

Acta Medica Okayama

Volume 40, Issue 6

1986

Article 5

DECEMBER 1986

Role of ammonia in the pathogenesis of brain edema.

Masachika Fujiwara*

*Okayama University,

Copyright ©1999 OKAYAMA UNIVERSITY MEDICAL SCHOOL. All rights reserved.

Role of ammonia in the pathogenesis of brain edema.*

Masachika Fujiwara

Abstract

The role of hyperammonemia in the pathogenesis of cerebral edema was investigated using mongrel dogs to develop a treatment for cerebral edema in acute hepatic failure. Intravenous infusion of ammonium acetate alone into dogs did not induce brain edema, although blood ammonia reached unphysiologically high levels. However, ammonium acetate infusion during mannitol-induced reversible (osmotic) opening of the blood-brain barrier (BBB) effectively induced cytotoxic brain edema. Pretreatment with a branched-chain amino acid (BCAA; valine, leucine and isoleucine) solution prevented an increase in intracranial pressure (ICP) and brain water content, and caused a decrease in brain ammonia content and an increase in brain BCAA and glutamic acid. The results suggest that ammonia plays an important role in the pathogenesis of cerebral edema during acute hepatic failure and that BCAAs accelerate ammonia detoxification in the brain.

KEYWORDS: brain edema, ammonia, blood-brain barrier, acute hepatic failure, branched-chain amino acid

*PMID: 3825594 [PubMed - indexed for MEDLINE]

Role of Ammonia in the Pathogenesis of Brain Edema

Masachika Fujiwara

First Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan

The role of hyperammonemia in the pathogenesis of cerebral edema was investigated using mongrel dogs to develop a treatment for cerebral edema in acute hepatic failure. Intravenous infusion of ammonium acetate alone into dogs did not induce brain edema, although blood ammonia reached unphysiologically high levels. However, ammonium acetate infusion during mannitol-induced reversible (osmotic) opening of the blood-brain barrier (BBB) effectively induced cytotoxic brain edema. Pretreatment with a branched-chain amino acid (BCAA; valine, leucine and isoleucine) solution prevented an increase in intracranial pressure (ICP) and brain water content, and caused a decrease in brain ammonia content and an increase in brain BCAA and glutamic acid. The results suggest that ammonia plays an important role in the pathogenesis of cerebral edema during acute hepatic failure and that BCAAs accelerate ammonia detoxification in the brain.

Key words : brain edema, ammonia, blood-brain barrier, acute hepatic failure, branched-chain amino acid

Acute hepatic failure shows a high incidence of mortality even when intensive therapeutic medicine is initiated rapidly. Complications of the disease, such as cerebral edema, sepsis, gastrointestinal bleeding and renal failure, develop frequently during the illness. Among these complications, cerebral edema is the most important (1, 2). In 9 cases (64%) of 14 hepatic failures in our clinic, cerebral edema was found during post-mortem examination of the brain (3). The low survival rate of patients with acute hepatic failure might be improved by treating and controlling cerebral edema. However, the pathogenesis of cerebral edema in this disorder has not yet been established. Previous reports have already mentioned an increase in BBB permeability in hepatectomized rats (4) and cytotoxic cerebral edema in patients with acute hepatic failure (5).

The present study was performed to clarify the pathogenesis of and develop a treatment for cerebral edema in acute hepatic failure by studying the changes in BBB permeability, the role of hyperammonemia in causing cytotoxic edema and the preventative effect of a BCAA solution.

Materials and Methods

Preparation of experimental animals. Adult mongrel dogs, weighing 10-15 kg each, were used after overnight fasting. Animals were anesthetized with ketamine hydrochloride (5.8 mg/kg body weight, intramuscularly; Sankyo Co., Tokyo, Japan) and relaxed with succinylcholine chloride (2 mg/kg body weight, intramuscularly; Yamano-uchi Pharmaceutical Co., Tokyo, Japan). The dogs were placed on a volume-cycled ventilator with a Harvard respiratory pump after intubation, and

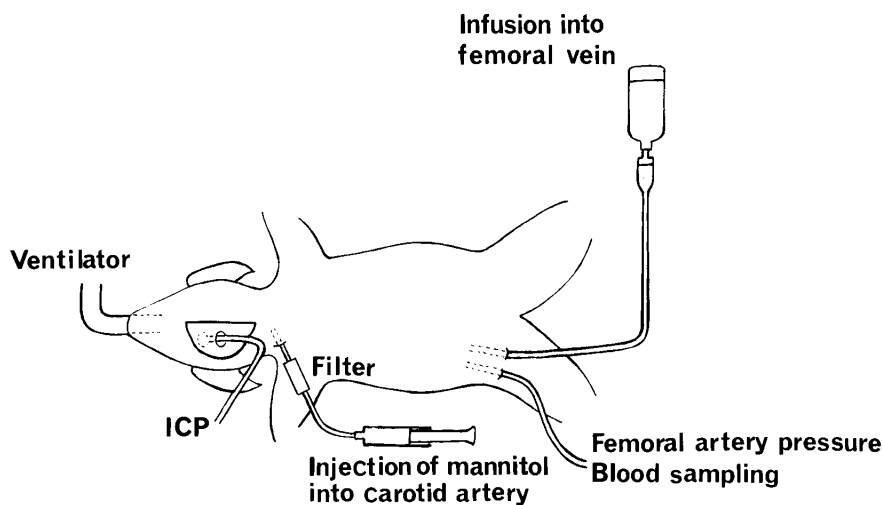


Fig. 1 Experimental set up. The dog was anesthetized and relaxed with intramuscular ketamine hydrochloride and succinylcholine chloride and placed on a volume-cycled ventilator after intubation. Arterial blood pressure was monitored, and blood was sampled from the femoral artery. An intracranial pressure transducer was inserted into the epidural space in the parietal region for monitoring ICP. An arterial catheter was introduced into the internal carotid artery through the common carotid artery for injecting mannitol solution. Drip infusion of 5% ammonium acetate, BCAA or saline solution was performed using the femoral vein.

anesthesia was maintained by administering the anesthetics described above at about 2-h intervals (Fig. 1). Cannulae were positioned in the femoral vein and artery. Arterial blood pressure was monitored with a strain gauge electromanometer (Nihon-Koden Model p-37, Tokyo, Japan), and blood was sampled from the femoral artery. Arterial gas analysis was conducted with a Corning Model 165/2 analyzer (Corning EEL, Essex, England). An arterial catheter (21 gauge needle with No. 15 tubing), filled with 0.9% NaCl and 1000 U/ml Na heparin, was introduced into the internal carotid artery through the common carotid artery for injecting mannitol solution.

A burr hole was made over the parietal region of the skull and an intracranial pressure transducer (Konigsberg Co., California, USA) was inserted into the epidural space to monitor ICP. The burr hole was sealed with a dental rubber base impression material (Surflex F, GC Dental Industrial Co., Tokyo, Japan) to ensure stable positioning of the instrument. The transducer lead was attached to a calibration unit with an amplifier (Konigsberg Co.). ICP was recorded at a paper speed of 0.5 cm/min on a VP-6521 W pen recorder (National Co., Osaka, Japan). Calibration

was checked before the experimental procedure. The experiments were started half an h after inserting the sensor into the epidural space, when ICP became stable. Changes in ICP were classified into four patterns: A) continuous increase throughout, B) one-time increase followed by a gradual decrease to 10 mmHg above the baseline value, stabilizing over half an h, C) similar to Pattern B, except for returning to approximately the baseline value, and D) no increase.

Physiological conditions such as body weight, body temperature, arterial blood pressure, arterial pH, PO_2 , PCO_2 and initial ammonia levels were not different between the test group and the control group.

Experiment 1. To induce acute hepatic failure in dogs, the abdomen was opened and dimethylnitrosamine (DMN, 25 mg/kg body weight; Wako Pure Chemical Industries Ltd., Osaka, Japan) was injected into the portal vein through the mesenteric vein in 5 dogs (AHF group). Liver function tests in the AHF group showed that the average serum GPT activities increased to 611 IU/l, prothrombin time was delayed (25.2 sec; control 13.5 sec) and blood ammonia concentrations increased up to 160 $\mu\text{g}/\text{dl}$ 12 h after the DMN injection.

Pathological findings of the liver in the AHF group demonstrated submassive hepatic necrosis. The four control animals were injected with physiological saline by a similar route. Twelve h after the portal DMN or saline injection, a hyperosmolar solution of mannitol (1.4 M, 2 ml/kg body weight (6), Wako Pure Chemical Industries Ltd., Osaka, Japan) was manually injected at a constant rate over 1 min through a 0.2 μ m filter (Nipro Co., Tokyo, Japan) into the internal carotid artery. The infusate was freshly prepared and warmed to 37°C to prevent crystallization (7). Immediately after the mannitol injection, 2% Evans blue (Nakarai Chemical Co., Kyoto, Japan) was injected intravenously to indicate the vasogenic state, and the animals were sacrificed 2 h later. The brain was removed and macroscopically and microscopically studied. The water content of the grey and white matter of the optical chiasma was measured gravimetrically after drying the tissue to constant weight in an oven (110°C) for 48 h (8). Blood sugar levels were determined with the Dextrometer System (Miles Laboratories Inc., Elkhart, USA).

Experiment 2. Five percent ammonium acetate solution (Kanto Chemical Co., Osaka, Japan) was infused into the femoral vein for two h at a rate of 2 ml/kg body weight/h while monitoring the ICP of the dogs. This solution produced blood ammonia levels of 200-400 μ g/dl (the Amitest

Meter System, Chugai Pharmaceutical Co., Tokyo, Japan)(9) after a one-h infusion. Following the infusion, a 1.4 M mannitol solution was injected into the carotid artery ($n=7$; test group). This mannitol concentration was just above the threshold for osmotic BBB disruption, which reversibility occurred only for 30 min (6). Two other groups were used as controls: Control 1, 5% ammonium acetate was infused without the intracarotid mannitol bolus injection ($n=4$), and Control 2, saline instead of ammonium acetate was infused with the mannitol injection ($n=5$). The animals were sacrificed when the ICP stabilized over half an h (2-3 h following the mannitol administration).

Experiment 3. A BCAA solution was made by dissolving 63 mM leucine, 42 mM isoleucine (Ishizu Pharmaceutical Co., Osaka, Japan) and 52 mM valine (Nakarai Chemical Co., Kyoto, Japan) (10) and infused into a femoral vein for 4 h at a rate of 10 ml/kg body weight/h ($n=12$, BCAA group). Saline was infused as a control ($n=9$) (Fig. 2). One h after initiating the infusion of the BCAA solution, 5% ammonium acetate was infused into a femoral vein for 2 h at a rate of 2 ml/kg body weight/h. One h after starting the ammonia infusion, hyperosmolar mannitol was injected into the carotid artery. After a 4-h infusion of BCAA, part of the cerebral tissue was biopsied with a freezing clamp (Natsume Industries Co., Tokyo, Japan) to measure brain ammonia contents, and

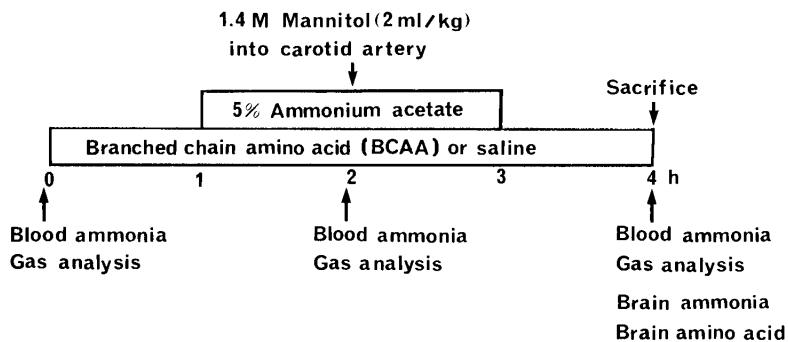


Fig. 2 Time course of Experiment 3. A BCAA solution was infused for 4 h at a rate of 10 ml/kg body weight/h. Saline was infused as the control. One h after initiating the infusion of the BCAA solution, 5% ammonium acetate was infused for 2 h at a rate of 2 ml/kg body weight/h. One h after start of the ammonia infusion, hyperosmotic mannitol was injected into the internal carotid artery to open the BBB permeability transiently. During the 4-h infusion of BCAA, brain tissue in the parietal region was biopsied with a freezing clamp to measure brain ammonia contents, and then the dogs were sacrificed by exsanguination. The brain was immediately removed to measure amino acid contents and for microscopic observation.

animals were sacrificed immediately. Brain ammonia contents were determined with an enzymatic kit (Kyowa Medex Co., Tokyo, Japan) according to Folbergrová *et al.* (11). After clear protein-free supernatants were made of the serum and brain by adding 5% sulfosalicylic acid, the serum and brain concentrations of amino acids were determined using a Nihon-Denshi liquid chromatograph (JCL-6 AH, Tokyo, Japan) as reported previously (12).

All of the differences were evaluated for statistical significance by the unpaired Student's *t*-test.

Results

Preliminary experiment. An intravenous infusion of ammonium acetate alone did not increase ICP, although blood ammonia levels reached 200–400 $\mu\text{g}/\text{dl}$, which are levels

commonly observed in patients with acute hepatic failure. When a large injection of ammonium acetate resulted in blood ammonia levels of 800 $\mu\text{g}/\text{dl}$, ICP finally increased to a mean level of 34 mmHg. However, this increase may have been due to severe metabolic acidosis ($\text{pH}=7.044$).

Experiment 1. To investigate changes in the permeability of the BBB in acute hepatic failure, both the AHF (portal DMN injection) and control groups were injected with the hyperosmotic mannitol solution. ICP was almost unchanged before the mannitol injection, but 2 h after the injection, ICP became much higher in the AHF group than in the control group (Table 1). Brain water content (only in the white matter) increased in the AHF group much more than

Table 1 Intracranial pressure and brain water content (Experiment 1)

Group	No. of dogs examined	Intracranial pressure (mmHg)		Brain water content (%)	
		Initial ^a	Final	Gray matter	White matter
Control	4	6.3 \pm 0.5 ^b	5.8 \pm 1.1	79.1 \pm 0.4	71.3 \pm 0.2
Acute hepatic failure	5	7.0 \pm 1.4	20.4 \pm 1.9**	80.2 \pm 0.8	72.0 \pm 0.2*

a: Twelve h after dimethylnitrosamine or saline administration (immediately before mannitol injection.)

b: Values are expressed as the mean \pm SE.

*, $p < 0.05$ ***, $p < 0.01$ against control.

Table 2 Intracranial pressure and brain water content (Experiment 2)

Group	No. of dogs examined	Intracranial pressure (mmHg)				Brain water content (%)	
		Initial ^a	1 h after ammonia ^b	Maximum ^c	Final	Gray matter	White matter
Control 1 ^d	4	3.8 \pm 0.5 ^e	5.0 \pm 0.6	—	5.5 \pm 0.7	80.7 \pm 0.9	71.1 \pm 0.2
Control 2 ^e	5	3.6 \pm 1.0	—	14.4 \pm 4.6	4.4 \pm 0.9	80.5 \pm 0.7	67.8 \pm 1.0
Test ^f	7	6.0 \pm 1.1	9.6 \pm 2.3	48.6 \pm 11.3 ^g	29.4 \pm 9.1*, #	84.2 \pm 0.9*, ##	77.5 \pm 1.7**, ##

a: Beginning of the experiment.

b: 1 h after infusion of 5% ammonium acetate solution.

c: Maximum value after mannitol bolus injection.

d: Ammonium acetate was infused without mannitol injection.

e: Saline instead of ammonium acetate was infused with mannitol.

f: Ammonium acetate was infused with mannitol.

g: Values are expressed as the mean \pm SE.

*, $p < 0.05$ **, $p < 0.01$ against control 1. #, $p < 0.05$ ##, $p < 0.01$ against control 2.

in the control group. The staining of Evans blue in the mannitol-injected side of the brain was also much more intense in the AHF group than in the control group. These findings indicate that the BBB in acute hepatic failure dogs was more easily altered by osmotic stimulus than in the control dogs.

Experiment 2. The effects of hyperammonemia during increased BBB permeability on ICP and brain water content were tested by drip infusion of an ammonium acetate solution. ICP and brain water content in the test group of dogs, which were injected with ammonium acetate under the osmotic BBB opening, were much higher than in the two control groups (Table 2). Although the test group showed ICP Pattern A or B, the two

control groups showed Pattern C or D (χ^2 -test, $p < 0.05$, data not shown). Macroscopic findings of the brain in the mannitol-injected side showed a swollen hemisphere with narrowed gyri and flattened convolutions. The light microscopic findings of the white matter in the injected side demonstrated a spongy texture. The brain was not stained with Evans blue 2 h after the mannitol injection, while ICP and brain water content remained increased. This result indicates that brain edema is cytotoxic in nature.

Experiment 3. The preventive effect of a BCAA solution on ammonia-induced brain edema was tested using the model described above. After an intracarotid bolus injection of mannitol, ICP and brain water content

Table 3 Intracranial pressure and brain water content (Experiment 3)

Group	No. of dogs examined	Intracranial pressure (mmHg)				Brain water content (%)	
		Initial ^a	1 h after ammonia ^b	Maximum ^c	Final	Gray matter	White matter
Saline	12	6.3	9.0	69.1	54.3	83.9	76.7
+ Ammonia ^d		$\pm 0.7^f$	± 1.4	± 14.7	± 16.2	± 0.6	± 1.0
BCAA	9	6.7	7.0	26.6	11.7	82.1	70.1
+ Ammonia ^e		± 0.8	± 1.1	$\pm 7.4^*$	$\pm 4.0^*$	± 0.5	$\pm 0.6^*$

a: Beginning of the experiment.

b: 1 h after infusion of ammonium acetate solution.

c: Maximum value after mannitol bolus injection.

d: 5% ammonium acetate solution was infused with mannitol bolus injection without BCAA-pretreatment.

e: Ammonium acetate was infused with mannitol injection with BCAA-pretreatment.

f: Values are expressed as the mean \pm SE.

*, $p < 0.05$ **, $p < 0.01$ against Saline + Ammonia.

Table 4 Brain ammonia and amino acid contents (Experiment 3)

Group	No. of dogs examined	Ammonia	Amino acids							
			BCAA			AAA				
			Leu	Ile	Val	Tyr	Phe	Gln	Glu	Ala
Saline	5	2.84	0.12	0.07	0.18	0.06	0.12	5.29	5.23	0.95
+ Ammonia ^a		$\pm 0.42^c$	± 0.03	± 0.01	± 0.05	± 0.01	± 0.02	± 1.00	± 0.45	± 0.22
BCAA	5	1.08	0.61	0.35	1.00	0.02	0.04	5.55	6.90	1.26
+ Ammonia ^b		$\pm 0.08^*$	$\pm 0.10^{**}$	$\pm 0.06^{**}$	$\pm 0.19^{**}$	$\pm 0.00^{**}$	$\pm 0.01^{**}$	± 1.00	$\pm 0.41^*$	± 0.11

a: 5% ammonium acetate solution was infused with mannitol bolus injection without BCAA-pretreatment.

b: Ammonium acetate was infused with mannitol injection with BCAA-pretreatment.

c: Values (mmoles/kg brain) are expressed as the mean \pm SE.

*, $p < 0.05$ **, $p < 0.01$ against Saline + Ammonia.

in the BCAA-pretreated dogs scarcely increased as compared with those in the saline-treated group (Table 3). The number of dogs with ICP Patterns of A, B, C and D was 0, 2, 2 and 5, respectively, in the BCAA group, which significantly differed from the control group (ICP Patterns A, B, C and D were observed in 5, 5, 1 and 1 dogs, respectively) (χ^2 -test, $p < 0.05$).

The brain ammonia and aromatic amino acid (AAA) contents were much lower in the BCAA-treated group, while the brain BCAA and glutamic acid levels were higher, than in the control group (Table 4). However, blood ammonia levels in the BCAA-treated group ($73.8 \pm 6.5 \mu\text{g}/\text{dl}$, Mean \pm SE) were not significantly low as compared to those in the control group (40.8 ± 8.6) at the time of sacrifice.

Discussion

The blood ammonia concentrations frequently increase in both acute and chronic hepatic failure (hepatic encephalopathy), indicating that ammonia is an important neurotoxin. Moreover, elevation of ammonia in the blood induces an increase in brain ammonia transport and a corresponding increase in brain ammonia content (13). These considerations suggest that hyperammonemia might be involved in the pathogenesis of hepatic encephalopathy and brain edema in acute hepatic failure.

Previous investigators demonstrated that the intravenous infusion of ammonium acetate solution (unphysiological levels of blood ammonia = $3938 \mu\text{g}/\text{dl}$) resulted in a rise in ICP in normal cats (14) and rabbits (15). They suggested that hyperammonemia induced an increase in cerebral blood flow and a remarkable fall in the resistance of the cerebrovascular bed (no brain edema, because brain water content did not increase). In the

present experiment, however, ICP did not rise at blood ammonia concentrations of 200–400 $\mu\text{g}/\text{dl}$, which were obtained by ammonium acetate infusion in dogs. These levels are found commonly in patients with hepatic failure. The additional ICP rise induced by infusing a larger dose of ammonium acetate seemed to be due to severe metabolic acidosis, and the blood ammonia levels obtained (about 800 $\mu\text{g}/\text{dl}$) were also beyond clinical significance.

In our laboratory, Shiota (13) previously demonstrated that brain ammonia contents in acute hepatic failure rats were very high as compared to those in normal rats even when similar blood concentrations in both groups were obtained by injecting ammonium acetate. This might be due to changes in permeability of the BBB in acute hepatic failure. The present study (Experiment 1) also showed that the BBB in acute hepatic failure dogs was easily opened by the stimulus of hyperosmotic solution. These findings strongly suggest that the cerebral influx of ammonia increases because of increased BBB permeability in acute hepatic failure.

In order to confirm this hypothesis, the BBB was transiently opened by an intracarotid infusion of hyperosmotic mannitol in Experiment 2 (6). During the BBB opening, an ammonium acetate solution was infused intravenously to obtain blood ammonia concentrations of 200–400 $\mu\text{g}/\text{dl}$. An increase in both ICP and brain water content with exclusion of Evans blue dye was confirmed, indicating that hyperammonemia produces cytotoxic brain edema only during increased BBB permeability.

Ionized ammonia (NH_4^+) does not penetrate the BBB, and 99.5% of the blood ammonia is ionized at physiological pH (16). On the contrary, un-ionized ammonia (gas form of ammonia, NH_3), which exists at less than 1% in the circulating blood, enters the brain easily by diffusion. However, under

increased permeability of the BBB and with an increased percentage of un-ionized ammonia due to metabolic alkalosis found in acute hepatic failure, both ionized and gaseous ammonia could enter the brain together. Once in the brain, they would inhibit glial and neuronal Na^+ - K^+ ATPase activities (17) and consequently induce brain Na^+ retention and finally produce cytotoxic brain edema. Pre-treatment with a BCAA solution could prevent ammonia-induced brain edema, because while the brain ammonia contents markedly decreased, both the BCAA and glutamic acid contents increased after the BCAA administration (Experiment 3). Therefore, the brain ammonia content appear to be closely involved in the pathogenesis of brain edema, supporting the hypothesis stated above.

In our laboratory, Takei (18) demonstrated that brain ammonia contents closely correlated with the increased activity of BCAA transaminase and branched chain α -ketoacid dehydrogenase in the brain mitochondrial fraction and also with the enhanced BCAA decarboxylation in the brain. Furthermore, administration of BCAAs resulted in decreased brain ammonia and increased brain glutamine, glutamic acid and alanine contents (19). The main inhibiting factor of the malate-aspartate shuttle (20) by ammonia loading would be a decrease in brain glutamic acid content. Therefore, if glutamic acid in the brain was supplied by BCAA administration, ammonia could be effectively detoxified and, thus, the neurotoxicity of ammonia could be reduced. Indeed, in the present experiment, brain glutamic acid contents significantly increased upon BCAA administration. The reason why brain glutamine did not increase in Experiment 3 might be the rapid efflux of glutamine and utilization of glutamine by neuronal cells.

A recent clinical study (21) also revealed that a BCAA-enriched solution infused for the treatment of hepatic encephalopathy effec-

tively reduced the ammonia concentration in the cerebrospinal fluid.

In summary, ammonia plays an important role in causing cerebral edema during acute hepatic failure, and BCAAs accelerate ammonia detoxification in the brain. However, the exact mechanism of the ammonia-induced brain edema is unclear and, thus, further study will be required. The substances that induce increased BBB permeability in acute hepatic failure must be investigated in the future.

Acknowledgement. I wish to express my sincere thanks to Prof. Hideo Nagashima and Associate Prof. Akiharu Watanabe for continuously guiding me and giving me valuable suggestions during this investigation.

References

1. Ware AJ, D'agostino A and Combes B: Cerebral oedema: a major complication of massive hepatic necrosis. *Gastroenterology* (1971) **61**, 877-884.
2. Canalese J, Gimson AES, Davis C, Mellon PJ, Davis M and Williams R: Controlled trial of dexamethasone and mannitol for the cerebral oedema of fulminant hepatic failure. *Gut* (1982) **23**, 625-629.
3. Fujiwara M, Watanabe A, Yamauchi Y, Hashimoto M, Nakatsukasa H, Kobayashi M, Higashi T and Nagashima H: Cerebral edema associated with acute hepatic failure. *Acta Med Okayama* (1985) **39**, 73-75.
4. Livingstone AS, Potvin M, Goresky CA, Finlayson MH and Hinchey J: Changes in the blood-brain barrier in hepatic coma after hepatectomy in the rat. *Gastroenterology* (1977) **73**, 697-704.
5. Ede R, Gimson AES, Canalese J and Williams R: Cerebral oedema and monitoring of intracranial pressure in fulminant hepatic failure. *Gastroenterol Jpn* (1982) **17**, 163-176.
6. Neuwelt EA, Frenkel EP, Rapoport S and Barnett P: Effect of osmotic blood-brain barrier disruption on methotrexate pharmacokinetics in the dog. *Neurosurgery* (1980) **7**, 36-43.
7. Tomiwa K, Hazama F and Mikawa H: Reversible osmotic opening of the blood-brain barrier. *Acta Pathol Jpn* (1982) **32**, 427-435.
8. Zimmermann V and Hossmann KA: Resuscitation of the monkey brain after one hour's complete ischemia. II. brain water and electrolytes. *Brain Res* (1975) **85**, 1-11.
9. Shiota T, Watanabe A, Takei N, Hayashi S, Obata T, Sakata T and Nagashima H: A simple and rapid

- method for determining blood ammonia levels. *Acta Med Okayama* (1983) **37**, 159-162.
10. Watanabe A, Higashi T and Nagashima H: An approach to nutritional therapy of hepatic encephalopathy by normalization of deranged amino acid patterns in serum. *Acta Med Okayama* (1978) **32**, 427-440.
 11. Folbergrová J, Passonneau JV, Lowry OH and Schulz DW: Glycogen, ammonia, and related metabolites in the brain during seizures evoked by methionine sulphoximine. *J Neurochem* (1969) **16**, 191-203.
 12. Watanabe A, Higashi T, Shiota T, Takei N, Sakata T, Fujiwara M, Nakatsukasa H, Kobayashi M and Nagashima H: Alterations in neutral amino acid transport across the blood-brain barrier in hepatic failure. *Res Exp Med* (1983) **183**, 11-18.
 13. Shiota T: Accelerated leucine decarboxylation in the rat brain in relation to increased blood ammonia levels during acute hepatic failure. *Acta Med Okayama* (1984) **38**, 219-225.
 14. Dempsey RJ and Kindt GW: Experimental acute hepatic encephalopathy; relationship of pathological cerebral vasodilatation to increased intracranial pressure. *Neurosurgery* (1982) **10**, 737-741.
 15. Brunner G, Windus G and Schmidt FW: Intracranial pressure and brain edema in experimental hyperammonemia. *Advances in Hepatic Encephalopathy and Urea Cycle Diseases*. Basel (1984) pp 325-330.
 16. Singh AK and Banister EW: Tissue ammonia and amino acids in rats at various oxygen pressures. *J Appl Physiol* (1983) **54**, 438-444.
 17. Schenker S, McCandless DW and Brophy E: Studies on intracerebral toxicity of ammonia. *J Clin Invest* (1967) **46**, 838-848.
 18. Takei N: Branched chain amino acid transaminase and branched chain alpha-ketoacid dehydrogenase activity in the brain, liver, and skeletal muscle of acute hepatic failure rats. *Acta Med Okayama* (1985) **39**, 1-10.
 19. Watanabe A, Shiota T, Takei N, Fujiwara M and Nagashima H: Ammonia detoxification by accelerated oxidation of branched chain amino acids in the brain of acute hepatic failure. *Biochem Med* (1985) (in press).
 20. Hindfelt B, Plum F and Duffy TE: Effect of acute ammonia intoxication on cerebral metabolism in rats with portacaval shunts. *J Clin Invest* (1985) **59**, 386-396.
 21. Rössle M, Herz R, Lehman G, Luft M and Gerok W: Therapie der hepatischen Enzephalopathie. Änderungen der Liquorkonzentrationen von Katecholamin-Neurotransmittern, Ammoniak und Aminosäuren im Verlaufe einer Infusionsbehandlung mit Verzweigtkettingen Aminosäuren. *Infusionsther Klin Ernähr* (1982) **9**, 256-258.

Received: July 14, 1986

Accepted: October 6, 1986

Reprint requests to:

Masachika Fujiwara

First Department of Internal Medicine

Okayama University Medical School

2-5-1 Shikata-cho

Okayama 700, Japan