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Acute Hyperammonemia in the Young Primate: Physiologic and Neuropathologic Correlates

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Summary

Infusion-induced acute (≤24 h) hyperammonemia to concentrations up to five times normal (0.19 ± 0.03 *versus* 0.90 ± 0.08 mM) was studied in eleven 6–9-month-old *Macaca mullata*. The young primates developed a progressive reduction of consciousness that correlated in severity directly with the elevation of blood ammonia concentration. Hyperventilation, electroencephalographic slowing, occasional seizure activity, and, eventually, apneustic breathing also occurred. Intracranial pressure rose from 76 ± 7 to 167 ± 12 mmH_2O. Arterial oxygen and blood pressure remained within normal limits. Neuropathologic examination showed early astrocytic changes, consisting primarily of swollen perikaryal cytoplasm and processes, and membranous whorls. The absence of neuronal pathology suggests that the acute, limited insult, as occurs in many of the childhood hyperammonemic syndromes, is fully reversible.

Abbreviations

EEG, electroencephalogram
FAM, formaldehyde, glacial acetic acid, and absolute methanol (1:1:8)
ICP, intracranial pressure
RER, rough endoplasmic reticulum

Hyperammonemia of infancy and childhood may occur in many settings: Reye's syndrome (18, 23, 39), hereditary deficiencies of urea cycle enzymes (10, 20), prematurity (5, 6, 15), birth asphyxia (19), and as a complication of urinary tract infections (38), valproic acid therapy (14), asparaginase therapy (30), and intravenous alimentation with amino acids or protein hydrolysates (21, 24). These illnesses share a common clinical picture, suggesting that hyperammonemia produces a specific constellation of signs and symptoms in infants and children, as in adults.

Ammonia intoxication has been studied in mature but not in developing animals. Acute, large increases in serum ammonia in fully grown rats cause seizures, coma, and astrocytic swelling (20, 31) whereas chronic, moderate elevations produce few behavioral changes but marked astrocytic hypertrophy (11, 17, 33, 44). Chronic hyperammonemia in mature primates causes intermittent lethargy and neuropathologic changes consistent with Alzheimer II astrocytosis (13, 27, 36).

In this study, we infused juvenile monkeys with ammonium acetate in order to examine the behavioral and neuropathologic effects of acute hyperammonemia in a young animal. The results indicate that isolated hyperammonemia is toxic to the immature nervous system and produces a sequential depression in the level of consciousness that correlates directly with the degree of elevation of the arterial ammonia concentration, but not with life-threatening elevations of intracranial pressure. The profound depression of the central nervous system, including coma, was found to be associated with morphologic changes in astrocytes but not in neurons, implying that the acute neurotoxicity of hyperammonemia may be wholly reversible.

MATERIALS AND METHODS

Animals. Macaca mullata of both sexes were housed individually in cages in compliance with the *Guide for the Care and Use of Laboratory Animals*, U.S. Public Health Service, 1972. The animals were fed fresh fruit and monkey chow, and had unlimited access to water. Their weights were 1.35–1.75 kg and ages, 6–9 months; the animals were approximately equivalent, neurodevelopmentally, to 2–3-year-old children.

Experimental procedures. The experiment was carried out in two stages. *Stage 1: Preparation of indwelling cannulae, electrodes, and epidural intracranial pressure monitors.* Surgery was performed aseptically under pentobarbital anesthesia (5 mg/kg). Indwelling cannulae were filled with heparinized saline to maintain patency, inserted into the femoral artery and vein, and buried beneath the sutured skin. The animal was placed in a stereotactic apparatus and the skull was exposed through a dorsal midline incision. Using the bregma as the center, three holes, 10 mm on each side of the sagittal suture and 15 mm apart, were made with a dental drill and threaded to accept nylon screws carrying Ag-AgCl ball electrodes for epidural EEG monitoring (9). A seventh hole, for the ground electrode, was placed on the right side, and the 7-electrode array was covered with a protective cap. An epidural acrylic plastic adaptor was inserted into a 0.5-cm fronto-parietal burr hole for subsequent monitoring of ICP (9). The animals received prophylactic penicillin-G (100,000 U/kg) and were returned to the animal quarters for 7–14 days. *Stage II: Infusion.* The animals were anesthetized lightly with ketamine (1 mg/kg), restrained in a semi-supine position with the legs immobilized, and the femoral catheters were exposed. While the animals were still under anesthesia, the femoral incisions were resutured and dressed with gauze that was moistened with sterile saline. The arterial catheter and ICP adaptor were connected to Statham pressure transducers for continuous monitoring of blood pressure and intermittent measurement of ICP. The protective electrode cap was replaced with a microelectrode adaptor that connected the six EEG leads and the ground electrode to a Beckman dynograph for continuous recording. A rectal thermistor was inserted to monitor core temperature, which was maintained at 37 ± 1°C by warming the animal with a heating lamp. After a minimum of 3.5 h, when the animals were judged to be awake by both clinical and EEG criteria, the test or control solution was infused into the femoral vein catheter via a Harvard pump.

Eleven experimental animals were infused with 0.19–1.5 M ammonium acetate dissolved in 5% dextrose. The rate of infusion was adjusted stepwise to induce a state of stupor or coma. Three control animals received infusions of 5% dextrose and 0.225% NaCl in water. The maximum rate of infusion was 6.7 ml/h (100 $ml \cdot kg^{-1} \cdot 24\ h^{-1}$). The EEG, electrocardiogram, blood pressure, ICP, heart rate, respiratory rate, and body temperature were monitored continuously in all animals, and intermittent arterial blood samples were obtained to measure blood gases and ammonia content.

At the conclusion of the infusion, the animals were anesthetized with pentobarbital (6 mg/kg, I.V.), and fully heparinized. After thoracotomy, the animals were perfused with physiologic saline at a pressure of 130 mmHg through the ascending aorta until the right atrial effluent was virtually clear, and then with the appropriate fixative for 20 min (37). Nine experimental animals were perfused with a mixture of FAM. Two experimental and two control animals were perfused with a solution of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3. The animals were decapitated and the heads were immersed in the appropriate fixative at room temperature for at least 2 h before the brains were removed.

Ammonia assay. Aliquots of whole blood were collected directly from the arterial line, immediately mixed with two volumes of ice cold 1.2 M perchloric acid and centrifuged at 0°C. The supernatant fluids were neutralized with 2 M $KHCO_3$ and stored at −80°C until analyzed by the fluorometric procedure of Folbergrová *et al* (16).

EEG analysis. The right parieto-occipital lead was evaluated according to the method of Laidlaw (28). In selected 50-sec epochs, frequencies ≤ 12 Hz were counted, and a histogram was constructed. The percentage of the tracing contributed by each frequency was then calculated. Because the dominant posterior rhythm of a 6–9-month-old rhesus monkey is 8–10 Hz (12), the proportion of each epoch spent at abnormally slow frequencies (≤7 Hz) was summated.

Neuropathologic examination. In the FAM-perfused animals, blocks from each occipital and temporal lobe, hippocampus, and cerebellar hemisphere were embedded in paraffin and in colloidin. Sections were stained with hematoxylin and eosin, and cresyl violet-luxol fast blue.

In the paraformaldehyde-glutaraldehyde-perfused animals, pieces of cortex, putamen, globus pallidus, centrum semiovale, cerebellar cortex, and cerebellar white matter were cut into 1-mm cubes, postfixed in osmium tetroxide, dehydrated with graded alcohols, and embedded in epon resin. Sections (1-μm thickness) were stained with toluidine blue and examined by light microscopy. Areas of cerebral cortex, putamen, and centrum semiovale were selected for electron microscopy, cut with an LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined with an RCA-G electron microscope.

RESULTS

Control infusion. The control infusions ranged from 19–21 h. During this period the animals attempted to free themselves from restraints, ate and drank eagerly, and vocalized. Except for rare moments of light sleep, they remained alert and active, and had normal EEGs. Pupillary light reflexes, extraocular movements, corneal responses, and plantar and palmar grasp reflexes were always present. There was no emesis, myoclonus, generalized or focal seizures, abnormal respiratory patterns, or cardiac arrhythmias. Baseline arterial ammonia concentrations in the control and experimental animals ranged from 0.14–0.22 mM, averaging 0.19 ± 0.03 mM (S.E.M.) (Table 1). The range of the control arterial ammonia values is comparable to that reported by Kline *et al.* (27) for normal adult rhesus monkeys. The initial respiratory alkalosis noted in all animals was probably due to the stress of restraint.

Experimental infusion. The experimental infusions lasted from 14–23.5 h (mean, 21.0 h) during which time the animals passed through four clinical stages, starting at baseline behavior (Stage 0). Stage I began at 0.25 h and lasted an average of 5 h (range,

Table 1. *Arterial ammonia concentration, blood pressure, $Paco_2$, pH, intracranial pressure, and EEG frequency during ammonia intoxication in the juvenile primate*[1]

	Behavioral stage				
	Baseline (12)	I (11)	II (11)	III (11)	IV (8)
Ammonium ion (mM)	0.19 ± 0.03	0.47 ± 0.05[2]	0.69 ± 0.03[2]	0.79 ± 0.06[2]	0.90 ± 0.08[2]
Mean arterial blood pressure (mmHg)	106 ± 4	110 ± 6	104 ± 2	106 ± 2	106 ± 3
$Paco_2$ (mmHg)	31 ± 1	28 ± 2	25 ± 2[4]	20 ± 3[3]	20 ± 2[3]
pH	7.51 ± 0.03	7.49 ± 0.04	7.50 ± 0.04	7.52 ± 0.05	7.56 ± 0.08
Intracranial pressure (mmH_2O)	76 ± 7	84 ± 3[4]	112 ± 7[2]	131 ± 10[2]	167 ± 12[2]
EEG (% activity ≤ 7 Hz)	21 ± 2	27 ± 4[2]	58 ± 6[2]	69 ± 5[2]	83 ± 6[2]

[1] Values are expressed as mean ± S.E.M. for the number of animals given in parentheses.
[2] The value is significantly different from the baseline value by the Mann-Whitney U test with $P < 0.001$.
[3] $P < 0.01$.
[4] $P < 0.05$.

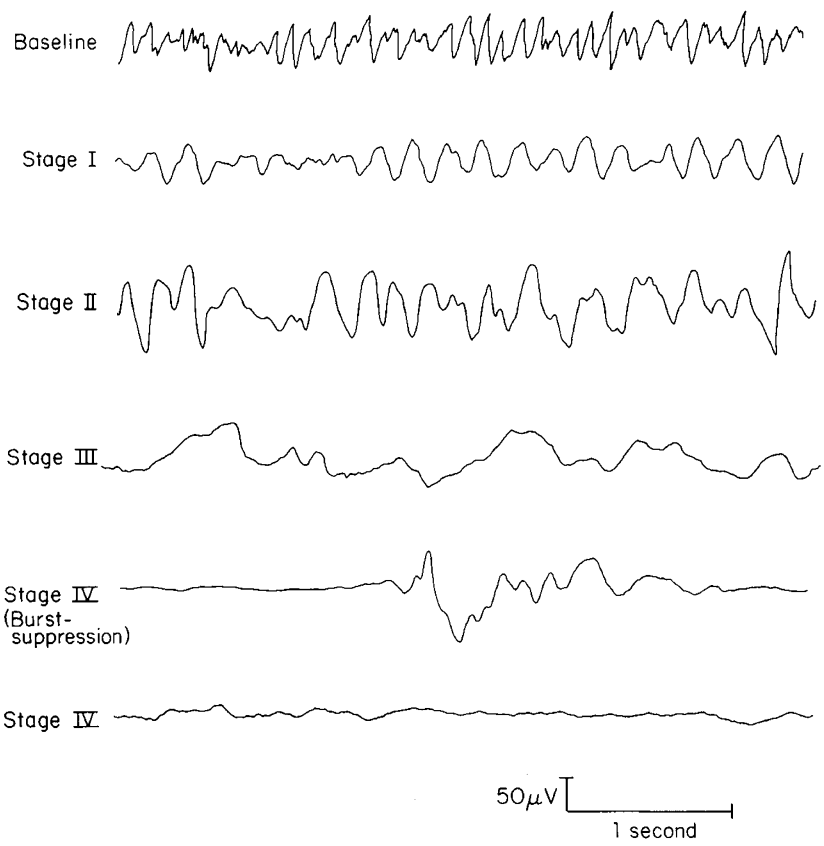

Fig. 1. Electroencephalogram recorded from the right parietal-occipital region demonstrates the progressive slowing that occurs at successive stages during an ammonium acetate infusion.

3.5–6.5 h). Animals in Stage I exhibited decreased spontaneous activity, disinterest in surroundings, lethargy, and recurrent vomiting. Mild hyperventilation and a slight rise in ICP occurred (Table 1). Arterial ammonia averaged 0.47 ± 0.05 mM during this phase, about twice the control values. Stage II lasted for approximately 9 h (range, 3.5–10.5 h) and was characterized by increasing lethargy alternating with brief periods of hyperactivity. Hyperventilation increased and mean ICP rose (Table 1). Arterial ammonia averaged 0.69 ± 0.03 mM.

Stage III, characterized by somnolence and loss of grasp reflex, lasted for approximately 4 h (range, 2.5–5 h). The arterial ammonia averaged 0.79 ± 0.06 mM; the $Paco_2$ fell to 20 ± 3 mmHg; and the mean ICP rose to 131 ± 10 mmH_2O (Table 1). Eight animals exhibited multifocal myoclonus without coincident EEG changes. Two animals in whom arterial ammonia levels transiently exceeded 1.0 mM had generalized seizures lasting less than 45 sec.

Eight animals, including the two that experienced generalized seizures, were comatose (Stage IV) for a mean of 4 h, during which time spontaneous movement and response to deep noxious stimuli disappeared. Corneal responses were depressed, oculocephalic responses became full and brisk, and pupils remained reactive. All eight comatose animals exhibited an apneustic respiratory pattern and three had short apneic spells. Arterial ammonia averaged 0.90 ± 0.08 mM. ICP averaged 167 ± 12 mmH_2O, and in three animals rose above 200 mmH_2O (Table 1). In all animals, mean arterial blood pressure remained above 100 mmHg, and Pao_2 above 80 mmHg. Blood electrolytes (Na^+, K^+, Cl^-, Ca^{++}, Mg^{++}, and phosphate), serum glucose, and indices of renal function (creatinine and blood urea nitrogen) similarly remained within control limits. These normal serum chemistries imply that significant hyperosmolality did not occur, presumably because acetate is rapidly metabolized, even in the presence of hyperammonemia (42). Indices of liver function, including bili-

rubin, total protein and globulin, and activities of lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase did not vary from control values during the infusions. The liver was examined microscopically in four experimental animals and was normal in each instance.

EEG. Frequencies of 7 Hz or less comprised 21 ± 2% of the control record whereas frequencies less than 4 Hz did not occur (Fig. 1). By Stage I, 37 ± 4% of the tracing was slower than 7 Hz and in the comatose animals, 83 ± 6% of the record was 7 Hz or less with 70% of the activity occurring at frequencies less than 4 Hz. EEG slowing correlated inversely with level of consciousness and directly with the elevated arterial ammonia concentration. In six of the eight animals that were in Stage IV, a burst-suppression pattern alternated with long periods of isoelectricity. The burst-suppression pattern on EEG was not associated with any behavioral changes. Triphasic waves, commonly reported in humans with hepatic encephalopathy (8), were not detected.

Neuropathology. Five of the eight animals in Stage IV showed a degree of brain swelling characterized by mild herniation of the cerebellar tonsils and flattening of the cortical gyri. Intracranial pressure in these five animals was significantly higher than that recorded in the Stage IV animals that did not exhibit tonsillar herniation (197 ± 7 mmH_2O *versus* 136 ± 12 mmH_2O, $P < 0.005$), and four of the five animals had shown burst-suppression activity on the EEG. Neither the duration of the ammonium acetate infusion nor the peak blood ammonia concentration differed significantly between animals with or without tonsillar herniation.

Light microscopic examination of paraffin-embedded sections were unremarkable; however, examination of 1-μ plastic-embedded sections showed astrocytic abnormalities confined to the grey matter. Perikarya and processes of the astrocytes were swollen and occasional clumps of coarse granules were noted in the expanded cytoplasm. The astrocytic nuclei were slightly vesicular and paired nuclei were seen. The cytoplasmic swelling was present in numerous astrocytes in the cerebral cortex, claustrum, and putamen, but was found only in occasional astrocytes in the globus pallidus and cerebellar cortex. Astrocytes within the white matter of centrum semiovale, external and extreme capsules, basal ganglia and cerebellum were unremarkable. One-micron plastic-embedded sections from control animals showed only occasional mild perivascular astrocytic swelling.

The ultrastructural appearance of control astrocytes (Figs. 2A and B) was normal and similar to previous reports (35). Astrocytic nuclei had evenly dispersed chromatin with a thin peripheral margin of condensed chromatin. The cytoplasm contained the usual organelles. Only occasional perivascular processes were dilated. In contrast, the ultrastructural appearance of astrocytes in the putamen and cortex of experimental animals showed swollen cytoplasm of the cell body and processes (Fig. 3A). The mitochondria appeared mildly pleomorphic, and membranous whorls were observed frequently both in the cell body and the

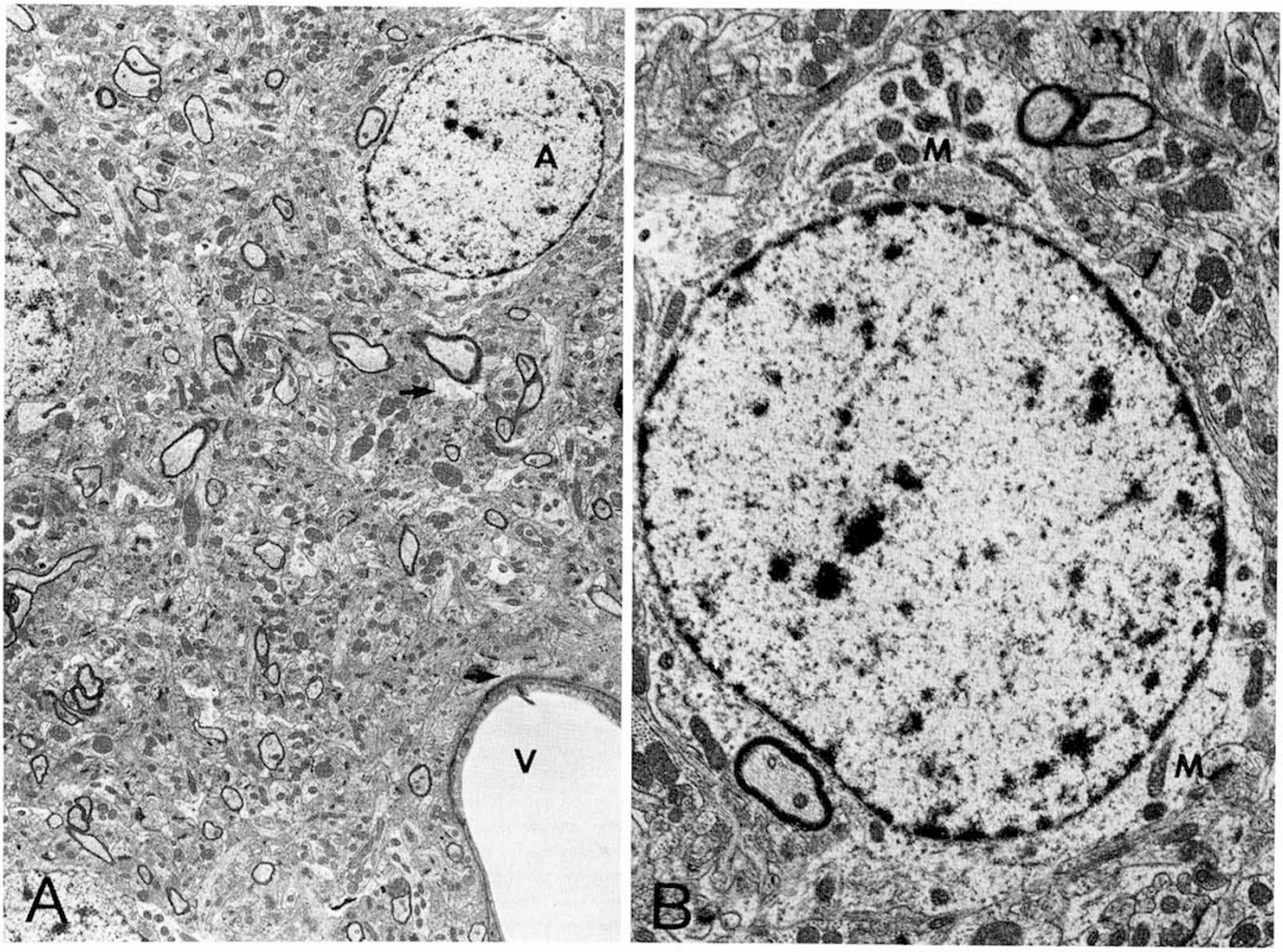

Fig. 2. A. *Control, putamen.* The astrocytic nucleus (*A*) is round and has a thin peripheral rim of condensed chromatin. Cytoplasm of the cell body is scant and the astrocytic processes within the neuropil and around the small blood vessel (*V*) are thin. *Arrows* indicate some of these processes (original magnification, ×2000). B Higher magnification of the astrocyte shows a thin rim of cytoplasm which contains scattered, round or elongated mitochondria (*M*) (original magnification ×5600).

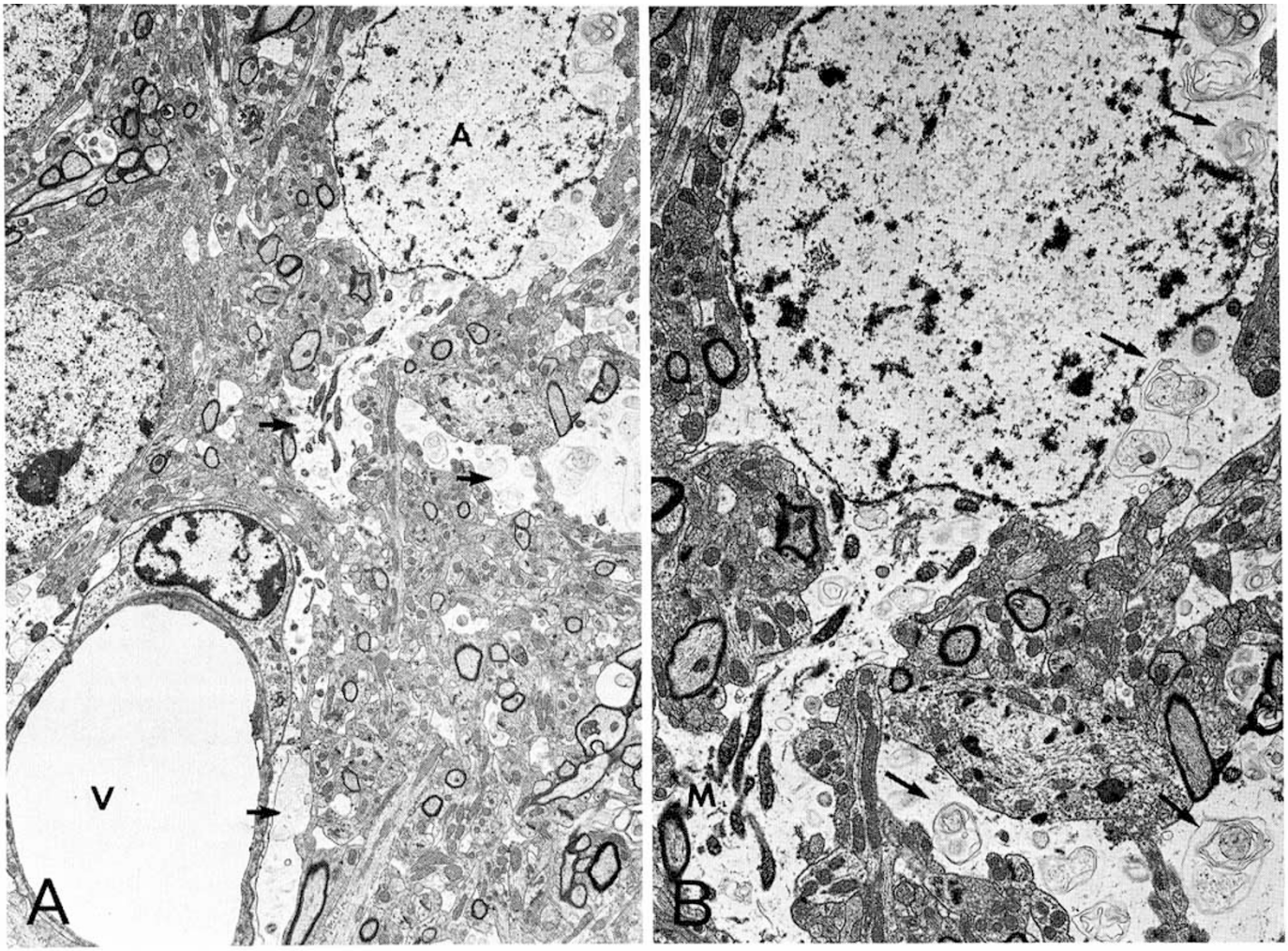

Fig. 3. A. *Experimental putamen.* The astrocytic nucleus (*A*) is enlarged and irregular, and the chromatin appears dispersed. The perikaryon and processes, including perivascular foot processes, are swollen. *Arrows* indicate some of these swollen processes. (Original magnification ×2000). B Higher magnification of the astrocyte shows swollen cytoplasm. Mitochondria (*M*) are slightly pleomorphic. Membrane whorls, some of which are indicated by arrows, are present in the perikaryon and processes (original magnification ×3800).

processes (Fig. 3B). Increases in RER were not noted. Astrocytic nuclei were enlarged and had a wavy, irregular outline. Neurons, axons, dendrites, and synapses appeared unremarkable.

DISCUSSION

These findings reproduce many aspects of the childhood hyperammonemic syndromes, without the complications of hypoxia, hypotension, hypoglycemia, and renal or hepatic failure. Vomiting, hyperventilation, EEG slowing, seizures, and coma characterize both the experimental and clinical setting. This confirms that ammonia exerts a specific toxicity on the immature nervous system.

The EEG changes we report add further physiologic evidence for this conclusion. The burst-suppression pattern is not frequently seen in adults with hepatic coma (8), but it almost always presages death in children with Reye's syndrome (4), usually due to cerebral herniation secondary to diffuse brain edema. In the present animals, the degree of brain-swelling was small, and unlikely to interfere with neurologic function. Other factors known to cause a burst-suppression pattern, particularly hypoxia, were absent; thus, hyperammonemia must be regarded as directly responsible for the neurophysiologic changes.

The mechanism of increased intracranial pressure in hyperammonemia has not been elucidated. One possibility may be a direct toxic effect on blood vessels with failure of cerebral autoregulation. Altenau *et al.* (2, 25, 26) induced raised intracranial pressure in mature primates and cats by infusion of ammonium acetate. These animals developed an increased cerebral blood volume and lost the normal hyperemic response to the inhalation of carbon dioxide. *In vitro* studies demonstrate that ammonium salts cause vasodilatation of human and rabbit cerebral arteries by inducing transient relaxation of the smooth muscle, independent of pH changes (3).

Another possible cause of increased intracranial pressure could be astrocytic swelling. Hyperammonemia has been shown to cause astrocytic changes in a variety of clinical and experimental circumstances, but in few instances have alterations been identified so early as in the present study. In individuals who die with chronic ammonia encephalopathy, the classic finding in postmortem or biopsy material fixed by immersion is the Alzheimer type II astrocyte, characterized by an enlarged vesicular nucleus with peripheral chromatin clumping (1, 11, 13). In perfusion-fixed brains of experimental animals, only minimal changes are visible by light microscopy, although more prominent alterations are seen with electron microscopy. In adult, hyperammonemic animals with either portal-systemic encephalopathy or methionine-sulfoximine intoxication (20, 33, 44), astrocytic changes consist of increased numbers of mitochondria and elevated amounts of RER and cytoplasmic glycogen. Partin *et al.* (34) have described similar astrocytic changes in biopsies from the brains of two children with Reye's syndrome; watery enlargement of the astrocytes was associated with increased numbers of organelles and cytoplasmic glycogen particles. At the time of bi-

opsy, both children were comatose and in one, the serum ammonia was seven times normal.

In the present study, hyperammonemia produced astrocytic changes similar to those previously described in experimental models of hepatic encephalopathy. The astrocytes had enlarged irregular nuclei and swollen cytoplasm containing pleomorphic mitochondria and membranous whorls. The proliferation of RER and mitochondria described in previous studies of hepatic encephalopathy were not present, perhaps reflecting the relatively short duration of ammonia intoxication.

The cause of the astrocytic pathology in syndromes of hyperammonemia remains uncertain. Zamora *et al.* (44) have suggested that the morphologic changes represent an increase in cerebral metabolic activity directed at the detoxification of ammonia. Glutamine synthetase and glutamate dehydrogenase, the enzymes primarily responsible for cerebral ammonia detoxification, are located within astrocytes (29, 32), and the cerebral uptake and conversion of ammonia to glutamine is increased in the presence of hyperammonemia (17). Alternatively, the astrocytic swelling may reflect an abnormal accumulation of monovalent ions in these cells. Astrocytes help to maintain Na^+ and K^+ homeostasis in the central nervous system via a membrane-bound Na^+,K^+-activated ATPase-dependent pump. At high concentrations, ammonium ions have been shown to promote intracellular Na^+, Cl^-, and water accumulation in brain tissue (7), possibly by altering the Na^+, K^+-activated ATPase activity of cell membranes (41).

The lack of pathologic change in the neurons in the present study is consistent with the reversibility of most acute, limited hyperammonemic syndromes, especially in children. When unassociated with other complications (*e.g.*, hypoglycemia, hypoxia, or hypotension), the prognosis for recovery from hyperammonemia with normal neurologic function is good (15, 40). Even in Reye's syndrome, when multiple metabolic abnormalities are present, the blood ammonia level on admission to hospital accurately predicts the outcome (23, 38). In addition, repeated episodes of hyperammonemic encephalopathy in children eventually lead to permanent neurologic dysfunction (22), and in adults, may contribute to the neuronal loss seen in non-Wilsonian chronic hepatocerebral degeneration (43). All evidence implies, therefore, that irrespective of cause, hyperammonemia should be treated aggressively.

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