpretation may be unfounded, as variability was greater in the MSUD group, and sample size smaller. This means that differences of a similar order as the normal group could be "masked" in the MSUD group. The only acids to show strong site variations were GABA and

phosphoethanolamine. Lower GABA levels were observed in the white matter of the cerebellum compared to the grey, the cerebellum and cortex compared to the basal ganglia and thalamus, the medulla and pons compared to the substantia nigra and to the white and grey matter of the cerebellum. Lower levels of both GABA and phosphoethanolamine were observed in the medulla and pons compared to the thalamus; and the hindbrain compared to the rest of the brain.

The interactions of site/region differences with differences between MSUD and normal brains are given in Tables 38, 39 and 40. These interactions can be assessed from data presented in Tables 17–37. For example, there was a significant interaction for GABA between genotypes and the medulla and pons sites. While the concentration of GABA was lower in the MSUD brains at both sites, the difference in concentration between MSUD and normal brains was greater in the pons than the medulla.

A significant interaction of taurine between genotypes and the grey and white matter of the cerebellum was complicated by an elevated level in the white matter of the cerebellum of ICM brains. A problem also arose with phosphoserine, as this amino acid was significantly higher for ICM brains in 6 of the 12 brain sites. Because of the significant ICM effect for taurine and phosphoserine at some sites, ICM brains could not be treated as "normal" at these sites, or regions that included these sites.

Some of the interactions of site/region differences with differences between MSUD and normal brains may be summarised as follows. As a consequence of MSUD :

- the depression of threonine and serine in the thalamus was greater than the substantia nigra, medulla, pons and basal ganglia,
- lower glutamate levels in the white matter of the cerebellum than the grey, and lower glutamate in the thalamus than the substantia nigra were observed,
- a smaller increase in glutamine was observed in the thalamus compared to the medulla and pons,
- glycine was depressed more than normal in the basal ganglia of MSUD calves compared to the cortex. In contrast, alanine levels were more depressed in the cortex compared to the basal ganglia,
- the difference in methionine concentration between the medulla and pons was reversed. Methionine levels were elevated in the frontal and temporal cortex compared to the occipital cortex, but levels were depressed in the basal ganglia compared to the whole cortex,

- the 3 BCAA's were more elevated in the substantia nigra than the basal ganglia, particularly in relation to the amygdala and caudate nucleus,
- a greater increase in isoleucine and leucine levels were observed in the substantia nigra compared to the thalamus,
- GABA was depressed by varying amounts for most sites and regions. This effect was greater in the pons than the medulla, which contributed to the interaction between genotypes and the medulla/pons and cerebellum. GABA levels in the cerebellum were more depressed than the cortex, which in turn was more depressed than the thalamus and substantia nigra. GABA concentrations for the basal ganglia were not significantly different from normal,
- arginine levels were lower for most sites, with the effect less in the amygdala than the rest of the basal ganglia, which in turn was less affected than the thalamus and substantia nigra. The effect was less in the cerebellum compared to the medulla and pons, and,
- phosphoethanolamine was elevated in all sites and regions. This effect was greater in the cortex than the cerebellum. The effect on the cerebellum and thalamus was similar, with both these sites affected more than the medulla and pons.

Table 41 summarises the clinical, pathological and biochemical findings associated with the ICM and MSUD genotypes. It is apparent from this table that the two conditions share few common features.

DISCUSSION.

Amino acid analyses of body fluids and tissues from Poll Hereford and Poll Hereford cross calves displaying dullness and opisthotonus, with status spongiosus of the CNS, has led to the discovery of the first aminoacidopathy in ruminants. Demonstration of a deficiency in the activity of the enzyme BCKAD in fibroblast cultures from calves with elevated levels of valine, isoleucine and leucine (G. Brown, unpublished data) has confirmed the condition as MSUD, the only model of this disease in man. The severity of the clinical signs and the biochemical observations recorded in this study indicate that the disease in calves is equivalent to the classical form of MSUD in humans (Tanaka and Rosenberg, 1985).

In the absence of serum/plasma or CSF, amino acid analyses of formalin-fixed tissues has enabled diagnoses of MSUD made on the basis of clinical and/or pathological observations. When suitable samples are available, more subtle metabolic variations such as nutritional effects can also be investigated. Comparison of amino acid concentrations between serum and plasma from clinically normal newborn calves revealed higher concentrations of serine, glutamate, taurine and arginine in serum. Red cells contain more taurine, aspartate, glutamate and glycine than plasma (Stein and Moore, 1954). Leaching may therefore account for these differences. As few amino acid concentration differences are observed in blood between the normal and ICM calves, analysis of plasma samples should be preferred to avoid the artifacts associated with leaching.

a) Inherited Congenital Myoclonus.

The possibility that "neuraxial oedema" consisted of more than one disease entity was considered prior to amino acid analyses, as there were inconsistencies in reported clinical signs and pathological findings. Our initial work on "neuraxial oedema" almost exclusively involved ICM calves. Results of breeding experiments comfirmed an autosomal recessive mode of inheritance (46 cases from 203 calves) and demonstrated that ICM calves have a shorter gestation period. The herd provided material for a detailed clinical and pathological description of the disease (Healy et al, 1985; Harper et al, 1986a). The clinical signs of bright, alert calves unable to stand from birth, displaying myoclonic spasms and whole body rigidity or

tetany on lifting, were very consistent (Figs. 1 and 2). None of the calves had "oedema of the neuraxis", though most had traumatic hip joint lesions. The chronic nature of the lesions in the hip joints proved expression of the disease in utero. Electromyographic studies on two affected calves and one normal calf revealed no differences in spontaneous electrical activity in the lumbar paraspinous muscles. Sciatic nerve conduction velocities were approximately 60m/sec and consistent responses were recorded at the distal site after repetitive nerve stimulation in each of the three calves. These findings suggested the ICM lesion was unlikely to be in the musculature, the neuromuscular junction or the peripheral nerves (Harper et al, 1986a).

Biochemical findings were unremarkable. Plasma and/or CSF concentrations of calcium, magnesium, phosphate, glucose, urea, protein and albumin (plasma only) did not differ between normal and ICM calves; neither did plasma activities of alkaline phosphatase, creatine kinase (CSF and plasma), aspartate amino transferase (CSF and plasma), gamma glutamyl transferase or ornithine carbamyl transferase.

In 1983, a stillborn male foetus was delivered with assistance, following prolonged labour. Post mortem examination of the calf revealed severe hydranencephaly affecting the fore- and mid-brain with a reduction in the size of the cerebellum. The brainstem and spinal cord were intact. Antibodies to akabane virus were detected in heart blood and cranial fluid, indicating that the calf was affected with Akabane disease (Hartley et al, 1977). Acute and chronic lesions were found in both hip joints. Previous reports of "neuraxial oedema" suggested that lesions in the coxae of newborn Poll Hereford calves could be considered sufficient evidence for a provisional diagnosis of the disease (Donaldson and Mason, 1984). This conclusion is supported by the results of our observations on ICM affected calves (Harper et al, 1986a; Healy et al, 1986). However, as not all ICM affected calves have lesions in the coxae, absence of that lesion does not preclude a diagnosis of ICM. As similar lesions are not a feature of Akabane disease, we concluded that this stillborn calf was affected with both Akabane disease and ICM. The presence of hip lesions secondary to myoclonic spasms in the absence of a fore- and mid-brain indicated that the lesion in ICM was posterior to the midbrain. The clinical features of spontaneous and stimulus responsive myoclonic jerks suggested that ICM could be the result of an abnormality in an inhibitory feedback mechanism in the brainstem or spinal cord, resulting in a failure to moderate motor responses. This hypothesis

prompted amino acid analyses with specific reference to inhibitory neurotransmitters - glycine, taurine and GABA. Glycine has been isolated in presynaptic nerve endings (Aprison and Werman, 1965), and shown to have an inhibitory effect on the spinal cord (Curtis et al, 1968). A familial disorder of glycine metabolism coincident with a spinal cord disorder has been reported (Bank and Morrow, 1972; Bank et al, 1978). No differences were observed in free glycine concentrations however in serum or CSF between normal and ICM calves.

Lower concentrations observed in the plasma from ICM calves for citrulline, valine, isoleucine, leucine, tyrosine and phenylalanine were not reflected in the CSF, suggesting the differences are a consequence of a visceral rather than CNS metabolic effect. The differences in the plasma may be a nutritional effect, as ICM calves cannot feed from their mothers, and so must be bottle fed. No differences between the two genotypes were observed in the concentrations of free amino acid neurotransmitters GABA, taurine, aspartate, glutamate, glycine and noradrenalin in body fluids.

In neither normal nor ICM-affected calves were there significant differences in free amino acids between dorsal and ventral regions of the spinal cord. In both dorsal and ventral regions of the lumbar cord of the cat, higher concentrations of the neurotransmitter amino acids are found in the grey matter than in the white matter (Graham et al, 1967; Johnston, 1968). In grey matter, Johnston (1968) found glycine and aspartate to be more concentrated in feline ventral cord than dorsal cord, while the reverse occurred with GABA, glutamine and glutamate. With the exceptions of glycine and aspartate, amino acids were found to be evenly distributed in the white matter of dorsal and ventral regions (Johnston, 1968). It is possible that similar amino acid differences between dorsal and ventral regions exist in bovine spinal cord, but may only be evident when the grey and white matter are separated.

Although it has been shown that glycine has an inhibitory effect on the spinal cord when applied iontophoretically, and is the most potent of the known inhibitory neurotransmitters (Curtis et al, 1968), there were no differences observed in glycine concentrations between the two genotypes. The most abundant amino acid found in the spinal cord of both genotypes was glutamate, as Gabrys and Konecki (1981) noted in their analysis of bovine spinal cord using gas chromatography. The reason for depressed threonine

concentrations in the dorsal and ventral regions of the spinal cord of ICM-affected calves is not known. This amino acid is not implicated as a neurotransmitter, nor is it structurally related to one (Curtis and Johnston, 1974). Depressed threonine concentrations were not observed in any of the 12 brain regions of ICM calves.

The concentrations of amino acids in the 12 brain sites were similar between normal and ICM calves (Tables 17-28). These findings are consistent with the results obtained from the body fluids and spinal cords. Only taurine, phosphoserine and glutamate concentrations differed at more than one site between normal and ICM calves; with only taurine and phosphoserine significantly different on an average of all the brain sites between the 2 genotypes.

The higher concentrations of valine and isoleucine in the caudate nucleus of ICM calves were the opposite of observations on amino acid differences in plasma. The other amino acids to show single site differences - alanine and aspartate - were not significantly different in the body fluids. There was no significant site by genotype interaction for valine. Alanine showed more than one significant interaction which may reflect nutritional differences between the normal and ICM genotypes. As valine, isoleucine, alanine and aspartate were not significantly different on an average of all the brain sites between normal and ICM calves, it is likely these finding reflect random fluctuations.

Glutamate was lower in the medulla oblongata of ICM calves, but higher in the caudate nucleus. This is the most abundant amino acid in the CNS, and regional variations in its distribution are thought to reflect roles as both a synaptic transmitter and a major brain metabolite (Johnson and Aprison, 1971). The changes observed in isolated regions of this proposed excitatory neurotransmitter however, may not be consistent enough to suggest a direct role in the pathogenesis of ICM. This is supported by only one significant brain site interaction (Table 29), due to lower glutamate levels in the medulla, pons and substantia nigra of ICM brains.

The higher concentrations of phosphoserine observed in 7 brain regions of ICM calves is difficult to interpret, as levels were not determined in body fluids. Although only one site by genotype interaction was noted, the consistently higher concentrations in CNS regions of ICM

calves, resulting in an overall brain difference between normal and ICM genotypes, suggests that phosphoserine levels may be altered as a consequence of ICM. As phosphoserine is not implicated as a neurotransmitter, it is unlikely to reflect the primary biochemical lesion.

There were 3 brain site interactions observed with taurine between normal and ICM calves, with the difference between the white and grey matter of the cerebellum significantly higher in ICM calves at the 1% level of significance. Taurine is considered to have a similar action to glycine (Curtis and Johnston, 1974), having a depressant effect on spinal neurones, brain-stem neurones and cortical neurones (Rassin, 1981). Although the concentration differences of taurine between the 2 genotypes were found in only 4 of the 12 brain sites, 3 of these 4 sites are located in the hindbrain. As this was the only part of the brain found in the Akabane/ICM-affected calf, the possibility that taurine is involved in the pathogenosis of ICM cannot be excluded.

Studies of receptor site function in synaptosome preparations from spinal cords of normal and ICM-affected calves revealed significant genotype differences (Gundlach et al, 1988). Binding experiments have shown a deficit of strychnine binding (glycine receptors) in ICM calves. On average, only 5% strychnine binding could be demonstrated in ICM cords, indicating a deficiency of functional glycine receptor sites. These findings are consistent with the neurological signs of myoclonic jerks and tetanic spasms being the result of a failure in the inhibitory feedback provided by the recurrent motor-axon collaterals that synapse with the interneurones in the spinal cord (Lewis, 1976). Furthermore, an increase in the rate of glycine uptake by synaptosomes from ICM-affected calves may potentiate this effect (Gundlach et al, 1988). These findings suggest the higher taurine concentrations observed in the 4 brain sites of ICM calves may be a secondary effect, either a direct consequence of the glycine receptor site abnormality, or a nutritional effect imposed by the ICM calf's inability to feed from its mother. With so few differences in concentrations between the 2 genotypes, the possibility of random fluctuations in the data cannot be ignored.

As few differences were found in the brain data between the normal and ICM genotypes, the two groups were pooled to provide reference material on amino acid distributions in the bovine CNS. The only other species where similar regions have been mapped for such a range of amino acid distributions

is man (Perry et al, 1971a,b). Most reports are concerned only with the putative neurotransmitters in brain regions of either man (Curtis and Johnston, 1974; Ellison et al, 1987) or rats (Erecinska et al, 1984; Chapman et al, 1986). The concentration of GABA in the calves was highest in the substantia nigra, a finding that is consistent in both man (Perry et al, 1971b) and rats (Chapman et al, 1986).

The least number of differences in amino acid concentrations were observed between the three cortical areas, suggesting homogeneity of these regions. A similar trend has been reported in the human cortex (Perry et al, 1971b). This finding is useful in interpretating the results of amino acid analyses of cerebrum in the diagnoses of bovine aminoacidopathies, where the cortical region submitted is not always specified.

The only region in this study where the white matter has been separated from the grey is the cerebellum, and concentration differences are evident in 12 of the 21 amino acids listed. This no doubt reflects the different physiological roles of the two regions, particularly with differences in neurotransmitter levels such as taurine, glutamate and GABA.

Concentrations of taurine, leucine, phenylalanine, arginine and phosphoserine were higher in the cerebellum compared to the cortex. With the exception of phosphoserine which was not studied, these results are consistent with findings in man (Perry et al, 1971b). Lower concentrations of serine and glutamate in the cerebellum of calves was not evident in the data presented by Perry et al (1971b) but have been reported in rats (Chapman et al, 1986). With the exception of aspartate, most amino acids are more concentrated in the basal ganglia than the cortex. As the basal ganglia plays a major role in the control of motor function, investigation of neurotransmitter levels in this region is essential in the study of myoclonic conditions such as ICM.

b) Maple Syrup Urine Disease.

The existence of a second neurological disease in newborn Poll Hereford calves was suspected due to inconsistencies in reports of "neuraxial oedema". In some instances, calves that were reported to be normal immediately after birth, were found dead or recumbent within the next 7 days. In contrast to ICM affected calves, these animals were found to have

vacuolation of the CNS as described by Cordy et al (1969). The first live case of this latter condition to be examined by Drs. Healy and Harper provided the material to distinguish the two syndromes (Healy et al, 1986). Clinical signs were different from those observed in ICM-affected calves. The calf was dull, lay in lateral recumbency, displaying opisthotonus and a paddling motion of the forelimbs. No hip joint abnormalities were evident, but a strong, sweet smell of the urine was detected. Histological examination revealed vacuolation of the white matter of the cerebellum and brain stem. Amino acid analysis of the plasma revealed elevated concentrations of the 3 BCAA's, valine, isoleucine and leucine. Levels of valine, isoleucine and leucine were also elevated in the CSF, urine, and fresh nervous tissue. Analysis of formalin-fixed cerebrum revealed elevated ratios of branched to straight chain amino acids. A 2,4 dinitrophenylhydrazine test of the urine indicated excretion of excessive amounts of BCKA's. Keto acids are reported to contain trace contaminants that smell like maple syrup (Snyderman et al, 1964). Although a deficiency of BCKAD activity had yet to be demonstrated, the evidence suggested a diagnosis of MSUD (Dancis and Levitz, 1978). A deficiency in activity of this enzyme would result in accumulation of the 3 BCKA's, KIVA, KMVA and KICA, as they could not be decarboxylated to their coenzyme A derivatives. Because the transamination step from BCAA's to BCKA's is reversible, the reaction is forced back in the direction of the BCAA's, thereby increasing their concentrations. Amino acid analyses of formalin-fixed cerebrum from 5 previous cases of "neuraxial oedema" with status spongiosus and similar clinical histories, also showed elevated ratios of branched to straight chain amino acids (Harper et al, 1986b).

As with the ICM herd, dams and sires of MSUD-affected calves were purchased to create an experimental herd. A limited number of obligate heterozygote cows were available, so multiple ovulation-embryo transfer programs were undertaken to obtain material for a clinical, pathological and biochemical study of this condition. As with ICM, MSUD was shown to be inherited in an autosomal recessive manner (8 cases from 29 calves). Affected calves were normal for the first day but consistently had an "unclean" appearance about the white parts of their faces (Fig. 3). On the second day of life, affected calves were dull and recumbent, deteriorating to opisthotonus by the second or third day (Fig. 4). Valine, isoleucine and leucine, and the corresponding BCKA's, KIVA, KMVA and KICA were elevated at birth in plasma (Fig. 6) and CSF. Elevated ratios of BCAA's to alanine were

also observed in fixed tissue from a stillborn calf that had histological lesions consistent with MSUD.

These findings are consistent with prenatal occurrence of the condition. This contrasts with the condition in humans where analyses of blood and urine at birth does not reveal significant abnormalities (Dancis and Levitz, 1978). Differences in the anatomical relationship of the maternal and foetal circulations between the species may explain this variation. In humans the close proximity of maternal and foetal circulation may facilitate the removal of metabolites from the foetus.

Plasma concentrations of the 3 BCAA's were approximately 10 times higher in MSUD calves (Fig. 7), while in CSF, concentrations were over 100 times higher. Such high CSF concentrations suggest the BCAA's may be considered the possible toxic components responsible for the neurological signs. An improvement in clinical signs has been reported in a patient suffering from the classical form of MSUD after plasma levels of leucine and KICA fell below 1mM (Shigematsu et al, 1983) Most reports however, implicate the BCKA's, particularly the leucine derivative, KICA as the toxic component. Howell and Lee (1963) demonstrated a 10–27% depression of oxygen utilisation in rat brain slices in the presence of KICA and KIVA. As BCAA's had no effect, it was concluded that CNS dysfunction in MSUD patients may be related to an accumulation of BCKA's. Silberberg (1969) demonstrated delayed myelination in the presence of KICA at concentrations of 1–2mM, while the BCAA's and the other two BCKA's did not affect myelin formation. This finding suggests KICA is at least partially responsible for the neurological damage resulting with MSUD in man, as myelin formation is incomplete at birth. Plasma concentrations of KICA and KIVA were found to be 300–400 times higher in MSUD–affected calves (Table 12), but a qualitative deficiency of myelin has not been observed (Harper et al, 1986b). The most significant electron microscopic lesion observed in MSUD in calves is one of myelin splitting, which results in an oedematous appearance of the CNS (Harper et al, 1986c). Although BCKA's are elevated at birth (Table 11), maternal circulation has restricted foetal concentrations approaching the critical mmolar levels. As myelin formation in bovine CNS is well advanced at birth, it is likely that the rises in BCKA's that occur after birth are of minimal consequence with respect to delaying myelination formation (Fig. 10).

The consistent finding of lower alanine concentrations in plasma and CSF from MSUD-affected calves may reflect a number of processes. Haymond et al (1973) noted that a fall in BCAA concentrations following a special MSUD diet restricting these amino acids, was accompanied with a rapid increase in the concentrations of glutamine and alanine. The conversion of BCAA's to their corresponding BCKA's is considered to account for approximately 10% of the total transaminases in the body (H. Oddy, pers. comm.). Low net transamination of the BCAA's may lead to reduced alanine and glutamine concentrations as these amino acids generally come from skeletal synthesis employing amine groups from the BCAA's (Haymond et al, 1978). Haymond and others (1978) also showed that alanine flux rates were lower in MSUD patients. They concluded that BCAA catabolism is an important rate-limiting event for alanine production in vivo. In severe neurological conditions such as MSUD, the calfs inclination and ability to feed deteriorates, resulting in less glucose available for alanine synthesis. Excessively high levels of leucine are also considered to depress blood glucose levels, even in normal subjects (DiGeorge et al, 1963). KICA added to brain homogenates has been shown to partially inhibit decarboxylation of pyruvate (Dreyfus and Prensky, 1967), further inhibiting the production of alanine.

Elevated levels of the BCAA's may disturb transport of other amines and distort the free amino acid pools (Carver, 1969). This is evidenced in the elevation of many straight-chain amino acids in the plasma and CSF of MSUD-affected calves. Citrulline and arginine were elevated in plasma, and glutamine and arginine were elevated in the CSF, suggesting a disturbance of the urea cycle is a consequence of the disease. The increased concentration of phenylalanine in plasma and CSF may indicate liver damage, as aromatic amino acids are elevated in such cases (G. Brown, pers. comm.).

The significance of elevated glycine in CSF is not understood. Hypoglycemic episodes occur in MSUD-affected children as a consequence of depressed blood glucose levels (MacKenzie and Woolf, 1959). Glycine levels varied considerably in plasma (CV:54%) and CSF (CV:74%), but they rarely dropped below normal values. In an extreme case, an MSUD calf born in the experimental herd had a plasma glycine concentration of 6576 umoles/l, which dropped to 934 umoles/l after 6 hours (Fig. 8). The high concentration was independently confirmed (Royal Childrens Hospital, Melbourne), but an explanation could not be found. A disorder of amino acid metabolism characterised by elevated glycine levels in the blood and urine, has been

described in man (Childs et al, 1961). Ketosis was an important feature, and clearly related to the metabolism of leucine, but the cause was undetermined. The reason for elevated levels of threonine, serine, methionine, tyrosine and histidine in the CSF of MSUD-affected calves is also not known.

In an attempt to relate BCAA concentrations to clinical signs, amino acid concentrations were determined on plasma taken at four hourly intervals over the three day life span of four MSUD-affected calves. Snyderman et al (1964) related the occurrence of acute symptoms of MSUD to an excess of leucine, rather than valine or isoleucine. Although a correlation between leucine concentration and severity of clinical signs could not be established, it was noted that the onset of clinical signs in the 4 calves coincided with the time the leucine:valine ratio exceeded one (12-16 hours), (Fig. 9).

Marked differences were observed in amino acid concentrations between normal and MSUD calves for the 12 brain regions, with concentrations often reflecting the patterns seen in plasma and CSF. On an average of all brain sites, valine, isoleucine, leucine and citrulline were elevated in MSUD calves and alanine was depressed, findings that are consistent with those seen in both plasma and CSF. As the concentrations of the BCAA's were elevated in all brain sites, it is possible they are partially responsible for the disturbances noted in other amino acids at each site. Phenylalanine and tyrosine were the only amino acids to remain within normal levels for the 12 brain regions.

Changes have been observed in the free amino acid patterns in foetal rat brain after prolonged exposure to elevated levels of each of the three BCAA's (Carver, 1969). Elevated levels of isoleucine were found to depress aspartate, methionine, tyrosine, phenylalanine and histidine. This may account for lower aspartate levels observed in all brain regions in MSUD calves, as well as lower methionine and histidine levels in isolated regions. Elevated levels of leucine and valine resulted in corresponding increases in arginine (Carver, 1969). This finding is reflected in the CSF and plasma results for MSUD calves, but arginine concentrations in the brain are considerably lower than normal. Arginine was only one of two amino acids to show a significantly different brain result from normal that was the reverse of that observed in the body fluids. The reason for this anomaly is not clear, but combined with elevated citrulline levels in the brain, plasma and

CSF of MSUD-affected calves, lower aspartate levels in all sites of the MSUD brain, and elevated glutamine in MSUD brain and CSF, it supports an earlier suggestion that a disturbance of the urea cycle is a consequence of the disease. A disturbance of the urea cycle may result in increased levels of ammonia. As glutamine synthesis provides a means of removing ammonia, the elevated levels of glutamine observed in the CNS of MSUD-affected calves would be expected. Glycine was the other amino acid to be lower in the brain of MSUD calves, but higher in the CSF. The brain result is consistent with hypoglycemic episodes observed in children with MSUD (MacKenzie and Woolf, 1959).

Glutamate was depressed in all regions of the MSUD brain, and GABA depressed in most regions. GABA is formed in the brain by enzymatic decarboxylation of glutamate via glutamate decarboxylase (GAD). Derivatives of phenylalanine, valine and leucine were found to inhibit in vitro activity of GAD in rat brain homogenates (Tashian, 1961). The inhibition was shown to be competitive and substrate dependent. It was suggested that metabolites of these amino acids, particularly valine and leucine which are formed in increased amounts in MSUD, may be responsible for the limited formation of GABA. Prensky and Moser (1966) noted similar findings in the frontal lobe of a 25 day old patient with MSUD. In contrast to the levels in the brains of MSUD calves however, Prensky and Moser reported lower glutamine concentrations and few changes with other amino acids. This was thought to reflect postmortem changes which would obscure any amino acid deficiencies that may have been present. It was suggested that depressed GABA and glutamate levels may indicate a disturbance of the Krebs cycle. Dreyfus and Prensky (1967) demonstrated reduced GABA and glutamate in MSUD patients, determined a normal GABA:glutamate ratio, and hence suggested the activity of GAD was probably normal. It was noted that decarboxylation of pyruvate was reduced in the presence of mmolar concentrations of KICA, supporting the theory that reduced GABA and glutamate concentrations are the result of a failure of the Krebs cycle.

Depressed alanine concentrations in most brain regions of MSUD calves is consistent with the findings in plasma and CSF, and with observations in man (Snyderman et al, 1964; Haymond et al, 1973; Haymond et al, 1978). As alanine is formed by the transamination of glutamate and pyruvate, it is likely that lower brain levels of glutamate, and the suppression of the decarboxylation of pyruvate due to elevated KICA

concentrations (Dreyfus and Prensky, 1966) are the main factors in inhibiting the production of alanine.

Phosphoethanolamine was considerably higher in all regions of the MSUD brain compared to normal levels by a similar order of magnitude as the BCAA's. Unfortunately this amino acid is rarely mentioned in reports of MSUD, so the significance of this finding is not understood.

As with the normal and ICM genotypes, the distributions of amino acids within the MSUD genotype were determined (Tables 35–37). Comparison with normal brain suggests a more homogeneous distribution between sites for MSUD brains, though smaller sample size and higher variability in the MSUD group restricts this interpretation.

The results presented in this thesis illustrates the profound effect MSUD has on metabolic processes other than BCAA metabolism. The mechanisms of these disturbances have not been elucidated as the toxic metabolite has yet to be identified, but the inability to metabolise three essential amino acids, and subsequent disturbances to the Krebs and urea cycles, undoubtedly contributes to the metabolic aberrations that result in the neurological dysfunction of MSUD.

Establishing normal amino acid concentrations in tissues and body fluids will be of value in the investigation of neurological conditions in cattle. Amino acid analyses has provided a more objective means of discriminating between ICM and MSUD than clinical evaluation and pathological observations. The probability that other aminoacidopathies exist in ruminants, as is the case in humans, has been confirmed in this laboratory by the finding of Citrullinaemia in Friesian calves (Harper et al, 1986d). Citrullinaemia is an autosomal recessive defect in man arising from a deficiency of argininosuccinate synthetase activity. This enzyme produces arginosuccinate from citrulline and aspartate in a cyclical process involving the removal of ammonia, a toxic metabolite of amino acid metabolism. Demonstration of elevated levels of citrulline in affected calves ultimately led to the definition of this disease in cattle. The fact that 50% of Friesians in Australia are descendants of an obligate heterozygote for citrullinaemia highlights the potential for widespread dissemination of genetic defects in livestock. It seems reasonable to predict that other

inborn errors of amino acid metabolism will emerge, and the data presented in this thesis will serve as reference material to aid in the diagnosis of such conditions.

TABLE 1.

CHARACTERISTICS OF GENOTYPES.

GENOTYPE	CLINICAL SIGNS	CNS PATHOLOGY	OTHER PATHOLOGY
Normal	Nil	Nil	Nil
ICM	Myoclonic jerks from birth.	Nil	Hip lesions
MSUD	Initially normal, opisthotonus.	Vacuolation in white matter of cerebellum	Nil

TABLE 2.

AMINO ACID STANDARDS

Variations in retention times, response factors and peak heights over five days for 22 amino acids, each at a concentration of 0.3nm/ul.

Amino Acid	Retention Times		Response Factors		Peak Heights	
	Mean	% CV	Mean	% CV	Mean	% CV
Taurine	4.37	5.5	2.21	5.0	121.2	6.1
Aspartate	15.92	5.5	0.53	6.2	28.8	6.7
Threonine	21.72	5.4	0.75	3.6	41.0	3.9
Serine	23.28	5.3	1.19	1.6	65.0	2.4
Asparagine	25.24	5.3	0.62	2.1	34.0	2.1
Glutamate	26.72	5.3	0.84	1.8	45.8	2.4
Glutamine	28.37	5.1	0.68	3.2	37.4	2.4
Glycine	41.00	3.8	1.05	4.5	58.0	6.2
Alanine	43.41	3.4	0.88	1.0	48.4	3.1
Citrulline	45.09	2.9	0.88	1.0	48.2	3.1
Valine	50.39	2.2	0.72	6.4	39.6	6.8
Methionine	57.95	1.3	0.92	2.8	50.6	3.3
Isoleucine	61.70	1.2	0.85	4.0	47.6	4.4
Leucine	63.65	1.2	0.96	0.9	53.0	3.2
Norleucine	65.41	1.2	1.00	0.0	54.8	3.0
Tyrosine	67.18	1.2	0.64	1.4	35.4	2.5
Phenylalanine	70.45	1.2	0.75	2.0	41.2	3.6
GABA	78.87	1.4	0.83	5.3	45.6	5.9
Histidine	89.55	0.6	1.31	5.1	72.0	5.6
Tryptophan	92.11	0.8	0.70	8.6	38.4	9.5
Lysine	96.89	0.6	2.47	2.7	134.0	3.6
Arginine	107.29	0.9	0.50	5.8	27.2	6.6

TABLE 3.

PRIMARY AMINO ACID CONCENTRATIONS (uM) IN SERUM AND PLASMA FROM NORMAL CALVES.

AMINO ACID	SERUM (n=7) Mean	SD	PLASMA (n=7) Mean	SD	p
Taurine	147	81	115	72	**
Aspartate	15	15	6	9	NS
Threonine	49	17	52	17	NS
Serine	124	45	113	44	*
Asparagine	61	33	48	20	NS
Glutamate	151	136	125	111	NS
Glutamine	645	309	555	290	*
Glycine	577	210	488	217	NS
Alanine	574	292	493	235	NS
Citrulline	114	46	96	37	NS
Valine	293	124	257	109	NS
Methionine	42	26	40	26	NS
Isoleucine	84	43	76	42	NS
Leucine	187	93	167	93	NS
Tyrosine	90	48	82	51	NS
Phenylalanine	85	26	77	25	NS
Histidine	150	58	133	67	NS
Tryptophan	9	6	9	8	NS
Lysine	163	71	148	75	NS
Arginine	198	72	129	75	***

NS : not significant
* : $p < 0.05$
** : $p < 0.02$
*** : $p < 0.01$

TABLE 4.

PRIMARY AMINO ACID CONCENTRATIONS (uM) IN PLASMA.

AMINO ACID	NORMAL (n=10)		ICM (n=10)		MSUD (n=10)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Tau	116	55	81	31	87	66
Asp	7	11	5	5	19	27
Thr	102	57	82	71	103	51
Ser	130	40	95	42	129	741
Asn	53	31	41	38	36	22
Glu	210	167	115	68	174	126
Gln	356	152	340	130	263	130
Gly	386	174	394	85	630	343
Ala	410	166	387	213	148*	91
Cit	75	23	43*	14	131*	31
Val	282	114	156*	74	2065*	856
Met	24	14	16	8	34	16
Iso	71	28	43*	28	775*	418
Leu	126	60	84*	52	2337*	982
Tyr	74	45	37*	10	102	50
Phe	65	21	49*	12	112*	44
Try	1	1	3	4	1	3
His	129	71	117	33	104	52
Lys	107	63	91	41	146	61
Arg	90	45	91	30	204*	95

* : $p<0.05$ between normal:ICM, and normal:MSUD.

TABLE 5.

PRIMARY AMINO ACID CONCENTRATION (uM) IN CEREBROSPINAL FLUID.

AMINO ACID	NORMAL (n=10)		ICM (n=10)		MSUD (n=5)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Tau	3.11	1.64	1.90	1.65	1.36	1.27
Asp	0.40	1.21	0.01	0.03	0.08	0.18
Thr	21.32	5.62	19.92	9.22	37.74*	12.54
Ser	54.25	9.03	59.41	15.76	72.12*	22.01
Asn	1.87	2.49	2.15	3.14	7.46*	4.41
Glu	0.52	1.54	0.42	0.95	ND	
Gln	423.74	104.27	463.24	217.26	1023.62*	390.95
Gly	1.95	1.79	2.89	2.31	10.06*	7.42
Ala	60.56	10.50	61.89	12.55	43.54*	17.68
Cit	0.46	0.65	0.44	0.72	5.19*	6.10
Val	3.86	1.77	3.99	1.55	452.59*	193.90
Met	2.27	1.77	2.30	1.80	10.00*	3.36
Iso	0.29	0.27	0.50	0.56	168.54*	75.05
Leu	2.17	1.38	2.14	1.38	557.14*	256.73
Tyr	3.65	2.34	5.56	6.12	22.19*	3.78
Phe	2.21	1.67	3.24	1.97	25.51*	12.85
Try	0.22	0.68	0.52	1.24	0.41	0.81
His	16.10	4.00	15.46	5.88	50.31*	7.82
Lys	28.97	5.70	28.51	8.37	26.41	7.49
Arg	42.11	11.51	39.69	13.51	71.67*	31.16

GABA concentrations > 0.01 umoles/l were detected in only one MSUD-affected calf (0.73 umoles/l).

* : $p<0.05$ between normal and ICM; and, normal and MSUD.

ND : not detected.

TABLE 6.

AMINO ACID RANGES (uM) IN NORMAL AND MSUD CALVES OVER THREE DAYS.

AMINO ACID	NORMAL (n=3) Range	Trend	MSUD (n=4) Range	Trend
Taurine	58 - 212		30 - 170	
Aspartate	12 - 47		0 - 25	
Threonine	12 - 204		34 - 161	
Serine	42 - 205		32 - 255	
Asparagine	22 - 118		11 - 168	
Glutamate	95 - 611		25 - 420	
Glutamine	14 - 486		51 - 454	
Glycine	40 - 652		187 - 1475	
Alanine	299 - 1239		49 - 294	
Citrulline	33 - 133		68 - 182	
Valine	192 - 529		482 - 2251	#
Methionine	14 - 57		10 - 43	
Isoleucine	30 - 132		241 - 811	#
Leucine	62 - 273		327 - 2399	#
Tyrosine	34 - 201		40 - 171	
Phenylalanine	34 - 180		55 - 125	
Histidine	78 - 301		51 - 188	
Lysine	45 - 262		29 - 178	
Arginine	57 - 201		30 - 280	#

: Trend of increasing concentration.

TABLE 7.

MEAN NORADRENALIN CONCENTRATIONS (nM) IN CSF FROM NORMAL CALVES AND ICM AFFECTED CALVES.

Amine	Normal (n=4)		ICM (n=4)	
	Mean	SD	Mean	SD
Noradrenalin	257.5	77.6	345.0	120.7

TABLE 8.

RATIOS OF VALINE, ISOLEUCINE AND LEUCINE TO ALANINE IN FLUIDS, FIXED TISSUE AND FIXATIVE FROM ONE MSUD CALF AND TWO TO SIX NORMAL CALVES.

AMINO ACIDS Calf 1	PLASMA	CSF	URINE	CNS TISSUE	FORMALIN
Val : Ala	22.7	11.6	6.2	1.2	1.8
Iso : Ala	6.8	3.9	1.1	0.5	0.6
Leu : Ala	25.9	14.6	9.1	1.5	1.9
NORMAL CALVES	n=6	n=6	n=2	n=4	n=4
Val : Ala	0.7	0.1	0.2	0.4	3.4
Iso : Ala	0.2	<0.1	0.1	0.2	0.4
Leu : Ala	0.3	<0.1	0.1	0.2	0.2

TABLE 9.

CLINICAL CHEMISTRY RESULTS IN PLASMA.

Mineral/enzyme		NORMAL (n=6) Mean	SD	ICM (n=6) Mean	SD
Calcium	(mmol/l)	2.85	0.15	2.55	0.21
Magnesium	(mmol/l)	0.99	0.11	1.02	0.16
Phosphorus	(mmol/l)	2.38	0.34	2.25	0.31
Protein	(g/l)	52	11	49	6
Albumin	(g/l)	24	2	23	3
Glucose	(mmol/l)	4.2	2.1	4.5	0.8
Urea	(mmol/l)	4.6	2.1	4.7	1.8
AP	(U/l)	432	204	434	232
CPK	(U/l)	88	115	202	182
GGT	(U/l)	10	5	17	7
GOT	(U/l)	84	43	96	32
OCT	(U/l)	21	38	8	7

TABLE 10.

CLINICAL CHEMISTRY RESULTS IN CSF.

Mineral/enzyme		NORMAL (n=14) Mean	SD	ICM (n=14) Mean	SD
Calcium	(mmol/l)	0.58	0.18	0.54	0.20
Magnesium	(mmol/l)	0.87	0.06	0.83	0.10
Phosphorus	(mmol/l)	0.55	0.07	0.62	0.12
Protein	(g/l)	0.24	0.06	0.24	0.08
Glucose	(mmol/l)	6.16	1.44	4.50	1.82
Urea	(mmol/l)	4.46	1.14	3.64	1.03
CPK #	(U/l)	14	-	11	25
GOT	(U/l)	16	3	16	4

Normal CPK : n=1.
ICM CPK : n=5.

TABLE 11.

MEAN BRANCHED CHAIN KETO ACID CONCENTRATIONS (uM) IN PLASMA FROM NORMAL CALVES AND MSUD AFFECTED CALVES.

KETO ACID	Normal (n=6)		MSUD (n=6)		
	Mean	SD	Mean	SD	
KIVA	1.0	0.6	321	60	*
KMVA	ND		303	121	*
KICA	2.2	3.0	805	170	*

* : $p<0.05$ between normal and MSUD.

TABLE 12.

BRANCHED CHAIN KETO ACID CONCENTRATIONS (uM) IN PRESUCKLE TO POSTMORTEM PLASMA FROM (A) ONE MSUD-AFFECTED AND (B) TWO NORMAL CALVES.

Hours after birth	KIVA		KMVA		KICA	
	A	B	A	B	A	B
0	82	0.9, 1.4	171	#	95	0.3, 0.8
4	160		213		265	
8	219		230		430	
12	182		233		449	
16	321	0.7, 1.0	272	#	678	0.6, 0.8
20	269		250		638	
24	296		251		694	
28	255		193		635	
32	301	0.6, 0.7	238	#	697	0.3, 0.6

: Levels too low to be accurately quantitated. Suffers partial coelution with 3-hydroxybutyrate.

TABLE 13.

RATIOS OF BRANCHED CHAIN AMINO ACIDS TO ALANINE IN FORMALIN-FIXED CEREBRUM FROM NORMAL, ICM AND MSUD CALVES.

RATIOS	NORMAL (n=5)		ICM (n=5)		MSUD (n=10)	
	Mean	SD	Mean	SD	Mean	SD
Val : Ala	0.36	0.07	0.37	0.07	4.09	2.67*
Ile : Ala	0.15	0.07	0.19	0.10	1.41	0.89*
Leu : Ala	0.21	0.12	0.28	0.25	3.19	1.93*

* : $p<0.05$ between normal and ICM; and, normal and MSUD.

TABLE 14.

RANGES OF RATIOS BRANCHED CHAIN AMINO ACIDS TO ALANINE IN FIXED CEREBRUM FROM NORMAL, ICM AND MSUD CALVES.

RATIOS	NORMAL RANGE (n=5)	ICM RANGE (n=5)	MSUD RANGE (n=10)
Val : ala	0.30 - 0.47	0.28 - 0.45	1.17 - 9.56
Ile : Ala	0.08 - 0.22	0.08 - 0.34	0.46 - 3.19
Leu : Ala	0.08 - 0.38	0.08 - 0.72	1.51 - 6.73

TABLE 15.

AMINO ACID CONCENTRATIONS (nmoles/g)+ IN DORSAL SPINAL CORD FROM NORMAL AND ICM CALVES.

Amino Acid	Normal calves (n=5)		ICM calves (n=5)	
	Mean	SD	Mean	SD
Tau	4160	962	3902	1130
Asp	3212	623	3543	366
Thr	743	145	397 *	88
Ser	1494	226	1433	143
Glu	12173	1059	11904	1026
Gln	4140	333	4546	455
Gly	5563	581	5405	326
Ala	3048	279	3285	540
Cit	204	40	190	40
Val	462	56	435	80
Met	145	23	139	24
Ile	186	21	191	30
Leu	403	35	435	74
Tyr	285	128	284	103
Phe	256	26	297	43
GABA	1756	441	1841	215
His	631	90	578	105
Lys	615	161	604	153
Arg	1149	77	997	180
Pse	140	22	151	52
Pea	581	269	649	489

* : $p<0.05$

+ : nmoles/g wet weight of tissue

TABLE 16.

AMINO ACID CONCENTRATIONS (nmoles/g)+ IN VENTRAL SPINAL CORD FROM NORMAL AND ICM CALVES.

Amino Acid	Normal calves (n=5)		ICM calves (n=5)	
	Mean	SD	Mean	SD
Tau	4190	942	4026	1280
Asp	2726	626	2956	386
Thr	711	149	406 *	54
Ser	1466	306	1422	179
Glu	11647	1327	11738	904
Gln	3629	945	3956	636
Gly	5702	597	5659	226
Ala	2967	312	3038	436
Cit	214	33	186	52
Val	453	65	451	83
Met	180	49	189	39
Ile	173	20	188	34
Leu	387	35	438	84
Tyr	295	114	253	76
Phe	243	26	306	63
GABA	1339	262	1230	188
His	603	54	566	93
Lys	643	139	515	230
Arg	1168	127	1075	182
Pse	125	25	143	45
Pea	693	339	899	621

* : $p<0.05$

+ : nmoles/g wet weight of tissue

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TABLE 17.

PRIMARY AMINO ACID CONCENTRATIONS (umoles/g)+ IN THE MEDULLA OBLONGATA.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean	S.D.
Tau	35.24	16.27	59.44 *	21.81	52.44	26.49
Asp	50.48	7.91	52.53	13.83	11.84 ***	4.27
Thr	5.68	2.25	4.49	1.53	3.85	2.95
Ser	13.45	3.59	14.38	1.92	14.98	7.80
Glu	95.97	9.00	84.94 *	7.87	35.06 ***	3.39
Gln	6.85	4.44	7.30	2.86	23.98 ***	9.74
Gly	32.72	6.03	26.10	7.08	38.15	10.34
Ala	27.67	7.23	28.41	11.95	11.85 ***	4.32
Cit	0.76	0.58	0.94	0.20	1.93 *	1.14
Val	3.26	1.58	3.50	1.12	28.90 ***	7.15
Met	0.85	0.77	0.79	0.54	1.15	0.79
Ile	1.92	1.43	2.30	1.88	10.91 ***	3.04
Leu	3.66	2.28	3.58	1.24	35.80 ***	12.06
Tyr	1.86	0.77	1.65	0.60	1.72	1.38
Phe	2.32	1.01	2.19	0.84	2.70	2.47
GABA	31.17	7.52	33.98	11.33	10.76 ***	3.42
His	2.95	1.10	2.91	0.88	3.00	2.29
Lys	10.38	5.09	17.25	7.02	6.31 **	6.27
Arg	10.55	2.72	9.72	2.26	5.02 ***	3.44
Pse	4.10	2.97	7.59 *	2.23	6.66	5.17
Pea	0.77	0.96	0.51	0.61	9.29 ***	5.46

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

+ : umoles/g wet weight of tissue

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TABLE 18.

PRIMARY AMINO ACID CONCENTRATIONS (umoles/g)+ IN THE PONS.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean	S.D.
Tau	41.97	13.41	70.11 *	22.10	56.55	41.29
Asp	78.32	30.28	43.80 *	20.96	12.68 ***	5.66
Thr	4.84	2.67	3.80	0.84	2.46 *	2.10
Ser	13.30	4.46	15.09	2.07	13.86	6.95
Glu	103.32	37.62	79.01	14.77	33.81 ***	8.69
Gln	4.92	6.40	3.84	3.11	19.08 **	9.85
Gly	25.50	12.27	25.00	3.95	23.14	6.82
Ala	24.34	5.62	24.02	4.20	11.72 ***	4.94
Cit	0.94	0.66	0.76	0.24	1.63	1.07
Val	3.25	2.26	2.38	0.67	21.96 ***	9.07
Met	1.41	1.05	0.98	0.22	0.75	0.91
Ile	1.61	1.13	1.22	0.37	8.60 ***	2.92
Leu	3.76	2.44	3.53	1.21	30.93 ***	13.18
Tyr	1.79	0.98	1.43	0.74	1.60	1.46
Phe	2.04	1.35	2.21	0.87	2.53	2.59
GABA	37.45	9.64	39.85	8.09	7.75 ***	5.61
His	3.39	1.56	3.00	0.49	2.83	1.84
Lys	15.11	5.02	20.80	3.73	8.71 **	10.82
Arg	10.34	3.41	10.54	1.76	5.49 **	3.76
Pse	4.93	2.81	8.76	4.23	7.04	4.93
Pea	0.68	0.62	0.43	0.26	7.12 ***	4.72

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

+ : umoles/g wet weight of tissue

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TABLE 19.

PRIMARY AMINO ACID CONCENTRATIONS (umoles/g)+ IN THE WHITE MATTER OF THE CEREBELLUM.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=5) Mean	S.D.
Tau	44.10	20.43	78.89 *	28.36	53.90	22.41
Asp	44.48	4.93	40.09	12.63	9.47 ***	3.69
Thr	6.56	2.36	5.43	1.45	3.04 **	1.73
Ser	14.22	2.34	16.71	1.92	15.76	11.13
Glu	104.46	16.23	100.47	17.09	30.89 ***	5.48
Gln	5.37	3.96	7.38	5.86	22.29 ***	10.31
Gly	22.62	2.77	22.68	4.13	15.38 ***	1.88
Ala	22.20	5.43	21.50	5.64	11.45 **	6.85
Cit	1.18	0.61	1.13	0.47	1.78	0.66
Val	3.51	1.32	3.71	1.38	29.87 ***	7.56
Met	1.50	0.86	1.36	0.57	1.22	0.56
Ile	2.13	1.60	2.11	1.36	11.28 **	4.94
Leu	4.43	2.17	4.39	1.73	38.29 ***	22.27
Tyr	2.25	1.39	2.11	0.62	2.10	0.84
Phe	3.04	1.23	3.06	0.51	2.78	2.15
GABA	32.02	7.35	31.53	5.06	11.28 ***	3.22
His	3.40	1.03	3.24	1.12	2.95	1.70
Lys	12.14	5.14	19.94	8.39	3.92 ***	2.90
Arg	10.44	2.57	10.38	1.25	7.12 *	3.68
Pse	4.36	3.03	9.20 *	2.57	9.45	10.08
Pea	1.69	1.49	1.44	0.93	12.10 ***	6.25

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

\+ : umoles/g wet weight of tissue

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TABLE 20.

PRIMARY AMINO ACID CONCENTRATIONS (umoles/g)+ IN THE GREY MATTER OF THE CEREBELLUM.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=5) Mean	S.D.
Tau	28.63	5.93	28.67	5.56	51.73 **	19.97
Asp	44.64	10.40	42.51	8.43	8.12 ***	3.29
Thr	6.37	2.67	5.75	1.34	3.70 *	2.12
Ser	13.43	2.39	15.13	2.41	12.73	2.60
Glu	115.57	25.51	113.55	15.87	57.58 ***	13.60
Gln	7.40	5.19	8.38	2.54	29.27 ***	11.96
Gly	22.58	5.50	25.67	4.82	18.23 *	4.79
Ala	26.92	5.49	26.90	7.16	12.10 ***	4.58
Cit	0.55	0.42	0.46	0.36	1.47 *	0.40
Val	4.45	1.90	4.27	1.37	27.37 ***	6.09
Met	0.97	0.61	0.89	0.77	1.42	0.74
Ile	1.77	1.01	2.15	1.14	9.65 ***	2.97
Leu	4.18	1.73	4.11	1.78	32.94 ***	12.99
Tyr	1.73	0.59	1.58	0.66	2.13	1.10
Phe	2.42	0.86	2.64	0.70	2.54	2.19
GABA	45.67	7.29	38.62	4.94	16.87 ***	4.43
His	3.29	1.10	3.57	0.66	2.35 *	1.03
Lys	12.54	5.68	16.90	7.72	3.84 ***	2.58
Arg	6.17	1.97	5.81	1.05	4.11 *	2.16
Pse	2.43	0.98	3.76 *	0.95	4.11	3.02
Pea	2.03	1.76	1.80	1.08	22.89 ***	12.63

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

+ : moles/g wet weight of tissue

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TABLE 21.

PRIMARY AMINO ACID CONCENTRATIONS (umoles/g)[+] IN THE FRONTAL CORTEX.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean	S.D.
Tau	20.76	6.26	28.50	8.67	35.57	19.64
Asp	60.97	10.63	63.29	11.20	10.33 ***	5.13
Thr	5.66	2.32	5.76	0.91	3.77 *	2.94
Ser	15.64	2.26	17.89	2.45	11.13 **	4.17
Glu	117.21	25.05	122.84	10.86	49.45 ***	17.02
Gln	6.67	3.39	8.06	2.62	14.62	8.21
Gly	19.30	4.76	25.56	9.32	20.98	5.79
Ala	21.08	5.62	26.38	9.61	10.63 ***	3.74
Cit	1.14	0.45	0.93	0.80	1.86	0.65
Val	3.42	1.54	3.67	1.08	27.14 ***	6.35
Met	0.77	0.51	0.64	0.58	1.05	0.61
Ile	1.38	0.82	2.04	1.53	10.26 ***	2.31
Leu	3.31	1.58	3.49	1.10	35.50 ***	10.22
Tyr	1.30	0.44	1.13	0.69	1.69	0.92
Phe	2.09	0.69	2.15	0.90	1.84	0.72
GABA	38.91	8.43	41.50	7.15	17.03 ***	4.17
His	2.88	0.77	3.41	0.90	2.89	1.97
Lys	13.01	7.86	16.72	8.62	4.86 **	4.45
Arg	4.85	1.73	4.84	1.77	3.19 *	1.07
Pse	2.41	1.41	4.36 *	1.24	3.54	2.68
Pea	1.13	0.52	1.59	1.44	22.02 ***	16.72

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

+ : umoles/g wet weight of tissue

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TABLE 22.

PRIMARY AMINO ACID CONCENTRATIONS (umoles/g)+ IN THE TEMPORAL CORTEX.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean		S.D.
Tau	20.85	5.97	25.68	7.03	37.03	*	16.63
Asp	65.34	18.33	56.05	11.34	8.62	***	4.84
Thr	5.52	2.02	5.19	0.79	3.07	**	1.66
Ser	15.71	3.14	16.49	2.78	10.44	**	4.04
Glu	119.21	18.20	120.52	21.48	46.63	***	12.68
Gln	5.61	3.03	6.67	1.35	15.26	**	9.67
Gly	21.03	7.16	24.61	5.73	19.67		5.49
Ala	20.48	6.28	23.29	8.35	10.82	***	3.28
Cit	1.17	0.67	0.97	0.50	2.11		0.66
Val	3.28	1.39	3.84	0.86	23.82	***	5.47
Met	0.80	0.48	0.74	0.40	0.99		0.54
Ile	1.34	0.40	1.95	0.82	9.52	***	2.24
Leu	3.30	1.65	3.30	0.55	33.41	***	9.99
Tyr	1.47	0.40	1.01	0.60	1.69		0.81
Phe	2.00	0.66	2.10	0.46	2.30		1.56
GABA	37.87	4.42	33.35	11.62	16.83	***	3.49
His	2.84	0.80	2.92	0.59	2.38		0.97
Lys	11.64	6.13	14.43	6.22	5.98	**	5.59
Arg	4.95	1.70	4.88	0.92	3.48	*	1.44
Pse	2.52	1.67	3.63	1.87	3.64		2.94
Pea	1.22	0.59	1.00	0.55	17.96	***	11.33

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

+ : umoles/g wet weight of tissue

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TABLE 23.

PRIMARY AMINO ACID CONCENTRATIONS (umoles/g)+ IN THE OCCIPITAL CORTEX.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean		S.D.
Tau	19.79	4.57	23.39	6.53	36.69	*	17.17
Asp	53.44	32.06	57.56	8.44	10.68	***	6.45
Thr	6.43	2.52	5.52	1.11	2.98	**	1.77
Ser	16.83	4.10	16.43	2.30	10.89	**	4.39
Glu	124.90	23.48	118.58	12.83	47.56	***	10.84
Gln	7.02	4.85	8.12	3.30	16.57	**	8.42
Gly	22.81	7.79	23.32	8.14	21.23		7.75
Ala	21.76	6.70	24.67	7.64	12.05	**	4.91
Cit	1.40	0.77	1.43	0.47	2.23	*	0.50
Val	3.60	0.94	3.93	1.24	24.75	***	8.13
Met	1.17	0.58	1.22	0.58	1.05		0.67
Ile	1.94	0.73	2.48	0.87	9.88	***	2.99
Leu	3.98	2.34	3.63	1.41	34.85	***	13.75
Tyr	1.65	0.50	1.46	0.76	2.02		1.63
Phe	2.34	0.82	2.35	0.84	2.26		1.68
GABA	39.10	6.15	33.50	5.18	17.84	***	4.49
His	3.25	1.90	3.04	0.85	2.48		1.64
Lys	20.25	23.02	14.95	6.18	5.84	**	5.55
Arg	5.22	2.63	4.88	1.22	3.40		1.62
Pse	2.52	1.85	3.68	1.81	3.85		2.44
Pea	1.33	0.82	1.21	0.63	19.97	***	12.97

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

+ : μmoles/g wet weight of tissue

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TABLE 24.

PRIMARY AMINO ACID CONCENTRATIONS (umoles/g)+ IN THE AMYGDALA.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean	S.D.
Tau	21.74	3.76	32.26	15.75	34.13	13.89
Asp	51.21	19.77	52.57	14.48	16.39 ***	17.54
Thr	6.84	3.02	6.17	1.52	3.40 **	1.72
Ser	16.42	4.86	17.79	3.62	12.30 *	6.69
Glu	136.22	45.33	131.22	19.50	40.62 ***	17.17
Gln	7.26	4.58	7.15	3.76	13.13	8.66
Gly	23.57	8.49	25.72	6.98	17.83	4.77
Ala	20.22	6.16	23.12	5.21	13.58 **	7.73
Cit	0.71	0.52	0.71	0.30	1.40	0.99
Val	3.98	1.98	4.30	1.52	26.68 ***	6.36
Met	1.19	0.65	1.25	0.65	0.70 *	0.70
Ile	1.90	1.04	2.40	1.13	10.58 ***	3.41
Leu	4.00	1.97	4.40	2.00	35.33 ***	11.67
Tyr	1.35	0.82	1.68	0.85	1.76	0.92
Phe	2.21	0.89	2.66	1.20	2.71	2.33
GABA	41.16	7.87	41.14	3.13	36.40	10.29
His	3.25	1.57	3.43	0.88	2.90	1.37
Lys	25.49	27.74	19.66	5.77	6.49 **	5.41
Arg	4.92	2.45	5.70	3.48	4.31	2.83
Pse	2.48	1.36	5.66 *	2.88	4.70	4.46
Pea	1.61	1.22	1.67	1.07	20.64 ***	24.65

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

+ : umoles/g wet weight of tissue

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TABLE 25.

PRIMARY AMINO ACID CONCENTRATIONS (umoles/g)+ IN THE THALAMUS.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean	S.D.
Tau	36.20	8.08	56.87	25.95	49.06	21.64
Asp	58.59	19.60	54.72	31.24	16.51 ***	16.14
Thr	8.34	2.57	6.77	2.24	2.92 **	1.97
Ser	21.37	5.59	20.43	4.08	12.31 **	7.93
Glu	137.93	27.38	126.23	25.27	39.32 ***	20.28
Gln	7.19	6.09	6.41	3.57	15.93 **	7.25
Gly	39.68	22.27	30.02	5.75	18.02 **	3.81
Ala	23.19	5.07	21.65	7.16	12.67 **	7.05
Cit	2.04	0.97	1.76	1.14	2.15	1.02
Val	4.13	0.96	3.96	1.55	30.10 ***	5.68
Met	2.11	0.57	2.04	0.89	1.01 *	0.70
Ile	2.71	0.52	3.07	1.94	11.55 ***	2.97
Leu	6.33	2.84	5.63	2.22	38.74 ***	10.95
Tyr	2.65	1.09	2.24	1.17	2.08	1.01
Phe	4.12	1.69	3.74	1.45	3.20	2.20
GABA	39.98	7.71	41.14	4.24	30.30 *	16.86
His	4.54	2.19	3.90	0.94	2.82 *	1.40
Lys	26.38	26.17	21.44	2.14	5.64 ***	5.14
Arg	12.46	4.20	10.42	3.10	4.76 ***	3.28
Pse	3.56	1.88	8.24 *	4.82	6.60	5.81
Pea	1.63	1.63	1.70	1.65	14.95 ***	8.32

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

+ : umoles/g wet weight of tissue

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TABLE 26.

PRIMARY AMINO ACID CONCENTRATIONS (umoles/g)[+] IN THE PUTAMEN.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=5) Mean		S.D.
Tau	28.64	6.65	32.96	8.56	32.76		13.59
Asp	57.27	30.27	45.56	22.00	19.39	**	23.79
Thr	7.68	3.27	7.37	1.71	4.56	*	2.38
Ser	21.44	6.46	21.18	2.19	15.00	**	4.71
Glu	147.61	46.77	143.10	20.02	59.77	***	17.50
Gln	6.04	6.14	6.04	2.59	21.98	***	7.44
Gly	31.47	6.38	33.11	2.83	20.60	***	7.28
Ala	19.78	4.47	21.52	3.59	17.16		11.82
Cit	1.22	0.58	1.39	0.48	2.32		1.66
Val	4.34	1.47	4.77	1.47	37.46	***	16.10
Met	2.30	0.93	2.25	0.69	1.25	*	0.68
Ile	2.63	1.12	2.97	0.67	14.81	***	8.58
Leu	6.11	3.25	6.27	1.56	45.08	***	27.18
Tyr	2.03	1.19	2.08	0.81	2.10		0.61
Phe	3.22	1.46	3.81	1.00	3.26		2.06
GABA	36.03	7.73	35.26	4.74	35.45		8.26
His	4.46	2.37	4.45	0.66	2.78	*	0.55
Lys	22.22	6.96	28.89	22.11	4.51	***	3.53
Arg	8.90	3.10	7.83	1.08	3.73	***	1.98
Pse	3.88	2.23	5.41	2.24	4.59		3.56
Pea	1.81	2.18	1.83	1.82	30.08	***	8.10

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

+ : umoles/g wet weight of tissue

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TABLE 27.

PRIMARY AMINO ACID CONCENTRATIONS (umoles/g)[+] IN THE CAUDATE NUCLEUS.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean	S.D.
Tau	25.17	4.02	31.37	9.75	32.90	12.64
Asp	38.35	13.85	43.90	14.61	8.25 ***	6.74
Thr	6.09	2.11	7.38	1.45	3.37 ***	1.61
Ser	18.32	3.62	20.58	2.41	12.73 **	5.62
Glu	120.14	33.10	148.00 *	4.72	47.46 ***	18.42
Gln	6.53	7.05	7.87	3.31	18.89 *	12.75
Gly	28.39	3.74	33.59	5.18	21.14 ***	4.94
Ala	18.41	3.65	22.96 *	3.39	11.91 ***	3.41
Cit	1.13	0.44	1.33	0.71	2.53 ***	0.56
Val	3.47	1.11	4.99 *	1.58	24.32 ***	6.97
Met	1.54	0.49	2.29	1.14	0.96 **	0.49
Ile	2.06	0.69	2.98 *	0.95	10.12 ***	2.37
Leu	4.80	2.37	6.03	2.08	32.00 ***	9.04
Tyr	1.86	0.80	1.93	0.89	1.50	0.70
Phe	2.58	0.77	3.24	0.39	2.50	1.24
GABA	31.64	13.48	30.44	7.18	30.77	5.91
His	3.81	1.48	3.81	0.35	2.73 *	0.77
Lys	20.19	2.89	19.96	7.05	5.45 ***	5.55
Arg	6.72	2.02	6.44	1.11	3.52 ***	1.21
Pse	2.90	1.38	4.73	2.25	3.78	3.33
Pea	1.74	1.93	2.14	2.17	23.01 ***	13.62

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

+ : umoles/g wet weight of tissue

For corrected page, see pocket in back

TABLE 28.

PRIMARY AMINO ACID CONCENTRATIONS (umoles/g)[+] IN THE SUBSTANTIA NIGRA.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean	S.D.
Tau	30.20	10.11	55.93 *	20.53	44.56	16.63
Asp	71.42	43.71	73.74	34.18	16.31 **	18.44
Thr	6.14	3.15	5.58	2.20	3.78	2.00
Ser	14.17	6.05	16.33	2.91	13.01	7.40
Glu	109.24	45.93	93.34	17.38	45.69 **	22.98
Gln	6.12	5.23	5.59	2.11	20.72 **	12.56
Gly	27.52	4.76	29.35	4.85	22.02 *	10.01
Ala	20.80	6.69	23.65	4.59	15.72	11.73
Cit	1.07	0.80	1.02	0.62	1.80 *	0.71
Val	3.21	1.29	3.25	1.18	29.59 ***	11.93
Met	1.44	0.57	1.88	0.75	0.96 *	0.52
Ile	1.82	0.69	1.88	0.60	12.43 ***	6.07
Leu	4.38	2.43	4.72	1.80	41.85 ***	20.18
Tyr	1.93	0.82	1.80	0.82	1.93	0.85
Phe	2.66	1.39	3.19	0.84	3.01	1.57
GABA	54.10	10.92	59.52	14.72	42.23 *	16.94
His	4.40	2.17	4.47	1.17	3.26	0.62
Lys	30.72	39.86	30.46	20.16	14.43 *	22.34
Arg	10.38	2.72	10.01	0.22	4.24 ***	1.50
Pse	3.52	2.30	7.34	4.15	5.50	4.76
Pea	0.81	0.82	0.85	0.71	19.03 ***	10.32

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

+ : umoles/g wet weight of tissue

KEY TO BRAIN SITES.

1	Medulla oblongata
2	Pons
3	White matter of the cerebellum
4	Grey matter of the cerebellum
5	Frontal cortex
6	Temporal cortex
7	Occipital cortex
8	Amygdala
9	Thalamus
10	Putamen
11	Caudate nucleus
12	Substantia nigra

KEY TO BRAIN REGIONS.

A1 : Hindbrain.

A2 : Cortex and basal ganglia.

A3 : Thalamus and substantia nigra.

A4 : Cortex, basal ganglia, thalamus and substantia nigra.

A5 : Hindbrain, cortex, basal ganglia, thalamus and substantia nigra.

B1 : Medulla oblongata, pons and thalamus.

B2 : Cerebellum and cortex.

B3 : Basal ganglia and substantia nigra.

B4 : Cerebellum, cortex, basal ganglia and substantia nigra.

B5 : Medulla oblongata, pons, thalamus, cerebellum, cortex, basal ganglia and substantia nigra.

C1 : Medulla oblongata, pons and substantia nigra.

C3 : Basal ganglia and thalamus.

C4 : Cerebellum, cortex, basal ganglia and thalamus.

C5 : Medulla oblongata, pons, substantia nigra, cerebellum, cortex, basal ganglia and thalamus.

TABLE 29.

INTERACTION OF THE NORMAL AND ICM GENOTYPES WITH AMINO ACID DIFFERENCES BETWEEN BRAIN SITES/REGIONS.

SITE/REGION DIFFERENCE	AMINO ACID						
	Tau	Asp	Thr	Ser	Glu	Gln	Gly
1 - 2							
3 - 4	**d						
5 - 6							
5+6 - 7							
10 - 11							
8 - 10+11							
1+2 - 3+4 (A1)	*d						
5+6+7 - 8+10+11 (A2)							
9 - 12 (A3)							
A2 - A3 (A4)							*e
A1 - A4 (A5)			*b		*b		
1+2 - 9 (B1)							
3+4 - 5+6+7 (B2)							
8+10+11 - 12 (B3)							
B2 - B3 (B4)							
B1 - B4 (B5)		*c	*b		**b		
1+2 - 12 (C1)							
8+10+11 - 9 (C3)							
B2 - C3 (C4)							
C1 - C4 (C5)	*d						

* : $p<0.05$; ** : $p<0.01$; *** : $p<0.001$.

Key for differences between sites/regions;

a. Normal > ICM : Both differences positive.
b. Normal > ICM : Both differences negative.
c. Normal > ICM : ICM differences negative.
d. ICM > Normal : Both differences positive.
e. ICM > Normal : Both differences negative.
f. ICM > Normal : Normal differences negative.

No asterisk indicates differences between sites/regions were similar for the two genotypes.

KEY TO BRAIN SITES.

1	Medulla oblongata
2	Pons
3	White matter of the cerebellum
4	Grey matter of the cerebellum
5	Frontal cortex
6	Temporal cortex
7	Occipital cortex
8	Amygdala
9	Thalamus
10	Putamen
11	Caudate nucleus
12	Substantia nigra

KEY TO BRAIN REGIONS.

A1 : Hindbrain.

A2 : Cortex and basal ganglia.

A3 : Thalamus and substantia nigra.

A4 : Cortex, basal ganglia, thalamus and substantia nigra.

A5 : Hindbrain, cortex, basal ganglia, thalamus and substantia nigra.

B1 : Medulla oblongata, pons and thalamus.

B2 : Cerebellum and cortex.

B3 : Basal ganglia and substantia nigra.

B4 : Cerebellum, cortex, basal ganglia and substantia nigra.

B5 : Medulla oblongata, pons, thalamus, cerebellum, cortex, basal ganglia and substantia nigra.

C1 : Medulla oblongata, pons and substantia nigra.

C3 : Basal ganglia and thalamus.

C4 : Cerebellum, cortex, basal ganglia and thalamus.

C5 : Medulla oblongata, pons, substantia nigra, cerebellum, cortex, basal ganglia and thalamus.

TABLE 30.

INTERACTION OF THE NORMAL AND ICM GENOTYPES ON AMINO ACID DIFFERENCES BETWEEN BRAIN SITES/REGIONS.

SITE/REGION DIFFERENCE	AMINO ACID						
	Ala	Cit	Val	Met	Ile	Leu	Tyr
1 - 2							
3 - 4							
5 - 6							
5+6 - 7							
10 - 11							
8 - 10+11							
1+2 - 3+4 (A1)							
5+6+7 - 8+10+11 (A2)							
9 - 12 (A3)							
A2 - A3 (A4)					*f		
A1 - A4 (A5)	*a						
1+2 - 9 (B1)							
3+4 - 5+6+7 (B2)							
8+10+11 - 12 (B3)							
B2 - B3 (B4)							
B1 - B4 (B5)	*a						
1+2 - 12 (C1)							
8+10+11 - 9 (C3)	*f						
B2 - C3 (C4)							
C1 - C4 (C5)							

* : $p<0.05$; ** : $p<0.01$; *** : $p<0.001$.

Key for differences between sites/regions;

a. Normal > ICM : Both differences positive.
b. Normal > ICM : Both differences negative.
c. Normal > ICM : ICM differences negative.
d. ICM > Normal : Both differences positive.
e. ICM > Normal : Both differences negative.
f. ICM > Normal : Normal differences negative.

No asterisk indicates differences between sites/regions were similar for the two genotypes.

KEY TO BRAIN SITES.

1	Medulla oblongata
2	Pons
3	White matter of the cerebellum
4	Grey matter of the cerebellum
5	Frontal cortex
6	Temporal cortex
7	Occipital cortex
8	Amygdala
9	Thalamus
10	Putamen
11	Caudate nucleus
12	Substantia nigra

KEY TO BRAIN REGIONS.

A1 : Hindbrain.

A2 : Cortex and basal ganglia.

A3 : Thalamus and substantia nigra.

A4 : Cortex, basal ganglia, thalamus and substantia nigra.

A5 : Hindbrain, cortex, basal ganglia, thalamus and substantia nigra.

B1 : Medulla oblongata, pons and thalamus.

B2 : Cerebellum and cortex.

B3 : Basal ganglia and substantia nigra.

B4 : Cerebellum, cortex, basal ganglia and substantia nigra.

B5 : Medulla oblongata, pons, thalamus, cerebellum, cortex, basal ganglia and substantia nigra.

C1 : Medulla oblongata, pons and substantia nigra.

C3 : Basal ganglia and thalamus.

C4 : Cerebellum, cortex, basal ganglia and thalamus.

C5 : Medulla oblongata, pons, substantia nigra, cerebellum, cortex, basal ganglia and thalamus.

TABLE 31.

INTERACTION OF THE NORMAL AND ICM GENOTYPES WITH AMINO ACID DIFFERENCES BETWEEN BRAIN SITES/REGIONS.

SITE/REGION DIFFERENCE	AMINO ACID						
	Phe	GABA	His	Lys	Arg	Pse	Pea
1 - 2							
3 - 4						*d	
5 - 6							*f
5+6 - 7							
10 - 11							
8 - 10+11							
1+2 - 3+4 (A1)							
5+6+7 - 8+10+11 (A2)							
9 - 12 (A3)	*a						
A2 - A3 (A4)							
A1 - A4 (A5)							*b
1+2 - 9 (B1)							
3+4 - 5+6+7 (B2)							
8+10+11 - 12 (B3)							
B2 - B3 (B4)							
B1 - B4 (B5)							
1+2 - 12 (C1)							
8+10+11 - 9 (C3)							
B2 - C3 (C4)							
C1 - C4 (C5)							

* : $p<0.05$; ** : $p<0.01$; *** : $p<0.001$.

Key for differences between sites/regions;

a. Normal > ICM : Both differences positive.
b. Normal > ICM : Both differences negative.
c. Normal > ICM : ICM differences negative.
d. ICM > Normal : Both differences positive.
e. ICM > Normal : Both differences negative.
f. ICM > Normal : Normal differences negative.

No asterisk indicates differences between sites/regions were similar for the two genotypes.

KEY TO BRAIN SITES.

1	Medulla oblongata
2	Pons
3	White matter of the cerebellum
4	Grey matter of the cerebellum
5	Frontal cortex
6	Temporal cortex
7	Occipital cortex
8	Amygdala
9	Thalamus
10	Putamen
11	Caudate nucleus
12	Substantia nigra

KEY TO BRAIN REGIONS.

A1 : Hindbrain.

A2 : Cortex and basal ganglia.

A3 : Thalamus and substantia nigra.

A4 : Cortex, basal ganglia, thalamus and substantia nigra.

A5 : Hindbrain, cortex, basal ganglia, thalamus and substantia nigra.

B1 : Medulla oblongata, pons and thalamus.

B2 : Cerebellum and cortex.

B3 : Basal ganglia and substantia nigra.

B4 : Cerebellum, cortex, basal ganglia and substantia nigra.

B5 : Medulla oblongata, pons, thalamus, cerebellum, cortex, basal ganglia and substantia nigra.

C1 : Medulla oblongata, pons and substantia nigra.

C3 : Basal ganglia and thalamus.

C4 : Cerebellum, cortex, basal ganglia and thalamus.

C5 : Medulla oblongata, pons, substantia nigra, cerebellum, cortex, basal ganglia and thalamus.

TABLE 32.

DIFFERENCES IN AMINO ACID CONCENTRATIONS BETWEEN BRAIN SITES/REGIONS.

SITE/REGION DIFFERENCE	AMINO ACID						
	Tau	Asp	Thr	Ser	Glu	Gln	Gly
1 - 2	*-		**+			**+	
3 - 4	***+			**+	**-	**-	
5 - 6						*+	
5+6 - 7	*+						
10 - 11				*+			
8 - 10+11				**-			**-
1+2 - 3+4 (A1)	**+	**+	***-		***-	*-	
5+6+7 - 8+10+11 (A2)	**-	*+	**-	*-	**-		***-
9 - 12 (A3)			*+	**+	*+		
A2 - A3 (A4)	***-						***-
A1 - A4 (A5)	***+		***-	***-	***-		
1+2 - 9 (B1)			***-	***-	***-		
3+4 - 5+6+7 (B2)	***+	***-		**-	**-		
8+10+11 - 12 (B3)	*-			*+	*+		
B2 - B3 (B4)			**-	*-			***-
B1 - B4 (B5)	***+		**-		***-	*-	*+
1+2 - 12 (C1)							
8+10+11 - 9 (C3)	**-						
B2 - C3 (C4)	*-		***-	***-	***-		***-
C1 - C4 (C5)	***+		***-	**-	***-	**-	

The normal and ICM groups were pooled, providing 13 subjects. Differences between sites/regions were established on log-transformed data and tested to determine significant differences from zero.

* : $p<0.05$; ** $p<0.01$; *** $p<0.001$.

+ : difference between two sites/regions is positive.

- : difference between two sites/regions is negative.

KEY TO BRAIN SITES.

1	Medulla oblongata
2	Pons
3	White matter of the cerebellum
4	Grey matter of the cerebellum
5	Frontal cortex
6	Temporal cortex
7	Occipital cortex
8	Amygdala
9	Thalamus
10	Putamen
11	Caudate nucleus
12	Substantia nigra

KEY TO BRAIN REGIONS.

A1 : Hindbrain.

A2 : Cortex and basal ganglia.

A3 : Thalamus and substantia nigra.

A4 : Cortex, basal ganglia, thalamus and substantia nigra.

A5 : Hindbrain, cortex, basal ganglia, thalamus and substantia nigra.

B1 : Medulla oblongata, pons and thalamus.

B2 : Cerebellum and cortex.

B3 : Basal ganglia and substantia nigra.

B4 : Cerebellum, cortex, basal ganglia and substantia nigra.

B5 : Medulla oblongata, pons, thalamus, cerebellum, cortex, basal ganglia and substantia nigra.

C1 : Medulla oblongata, pons and substantia nigra.

C3 : Basal ganglia and thalamus.

C4 : Cerebellum, cortex, basal ganglia and thalamus.

C5 : Medulla oblongata, pons, substantia nigra, cerebellum, cortex, basal ganglia and thalamus.

TABLE 33.

DIFFERENCES IN AMINO ACID CONCENTRATIONS BETWEEN BRAIN SITES/REGIONS.

SITE/REGION DIFFERENCE	AMINO ACID						
	Ala	Cit	Val	Met	Ile	Leu	Tyr
1 - 2			*+				
3 - 4	***-	**+	**-	*+			
5 - 6							
5+6 - 7				**-	**-		
10 - 11							
8 - 10+11		**-		**-	*-	***-	*-
1+2 - 3+4 (A1)			***-		*-	*-	
5+6+7 - 8+10+11 (A2)			*-	***-	**-	***-	
9 - 12 (A3)		***+	**+	*+	**+	***+	**+
A2 - A3 (A4)				**-		**-	*-
A1 - A4 (A5)	***+	*-	*-	*-		***-	
1+2 - 9 (B1)	**+	**-	**-	**-	**-	***-	***-
3+4 - 5+6+7 (B2)						*+	
8+10+11 - 12 (B3)			**+		**+	*+	
B2 - B3 (B4)				**-	*-	***-	
B1 - B4 (B5)	**+		**-				
1+2 - 12 (C1)				**-		***-	
8+10+11 - 9 (C3)		**-				*-	**-
B2 - C3 (C4)		*-		**-	**-	***-	*-
C1 - C4 (C5)			***-		*-	**-	

The normal and ICM groups were pooled, providing 13 subjects. Differences between sites/regions were established on the log-transformed data and tested to determine significant differences from zero.

* : $p<0.05$; ** $p<0.01$; *** $p<0.001$.

+ : differences between two sites/regions is positive.

- : differences between two sites/regions is negative.

KEY TO BRAIN SITES.

1	Medulla oblongata
2	Pons
3	White matter of the cerebellum
4	Grey matter of the cerebellum
5	Frontal cortex
6	Temporal cortex
7	Occipital cortex
8	Amygdala
9	Thalamus
10	Putamen
11	Caudate nucleus
12	Substantia nigra

KEY TO BRAIN REGIONS.

A1 : Hindbrain.

A2 : Cortex and basal ganglia.

A3 : Thalamus and substantia nigra.

A4 : Cortex, basal ganglia, thalamus and substantia nigra.

A5 : Hindbrain, cortex, basal ganglia, thalamus and substantia nigra.

B1 : Medulla oblongata, pons and thalamus.

B2 : Cerebellum and cortex.

B3 : Basal ganglia and substantia nigra.

B4 : Cerebellum, cortex, basal ganglia and substantia nigra.

B5 : Medulla oblongata, pons, thalamus, cerebellum, cortex, basal ganglia and substantia nigra.

C1 : Medulla oblongata, pons and substantia nigra.

C3 : Basal ganglia and thalamus.

C4 : Cerebellum, cortex, basal ganglia and thalamus.

C5 : Medulla oblongata, pons, substantia nigra, cerebellum, cortex, basal ganglia and thalamus.

TABLE 34.

DIFFERENCES IN AMINO ACID CONCENTRATIONS BETWEEN BRAIN SITES/REGIONS

SITE/REGION DIFFERENCE	AMINO ACID						
	Phe	GABA	His	Lys	Arg	Pse	Pea
1 - 2				*-			
3 - 4	**+	***-			***+	***+	
5 - 6		*+					
5+6 - 7	*-						
10 - 11		*+	*+		***+	*+	
8 - 10+11	*-	**+	**-		**-		
1+2 - 3+4 (A1)	**-				***+	**+	***-
5+6+7 - 8+10+11 (A2)	***-		***-	**-	**-	*-	
9 - 12 (A3)	**+	**-					**+
A2 - A3 (A4)	**-	***-	***-	*-	***-	*-	*+
A1 - A4 (A5)	*-	*-	*-	*-	***+	***+	***-
1+2 - 9 (B1)	***-	*-	***-	*-			***-
3+4 - 5+6+7 (B2)	***+				***+	**+	
8+10+11 - 12 (B3)		***-			***-		**+
B2 - B3 (B4)	***-		***-	**-	**-		
B1 - B4 (B5)					***+	***+	***-
1+2 - 12 (C1)	***-	***-	***-	*-			
8+10+11 - 9 (C3)	**-	**-			***-	*-	
B2 - C3 (C4)	***-		***-	**-	**-	*-	
C1 - C4 (C5)		*+			***+	**+	***-

The normal and ICM groups were pooled, providing 13 subjects. Differences between sites/regions were established on the log-transformed data and tested to determine significant differences from zero.

* : $p<0.05$; ** $p<0.01$; *** $p<0.001$.

+ : differences between two sites/regions is positive.

- : differences between two sites/regions is negative.

KEY TO BRAIN SITES.

1	Medulla oblongata
2	Pons
3	White matter of the cerebellum
4	Grey matter of the cerebellum
5	Frontal cortex
6	Temporal cortex
7	Occipital cortex
8	Amygdala
9	Thalamus
10	Putamen
11	Caudate nucleus
12	Substantia nigra

KEY TO BRAIN REGIONS.

A1 : Hindbrain.

A2 : Cortex and basal ganglia.

A3 : Thalamus and substantia nigra.

A4 : Cortex, basal ganglia, thalamus and substantia nigra.

A5 : Hindbrain, cortex, basal ganglia, thalamus and substantia nigra.

B1 : Medulla oblongata, pons and thalamus.

B2 : Cerebellum and cortex.

B3 : Basal ganglia and substantia nigra.

B4 : Cerebellum, cortex, basal ganglia and substantia nigra.

B5 : Medulla oblongata, pons, thalamus, cerebellum, cortex, basal ganglia and substantia nigra.

C1 : Medulla oblongata, pons and substantia nigra.

C3 : Basal ganglia and thalamus.

C4 : Cerebellum, cortex, basal ganglia and thalamus.

C5 : Medulla oblongata, pons, substantia nigra, cerebellum, cortex, basal ganglia and thalamus.

TABLE 35.

DIFFERENCES IN AMINO ACID CONCENTRATIONS BETWEEN BRAIN SITES/REGIONS WITHIN THE MSUD GENOTYPE.

SITE/REGION DIFFERENCE	AMINO ACID						
	Tau	Asp	Thr	Ser	Glu	Gln	Gly
1 - 2							
3 - 4			*-		**-		
5 - 6		**+					
5+6 - 7						*-	
10 - 11							
8 - 10+11							
1+2 - 3+4 (A1)		**+					**+
5+6+7 - 8+10+11 (A2)							
9 - 12 (A3)							
A2 - A3 (A4)	*-						
A1 - A4 (A5)	*+						
1+2 - 9 (B1)							*+
3+4 - 5+6+7 (B2)	*+						
8+10+11 - 12 (B3)							
B2 - B3 (B4)							
B1 - B4 (B5)	*+	**+			*-		*+
1+2 - 12 (C1)							*+
8+10+11 - 9 (C3)							
B2 - C3 (C4)							
C1 - C4 (C5)	**+					*+	*+

The MSUD group comprised five or six subjects. Differences between sites/regions were established on log-transformed data and tested to determine significant differences from zero.

* : $p<0.05$; ** $p<0.01$; *** $p<0.001$.

+ : difference between two sites/regions is positive.

- : difference between two sites/regions is negative.

KEY TO BRAIN SITES.

1	Medulla oblongata
2	Pons
3	White matter of the cerebellum
4	Grey matter of the cerebellum
5	Frontal cortex
6	Temporal cortex
7	Occipital cortex
8	Amygdala
9	Thalamus
10	Putamen
11	Caudate nucleus
12	Substantia nigra

KEY TO BRAIN REGIONS.

A1 : Hindbrain.

A2 : Cortex and basal ganglia.

A3 : Thalamus and substantia nigra.

A4 : Cortex, basal ganglia, thalamus and substantia nigra.

A5 : Hindbrain, cortex, basal ganglia, thalamus and substantia nigra.

B1 : Medulla oblongata, pons and thalamus.

B2 : Cerebellum and cortex.

B3 : Basal ganglia and substantia nigra.

B4 : Cerebellum, cortex, basal ganglia and substantia nigra.

B5 : Medulla oblongata, pons, thalamus, cerebellum, cortex, basal ganglia and substantia nigra.

C1 : Medulla oblongata, pons and substantia nigra.

C3 : Basal ganglia and thalamus.

C4 : Cerebellum, cortex, basal ganglia and thalamus.

C5 : Medulla oblongata, pons, substantia nigra, cerebellum, cortex, basal ganglia and thalamus.

TABLE 36.

DIFFERENCES IN AMINO ACID CONCENTRATIONS BETWEEN BRAIN SITES/REGIONS WITHIN THE MSUD GENOTYPE.

SITE/REGION DIFFERENCE	AMINO ACID						
	Ala	Cit	Val	Met	Ile	Leu	Tyr
1 - 2							
3 - 4							
5 - 6			*+				
5+6 - 7							
10 - 11							
8 - 10+11							
1+2 - 3+4 (A1)							
5+6+7 - 8+10+11 (A2)							
9 - 12 (A3)							
A2 - A3 (A4)					*-	*-	
A1 - A4 (A5)							
1+2 - 9 (B1)			*-		*-	*-	
3+4 - 5+6+7 (B2)		*-					
8+10+11 - 12 (B3)					*-		
B2 - B3 (B4)	*-						
B1 - B4 (B5)							
1+2 - 12 (C1)						*-	
8+10+11 - 9 (C3)							*-
B2 - C3 (C4)							
C1 - C4 (C5)							

The MSUD group comprised six subjects. Differences between sites/regions were established on log-transformed data and tested to determine significant differences from zero.

* : $p<0.05$; ** $p<0.01$; *** $p<0.001$.

+ : difference between two sites/regions is positive.

- : difference between two sites/regions is negative.

KEY TO BRAIN SITES.

1	Medulla oblongata
2	Pons
3	White matter of the cerebellum
4	Grey matter of the cerebellum
5	Frontal cortex
6	Temporal cortex
7	Occipital cortex
8	Amygdala
9	Thalamus
10	Putamen
11	Caudate nucleus
12	Substantia nigra

KEY TO BRAIN REGIONS.

A1 : Hindbrain.

A2 : Cortex and basal ganglia.

A3 : Thalamus and substantia nigra.

A4 : Cortex, basal ganglia, thalamus and substantia nigra.

A5 : Hindbrain, cortex, basal ganglia, thalamus and substantia nigra.

B1 : Medulla oblongata, pons and thalamus.

B2 : Cerebellum and cortex.

B3 : Basal ganglia and substantia nigra.

B4 : Cerebellum, cortex, basal ganglia and substantia nigra.

B5 : Medulla oblongata, pons, thalamus, cerebellum, cortex, basal ganglia and substantia nigra.

C1 : Medulla oblongata, pons and substantia nigra.

C3 : Basal ganglia and thalamus.

C4 : Cerebellum, cortex, basal ganglia and thalamus.

C5 : Medulla oblongata, pons, substantia nigra, cerebellum, cortex, basal ganglia and thalamus.

TABLE 37.

DIFFERENCES IN AMINO ACID CONCENTRATIONS BETWEEN BRAIN SITES/REGIONS WITHIN THE MSUD GENOTYPE.

SITE/REGION DIFFERENCE	AMINO ACID						
	Phe	GABA	His	Lys	Arg	Pse	Pea
1 - 2							
3 - 4		***-					
5 - 6							
5+6 - 7							
10 - 11							
8 - 10+11							
1+2 - 3+4 (A1)		***-					
5+6+7 - 8+10+11 (A2)		**-					
9 - 12 (A3)				*-			
A2 - A3 (A4)	**-		*-	*-			
A1 - A4 (A5)		***-				*+	***-
1+2 - 9 (B1)		**-					***-
3+4 - 5+6+7 (B2)							
8+10+11 - 12 (B3)			*-	*-		*-	
B2 - B3 (B4)		**-		**-			
B1 - B4 (B5)		**-					**-
1+2 - 12 (C1)		***-					
8+10+11 - 9 (C3)							*+
B2 - C3 (C4)		***-					
C1 - C4 (C5)		**-				*+	*-

The MSUD group comprised six subjects. Differences between sites/regions were established on log-transformed data and tested to determine significant differences from zero.

* : $p<0.05$; ** $p<0.01$; *** $p<0.001$.

+ : difference between two sites/regions is positive.

- : difference between two sites/regions is negative.

KEY TO BRAIN SITES.

1	Medulla oblongata
2	Pons
3	White matter of the cerebellum
4	Grey matter of the cerebellum
5	Frontal cortex
6	Temporal cortex
7	Occipital cortex
8	Amygdala
9	Thalamus
10	Putamen
11	Caudate nucleus
12	Substantia nigra

KEY TO BRAIN REGIONS.

A1 : Hindbrain.

A2 : Cortex and basal ganglia.

A3 : Thalamus and substantia nigra.

A4 : Cortex, basal ganglia, thalamus and substantia nigra.

A5 : Hindbrain, cortex, basal ganglia, thalamus and substantia nigra.

B1 : Medulla oblongata, pons and thalamus.

B2 : Cerebellum and cortex.

B3 : Basal ganglia and substantia nigra.

B4 : Cerebellum, cortex, basal ganglia and substantia nigra.

B5 : Medulla oblongata, pons, thalamus, cerebellum, cortex, basal ganglia and substantia nigra.

C1 : Medulla oblongata, pons and substantia nigra.

C3 : Basal ganglia and thalamus.

C4 : Cerebellum, cortex, basal ganglia and thalamus.

C5 : Medulla oblongata, pons, substantia nigra, cerebellum, cortex, basal ganglia and thalamus.

TABLE 38.

INTERACTION OF THE MSUD GENOTYPE, RELATIVE TO THE NORMAL AND ICM GENOTYPES, ON DIFFERENCES IN AMINO ACID CONCENTRATIONS BETWEEN BRAIN SITES/REGIONS.

SITE/REGION DIFFERENCE	AMINO ACID						
	Tau	Asp	Thr	Ser	Glu	Gln	Gly
1 - 2							
3 - 4	**a		**a		***d		
5 - 6							
5+6 - 7							
10 - 11							
8 - 10+11							
1+2 - 3+4 (A1)	**a						**d
5+6+7 - 8+10+11 (A2)							**f
9 - 12 (A3)			*c	*c	*c		
A2 - A3 (A4)							
A1 - A4 (A5)				***f			
1+2 - 9 (B1)			*f	***f		*f	**f
3+4 - 5+6+7 (B2)				**f			*c
8+10+11 - 12 (B3)							
B2 - B3 (B4)							*e
B1 - B4 (B5)	**a		*b				
1+2 - 12 (C1)							**f
8+10+11 - 9 (C3)			*f	*f			
B2 - C3 (C4)					**f		**e
C1 - C4 (C5)	**a			*f		**f	*d

* : p<0.05 ; ** : p<0.01 ; *** : p<0.001.

Key for differences between sites/regions;

a) Normal+ICM > MSUD : Both differences positive.

b) Normal+ICM > MSUD : Both differences negative.

c) Normal+ICM > MSUD : MSUD difference negative.

d) MSUD > Normal+ICM : Both differences positive.

e) MSUD > Normal+ICM : Both differences negative.

f) MSUD > Normal+ICM : Normal+ICM differences negative.

KEY TO BRAIN SITES.

1	Medulla oblongata
2	Pons
3	White matter of the cerebellum
4	Grey matter of the cerebellum
5	Frontal cortex
6	Temporal cortex
7	Occipital cortex
8	Amygdala
9	Thalamus
10	Putamen
11	Caudate nucleus
12	Substantia nigra

KEY TO BRAIN REGIONS.

A1 : Hindbrain.

A2 : Cortex and basal ganglia.

A3 : Thalamus and substantia nigra.

A4 : Cortex, basal ganglia, thalamus and substantia nigra.

A5 : Hindbrain, cortex, basal ganglia, thalamus and substantia nigra.

B1 : Medulla oblongata, pons and thalamus.

B2 : Cerebellum and cortex.

B3 : Basal ganglia and substantia nigra.

B4 : Cerebellum, cortex, basal ganglia and substantia nigra.

B5 : Medulla oblongata, pons, thalamus, cerebellum, cortex, basal ganglia and substantia nigra.

C1 : Medulla oblongata, pons and substantia nigra.

C3 : Basal ganglia and thalamus.

C4 : Cerebellum, cortex, basal ganglia and thalamus.

C5 : Medulla oblongata, pons, substantia nigra, cerebellum, cortex, basal ganglia and thalamus.

TABLE 39.

INTERACTION OF THE MSUD GENOTYPE, RELATIVE TO THE NORMAL AND ICM GENOTYPES, ON DIFFERENCES IN AMINO ACID CONCENTRATIONS BETWEEN BRAIN SITES/REGIONS.

SITE/REGION DIFFERENCE	AMINO ACID						
	Ala	Cit	Val	Met	Ile	Leu	Tyr
1 - 2				**f		*f	
3 - 4			**f				
5 - 6							
5+6 - 7				*e	*f		
10 - 11							
8 - 10+11						*d	
1+2 - 3+4 (A1)							
5+6+7 - 8+10+11 (A2)	*c			**f		**e	
9 - 12 (A3)					*c	**c	
A2 - A3 (A4)							
A1 - A4 (A5)	***c						
1+2 - 9 (B1)						**e	
3+4 - 5+6+7 (B2)							
8+10+11 - 12 (B3)			*c		**c	**c	
B2 - B3 (B4)	**c			**f		*e	
B1 - B4 (B5)	*c						
1+2 - 12 (C1)							
8+10+11 - 9 (C3)							
B2 - C3 (C4)	**c			**f		**e	
C1 - C4 (C5)			*e				

* : $p<0.05$; ** : $p<0.01$; *** : $p<0.001$.

Key for differences between sites/regions;

a) Normal+ICM > MSUD : Both differences positive.

b) Normal+ICM > MSUD : Both differences negative.

c) Normal+ICM > MSUD : MSUD difference negative.

d) MSUD > Normal+ICM : Both differences positive.

e) MSUD > Normal+ICM : Both differences negative.

f) MSUD > Normal+ICM : Normal+ICM differences negative.

KEY TO BRAIN SITES.

1	Medulla oblongata
2	Pons
3	White matter of the cerebellum
4	Grey matter of the cerebellum
5	Frontal cortex
6	Temporal cortex
7	Occipital cortex
8	Amygdala
9	Thalamus
10	Putamen
11	Caudate nucleus
12	Substantia nigra

KEY TO BRAIN REGIONS.

A1 : Hindbrain.

A2 : Cortex and basal ganglia.

A3 : Thalamus and substantia nigra.

A4 : Cortex, basal ganglia, thalamus and substantia nigra.

A5 : Hindbrain, cortex, basal ganglia, thalamus and substantia nigra.

B1 : Medulla oblongata, pons and thalamus.

B2 : Cerebellum and cortex.

B3 : Basal ganglia and substantia nigra.

B4 : Cerebellum, cortex, basal ganglia and substantia nigra.

B5 : Medulla oblongata, pons, thalamus, cerebellum, cortex, basal ganglia and substantia nigra.

C1 : Medulla oblongata, pons and substantia nigra.

C3 : Basal ganglia and thalamus.

C4 : Cerebellum, cortex, basal ganglia and thalamus.

C5 : Medulla oblongata, pons, substantia nigra, cerebellum, cortex, basal ganglia and thalamus.

TABLE 40.

INTERACTION OF THE MSUD GENOTYPE, RELATIVE TO THE NORMAL AND ICM GENOTYPES, ON DIFFERENCES IN AMINO ACID CONCENTRATIONS BETWEEN BRAIN SITES/REGIONS.

SITE/REGION DIFFERENCE	AMINO ACID						
	Phe	GABA	His	Lys	Arg	Pse	Pea
1 - 2		*f					
3 - 4							
5 - 6							
5+6 - 7						*d	
10 - 11							
8 - 10+11					**a		
1+2 - 3+4 (A1)		***b			*c		*e
5+6+7 - 8+10+11 (A2)		***c					
9 - 12 (A3)							
A2 - A3 (A4)					***e		
A1 - A4 (A5)		***a					*b
1+2 - 9 (B1)		***b		*c			*e
3+4 - 5+6+7 (B2)		*b					*c
8+10+11 - 12 (B3)		*e			**e		
B2 - B3 (B4)		***b			*f		
B1 - B4 (B5)		***b			**a		
1+2 - 12 (C1)		***b					
8+10+11 - 9 (C3)		*f			*e		
B2 - C3 (C4)		***c			*f		
C1 - C4 (C5)		***c			***a		*e

* : p<0.05 ; ** : p<0.01 ; *** : p<0.001.

Key for differences between sites/regions;

a) Normal+ICM > MSUD : Both differences positive.
b) Normal+ICM > MSUD : Both differences negative.
c) Normal+ICM > MSUD : MSUD difference negative.
d) MSUD > Normal+ICM : Both differences positive.
e) MSUD > Normal+ICM : Both differences negative.
f) MSUD > Normal+ICM : Normal+ICM differences negative.

TABLE 41.

DISTUINGUISHING FEATURES OF THE ICM AND MSUD GENOTYPES.

	Inherited Congenital Myoclonus	Maple Syrup Urine Disease
Onset of clinical signs	Prior to birth	1-3 days after birth
Most consistent clinical signs	Stimulus-responsive myoclonic spasms	Dullness and opistothonus
Post mortem observations	Traumatic lesions of hip joints	Burnt sugar aroma of urine
Histological lesions in CNS	None	Status spongiosus
Biochemical findings	Glycine receptor site abnormality with no effect on free amino acid concentrations	Deficient BCKAD activity resulting elevated levels of val, ile, leu, KIVA, KMVA and KICA
Features common to both genotypes	Poll hereford and Poll hereford cross calves affected Autosomal recessive mode of inheritance	

APPENDIX 1.

PLASMA AMINO ACID CONCENTRATIONS (uM) IN VARIOUS SPECIES.

Amino Acid	Man (a)		Rat (b)		Calves (c)	
	Mean	SD	Mean	SD	Mean	SD
Taurine	70	25	309	20	74	19
Aspartate	9	4	53	25	26	16
Threonine	122	41	74	24	137	45
Serine	132	31	614	183	123	38
Asparagine	49	17	97	28		
Glutamate	46	16	127	16	191	107
Glutamine	571	119	949	372		
Glycine	225	59	434	145	271	68
Alanine	360	120	584	179	211	39
Citrulline					77	10
Valine	306	87	96	34	344	93
Methionine	23	10	30	8	52	32
Isoleucine	64	29	41	15	148	22
Leucine	138	54	87	25	238	43
Tyrosine	69	25	27	10	90	18
Phenylalanine	62	18	43	9	104	20
Histidine	91	22	181	42	76	26
Tryptophan			24	6	77	-
Lysine	168	51	325	124	280	68
Arginine	62	25	138	56	186	48

(a) : Gregory et al, 1986. n=52. Values represent children:mean of 8 years. (AA analyser).

(b) : Gustafson et al, 1986. n=6 (AA analyser).

(c) : Williams et al, 1980. Values represent one preruminant plasma analysed by four laboratories. Only one result for tryptophan (AA analyser).

APPENDIX 2.

PLASMA AMINO ACID CONCENTRATIONS (uM) IN VARIOUS SPECIES.

Amino Acid	Sheep (d)		Rabbit (e)		Chick (f)	
	Mean	SD	Mean	SD	Mean	SD
Taurine					210	65
Aspartate	250	132	145		58	25
Threonine	400	299	183		426	139
Serine	160	100	526		253	74
Asparagine					34	25
Glutamate	1400	465	490		76	20
Glutamine					605	93
Glycine	170	66	2534		209	37
Alanine	120	33	724		133	37
Citrulline						
Valine	360	132	308		311	74
Methionine	30	0	70		43	17
Isoleucine	180	66	108		175	34
Leucine	200	66	192		167	31
Tyrosine	30	0	137		61	11
Phenylalanine	70	33	78		58	8
Histidine	230	132			35	8
Tryptophan					38	3
Lysine	120	100	379		211	68
Arginine	80	33			106	14

(d) : Krishnamurti et al, 1984. n=11. Adult ewe. (GC).

(e) : Bauer et al, 1986. n=145. Adult rabbits. (GC). Values represent 97.5% percentile. No SD's given.

(f) : Frampton et al, 1986. n=8. Newborn chick. (RP-HPLC).

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the medulla, grey and white matter of the cerebell
amygdala and thalamus. The other amino acids to
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Glutamate concentration was greater in
the medulla. Aspartate was th
brains (pons).

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the medulla, grey and white matter of the cerebellum, frontal cortex, amygdala and thalamus. The other amino acids to have higher concentrations in ICM brains were valine, isoleucine and alanine (caudate nucleus). Glutamate concentration was greater in the caudate of ICM calves but lower in the medulla. Aspartate was the only other amino acid to be lower in ICM brains (pons).

On an average of all the brain sites, only taurine and phosphoserine concentrations were significantly different ($p<0.05$) between the two genotypes, both being higher in ICM calves.

Differences between brain sites/regions were compared between the normal and ICM genotypes (Tables 29, 30 and 31). No interactions were observed between any combination of sites for serine, glutamine, citrulline, valine, methionine, leucine, tyrosine, GABA, histidine, lysine or arginine.

While the concentration of taurine was higher in the white matter of the cerebellum than the grey for both normal and ICM calves, and taurine concentration in the grey matter was similar for both genotypes, the concentration of taurine in the white matter of the cerebellum of ICM calves (7889 nmoles/g) was much higher than for normal calves (4410 nmoles/g). Comparison of the medulla and pons with the cerebellum showed a significant interaction that suggested the concentration of taurine in the grey matter of the cerebellum of ICM calves was lower than would be expected given the pattern at the other 3 sites for both genotypes. One other significant interaction of site by genotype for taurine involved all the brain regions, suggesting a reflection of the previous 2 interactions.

The only interaction of site by genotype for phosphoserine was between the white and grey matter of the cerebellum. As was the finding with taurine, analysis of patterns within the matrix showed the level of phosphoserine of ICM calves to be much higher than normal in the white matter of the cerebellum. The remaining 13 significant interactions involved amino acids that were not different on average over all the brain sites. Having established few concentration differences within brain sites between normal and ICM calves and few interactions of sites/regions with these genotypes, data for the normal and ICM calves were pooled to assess differences in amino acid concentrations between sites/regions in the brain.

TABLE 17.

PRIMARY AMINO ACID CONCENTRATIONS (nmoles/g) IN THE MEDULLA OBLONGATA.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean	S.D.
Tau	3524	1627	5944 *	2181	5244	2649
Asp	5048	791	5253	1383	1184 ***	427
Thr	568	225	449	153	385	295
Ser	1345	359	1438	192	1498	780
Glu	9597	900	8494 *	787	3506 ***	339
Gln	685	444	730	286	2398 ***	974
Gly	3272	603	2610	708	3815	1034
Ala	2767	723	2841	1195	1185 ***	432
Cit	76	58	94	20	193 *	114
Val	326	158	350	112	2890 ***	715
Met	85	77	79	54	115	79
Ile	192	143	230	188	1091 ***	304
Leu	366	228	358	124	3580 ***	1206
Tyr	186	77	165	60	172	138
Phe	232	101	219	84	270	247
GABA	3117	752	3398	1133	1076 ***	342
His	295	110	291	88	300	229
Lys	1038	509	1725	702	631 **	627
Arg	1055	272	972	226	502 ***	344
Pse	410	297	759 *	223	666	517
Pea	77	96	51	61	929 ***	546

* : $p<0.5$
** : $p<0.1$
*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

TABLE 18.

PRIMARY AMINO ACID CONCENTRATIONS (nmoles/g) IN THE PONS.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean	S.D.
Tau	4197	1341	7011 *	2210	5655	4129
Asp	7832	3028	4380 *	2096	1268 ***	566
Thr	484	267	380	84	246 *	210
Ser	1330	446	1509	207	1386	695
Glu	10332	3762	7901	1477	3381 ***	869
Gln	492	640	384	311	1908 **	985
Gly	2550	1227	2500	395	2314	682
Ala	2434	562	2402	420	1172 ***	494
Cit	94	66	076	24	163	107
Val	325	226	238	67	2196 ***	907
Met	141	105	98	22	75	91
Ile	161	113	122	37	860 ***	292
Leu	376	244	353	121	3093 ***	1318
Tyr	179	98	143	74	160	146
Phe	204	135	221	87	253	259
GABA	3745	964	3985	809	775 ***	561
His	339	156	300	49	283	184
Lys	1511	502	2080	373	871 **	1082
Arg	1034	341	1054	176	549 **	376
Pse	493	281	876	423	704	493
Pea	68	62	43	26	712 ***	472

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

TABLE 19.

PRIMARY AMINO ACID CONCENTRATIONS (nmoles/g) IN THE WHITE MATTER OF THE CEREBELLUM.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=5) Mean	S.D.
Tau	4410	2043	7889 *	2836	5390	2241
Asp	4448	493	4009	1263	947 ***	369
Thr	656	236	543	145	304 **	173
Ser	1422	234	1671	192	1576	1113
Glu	10446	1623	10047	1709	3089 ***	548
Gln	537	396	738	586	2229 ***	1031
Gly	2262	277	2268	413	1538 ***	188
Ala	2220	543	2150	564	1145 **	685
Cit	118	61	113	47	178	66
Val	351	132	371	138	2987 ***	756
Met	150	86	136	57	122	56
Ile	213	160	211	136	1128 **	494
Leu	443	217	439	173	3829 ***	2227
Tyr	225	139	211	62	210	84
Phe	304	123	306	51	278	215
GABA	3202	735	3153	506	1128 ***	322
His	340	103	324	112	295	170
Lys	1214	514	1994	839	392 ***	290
Arg	1044	257	1038	125	712 *	368
Pse	436	303	920 *	257	945	1008
Pea	169	149	144	93	1210 ***	625

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

TABLE 20.

PRIMARY AMINO ACID CONCENTRATIONS (nmoles/g) IN THE GREY MATTER OF THE CEREBELLUM.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=5) Mean	S.D.
Tau	2863	593	2867	556	5173 **	1997
Asp	4464	1040	4251	843	812 ***	329
Thr	637	267	575	134	370 *	212
Ser	1343	239	1513	241	1273	260
Glu	11557	2551	11355	1587	5758 ***	1360
Gln	740	519	838	254	2927 ***	1196
Gly	2258	550	2567	482	1823 *	479
Ala	2692	549	2690	716	1210 ***	458
Cit	55	42	46	36	147 *	40
Val	445	190	427	137	2737 ***	609
Met	97	61	89	77	142	74
Ile	177	101	215	114	965 ***	297
Leu	418	173	411	178	3294 ***	1299
Tyr	173	59	158	66	213	110
Phe	242	86	264	70	254	219
GABA	4567	729	3862	494	1687 ***	443
His	329	110	357	66	235 *	103
Lys	1254	568	1690	772	384 ***	258
Arg	617	197	581	105	411 *	216
Pse	243	98	376 *	95	411	302
Pea	203	176	180	108	2289 ***	1263

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

TABLE 21.

PRIMARY AMINO ACID CONCENTRATIONS (nmoles/g) IN THE FRONTAL CORTEX.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean	S.D.
Tau	2076	626	2850	867	3557	1964
Asp	6097	1063	6329	1120	1033 ***	513
Thr	566	232	576	91	377 *	294
Ser	1564	226	1789	245	1113 **	417
Glu	11721	2505	12284	1086	4945 ***	1702
Gln	667	339	806	262	1462	821
Gly	1930	476	2556	932	2098	579
Ala	2108	562	2638	961	1063 ***	374
Cit	114	45	93	80	186	65
Val	342	154	367	108	2714 ***	635
Met	077	51	64	58	105	61
Ile	138	82	204	153	1026 ***	231
Leu	331	158	349	110	3550 ***	1022
Tyr	130	44	113	69	169	92
Phe	209	69	215	90	184	72
GABA	3891	843	4150	715	1703 ***	417
His	288	77	341	90	289	197
Lys	1301	786	1672	862	486 **	445
Arg	485	173	484	177	319 *	107
Pse	241	141	436 *	124	354	268
Pea	113	52	159	144	2202 ***	1672

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

TABLE 22.

PRIMARY AMINO ACID CONCENTRATIONS (nmoles/g) IN THE TEMPORAL CORTEX.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean		S.D.
Tau	2085	597	2568	703	3703	*	1663
Asp	6534	1833	5605	1134	862	***	484
Thr	552	202	519	79	307	**	166
Ser	1571	314	1649	278	1044	**	404
Glu	11921	1820	12052	2148	4663	***	1268
Gln	561	303	667	135	1526	**	967
Gly	2103	716	2461	573	1967		549
Ala	2048	628	2329	835	1082	***	328
Cit	117	67	97	50	211		66
Val	328	139	384	86	2382	***	547
Met	80	48	74	40	99		54
Ile	134	40	195	82	952	***	224
Leu	330	165	330	55	3341	***	999
Tyr	147	40	101	60	169		81
Phe	200	66	210	46	230		156
GABA	3787	442	3335	1162	1683	***	349
His	284	80	292	59	238		97
Lys	1164	613	1443	622	598	**	559
Arg	495	170	488	92	348	*	144
Pse	252	167	363	187	364		294
Pea	122	59	100	55	1796	***	1133

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

TABLE 23.

PRIMARY AMINO ACID CONCENTRATIONS (nmoles/g) IN THE OCCIPITAL CORTEX.

AMINO ACID	CONTROL (n=7)		ICM (n=6)		MSUD (n=6)		
	Mean	S.D.	Mean	S.D.	Mean		S.D.
Tau	1979	457	2339	653	3669	*	1717
Asp	5344	3206	5756	844	1068	***	645
Thr	643	252	552	111	298	**	177
Ser	1683	410	1643	230	1089	**	439
Glu	12490	2348	11858	1283	4756	***	1084
Gln	702	485	812	330	1657	**	842
Gly	2281	779	2332	814	2123		775
Ala	2176	670	2467	764	1205	**	491
Cit	140	77	143	47	223	*	50
Val	360	94	393	124	2475	***	813
Met	117	58	122	58	105		67
Ile	194	73	248	87	988	***	299
Leu	398	234	363	141	3485	***	1375
Tyr	165	50	146	76	202		163
Phe	234	82	235	84	226		168
GABA	3910	615	3350	518	1784	***	449
His	325	190	304	85	248		164
Lys	2025	2302	1495	618	584	**	555
Arg	522	263	488	122	340		162
Pse	252	185	368	181	385		244
Pea	133	82	121	63	1997	***	1297

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

TABLE 24.

PRIMARY AMINO ACID CONCENTRATIONS (nmoles/g) IN THE AMYGDALA.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean	S.D.
Tau	2174	376	3226	1575	3413	1389
Asp	5121	1977	5257	1448	1639 ***	1754
Thr	684	302	617	152	340 **	172
Ser	1642	486	1779	362	1230 *	669
Glu	13622	4533	13122	1950	4062 ***	1717
Gln	726	458	715	376	1313	866
Gly	2357	849	2572	698	1783	477
Ala	2022	616	2312	521	1358 **	773
Cit	71	52	71	30	140	99
Val	398	198	430	152	2668 ***	636
Met	119	65	125	65	70 *	70
Ile	190	104	240	113	1058 ***	341
Leu	400	197	440	200	3533 ***	1167
Tyr	135	82	168	85	176	92
Phe	221	89	266	120	271	233
GABA	4116	787	4114	313	3640	1029
His	325	157	343	88	290	137
Lys	2549	2774	1966	577	649 **	541
Arg	492	245	570	348	431	283
Pse	248	136	566 *	288	470	446
Pea	161	122	167	107	2064 ***	2465

* : $p<0.5$

** : $p<0.1$

*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

TABLE 25.

PRIMARY AMINO ACID CONCENTRATIONS (nmoles/g) IN THE THALAMUS.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean	S.D.
Tau	3620	808	5687	2595	4906	2164
Asp	5859	1960	5472	3124	1651 ***	1614
Thr	834	257	677	224	292 **	197
Ser	2137	559	2043	408	1231 **	793
Glu	13793	2738	12623	2527	3932 ***	2028
Gln	719	609	641	357	1593 **	725
Gly	3968	2227	3002	575	1802 **	381
Ala	2319	507	2165	716	1267 **	705
Cit	204	97	176	114	215	102
Val	413	96	396	155	3010 ***	568
Met	211	57	204	89	101 *	70
Ile	271	52	307	194	1155 ***	297
Leu	633	284	563	222	3874 ***	1095
Tyr	265	109	224	117	208	101
Phe	412	169	374	145	320	220
GABA	3998	771	4114	424	3030 *	1686
His	454	219	390	94	282 *	140
Lys	2638	2617	2144	214	564 ***	514
Arg	1246	420	1042	310	476 ***	328
Pse	356	188	824 *	482	660	581
Pea	163	163	170	165	1495 ***	832

* : $p<0.5$
** : $p<0.1$
*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

TABLE 26.

PRIMARY AMINO ACID CONCENTRATIONS (nmoles/g) IN THE PUTAMEN.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=5) Mean		S.D.
Tau	2864	665	3296	856	3276		1359
Asp	5727	3027	4556	2200	1939	**	2379
Thr	768	327	737	171	456	*	238
Ser	2144	646	2118	219	1500	**	471
Glu	14761	4677	14310	2002	5977	***	1750
Gln	604	614	604	259	2198	***	744
Gly	3147	638	3311	283	2060	***	728
Ala	1978	447	2152	359	1716		1182
Cit	122	58	139	48	232		166
Val	434	147	477	147	3746	***	1610
Met	230	93	225	69	125	*	68
Ile	263	112	297	67	1481	***	858
Leu	611	325	627	156	4508	***	2718
Tyr	203	119	208	81	210		61
Phe	322	146	381	100	326		206
GABA	3603	773	3526	474	3545		826
His	446	237	445	66	278	*	55
Lys	2222	696	2889	2211	451	***	353
Arg	890	310	783	108	373	***	198
Pse	388	223	541	224	459		356
Pea	181	218	183	182	3008	***	810

* : $p<0.5$
** : $p<0.1$
*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

TABLE 27.

PRIMARY AMINO ACID CONCENTRATIONS (nmoles/g) IN THE CAUDATE NUCLEUS.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean	S.D.
Tau	2517	402	3137	975	3290	1264
Asp	3835	1385	4390	1461	825 ***	674
Thr	609	211	738	145	337 ***	161
Ser	1832	362	2058	241	1273 **	562
Glu	12014	3310	14800 *	472	4746 ***	1842
Gln	653	705	787	331	1889 *	1275
Gly	2839	374	3359	518	2114 ***	494
Ala	1841	365	2296 *	339	1191 ***	341
Cit	113	44	133	71	253 ***	56
Val	347	111	499 *	158	2432 ***	697
Met	154	49	229	114	96 **	49
Ile	206	69	298 *	95	1012 ***	237
Leu	480	237	603	208	3200 ***	904
Tyr	186	80	193	89	150	70
Phe	258	77	324	39	250	124
GABA	3164	1348	3044	718	3077	591
His	381	148	381	35	273 *	77
Lys	2019	289	1996	705	545 ***	555
Arg	672	202	644	111	352 ***	121
Pse	290	138	473	225	378	333
Pea	174	193	214	217	2301 ***	1362

* : $p<0.5$
** : $p<0.1$
*** : $p<0.01$
Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.

TABLE 28.

PRIMARY AMINO ACID CONCENTRATIONS (nmoles/g) IN THE SUBSTANTIA NIGRA.

AMINO ACID	CONTROL (n=7) Mean	S.D.	ICM (n=6) Mean	S.D.	MSUD (n=6) Mean	S.D.
Tau	3020	1011	5593 *	2053	4456	1663
Asp	7142	4371	7374	3418	1631 **	1844
Thr	614	315	558	220	378	200
Ser	1417	605	1633	291	1301	740
Glu	10924	4593	9334	1738	4569 **	2298
Gln	612	523	559	211	2072 **	1256
Gly	2752	476	2935	485	2202 *	1001
Ala	2080	669	2365	459	1572	1173
Cit	107	80	102	62	180 *	71
Val	321	129	325	118	2959 ***	1193
Met	144	57	188	75	96 *	52
Ile	182	69	188	60	1243 ***	607
Leu	438	243	472	180	4185 ***	2018
Tyr	193	82	180	82	193	85
Phe	266	139	319	84	301	157
GABA	5410	1092	5952	1472	4223 *	1694
His	440	217	447	117	326	62
Lys	3072	3986	3046	2016	1443 *	2234
Arg	1038	272	1001	22	424 ***	150
Pse	352	230	734	415	550	476
Pea	81	82	85	71	1903 ***	1032

* : $p<0.5$
** : $p<0.1$
*** : $p<0.01$

Significance levels based on differences between normal and ICM; and between normal plus ICM and MSUD.